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Geographic Variations in Four Freshwater Crab (*Eriocheir sinensis*) Populations throughout Its Distribution Range

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분포지역에 따른 민물가재 4집단(*Eriocheir sinensis*)의 지리적 변이

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ABSTRACT : Genomic DNA samples isolated from four geographical freshwater crab (*Eriocheir sinensis*) populations collected in the inland of the Korean Peninsula (Gunsan, Paju, and Nampo) and a Chinese site, were used for PCR amplification. Seven decamer primers generated 19 specific loci (19/243 loci, 7.81%) in the Gunsan population, 32 (32/215 loci, 14.88%) in the Paju population, 19 (19/231 loci, 8.23%) in the Nampo population and 62 (62/340 loci, 18.24%) in a Chinese population. The average 8.9 specific loci exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms in the Chinese population. The number of unique shared loci to each population and number of shared loci by the four populations were generated by molecular analysis using seven primers in four populations. 35 unique shared loci to each population, with an average of 5.0 per primer, were observed in the Gunsan population, and 50 loci, with an average of 7.1 per primer, were observed in the Chinese population. The hierarchical dendrogram indicates three main branches: cluster 1 (GUNSAN 01~GUNSAN 05, PAJU 06~PAJU 10 and NAMPO 11~NAMPO 15) and cluster 2 (CHINESE 16, 17, 18, 19 and 20). Conclusively individual no. 20 of the PAJU 10 freshwater crab was most distantly related to CHINESE no. 20 (genetic distance = 0.667). Taken together, these results demonstrate the potential of RAPD analysis to identify diagnostic markers for the identification of four freshwater crab populations.

Key words : *Eriocheir sinensis*, Dendrogram, Chinese mitten crab, Freshwater crab, Genetic distance.

INTRODUCTION

Crabs are the most popular marine products in Korea because of their taste and nutritional value, and Koreans consume them in large quantities. Among crabs, the Korean freshwater crab (*Eriocheir sinensis*) and Chinese freshwater crab (*E. sinensis*), also known as Chinese mitten crab, are an economically important aquacultural species that belongs to the family Grapsidae. Gunsan freshwater crab (GFC) and Chinese freshwater crab (CFC) are widely distributed in the entirety of ponds, brooks, rivers, estuary, brackish-water habitats and seawater areas of the West Sea in the Korean Peninsula, as well as in several areas in China. However, in spite of their economic and scientific consequences, a little information currently exist regarding the physiological and ecological levels only of crab species in Korea (Koo et al., 2004). As the crab preservation increases, the understanding of the genetics of this crab species becomes more necessary to evaluate the potential genetic effects. Research in crab fisheries has progressed steadily in many aspects, over-fishing, and water pollution by industries and city sewage. In the present study, to elucidate the genetic distances and differences among geographical

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freshwater crab populations, author performed a clustering analysis of various geographical crab populations collected from Korea and China. The present study was also undertaken to confirm that the population relationships identified by RAPD analysis are consistent with previously obtained data using morphological and/or ecological affinities.

MATERIALS AND METHODS

Populations of freshwater crab (*E. sinensis*) were obtained from Gunsan, Paju and Nampo district in the vicinity of the West Sea of Korea and a crab population, known as Chinese mitten crab, was imported from China. Crab muscle was collected in sterile tubes, immediately placed on dry ice, and stored at −40℃ until the genomic DNA extraction. Genomic DNA was extracted and purified under the conditions described previously (Yoon & Kim, 2004). The concentration of the extracted genomic DNA was measured by absorbance ratio at 260 ㎚ by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK). Seven primers (BION-03, BION-10, BION-19, BION-21, BION-25, BION-30 and BION-34) were shown to generate the shared loci, specific loci, unique shared loci to each population and shared loci by the four populations which could be clearly scored. 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; Yoke-Kqueen & Radu, 2006). Genetic distances within- and between-population were also calculated by complete linkage method with the help of the hierarchical dendrogram program Systat version 10 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Seven arbitrarily selected primers, BION-03, BION-10, BION-19, BION-21, BION-25, BION-30 and BION-34, were used to generate the shared loci, specific, unique shared loci to each population and shared loci by the four populations, with a DNA fragment size ranging from 150 bp to 2,500 bp, as shown in Fig. 1. In this study, on average, a decamer primer generated 34.7 amplified products per primer in the Gunsan population, and 48.6 in a Chinese population (Table 1). Seven decamer primers generated 19 specific loci (19/243 loci, 7.81%) in the Gunsan crab population, 32 (32/215 loci, 14.88%) in the Paju population, 19 (19/231 loci, 8.23%) in the Nampo population and 62 (62/340 loci, 18.24%) in a Chinese population (Table 1). The average 8.9 specific loci exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms in the Chinese population, as shown in Fig. 2. The number of unique shared loci to each population and number of shared loci by the four populations generated by RAPD analysis using 7 primers in Gunsan, Paju, Nampo and the Chinese populations. 35 unique shared

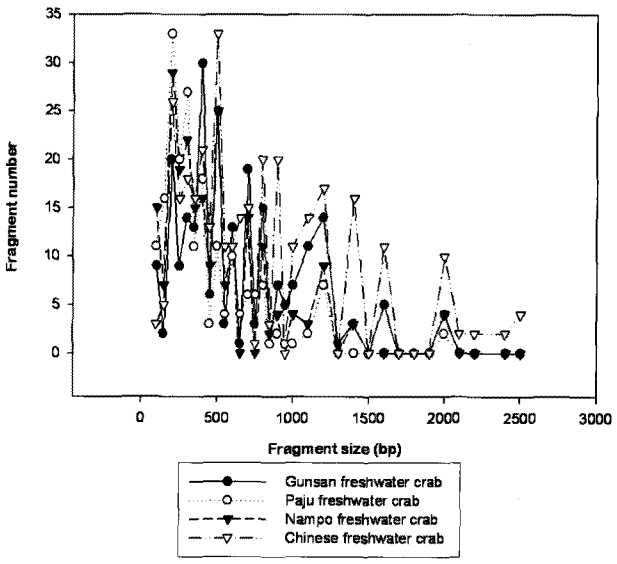

Fig. 1. Distribution of fragment sizes of four freshwater crab populations. *Circles: Gunsan freshwater crab population.* Blank circles: Paju freshwater crab population. Triangles: Nampo freshwater crab population. Blank triangles: the Chinese freshwater crab population. The fragment numbers in each size interval have been computed from the pooled fragments obtained with all the primers.

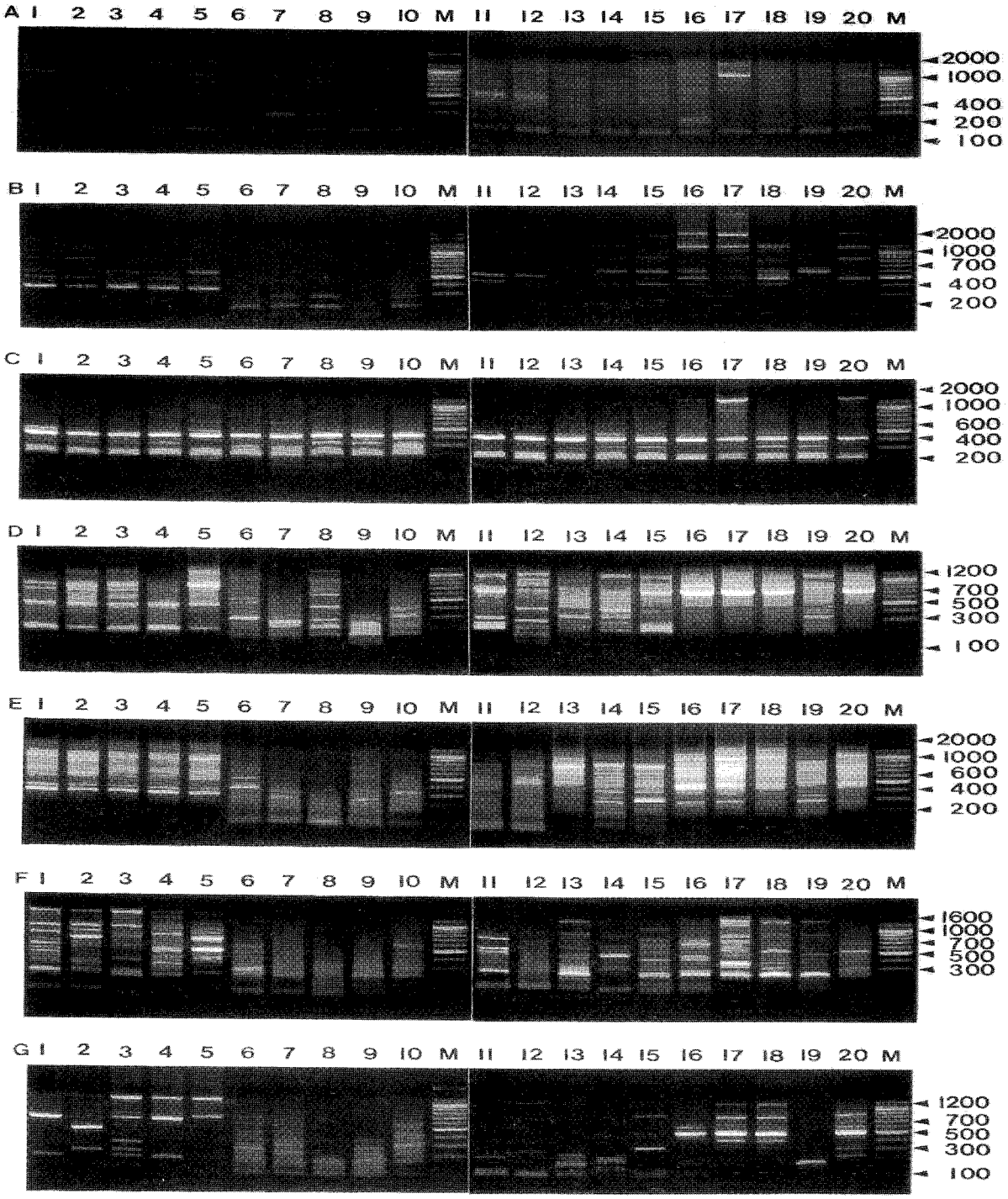

Fig. 2. RAPD-based agarose electrophoretic profiles of individual crab (*E. sinensis*). Each lane shows DNA samples extracted from 20 individuals. DNA isolated from Gunsan (lane 1~5), Paju (lane 6~10), Nampo (lane 11~15) and a Chinese site (lane 16~20) were amplified by random primers BION-03 (A), BION-10 (B), BION-19 (C), BION-21 (D), BION-25 (E), BION-30 (F) and BION-34 (G). Amplification products were generated via electrophoresis on 1.4% agarose gel. The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a Photoman direct copy system. M, 100 bp Ladder DNA marker.

Table 1. Total, average, shared, and specific loci generated by RAPD analysis using 7 decamer primers in freshwater crab (*E. sinensis*) from Gunsan and Paju of South Korea, Nampo of North Korea and a Chinese site

Item	No. of average loci per lane				No. of shared loci				No. of specific loci			
Population / Primer	Gunsan	Paju	Nampo	Chinese	Gunsan	Paju	Nampo	Chinese	Gunsan	Paju	Nampo	Chinese
BION-03	6.0(30)	3.8(19)	6.2(31)	5.0(45)	15	5	10	20	3	1	2	7
BION-10	3.4(17)	3.4(17)	3.4(17)	8.8(44)	10	15	0	5	2	1	4	25
BION-19	5.2(26)	6.4(32)	7.0(35)	9.4(47)	15	15	25	25	2	4	3	14
BION-21	8.4(42)	8.4(42)	7.6(38)	9.6(48)	20	0	5	35	4	10	2	2
BION-25	11.8(59)	7.0(35)	10.8(54)	15.4(77)	50	20	35	55	0	0	4	2
BION-30	10.2(51)	7.6(38)	7.8(39)	10.6(53)	10	30	20	15	3	3	2	7
BION-34	3.6(18)	6.4(32)	3.4(17)	5.2(26)	5	10	5	0	5	13	2	5
Total no.	48.6(243)	43.0(215)	46.2(231)	68.0(340)	125	95	100	155	19	32	19	62
Average no. per primer	34.7	30.7	33.0	48.6	17.9	13.6	14.3	22.1	2.7	4.6	2.7	8.9

Total number of loci generated by a primer in freshwater crab obtained Gunsan and Paju of South Korea, Nampo of North Korea and a Chinese site, respectively, is shown in parentheses.

Table 2. Number of unique shared loci to each population, and shared loci in the four populations generated by RAPD analysis using 7 oligonucleotide primers in freshwater crab (*E. sinensis*) from Gunsan and Paju of South Korea, Nampo of North Korea and a Chinese site

Item	No. of unique shared loci to each population				No. of shared loci by the four populations
Population / Primer	Gunsan	Paju	Nampo	a Chinese site	Four locales
BION-03	5	0	0	5	5
BION-10	5	15	0	0	0
BION-19	0	0	0	0	15
BION-21	10	5	5	25	0
BION-25	5	10	0	15	0
BION-30	5	5	5	5	0
BION-34	5	5	0	0	0
Total no.	35	40	10	50	20
Average no. per primer	5.0	5.7	1.4	7.1	2.9

loci to each population, with an average of 5.0 per primer, were observed in the Gunsan population, and 50 loci, with an average of 7.1 per primer, were observed in the Chinese population (Table 2). 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), shrimp populations (Yoon & Kim, 2003), deep sea lobster (*Puerulus sewelli*) (Park et al., 2005) and swimming crab (*Portunus trituberculatus*) (Yoon, 2006).

The hierarchical dendrogram, generated according to the bandsharing values, indicates three main branches: cluster 1 (GUNSAN 01～GUNSAN 05, PAJU 06～PAJU 10 and

Table 3. Similarity matrix, including bandsharing values (above the diagonal) and genetic differences (under the diagonal) calculated with the number of bands shared for sample pairs in Gunsan and Paju of South Korea, Nampo of North Korea and the Chinese freshwater crab (*E. sinensis*)

			Bandsharing values of freshwater crab																			
			From Gunsan					From Paju					From Nampo					From a Chinese site				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Genetic differences of freshwater crab	From Gunsan	1		0.716	0.703	0.823	0.694	0.346	0.254	0.300	0.374	0.310	0.581	0.458	0.441	0.507	0.486	0.505	0.512	0.536	0.571	0.495
		2	0.284		0.683	0.768	0.689	0.394	0.297	0.337	0.346	0.362	0.573	0.501	0.464	0.493	0.512	0.524	0.522	0.548	0.471	0.503
		3	0.297	0.317		0.743	0.687	0.377	0.288	0.286	0.401	0.372	0.490	0.307	0.335	0.370	0.394	0.409	0.462	0.411	0.419	0.423
		4	0.177	0.232	0.257		0.770	0.357	0.269	0.293	0.402	0.331	0.631	0.483	0.405	0.433	0.514	0.476	0.497	0.470	0.466	0.513
		5	0.306	0.311	0.313	0.230		0.315	0.245	0.269	0.317	0.297	0.504	0.365	0.404	0.390	0.413	0.404	0.476	0.477	0.467	0.461
	From Paju	6	0.654	0.606	0.623	0.643	0.685		0.635	0.671	0.682	0.648	0.385	0.450	0.456	0.440	0.334	0.330	0.299	0.251	0.345	0.384
		7	0.746	0.703	0.712	0.731	0.755	0.365		0.640	0.667	0.651	0.346	0.400	0.426	0.416	0.337	0.298	0.260	0.215	0.318	0.333
		8	0.700	0.663	0.714	0.707	0.731	0.329	0.360		0.704	0.676	0.421	0.433	0.476	0.441	0.334	0.357	0.298	0.272	0.418	0.338
		9	0.626	0.654	0.599	0.598	0.683	0.318	0.333	0.296		0.654	0.449	0.474	0.489	0.468	0.407	0.308	0.274	0.237	0.395	0.345
		10	0.690	0.638	0.628	0.669	0.703	0.352	0.349	0.324	0.346		0.388	0.348	0.410	0.416	0.403	0.393	0.355	0.305	0.360	0.436
	From Nampo	11	0.419	0.427	0.510	0.369	0.496	0.615	0.654	0.579	0.551	0.612		0.666	0.556	0.678	0.692	0.448	0.404	0.414	0.469	0.455
		12	0.542	0.499	0.693	0.517	0.635	0.550	0.600	0.567	0.526	0.652	0.334		0.639	0.593	0.570	0.396	0.399	0.431	0.411	0.401
		13	0.559	0.536	0.665	0.595	0.596	0.544	0.574	0.524	0.511	0.590	0.444	0.361		0.600	0.475	0.433	0.404	0.391	0.426	0.422
		14	0.493	0.507	0.630	0.567	0.610	0.560	0.584	0.559	0.532	0.584	0.322	0.407	0.400		0.621	0.424	0.381	0.460	0.579	0.423
		15	0.514	0.488	0.606	0.486	0.587	0.560	0.663	0.666	0.593	0.597	0.308	0.430	0.525	0.379		0.537	0.505	0.499	0.503	0.495
	From a Chinese site	16	0.495	0.476	0.591	0.524	0.596	0.670	0.740	0.643	0.692	0.607	0.552	0.604	0.567	0.576	0.463		0.781	0.692	0.558	0.741
		17	0.488	0.478	0.538	0.503	0.524	0.701	0.740	0.702	0.726	0.645	0.596	0.601	0.596	0.619	0.495	0.219		0.819	0.506	0.693
		18	0.464	0.452	0.589	0.530	0.523	0.749	0.785	0.728	0.763	0.695	0.586	0.569	0.609	0.540	0.501	0.308	0.181		0.556	0.688
		19	0.429	0.529	0.581	0.534	0.533	0.655	0.682	0.582	0.605	0.640	0.429	0.589	0.574	0.421	0.497	0.442	0.494	0.444		0.460
		20	0.505	0.497	0.577	0.487	0.539	0.616	0.667	0.662	0.655	0.564	0.545	0.599	0.578	0.577	0.505	0.259	0.307	0.312	0.540	

NAMPO 11~NAMPO 15) and cluster 2 (CHINESE 16, 17, 18, 19 and 20) (Table 3, Fig. 3). The shortest genetic distance displaying significant molecular difference was between individuals' GUNSAN no. 04 and GUNSAN no. 01 (0.062) (Fig. 3). Ultimately, individual no. 20 of the PAJU 10 freshwater crab was most distantly related to CHINESE no. 20 (genetic distance = 0.667). The genetic distance between the Indian Ocean lobster and the Korean Slipper lobster species ranged between 0.040 and 0.612 (Park et al., 2005). In particular, the longest genetic distance displaying significant molecular differences was determined to exist between individuals in the two lobster species, namely between individuals SLIPPER no. 04 of the Korea lobster species and DEEPSEA no. 16 of the Indian Ocean lobster species (genetic distance = 0.612).

From what has been said above, the potential of RAPD analysis to identify diagnostic markers for the identification of four freshwater crab populations has been demonstrated. Generally speaking, using a variety of arbitrary primers, RAPD-PCR has been applied to identify polymorphic/specific markers particular to line, species and geographical population, as well as genetic diversity/polymorphism in diverse species of organisms (McCormack et al., 2000; Yoon & Kim, 2004). The classification of geographical

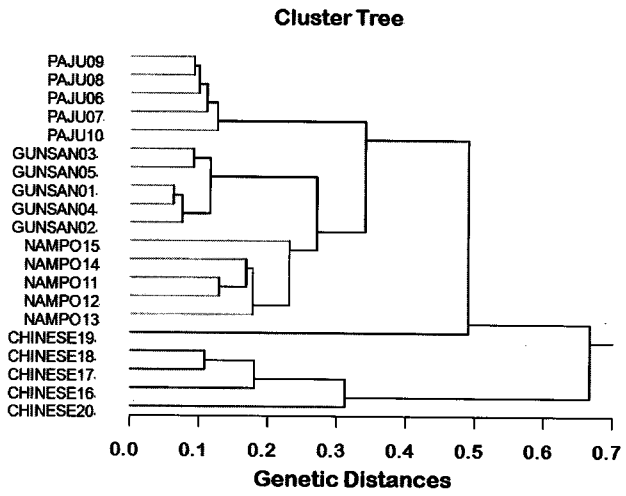

Fig. 3. Representation of hierarchical dendrogram of genetic distances, obtained from four geographical populations of crab (*E. sinensis*). The relatedness between different individuals in the crab populations of Gunsan (GUNSAN 01~GUNSAN 05), Paju (PAJU 06~PAJU 10), Nampo (NAMPO 11~NAMPO 15) and a Chinese site (CHINESE 16~CHINESE 20) was generated according to the band-sharing values and similarity matrix (see Table 3).

populations of crab is based on morphological variations in antennae, compound eyes, cephalothorax, big claws called chelipeds, body color and hardness of carapace. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga et al., 2004). In the future, diagnostic DNA markers will be necessary for characterization of the different geographical crab species to correlate with the morphological traits and for clarification of the ambiguity among species and/or geographic populations.

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