# Variation of Shell Color in Three Geographic White Clam (*Meretrix lusoria*) Populations of the Yellow Sea

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**ABSTRACT** : Genomic DNAs (gDNAs) were isolated from the hard clam (*Meretrix lusoria*, Röding, 1798) populations of Gunsan located in the Yellow Sea of the Korean peninsula. Genetic distances among different individuals of the LSCP (light shell color population) population of the hard clam (lane 1-11), GSCP (grey shell color population) population of the hard clam (lane 12-22) and DSCP (dark shell color population) population of the hard clam (lane 23-33), respectively, were generated using Systat version 10 according to the bandsharing values and similarity matrix. The dendrogram, generated by seven reliable oligonucleotides primers, indicates 3 genetic clusters. LSCP population could be evidently discriminated with the other two populations among three populations. The longest genetic distance (0.801) was found to exist between individuals in the two populations, between individuals' no. 33 of the DSCP population and no. 06 of the LSCP population. The higher fragment sizes (>2,000 bp) are much more observed in the GSCP population. Three hard clam populations can be clearly distinguished, especially, by their morphological characters and PCR-based approach.

**Key words** : Genetic distance, Hard clam, *Meretrix lusoria*, Morphological variation, Shell color

## INTRODUCTION

The hard clam (*Meretrix lusoria*, 1798), which belongs to the family Veneridae, is widely distributed in the estuary flat, the intertidal zone and the 10-meter depth of seawater areas of the Yellow Sea and the East Sea in the Korean Peninsula. In general, the size, type, stripe pattern, and color of the hard clam vary in accordance with water depth, turbidity, nutrition, growth period, water temperature, and other environmental aspects (Jung et al., 2004). Research for clam artificial production has progressed steadily in many aspects, over-catching, and water pollution by industries and city sewage. As the clam culture industry grows, so doe's interest into the genetics of the hard clam. However, little information currently exists regarding the genetics of the hard clam. In the present study, we performed a clustering analysis to elucidate the genetic differences among individuals of the LSCP, GSCP and DSCP populations of the hard clam, respectively, by PCR analysis based on the samples collected from Gunsan area.

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## MATERIALS AND METHODS

### 1. Extraction of Genomic DNA, Decamer Primers and Amplification Stipulations

Genomic DNAs were isolated from 33 individuals of three hard clam (*M. lusoria*) populations of Gunsan located in the Yellow Sea of Korean peninsula. DNA extraction and/ or purification was performed as described previously (Park & Yoon, 2008). After several washings, lysis buffer Ⅰ (155 mM $NH_4Cl$; 10 mM $KHCO_3$; 1 mM EDTA) was added to the samples, and the mixture tubes were gently inverted. Ice-cold 70% ethanol was added, and then the samples were centrifuged at 19,621 g for 5 minutes to extract the DNA from the lysates. The DNA pellets were then incubation-dried for more than 10 hours, maintained

at −40℃ until analysis, then dissolved in the ultra-pure water (JW Pharmaceutical, Seoul, Korea). The concentration of the extracted genomic DNA was measured by its absorbance ratio at 260 nm, with a spectrophotometer (Beckman DU 600 series, UK).

Seven primers, OPA-02 (5'-TGCCGAGCTG-3'), OPA-13 (5'-CAGCACCCAC-3'), OPA-17 (5'-GACCGCTTGT-3'), OPB-15 (5'-GGAGGGTGTT-3'), OPB-17 (5'-AGGGAACGAG-3'), OPB-20 (5'-GGACCCTTAC-3') and OPC-01 (5'-TTCG AGCCAG-3') were exposed to generate the unique loci to each population and number of shared loci by 3 populations of the hard clam which could be clearly scored. Amplified polymorphic DNA analysis was performed using two Programmable DNA Thermal Cyclers (MJ Research Inc., Waltham, MA, USA). Amplification products were generated via electrophoresis on 1.4% agarose (Bioneer Corp., Daejeon, Korea) gel containing TBE (90 mM Tris, pH 8.5; 90 mM borate; 2.5 mM EDTA). 100 bp ladder marker (Bioneer Corp., Daejeon, Korea) was utilized as a DNA molecular weight marker. Bands were detected by ethidium bromide staining. The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a Photoman direct copy system (PECA Products, Beloit, WI, USA). The degree of variability was calculated by use of the Dice coefficient ($F$), which is given by the formula: $F = 2\ n_{ab} / (n_a+n_b)$, where $n_{ab}$ is the number of bands shared between the samples $a$ and $b$, $n_a$ is the total number of bands for sample $a$ and $n_b$ is the total number of bands for sample $b$ (Jeffreys & Morton, 1987; Yoon & Kim, 2004; Yoke-Kqueen & Radu, 2006). The relatedness between different individuals in 3 population of the hard clam was generated according to the bandsharing values and similarity matrix. The hierarchical clustering tree was analyzed by the similarity matrices to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

The number of unique loci to each population and number of shared loci by the two populations generated by PCR using 7 decamer primers in the hard clam (*M. lusoria*) population of Gunsan, as illustrated in Table 1. Thirty three numbers of unique loci to each population, with an average of 4.7 per primer, were observed in the LSCP population. 271 unique loci, with an average of 38.7 per primer, were identified in the GSCP population. 165 unique loci, with an average of 23.6 per primer, were identified in the GSCP population. Especially, 33 numbers of shared loci by the 3 populations, with an average of 4.7 per primer, were observed in the three hard clam populations. Diagnostic markers that are found to be present in the 3 populations of an eel-loach (*Pangio* sp.) are also considered to be species-specific markers, whereas the other bands were considered to be population-specific markers (Siti Azizah et al., 2005). The higher fragment sizes (>2,000 bp) are much more observed in the GSCP population, as shown in Fig. 1. The size of the DNA fragments also varied wildly, from 50 to 2,700 bp, as shown in Fig. 2. The dendrogram, generated by seven reliable oligonucleotides primers, indicates

**Table 1. The number of unique loci to each population and number of shared loci by the three populations generated by PCR analysis using 7 decamer primers in G1 (LSCP), G2 (GSCP) and G3 (DSCP) population of hard clam (*Meretrix lusoria*)**

| Item | No. of unique loci to each population | | | No. of shared loci by the three populations |
|---|---|---|---|---|
| Population / Primer | G1 | G2 | G3 | Three populations (11 individuals per population) |
| OPA-02 | 22 | 66 | 55 | 33 |
| OPA-13 | 0 | 88 | 55 | 0 |
| OPA-17 | 0 | 44 | 11 | 0 |
| OPB-15 | 11 | 44 | 0 | 0 |
| OPB-17 | 0 | 44 | 11 | 0 |
| OPB-20 | 0 | 0 | 0 | 0 |
| OPC-01 | 0 | 44 | 33 | 0 |
| Total no. | 33 | 271 | 165 | 33 |
| Average no. per primer | 4.7 | 38.7 | 23.6 | 4.7 |

LSCP: Light shell color population, GSCP: Grey shell color population, DSCP: Dark shell color population.

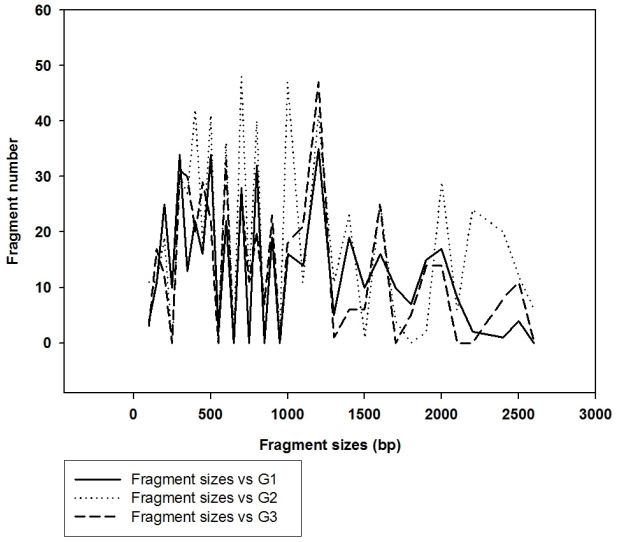


**Fig. 1. Distribution of fragment sizes of G1 (LSCP), G2 (GSCP) and G3 (DSCP) population of the hard clam (*Meretrix lusoria*).** The fragment numbers in each size interval have been computed from the pooled fragments obtained with all the primers. The higher fragment sizes (>2,000 bp) are much more observed in the G2 population. Solid lines: G1 population. Dotted lines: G2 population. Thick dotted lines: G3 population.

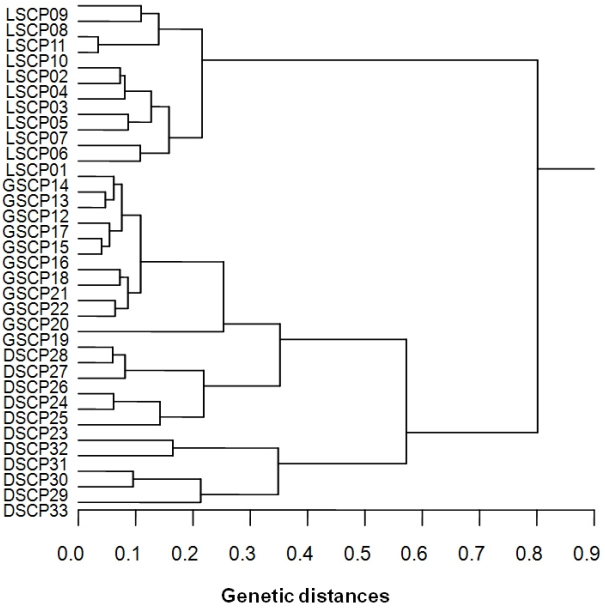


**Fig. 3. Hierarchical dendrogram of genetic distances, obtained from the 3 populations of hard clam (*Meretrix lusoria*).** The relatedness among different individuals in the hard clam populations of shell color from Gunsan of Korean peninsula were generated according to the bandsharing values and similarity matrix.

three genetic clusters, as shown in Figure 3. LSCP population could be evidently discriminated with the other 2 populations among three populations. The longest genetic distance (0.801) was found to exist between individuals' no. 33 of the DSCP population and no. 06 of the LSCP population. In this study, PCR analysis has revealed a significant genetic distance among 3 hard clam populations. Three hard clam populations can be clearly distinguished, especially, by their morphological characters and PCR-based approach. In general, the population classification of the hard clam is based on morphological variations in shell type, shell color, shell length, body weight and shell size. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga et al., 2004). As stated above, the potential of random amplified polymorphic DNAs to identify diagnostic markers for breed, species and population identification in teleosts (Callejas & Ochando, 1998; Yoon & Kim, 2004; Yoon, 2008), and in shellfish (McCormack et al., 2000; Kim et al., 2004; Park & Yoon, 2008) has also been well established. Thus, this PCR analysis disclosed a considerable genetic distance among the 3 hard clam (*M. lusoria*) populations.

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## REFERENCES

Callejas C, Ochando MD (1998) Identification of Spanish

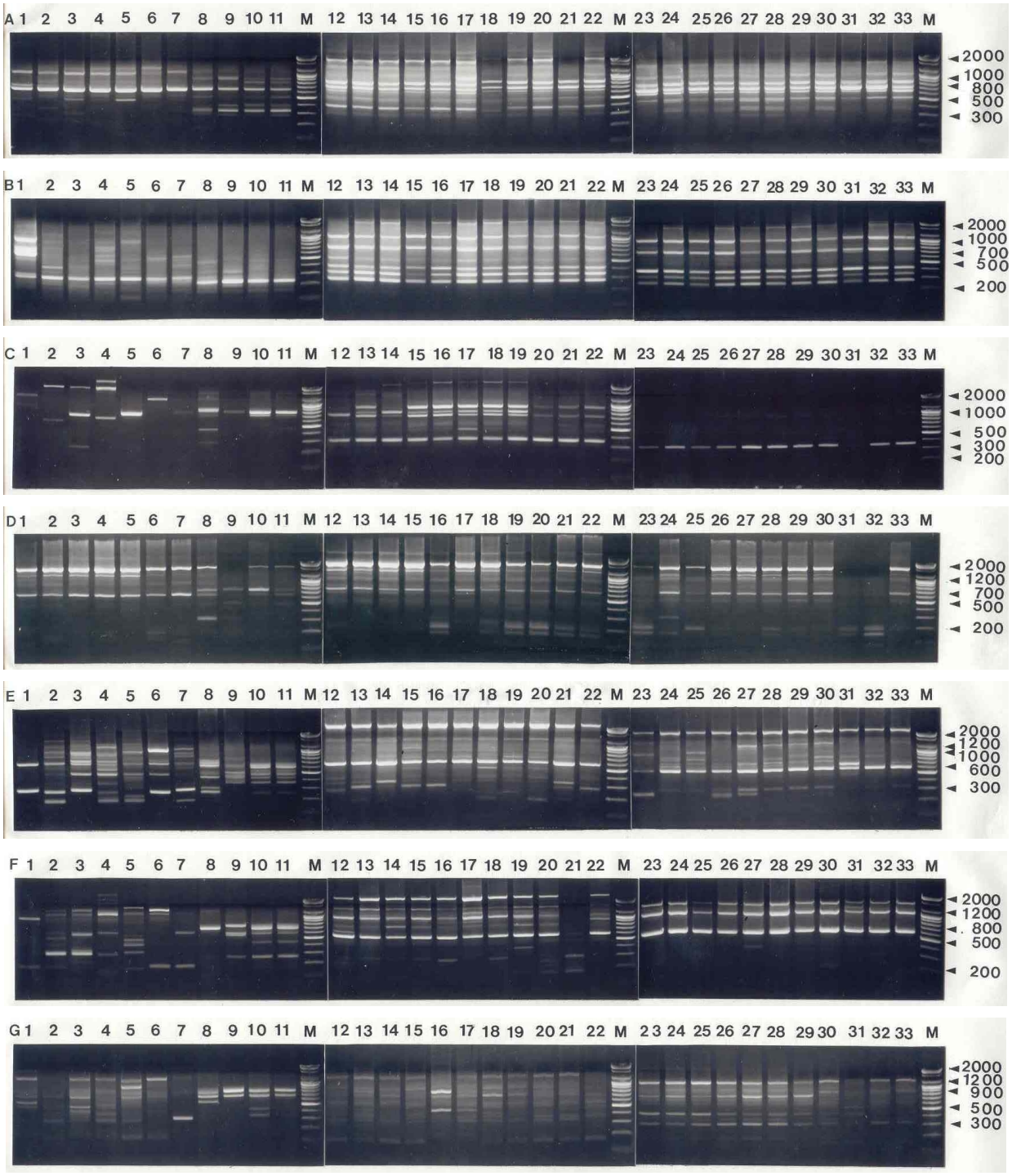


**Fig. 2. PCR-generated electrophoretic profiles of individual hard clam (*Meretrix lusoria*).** DNA isolated from G1 (LSCP) population of the hard clam (lane 1-11), G2 (GSCP) population of the hard clam (lane 12-22) and G3 (DSCP) population of the hard clam (lane 23-33) were amplified by random primers OPA-02 (A), OPA-13 (B), OPA-17 (C), OPB-15 (D), OPB-17 (E), OPB-20 (F) and OPC-01 (G). The PCR products were separated by 1.4% agarose gel electrophoresis and detected by ethidium bromide staining. Each lane shows DNA samples extracted from 33 individuals.

barbel species using the RAPD technique. J Fish Biol 53:208-215.

Chenyambuga SW, Hanotte O, Hirbo J, Watts PC, Kemp SJ, Kifaro GC, Gwakisa PS, Petersen PH, Rege JEO (2004) Genetic characterization of indigenous goats of sub-Saharan Africa using microsatellite DNA markers. Asian-Aust J Anim Sci 17:445-452.

Jeffreys AJ, Morton DB (1987) DNA fingerprints of dogs and cats. Animal Genetics 18:1-15.

Jung HT, Kim J, Shin JA, Sohn HY, Choi SD (2004) Genetic relationship of the five venerid clams (Bivalvia, Veneridae) in Korea. J Aquacult 17:251-257.

Kim JY, Park CY, Yoon JM (2004) Genetic differences and DNA polymorphism in oyster (*Crassostrea* spp.) analysed by RAPD-PCR. Korean J Genet 26:123-134.

McCormack GC, Powell R, Keegan B (2000) Comparative analysis of two populations of the brittle star *Amphiura filiformis* (Echinodermata: Ophiuroidae) with different life history strategies using RAPD markers. Mar Biotechnol 2:100-106.

Park GS, Yoon JM (2008) Geographic variations between Jedo Venus Clam (*Protothaca jedoensis* Lischke) populations of Boryeong and Wonsan of Korea. Korean J Malacol 24:13-26.

Siti Azizah MN, Ruzainah A, Patimah I (2005) Development of RAPD markers in the eel-loach (*Pangio* spp.) for genetic discrimination and monitoring of wild and cultured populations. World Aquacul 36:37-43.

Yoke-Kqueen C, Radu S (2006) Random amplified polymorphic DNA analysis of genetically modified organisms. J Biotechnol 127:161-166.

Yoon JM, Kim JY (2004) Genetic differences within and between populations of Korean catfish (*S. asotus*) and bullhead (*P. fulvidraco*) analysed by RAPD-PCR. Asian-Aust J Anim Sci 17:1053-1061.

Yoon JM (2008) Variability in two species of Osmeridae (*Hypomesus nipponensis* and *Mallotus villosus*). Dev Reprod 12:151-158.

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