



Isolation and characterization of novel polymorphic microsatellite markers for *Epinephelus akaara*

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ABSTRACT. In the present study, ten novel microsatellite markers were developed from an enriched-(CA)₁₃ genomic library of *Epinephelus akaara*. The mean number of alleles per locus was 21.6, with a range of 12 to 33. Observed heterozygosity ranged from 0.767 to 0.967, and expected heterozygosity ranged from 0.831 to 0.975, with mean values of 0.877 and 0.923, respectively. Among the ten loci, three loci deviated from Hardy-Weinberg equilibrium after sequential Bonferroni's correction. These polymorphic microsatellite markers may be useful for studies on the population genetics of *E. akaara*.

Key words: Microsatellite marker; *Epinephelus akaara*

INTRODUCTION

Epinephelus akaara (Red-spotted grouper) belongs to the subfamily *Epinephelinae*, and mainly inhabits temperate and subtropical regions of the western Pacific Ocean (Watanabe et al., 2011). Due to its delicious flavor and high market demand, *E. akaara* is recognized as one of the major economically important marine fish species in the Asia-Pacific region. In recent years, wild populations of *E. akaara* have largely declined through overfishing and habitat destruction. However, there is little information about the genetic diversity and population structure of this species. Microsatellite markers are highly polymorphic and have been widely used in genome mapping and population genetic studies in fish species (Yue, 2014). Therefore, development of a sufficient number of polymorphic microsatellite markers to enable assessments of genetic diversity and linkage map construction in *E. akaara* are essential. In this study, our aim was to isolate and characterize polymorphic microsatellite loci in *E. akaara* to enable further investigation of the population genetic structure of this species.

MATERIAL AND METHODS

Thirty individuals of *E. akaara* were collected from the Fisheries Research Institute of Fujian, Xiamen, China. Genomic DNA was extracted from fin tissue using the salting-out procedure (Howe et al., 1997). A genomic library enriched for CA repeats was developed as described by Xie et al. (2014). Briefly, genomic DNA was digested with the MseI restriction enzyme and ligated to MseI adaptors, oligo A (5'-TACTCAGGACTCAG-3'), and oligo B (5'-GACGATGAGTCCTGAG-3'), using T4 DNA ligase (New England Biolabs, USA).

DNA fragments ranged in length from 400 to 1000 bp and were subsequently amplified with adaptor-specific primers (5'-GATGAGTCCTGAGTAAN-3', MseI-N). PCR products were hybridized with biotin-labeled (CA)₁₃ probes. Streptavidin-coated magnetic beads (Promega, USA) were used to selectively capture sequences containing TG repeats. The specific DNA was eluted from the beads and then the eluted DNA was amplified again. After purification using the Wizard PCR clean-up system (Promega), DNA products were cloned into the pMD18-T vector (TaKaRa, Japan) and transformed into *Escherichia coli* DH5 α competent cells. Positive clones were randomly selected and sequenced using an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, USA).

Primer pairs were designed using the online software PRIMER 3 (<http://www.frodo.wi.mit.edu/>). PCR amplification was performed in a 20 μ L reaction volume containing 1 μ M each primer, 10X PCR buffer, and 100 ng template DNA. PCR conditions consisted of initial denaturation at 94°C for 5 min, 35 cycles of 45 s at 94°C, 40 s at the locus-specific annealing temperature (Table 1), 40 s at 72°C, and a final step of 5 min at 72°C. Amplified products were separated on an 8% polyacrylamide gel and visualized using silver staining.

Allele size was estimated according to the pBR322 DNA/MspI marker (Tiangen, China). The number of alleles at each locus, expected and observed heterozygosities, together with deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium, were calculated using GENEPOP 4.0 (Raymond and Rousset, 1995). Null allele frequencies were calculated using MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). Significant values for all multiple tests were corrected using the sequential Bonferroni procedure (Rice, 1989).

Table 1. Characteristics of ten microsatellite loci for *Epinephelus akaara*.

Locus	Accession No.	Repeat motif	Primer sequences (5'-3')	N_A	Size range (bp)	Ta (°C)	H_O	H_E	P_{HWE}
MD-1*	KP644309	(CA) ₄₀	F: GGCTGTCACTGCAACTGAAG R: ACAAAGTTGCAATCCACCACT	24	150-214	55	0.800	0.956	0.012*
MD-2	KP644310	(GT) ₂₇	F: GAGAACTGCTGTCTAGGAATGT R: TTGGAAGTGGAGACTCGACC	29	150-264	55	0.933	0.897	0.579
MD-3	KP644311	(GT) ₂₆	F: AGTGTGGTCTGCGTGATGTA R: GCTTCCGCTCTGTCTCATTG	20	117-189	56	0.867	0.967	0.137
MD-4	KP644312	(GT) ₄₆	F: GACCAGAGCCAAACCCCAA R: AATGTTCAAAGACACATTGCGCA	21	132-210	55	0.867	0.912	0.524
MD-5*	KP644313	(CA) ₁₇	F: AGGCTTCCTTTGACTGCACT R: CGTGGTTCGTGTTTGTGTTA	23	162-222	56	0.767	0.890	0.017*
MD-6	KP644314	(AC) ₂₇	F: GTCCTGAGTAAGCCAGAGG R: AGTAACGTGAGCAACATGGC	21	133-195	56	0.967	0.948	0.310
MD-7	KP644315	(CA) ₂₇	F: ACTTGCAAGTAAACAGGGT R: AGGAGCTTCATGTCTCTGCA	19	145-215	55	0.967	0.948	0.768
MD-8	KP644316	(TG) ₁₆	F: AGTGGAGCCGACAAGTACAT R: TTTGGAGTGTGACCCCTGACC	14	183-221	56	0.900	0.831	0.925
MD-9	KP644317	(GT) ₁₄	F: GAGAACTGCTGTCTAGGAATGT R: TTGGAAGTGGAGACTCGACC	33	180-264	56	0.833	0.902	0.447
MD-10*	KP644318	(GT) ₂₆	F: AGCCATGCATGAACACACAG R: GTTGAAGCATGTGACCCTG	12	177-201	54	0.867	0.975	0.036*

Ta = optimized annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HWE} = Hardy-Weinberg probability. *Locus deviated from Hardy-Weinberg equilibrium (adjusted P value < 0.036). *Locus may harbor null alleles (null allele frequency >5%).

RESULTS AND DISCUSSION

In this study, 10 polymorphic microsatellite loci were successfully isolated from *E. akaara*. The mean number of alleles per locus was 21.6 and ranged from 12 to 33. The observed heterozygosity ranged from 0.767 to 0.967, and the expected heterozygosity ranged from 0.831 to 0.975, with mean values of 0.877 and 0.923, respectively (Table 1). Among the ten loci, three loci (MD-1, MD-5, MD-10) deviated from HWE ($P < 0.05$) after sequential Bonferroni correction (adjusted P value < 0.036), likely due to the presence of null alleles.

The present study provides 10 novel, highly polymorphic microsatellite markers that can be employed to investigate the population genetics of *E. akaara*.

Conflicts of interest

The authors declare no conflict of interest.

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