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# Genetic Variations of Three *Tegillarca granosa* Populations Investigated by PCR Technique

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## ABSTRACT

The selected seven oligonucleotides primers BION-32, BION-33, BION-35, BION-38, BION-40, BION-46 and BION-58 generated the shared loci, specific loci, unique shared loci to each population and shared loci by the three *T. granosa* populations in Beolgyo, a Chinese site and Wonsan, respectively. The bandsharing value between individuals' no. 03 and no. 04 was 0.816, which was the highest value identified within the Beolgyo population. The primer BION-35 generated the most loci (a total of 70), with an average of 10.0 in the Wonsan population. On average, seven oligonucleotides primers generated 16.1 specific loci in the Beolgyo population, 22.3 in the Chinese population and 39.3 in the Wonsan population. 126 unique shared loci to each population, with an average of 18 per primer, were observed in the Beolgyo population, 63 loci, with an average of 9 per primer, were observed in the Chinese population, and 49 loci, with an average of 7 per primer, and were observed in the Wonsan population. The oligonucleotides primer BION-32 generated 14 unique loci to each population, which were identifying each population in the Beolgyo population. Interestingly, every primer had not distinguished the shared loci by the three populations, major and/or minor fragments of sizes, which were identical in almost all of the samples. As regards average bandsharing value (BS) results, individuals from Beolgyo population (0.717 ± 0.057) exhibited higher BS values than did those from Wonsan population (0.552 ± 0.104) ($P < 0.05$). The dendrogram resulted from truthful seven oligonucleotides primers, representing three genetic clusters comprising group I (BEOLGYO 01, 02, 03, 04, 05, 06 and 07), group II (CHINESE 08, 09, 10, 11, 12, 13 and 14) and group III (WONSAN 15, 16, 17, 18, 19, 20 and 21). In three *T. granosa* populations, the longest genetic distance (0.874) displaying significant molecular difference was also between individual no. 02 within the Beolgyo population and individual no. 12 within the Chinese population. Relatively, individuals of the CHINESE population were fairly closely related to those of the WONSAN population.

**Key words:** Granulated ark shell, *Tegillarca granosa,* Genetic distance, Geographic variations, Specific loci.

## INTRODUCTION

*Tegillarca granosa* is, ecologically warmwater bivalve species, belonging to the class Bivalvia, and family Arcidae, broadly distributed on the southern sea, the Yellow sea and the several sea in the Korean Peninsula and the various sea zones in Japan, China and the Southeast Asia under the natural ecosystem. The granulated ark shell inhabits a sandy mud bottom comprising of a lot of mud and slime from intertidal zone to 10m in depth. The breeding seasons are July to August. The coarse surface of this shellfish species is light gray, covered with black-hair, and the inside is white under the natural conditions. Inner feet are short, triangular, and white-gray. Principally, there are marked differences of the weight, size, color and shape in *T. granosa* in keeping with the environmental conditions of habitat such as feed, water temperature and winterization period. Nevertheless, these kinds of Korean bivalve, which are recognized important morphologically (Chang *et al*., 2006), environmentally (Kang *et al*., 2000), physiologically (Kang, 2004; Kim *et*

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*al*., 2009; Moon and Shin, 2010; Shin *et al*., 2015), reproductively (Lee, 1997; Kim *et al*., 2009), as well as histologically (Ku *et al*., 2015), are not genetically and/or molecular-biologically studied like other shellfishes. There is an essential to understand the genetic traits and structure of this bivalve in order to evaluate precisely the manifest genetic effect. In this study, to elucidate the genetic distances and differences among three *T. granosa* geographical populations, the author accomplished a clustering analysis of three granulated ark shell populations collected from Beolgyo, a Chinese site and Wonsan of the Korean Peninsula, by means of PCR investigation.

## MATERIALS AND METHODS

### 1. Sampling, isolation and/or extraction of genomic DNA

Genomic DNAs were separated from 21 individuals of three *T. granosa* populations of Beolgyo, a Chinese site and Wonsan of the Korean Peninsula, respectively. The granulated ark shell muscle was collected in sterile tubes, placed on ice immediately, and stored at - 40℃ until analysis. DNA extraction/purification should be carried out in keeping with the methods previously described (Yoon and Kim, 2004). So as to attain good results, DNA extraction should be accomplished with highest reagent (Bioneer Corp., Daejeon, Korea) and according to standard procedures with minor modification. After washings several times, 3 volumes of lysis buffer Ⅰ (155 mM $NH_4Cl$; 10 mM $KHCO_3$; 1 mM EDTA) was added and mixed gently by inverting the tubes. The DNA pellets were incubation-dried for 2 hrs, held at - 40℃ until needed, 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. Oligonucleotides primers, amplification conditions and data analyses

The author used the oligonucleotides primers to certify the genetic distances and geological variations of *T. granosa* individuals. Seven oligonucleotides primers, BION-32 (5'-GACCAGCGAA-3'), BION-33 (5'-ACATCCTGCG-3'), BION-35 (5'-AGCGGCTAGG-3'), BION-38 (5'-GGTCCCTGAC-3'), BION-40 (5'-GTTGCGATCC-3'), BION-46 (5'-TTCCCGGAGC-3'), and BION-58 (5'-AGCCTGTGTC-3') were shown to generate the shared loci, specific loci, unique shared loci to each population and shared loci by the three *T. granosa* populations which could be clearly scored. Thus, the author used the primers to study the genetic variations and DNA polymorphisms of the granulated ark shell. PCR was performed using Programmable DNA Thermal Cycler (MJ Research Inc., Waltham, MA, USA). Optimal DNA concentrations for amplification were determined by testing quite a few dilutions, one of which was taken as the standard for every successive amplification. Amplification products were separated by electrophoresis in 1.4% agarose gels (Bioneer Corp., Daejeon, Korea) with TBE (0.09 M Tris, pH 8.5; 0.09 M borate; 2.5 mM EDTA). The 100 bp DNA ladder (Bioneer Corp., Daejeon, Korea) was used as a DNA molecular weight marker. The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a photoman direct copy system (PECA Products, Beloit, WI, USA). Bandsharing (BS) values were calculating according to the presence/absence of amplified products at specific positions in the same gel from the PCR profiles. Absence of bands indicates that the priming site is not present, presumably as a result of some alteration in the DNA sequence. The degree of variableness was calculated by use of the Dice coefficient (*F*), which is given by the formula: $F$ (BS) = 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 and Morton, 1987; Yoke-Kqueen and Radu, 2006). The average within-population similarity was calculated by pairwise comparison between individuals within a population. The levels of affinity among different individuals from Beolgyo (BEOLGYO01-BEOLGYO07), a Chinese site (CHINESE08-CHINESE14) and Wonsan (WONSAN15-WONSAN21) population of the Korean Peninsula, respectively, were generated according to the bandsharing values and similarity matrix. The

**Table 1**. Similarity matrix including bandsharing values (BS) and genetic differences calculated using Nei and Li's index of the similarity of *T. granosa* from Beolgyo, a Chinese site and Wonsan of Korea, respectively

| Bandsharing values of *Tegillarca granosa* | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| from Beolgyo | | | | | | | from a Chinese site | | | | | | | from Wonsan | | | | | | | |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | |
| - | 0.788 | 0.629 | 0.712 | 0.665 | 0.701 | 0.653 | 0.548 | 0.443 | 0.619 | 0.377 | 0.485 | 0.486 | 0.515 | 0.176 | 0.238 | 0.369 | 0.126 | 0.298 | 0.242 | 0.153 | **1** |
| | - | 0.733 | 0.768 | 0.779 | 0.765 | 0.656 | 0.553 | 0.456 | 0.552 | 0.423 | 0.441 | 0.458 | 0.439 | 0.209 | 0.250 | 0.335 | 0.194 | 0.290 | 0.241 | 0.213 | **2** |
| | | - | 0.816 | 0.694 | 0.770 | 0.696 | 0.423 | 0.420 | 0.462 | 0.464 | 0.409 | 0.327 | 0.413 | 0.163 | 0.195 | 0.255 | 0.189 | 0.232 | 0.214 | 0.198 | **3** |
| | | | - | 0.692 | 0.707 | 0.683 | 0.470 | 0.434 | 0.501 | 0.465 | 0.453 | 0.434 | 0.489 | 0.145 | 0.229 | 0.303 | 0.184 | 0.292 | 0.269 | 0.200 | **4** |
| | | | | - | 0.756 | 0.614 | 0.543 | 0.507 | 0.569 | 0.383 | 0.454 | 0.511 | 0.452 | 0.182 | 0.241 | 0.403 | 0.253 | 0.361 | 0.313 | 0.232 | **5** |
| | | | | | - | 0.781 | 0.527 | 0.524 | 0.508 | 0.524 | 0.519 | 0.422 | 0.507 | 0.183 | 0.217 | 0.327 | 0.197 | 0.350 | 0.288 | 0.195 | **6** |
| | | | | | | - | 0.510 | 0.486 | 0.486 | 0.462 | 0.433 | 0.432 | 0.568 | 0.201 | 0.313 | 0.392 | 0.302 | 0.388 | 0.330 | 0.212 | **7** |
| | | | | | | | - | 0.670 | 0.652 | 0.434 | 0.579 | 0.628 | 0.567 | 0.150 | 0.246 | 0.340 | 0.158 | 0.294 | 0.285 | 0.190 | **8** |
| | | | | | | | | - | 0.697 | 0.616 | 0.639 | 0.445 | 0.527 | 0.110 | 0.162 | 0.311 | 0.159 | 0.292 | 0.224 | 0.139 | **9** |
| | | | | | | | | | - | 0.514 | 0.613 | 0.552 | 0.595 | 0.096 | 0.221 | 0.374 | 0.198 | 0.391 | 0.320 | 0.225 | **10** |
| | | | | | | | | | | - | 0.758 | 0.457 | 0.503 | 0.097 | 0.119 | 0.169 | 0.104 | 0.185 | 0.117 | 0.058 | **11** |
| | | | | | | | | | | | - | 0.660 | 0.662 | 0.104 | 0.154 | 0.263 | 0.144 | 0.285 | 0.229 | 0.099 | **12** |
| | | | | | | | | | | | | - | 0.648 | 0.249 | 0.252 | 0.370 | 0.267 | 0.328 | 0.297 | 0.242 | **13** |
| | | | | | | | | | | | | | - | 0.178 | 0.268 | 0.392 | 0.276 | 0.320 | 0.243 | 0.221 | **14** |
| | | | | | | | | | | | | | | - | 0.498 | 0.409 | 0.429 | 0.433 | 0.455 | 0.432 | **15** |
| | | | | | | | | | | | | | | | - | 0.519 | 0.580 | 0.578 | 0.529 | 0.496 | **16** |
| | | | | | | | | | | | | | | | | - | 0.520 | 0.733 | 0.633 | 0.497 | **17** |
| | | | | | | | | | | | | | | | | | - | 0.590 | 0.545 | 0.611 | **18** |
| | | | | | | | | | | | | | | | | | | - | 0.788 | 0.616 | **19** |
| | | | | | | | | | | | | | | | | | | | - | 0.706 | **20** |
| | | | | | | | | | | | | | | | | | | | | - | **21** |

hierarchical clustering tree was examined by the similarity matrices to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., Chicago, IL, USA). The Systat software was also accomplished to observe genetic differences, Euclidean genetic distances within and between populations, means, standard errors, and *t*-test scores.

## RESULTS AND DISCUSSION

At this point, the complexity of the banding patterns varied dramatically between the oligonucleotides primers from the three localities. DNA fragments obtained by seven oligonucleotides primers ranged in size from 50 to 2,000 bp in the granulated ark shell. The selected seven primers BION-32, BION-33, BION-35, BION-38, BION-40, BION-46 and BION-58 generated the shared loci, specific loci, unique shared loci to each population and shared loci by the three *T. granosa* populations in Beolgyo, a Chinese site and Wonsan, respectively. Similarity matrix including bandsharing values (BS) was calculated using Nei and Li's index of the similarity of the individuals from the three granulated ark shell populations, as established in Table 1. Founded on the average bandsharing values of all samples, the similarity matrix extended from 0.614 to 0.816 in the Beolgyo population, from 0.434 to 0.758 in the Chinese population and from 0.409 to 0.788 in the Wonsan population, as illustrated in Table 1. The bandsharing value between individuals' no. 03 and no. 04 was 0.816, which was the highest value identified within the Beolgyo

**Table 2**. The number of average loci per lane and specific loci by PCR analysis using 7 primers in of *T. granosa* from Beolgyo, a Chinese site and Wonsan of Korea, respectively

| Item | No. of average loci per lane | | | No. of specific loci | | |
|---|---|---|---|---|---|---|
| Primer | Beolgyo | Chinese | Wonsan | Beolgyo | Chinese | Wonsan |
| BION-32 | 4.3 (30) | 3.6 (25) | 6.0 (42) | 16 | 25 | 42 |
| BION-33 | 4.3 (30) | 3.7 (26) | 5.7 (40) | 16 | 12 | 26 |
| BION-35 | 6.6 (46) | 5.9 (41) | 10.0 (70) | 25 | 27 | 56 |
| BION-38 | 4.6 (32) | 5.4 (38) | 6.0 (42) | 11 | 31 | 35 |
| BION-40 | 3.9 (27) | 3.1 (22) | 4.4 (31) | 13 | 8 | 24 |
| BION-46 | 4.4 (31) | 6.0 (42) | 6.6 (46) | 17 | | 39 |
| BION-58 | 6.1 (43) | 3.6 (25) | 7.6 (53) | 15 | 18 | 53 |
| Total no. | 34.1 (239) | 31.3 (219) | 46.3 (324) | 112 | 156 | 275 |
| Average no. per primer | 34.1 | 31.3 | 46.3 | 16.1 | 22.3 | 39.3 |

population. Oppositely, the bandsharing value between individuals' no. 15 of Wonsan and no. 10 of Chinese was 0.816, which was the lowest value. Seven oligonucleotides primers were shown to generate the shared loci, specific loci, which could be apparently scored, as demonstrated in Table 2. In this study, 7 oligonucleotides primers generated 239 total loci in the Beolgyo, 219 in the Chinese and 324 in the Wonsan population, respectively. Also, these oligonucleotides primers made 122 specific loci in the Beolgyo, 156 in the Chinese and 276 in the Wonsan population, respectively. The primer BION-35 generated the most loci (a total of 70), with an average of 10.0 in the Wonsan population, as illustrated in Table 2. The oligonucleotides primer BION-40 generated the least loci (a total of 22), with an average of 3.1 in the Chinese population, in contrast to the other primers used. On average, 7 oligonucleotides primers generated 16.1 specific loci in the Beolgyo population, 22.3 in the Chinese population and 39.3 in the Wonsan population. The specific loci generated by oligonucleotides primers exhibited inter-species-specific characteristics, thus revealing DNA polymorphisms. Here, the seven oligonucleotides primers were used to generate the unique loci to each population and shared loci by the three populations, as illustrated in Table 3.

**Table 3**. The number of unique loci to each population and number of shared loci by the three populations generated by PCR analysis using 7 oligonucleotides oligonucleotides primers in *T. granosa* from Beolgyo, a Chinese site and Wonsan population of Korea, respectively

| Item | No. of unique loci to each population | | | No. of shared loci by the three populations |
|---|---|---|---|---|
| Primer \ Population | Beolgyo | Chinese | Wonsan | Three populations (7 individuals per population) |
| BION - 32 | 14 | 0 | 0 | 0 |
| BION - 33 | 14 | 14 | 14 | 0 |
| BION - 35 | 21 | 14 | 14 | 0 |
| BION - 38 | 21 | 7 | 7 | 0 |
| BION - 40 | 14 | 14 | 7 | 0 |
| BION - 46 | 14 | 7 | 7 | 0 |
| BION - 58 | 28 | 7 | 0 | 0 |
| Total no. | 126 | 63 | 49 | 0 |
| Average no. per primer | 18 | 9 | 7 | 0 |

**Table 4**. Several comparisons of average bandsharing values among three *T. granosa* populations were generated according to the bandsharing values and similarity matrix

| Population | Beolgyo | Chinese | Wonsan |
|---|---|---|---|
| Beolgyo | $0.717 \pm 0.057^{f}$ | $0.475 \pm 0.056^{cd}$ | $0.251 \pm 0.070^{a}$ |
| Chinese | - | $0.591 \pm 0.087^{d}$ | $0.223 \pm 0.088^{a}$ |
| Wonsan | - | - | $0.552 \pm 0.104^{d}$ |

[a-f]: Values with different superscript are significantly different, $P < 0.05$
Each value is a result of three different experiments.

126 unique shared loci to each population, with an average of 18 per primer, were observed in the Beolgyo population, 63 loci, with an average of 9 per primer, were observed in the Chinese population, and 49 loci, with an average of 7 per primer, and were observed in the Wonsan population. The oligonucleotides primer BION-35 generated 21 unique loci to each population, approximately 150 bp, 200 bp and 350 bp, respectively, in the Beolgyo population. The oligonucleotides primer BION-58 generated 28 unique loci to each population in the Beolgyo population. Especially, the oligonucleotides primer BION-32 generated 14 unique loci to each population, which were identifying each population in the Beolgyo population. Interestingly, every primer had not distinguished the shared loci by the three populations, major and/or minor fragments of sizes, which were identical in almost all of the samples. Several investigators researched the sizes of DNA fragments in the PCR outlines of five species of Eastern Pacific abalone (genus *Haliotis*) (Muchmore *et al*., 1998), black tiger shrimp (*Penaeus monodon*) (Tassanakajon *et al*., 1998), the brittle star (*Amphiura filiformis*) (McCormack *et al*., 2000) and shrimp populations (Yoon and Kim, 2003).

With regard to average bandsharing value (BS) results, individuals from Beolgyo population ($0.717 \pm 0.057$) exhibited higher BS values than did those from Wonsan population ($0.552 \pm 0.104$) ($P < 0.05$), as summarized in Table 4. The dendrogram resulted from truthful seven oligonucleotides primers, representing three genetic clusters composed of group I (BEOLGYO 01, 02, 03, 04, 05, 06 and 07), group II (CHINESE 08, 09, 10, 11, 12, 13 and 14) and group III (WONSAN 15, 16, 17, 18, 19, 20 and 21), as revealed in Fig. 1. The genetic distance among the three granulated ark shell populations extended from 0.073 to 0.874. In three ark shell populations, the longest genetic distance (0.874) exhibiting significant molecular difference was also between individual no. 02 within the Beolgyo population and individual no. 12 within the Chinese population. Reversely, the shortest genetic distance (0.073) demonstrating significant molecular difference was also between individual no. 19 and no. 20 within the Wonsan population. Comparatively, individuals of the CHINESE population were fairly closely related to

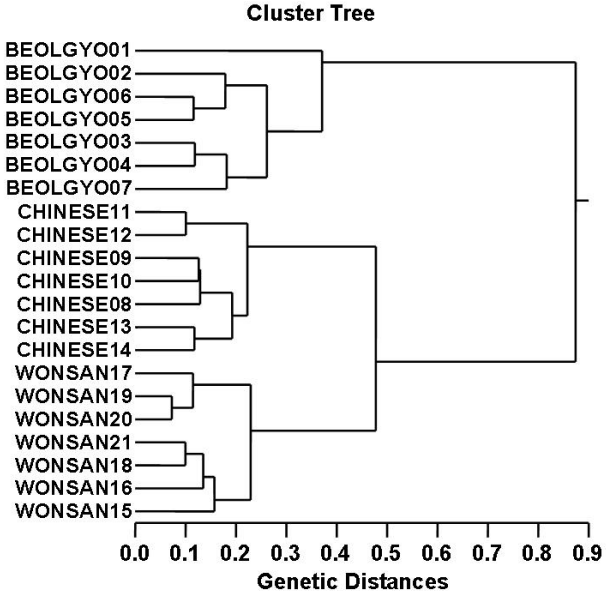


**Fig. 1**. Hierarchical dendrogram of genetic distances, attained from three populations of *T. granosa*. The relatedness among different individuals in three populations of the granulated arkshell from Beolgyo (BEOLGYO01-BEOLGYO07), a Chinese site (CHINESE0-CHINESE14) and Wonsan (WONSAN15-WONSAN21) population of the Korean Peninsula, respectively, were generated according to the bandsharing values and similarity matrix.

those of the WONSAN population.

Above-mentioned, a dendrogram disclosed close relationships between individual identities within three geographical bivalve populations (McCormack *et al*., 2000). 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 granulated ark shell populations can be obviously distinguished by PCR-based system. The prospective of oligonucleotides amplified polymorphic DNAs to determine diagnostic markers for line, species and population identification in crustacean has also been well recognized (Tassanakajon *et al*., 1998; McCormack *et al*., 2000; Kim *et al*., 2004; Park *et al*., 2005; Song and Yoon, 2013). PCR fragments revealed of in the present study may be useful as a DNA marker the three regional populations to discriminate. As a whole, the population grouping of *T. granosa* is recognized on morphological variations in shell type, shell color, shell body weight, shell length, hair color and hair length. It is associated that differences in such characters reflect distinctive origins or genetic identity (Chenyambuga *et al*., 2004). If Korean *T. granosa* systematic investigation was in additional advancement, this study could be used as fundamental data. Henceforth, further more research is prerequisite for more profound population/species identification in shellfish.

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