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TECHNICAL NOTE

Isolation and characterization of eleven microsatellite loci in small abalone, *Haliotis diversicolor* Reeve

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Abstract Eleven novel microsatellite markers were isolated from small abalone, *Haliotis diversicolor* Reeve. These loci were tested on 22 individuals from two different geographic populations. We identified a total of 162 alleles from the 11 microsatellite loci. All of the loci were highly polymorphic. Polymorphism information content (PIC) is ranging from 0.7276 to 0.9163. Observed and expected heterozygosities ranged from 0.2727 to 1.0000 and from 0.7738 to 0.9429, respectively. Three loci deviated significantly from Hardy–Weinberg equilibrium. No pairs of loci displayed linkage disequilibrium. These polymorphic markers will be used to analyze population structure, genetic diversity and construct a genetic linkage map.

Keywords Magnetic beads enrichment · Microsatellite markers · *Haliotis diversicolor* Reeve · Genetic linkage map

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Introduction

Small abalone (*Haliotis diversicolor* Reeve) is a high-valued shellfish in China, and has been playing an economically important role in aquaculture industries of China in recent years. Culture and ecology of *H. diversicolor* have been frequently studied (Zhongbao and Changsheng 2004), but genetic markers for population assessment is lacking. Here, we describe 11 microsatellite loci isolated from *H. diversicolor*, which will be used to assess population structure, gene flow, and genetic diversity of this species.

Materials and methods

Foot muscles were taken from six abalones. Genomic DNA was extracted with the Genomic DNA Mini Preparation Kit (Beyotime, China). The six genomic DNA samples were pooled and fragmented by sonication. After agarose gel electrophoresis, fragments of 750–1,000 bp were excised and recovered using the QIAquick Gel Extraction Kit (Qiagen, USA).

The purified fragments were mixed with SNX linker (Hamilton et al. 1999) (SNX forward, 5′-CTAAGGCCTT GCTAGCAGAAGC-3′ and SNX reverse, 5′-pGCTTCTGC TAGCAAGGCCTTAGAAAA-3′) and T4 DNA ligase (NEB, USA) for 16 h at 14°C. The ligation products were examined by polymerase chain reaction (PCR) using the SNX forward sequence as primer with the annealing temperature 62°C in a 2720 thermal cycler (Applied Biosystems, USA). The PCR products appeared as a smear at 750–1,000 bp. The examined ligation products were denatured by incubating in 95°C for 15 min then on ice for 5 min, and hybridized to biotin-labeled oligonucleotides (Jin et al. 1994) that included the dimers $(AC)_{12}$, $(AT)_{12}$,

Table 1 Characterization of 11 polymorphic microsatellite loci genotyped in 22 individuals of small abalones *H. diversicolor* Reeve from two natural populations

Locus	Primer sequence (5′–3′)	Fluorescent dye	Repeat motif	T_a	GenBank number	N	R_A	PIC	H_o	H_e	HWE (P value)
aba100	F: CATCACTCACAGCGAACAGTG R: TGTATTGACACAACTTGATGCCT	FAM	$(CT)_6t(TC)_8(TG)_{25}$	57	FJ167863	18	145–197	0.9093	1.0000	0.9366	1.0000
aba104	F: TGCCTGATCTATCCAACCATC R: CTTAAGTGACGTGGTGAGGTTC	FAM	$(AC)_{34}$	57	FJ167864	15	112–186	0.8714	0.9091	0.9027	0.1247
aba128	F: CGTAGAACAAGTTCCAGGTCG R: GTCATAGGTGTGTTGTGGATATGAA	HEX	$(CA)_{39}$	59	FJ167865	14	182–236	0.8386	0.4545	0.8732	0.0000[a]
aba131	F: TCGTTGTTGACAGGTCTTCAT R: CTAGTTAGATGCTGGCGTTGG	FAM	$(AC)_{31}$tgccgtccacacacacctctgccatccacacacac ctctgcc gtc$(CA)_6$cctctgctgtcct$(CA)_8$cctctgccat ccacacacacttc tgccgtcctcacacacacctctgccgtc$(CA)_6$	57	FJ167866	16	286–352	0.8977	1.0000	0.926	1.0000
aba132	F: GGATTGTCTGGTGAAGATTCG R: TTCATTACGAGTAGTGCGTGC	FAM	$(AC)_{12}$gaacgaacacaaacacacgcacg$(CA)_6$	57	FJ167867	8	163–177	0.8221	0.8182	0.8615	0.2513
aba223	F: AATAATAGCGTGTAGGCGTGC R: ACAGCTTCGTCTTGCATTGTT	HEX	$(TG)_6$ta$(TG)_8$tttgtgtgtgtatgtgtatgtgtgtt$(TG)_8$ tttgtttgtgtgtgtt $(TG)_9$tttgtttgtgtatgtt$(TG)_{10}$tttgtt$(TG)_6$tt$(TG)_6$c$(GT)_{17}$	57	FJ167868	17	262–328	0.9046	0.9545	0.9323	0.5500
aba231	F: TCACTTCCTTCCTCCACACTG R: TAGCCACATACACGACAGAGG	FAM	$(CA)_{31}$	57	FJ167869	18	194–264	0.9061	1.0000	0.9334	1.0000
aba235	F: CTGGAATCGACAGTGTCACAA R: TGCTGTTGATGATGATGGAAG	HEX	$(TCA)_{11}$tcctttaatcttacataaaccatctccatcaccattg$(TCA)_{10}$	57	FJ167870	9	233–263	0.7276	0.7273	0.7738	0.4739
aba250	F: ACACAGGCACAAGCACAT R: GAGGCTCAAGAGAGACTGG	HEX	$(CA)_6$tggacacatgcatgcacgcgcgcg$(CA)_{42}$	59	FJ167871	11	227–289	0.8012	0.2727	0.8404	0.0000[a]
aba259	F: TGTGTGTATGGTTGTGTGCGT R: CCTCACATTACAGCACGCC	FAM	$(TG)_{11}(AG)_{15}$acagacagacagacagt$(CAGA)_5$	59	FJ167872	19	224–298	0.9163	0.9091	0.9429	0.0653
aba266	F: GGTCACATGTGCTCGTTGTAG R: GTAACGGACAGTGATGCTGGT	FAM	$(CT)_{32}$	59	FJ167873	17	204–298	0.8528	0.4545	0.8858	0.0000[a]

Shown are the locus name, primer sequences, fluorescent dye, repeat motif, T_a is locus-specific annealing temperature, GenBank accession numbers for DNA sequences of loci

N the number of individuals genotyped, R_A allele size ranges, H_o and H_e observed and expected heterozygosities, respectively, *PIC* polymorphic information content of each locus

[a] Denotes significant departure from HWE

$(AG)_{12}$ and $(GC)_{12}$, and the trimers $(AAT)_8$, $(AAC)_8$, $(AAG)_8$, $(ATC)_8$, $(ACG)_8$, $(ACT)_8$, $(AGC)_8$, $(GCC)_8$, $(AGG)_8$ and $(ACC)_8$ probes (Sangon, China) in 30% 20× SSC (total volume is 100 µl) overnight at the probes' annealing temperatures. The probes with close annealing temperature were grouped in the same tube. Five tubes were grouped with annealing temperatures at 40, 53, 60, 65 and 70°C, respectively. The hybridization mixtures were enriched by anti-biotin magnetic bead in hybridization buffer (6× SSC, 0.1% SDS) for 3 h at 43°C in the shaking table, then washed three times with washing buffer at three temperatures to remove unbound fragments (2× SSC, 0.1% SDS at room temperature for 5 min, 1× SSC, 0.1% SDS at 45°C for 5 min, 1× SSC, 0.1% SDS at 60°C for 5 min). Binding fragments were eluted by suspending the beads in 60 µl ddH_2O, heating them at 95°C for 10 min and extracting the supernatant when the samples were placed near a magnet. Elution steps were repeated once and eluted fragments were combined and precipited. Purified DNA were resuspended in 30 µl ddH_2O and amplified by PCR, which was carried out in 50 µl reaction volume with SNX forward primer and 62°C annealing temperature. The PCR products between 750 and 1,000 bp were isolated and ligated with pGEM-T Vector (TaKaRa, Japan), following transformed to *Escherichia coli* DH10B competent cells (TaKaRa).

Five hundred and forty four colonies were sequenced and 504 contigs were generated after analyzed by Phred/Phrap/Consed (http://www.phrap.org) software. 429 pairs of primers were designed using the software PRIMER PREMIER 3.0 (Premier Biosoft International) and 175 were consistent amplifications. Among them, 11 of them (Table 1) were labeled by FAM and HEX for population study.

We collected 22 abalones *H. diversicolor* Reeve, 12 from Taiwan population and the other 10 from Vietnam population. Genomic DNA was extracted from foot muscle tissues using the standard phenol–chloroform method (Sambrook et al. 1989). PCRs were used the following conditions in a 10 µl reaction volume: approximately 100 ng genomic DNA, 1× PCR buffer, 0.08 µM 5′-labeled (FAM or HEX) forward primer (Table 1), 0.08 µM unlabeled reverse primer (Table 1), 1.5 mM $MgCl_2$, 0.175 mM of dNTP and 0.4U *Taq* DNA Polymerase (TaKaRa). Thermal cycling conditions were 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 45 s at primer-specific temperature (Table 1) and 45 s at 72°C, with a final extension step of 30 min at 72°C. The PCR products were genotyped on 3730 DNA Analyzer (Applied Biosystems) and performed with GeneMarker 1.7 (Applied Biosystems). The expected and observed heterozygosities, polymorphism information content (PIC) (Table 1) were calculated with MS_tools (http://www.animalgenomics.ucd.ie/sdepark/ms-toolkit/), Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were analyzed with GENEPOP v3.4 software (Raymond and Rousset 2003) and FSTAT (Goudet 1995, 2001), respectively.

Results

We identified a total of 162 alleles from the 11 microsatellite loci. The mean number of alleles per marker was 14.7 (range: 8–19, Table 1). Observed and expected heterozygosity values ranged from 0.2727 to 1.0000, and from 0.7738 to 0.9429, respectively. HWE was estimated with the Markov chain method 10,000 dememorization, 100 batches, 5,000 iterations per batch. Three loci (aba128, aba250 and aba266, Table 1) showed significant heterozygote deficit, and all of them showed the presence of null alleles after detected with MICRO-CHECKER v2.2.3 software (van Oosterhout et al. 2004). No pairs of loci displayed linkage disequilibrium following a Bonferroni correction in FSTAT. We anticipate that the microsatellite markers reported here will be useful to evaluate genetic diversity, population structure analyses and the construction of genetic linkage map of *H. diversicolor* in the near future.

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References

Goudet J (1995) FSTAT, a computer program to test *F*-statistics. J Hered 86:485–486

Goudet J (2001) FSTAT: a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995)

Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27(3):500–502 504–507

Jin L, Zhong Y, Chakraborty R (1994) The exact numbers of possible microsatellite motifs. Am J Hum Genet 55:582

Raymond M, Rousset F (2003) GENEPOP (version 3.4): population genetics software for exact tests and ecumenicism. Updated from Raymond and Rousset (1995)

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. doi:10.1111/j.1471-8286.2004.00684.x

Zhongbao L, Changsheng C (2004) Genetic structure of cultured *Haliotis diversicolor* supertexta (Reeve) populations. J Shellfish Res 23:1135–1137