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Bandsharing Values and Genetic Distances of Two Wild Shortnecked Clam, *Ruditapes philippinarum* Populations from the Yellow Sea Assessed by Random Amplified Polymorphic DNAs-Polymerase Chain Reaction

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Genomic DNAs were extracted from the muscle of twenty-two specimens of two shortnecked clam, *Ruditapes philippinarum* populations collected in Anmyeondo and Seocheon. Genetic differences within and between populations were analysed by random amplified polymorphic DNAs-polymerase chain reaction (RAPD-PCR) using twenty arbitrary decamer primers. Out of 20 primers, 6 generated a total of 1,111 major and minor RAPD bands from individuals of two sites, producing approximately 4.2 average polymorphic bands per primer in individuals from Anmyeondo and ranging in size from less than 50 to larger than 1,500 base pairs (bp). The electrophoretic analysis of RAPD products amplified showed moderate levels of similarity among the different individuals in Seocheon population. The average bandsharing values (BS value) of the samples within population from Anmyeondo ranged from 0.155 to 0.684, whereas it was 0.143~0.782 within population from Seocheon. The average BS value between individuals No. 13 and No. 14 from Seocheon was 0.782 which was higher than that of those from Anmyeondo. The single linkage dendrogram resulted from three primers (OPA-08, -09 and -20), indicating six genetic groupings composed of group 1 (No. 4, 8 and 10), group 2 (No. 18), group 3 (No. 2, 5 and 7), group 4 (No. 1, 3, 6, 9, 11, 12, 13, 14, 15 and 17), group 5 (16, 19 and 20) and group 6 (No. 21 and 22). In the Seocheon population, the individual No. 18 clustered distinctly from the others of this population. The observed genetic distance between the two populations from Anmyeondo and Seocheon was more than 0.209 (0.247 and 0.275). The shortest genetic distance (0.094) displaying significant molecular differences was between individuals No. 13 and No. 14. Especially, the genetic distance between individuals No. 22 and the remnants among individuals in two geographical populations was highest (0.275). This result illustrated that individual No. 22 is distinct from other individuals within two shortnecked populations. The different geographical features of two sites may have caused the genetic diversity in two shortnecked clam populations.

Keywords: Bandsharing value, Genetic distance, Decamer primer, RAPD markers, *Ruditapes philippinarum*, Shortnecked clam, Similarity matrix

Introduction

The polymorphic DNA markers that were shown to genetically link to a trait of interest have been used for individual identification, population genetics, genetic diagnostics, and for trait improvement in life genetics and breeding programs. There were so far used various molecular biological methods including restriction fragment length polymorphism (RFLP) (Clifford et al., 1998) and random amplified polymorphic DNAs (RAPD) (Johnson et al., 1994; Partis and Wells, 1996; Lilley et al., 1997; Han et al., 1998; Das et al., 1999; Iyengar et al., 2000; Kim et al., 2000; Mohd-Azmi et al., 2000; Yoon and Kim, 2001) based on the polymerase chain reaction (PCR).

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Geographic populations were distinguished by RFLP-PCR method in various fishes (Clifford et al., 1998). Especially, there were the relative advantages in the use of RAPD in the identification of genetic differences between invertebrate populations as compared to other studies such as mtDNA RFLP and allozymes on fish (Tassanakajon et al., 1998; Klinbunga et al., 2000). Polymorphic bands generated by PCR amplification of DNA using arbitrary primers had good merits for detecting DNA diversity and similarity between organisms (Dias Neto et al., 1993; Gwakisa et al., 1994; Castagna et al., 1997; Spooner et al., 1997; Liu et al., 1998; McCormack et al., 2000). The various primers can detect polymorphisms in the presence or absence of specific nucleotide sequence information, and the polymorphisms can function as genetic markers (Smith et al., 1997). Many genetic and molecular researches

were made because RAPD-PCR is a simple and rapid method for determining genetic diversity and similarity in various organisms with the advantage that no prior knowledge of the genome under research is needed (Fischer et al., 2000; Klinbunga et al., 2000).

Shortnecked clam is a commercially important mollusk species, which is distributed all over the Yellow Sea. As in bivalve species, population/density of the wild shortnecked clam is decreased significantly owing mainly to imprudent tidal land reclamation project and reckless development during the last four decades. There is a need to understand the genetic composition of wild shortnecked clam populations in order to evaluate exactly the latent genetic effects induced by seed production operations. In spite of its economic and scientific importance, little information is available on the genetic characteristics of the wild shortnecked clam populations in Korea. Thus, the applications of RAPD to aquaculture had been made to identify genetic similarity and diversity between a few of fish species and mollusks apart from geographic sites (Smith et al., 1997; Callejas and Ochando, 1998; Tassanakajon et al., 1998; Hamm and Burton, 2000; Kim et al., 2000; Klinbunga et al., 2000; McCormack et al., 2000; Yoon, 2001).

In the present work, genetic differences and distances within and between two populations of wild shortnecked clam, *R. philippinarum* from the Yellow Sea were determined by analyzing band patterns obtained from RAPD-PCR with band-sharing analysis and single linkage cluster analysis.

Materials and Methods

Muscle collection

DNA samples of wild shortnecked clam, *R. philippinarum* were obtained from two sites in the periphery of Anmyeondo and Seocheon at an approximately 120-km geographic distance. RAPD-PCR analysis was performed on genomic DNA samples from a total of 22 shortnecked clam using twenty different random decamers. Muscle samples collected from shortnecked clam were refrigerated at -70°C until use.

Purification of genomic DNA

DNA extraction was carried out according to the separation and extraction methods (Yoon and Kim, 2001). Samples of sliced muscle were placed into 10 ml test tubes, to which 4 volumes of lysis buffer I (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA) was added, and the mixture tubes were gently inverted several times. The samples were incubated on ice for 30 min, centrifuged at 1,750 g for 10 min. at 4°C. The supernatants were decanted and the pellets were resuspended with lysis buffer I. The pellets were transferred to 1.5 ml Eppendorf tubes with lysis buffer I, and then mixtures were centrifuged at 22,388 g for 1 min. The precipitates were resuspended with 0.8 ml lysis buffer II (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS). Aqueous phase was transferred to 1.5 ml Eppendorf tubes and added 15 μl proteinase K solution (10 mg/ml). The mixtures were gently inverted and incubated at 65 for overnight. After incubation, 300 μl of 6M NaCl was added and gently pipetted for a few min. 600 μl of chloroform was added to the mixture and then inverted (no phenol). Samples were spun down at 22,388 g for 5 min. The cleared lysates were extracted with 2 volume of ice-cold 70% ethanol, and centrifuged at 6,289 g for 5 min. The DNA pellets were air-dried for 30 min, and then used after dissolving in pure water. Purity and concentration of DNA was measured with a spectrophotometer (Shimadzu, Australia). Purity was estimated by calculating the ratios of the absorbance (O.D. value) measured at 260~280 nm and the final concentration was estimated by agarose electrophoresis and ethidium staining.

Primer, marker and amplification conditions

The primers, chosen arbitrarily for these experiments, were obtained from Operon Technologies, USA. All of these decamer random primers had a G+C content in the range 60~70%. The genomic DNAs were amplified using PCR with twenty decamer primers (5' to 3') in a DNA Thermal Cycler (Perkin Elmer Cetus, USA).

Amplification reactions were carried out in volumes of 20 μl contained 10 ng of template DNA, 20 μl AccuPower premix (Super-Bio Co., Korea) and 1.0 unit primer (Operon Technologies, USA). This mixture was pre-denaturated at 94°C for 5 min. The thermal cycler was programmed for 45 cycles at 94°C for 1 min for denaturation, at 36°C for 1 min for annealing, at 72°C for 1 min for extension, at 72°C for 5 min for post-extension, using the fastest available transition between each temperature.

Amplification products were separated by electrophoresis with ΦX174 DNA/*Hae*III marker (Promega Co., USA) in 1.4% agarose gels with TBE (0.09 M Tris, pH 8.5; 0.09 M borate; 2.5 mM EDTA) and detected by staining with ethidium bromide. The gels were illuminated with UV-ray and photographed by photoman direct copy system (PECA products, USA).

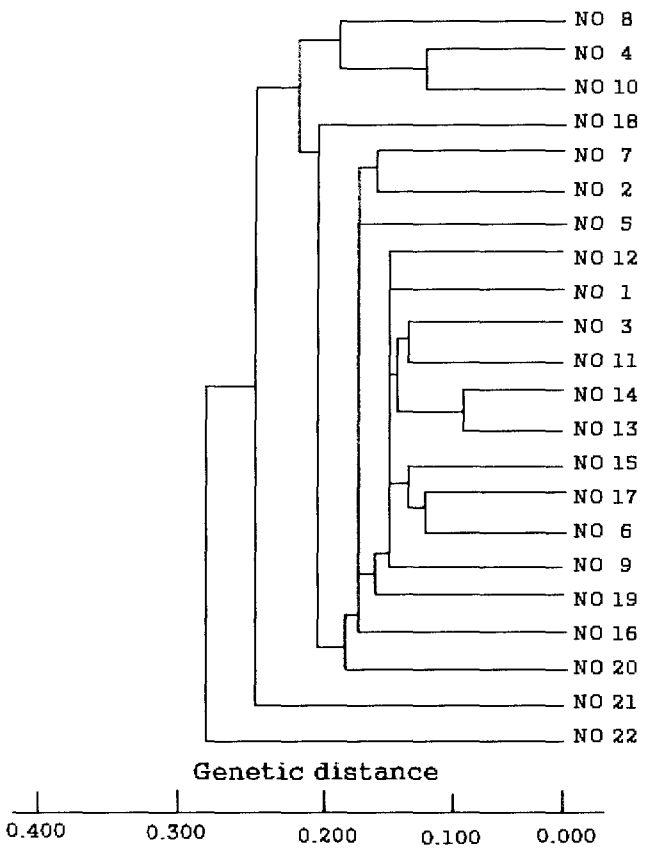

Fig. 1. Hierarchial dendrogram of genetic distances (Euclidean distances) showing the relatedness among different individuals from Anmyeondo (No. 1~No. 11) and individuals from Seocheon (No. 12~No. 22) in wild shortnecked clam, *R. philippinarum* populations generated according to the bandsharing values and similarity matrix in Table 4.

Analytical methods

Bandsharing calculation was slightly modified from the formula of Nei and Li (1979), Jeffreys and Morton (1987) and Mohd-Azmi et al. (2000): BS=N (Ba.....n)/(Ba+Bb+..... +Bn). Where Nabc is the number of bands shared by individuals a, b and c, Na is the total number of bands for individual a, Nb is the total number of bands for individual b and Nc is the total number of bands for individual c. If the comparison between the three lanes, the formula would be: BS=3 (Nabc)/(Na+Nb+Nc) and so on. Only bands that were readily visible were scored. An average of whin-population similarity is calculated across all pairwise comparisons among individuals within a population. Single linkage cluster analysis was performed on the similarity matrices in order to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., USA). Genetic differences and distances of individuals within and between populations were calculated with dendrograms performed by Systat version 10. BS values were scored by the presence or absence of an amplified product at specific positions in the same gel from the RAPD profiles. PCR amplification and bandsharing experiments on the same DNA sample were carried out to examine the efficiency and then the data obtained were used in this experiment and data analyses above-mentioned.

Results

Within-population variations

Of the 20 arbitrarily selected primers, six random primers (OPA-08, -09, -11, -16, -18 and -20) generated common, specific and polymorphic bands when DNA isolated from the muscle of 22 individuals from Anmyeondo (lanes 1~11) and Seocheon (lanes 12~22) were amplified. Six primers were used generating a total of 1,111 scorable bands in wild populations, ranging in size from less than 50 to larger than 1,500 bp (Table 1) (Fig. 2A, B, C, D, E and F).

In wild shortnecked clam, *R. philippinarum* from Anmyeondo, the intra-population variation was revealed in the band patterns ranged from smaller than 0.15 to approximately 1.08 kilobase pairs (kb) produced by random primer OPA-08 (GTGACGTAGG) (Fig. 2A) (Table 1). The identical band patterns (nine bands out of eleven bands) were observed in approximately 0.23 kb. Especially, this primer showed a great variety of specific minor bands even if there were not identified dense bands in comparison with other primers. The specific minor band patterns in the molecular size in smaller than 0.19 kb were observed from lane 8 and 9, specially, the band patterns identifying individuals. The specific minor band patterns in the molecular size in 0.60 kb were also observed from lane 8. Additionally, the band patterns identifying DNA polymorphism were observed from 1.08 kb (in lane 4). Also, the polymorphic minor band patterns were observed from 0.31 to 0.60 kb. The RAPD profiles obtained by a primer with pooled DNAs of individuals were different. The common band patterns of RAPD-PCR products by random decamer OPA-09 (GGGTAACGCC) were observed in larger than 0.31 kb (Fig. 2B). Also, the specific major band in the molecular size in approximately 1.08 kb was observed in lane 5 and 7. Also, the specific band pattern was observed in lane 5 (molecular size, 0.23 kb). The other primers OPA-11 (CAATCGCCGT) generated specific and polymorphic RAPD band patterns showing individuals identification and DNA polymorphisms (Fig. 2C). The common band pattern in the molecular size of 0.28 kb was observed in every lane except

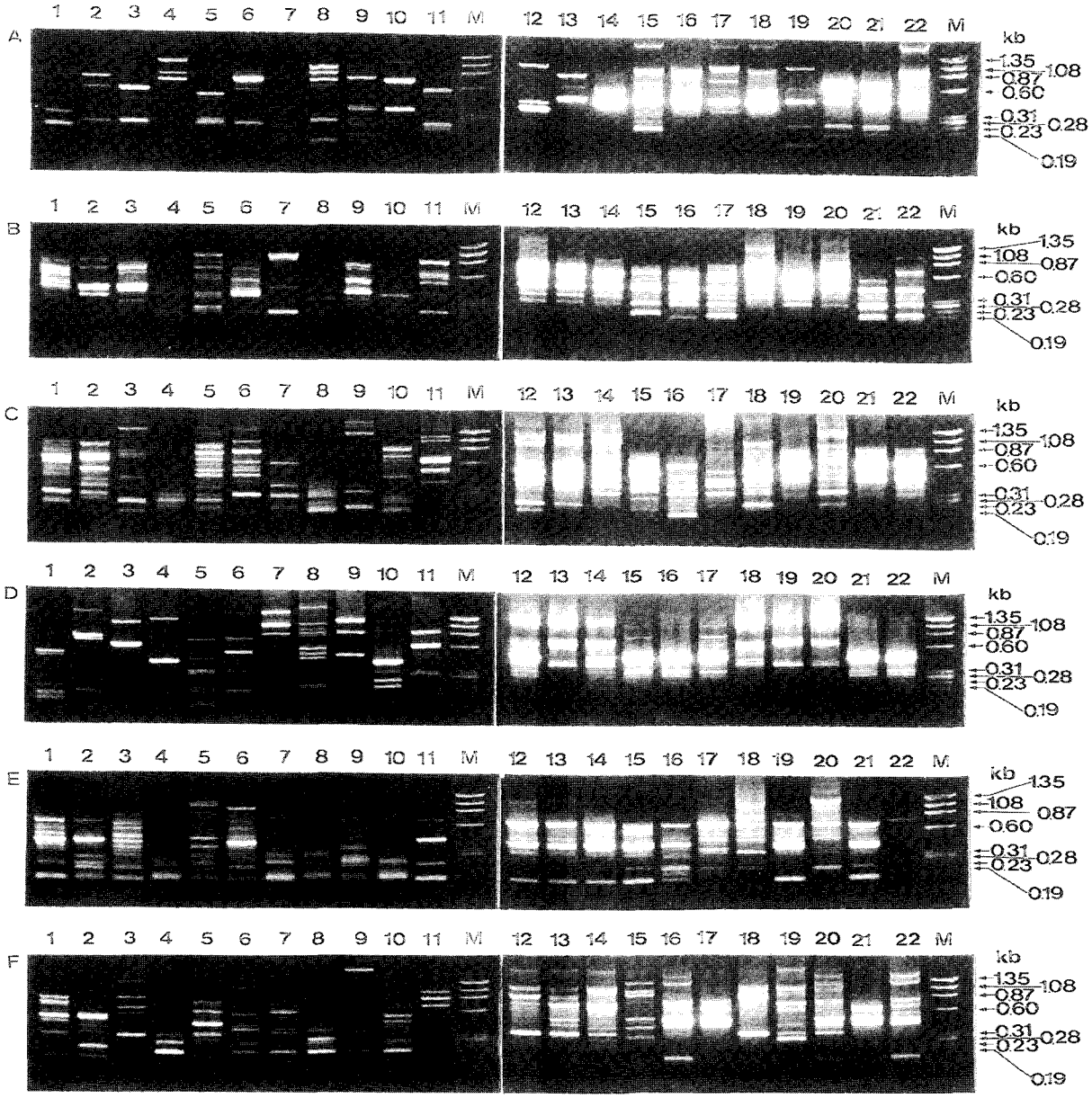

Fig. 2. RAPD-PCR fragments of shortnecked clam, *R. philippinarum* amplified by arbitrary primer OPA-08 (A), OPA-09 (B), OPA-11 (C), OPA-6 (D), OPA-18 (E) and OPA-20 (F). Amplification products were electrophoresed on a 1.4% agarose gel with TBE and detected by staining using ethidium bromide. Each lane (1~22) shows different individual DNA samples (lanes 111 from Anmyeondo and lanes 12~22 from Seo-heon). M: ΦX174 DNA molecular marker digested with restriction enzyme *Hae*III.

or lane 11. Also, the identical band in 0.60 kb generated by his primer was observed in remaining 8 lanes except for lane 4, 8 and 9. Especially, the specific band pattern was observed n lane 3 and 9 (molecular size, larger than 1.35 kb). Also, he specific band in the molecular size of approximately 0.87 b was observed in lane 6. The fragments ranged from 0.50 o 1.50 kb were produced by primer OPA-16 (AGCCAGCGAA) Fig. 2D). The specific band in the molecular size of approx-mately 1.35 kb was observed in lane 8. Also, the specific band (molecular size of 1.08 kb) was observed in lane 4, 7 and 9. The specific bands exhibited the inter-individual-specific characteristics and showed DNA polymorphisms were present in lane 2 and 6 from 0.60 to 0.87 kb, which were polymorphic. Also, the specific major band in the molecular size in approximately 0.60 kb was observed in lane 3 and 11 and also in lane 4 and 10 (molecular size, 0.31 kb). Especially, only a specific band (0.23 kb) identifying individuals was observed in lane 10. The Intra-population variation was revealed in the band patterns identified by this primer. Another primer, OPA-18 (AGGTGACCGT), detected 2 kinds of com-

Table 1. Average length of RAPD-PCR fragments on individuals from Anmyeondo and Seocheon in wild shortnecked clam, *R. philippinarum* populations.

Primer	Population	Average length of RAPD-PCR fragments																
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
OPA-08	Anmyeondo	1080	870	800	700	650	600	550	500	350	310	230	190	150				
	Seocheon	1500	1000	870	750	700	600	500	450	350	310	280	230	190				
OPA-09	Anmyeondo	1080	870	600	450	400	310	280	230	190	150							
	Seocheon	1400	1200	1080	870	700	600	450	400	350	310	280	230	190				
OPA-11	Anmyeondo	1400	1200	1080	870	700	600	500	400	350	310	280	230	190				
	Seocheon	1400	1200	1080	870	800	700	600	500	400	350	310	280	230	190	150		
OPA-16	Anmyeondo	1500	1400	1350	1080	870	700	600	450	400	310	280	230	190	150	100	50	
	Seocheon	1500	1400	1350	1080	870	700	650	600	500	450	400	350	310	280	230	190	150
OPA-18	Anmyeondo	1080	900	800	700	600	500	450	400	350	310	280	230	190	150	100	50	
	Seocheon	1500	1350	1080	870	800	700	600	500	400	350	310	280	230	190	150	100	50
OPA-20	Anmyeondo	1500	1350	1080	870	800	700	600	500	400	350	310	280	230	190	150		
	Seocheon	1500	1350	1080	870	800	700	600	500	400	350	310	280	230	190	150		

mon bands in molecular size of 0.19 and 0.23 kb, which were identical (Fig. 2E). Also, the DNA bands of 0.28 kb in remaining other lanes except for lane 1, 10 and 11 were identical. The common bands from 0.31 to 0.60 kb were present in every individuals except for lane 4, 7, 8 and 10. The specific band patterns of RAPD-PCR products were observed in lane 5 and 9 (approximately 1.08 kb). Also, the specific bands from 0.87 to 1.08 kb were present in lane 6, which were polymorphic. The identical band of 0.19 kb generated by random primer OPA-20 (GTTGCGATCC) identified in remaining 8 lanes except for lane 3, 5 and 11 (Fig. 2F). The RAPD polymorphism generated by this random primer showed polymorphic bands. The sizes of the specific bands recorded ranged from 0.31 to 0.60 kb in lane 5, 0.87 kb in lane 11, 1.08 kb in lane 6 and larger than 1.35 kb in lane 9, respectively, which may be useful for identifying the individuals. In wild shortnecked clam, *R. philippinarum* from Seocheon, the Intra-population variation was revealed in the band patterns ranged from less than 0.19 to 1.50 kb produced by random primer OPA-08 (Fig. 2A) (Table 1). Primer OPA-08 generated the lowest number of fragments in shortnecked clam population from Seocheon. The same major band in the molecular size from 0.31 to 0.60 kb produced by random primer OPA-08 was observed. The only specific band patterns identifying individuals were observed in 0.28 kb. This primer also produced the sizes of polymorphic DNA bands (less than 0.19 kb in lane 19, 0.31 kb in lane 12 and 17). The identical band from 0.31 kb to 0.60 kb generated by random primer OPA-09 was analyzed (Fig. 2B). This primer also produced the sizes of common DNA major bands ranged from 0.31 kb to 0.60 kb. Also, the identical bands from 0.28 kb to 0.31 kb were present in every individuals. Especially, this primer produced specific RAPD profiles of polymorphic bands of 0.23 kb in lane 17. The size of the specific band also recorded 1.08 kb in lane 12. The specific band patterns of RAPD products were observed in lane 12 and 20 (larger than 1.35 kb). The identical band ranged from 0.31 to larger than 0.60 kb generated by random primer OPA-11 identified in every individuals (Fig. 2C). Especially, this primer generated the highest number of fragments (a total of 133) in comparison with other primers used, with an average of 12.1 (Table 2). The specific major band pattern produced by this random primer in lane 16, which showed individual identification, in the range of 0.19 kb. The sizes of the specific bands also recorded less than 1.35 kb in lane 20 and larger than 1.35 kb in lane 18.

The identical band from 1.08 kb to 1.35 kb generated by random primer OPA-16 was analyzed (Fig. 2D). This primer also produced the sizes of common DNA major bands ranged from 0.31 kb to 0.60 kb in every lanes. Additionally, the same major band in the molecular size from 0.28 kb to 0.31 kb produced by this random primer was observed. Especially, this primer produced the sizes of polymorphic DNA bands (larger than 1.35 kb in lane 12, 13 and 18, from 0.60 kb to 0.87 kb in lane 15 and 17). The identical band patterns in 10 out of 11 lanes generated by random primer OPA-18 were observed from 0.31 kb to 0.60 kb (Fig. 2E). The DNA bands of 0.23 kb were present in every individuals, however, a corresponding band was absent in lane 22. Only single band pattern, which were polymorphic (lane 22), were obtained for primer OPA-18. This primer detected two specific RAPD minor bands in 0.19 kb, which were identifying individuals (lane 16 and 17). The identical band of 0.31 kb generated by

random primer OPA-20 were also observed in every individuals (Fig. 2F). Additionally, the common band ranged from 0.31 kb to 0.60 kb identified in 10 out of 11 lanes, but a corresponding band was absent in lane 12. This primer detected the specific RAPD major and minor bands of less than 0.19 kb in three lanes (lane 16, 20 and 22). The specific bands of 0.23 kb were present in only lane 14 and also 0.28 kb in this lane.

Between-populations variations

Out of 20 primers, 6 generated a total of 1,111 major and minor RAPD bands, especially producing approximately 7.23 average products per primer in shortnecked clam population from Anmyeondo (Table 2). In the concrete, six primers also yielded a total of 533 polymorphic bands, of which 278 bands in shortnecked clam from Anmyeondo and 255 in that from Seocheon (Table 3). This primer produced a total of 78 specific bands, of which 48 bands in shortnecked clam from Anmyeondo and 30 in that from Seocheon. The specific bands from 0.31 to 0.60 kb were present, which were polymorphic. In these ranges, the band patterns were highly reproducible and also very different between two populations. The observed bands exhibited the inter-population-specific characteristics. Overall, the bands generated by six primers in individuals from Seocheon were comparatively denser than those from Anmyeondo. The unique properties of the genomic DNA were used to investigate the features of two populations of this species. Six random primers produced amplified fragments that were consistently polymorphic among individuals from Anmyeondo and that from Seocheon. In this study, 30% of the random primers appeared to amplify polymorphic bands (Table 3). These random primers produced polymorphic DNA bands whose sizes ranged from approximately 190 to 1,080 bp within shortnecked clam from Anmyeondo and also from 190 to larger than 1,350 bp within that from Seocheon. A total of

Table 2. Total and average number of products generated with 6 primers from Anmyeondo and Seocheon shortnecked clam, *R. philippinarum* populations (11 bivalves/primer, respectively).

Primer	Total no. of products		Average no. of products	
	Anmyeondo	Seocheon	Anmyeondo	Seocheon
OPA-08	63	74	5.7	6.7
OPA-09	72	95	6.5	8.6
OPA-11	104	133	9.5	12.1
OPA-16	78	103	7.1	9.4
OPA-18	89	117	8.1	10.6
OPA-20	71	112	6.5	10.2
Subtotal	477	634	43.5	57.6
Total	1111		101.1	
Average	79.5	105.7	7.23	9.6

25.2 average number of polymorphic products were observed in shortnecked clams from Anmyeondo and also 23.2 in those from Seocheon (Table 3). An average of polymorphic bands were 4.2 in shortnecked clams from Anmyeondo and also 3.9 in those from Seocheon. The average level of polymorphic bands generated by primer OPA-11 in shortnecked clam population from Anmyeondo was 6.0 that were higher than any other primers. The average of value (23.2) of total polymorphic bands (278) were approximately 9.06% and also polymorphic to shortnecked clam population from Anmyeondo. There were shown the most fragments for OPA-20 than any other primers even if there was observed the least for OPA-09.

Overall, the remaining primer OPA-08, OPA-11, OPA-16, and OPA-18 generated an average of 2.5, 5.0, 4.9, and 2.7, respectively (Table 3). On average, each primer RAPD primer produced 0.73 amplified products. Especially, the average level of specific bands generated by random primer OPA-16 in population from Anmyeondo was 1.82 that was higher than any other primers and in the same manner the highest in those from Seocheon. An average of 0.73 bands per lane in shortnecked clams from Anmyeondo were observed, giving a total of 48 different fragments and there were identified the

Table 3. Number and average of polymorphic products and specific products generated by 6 random primers of shortnecked clam, *R. philippinarum* from two sites (11 bivalves/primer).

Primer	Number of polymorphic products		Average number of polymorphic products		Number of specific products		Average number of specific products	
	Anmyeondo	Seocheon	Anmyeondo	Seocheon	Anmyeondo	Seocheon	Anmyeondo	Seocheon
OPA-08	23	27	2.1	2.5	9	4	0.82	0.36
OPA-09	13	26	1.2	2.4	5	5	0.45	0.45
OPA-11	66	55	6.0	5.0	3	3	0.27	0.27
OPA-16	61	54	5.5	4.9	20	9	1.82	0.82
OPA-18	52	30	4.7	2.7	6	3	0.55	0.27
OPA-20	63	63	5.7	5.7	5	6	0.45	0.55
Subtotal	278	255	25.2	23.2	48	30	4.36	2.72
Total	533		48.4		78		7.08	
Average	46.3	42.5	4.2	3.9	8	5	0.73	0.45

Table 4. Similarity matrix including bandsharing values and genetic differences calculated using Nei and Li's index of similarity for wild shortnecked clam, *R. philippinarum*.

		Bandsharing values of shortnecked clam from Anmyeondo											Bandsharing values of shortnecked clam from Seocheon										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Genetic differences of individuals from Anmyeondo	1	-	0.339	0.500	0.358	0.445	0.475	0.457	0.367	0.541	0.439	0.597	0.634	0.477	0.525	0.599	0.500	0.535	0.371	0.622	0.460	0.677	0.451
	2	0.661	-	0.528	0.310	0.417	0.657	0.450	0.367	0.454	0.234	0.508	0.238	0.393	0.510	0.517	0.271	0.449	0.414	0.419	0.385	0.245	0.419
	3	0.500	0.472	-	0.222	0.356	0.550	0.578	0.388	0.473	0.462	0.684	0.477	0.507	0.566	0.584	0.523	0.606	0.522	0.702	0.712	0.536	0.536
	4	0.642	0.690	0.778	-	0.188	0.437	0.250	0.443	0.334	0.606	0.215	0.310	0.417	0.154	0.267	0.377	0.467	0.304	0.356	0.377	0.508	0.369
	5	0.555	0.583	0.644	0.812	-	0.472	0.259	0.249	0.413	0.225	0.565	0.291	0.341	0.461	0.463	0.425	0.502	0.458	0.566	0.467	0.436	0.484
	6	0.525	0.343	0.445	0.563	0.528	-	0.286	0.343	0.405	0.265	0.620	0.261	0.389	0.367	0.516	0.604	0.702	0.523	0.516	0.624	0.641	0.643
	7	0.543	0.550	0.422	0.750	0.741	0.714	-	0.259	0.478	0.293	0.397	0.365	0.299	0.331	0.489	0.143	0.498	0.325	0.399	0.390	0.308	0.347
	8	0.633	0.633	0.612	0.557	0.751	0.657	0.741	-	0.372	0.294	0.167	0.177	0.286	0.182	0.492	0.478	0.398	0.254	0.432	0.300	0.439	0.374
	9	0.459	0.546	0.527	0.666	0.587	0.595	0.522	0.628	-	0.155	0.412	0.233	0.344	0.313	0.612	0.566	0.705	0.487	0.548	0.511	0.513	0.467
	10	0.561	0.766	0.538	0.394	0.775	0.735	0.707	0.706	0.845	-	0.279	0.434	0.467	0.227	0.415	0.291	0.515	0.256	0.500	0.388	0.450	0.388
	11	0.403	0.492	0.316	0.785	0.435	0.380	0.603	0.833	0.588	0.721	-	0.151	0.459	0.622	0.647	0.565	0.544	0.494	0.567	0.597	0.517	0.604
Genetic differences of individuals from Seocheon	12	0.366	0.762	0.523	0.690	0.709	0.739	0.635	0.823	0.767	0.566	0.849	-	0.487	0.604	0.450	0.513	0.550	0.473	0.568	0.546	0.382	0.403
	13	0.523	0.607	0.493	0.583	0.659	0.611	0.701	0.714	0.656	0.533	0.541	0.513	-	0.782	0.516	0.573	0.516	0.565	0.662	0.581	0.436	0.534
	14	0.475	0.490	0.434	0.846	0.539	0.633	0.669	0.818	0.687	0.773	0.378	0.396	0.218	-	0.438	0.544	0.585	0.500	0.651	0.571	0.558	0.521
	15	0.401	0.483	0.416	0.733	0.537	0.484	0.511	0.508	0.388	0.585	0.353	0.550	0.484	0.562	-	0.566	0.758	0.623	0.548	0.458	0.576	0.725
	16	0.500	0.729	0.477	0.623	0.575	0.396	0.857	0.522	0.434	0.709	0.435	0.487	0.427	0.456	0.434	-	0.583	0.542	0.525	0.467	0.494	0.486
	17	0.465	0.551	0.394	0.533	0.498	0.298	0.502	0.602	0.295	0.585	0.456	0.450	0.484	0.415	0.242	0.417	-	0.512	0.646	0.657	0.552	0.628
	18	0.629	0.586	0.478	0.696	0.542	0.477	0.675	0.745	0.413	0.544	0.506	0.527	0.435	0.500	0.373	0.458	0.488	-	0.486	0.497	0.415	0.468
	19	0.378	0.581	0.298	0.644	0.434	0.484	0.601	0.568	0.452	0.500	0.433	0.432	0.338	0.349	0.452	0.475	0.354	0.514	-	0.584	0.536	0.569
	20	0.540	0.615	0.288	0.623	0.533	0.376	0.610	0.700	0.489	0.612	0.403	0.454	0.419	0.429	0.542	0.533	0.343	0.503	0.416	-	0.651	0.589
	21	0.323	0.755	0.464	0.492	0.564	0.359	0.692	0.561	0.487	0.550	0.483	0.618	0.564	0.442	0.424	0.506	0.448	0.585	0.464	0.349	-	0.594
	22	0.549	0.581	0.464	0.631	0.516	0.357	0.653	0.626	0.533	0.612	0.396	0.597	0.466	0.479	0.275	0.514	0.372	0.532	0.431	0.411	0.406	-

range from 0.27 to 0.82 fragments per lane in population from Seocheon. The average of value (4.36) of total polymorphic bands (48) were approximately 9.08% and also specific to in shortnecked clam population from Anmyeondo. The average of value (2.72) of total polymorphic bands (30) were also approximately 9.07% and also specific to shortnecked clam population from Seocheon.

Bandsharing values and genetic distances

In the present study, the average bandsharing values of all of the samples within population from Anmyeondo ranged from 0.155 to 0.684, whereas 0.143~0.782 within population from Seocheon (Table 4). The bandsharing value between individuals No. 3 and No. 11 showed the highest level within population from Anmyeondo, whereas the bandsharing value between individuals No. 9 and No. 10 showed the lowest level. The average level of BS value between individuals No. 7 and No. 16 from Seocheon was 0.782, which was higher than that in that from Anmyeondo. Also, the BS value between individuals No. 13 and No. 14 showed the lowest level in shortnecked clam population from Seocheon. Also, the bandsharing value between individuals No. 7 and No. 16 showed the lowest level within population from Seocheon, whereas the bandsharing value between individuals No. 13 and No. 14 showed the highest level. A similarity matrix based on Nei and Li's index of similarity was used to perform single linkage cluster analysis in order to obtain the Euclidean distances and dendrogram (Fig. 1). The single linkage dendrogram resulted from three primers (OPA-08, -09 and -20), indicating six genetic groupings composed of group 1 (No. 4, 8 and 10), group 2 (No. 18), group 3 (No. 2, 5 and 7), group 4 (No. 1, 3, 6, 9, 11, 12, 13, 14, 15 and 17), group 5 (16, 19 and 20) and group 6 (No. 21 and 22). Among individuals of within-population from Seocheon, the individual No. 18 clustered distinctly from the remnants of this population. The observed genetic distance between two populations from Anmyeondo and Seocheon was more than 0.209 (0.247 and 0.275). The geographical distance (120 km) between two sampling sites displayed significant molecular differences based on RAPD method. The genetic distances between two populations ranged from 0.094 to 0.275. The shortest genetic distance (0.094) displaying significant molecular differences was between individuals No. 13 and No. 14. Especially, the genetic distance between individuals No. 22 and the remnants among individuals in two geographical populations was highest (0.275).

Discussion

Polymorphic bands, specific bands, bandsharing values and genetic distances

Up to date, the RAPD-PCR method has been applied to detect DNA polymorphisms in various animals/plants/microbes using a great number of random primers (Vierling et al., 1994; Spooner et al., 1997; Callejas and Ochando, 1998; Das et al., 1999; Huang et al., 2000). Polymorphisms were scored by the presence or absence of the band pattern of amplified products at specific positions expressed by various primers (Smith et al., 1997; Tassanakajon et al., 1998).

In this study, six random primers (OPA-08, -09, -11, -16, -18 and -20) generated a number of common/specific/polymorphic fragments when genomic DNAs isolated from the muscle of 22 individuals in two shortnecked clam, *R. philippinarum* populations from Anmyeondo (lanes 1~11) and Seocheon (lanes 12~22) were amplified by RAPD-PCR and analyzed by bandsharing method, as shown in Fig. 2 (A, B, C, D, E and F) and summarized in Table 1~4. It were used DNAs extracted from shortnecked clam muscle which had the genome sizes of from larger than 50 to less than 1,500 bp, as summarized in Table 1. This indicated that the genome size of shortnecked clam was similar to that of blue catfish analyzed by Liu et al. (1998). Generally, the size and number of the fragments produced strictly depended on the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific DNA fragment (Welsh et al., 1991).

The band patterns of Anmyeondo population (lanes 1~11) were considerably varied in comparison to those from Seocheon (lanes 12~22). In the concrete, a total of 1,111 amplification products were produced of which 533 were polymorphic (48.0%), as summarized in Table 2 and 3. The percentages of polymorphic bands of the five geographic populations investigated in black tiger shrimp, *Penaeus monodon* varied from 51.5 to 57.7% (Tassanakajon et al., 1998). In this study, a number of polymorphic bands were fairly identified, as shown in Fig. 2. Namely, on average, 4.2 amplified bands were found to be polymorphic in population from Anmyeondo. Random primer OPA-11 produced a very high level of total polymorphic bands and average number of polymorphic bands (66 and 6.0, respectively) in population from Anmyeondo, as summarized in Table 3. Also, on average, 5.3 fragments obtained by this primer were found to be polymorphic in population from Seocheon. This random primer generated the highest number of fragments among the primers (the total

of 133, the average of 12.1) in population from Seocheon, as shown in Table 3. On the contrary, arbitrary primer OPA-09 generated the lowest number of products, with the average of 1.2 out of 13 in population from Anmyeondo and also the lowest number of fragments, with the average of 2.4 out of 26 in population from Seocheon, as shown in Table 3. These results that various polymorphic bands in a collection site were identified, implies the genetic variation by segregation with populations from different geographic regions, as compared with the other. A high level of genetic difference/variation within the populations deemed sufficient to separate the individuals within each population. RAPD markers produced by primers were effective in determining polymorphism between sorghum lines (Vierling et al., 1994). Till now, polymorphic bands generated by RAPD-PCR using arbitrary primers had good merits for detecting DNA similarity and diversity between life organisms (Gwakisa et al., 1994; Liu et al., 1998; McCormack et al., 2000; Nebauer et al., 2000).

In this study, the number of specific RAPD fragments was shared between the individuals of each population. Primer OPA-16 produced a very high level of total specific bands and average number of specific bands (20 and 1.82, respectively) in population from Anmyeondo, as summarized in Table 3. Also, this primer generated the highest value in population from Seocheon (9 in total number of specific bands and 0.82 in average number of specific bands, respectively). The random primer (OPA-18) detected two specific RAPD minor bands in 0.19 kb, which were identifying individuals (lane 16 and 17) from Seocheon, as shown in Fig. 2E. The primer (OPA-20) detected the specific RAPD major and minor bands of less than 0.19 kb in three lanes (lane 16, 20 and 22), as shown in Fig. 2F. The specific bands of 0.23 kb were present in only lane 14 and also 0.28 kb in this lane. This specific primer was also found to be useful in the individual identification, resulting from the different DNA polymorphism among individuals (Liu et al., 1998). Also, there were population-related RAPD fragments in catfish and there were differences in frequencies of six primer fragments, as have been reported in catfish (Liu et al., 1998). Additionally, it was revealed that at least some of the outbreaks of white-clawed crayfish, *Austropotamobius pallipes* plague in England resulted from imports of crayfish, *Pacifastacus leniusculus* from northern Europe after, or directly from North America by means of RAPD method (Lilley et al., 1997). A total of 73.7 average number of polymorphic products were observed between common carp and Israeli carp (Yoon, 2001). On average, each random decamer primer generated 4.1 amplified products in cultured population. Each random RAPD primer produced an average of amplified 10 fragments from 2.0 to 5.9 bands in cultured population. In other words, intra-specific or inter-strain variations in the pattern were observed for each primer and also such data should be of value not only in the discrimination of the correlation with the economic traits but also in the construction of phylogenetic trees or genetic relationships (Orozco-Castillo et al., 1994; Spooner et al., 1997; Hamm and Burton, 2000).

Some intra-specific or intra-strain RAPD variations were observed for different strains, for individuals or for populations (Liu et al., 1998; Fischer et al., 2000). Particularly, Liu et al. (1998) screened a collection of RAPD markers in catfish and identified 22 primers that revealed 171 strain-specific genetic markers. Also, Johnson et al. (1994) referred a collection of RAPD markers in zebrafish and identified 116 primers that revealed 721 strain-specific genetic markers. These workers have found more variation within a strain or population than between strains or populations considered as a whole. Besides, this analysis method could be used as an potential genetic marker for linkage analysis with economically important traits in fish (Smith et al., 1997; Tassanakajon et al., 1998). They suggested that RAPD marker would be particularly useful in marker-assisted selection (MAS) programs in these important aquaculture species. In this study, the bandsharing values altered from 0.155 to 0.684 in population from Anmyeondo and also from 0.143 to 0.782 in population from Seocheon, as summarized in Table 4. The RAPD profiles obtained from individuals DNA of two populations were fairly different for reference to BS values. In the concrete, the bandsharing value between individuals No. 3 and No. 11 showed the highest level within population from Anmyeondo, whereas the bandsharing value between individuals No. 9 and No. 10 showed the lowest level. Also, the average level of BS value between individuals No. 13 and No. 14 from Seocheon was 0.782 that was higher than any other that in those from Anmyeondo. On the contrary, the BS value between individuals No. 7 and No. 16 from Seocheon was 0.143 that was lower than any other that in those from Anmyeondo. The bandsharing values of individuals in population from Anmyeondo showed higher than those in population from Seocheon. This result showed that individuals from Anmyeondo have held much higher genetic variations than those from Seocheon. This result also revealed that the higher genetic sim-

ilarity might be identified due to raise in the same environmental conditions or to inbreeding within a population of cultured Korean catfish (Yoon and Kim, 2001).

Generally, the varying degrees of genetic differences among shortnecked clam populations were of relevance to the isolation of wild bivalve. The bandsharing values were calculated as an expression of similarity of RAPD fingerprints of animals from either the same or different breeds (Jeffreys and Morton, 1987; Mohd-Azmi et al., 2000). Also, genetic similarity and co-phenetic values calculated with RAPD markers were very similar to those calculated with RFLP markers for the intra-specific comparisons, but not for the inter-specific comparisons in wheat *Triticum urartu* (Castagna et al., 1997). In addition, the higher genetic variation detected by AFLP in comparison to RAPD was reflected in the topography of the phenetic dendrograms obtained (Das et al., 1999). However, polymorphism was detected with 77% of the random primers, compared to 61% of the RFLP probes using four restriction enzymes (Vierling et al., 1994). Up to date, the potential of RAPDs to identify diagnostic markers for strain or species identification in livestock (Gwakisa et al., 1994; Han et al., 1998; Mohd-Azmi et al., 2000), in parasites or pathogens (Dias Neto et al., 1993; Lilley et al., 1997), in plants (Orozco-Castillo et al., 1994; Vierling et al., 1994; Castagna et al., 1997; Nebauer et al., 2000) and in aquatic organisms (Partis and Wells, 1996; Callejas and Ochando, 1998; Iyengar et al., 2000; Kim et al., 2000) has also been demonstrated.

As shown in Fig. 1, the single linkage dendrogram resulted from three primers such as OPA-08, -09 and -20), indicating six genetic groupings composed of group 1 (No. 4, 8 and 10), group 2 (No. 18), group 3 (No. 2, 5 and 7), group 4 (No. 1, 3, 6, 9, 11, 12, 13, 14, 15 and 17), group 5 (16, 19 and 20) and group 6 (No. 21 and 22). As expected, the dendrogram revealed close relationships between individual identities within two geographical populations and individuals seemed to derive from the similar ancestor. However, the genetic distances between two populations ranged from 0.094 to 0.275. The shortest genetic distance (0.094) displaying significant molecular differences was between individuals No. 13 and No. 14. Especially, the genetic distance between individuals No. 22 and the remaining individuals in two geographical populations was the highest (0.275). These results illustrated that individual No. 22 be distinct from other individuals within two shortnecked populations. The geographical distance (120 km) between two sampling sites displayed significant molecular differences based on RAPD method.

RAPD-PCR technique was employed to identify three endemic Spanish barbel species (Callejas and Ochando, 1998). They illustrated that the species *Barbus bocagei* and *B. graellsii* be more related to each other than *B. sclateri* by means of the cluster analysis of the genetic similarity values obtained from RAPD data. RAPD analysis has also revealed genetic distances among population pairs and their geographic distances to be related (Huang et al., 2000). The shortest distance (55 km) displayed significant molecular differences based on RAPD data. Undoubtedly, all population pairs separated by distances >55 km displayed significant molecular variation. In the concrete, Hegde et al. (2000) reported that diploid wheats appeared to be related to their geographical distances as genetic distances between 2 populations nearly reflected their geographical distances. However, Hamm and Burton (2000) suggested that genetic distance was found to be independent of geographic distance over the long-distance (300 km) sampling range.

Overall, there was significant genetic variation between two populations apart from each other. The genetic variations in intra-populations could be diagnostic of specific strains and their relatedness. In the long run, besides gene mapping and breeding applications, RAPD-PCR system could be very useful for the rapid certification and quality control of seed production and for all projects based on PCR amplification of specific bivalve/fish DNA fragments (Tassanakajon et al., 1998). Further analyses and more sampling sites are required to identify sufficient polymorphic or specific fragments shared by the species in order to accomplish a quantitative analysis. Also, it seems to be essential to get more data on the genetic distances among a large number of shortnecked clam populations. In future, thorough comparisons need to be made by other additional DNA analyses methods such as simple sequence repeats (SSR) based on microsatellite sequences, minisatellite and amplified fragment length polymorphism (AFLP) and so on.

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