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Development of new polymorphic microsatellite markers in topmouth culter (*Culter alburnus*) and determination of their applicability in *Culter mongolicus*

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ABSTRACT. Fifteen new polymorphic microsatellite markers were developed for *Culter alburnus*. In 32 individuals representing a wild population of the Danjiangkou Reservoir, Hubei, China, the number of alleles at these loci varied between 2 and 10, with an average of 5.5. The average observed and expected heterozygosities were 0.664 and 0.681, respectively. The polymorphism information content of 11 loci was more than 0.5 whereas that of the other 4 loci was less than 0.5 but more than 0.25. In addition, the genomes of 30 *C. mongolicus* individuals were successfully amplified with these primer pairs, indicating that the primer pairs were applicable for the related species, *C. mongolicus*.

Key words: *Culter alburnus*; *Culter mongolicus*; Microsatellite; Cross-species amplification

INTRODUCTION

Topmouth culter (*Culter alburnus* Basilewsky) belonging to the family Cyprinidae widely inhabits major rivers, lakes, and reservoirs of China (Luo and Chen, 1998). Among the various species of the genus *Culter*, topmouth culter has the longest body. For a long time, *C. alburnus*, *Trachidermus fasciatus*, Yellow River carp, and Songhua River salmon have been considered the four most famous fresh water fish species because of their delicious taste and tender meat (Wang, 2007). As one of the most important commercial freshwater fish species in China, the annual culturing yield of *C. alburnus* has continuously increased over the past decades to approximately 1500 tons in recent years. Previous studies have found that *C. alburnus* lays two types of egg: fish living in rivers lay floating eggs and those living in lakes and reservoirs lay sticky eggs (Cheng, 2006). However, many questions remain unclear, including the reasons for laying different types of eggs. We hypothesized that the genomes of fish inhabiting different environments are differentiated.

Microsatellites or simple sequence repeats (SSRs) have been widely used in deciphering population structures as well as determining parentage and kinship (O'Connell and Wright, 1997), constructing linkage maps, further understanding the genetic bases of important traits, and selecting elite varieties of diverse fish species (Brown et al., 2007; Guo et al., 2009; Wang et al., 2011). A few microsatellites are available for *C. alburnus* (Chen et al., 2009; Li et al., 2010), but unfortunately, the number of SSRs available for this species is limited. In this study, we identified 15 new SSR markers for *C. alburnus* and determined their applicability in *C. mongolicus*.

MATERIAL AND METHODS

Genomic DNA was isolated from the dorsal fin using the traditional proteinase K digestion and phenol-chloroform extraction method in combination with RNase treatment. The DNA was digested with *Tru*II into 400-1000-bp fragments, which were ligated with a *Tru*II adapter. The fragments were hybridized with a biotin-$(CA)_{12}$ probe and bound to streptavidin-coated beads (Dynal Biotech, Oslo, Norway). The eluted strands were amplified with adapter-specific primers, inserted into the pMD18-T vector (TaKaRa), and transformed into DH5α competent cells. Recombinant clones were screened for SSR-containing inserts, and positive clones were sequenced with the T7 primer using an ABI PRISM 3730 automated sequencer. After removing the vector sequences by comparison to vector sequences deposited in the GenBank (searched by BLAST on the NCBI website), the trimmed sequences were screened for microsatellites using SSR Hunter. Primers were designed on the basis of the identified SSRs and appropriate flanking regions by using PRIMER 3 (Rozen and Skaletsky, 2000). Using a subset of templates (5 fishes), the annealing temperature of each pair of primers was optimized.

The polymorphism of each microsatellite was assayed by 8% non-denaturing polyacrylamide gel electrophoresis with 32 specimens of *C. alburnus*, representing the wild population of the Danjiangkou Reservoir, and 30 individuals of *C. mongolicus*. The number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) were calculated with POPGENE 1.32 (Yeh and Boyle, 1997). MICRO-CHECKER (Van Oosterhout et al., 2004) was used to infer the most probable technical cause of Hardy-Weinberg equilibrium (HWE) departures, and the significance was adjusted using the sequential Bonferroni's correction (Rice, 1989). The polymorphism information content (PIC) was calculated by the Botstein method:

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

where q_i and q_j represent the allele frequencies of the i^{th} and j^{th} alleles, respectively, and n is the number of alleles (Botstein et al., 1980).

RESULTS AND DISCUSSION

In all, 58 sequences containing microsatellites were obtained in this study. Thirty-five sequences contained appropriate flanking regions, and from these sequences, 35 microsatellite markers (35 pairs of primers) were designed. The genomes of 32 *C. alburnus* individuals (representing the wild population of the Danjiangkou Reservoir) were successively amplified with 24 designed primer pairs. Polymorphism was detected in *C. alburnus* with 15 primer pairs (Table 1) by using the optimized annealing temperatures listed in Table 1.

Table 1. Characterization of 15 polymorphic microsatellite markers in *Culter alburnus*.

Locus	Accession No.	Primer sequence (5'-3')	Repeat motif	Ta (°C)	S (bp)
QZ194	JQ965647	F: CCCATCCAAGTTGGTGTGTA R: TCAGCAGCTCAGAAATCGAGAA	$(CA)_{45}$	62	198-256
QZ91	JQ965648	F: CTCCTGCATTTTTTTCCACTTGC R: TTCTTAGAGAAGAGGCAGCGG	$(TC)_{10}$	59	285-335
QZ126	JQ965649	F: CATTTTCAGTTCACAGTCCAAGA R: CATTACAGACGCATGAGCAACA	$(GT)_{12}GA(GT)_{21}$	63	249-301
QZ150	JQ965650	F: TACCACTGGAACCACAGTCTCCTG R: AATGACATCGCTCTCCTGCAA	$(TG)_{15}$	65	258-296
QZ188	JQ965651	F: CACAAACACATTCACAATTCTGGG R: GTCAATCAAAAACACTGACATTT	$(GT)_{7}$	64	259-281
QZ68	JQ965652	F: GTGATCATGAAAACTGACTGAAC R: CGAGCGTGAAGATGTACAGAATGA	$(TG)_{10}G(TA)_{5}$	64	218-256
QZ73	JQ965653	F: CTGAAGGTTATATTTGGGGTGAG R: TCAGCAGATAAAGTAGAGAGGTCA	$(TG)_{10}\ldots(AG)_{11}$	65	224-318
QZ133	JQ965654	F: TAAAACGAGGGAGGCATGAAGA R: GTATGCGATATACGGTCATTCACC	$(GT)_{17}$	62	285-309
QZ175	QZ175	F: CACCGACGGCACAGACAT R: TGTTACCGCTGAAAACACACA	$(TA)_{5}\ldots(TG)_{33}\ldots(AC)_{5}AT(AC)_{9}$	67	255-367
QZ214	JQ965656	F: AGCGGTTGAGGAGCTGTATTA R: TCCCTGAATGTTGTAAGACCA	$(CA)_{7}$	59	308-330
QZ11	JQ965657	F: TTCATTACGGTCGAACCACC R: ATGCGGATGGTTCCTGGATAA	$(GT)_{7}GC(GT)_{9}$	65	186-212
QZ119	JQ965658	F: GCAAAGAGGGGTTCAGTGAAT R: TGATCTGCTCTGATGCTGGAT	$(GT)_{6}$	65	174-228
QZ204	JQ965659	F: TCGATCAGACTGTCCATGGGT R: TGATTGGCAACAGCTGGAGA	$(AC)_{10}$	65	128-225
QZ94	JQ965660	F: TCACCCGTGGGTCTCTGAAAT R: AGTGCTCTAAGCTGAGCGGA	$(CA)_{51}$	60	224-316
QZ19	JQ965661	F: CAGGCTCCATATAATGACAATGA R: TGACACGTGTTTGGGTAATGACTG	$(AC)_{11}\ldots(CT)_{12}$	63	234-286

Ta = annealing temperature; S = allele size range.

The N_A at the 15 polymorphic microsatellite loci ranged from 2 to 10 with an average of 5.5. The values of H_O and H_E ranged from 0.418 to 0.913 (average of 0.664) and from 0.457 to 0.912 (average of 0.681), respectively (Table 2). Significant deviation from HWE was observed

at *QZ91*, *QZ188*, and *QZ11*. No significant linkage disequilibrium was found among the 15 polymorphic loci (P < 0.003). The PIC of 11 of the loci was more than 0.5, and the PIC of the remaining 4 loci (*QZ91*, *QZ133*, *QZ11*, and *QZ119*) was less than 0.5 but more than 0.25.

Table 2. Statistics of the loci developed in this study.

Locus	*Culter alburnus*				*Culter mongolicus*			
	N_A	H_O	H_E	PIC	N_A	H_O	H_E	PIC
QZ194	6	0.685	0.661	0.682	8	0.688	0.747	0.621
QZ91	4	0.563	0.628	0.265*	7	0.478	0.546	0.598
QZ126	9	0.897	0.912	0.813	6	0.944	0.617	0.527
QZ150	7	0.732	0.748	0.714	5	0.378	0.428	0.395
QZ188	3	0.431	0.485	0.546*	5	0.673	0.735	0.564
QZ68	4	0.574	0.561	0.615				
QZ73	9	0.831	0.858	0.735	8	0.768	0.835	0.724
QZ133	3	0.643	0.677	0.425	4	0.482	0.568	0.576
QZ175	8	0.798	0.763	0.675	6	0.845	0.728	0.735
QZ214	3	0.418	0.457	0.586				
QZ11	2	0.513	0.564	0.328*				
QZ119	2	0.485	0.474	0.352	3	0.375	0.476	0.654*
QZ204	10	0.913	0.908	0.885	6	0.875	0.748	0.824
QZ94	8	0.846	0.877	0.713	5	0.568	0.497	0.726
QZ19	5	0.624	0.643	0.617	6	0.673	0.728	0.619

N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content. *Deviation from Hardy-Weinberg equilibrium (P < 0.03) after Bonferroni's correction.

The genomes of 30 *C. mongolicus* individuals were successively amplified with the 15 primer pairs that detected polymorphisms in *C. alburnus* (Table 2). The number of alleles per loci ranged from 3 to 8 (average of 5.8). The value of H_E ranged from 0.428 to 0.835 (average of 0.646), and the value of H_O varied between 0.375 and 0.944 (average of 0.638). The microsatellites developed in this study are applicable for assessing the genetic diversity and genetic structure of both *C. alburnus* and *C. mongolicus*, and they facilitate the identification of *C. alburnus* that lay different types of eggs.

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