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Short Communication

Characterization of novel microsatellite loci for clam *Moerella iridescens* (Benson)

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From the $(CA)_{15}$ enriched genomic libraries of *Moerella iridescens*, eleven novel polymorphic microsatellite loci were isolated and characterized in *M. iridescens* (Benson), an important species of marine economic shellfish. The mean number of observed alleles per locus was 18 (range 5 – 34). The observed and expected heterozygosity values ranged from 0.043 to 0.979 and from 0.162 to 0.966 respectively. Three loci showed significant departure from Hardy-Weinberg equilibrium and non-significant linkage disequilibrium was found among all loci. These highly informative microsatellite markers should be useful for population genetic analyses of *M. iridescens*.

Key words: Microsatellite, *Moerella iridescens,* genetic diversity, polymorphism.

INTRODUCTION

Moerella iridescens (Benson) is widely distributed throughout the Northwest Pacific, China, Korea and Japan. As a high-valued marine mollusca species, *M. iridescens* had been studied about the fauna systematics (Xu, 1997), morphology characteristics (Cai et al., 1991; Ji et al., 2007) and environmental toxicology (Wang et al., 2007). But the population genetic diversity and genetic structure of the species rarely had been analyzed (Yu et al., 2009). In recent years, the resource of *M. iridescens* have been in decline due to unsustainable exploitation and deterioration of ecological environment, so more understanding of its population genetics is becoming increasingly urgent. To date, no study has utilized microsatellite markers to understand the population structure of this species. Because of their high abundance, high polymorphism content and codominant inheritance, microsatellite markers are powerful tools in population genetic studies (Zane et al., 2002). In this note, we first reported the isolation and characterization of eleven microsatellite loci in *M. iridescens*.

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MATERIALS AND METHODS

The samples of *M. iridescens* were collected from Zhoushan Island (Zhejiang Province, China). Genomic DNA was isolated from adductor muscle of five *M. iridescens* individuals according to standard phenol-chloroform protocols. Then extracted genomic DNA was mixed. The enriched library protocol for isolating CA repeat motifs followed Hua (2007), though some additional modifications were made. The mixed genomic DNA was digested with restriction enzyme MboI and the fragments of 300 – 800 bp were selected on an agarose gel and recovered using DNA purification kit (Tiangen). Fragments were then ligated to a blunt-end adapter (SAULA: GCGGTACCCGGGAAGCTTGG, SAULB: GATCCCAAGCTTCCCGGGTACCGC) with T4 DNA ligase (Takara). Using the linker sequences as specific primers, the ligation products were amplified and the amplified products were hybridized to a biotin-labeled dinucleotide repeat $(CA)_{15}$ probe at 50°C in sodium phosphate (0.5 M sodium phosphate, 0.5% SDS, pH 7.4) for 18 h. The hybridization mixture was incubated with VECTREX Avidin D (Vector Laboratories) at 37°C and washed with binding buffer four times at different temperatures to remove unbound fragments. Bound fragments were eluted with ddH_2O and recovered with PCR. Then these targeted fragments were ligated with pMD19-T vector (Takara), which was then used to transform DH5*a* competent cells. Fifty-five positive clones were identified from 84 recombinant clones through PCR with SAULA as primers. They were sequenced with M13 universal primers (Invitrogen) and 35 sequences containing CA/TG repeats were selected for primer design using the software PRIMER PREMIER 5.0 software (PREMIER Biosoft International).Through the gradient PCR, 11 loci

Table 1. Characterization of eleven microsatellite loci developed for *M. iridescens.*

Locus	GenBank accession no.	Repeat motif	Primer sequences(5'-3')	T_a (℃)	N_a	Size range (bp)	H_O/H_E	*P*
MG9A	GU980144	$(TG)_{100}$	F: GTGTCTGGACACCACTGT R: CTTACTCACATTCTCCCAG	46	5	180-268	0.043/0.162	0.0013*
MH19A	GU980145	$(TG)_{28}$	F: GTGAGCAGGAATCAAAGGTG R: CTCCGCTCTGTTTGCCTAT	55	17	105-145	0.869/0.890	0.9995
Mi44A	GU980146	$(TG)_{61}$	F: CCTCGGAGACCATTCGCTAC R: TGCTTTTCTATGACAACCCT	52	6	085-101	0.707/0.586	0.0175
MW15A	GU980147	$(CA)_{24}$	F: GATCAAAATTGACAAGGCT R: AAGACAAACACGGATGGT	46	12	088-150	0.717/0.800	0.5534
MW32A	GU980148	$(TG)_7(GG)_6GTTATTGG(GT)_9$	F: GGGGCGGACACTCTAACA R: CCAGTCTCCTGCCACCAT	52	26	100-190	0.787/0.936	1.0000
MW33A	GU980149	$(TG)_{13}TA(TG)_5(CG)_2TGCG$	F: TTCCTATCCTTACCCTTG R: CTGACTGGAAACTCAACAC	48	17	111-171	0.979/0.833	0.9918
MX39C	GU980150	$TGTT(TG)_6(GG)_4GT$	F: CCCAACCAGAATAATACCA R: TCCAACAAAGGAATACGATA	48	11	200-220	0.192/0.852	0.0000*
MX47A	GU980151	$(TG)_{13}CG(TG)_3CG(TG)_4$	F: GTAATCTGCACAACCCTG R: ATGCCAATGTGACAAGAC	50	31	202-326	0.500/0.966	1.0000
MX55A	GU980152	$(TG)_5TA\ (TG)_{17}$	F: GAATACAGCCCGCAGATG R: TATGTCGGGGAGTATTAGCA	55	21	137-191	0.307/0.949	0.7751
MY36B	GU980153	$(TG)_7(GG)_3$	F: CCGTTGGTAAAGACGATAT R: TGGTTGCGAGTTGGACAC	58	14	251-283	0.170/0.911	0.0000*
MZT46B	GU980154	$(TG)_{75}$	F: GACATAAAGGTTGTAGGGA R: ATGGTAGTGATGATGCTTG	46	34	151-283	0.425/0.964	1.0000

Tm, Annealing temperature of primer pairs; Na, number of alleles; Ho, observed heterozygosity; HE, expected heterozygosity. * indicates significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction for multiple comparisons ($P < 0.0045$).

were amplified successfully and the optimal annealing temperature were determined (Table 1).

To test the level of genetic polymorphism for these loci,we sampled 42 individuals from a natural population in Zhoushan Island (Zhejiang Province, China) . A total of 15 µL reaction volume contained 50 – 100 ng genomic DNA, 0.2 µM forward primer (5' modified with FAM, HEX or TAMRA fluorescent dye), 0.2 µM reverse primer, 0.2 mM of each dNTP, 0.25 U Hotstar Taq DNA polymerase (Qiagen) and 1.5 mM $MgCl_2$. Typical PCR amplifications were performed with an initial denaturation at 95℃ for 15 min, followed by 34 cycles of 94℃ for 30 s, T_a (the optimal annealing temperatures, Table 1) for 30 s, 72℃ for 30 s, and a further extension step of 72℃ for 20 min. The PCR products were genotyped on an ABI 3730 sequencer (Applied Biosystems) and analyzed with GeneMapper v4.0 software (Applied Biosystems). Observed and expected heterozygosity values, and tests for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed using GENEPOP 3.4 software (Raymond and Rousset, 1995).

RESULTS AND DISCUSSION

Details for the newly developed 11 novel microsatellite loci and variability measures across 48 individuals are summarized in Table 1. In total, 11 of 35 amplicons from the microsatellite-enriched genomic libraries were successfully amplified and shown to be polymorphic in the *M. iridescens*. The remaining 24 loci were monomorphic and failed to amplify. The 11 sequences containing microsatellite loci were deposited in GenBank (GU980144- GU980154).

No similarity was found between the 11 microsatellites and the published sequences in GenBank. The observed numbers of alleles per locus ranged from 5 to 34 (mean 18). Observed heterozygosity ranged from 0.043 to 0.979 and expected heterozygosity ranged from 0.162 to 0.966. Three of these loci (MG9X, MX39C and MY36B) deviated significantly from Hardy-Weinberg equilibrium after Bonferroni correction (adjusted P value = 0.0045), the remaining 8 loci conformed to HWE. MG9X, MX39C and MY36B deviated from the HWE possibly due to the presence of null alleles or the existence of subpopulations. Null alleles were found in 7 loci (MX39C, MZT46B, MY36B, MX55A, MX47A, MW32A and MG9A) and stuttering errors were found in two locus (MX39C and MY36B) using MICRO-CHECKER (Van Oosterhout et al., 2004) (Bonferroni correction), but no evidence of allelic dropout was found in any of the loci (Bonferroni correction). In total, all pair-wise tests for linkage disequilibrium among the 11 loci were non-significant. These polymorphic microsatellite loci in *C. sinensis* will be useful in studies of conservation genetics and population genetic structure of *M. iridescens* as well as other species of this genus.

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REFERENCES

Xu FS (1997). Bivalve Mollusca of China Seas, Beijing, Science Press China, pp. 164-165.

Cai RX, Huang WH (1991). Mollusca of Zhejiang Province, Hangzhou, Zhejiang Science and Technology Press China, pp. 265-266.

Ji YB, Li TW, Su XR (2007). The Biological Characteristics of Clam *Moerella iridescens* Inshore in Zhejiang Province. Fish Sci., 26(9): 494-496.

Wang ZZ, Wang WD, Yang Y, Lu ZF (2007)Acute toxic effects of four heavy metals on *Moerella iridescens*. Oceanol. Limnol. Sin., 38(4): 373-378.

Yu H, Xue LY , Zhang JF (2009). Genomic DNA isolation and RAPD analysis on *Moerella iridescens*. Mar. Sci., 33(3): 4-9.

Zane L, Bargenoni L, Patarnello T (2002). Strategies for microsatellites isolation: A review. Mol. Ecol., 11: 1-16.

Hua PY, Chen JP, Zhang LB et al (2007) Isolation and characterization of microsatellite loci in the flat-headed bat (*Tylonycteris pachypus*). Mol. Ecol. Notes, 7: 486-488.

Raymond M, Rousset F (1995). GENEPOP (version 3.3): Updated version of GENEPOP (v.1.2) Population genetics software for exact tests and ecumenicism. J. Hered., 86:248-249