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TITLE

Isolation and characterization of 13 polymorphic microsatellites for the Hokkai Shrimp, *Pandalus latirostris*.

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Abstract

We have developed and characterized 13 novel polymorphic microsatellite markers for Hokkai shrimp, *Pandalus latirostris*, to provide an effective tool for conducting genetic studies on this species. No linkage disequilibria and no deviation from HWE were detected in these markers. In 32 individuals from Notoro Lagoon in Hokkaido, Japan, the number of alleles and expected heterozygosities ranged from 7 to 22 and from 0.84 to 0.95, respectively, suggesting the availability of these markers for ecological studies and conservation genetics in this species.

Hokkai Shrimp, *Pandalus latirostris* Rathbum (Decapoda, Pandalidae) occurs in coastal water along northern Japan and Primorye, Russia, and has been commercially exploited as an endemic fishery product (Bergström 2000). Yields, however, have been unstable or decreasing in most of the exploited populations (Hokkaido Research Organization 2010, Chiba personal communications), and high fishing intensity **might collapse some local populations**. Though transplantation between populations is being planned to sustain shrimp stocks in some populations, this may disturb the natural population structure and genetic diversity of the shrimp, in which genetic differentiation between local populations is probably high. Since the species has no planktonic larval period and establishes discrete habitats exclusively in seagrass areas (Chiba et al 2000), the dispersal ability between local populations would be low. **Therefore**, local populations would be genetically very different from each other. The genetic background should be assessed before transplantation is conducted. Genetic studies are also important in this species to quantify the effect of skewed sex ratios on reproductive efficiency. This shrimp changes sex from male to female during their lifetime (Bergström 2000), and because larger shrimps are generally sex-changed females (Nishihama et al. 1997), large-selective fishing causes the adult sex ratio to be strongly biased toward male. Excessively biased sex ratios are acknowledged as an important concern in the conservation of wild animal resources (Rowe and Hutchings 2003; Allendorf and Hard 2009; Zhou et al. 2010). **We developed microsatellite markers, that are essential to investigate population genetics and the skew of reproductive efficiency, and examined utility of the markers.**

Genomic DNA was extracted from the legs of ethanol fixed shrimp samples from Notoro Lagoon (44°03N, 144°10E) with DNeasy Blood & Tissue Kit (Qiagen). A microsatellite-enriched library was constructed using the methods outlined by Glenn and Schable (2005). Approximate 5 µg of genome DNA from an individual was digested with either *Rsa* I or *Alu* I (NEB), and DNA fragments were ligated to Super SNX24 linkers (forward, 5'- GTT TAA GGC CTA GCT AGC AGA ATC -3'; reverse, 5'- p GAT TCT GCT AGC TAG GCC TTA AAC AAA A -3'). The fragments with linkers were enriched for microsatellite repeats by hybridization to 3' biotinylated oligonucleotides ((GT)₁₂ and (AG)₁₂) followed by capture on streptavidin-coated magnetic beads. Captured fragments were then amplified by PCR with SNX24F primer. Purified PCR products were ligated into pT7Blue T-vector (Novagen) and inserted into *ECOS* Competent *E. coli* JM109 (NIPPON GENE). Cloned inserts were amplified and sequenced using universal primers, P7 (5'- CGC CAG GGT TTT CCC AGT CAG GAC -3') and P8 (5'- AGC GGA TAA

CAA TTT CAC ACA GGA AA -3'), and ABI BigDye™ Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems) on Genetic Analyzer ABI 3730xl (Applied Biosystems). The 155 pairs of primer were designed using Primer3 (Rozen and Skaletsky 2000) and amplification was tested for 16 individuals. PCR was carried out using Qiagen Multiplex PCR Kit (Qiagen) in a total volume of 10 µL following the manufacture's instructions. The thermal profile included pre-cycling denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 90 s and extension at 72°C for 30 s and post-cycling extension at 72°C for 10 min. PCR products were roughly sized on agarose electrophoresis. A total of 53 primer sets, that were amplified in all 16 samples and showed length **polymorphism**, were selected, and the F or R primer of each was fluorescently labeled with Beckman Dye 2 - 4. PCR with fluorescent primer was carried out using 8-16 individuals, and products were sized by CEQ8000 DNA sequencer (Beckman Coulter). Finally we selected 14 of the primer sets (loci) and the optimal annealing temperature was determined for each primer set by gradient PCR. Then, PCR and fragment analysis with 32 individuals from Notoro Lagoon were carried out in the same manner although annealing temperature was modified in each locus (Table 1). The number of alleles and the observed and expected heterozygosities were calculated with GENEPOP ON THE WEB (Raymond and Rousset 1995, Rousset 2008), and the fit of genotype frequencies to Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium was also tested on the same website.

Multilocus amplification (three peaks) was observed in 9 individuals at one of 14 loci, but the remaining 13 loci were well scored (Table 1). The number of alleles ranged from 7 in Pal-152 to 22 in Pal-2, and the expected heterozygosity ranged from 0.84 in Pal-178 to 0.95 in Pal-2. No linkage disequilibrium was significant among all loci before and after Bonferroni correction. Pal-166 showed almost significant deviation from HWE ($p = 0.013$) before Bonferroni correction, but all were far from significant after Bonferroni correction, suggesting no occurrence of null alleles in all 13 of the tested loci.

The developed microsatellite markers are expected to be available for population genetics, parental assignment, kinship estimation and other ecological study to provide useful information for conservation of this species.

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Table 1. Primer sequences, repeat motif from the original clone, the annealing temperature, size range and observed number of alleles (N_A), expected and observed heterozygosity (H_E and H_O) and GeneBank accession number for original sequence for 13 polymorphic microsatellite loci in *Pandalus latirostris*.

Locus name	Primer sequence (5'-3')	Repeat motif	T_a (°C)	Allele size range (bp)	N_A	H_O/H_E	GeneBank accession no.
<i>Pal-123</i>	F: GAATGGGAAATCCACACACC R: GTTTGAAATCATAATGCAACCAATCATAACAGGC	(GT) ₂₁	65	217 - 252	17	0.84/0.92	AB608994
<i>Pal-144</i>	F: ATTGCCTCATGATCTCTGAGC R: AAAATGCATCACCCCAATA	(CA) ₂₀	65	187 - 216	11	0.81/0.85	AB608993
<i>Pal-152</i>	F: AAGAACTTCTAGACTAAACGGGGTATATGAAG R: ACACATACTCGTGCACATGCT	(CAA) ₁₆ N ₁₂ (CAA) ₁₆	60	289 - 308	7	0.90/0.85	AB608995
<i>Pal-166</i>	F: CGAAATAAATCGCTTCTGTGTTACGATATAC R: TTTGACCCATAAACAACAACA	(GT) ₃₅	65	145 - 177	16	0.94/0.90	AB608996
<i>Pal-178</i>	F: CTAATACATATCCATGGGTCTTGTGTGG R: TTTTGTTTAGAAGTATAAGGAATAGGGATCG	(CA) ₄ N ₆ (CA) ₂₀ (CG) ₅	60	213 - 236	11	0.84/0.84	AB608997
<i>Pal-203</i>	F: ACAAGACATAATAGATAAAGAGGGAGACTGC R: AATTCTATAATTTCTGGGTAGAGTCAAGTC	(CT) ₁₅ CC(CT) ₄ N ₂₆ (GAA) ₂₉	60	492 - 530	13	0.88/0.87	AB608999
<i>Pal-216</i>	F: CACAGTGATCTTTAGCGAAAATATTGATATAC R: ATAACAGAGTATCTGCATTGTGTATGCTTAGT	(GT) ₂₄	65	240 - 269	14	0.94/0.87	AB608998
<i>Pal-147</i>	F: AATCGTGGCTTCCTCTTTGA R: GTTATATAAAAGAGAAAAGCAATTGGTCGTTAAG	(GA) ₂₉	65	131 - 169	17	0.91/0.93	AB609000
<i>Pal-226</i>	F: TAGGTCAATCGATCTAATCTAATCTCTCAGG R: ATAACATCATCAAAGACAATTATATCCTTCCAG	(TC) ₁₇	65	357 - 384	15	0.78/0.94	AB609001
<i>Pal-232</i>	F: ATGATCTCACCAGTCGAATAAAGGTTAC R: GGCATATTAAATGAACCTTATCGCTTAC	(CA) ₃₀	60	338 - 378	16	0.94/0.90	AB609002
<i>Pal-1</i>	F: GTGTATCTGAAGGAACAGGTT R: CCTCATCAGATTGGGTTTCATTTCAGGAG	(GA) ₂₅ (GT) ₈	63	130 - 172	20	0.94/0.91	AB609003
<i>Pal-2</i>	F: ACGGTGAAAACCATCGCTAA R: GGAGAAACATTTTAAAGAGTGCCAGG	(CT) ₂₁	60	120 - 224	22	0.97/0.95	AB609004
<i>Pal-3</i>	F: GAGCAGAGAGGCCAAAATTAA R: CGGTCTGAGTCAGGAAGTCTCATC	(GA) ₄₂	62	206 - 246	20	0.97/0.94	AB609005