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Isolation and characterization of new polymorphic microsatellite markers from the cuttlefish *Sepiella maindroni* (Cephalopoda; Sepiidae)

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ABSTRACT. Fifteen new polymorphic microsatellite loci were developed for the cuttlefish *Sepiella maindroni*. In 32 individuals from a wild population of coastal Ningde, Fujian Province, China, the number of alleles at these loci varied between 2 and 12, with an average of 5.86. The mean observed and expected heterozygosities were 0.6917 and 0.5993, respectively. Among these polymorphic microsatellite loci, 4 (SM2, SM19, SM40, and SM81) significantly deviated from Hardy-Weinberg equilibrium after sequential Bonferroni's correction. All of them were in linkage equilibrium. These microsatellite loci would be useful for evaluating the effect of releasing on extant *S. maindroni* populations as well as for investigating genetic diversity and population structure of this species.

Key words: *Sepiella maindroni*; Microsatellite DNA; Stock enhancement; Genetic diversity

INTRODUCTION

Sepiella maindroni de Rochebrone (Cephalopoda, Sepiidae) is the only cuttlefish species native to coastal China (Dong, 1988). The species is naturally distributed along the coast waters of Eastern Russia, Japan, South Korea, and China (Nesis, 1987; Dong, 1991; Okutani, 1995). The value of cuttlefish used as food and officinal is quite high, and thus, cuttlefish is an important and valuable fishery species and considered 1 of the 4 most famous marine products in China. Unfortunately, the wild population has dramatically declined and become nearly extinct presumably due to over exploitation (Wu et al., 2006). Strategies for the appropriate conservation and sustainable exploitation of this species have to be developed soon. Our research team is making efforts for enhancement of the stocks of this valuable cuttlefish to recover resources. Data reported by Dong (2010) indicated that the *S. maindroni* population apparently recovered. We found no morphological difference between wild and released *S. maindroni*. It is difficult to analyze how effective the supplement is, and therefore, evaluation of the effect of release is the key issue. Microsatellite DNA loci were shown to be highly polymorphic and useful molecular markers in population genetics (O'Connell and Wright, 1997) and parentage assignment (Jackson et al., 2003; Vandeputte et al., 2004). Even though microsatellite markers are available for *S. maindroni* (Wu et al., 2010), these loci are not enough for carrying out a population genetic study and kinship analysis of *S. maindroni* on a large scale. In this report, we described the development and characterization of 15 novel microsatellite loci to evaluate the enhancement effect on this valuable cuttlefish.

MATERIAL AND METHODS

Genomic DNA was isolated from mantle muscles using a traditional proteinase-K digestion and phenol-chloroform extraction method in combination with RNase treatment. The DNA was digested with Tru II into 400- to 1000-bp fragments, which were ligated with Tru II adaptor. The fragments were hybridized with biotin-$(CA)_{12}$ probe and bound to streptavidin-coated beads (Dynal Biotech). The eluted strands were amplified with adapter-specific primer, inserted into pMD18-T vector (TaKaRa) and transferred to DH5α competent cells. Recombinant clones were screened for the existence of SSR-containing inserts with positive clones sequenced with T7 primer using an ABIPRISM 3730 automated sequencer. After the vector sequences were removed, the remnant sequences were screened for microsatellites with SSR Hunter, and primers were designed from those containing SSR and appropriate flanking regions with PRIMER 3 (Rozen and Skaletsky, 2000). With a subset of templates (5 cuttlefish individuals), the annealing temperature of each pair of primers was optimized.

The polymorphism for each microsatellite was assayed with 32 individuals of *S. maindroni* representing a wild population of coastal Ningde, Fujian Province, China. Electrophoresis was carried out using 8% non-denaturing polyacrylamide gels. The number of alleles (N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated with the POPGENE 1.32 software (Yeh and Boyle, 1997). MICRO-CHECKER (Van Oosterhout et al., 2004) was employed to infer the technical probability of departure from Hardy-Weinberg equilibrium. Statistical significance was adjusted using sequential Bonferroni's correction (Rice, 1989). Polymorphism information content (PIC) was calculated according to Botstein et al. (1980) using 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 frequency of the i[th] and j[th] alleles, respectively, and n is the number of alleles.

RESULTS AND DISCUSSION

In total, 62 sequences containing microsatellites were obtained in this study. Forty-one of the sequences contained appropriate flanking regions, from which 37 microsatellite markers (37 primer pairs) were designed. The 32 individuals of *S. maindroni* representing a wild population from the coast of Ningde were successfully amplified using the 37 primer pairs. Fifteen primer pairs detected polymorphism in *S. maindroni*. Their optimized annealing temperatures are listed in Table 1.

Table 1. Characterization of 15 polymorphic microsatellite markers in *Sepiella maindroni*.

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Size (bp)	N_A	H_O	H_E	PIC
SM2*	F: ACTTTGGCCCTCTCCCTCTA R: GGAAATGTGGGAAGGTTGAA	$(CA)_{16}$	60	158-216	9	0.9375	0.8953	0.6210
SM 3	F: GTATGTGCGTGCGTTCGTAT R: TTATTCACCGCTGAGAAACG	$(TG)_{29}$	62	235-315	7	0.7500	0.7450	0.6944
SM 5	F: AGCGTGCACCCTTACACAC R: GTCCCGTGCTTCGATTTTTA	$(GT)_{10}$	60	228-298	3	0.2500	0.2297	0.2533
SM9	F: CACAGACACGCACTACAAAAA R: CAGGGATTTCCACCAAACTC	$(CA)_9$	60	258-296	4	1.0000	0.6329	0.5520
SM19*	F: TGCTTTTCATTTCCGCTACA R: TGGCTGACTCACTTGACCTG	$(GT)_7$	59	217-261	2	0.2812	0.2455	0.2524
SM28	F: GATGGCGTCTATTCCGTTTG R: CTACCGTTTGCGATGGATTT	$(TG)_{10}$	60	218-256	3	0.1250	0.2297	0.2633
SM31	F: TACGTCCTGAAGGGATACGG R: TCTAGACCCCCGCCTTTAGT	$(AAC)_7$	60	204-268	5	1.0000	0.6612	0.4126
SM38	F: CTCACACTTCTCCCCCTCTTT R: AATGGATCGAGAGGAAAGGA	$(TC)_{13}$	60	150-226	6	0.8125	0.5967	0.5429
SM40*	F: TTTGCACCAGGCCTCTGTAT R: TAAACAAGACGCGCACGATA	$(CA)_9$	60	338-367	4	1.0000	0.5079	0.3750
SM53	F: CTTTCTTTTTCTCTCACACC R: GCGTGTGTGTTTGAGTGTTTG	$(CA)_{45}$	59	152-190	4	0.6250	0.6974	0.5243
SM66	F: GAGTGAGAAAGCGAGGGAGA R: TAACAGAGCGAGCGACACAG	$(TG)_{46}$	56	186-218	6	0.5000	0.3810	0.3047
SM81*	F: TATACACCTGCGTGCGAGAA R: CATGCAGAGAGAGCGCAATA	$(GT)_6$	53	174-228	5	0.1875	0.5898	0.4948
SM82	F: ATGGCGACAAAGATGAGGAC R: CACGTGCATATTCACACACG	$(TG)_{30}$	62	158-280	10	0.9375	0.8805	0.8529
SM85	F: CGTTTCATCCCACCAACATA R: TCAGTTCGACATTCCAACCA	$(TG)_{15}$	60	224-316	8	0.9688	0.8105	0.7735
SM87	F: TCGCCTTCTTTAGTGTACCTGT R: GCCCCACCCCACTAAAAA	$(TG)_{23}$	53	150-286	12	1.0000	0.8869	0.8615

Ta = annealing temperature; Size = allele size range; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content. *The locus deviates from HWE.

The N_A at 15 polymorphic microsatellite loci ranged from 2 to 12, with an average of 5.86. H_O and H_E ranged from 0.1250 to 1.0000 (average of 0.6640) and from 0.2297 to 0.8953 (average of 0.5993), respectively (Table 1). Significant deviation from Hardy-Weinberg equi-

librium was observed at SM2, SM19, SM40, and SM81. No significant linkage disequilibrium was found at the 15 polymorphic loci. The PIC of 8 loci was more than 0.5, and that of the other 7 was between 0.25 and 0.50. These microsatellite loci could be useful for evaluating the effect of release on *S. maindroni*, as well as investigating its genetic diversity and population structure.

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