Analysis of Microsatellite Loci for Swimming Crab *Portunus trituberculatus* Populations in the Korean Side of the Yellow Sea

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The swimming crab, *Portunus trituberculatus*, inhabits seafloor habitats containing sand or pebbles and is widely distributed throughout the world. The present study investigated genetic polymorphisms of 10 microsatellites in 281 samples of *P. trituberculatus* collected from four locations along the coastal water of the Korean side of the Yellow Sea (Yeonggwang, Taean, Sorea, and Yeonpyeong-do Island). The number of alleles per locus ranged from 50 to 129, with a mean of 69.5. The observed and expected hetrozygosity varied from 0.111 to 1.000 and from 0.609 to 0.979, respectively. The inbreeding coefficients (Fis) varied among the loci from -0.0207 to 0.8175. The genetic differentiation (Fst) was less than 0.05 (range 0.0020-0.0124). Therefore, the four groups of *P. trituberculatus* appeared to exhibit little genetic differentiation. The lack of differentiation was confirmed in a phylogenetic tree constructed by the unweighted pair group method with the arithmetic average (UPGMA). The hypervariation between the populations and the lack of genetic differentiation may reflect active gene flow among the Yellow Sea populations and the absence of geographical boundaries. The highly polymorphic microsatellite loci will be useful for molecular and phylogenetic studies, as well as stock management, of swimming crab, which is an important fishery resource.

Key words : Microsatellite, polymorphism, *Portunus trituberculatus*, swimming crab, Yellow Sea

Introduction

The swimming crab, *Portunus trituberculatus* (Crustacea: Decapoda: Brachyura) belongs to the crustacean brachyuran species, which inhabit both seawater and freshwater worldwide, comprising about 6,000 species [3]. *P. trituberculatus* is distributed in coastal waters of East Asia with sand or pebble habitats. Because *P. trituberculatus* is one of the most common edible crab species in East Asia, it is considered to be a commercially important fishery resource. Thus, there have been performed considerable efforts to increase the aquacultural yield of *P. trituberculatus* by artificial propagation and the release of young swimming crabs.

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Based on the analyses of mitochondrial DNA (mtDNA), several molecular approaches have been taken to understand the population genetic structures of *P. trituberculatus*. Imai et al [9] investigated the restriction fragment length polymorphisms (RFLP) in the whole mtDNA of Japanese samples. Liu et al [11] and Xu et al [23] studied the genetic differentiation among geographical populations captured from several coastal areas of China, based on the mitochondrial 16S rDNA and cytochrome c oxidase subunit 1 (*COI*) genes. Particularly, Cho et al [5] performed the phylogenetic analysis using haplotypes of the mtDNA control region in subject with *P. trituberculatus* collected in the Korean coastal area of the Yellow Sea. However, these mtDNA markers have been shown to have some restriction in application to the various studies of phylogenetic analysis, molecular breeding and individual identification and forensics. Therefore, more effective molecular markers for population genetic studies are required.

Microsatellites, also called short tandem repeats (STRs), have been regarded to be effective molecular markers for population genetic study due to their high level of in-

formativity and wide distributions in the genome [7, 20]. Microsatellites have been widely applied for chromosomal mapping and identification of quantitative trait loci [18, 19], phylogenic study [8], and forensic application [2, 12, 25] as well as fishery study [1]. Microsatellites have characteristics of co-dominant and multiallelic inheritance. Microsatellites in *P. trituberculatus* have very recently been studied in samples collected from the East China Sea [6, 24] and the Yellow Sea in Korea [10].

Because of their phylogeographic distribution and importance as a fishery resource in East Asia, the determination of population genetic structure in *P. trituberculatus* is important. Most molecular genetic studies for *P. trituberculatus* have been performed in samples collected from coastal waters near the China and Japan. However, little phylogenetic study has been performed in crabs from Korean coastal water, although genetic polymorphisms of mtDNA [5] and microsatellites [10] have been studied in samples from the Yellow Sea.

In the present study, we identified many microsatellite motifs from *P. trituberculatus* genome, and 10 markers (PS27, PS108, PS247, PS260, PS385, PS747, PS756, PS400, PS703, and PS704) were analyzed for their genetic variation and population genetic structure among four populations collected

from coastal waters of the Yellow Sea in Korea.

Materials and Methods

Sample collection and extraction of genomic DNA

Samples of *P. trituberculatus* (n=281) were collected from four locations along the coastal water of the Korean side of the Yellow Sea: Yeonggwang (Jeonnam, n=83), Taean (Chungnam, n=93), Sorea (Incheon, n=78), and Yeonpyeong-do Island (Incheon, n=27) during June to October, 2010 (Fig. 1). Crab tissues (usually leg muscle) were disrupted by a TissueLyser II (Qiagen, Hilden, Germany), and then used for genomic DNA purification. DNA was purified using a QIAamp DNA Micro kit (Qiagen, Hilden, Germany). The purified DNA was quantified by a spectrophotometer (Smart Spec 3000, BIO-RAD, USA) and stored at -20°C until use.

Preparation of locus-specific primers

The flanking sequences of DNA fragments with the microsatellite repeat motifs were used to design locus-specific primers. The primer pairs were designed according to the following criteria by using the PRIMER 3 program (ver. 0.4.0, http://fokker.wi.mit.edu/primer3/): length, 20-26 bp; melting temperature, 55°C or higher, and PCR product size,
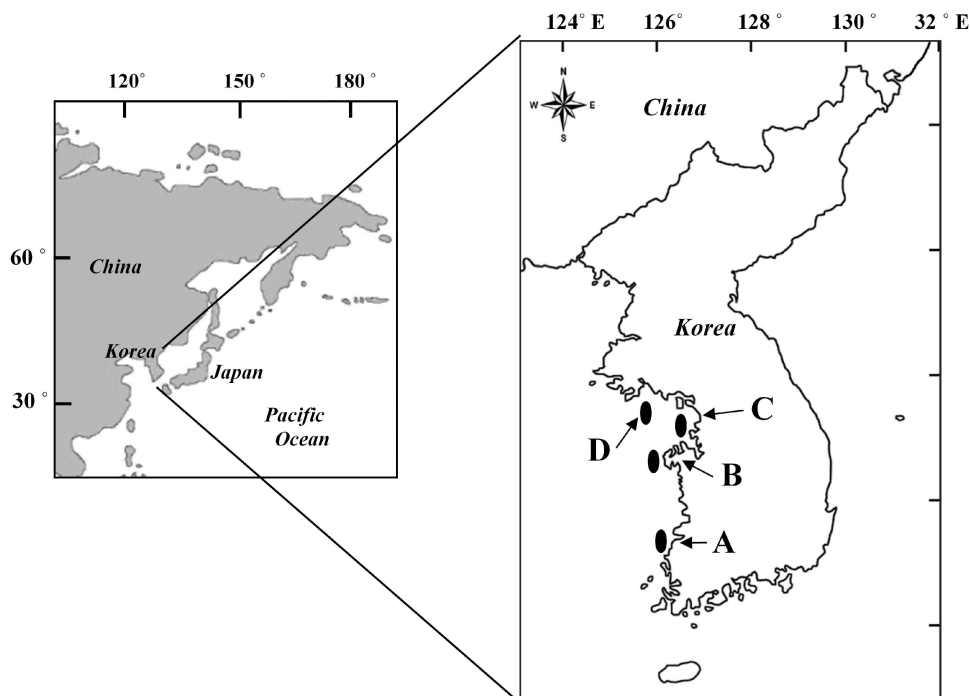

Fig. 1. Sampling locations of swimming crab, *P. trituberculatus*. Swimming crabs were collected from four sites along the coast of the Yellow Sea in Korea: Yeonggwang (A), Taean (B), Sorea (C), and Yeonpyeong-do Island (D) during June to October, 2010.

100 - 350 bp. Particularly, all forward primers (designated as M13F-forward primer) were prepared to have M13 universal primer sequence at the 5'-upstream of locus-specific sequence (5'-TGTAAAACGACGGCCAGT-locus specific forward primer-3').

PCR and genotyping of microsatellites

PCR was performed as described by Schuelke [16] with minor modifications. The reaction mixture contained 0.25 pmol of reverse primer, 1 pmol of each M13F-forward primer and FAM-labeled M13F primer (FAM-5'-TGTAAAACG-ACGGCCAGT-3'), 10-20 ng of genomic DNA, 0.25 unit of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA, USA) and 1X buffer with dNTPs in a total volume of 10 μl. Thermal cycling was conducted on a PTC-200 DNA engine (MJ Research, Waltham, MA) using the following condition: 95°C for 11 min, 20 cycles of 95°C for 30 s; 45 s at a given annealing temperature for each marker; 72°C for 1 min, 15 cycles of 95°C for 30 s; 45 s at 53°C; 72°C for 1 min with a final elongation of 68°C for 60 min. Primer sequences, GenBank accession number, annealing temperature and repeat unit of each locus are listed in Table 1. Each PCR product was separated by an ABI 3100 genetic analyzer (Applied Biosystems). The Liz-500 was used for internal standard. Genotypes were determined using the Genotyper program, ver. 3.7 (Applied Biosystems). The nucleotide sequences for microsatellite markers were determined by using a Chromas program, ver. 2.33 (Technelysium Pty, Australia).

Statistical analyses

Allele frequencies were calculated from the observed genotypes. Genotyping errors (i.e. null alleles, stuttering and large allele dropout) which can cause deviations from Hardy-Weinberg proportions were determined using MICRO-CHECKER version 2.2.3 software [21]. Corrections of the significance level for multiple tests were adjusted using the Bonferroni procedure [13]. The diversity statistics (observed and expected heterozygosities), the Hardy-Weinberg equilibrium (HWE) for each locus, tests for genotypic linkage disequilibrium, the inbreeding coefficient ($F$is) per locus and sample [22], and assessments of multi-locus Fst values were carried out using GENEPOP software, ver. 4.0 [15]. A phylogenetic dendrogram using allele frequencies was constructed by the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of

Table 1. Primer sequences, annealing temperatures and allelic structures of the polymorphic microsatellite markers in *P. trituberculatus*

Locus	GenBank Acc. No.	Primer sequence (5'-3')[a]	Tm. (℃)	Size (bp)	Allele No.[b]	Repeat motif
PS27	JN803923	AGGTGTTATGATAAGCAGGAAGG CCTCCTCTCCCTTCTCAATTC	60	211-339	70	(AGT)n (AGT)n
PS108	JN803925	TCAACACTAAGAACGCTGAGGA TTGACTGTTTCTGCTACTAGGTTTTC	55	99-326	129	(AAG)n (AAG)n
PS247	JN803926	TCCTACCCTGCCCTACCTTAC ACTCTCCAGGCTTACACACCA	60	109-211	50	(AG)n (AG)n
PS260	JN607375	GCACGAGAGAAAGACGAA AGTGAGGGATGGTGATTATAAG	60	99-203	55	(AC)n (AC)n
PS385	JN607395	CATCTTTAAAAACCCGTGTGC AATTTTCCCAGTCGTTGTCCT	60	118-198	52	(AG)n (AG)n
PS747	JN803933	CCGCTGAATCGTTATTGAATG CCATAGGCCCTTCCTAATGTG	60	117-252	64	(AG)n (AG)n
PS756	JN803934	CTGGGGAAATCCTAATGGTGT TCCTTTCATTCTCCCTCCACT	60	169-220	51	(AG)n (AG)n
PS400	JN803927	GTCGAGCGTGCTAACCACTAC GCGGCATGTAATATTGAGGAA	60	121-223	75	(AC)n (AC)n
PS703	JN803930	AGATAGGGAGGACAAGGAGAAG CTGTGTAGCACCTGTCTTGCT	60	111-220	72	(AG)n (AG)n
PS704	JN803931	CGCACAGGGATTAATGCATAC TCGCAAAATTCAGGTTACTGC	60	148-240	77	(AG)n (AG)n

[a]Forward primers represent only locus-specific sequence (5'-M13 sequence was not shown).
[b]Observed allele numbers were obtained from 281 samples collected from 4 different localities.

NTSYS-pc, ver. 2.1 [14]. The gene diversity (GD) was determined using the formula $GD = n(1 - \Sigma P_i^2)/(n-1)$, where n represents the number of samples, and Pi is the allele frequencies in a given population sample. The polymorphism information content (PIC), power of exclusion (PE), and power of discrimination (PD) were calculated by the PowerStatsV12 program (Promega, Madison, WI, USA).

Results

Isolation of polymorphic microsatellite loci

We have previously identified 684 microsatellite motifs from the *P. trituberculatus* genome [10]. From the isolated microsatellite motifs, 87 locus-specific primer pairs were prepared and used for PCR amplification and genotyping on the genome of *P. trituberculatus*. PCR amplification was distinctly achieved at 60 primer sets, however, 27 primer pairs failed to obtain a clear PCR product or genotyping result from the PCR product. Genotyping of 60 loci revealed that 52 loci were polymorphic from 30 *P. trituberculatus* samples collected from the Korean side of the Yellow Sea (86.7%),

whereas 8 loci showed monomorphic. Most polymorphic loci were determined to be highly informative. Forty four loci exhibited observed allele numbers of 10 or more. Moreover, 11 loci showed more than 30 alleles.

Population genetic study

From the polymorphic loci, 10 loci (PS27, PS108, PS247, PS260, PS385, PS747, PS756, PS400, PS703, and PS704) were chosen, and were examined for their polymorphism and phylogenetic analysis of *P. trituberculatus* populations. We analyzed a total of 281 *P. trituberculatus* samples collected from the west side of Korea including Yeonggwang (WOJ, n=83), Taean (WOT, n=93), Sorea (WOP, n=78), and Yeonpyeong-do Island (WOY, n=27) (Fig. 1).

The genotyping results revealed that all the populations of *P. trituberculatus* were highly polymorphic with a total observed allele number of 695. The number of alleles per locus ranged from 50 (PS247) to 129 (PS108) with a mean number of 69.5 (Table 1). The observed and expected heterozygosities varied from 0.111 to 1.000 and from 0.609 to 0.979, respectively. Significant deviations of the Hardy-Weinberg

Table 2. Summary of variation across 10 microsatellite loci in swimming crab (*P. trituberculatus*) from four natural populations

Population (n)[a]	Item[b]	Microsatellite loci									
		PS27	PS108	PS247	PS260	PS385	PS747	PS756	PS400	PS703	PS704
WOJ (n=83)	A	45	77	39	36	43	49	31	47	43	51
	H_O	0.7108	0.8675	0.8193	0.9759	0.8675	0.9036	0.8675	0.6747	0.7590	0.6265
	H_E	0.8856	0.9793	0.9662	0.9561	0.9683	0.9684	0.9535	0.8096	0.8416	0.8233
	HWE	0.0000	0.7658	0.0131	0.1099	0.1425	0.3204	0.0023	0.0949	0.0000	0.0000
	F_{IS}	0.1973	0.1142	0.1521	-0.0207	0.1041	0.0669	0.0902	0.1666	0.0981	0.2390
WOT (n=93)	A	48	69	40	33	37	40	37	50	43	54
	H_O	0.7957	0.6774	0.5914	0.9247	0.8710	0.8817	0.8817	0.8925	0.9570	0.8710
	H_E	0.9524	0.9602	0.8723	0.9543	0.9568	0.9376	0.9562	0.9385	0.9326	0.9154
	HWE	0.0000	0.0000	0.0000	0.0157	0.0042	0.0000	0.0000	0.0032	0.2394	0.0709
	F_{IS}	0.1645	0.2945	0.3220	0.0310	0.0897	0.0596	0.0779	0.0490	-0.0262	0.0485
WOP (n=78)	A	36	54	32	39	32	44	38	43	38	39
	H_O	0.5513	0.5641	0.3718	0.8077	0.8974	0.7949	0.8205	0.5641	0.6410	0.5513
	H_E	0.8531	0.9587	0.9228	0.9548	0.9555	0.9591	0.9544	0.7485	0.7796	0.7821
	HWE	0.0025	0.0000	0.0000	0.0000	0.0001	0.0052	0.0000	0.0000	0.0000	0.0004
	F_{IS}	0.3538	0.4116	0.5971	0.1541	0.0608	0.1712	0.1403	0.2464	0.1778	0.2951
WOY (n=27)	A	25	28	15	24	23	25	19	26	30	37
	H_O	0.9259	0.4444	0.1111	0.8519	0.7407	0.7778	0.7407	1.0000	0.9259	0.9630
	H_E	0.9580	0.9757	0.6088	0.9636	0.9636	0.9687	0.9181	0.9666	0.9751	0.9497
	HWE	0.2784	0.0000	0.0000	0.0537	0.0182	0.0024	0.0102	0.4114	0.0607	0.0692
	F_{IS}	0.0335	0.5445	0.8175	0.1160	0.2313	0.1971	0.1932	-0.0346	0.0504	-0.0140
	F_{ST}	0.0075	0.0042	0.0028	0.0041	0.0020	0.0027	0.0101	0.0062	0.0056	0.0124

[a]Yeonggwang (WOJ), Taean (WOT), Sorea (WOP), and Yeonpyeong-do Island (WOY).
[b]Number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), *P*-values for the Hardy-Weinberg equilibrium (HWE) tests, inbreeding coefficient (F_{IS}), and genetic differentiation between populations (F_{ST}).

equilibrium (HWE) were detected in three loci in Yeonggwang, five loci in Taean, eight loci in Sorea, and two loci in Yeonpyeong-do Island population (Table 2). The inbreeding coefficients (F_{is}) varied among loci from -0.0207 to 0.2390 in Yeonggwang, from -0.0262 to 0.3220 in Taean, from 0.0608 to 0.4116 in Sorea, and from -0.0346 to 0.8175 in the Yeonpyeong-do Island population (Table 2). In addition, linkage disequilibrium was revealed between six of the locus pairs (PS27 and PS385, PS260 and PS400, PS385 and PS400, PS747 and PS400, PS747 and PS704, PS756 and PS704) after Bonferroni correction ($p < 0.00357$). We estimated the genetic differentiation (F_{st} value) between these populations. From the results, the F_{st} over all samples per locus ranged from 0.0020 to 0.0124 ($p < 0.05$) (Table 2). The phylogenetic tree constructed by using the UPGMA also suggested that there is no significant genetic difference between Yeonggwang, Taean, Sorea, and Yeonpyeong-do Island populations (Fig. 2).

Forensic analysis

Several forensic parameters revealed that all the analyzed loci are highly useful for individual identification and paternity test (Table 3). High levels of gene diversity (GD) were calculated from all the loci ranging from 0.820 at PS108 (Taean) to 0.945 at PS260 and PS704 (Yeonpyeong-do Island). The power of exclusion (PE) and discrimination (PD) indexes
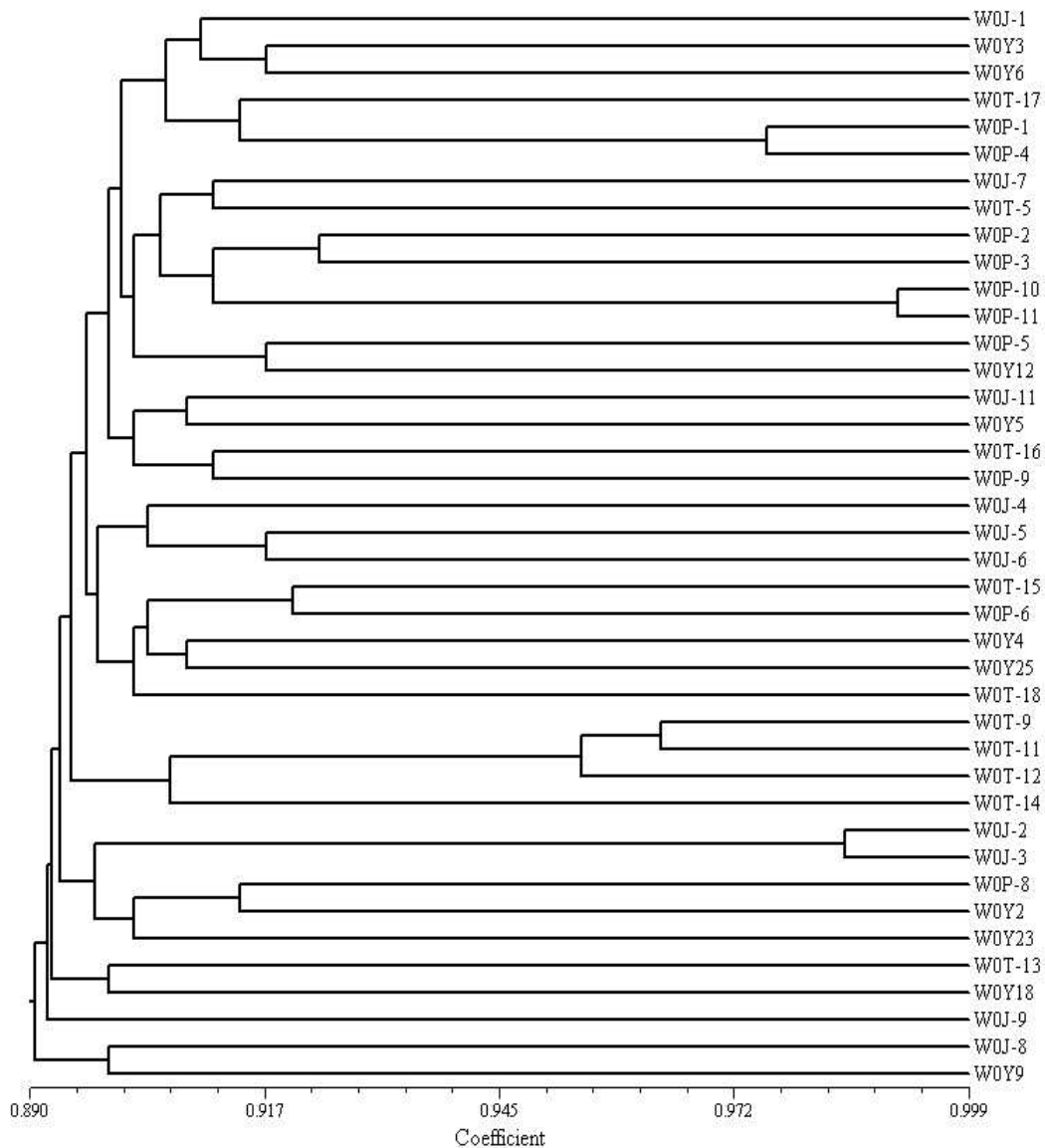

Fig. 2. Phylogenetic relationship of 40 *P. trituberculatus* samples based on the microsatellite marker typing. The dendrogram was constructed by the unweighted pair group method with arithmetic average (UPGMA).

Table 3. Genetic diversity and forensic parameters of 10 microsatellite loci of *P. trituberculatus*

Loci	GD[a]				PIC[b]				PE[c]				PD[d]			
	WOJ	WOT	WOP	WOY	WOJ	WOT	WOP	WOY	WOJ	WOT	WOP	WOY	WOJ	WOT	WOP	WOY
PS27	0.857	0.860	0.837	0.883	0.850	0.850	0.820	0.880	0.188	0.795	0.114	0.795	0.900	0.900	0.860	0.900
PS108	0.925	0.820	0.885	0.900	0.920	0.800	0.870	0.890	1.000	0.188	0.599	0.114	0.860	0.840	0.880	0.880
PS247	0.895	0.880	0.870	0.860	0.890	0.870	0.860	0.840	0.428	0.599	0.188	0.000	0.880	0.840	0.860	0.860
PS260	0.900	0.890	0.885	0.945	0.890	0.880	0.880	0.940	1.000	1.000	0.188	1.000	0.880	0.880	0.860	0.900
PS385	0.900	0.895	0.870	0.930	0.890	0.890	0.860	0.930	0.795	1.000	0.795	0.795	0.880	0.840	0.880	0.900
PS747	0.895	0.875	0.910	0.895	0.890	0.860	0.900	0.890	0.795	0.599	0.599	0.428	0.880	0.820	0.860	0.880
PS756	0.830	0.910	0.850	0.880	0.810	0.900	0.840	0.870	0.599	0.599	0.795	0.291	0.880	0.900	0.860	0.880
PS400	0.915	0.920	0.895	0.920	0.910	0.910	0.890	0.910	1.000	1.000	0.795	1.000	0.880	0.880	0.880	0.900
PS703	0.895	0.895	0.900	0.890	0.890	0.890	0.890	0.880	0.795	1.000	0.795	0.795	0.840	0.900	0.880	0.900
PS704	0.925	0.858	0.900	0.945	0.920	0.840	0.890	0.940	1.000	1.000	0.599	1.000	0.900	0.815	0.880	0.900
Mean	0.894	0.880	0.880	0,905	0.886	0.869	0.870	0.897	0.760	0.778	0.547	0.622	0.878	0.862	0.870	0.890

[a]GD: gene diversity, [b]PIC: polymorphism information contents, [c]PE: power of exclusion, and [d]PD: power of discrimination.

ranged from 0.114 at PS27 (Sorea) to 1.000 at several loci and 0.815 at PS704 (Taean) to 0.900 at several loci, respectively. The polymorphism information contents (PIC) was also very high ranging from 0.800 at PS108 (Taean) to 0.940 at PS704 (Yeonpyeong-do Island). Microsatellites are usually called highly informative loci, if the PIC values are higher than 0.7.

Discussion

The swimming crab, *P. trituberculatus* is one of the most important fishery resources in the East Asian countries. However, the population genetic structure of this species was less understood at the molecular level. Although several molecular phylogenetic analyses have been taken, most studies have been performed on the mtDNA polymorphisms [5, 9, 11, 23], and fewer studies were done for microsatellite loci [6, 10, 24].

The present study identified 52 polymorphic microsatellite loci. Of them, 10 hypervariable loci were chosen, and were examined for their polymorphism and phylogenetic analysis of four *P. trituberculatus* populations. The genotyping results revealed that all the analyzed loci were highly informative for four examined populations with high level of the PIC (≥0.7). The mean number of alleles per locus, PIC, observed and expected hetrozyosities were 69.5, 0.881, 0.762 and 0.916, respectively. The PD and PE, which are important indexes for individual identification and paternity examination, also showed high values with means of 0.863 and 0.677, respectively. The high polymorphism found in this study is most likely due to the large number of alleles found in *P. trituberculatus*. Besides, most microsatellite loci

are dinucleotides, which typically show higher levels of polymorphism [7]. The number of alleles per locus is positively correlated with the number of repeat motifs [4, 7].

Significant deviations of the Hardy-Weinberg equilibrium (HWE) were detected in three loci in Yeonggwang, five loci in Taean, eight loci in Sorea, and two loci in Yeonpyeong-do Island population. The observed heterozygosities in four populations were significantly lower than the heterozygosity expected value, especially in Taean and Sorea populations. Similarly, previous study for Wonsando Island and Jeonjangpo *P. trituberculatus* populations also showed deviations from HWE in many loci [10]. The departure from HWE in several loci may due to small sample sizes and hyper-polymorphisms, as well as heterozygote deficiency by null alleles, stuttering or large allele dropout [17].

The genetic differentiation (Fst value) showed less than 0.05 (range 0.0020-0.0124), therefore, we suggested that four groups of *P. trituberculatus* were considered to indicate little genetic differentiation. We also confirmed this suggestion from the phylogenetic analysis. We could not find any statistically significant geographical structure from a neighbor-joining tree, in which there is no apparent relationship between the location where a given sample was located and its genetic relationship with the other samples. The high level of genetic diversity and little genetic differentiation between populations may reflect active gene flow among the Yellow Sea populations of *P. trituberculatus*. The low genetic differentiation of *P. trituberculatus* is consistent with other studies. The mtDNA haplotype study also suggested that three *P. trituberculatus* groups from the Korean side of the Yellow Sea have no significant geographical structure [5]. Populations of *P. trituberculatus* in the East China Sea and

in the Korean Yellow Sea also revealed low genetic differentiation between the populations from the F-statistic analysis using microsatellites [10, 24]. The swimming crab, *P. trituberculatus* in the Yellow Sea may go on an excursion with a long migration track.

Because swimming crab is a commercially very important fishery resource, more than 10 million young crabs are stocked in the Yellow Sea in each year, to increase the amount of catch in Korea. However, the economic and ecological effects of crab stocking have not been exactly determined. Several forensic parameters indicated that the examined loci are suitable for individual identification and paternity test, thus the developed microsatellite loci may be available for determination of stocking effect by a maternity test between parent (female) and recaptured crabs. Although this study did not examine the cross-species amplification, the developed loci may be applicable to other related species, since several *P. trituberculatus* microsatellites have been applied in two other portunid species, *P. pelagicus* and *P. sanguinolentus* [24]. Altogether, we consider that these highly polymorphic microsatellite markers will provide a useful tool for the population structure and ecogenetic studies as well as management of swimming crab, *P. trituberculatus.*

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References

1. An, H. S., Lee, J. H., Noh, J. K., Kim, H. C., Park, C. J., Min, B. H., and Myeong, J. I. 2010. Ten new microsatellite markers in cutlassfish *Trichiurus lepturus* derived from an enriched genomic library. *Anim Cells Sys* **14**, 169-174.
2. Blouin, M. S., Parsons, M., Lacaille, V. and Lotz, S. 1996. Use of microsatellite loci to classify individuals by relatedness. *Mol Ecol* **5**, 393-401.
3. Bowman, T. E. and Abele, L. G. 1982. Classification of the recent Crustacea, pp. 1-92. In: Vol. 1: Systematics, the Fossil Record, and Biogeography, L.G. Abele (ed.), *The Biology of Crustacea.* Academic Press, New York, NY.
4. Brandstrom, M. and Ellegren, H. 2008. Genome-wide analysis of microsatellite polymorphism in chicken circumventing the ascertainment bias. *Genome Res* **18**, 881-887.
5. Cho, E. M., Min, G. S., Kanwal, S., Hyun, Y. S., Park, S.

W. and Chung, K. W. 2009. Phylogenetic analysis of mitochondrial DNA control region in the swimming crab, *Portunus trituberculatus. Anim Cells Sys* **13**, 305-314.
6. Cui, Z., Liu, Y., Wang, H., Wu, D., Luan, W., Tan, F. and Huang, M. 2012. Isolation and characterization of microsatellites in *Portunus trituberculatus. Conservation Genet Resour* **4**, 251-255.
7. Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. *Nat Rev Genet* **5**, 435-445.
8. Goldstein, D. B., Linares, A. R., Cavalli-Sforza, L. L. and Feldman, M. W. 1995. An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463-471.
9. Imai, H., Fujii, Y. and Karakawa, J. 1999. Analysis of the population structure of the swimming crab, *Portunus trituberculatus* in the coastal waters of Okayama Prefecture, by RFLPs in the whole region of mitochondrial DNA. *Fish Sci* **65**, 655-656.
10. Lee, H. J., Lee, D. H., Yoon, S. H., Kim, D. H., Kim, S. G., Hyun, Y. S., Min, G. S. and Chung, K. W. 2013. Characterization of 20 microsatellite loci by multiplex PCR in swimming crab, *Portunus trituberculatus. Genes Genom* **35**, 77-85.
11. Liu, Y., Liu, R., Ye, L., Liang, J., Xuan, F. and Xu, Q. 2009. Genetic differentiation between populations of swimming crab *Portunus trituberculatus* along the coastal waters of the East China Sea. *Hydrobiologia* **618**, 125-137.
12. Madsen, B. E., Villesen, P. and Wiuf, C. 2010. Short tandem repeats and genetic variation. *Methods Mol Biol* **628**, 297-306.
13. Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**, 223-225.
14. Rohlf, F. J. 2002. *NTSYSPC: Numerical Taxonomy System, ver. 2.1.* Exeter Publishing, Ltd. Setauket, New York.
15. Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resour* **8**, 103-106.
16. Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragment. *Nat Biotechnol* **18**, 233-234.
17. Sekino, M., Hara, M. and Taniguchi, N. 2002. Loss of microsatellite and mitochondrial DNA variation in hatchery strains of Japanese flounder *Paralichthys olivaceus. Aquaculture* **213**, 101-122.
18. Suji, K. K., Biji, K. R., Poornima, R., Prince, K. S., Amudha, K., Kavitha, S., Mankar, S. and Babu, R. C. 2012. Mapping QTLs for plant phenology and production traits using Indica rice (*Oryza sativa* L.) lines adapted to rainfed environment. *Mol Biotechnol* **52**, 151-160.
19. Tanksley, S. D., Grandillo, S., Fulton, T. M., Zamir, D., Eshed, Y., Petiard, V., Lopes, J. and Beck-Bunn, T. 1996. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium. Theor Appl Genet* **92**, 213-224.
20. Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res* **17**, 6463-6471.
21. van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data.

Mol Ecol Notes **4**, 535-538.

22. Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

23. Xu, Q., Liu, R. and Liu, Y. 2009. Genetic population structure of the swimming crab, *Portunus trituberculatus* in the East China Sea based on mtDNA 16S rRNA sequences. *J Exp Mar Biol Ecol* **371**, 121-129.

24. Xu, Q. and Liu, R. 2011. Development and characterization of microsatellite markers for genetic analysis of the swimming crab, *Portunus trituberculatus*. *Biochem Genet* **49**, 202-212.

25. Yoo, S. Y., Cho, N. S., Park, M. J., Seong, K. M., Hwang, J. H., Song, S. B., Han, M. S., Lee, W. T. and Chung, K. W. 2011. A large population genetic study of 15 autosomal short tandem repeat loci for establishment of Korean DNA profile database. *Mol Cells* **32**, 15-19.

초록 : 서해안에서 채집된 꽃게(*Portunus trituberculatus*) 집단에 대한 microsatellite 좌위의 분석

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꽃게(*Portunus trituberculatus*)는 세계적으로 넓게 분포하는 갑각류로 모래나 돌멩이가 있는 해저에 서식한다. 본 연구는 서해의 4개 지점(영광, 태안, 소래, 연평도)에서 채집된 *P. trituberculatus* 281 개체에 대해 10 종류 microsatellite 좌위의 유전적 다형성을 조사하였다. 좌위당 대립유전자 수는 50-129개로 평균 69.5개였으며, 관측 및 예상 이형접합도는 각각 0.111-1.000 및 0.609-0.979 범위에 있었다. 좌위별 근친계수((F_{is})는 -0.0207에서 0.8175 범위였다. 유전적 분화도(F_{st})는 0.05보다 낮게 나타났는데, 이것은 4 꽃게 간의 유전적 분화(genetic differentiation)가 매우 낮은 이루어진 것으로 추정하게 한다. UPGMA을 이용한 계통도 작성에서도 4 그룹 간의 유전적 거리는 매우 가깝다는 결과를 얻을 수 있었다. 매우 높은 다형성과 집단간의 낮은 유전적 분화는 서해안의 꽃게 집단은 활발한 유전적 흐름(gene flow)이 일어나며, 그룹간 지리적 경계가 없음을 제시한다.