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Development of polymorphic expressed sequence tag-single sequence repeat markers in the common Chinese cuttlefish, *Sepiella maindroni*

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ABSTRACT. The common Chinese cuttlefish (*Sepiella maindroni)* is one of the popular edible cephalopod consumed across Asia. To facilitate the population genetic investigation of this species, we developed fourteen polymorphic microsatellite makers from expressed sequence tags of *S. maindroni.* The number of alleles at each locus ranged from 6 to 10 with an average of 7.9 alleles per locus. The ranges of observed and expected heterozygosity were from 0.615 to 0.962 and 0.685 to 0.888, respectively. Four loci were found deviated significantly from Hardy-Weinberg equilibrium. The polymorphism information content ranged from 0.638 to 0.833. These polymorphic microsatellite loci will be helpful for the population genetic, genetic linkage map, and other genetic studies of *S. maindroni.*

Key words: Expressed sequence taq; Microsatellite marker; Common Chinese cuttlefish; *Sepiella maindroni*

INTRODUCTION

The common Chinese cuttlefish *Sepiella maindroni* is distributed widely along the coast of Eastern Russia, Japan, North Korea, China, Malaysia, and Philippine Islands (Zheng et al., 2001; Wu et al., 2006). As a popular edible cuttlefish, it is one of the most high-productive economic cephalopod harvested from the sea. Basic research, including physiology, reproductive biology, and embryology, of this species has been performed (Jiang et al., 2011; Wu et al., 2012). Further, the investigation of the medicinal value of the ink extracts of *S. maindroni* is in progress (Liu et al., 2008). However, only limited information is available regarding the genetic diversity and population structure of this species (Zheng et al., 2003), which is very important for the conservation and sustainable exploitation of *S. maindroni* resources.

Microsatellite or simple sequence repeat (SSR) markers represent one of the most powerful tools for the analysis of population structure and genetic diversity due to their genetic co-dominance, relative abundance, multi-allelic variation, and high reproducibility (Rajwant et al., 2011). To date, 10 microsatellite markers from *S. maindroni* have been reported by Wu et al. (2010). Additional loci will be needed to benefit the research of population genetics, genetic diversity, and genetic linkage map construction.

The SSRs derived from expressed sequence tag (EST) have many intrinsic advantages over genomic microsatellites for their higher transferability among related species, lower cost for development, and higher proportion of high-quality markers (Varshney et al., 2005). In this study, we developed 14 highly polymorphic microsatellite markers from ESTs of *S. maindroni* that will be useful for genetic research of this species.

MATERIAL AND METHODS

In all, 9439 EST sequences of *S. maindroni* were obtained, of which 899 sequences were downloaded from the GeneBank and 8540 were collected from the juvenile cDNA library constructed by our laboratory (data unpublished). Putative EST-SSR markers were screened using the SSRHUNTER 1.3 software (http://www.biosoft.net/dna/SSRHunter.htm). The criteria used in SSRHUNTER to identify microsatellites were as follows: 5 repeats for di-, tri-, and tetranucleotide repeats. Primers flanking microsatellites were designed using the PRIMER 5.0 program (http://www.premierbiosoft. com/).

Polymorphism evaluation was tested using 30 wild individuals of *S. maindroni* collected from Liuheng, Zhejiang Province, China. Genomic DNA of each specimen was extracted from the muscle tissue by using a genomic DNA extraction kit (BioTeke, Beijing, China) following manufacturer protocols. Polymerase chain reaction (PCR) was performed in 10-μL volumes containing 5 μL 2x Power Taq PCR Master Mix (BioTeke), 1 μM each primer set, and about 100 ng template DNA. PCR was performed on a Master-cycler gradient thermal cycler (Eppendorf) with the following program: 3 min at 94°C; 35 cycles of 1 min at 94°C, annealing (see Table 1 for annealing temperatures) for 1 min, 72°C for 1 min per cycle; followed by 5 min at 72°C. Amplification products were resolved on 8% denaturing polyacrylamide gel and visualized by silver staining. A 10-bp DNA ladder (Invitrogen Inc.) was used as a reference marker for allele size determination.

Allele number (N_A), polymorphism information content (PIC), expected and observed heterozygosities (H_E and H_O, respectively) were analyzed using the CERVUS 3.0 software (Kalin-

owski et al., 2007). Tested for linkage disequilibrium (LD) and deviations from Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP 4.0.10 (Raymond and Rousset, 1995). Sequential Bonferroni corrections (Rice, 1989) were applied for all multiple tests ($P < 0.05$).

Table 1. Characterization of 14 polymorphic microsatellite loci in *Sepiella maindroni.*

Locus	Repeat motif	Primer sequence (5'-3')	T_A (°C)	N_A	Expected size (bp)	H_O	H_E	PIC	P_{HWE}	Accession No.
SMA6	$(AAG)_{21}$	F: TCTGCGATTAGAGGTAGAGGG R: GGTATAGACGGTGGAGGAGG	56	7	270-291	0.800	0.776	0.730	0.560	KC478084
SMA8	$(GAT)_{20}$	F: TGTTCATTGTGGGTTGCT R: ATCGTTAAAAACAATATCATAGTCA	53	7	310-340	0.920	0.827	0.783	0.054	KC478087
SMA10	$(AAG)_{20}$	F: TGCCAAAGGTGGTGAGAA R: TTGCAGCATCTACCAATACG	55	8	207-237	0.960	0.803	0.759	0.002*	KC478086
SMA20	$(TA)_8$	F: GTTAGCAGCCGGAGTGTT R: AAGTCAGTCTCGCTTGGT	52	6	270-290	0.720	0.718	0.671	0.622	GT618356
SMA21	$(CTC)_7$	F: TTCTCCGCCTCATTCACC R: CGATAGTAACCCGTCATTTT	55	6	187-205	0.760	0.788	0.740	0.646	GT618547
SMA36	$(AGC)_5$	F: AGTAGAGTTGCTATTAAGCAGG R: GCAGAACCCAAGAAACGA	53	6	467-494	0.680	0.685	0.638	0.629	GT618088
SMA39	$(TC)_{14}$	F: GGGGAAGAAATCTCAGGAACA R: GTAACAATGGCCGTGATGAA	57	9	212-270	0.654	0.857	0.822	0.004	KC869652
SMA40	$(AC)_{14}$	F: ATGTTGAAACCAAGTAGATGCA R: TATTTTCTTTTGGCGGGAG	56	10	185-220	0.962	0.800	0.756	0.564	KC869653
SMA41	$(AC)_{14}$	F: CAGATGACCCCTTGAAATGA R: CACCCAGGATGATGACTATTTT	56	10	130-166	0.880	0.862	0.828	0.003*	KC869654
SMA43	$(CA)_{13}$	F: AGTTGACGCAGGAGAAAGTGT R: CAATTCCACAAGCAAACCAT	57	8	108-138	0.833	0.888	0.833	0.000*	KC869655
SMA47	$(TATC)_{13}$	F: TTCAACCTTTATGACCGACTA R: CAGGTAGGTGTACGAGCAAA	53	6	176-204	0.789	0.797	0.743	0.030	KC869657
SMA54	$(TAC)_{12}$	F: AGGTGGGGTCCTCATCTGTT R: TGTTCCTGCCTGATAAAAGCA	58	9	173-197	0.850	0.850	0.808	0.000*	KC869656
SMA55	$(ACT)_{12}$	F: TGAAAAGAGGATGGAAGAGACT R: TGTTATTACCACAGAGCAGGA	54	10	135-168	0.615	0.852	0.818	0.011	KC869658
SMA58	$(CA)_{12}$	F: CACACCAAATTCTAACCTTCA R: GTTTCCTGTTAAATTGATATGAGT	53	8	190-216	0.800	0.819	0.775	0.980	KC869659

T_A = annealing temperature; N_A = observed number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content. *Significant deviation from HWE ($P < 0.05$).

RESULTS AND DISCUSSION

A total of 1219 microsatellite-containing EST sequences were detected from 9439 ESTs of *S. maindroni*. Primers were designed for 58 microsatellites because of repetition times and flaking sequence priority. Twenty-eight of the 58 primers were successfully amplified, and 14 loci were shown to be polymorphic in *S. maindroni* (Table 1).

The number of alleles at each locus ranged from 6 to 10 with an average of 7.9 alleles per locus. The ranges of H_O and H_E were from 0.615 to 0.962 and 0.685 to 0.888, respectively. The PIC ranged from 0.638 to 0.833. Significant departure from HWE was found at 4 loci after correction for multiple tests, and no significant linkage disequilibrium was found between all these loci.

The EST-SSR markers developed in this study will facilitate the elucidation of the population structure, genetic diversity, and genetic linkage map of *S. maindroni*.

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