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Geographic Variations of Three *Fulvia mutica* Populations

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ABSTRACT

In the present study, the seven primers BION-33, BION-34, BION-37, BION-41, BION-44, BION-45 and BION-42 generated the total number of loci, average number of loci per lane and specific loci in Hongseong, Yeosu and Goheung population of *F. mutica*, respectively. 7 primers generated 19 specific loci in the Hongseong population, 29.3 in the Yeosu population and 23.1 in the Goheung population, respectively. Especially, the decamer primer BION-37 generated 7 unique loci to each population, which were identifying each population, approximately 700 bp in Hongseong population. In this study, the dendrogram obtained by the seven primers indicates three genetic clusters: cluster 1 (HONGSEONG 01-HONGSEONG 07), cluster 2 (YEOSU 08-YEOSU 14) and cluster 3 (GOHEUNG 15-GOHEUNG 21). Among the twenty one cockles, the shortest genetic distance that displayed significant molecular differences was between individuals 17 and 19 from the Goheung population (genetic distance = 0.051), while the longest genetic distance among the twenty-one cockle individuals that displayed significant molecular differences was between individuals HONGSEONG no. 03 and YEOSU no. 12 (genetic distance = 0.616). Relatively, individuals of YEOSU population were fairly closely related to that of GOHEUNG population. Ultimately, PCR fragments revealed of in this study may be useful as a DNA marker the three geographic populations to distinguish.

Key words: Cockles, *Fulvia mutica*, Genetic distance, Geographic variations, Specific loci

INTRODUCTION

Fulvia mutica is, ecologically warmwater bivalve species, belonging to the class Bivalvia, and family Cardiidae, widely distributed on the coast of the Yellow Sea, southern sea and Jeju Island in the Korean peninsula and the several sea areas in Japan and China under the natural ecosystem. The cockles dwell in a sandy mud bottom consisting of a lot of sand, mud and slime from 10 to 50m in depth. The breeding seasons are February to June, and August to November. The name is from their fairly long inner feet that look like beaks of birds when they are peeled. The cockles are yellowish brown and coarse in the shell surface, and the inside is pink under natural conditions. There are 40-50 thin and slim radial-shaped wrinkles on surfaces. Shell surface is covered with light brown epidermis, and the inside is pink. Inner feet are long, triangular, and dark brown. Basically, there are marked differences of the size, color and shape in *F. mutica* according to the environmental conditions of habitat such as feed and winterization period. However, these kinds of Korean bivalve, which are recognized important physiologically (Yang, 2006), biochemically (Mahmoud *et al*., 2010) as well as economically (Kim *et al*., 2001), are not genetically studied or researched like other shellfishes. There is a need to understand the genetic traits and composition of this bivalve in order to evaluate exactly the patent genetic effect. We perform clustering analyses to elucidate the genetic distances and

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Fig.1. Map illustrating bivalve sampling localities. Genomic DNA samples isolated from three geographical *F. mutica* populations collected in Hongseong of the Yellow Sea, Yeosu and Goheung of the southern sea, off the Korean Peninsula.

geological variations among three *Fulvia mutica* geographical populations from Hongseong, Yeosu and Goheung of the Korean peninsula, using PCR method.

MATERIALS AND METHODS

1. Sample collection and purification of genomic DNA

Three geographic populations of the *F. mutica* were obtained from three different regions of Hongseong, Yeosu and Goheung in Korea, respectively, as shown in Fig. 1. Cockle muscle was collected in sterile tubes, placed on ice immediately, and stored at - 40℃ until needed. PCR analysis was performed on DNA samples extracted from a total of 21 individuals using seven decamer primers. DNA extraction/purification should be carried out according to the methods previously described (Yoon and Kim, 2004). The DNA pellets were incubation-dried for 2 hrs, held at - 40℃ 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

We used the decamer primers to clarify the genetic distances and geological variations of *F. mutica* individuals. Seven primers, BION-33 (5'-ACATCCTGCG-3'), BION-34 (5'-GCCGCTACTA-3'), BION-37 (5'-AGTCAGCCAC-3'), BION-41 (5'-ATCGGGTCCG-3'), BION-44 (5'-TGCCGAGCTG-3'), BION-45 (5'-CAAACGTCGG-3'), and BION-42 (5'-TACGATGACG-3') were shown to generate the unique shared loci to each population and shared loci by the three *F. mutica* populations which could be clearly scored. Thus, we used the primers to study the genetic variations and DNA polymorphisms of the cockle. PCR was performed using Programmable DNA Thermal Cycler (MJ Research Inc., Waltham, MA, USA). Optimal DNA concentrations for amplification were determined by testing several dilutions, one of which was taken as the standard for every subsequent 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 relatedness among different individuals from Hongseong population (HONGSEONG 01-HONGSEONG 07), Yeosu population (YEOSU 08-YEOSU 14) and Goheung population (GOHEUNG 15-GOHEUNG 21) were generated according to the

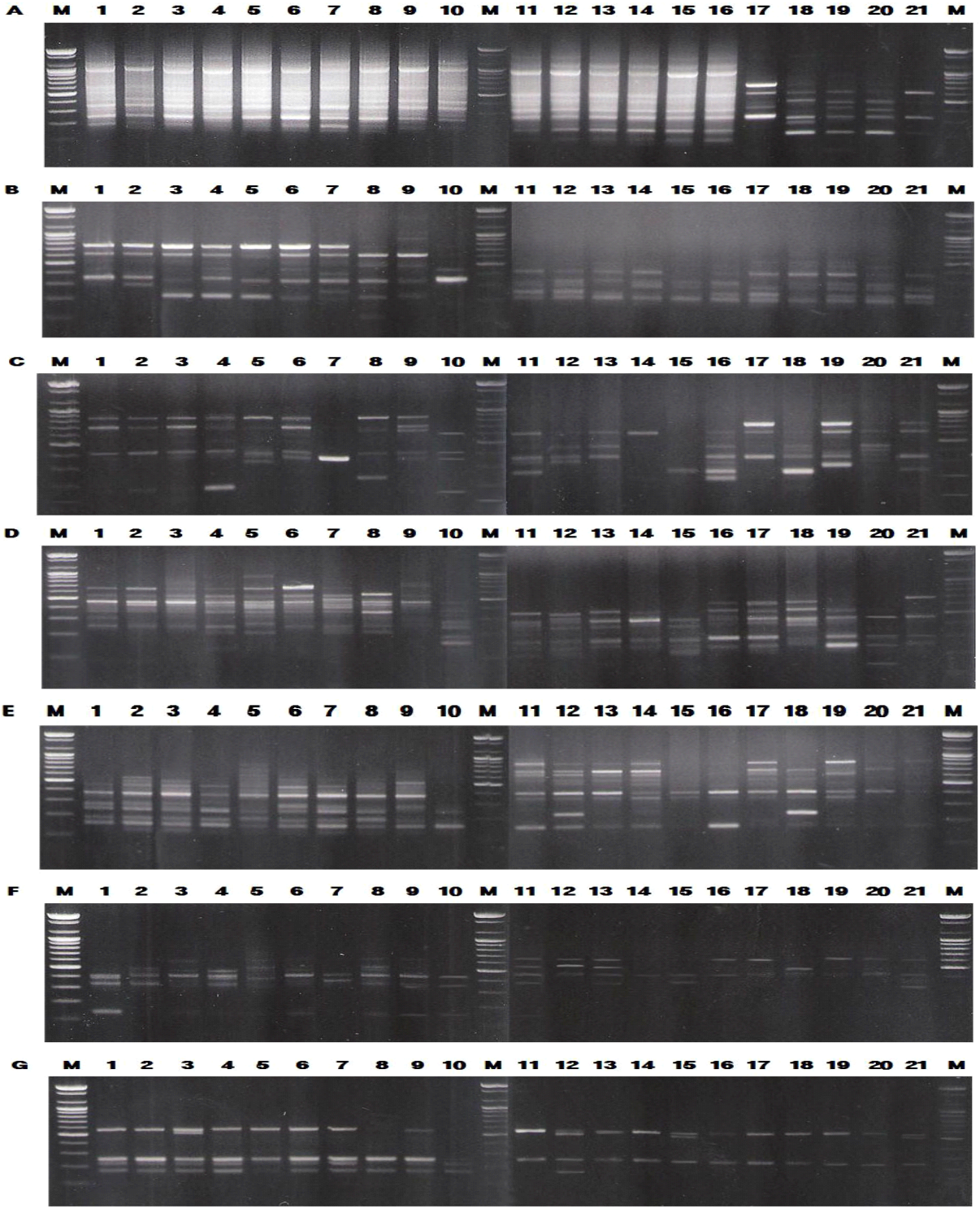

Fig. 2. PCR-based electrophoretic profiles of individuals *F. mutica*. Each lane shows DNA samples extracted from 21 individuals. The DNA isolated from Hongseong population (lane 1-7), Yeosu population (lane 8-14) and Goheung population of *F. mutica* (lane 15-21) were amplified by decamer primers BION-33 (**A**), BION-34 (**B**), BION-37 (**C**), BION-41 (**D**), BION-44 **(E**), BION-45 (**F**) and BION-42(**G**). The PCR products were separated by 1.4% agarose gel electrophoresis, and stained with ethidium bromide. 100 bp ladder (M) was used as a DNA molecular weight marker.

Table 1. Similarity matrix including bandsharing values (BS) and genetic differences calculated using Nei and Li's index of the similarity of *F. mutica* from Hongseong, Yeosu and Goheung of Korea, respectively

	BS from Hongseong							BS from Yeosu							BS from Goheung						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	0.890	0.894	0.854	0.796	0.825	0.835	0.712	0.718	0.656	0.535	0.611	0.591	0.532	0.581	0.482	0.384	0.505	0.462	0.411	0.406
2		-	0.815	0.783	0.742	0.863	0.752	0.722	0.702	0.673	0.600	0.693	0.624	0.636	0.611	0.510	0.410	0.576	0.428	0.390	0.410
3			-	0.820	0.800	0.832	0.813	0.710	0.710	0.645	0.544	0.672	0.600	0.530	0.526	0.543	0.508	0.549	0.526	0.480	0.526
4				-	0.768	0.760	0.742	0.666	0.682	0.698	0.444	0.541	0.471	0.460	0.477	0.417	0.406	0.468	0.407	0.359	0.385
5					-	0.846	0.776	0.749	0.751	0.595	0.610	0.678	0.626	0.559	0.468	0.538	0.534	0.590	0.494	0.533	0.465
6						-	0.876	0.782	0.764	0.618	0.618	0.707	0.665	0.596	0.492	0.585	0.511	0.604	0.534	0.470	0.459
7							-	0.740	0.734	0.619	0.564	0.621	0.528	0.513	0.499	0.504	0.479	0.532	0.523	0.432	0.474
8								-	0.725	0.623	0.552	0.790	0.557	0.497	0.527	0.496	0.418	0.503	0.493	0.419	0.359
9									-	0.625	0.558	0.637	0.538	0.487	0.440	0.406	0.373	0.498	0.400	0.440	0.357
10										-	0.459	0.518	0.499	0.479	0.472	0.383	0.300	0.395	0.346	0.384	0.381
11											-	0.832	0.827	0.701	0.558	0.692	0.580	0.598	0.594	0.527	0.546
12												-	0.821	0.734	0.528	0.672	0.634	0.604	0.617	0.567	0.528
13													-	0.800	0.604	0.671	0.583	0.619	0.535	0.559	0.553
14														-	0.629	0.621	0.563	0.598	0.562	0.512	0.471
15															-	0.640	0.503	0.522	0.495	0.457	0.628
16																-	0.771	0.682	0.750	0.689	0.669
17																	-	0.725	0.852	0.772	0.678
18																		-	0.677	0.657	0.573
19																			-	0.776	0.683
20																				-	0.697
21																					-

bandsharing values and similarity matrix. A hierarchical clustering tree was assembling using similarity matrices to produce a dendrogram, which was facilitated by the Systat version 10 software (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 amplified products were separated by agarose gel electrophoresis (AGE) with oligonucleotides decamer primers, and stained with ethidium bromide. Similarity matrix including bandsharing values (BS) and genetic differences was calculated using Nei and Li's index of the similarity of cockle individuals from Hongseong, Yeosu and Goheung of the Korean peninsula, respectively, as illustrated in Table 1. The seven selected primers BION-33, BION-34, BION-37, BION-41, BION-44, BION-45 and BION-42 generated the total number of loci, average number of loci per lane and specific loci in Hongseong, Yeosu and Goheung population, as summarized in Table 2. Here, the complexity of the banding patterns varied dramatically between the primers from the three locations. The size of the DNA fragments also varied wildly, from 100 to 1,600 bp, as shown in Fig. 1. The primer BION-33 generated the most loci (a total of 70), with an average of 10.0 in the Yeosu population, as illustrated in Table 2. The decamer primer BION-45 generated the least loci (a total of 15), with an average of 2.14 in the Goheung population, in comparison to the other primers used. In the present study, 7 primers generated 19 specific loci in the Hongseong population, 29.3 in the Yeosu population and 23.1 in the Goheung population. The specific loci generated by decamer primers exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms. Many researchers studied the sizes of DNA fragments in the PCR profiles 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), marsh clams (*Corbicula* spp.) (Yoon and Kim, 2003a) and shrimp populations (Yoon

Table 2. The number of average loci per lane and specific loci by PCR analysis using 7 primers in *F. mutica* in Hongseong, Yeosu and Goheung of Korea

Item	No. of average loci per lane			No. of specific loci		
Primer	HS	YS	GH	HS	YS	GH
BION-33	9.86 (69)	10.0 (70)	7.43 (52)	13	14	31
BION-34	6.29 (44)	5.29 (37)	5.43 (38)	23	37	31
BION-37	4.14 (29)	4.00 (28)	4.86 (34)	22	28	34
BION-41	9.14 (64)	7.00 (49)	5.71 (40)	15	35	33
BION-44	7.86 (55)	8.43 (59)	5.29 (37)	27	52	23
BION-45	5.71 (40)	3.86 (27)	2.14 (15)	26	20	8
BION-42	6.00 (42)	3.71 (26)	2.29 (16)	7	19	2
Total No.	49 (343)	42.3 (296)	33.1 (232)	133	205	162
Average No. per primer	49	42.3	33.1	19	29.3	23.1

The total number of loci generated by 7 primers in *F. mutica* obtained from Hongseong, Yeosu and Goheung is shown in parentheses. HS: Hongseong, YS: Yeosu, GH: Goheung

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 decamer primers in Hongseong, Yeosu and Goheung population of *F. mutica*, respectively

Item	No. of unique loci to each population			No. of shared loci by the three populations
Primer \ Population	Hongseong	Yeosu	Goheung	Three populations (7 individuals per population)
BION - 33	56	56	21	42
BION - 34	21	0	7	0
BION - 37	7	0	0	0
BION - 41	42	14	7	0
BION - 44	28	7	14	21
BION - 45	14	7	7	21
BION - 42	35	7	14	21
Total no.	203	91	70	105
Average no. per primer	29	13	10	15

and Kim, 2003b).

The seven selected primers BION-33, BION-34, BION-37, BION-41, BION-44, BION-45 and BION-42 were used to generate unique shared loci to each population and shared loci by the four populations, as summarized in Table 3. The decamer primer BION-33 generated 56 unique loci to each population, approximately 250 bp, 350 bp, 500 bp, 600 bp, 700 bp, 1000 bp, 1200 bp and 1600 bp, respectively, in the Hongseong population. Especially, the decamer primer BION-37 generated 7 unique loci to each population, which were identifying each population, approximately 700 bp in Hongseong population, as summarized in Table 3. Interestingly, the primer BION-33 detected 42 shared loci by the three populations, major and/or minor fragments of sizes 350 bp and 600 bp, respectively, which were identical in all samples. As regards average bandsharing value (BS) results, individuals from Hongseong population (0.813) exhibited higher bandsharing values than did individuals from Yeosu population (0.631), as summarized in Table 4. In this study, the dendrogram obtained by the seven decamer primers indicates three genetic clusters: cluster 1 (HONGSEONG 01-HONGSEONG 07), cluster 2 (YEOSU 08-YEOSU 14) and cluster 3 (GOHEUNG 15-GOHEUNG 21). Among

Table 4. Multiple comparisons of average bandsharing values among Korean *F. mutica* populations from three regions were generated according to the bandsharing values and similarity matrix

Population	Hongseong	Yeosu	Goheung
Hongseong	0.813	0.633	0.487
Yeosu	-	0.631	0.513
Goheung	-	-	0.662

the twenty-one cockle, the shortest genetic distance that displayed significant molecular differences was between individuals 17 and 19 from the Goheung population (genetic distance = 0.051), while the longest genetic distance among the twenty-one *F. mutica* individuals that demonstrate d significant molecular differences was between individuals HONGSEONG no. 03 and YEOSU no. 12 (genetic distance = 0.616). Relatively, individuals of YEOSU population were fairly closely related to that of GOHEUNG population. From what has been said above, a dendrogram disclosed close relationships between individual characteristics 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).

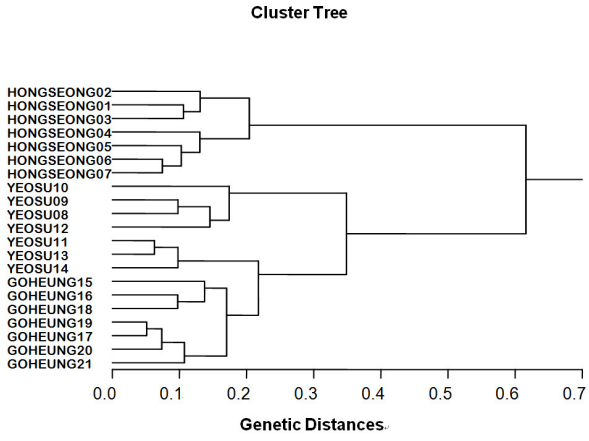

Fig. 3. Hierarchical dendrogram of genetic distances, obtained from three populations of *F. mutica*. The relatedness among different individuals in the three cockle populations from Hongseong, Yeosu and Goheung of the Korean peninsula were generated according to the bandsharing values and similarity matrix.

Above-mentioned, a dendrogram disclosed close relationships between individual identities within three geographical bivalve populations (McCormack *et al*., 2000). Three *F. mutica* populations can be clearly distinguished, by PCR-based approach. The potential of oligonucleotides amplified polymorphic DNAs to discover diagnostic markers for breed, species and population identification in shellfish (McCormack *et al*., 2000; Kim *et al*., 2004; Park *et al*., 2005) has also been well established. PCR fragments discovered of in this study may be useful as a DNA marker the three regional populations to distinguish. In general, the population classification of *F. mutica* is founded on morphological variations in shell body weight, shell type, shell color, shell length and feet length. It is implicated that differences in such characters reflect distinctive origins or genetic identity (Chenyambuga *et al*., 2004). If Korean *F. mutica* systematic study was in supplemental progress, which could be utilized as fundamental data.

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