

Geographical Variations and Genetic Distances of Three *Saxidomus purpuratus* Populations ascertained by PCR Analysis

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ABSTRACT : Genomic DNA samples isolated from geographical purplish Washington clam (*Saxidomus purpuratus*) were obtained from three different regions in the Korean Peninsula: Geoje (Geoje population; GJP), Gunsan (Gunsan population; GSP) and a site of North Korea (North Korea population; NKP). The seven primers generated the total 369 loci that can be scored from the GSP clam population. 356 fragments were generated from the NKP clam population. The complexity of the banding patterns varies dramatically between the primers and three localities. In this study, 319 loci were identified in the purplish Washington clam from Geoje and 369 in the clam population from Gunsan: 221 specific loci (69.3%) in the GJP clam population and 300 (81.3%) in the GSP population. These results demonstrate that the primer detected a large quantity of specific fragments, suggesting that the genetic variation in the GSP is higher than in the GJP population. In particular, the BION-28 primer gave DNA profiles with more fragments than the other six primers in the NKP population. The oligonucleotides primer BION-75 produced 21 unique loci to each population, which were ascertaining each population, approximately 250 bp, 300 bp and 400 bp, in the GJP population. Outstandingly, the primer BION-50 detected 21 shared loci by the three populations, major and/or minor fragments of sizes 150 bp, which were matching in all samples. With regard to average bandsharing value (BS) results, individuals from GJP population (0.743) displayed higher bandsharing values than did individuals from GSP population (0.606). In the present study, the dendrogram gained by the seven oligonucleotides primers indicates three genetic clusters: cluster 1 (GEOJE 01 ~ GEOJE 07), cluster 2 (GUNSAN 08 ~ GUNSAN 14), cluster 3 (N.KOREA 15 ~ N.KOREA 21). Among the twenty one clams, the shortest genetic distance that revealed significant molecular differences was between individuals 08 and 09 from the NKP population (genetic distance = 0.073), while the longest genetic distance among the twenty-one individuals that demonstrated significant molecular differences was between individuals GEOJE no. 03 and GUNSAN no. 09 (genetic distance = 0.669). Comparatively, individuals of GJP population were properly closely related to that of NKP population, as revealed in the hierarchical dendrogram of genetic distances. In due course, PCR analysis has revealed the significant genetic distance among three purplish Washington clam populations. PCR fragments discovered in this study could be valuable as a DNA marker of the three geographical clam populations to distinguish.

Key words : Genetic cluster, Genetic distance, Geographical variation, purplish Washington clam, *Saxidomus purpuratus*

INTRODUCTION

Saxidomus purpuratus is, environmentally warmwater bivalve species, belonging to family Veneridae, widely distributed on the coast of the Yellow Sea, southern sea and Jeju Island

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in the Korean Peninsula and the Bo Hai of China under the natural ecosystem. Their larvae eat sessile diatoms, microscopic algae and roughly debris (Min, 2001). Genomic DNA samples isolated from geographical purplish Washington clam (*Saxidomus purpuratus*) were achieved from three different regions in the Korean Peninsula: Geoje (Geoje population; GJP), Gunsan (Gunsan population; GSP) and a site of North Korea (North Korea population; NKP). Under the natural ecosystem, the clams inhabit in the estuary flats consisting of a lot of mud, silt and slime in the coastal tidal marshland where the freshwater is flowed transitorily. Like other clams fundamentally, the rate at which the clam grows depends very much on water quality. Predominantly, there are marked differences of the size, color and shape in purplish Washington clam along with the ecological circumstances of habitat such as nurture and rigid period. However, these kinds of Korean bivalve, which are recognized important reproductively (Shin et al., 2007), ultrahistologically (Ju & Lee, 2011), resources-ecologically (Jin et al., 2011), genotoxicologically (Kim et al., 2011) as well as stock-economically (Kim et al., 2007), are not genetically studied or researched like other shellfishes. There is a requisite to understand the genetic traits and structures of this mollusk population in order to evaluate precisely the conspicuous genetic consequence. The author undertook clustering analyses to disclose the Euclidean distances among three purplish Washington clam (*Saxidomus purpuratus*) populations from Geoje, Gunsan and a site of North Korea in the Korean peninsula.

MATERIALS AND METHODS

1. Sample collection and purification of genomic DNA

Adductor muscle tissues were collected separately from three GJP, GSP and NKP populations, respectively. This clam muscle was collected in sterile tubes, placed on ice immediately, and stored at -40°C until needed. DNA samples extracted from a total of 21 individuals. DNA extraction should be carried out according to the separation and

extraction methods (Yoon & Kim, 2004; Park et al., 2005). After washing several times, samples of muscle tissues were placed into 10 test tubes, to which 3 volumes of lysis buffer (155 mM NH_4Cl , 10 mM KHCO_3 , 1 mM EDTA) was added, and the mixture tubes were lightly overturned. The precipitates obtained were diffused with lysis buffer (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS). Samples were added 15 μL proteinase K solutions (10 mg/mL). After incubation, there was added 300 μL of 6 M NaCl and softly pipetted for a few of min. 600 μL of chloroform was added to the mixture and then overturned (no phenol). The DNA pellets were incubation-dried for 2 hrs, held at -40°C until analysis, and then dissolved in the TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). The concentrations of the extracted genomic DNA samples were estimated based on the absorbance at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

2. Amplification conditions and data analyses

Amplification products were separated by electrophoresis in 1.4% agarose gels with TBE, using 100 bp DNA ladder (Bioneer Corp., Daejeon, Korea) as DNA molecular weight marker and detected by staining with ethidium bromide. The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a photoman direct copy system (PECA Products, Beloit, WI, USA). The author used the oligonucleotides primers to elucidate the genetic distances and geological variations of *S. purpuratus* individuals. Seven primers, BION-22 (5'-GGTACTCCAC-3'), BION-24 (5'-TGACGCGCTC-3'), BION-28 (5'-CCC GCCGTTG-3'), BION-68 (5'-CCTTGACGCA-3'), BION-73 (5'-CAGCACCCAT-3'), BION-74 (5'-CCTCTGACTG-3'), and BION-75 (5'-GAGGTCCACA-3') were displayed to create the unique shared loci to each population and shared loci by the three *S. purpuratus* populations which could be obviously counted. Thus, the author used the oligonucleotides primers to determine the genetic differences and geographical variations of the purplish Washington clam. PCR was

performed using programmable DNA Thermal Cycler (MJ Research Inc., Waltham, MA, USA). Similarity matrix including bandsharing values between different individuals in the three purplish Washington clam populations was generated allowing formula of Jeffreys and Morton (1987) and Yoke-Kqueen and Radu (2006). The average of within-population similarity was calculated by pairwise comparison between individuals within a population. A hierarchical clustering tree was assembled using similarity matrices to produce a dendrogram, which was assisted by the Systat version 10 (SPSS Inc., Chicago, IL, USA). The Systat software was also used to analyze genetic differences, Euclidean genetic distances within and between populations, means, standard errors, and *t*-test scores.

RESULTS AND DISCUSSION

The number of total loci, average loci per lane and specific loci was calculated by analytical method using 7 oligonucleotides primers from three purplish Washington clam individuals from GJP, GSP and NKP population, respectively, as showed in Table 1. The seven selected

primers BION-22, BION-24, BION-28, BION-68, BION-73, BION-74 and BION-75 generated the total number of loci, average number of loci per lane and specific loci in GJP, GSP and NK population. Here, the intricacy of the banding patterns varied dramatically between the primers from the three sites. In this study, the seven oligonucleotides primers generated the total 369 loci that can be scored from the GSP clam population. 356 fragments were generated from the NKP clam population. In this study, total 319 loci were identified in the purplish Washington clam from Geoje and 369 in the clam population from Gunsan: 221 specific loci (69.3%) in the GJP clam population and 300 (81.3%) in the GSP population. In particular, the BION-28 primer gave DNA profiles with more fragments than the other six primers in the NKP population, as shown in Table 1. These results demonstrate that the primer detected a large quantity of specific fragments, suggesting that the genetic variation in the GSP is higher than in the GJP population. In the marsh clam from Gochang (*Corbicula* spp.), 7 primers generated 585 major and minor fragments from three geographic sites, producing approximately 6.6 products per primer on average (Yoon & Kim, 2003). The

Table 1. The number of total loci, average loci per lane, specific loci by PCR analysis using 7 oligonucleotides primers from three purplish Washington clam population in the Korean Peninsula

Primer	Item	No. of average loci per lane			No. of specific loci		
		GJP	GSP	NKP	GJP	GSP	NKP
BION-22		6.6(46)	6.9(48)	9.6(67)	39	0	46
BION-24		5.9(41)	9.6(67)	7.1(50)	20	46	29
BION-28		8.6(60)	8.7(61)	10.3(72)	46	61	72
BION-68		5.7(40)	7.9(50)	8.9(62)	19	50	41
BION-73		9.1(64)	7.4(52)	3.0(21)	64	52	7
BION-74		5.6(39)	4.7(33)	6.1(43)	25	33	29
BION-75		4.1(29)	8.3(58)	5.9(41)	8	58	41
Total no.		319	369	356	221	300	265
Average no. per primer		45.6	52.7	50.9	31.6	42.9	37.9

The total number of loci generated by 7 primers in *S. purpuratus* obtained from Geoje population (GJP), Gunsan population (GSP), North Korea population (NKP).

number of unique shared loci to each clam population and number of shared loci by the three clam population generated by PCR analysis using 7 oligonucleotides primers in the three purplish Washington clam population, respectively, as summarized in Table 2. The primer BION-75 generated 21 unique loci to each population, which were ascertaining each population, approximately 250 bp, 300 bp and 400 bp, in the GJP population. Remarkably, the primer BION-24 detected 21 shared loci by the three populations, major and/or minor fragments of sizes 150 bp, which were matching in all samples. The primer BION-73 also generated 14 unique loci to each population, which were determining each population, approximately 500 bp and 700 bp, in the NKP population. Markedly, the only BION-24 of the other oligonucleotides primers amplified the fragments specific to the GSP population. The amplified DNA fragments ranging in size from 150bp, 250bp and 600bp, respectively, were found to be specific to GSP population. Generally, the specific oligonucleotides primer could be used for detecting genetic similarity/diversity/polymorphisms among various organisms (Welsh et al., 1991; McCormack et al., 2000;

Table 3. Multiple comparisons of average bandsharing values among three purplish Washington clam population were created according to the band-sharing values and similarity matrix

Population	GJP	GSP	NKP
GJP	0.743±0.158 ^a	0.441±0.052 ^c	0.460±0.050 ^c
GSP	-	0.606±0.247 ^b	0.459±0.071 ^c
NKP	-	-	0.726±0.171 ^a

GJP: Geoje population, GSP: Gunsan population, NKP: North Korea population.

Ramesha et al., 2002; Yoon & Kim, 2003; Song & Yoon, 2013). As regards average bandsharing value (BS) results, individuals from GJP population (0.743) exhibited higher bandsharing values than did individuals from GSP population (0.606) ($P<0.05$), as demonstrated in Table 3.

In the present study, the dendrogram obtained by the seven oligonucleotides primers indicates three genetic clusters: cluster 1 (GEOJE 01 ~ GEOJE 07), cluster 2 (GUNSAN 08 ~ GUNSAN 14), cluster 3 (N. KOREA 15 ~ N. KOREA 21), as demonstrated in Fig. 1. Among the twenty one clams,

Table 2. The number of unique shared loci to each clam population and number of shared loci by the three clam population generated by PCR analysis using 7 oligonucleotides primers in the three purplish Washington clam population, respectively

Primer	Population	No. of unique loci to each population			No. of shared loci by the three populations
		GJP	GSP	NKP	Three populations (7 individuals per population)
BION - 22		7	0	21	0
BION - 24		21	21	21	7
BION - 28		14	0	0	0
BION - 68		21	0	21	0
BION - 73		0	0	14	0
BION - 74		14	0	14	0
BION - 75		21	0	0	0
Total no.		98	21	91	7
Average no. per primer		14	3	13	1

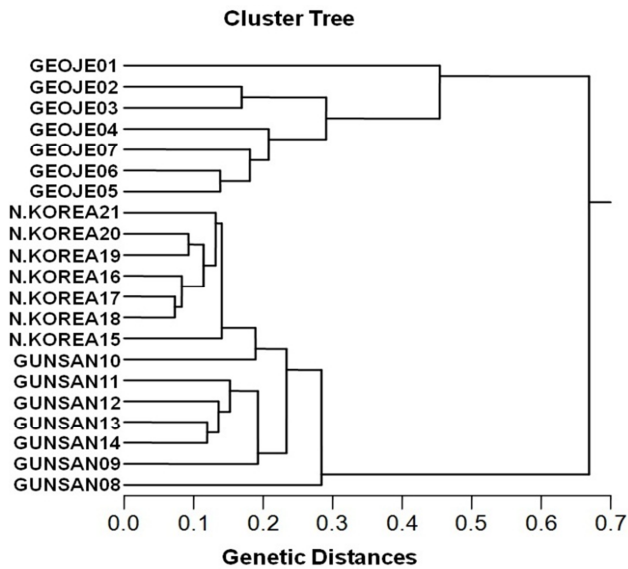


Fig. 1. Hierarchical dendrogram of genetic distances obtained from three purplish Washington clam population. The relatedness among different individuals of three purplish Washington clam population from Geoje clam population (GEOJE 01 ~ GEOJE 07), Gunsan clam population (GUNSAN 08 ~ GUNSAN 14) and North Korea clam population (N.KOREA 15 ~ N.KOREA 21) generated according to the band-sharing values and similarity matrix.

the shortest genetic distance that displayed significant molecular differences was between individuals 08 and 09 from the NKP population (genetic distance = 0.073), while the longest genetic distance among the twenty-one individuals that displayed significant molecular differences was between individuals GEOJE no. 03 and GUNSAN no. 09 (genetic distance = 0.669). Relatively, individuals of GJP population were properly closely related to that of NKP population, as shown in the hierarchical dendrogram of genetic distances. Aforementioned, a dendrogram revealed close relationships between individual structures within three geographical bivalve populations (McCormack et al., 2000). Cluster analysis showed a similar pattern to that illustrated by Yoon & Kim (2004). They reported that single linkage cluster analysis, which indicated four genetic groupings, and the dendrogram revealed a close relation-

ship between the individual identities within two geographical populations. In invertebrates, cluster analysis of the pairwise population matrix, generated from genetic data, showed that geographically close populations be inclined to cluster together in the blacklip abalone (Huang et al., 2000).

Three *S. purpuratus* populations can be evidently discriminated, by PCR-based approach. The potential of oligonucleotides amplified polymorphic DNAs to discover diagnostic markers for breed, species and population identification in shellfish (Huang et al., 2000; McCormack et al., 2000; Yoon & Kim, 2003; Kim et al., 2004; Park et al., 2005; Song & Yoon, 2013) has also been well established. In due course, PCR analysis has revealed the significant genetic distance among three purplish Washington clam populations. PCR fragments revealed of in this study may be valuable as a DNA marker the three regional populations to differentiate. As a whole, the population grouping of *S. purpuratus* is founded on morphological variations in shell color, shell type, shell length, feet length, mantle edge and etc. It is implicated that differences in such traits reflect distinctive origins or genetic identity (Chenyambuga et al., 2004). High levels of a significant genetic distance among three purplish Washington clam populations showed this PCR approach is one of the most apt tools for individuals and/or populations biological DNA studies (Tassanakajon et al., 1998; Yoon & Kim, 2003). Therefore, this process can also be applied to other species of Veneridae and make technically-convenient the analysis of many samples in a short time.

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