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Identification of 15 novel polymorphic microsatellite loci in pearl oyster (*Pinctada fucata*)

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ABSTRACT. The pearl oyster *Pinctada fucata* is a commercially important marine shellfish. As a result, genetic improvement and selective-breeding program have been conducted for this species. Polymorphic microsatellites are effective molecular markers to investigate molecular marker-assisted selection and genetic variance. In this study, microsatellite DNAs were screened and characterized based on the partial genome sequence of *P. fucata*. We identified 111 microsatellite DNA motifs through mining the published draft genome sequence of *P. fucata*. Forty-two loci were screened with 8 *P. fucata* individuals, and 15 were found to be polymorphic and were therefore

further evaluated using 40 wild individuals from the Daya Bay, Shenzhen City, Guangdong Province, China. The number of alleles per locus ranged from 3 to 8, with an average of 5.2667 for the 15 polymorphic loci. Observed and expected heterozygosities ranged from 0.1154 to 0.6216 (0.3321 on average) and 0.4950 to 0.8491 (0.6768 on average), respectively. Of the 15 polymorphic loci, 12 loci deviated from Hardy-Weinberg equilibrium after Bonferroni correction ($P < 0.0033$). Polymorphism information content ranged from 0.44 to 0.83 with a mean value of 0.63. The results suggest that the markers isolated in this study can be used for research on molecular marker-assisted selection and genetic variance of *P. fucata*.

Key words: *Pinctada fucata*; Polymorphism; Microsatellite; Genetic diversity

INTRODUCTION

The pearl oyster *Pinctada fucata* (Gould, 1850) is a commercially important marine shellfish cultured for producing marine pearls, mainly in China and Japan (Yu and Chu, 2006). It is common in tropical and subtropical oceans and seas in the Pacific and Indian regions. In China, the first successful artificial propagation of this species was conducted in Guangxi Province in 1965, which allowed the pearl culture practice to subsequently expand rapidly to the neighboring Guangdong and Hainan Provinces (Meng et al., 1996). The pearls produced by these animals are referred to as "South Pearl", and they account for over 90% of the total marine pearls produced in China. Yet, because of overfishing, coastal water pollution, and inbreeding depression by years of artificial propagation without recording of their background, some traits of *P. fucata* appear to have degenerated, which hampers the development of the pearl industry (Niu et al., 2015). Thus, genetic improvement and selective breeding of elite varieties should be conducted to prevent the growth traits of the animal from degenerating to maintain pearl quality (Wada and Jerry, 2008).

Because of their co-dominance, high polymorphism, and genome-wide abundance, microsatellites (simple sequence repeats, SSRs) are effective molecular markers used in molecular marker-assisted selection and to maintain genetic variance in shellfish (Li et al., 2003; Sato et al., 2005; Evans et al., 2006; Andrea et al., 2014; Silva Neta et al., 2015). Although some polymorphic microsatellite loci have been isolated for *P. fucata* (Kuang et al., 2009; Fan et al., 2014), more SSRs are needed for genetic research. Developing SSRs from published data, such as ESTs and genome sequences, is low-cost and highly effective (Guo et al., 2013; Andrea et al., 2014; Tan et al., 2014). Genomic SSRs are more likely to be polymorphic than EST-SSRs (Kong et al., 2014). The publication of the draft genome sequence of *P. fucata* (Takeuchi et al., 2012) has made it possible to mine SSR motifs from its genomic sequence. In this study, 111 microsatellite loci were screened from part of the genomic data of *P. fucata*, and 15 polymorphic microsatellite loci were identified that would be useful in molecular marker-assisted selection and the maintenance of genetic variance in *P. fucata*.

MATERIAL AND METHODS

Wild adult individuals (N = 40) of *P. fucata* (shell length: 4-5 cm) were collected

randomly from Daya Bay, Shenzhen (22.55°S, 114.52°E) and transported to our laboratory in Guangzhou. Adductor muscle were dissected and preserved in 95% ethanol and stored at -20°C. DNA from the adductor muscle tissue was extracted immediately using the TIANamp Marine Animals DNA Kit (Tiangen Products, Beijing, China) according to the manufacturer specification. Partial genomic sequences of *P. fucata*, including scaffold1.1, scaffold2.1, and scaffold3.1, were downloaded from the *P. fucata* genome platform (http://marinegenomics.oist.jp/pinctada_fucata). Microsatellite sequence motifs were discovered with SSR Hunter 1.3 (Li and Wan, 2005) and di-, tri-, tetra-, penta-, and hexanucleotide motifs were identified with a minimum of six, five, four, four, and four repeats, respectively. SSR-containing sequences with sufficient flanking sequences (no less than 150 bp) were selected for characterization. For selected microsatellite loci, 42 primer pairs were designed using Primer Premier 5.0 (Premier Biosoft International, USA) (Table 1).

Forty-two primers were synthesized and verified by PCR in eight *P. fucata* individuals. PCR amplification was performed in 20-μL reactions containing 30-50 ng genomic DNA, 1 U rTaq polymerase (Takara, Japan), 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 0.2 μM each primer. The amplification conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, a primer-specific annealing temperature for 30 s, and 72°C for 1 min, with a final extension at 72°C for 7 min. The Eppendorf MasterCycler® gradient S (Hamburg, Germany) was utilized for PCR. PCR products were examined with electrophoresis on an 8% non-denaturing polyacrylamide gel (200 V, 150 min) and visualized with silver staining. Successfully amplified primers were further characterized with 40 individuals of *P. fucata*. PCR amplification and profile were the same as above.

Genetic variation in terms of number of alleles (N_A), number of effective alleles, observed (H_O) and expected (H_E) heterozygosities, and the probability of a deviation from Hardy-Weinberg equilibrium (HWE) were calculated using Popgene 1.32 (Yeh and Boule, 2000). Polymorphism information content (PIC) was calculated using the PIC Calc online software (Nagy et al., 2012).

RESULTS AND DISCUSSION

SSR locus screening from published whole-genome data was low-cost, time-saving, and highly efficient. Nearly 1,162,212 nucleotides were downloaded from the *P. fucata* genome platform, and 49, 43, and 19 SSR motifs were found in scaffold1.1, scaffold2.1, and scaffold3.1, respectively. Among others, primer pairs of 42 motifs were designed and verified in 8 individuals of *P. fucata*. As a result, 13 failed to amplify any PCR products and 29 produced clear and specific bands, of which 15 showed to be polymorphic and were deposited in GenBank (accession Nos. KT714056-KT714070) (Table 1).

The 15 polymorphic loci were further characterized using 40 wild *P. fucata* individuals from the Daya Bay, Shenzhen (Guangdong Province, China). As shown in Table 2, the N_A per locus ranged from 3 to 8 with an average of 5.2667. H_O and H_E varied from 0.1154 to 0.6216 (0.3321 on average) and 0.4950 to 0.8491 (0.6768 on average), respectively. Among the 15 polymorphic loci, 12 loci deviated from HWE after Bonferroni correction, possibly because of a heterozygote deficit (Kuang et al., 2009) or the occurrence of a non-amplifying null allele (Qiu et al., 2013). The PIC ranged from 0.44 to 0.83 with an average of 0.63. The informativeness value for these genomic SSR markers (0.63) was higher than the genomic SSR marker value (0.37) for *P. fucata* reported by Kuang et al. (2009).

Table 1. Characteristics of 15 microsatellite loci in *Pinctada fucata*.

Locus	Primer sequences (5'-3')	Repeat	Ta (°C)	Size (bp)	GenBank accession No.
scd1.1-1	F:CCGGTGTCACATTAAATTAAGAGC	$(GTCC)_4$	55	176-250	KT714056
	R:TAACAGACTAAACACTACATGGCCG				
scd1.1-3	F:TAGTGGAAAGCCCAGAGGTAAT	$(TCTG)_7$	55	242-250	KT714057
	R:AGAGGGGATAGACCTCAGACAA				
scd1.1-4	F:GGGTGGGAGACAGAATAGCATA	$(TAGA)_4$	57	360-400	KT714058
	R:TAATTCACATAGGTCGCCATCA				
scd1.1-12	F:TCAGGCAATATCCATATCCACC	$(AT)_9$	55	180-250	KT714059
	R:TGGAGTTCAGTCCCCTTCTTGT				
scd1.1-15	F:TATCGGAAGTTTTGCGAGACC	$(AAC)_{12}$	53	170-200	KT714060
	R:ATGGCAAGTGTTCCTCAAAGTG				
scd2.1-10	F:GTATCTGAAAGAAACGATCTG	$(GAT)_{14}$	49	120-200	KT714061
	R:CACTAGAATAAATGTGGAAGC				
scd2.1-15	F:TGAGATGGATTTTCAGACGC	$(AT)_7$	55	100-120	KT714062
	R:CCTGGAAGAGTTTGTAGTAAACACG				
scd2.1-17	F:TTTATCGGGCTTATCGCTTTC	$(TGTT)_7$	55	200-230	KT714063
	R:CAACCTGGAGTATCCTGAAAGAAC				
scd3.1-1	F:TCGCACTATTTCAAGACTAT	$(AT)_7$	49	190-220	KT714064
	R:TTCATACCTGATTAAGCATC				
scd3.1-2	F:CGATGAAGACGGCATACCTG	$(TA)_6$	55	280-300	KT714065
	R:TCCCCTCCTGTGACCCATTA				
scd3.1-3	F:TCTGTACCTCCACATGACTG	$(ATGA)_8$	53	200-250	KT714066
	R:CAGCCATTTGGAGACATAGA				
scd3.1-6	F:ATCAAGGGGCACTTTGGTTC	$(AGG)_5$	53	300-330	KT714067
	R:TCAAATGTTTACGAAGTAGGGG				
scd3.1-11	F:TTCATTGGGTGTTGAGGCTTAT	$(GACA)_4$	55	260-400	KT714068
	R:ATTCGGAACAGCGGAAGTCG				
scd3.1-12	F:TGCCAAACTTGTGCTCCTTC	$(CAA)_6$	55	150-200	KT714069
	R:AGATTGCGTCGTCGGAACAT				
scd3.1-14	F:TAATGTCTCAGTGCTGTTCT	$(TA)_6$	50	320-390	KT714070
	R:GTGCCATTGGATACCAGT				

Ta: annealing temperature.

Table 2. Result of screening 15 polymorphic microsatellite loci with 40 individuals of *Pinctada fucata*.

Locus	N_A	N_E	H_O	H_E	PIC
scd1.1-1[a]	6	4.8809	0.1154	0.7951	0.77
scd1.1-3[a]	7	4.8855	0.6000	0.7953	0.76
scd1.1-4[a]	5	2.5423	0.2895	0.6066	0.57
scd1.1-12	6	3.8216	0.5641	0.7383	0.70
scd1.1-15	6	4.4520	0.6216	0.7754	0.74
scd2.1-10[a]	8	5.3963	0.5000	0.8147	0.79
scd2.1-15[a]	4	2.8089	0.2308	0.6440	0.58
scd2.1-17[a]	4	2.1744	0.2051	0.5401	0.48
scd3.1-1[a]	3	2.3038	0.1500	0.5659	0.50
scd3.1-2[a]	3	1.9802	0.0667	0.4950	0.44
scd3.1-3[a]	8	6.6253	0.2500	0.8491	0.83
scd3.1-6[a]	4	3.0124	0.1515	0.6680	0.61
scd3.1-11	6	2.4336	0.4872	0.5891	0.54
scd3.1-12[a]	4	2.1462	0.2500	0.5341	0.47
scd3.1-14[a]	5	3.8610	0.5000	0.7410	0.70
Mean	5.2667	3.5550	0.3321	0.6768	0.632

N_A, number of alleles; N_E, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; HWE, Hardy-Weinberg equilibrium; PIC, polymorphism information content. [a]Statistically significant deviation from HWE after Bonferroni correction ($P < 0.0033$).

In conclusion, 15 polymorphic microsatellite loci were screened and identified from the published genomic sequence of *P. fucata*. These microsatellites were effective for investigating the population genetics of *P. fucata*. They would facilitate research on molecular marker-assisted selection and genetic variance in *P. fucata*.

Conflicts of interest

The authors declare no conflict of interest.

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