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Development of microsatellite markers for *Mytilus coruscus* (Mytilidae), an economically important mussel in the East China Sea

B.-Y. Guo[1], C.-W. Wu[1], C.-L. Liu[2], A.-Y. Zhu[1] and Y.-Y. Ye[1]

[1]Zhejiang Ocean University,
National Engineering Research Center of Marine Facilities Aquaculture,
Zhoushan, China
[2]Key Laboratory for Sustainable Utilization of Marine Fisheries Resources,
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences, Qingdao, China

Corresponding author: C.-W. Wu
E-mail: wucw2012@yahoo.cn

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ABSTRACT. Twelve new polymorphic microsatellite loci were developed for the hard-shelled mussel, *Mytilus coruscus*. In 32 individuals from a wild population of coastal Zhoushan, Zhejiang Province, China, the number of alleles at these loci varied from 3 to 15, with a mean of 5.667. The mean observed and expected heterozygosities were 0.6927 and 0.6591, respectively. Among these polymorphic microsatellite loci, three (MC42, MC129, and MC180) significantly deviated from Hardy-Weinberg equilibrium after sequential Bonferroni's correction. All other microsatellite loci were in linkage equilibrium. These microsatellite loci will be useful for detecting genetic differences and for planning aquaculture management of *M. coruscus*.

Key words: *Mytilus coruscus*; Microsatellite DNA; Genetic diversity; Population structure

INTRODUCTION

The hard-shelled mussel *Mytilus coruscus* (Mollusca, Bivalvia) is an economically important mussel widely distributed along the coasts of China, Japan, and Korea (Wang, 1997). The culture of *M. coruscus* has been carried out throughout the coastal areas of China, and Shengsi Island in the East China Sea is especially famous for the production of this mussel, demonstrating the largest culture of *M. coruscus* in China. Presently, the juveniles of *M. coruscus* for aquaculture mainly originate from the collection of the wild population. However, in recent years, the wild resources of *M. coruscus* have dramatically declined mainly due to over exploitation. For the purpose of developing rational strategies to protect genetic resources and utilize the valuable resources sustainably, it is critical to accurately detect the genetic characteristics of the wild populations. Microsatellite markers have been widely used in genetics and ecology studies such as genetic identification, parentage, and population variability, and have been considered to be one of the best genetic markers (Liu and Cordes, 2004). Although a few microsatellite markers are available for *M. coruscus* (Xu et al., 2010), these loci have not been enough for population genetic study on *M. coruscus* on a large scale. In this study, we developed 12 new polymorphic SSR markers to assist in the breeding of *M. coruscus*.

MATERIAL AND METHODS

Genomic DNA was extracted from the gills with the Genomic DNA mini kit (Tiangen). A partial genomic DNA library enriched for CA-repeats was constructed using the enrichment technique described by Yue et al. (2000). The DNA was digested with *Tru*II into 400- to 1000-bp fragments, which were ligated with *Tru*II adapters. 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, with primers designed from those containing SSR and appropriate flanking regions with PRIMER 3 (Rozen and Skaletsky, 2000). With a subset of templates (5 hard-shelled mussel individuals), the annealing temperature for each pair of primers was optimized.

The polymorphism of each microsatellite was assayed with 32 individuals of *M. coruscus* representing a wild population of the coastal waters of Zhoushan, Zhejiang Province, China. Electrophoresis was performed using 8% non-denaturing polyacrylamide gels. The number of alleles, the observed heterozygosity, and the expected heterozygosity were calculated with the POPGENE 1.32 software (Yeh and Boyle, 1997). MICRO-CHECKER (Van Oosterhout et al., 2004) was employed to infer the most probable technical cause of departure from Hardy-Weinberg equilibrium with significance adjusted using sequential Bonferroni's correction (Rice, 1989). Polymorphism information content (PIC) was calculated according to Botstein et al. (1980) with 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, respectively, the frequency of the ith and jth alleles and n is the number of alleles.

RESULTS AND DISCUSSION

In total, 76 sequences containing microsatellites were obtained in this study. Of them, 48 sequences contained appropriate flanking regions, and of these sequences, 40 microsatellite markers (40 primer pairs) were designed. The genomes of 32 individuals of *M. coruscus* representing a wild population of the coast of Zhoushan, Zhejiang Province, were successively amplified with 40 primer pairs. Polymorphism was detected in *M. coruscus* by 12 primer pairs with the optimized annealing temperatures listed in Table 1.

Table 1. Characterization of 12 polymorphic microsatellite markers in *Mytilus coruscus*.

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Size (bp)	N_A	H_O	H_E	PIC
MC42*	F: AAGGGAAAGCTGGAATTGAG R: TAGAGTGCGAAAACCCATGC	$(CTG)_6$	60	193	6	0.9688	0.7644	0.7112
MC64	F: TGTCAAGGTAAGATGCTGTGC R: AAACATCCCTGCTGAACGAC	$(TG)_{13}$	62	236	7	0.7812	0.7614	0.7131
MC83	F: CAAGGTCAATGATGCTTTCG R: AAAGATCGTCTTCAGAATGG	$(CACAT)_7$	60	234	5	0.4062	0.7594	0.7103
MC100	F: AACTATTTGCCATTGGACGTT R: CGATGCCTCATGCAGAAGT	$(TG)_{20}$	60	195	6	0.5625	0.8214	0.7822
MC111	F: AAGCTGTGTAAACCACCATCC R: TGGGGAGATAATCTGATTCG	$(CA)_{50}$	59	318	15	1.0000	0.9211	0.8991
MC118	F: ATACCTCTGGCGAATGTGGA R: GTACTAGTTTATCACCATAGG	$(CA)_{60}$	60	226	3	0.1562	0.3497	0.3176
MC122	F: TCCCGACATTGGACGTTTAG R: ACACACAGTCGATGCCTCAT	$(CAAT)_5$	60	217	5	0.7812	0.7584	0.7022
MC124	F: CTCACACTTCTCCCCCTCTTT R: AATGGATCGAGAGGAAAGGA	$(CA)_{15}$	60	369	6	1.0000	0.6463	0.5678
MC129*	F: GAACTATTTGCCATTGGACGTT R: CGATGCCTCATGCAGAAGT	$(TG)_9$	60	195	3	0.8438	0.5312	0.4268
MC138	F: CACCCCATCCAAAACCAC R: GCGTGTGTGTTTGAGTGTTTG	$(CA)_{20}$	59	178	5	0.2188	0.4975	0.4536
MC168	F: AGCTTGCATTTCCGTTGGT R: ACCAACTCGTGATGGTGTCA	$(TTG)_{23}$	56	176	3	0.9688	0.6057	0.5181
MC180*	F: CGTTGGATAAAAACCGCAAT R: TTGCCTCTAAAATGGCCTGT	$(CA)_{12}$	53	188	4	0.6250	0.4926	0.4439

Ta = annealing temperature (°C); Size = allele size range; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content. *Locus that deviates from Hardy-Weinberg equilibrium.

The number of alleles at 12 polymorphic microsatellite loci ranged from 3 to 15, with an average of 5.667. Observed and expected heterozygosities varied from 0.1562 to 1.0000 (average of 0.6927) and from 0.3497 to 0.9211 (average of 0.6591), respectively (Table 1). Significant deviation from Hardy-Weinberg equilibrium was observed at MC42, MC129, and MC180. No significant linkage disequilibrium was found among the 12 polymorphic loci. The PIC of 8 loci was more than 0.5, and that of the other 4 was between 0.25 and 0.5. These microsatellite loci could be useful for detecting genetic characteristics and guiding the aquaculture of *M. coruscus*.

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