한국어병학회지 제 19 권 제 2 호 (2006)
J. Fish Pathol., 19(2) : 141 ~ 153 (2006)

Genetic Variation in Geographic Crayfish (*Cambaroides similis*) Populations

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Genomic DNA samples isolated from two geographical crayfish (*Cambaroides similis*) populations in the inland of the Korean Peninsula, at Jeonju (Jeonju crayfish; JJC) and Jeongup (Jeongup crayfish; JUC), were PCR-amplified repeatedly. The six arbitrarily selected primers OPC-03, OPC-06, OPC-09, URP-02, URP-07 and URP-09 generated the common, specific, and polymorphic fragments. The sizes of DNA fragments also varied widely, from 100 bp ~ 2,600 bp. Here, 521 fragments were identified in the JJC population, and 354 in the JUC population: 6 primers generated 60 specific fragments (60/521 fragment, 11.5%) in the JJC population, and 90 (90/354 fragments, 25.4%) in the JUC population. These primers produced 42 polymorphic fragments (8.1%) in the JJC population, and 18 (5.1%) in the JUC population. Especially these results demonstrate that the primers detected numerous specific fragments. Especially, the decamer primer OPC-06 generated inter-population-common DNA fragments, approximately 400 and 800 bp, respectively, in both the JJC and JUC populations. The universal primer URP-02 also generated inter-population-identical DNA fragments, approximately 350 bp and 600 bp, between the two geographical crayfish populations. Based on the average bandsharing values of all samples, the bandsharing value of individuals within the JJC population was much higher than in the JUC population. The bandsharing value between individuals no. 10 and no. 15 was 0.683, which was the highest between the two geographical populations. The dendrogram obtained by the six primers indicates two genetic clusters: cluster 1 (CRAYFISH 01 ~ CRAYFISH 11), and cluster 2 (CRAYFISH 12 ~ CRAYFISH 22). The genetic distance between the two geographical populations ranged from 0.053 to 0.605. Ultimately, the longest genetic distance displaying significant molecular differences was found to exist between individuals in the two crayfish populations, between individuals CRAYFISH no. 02 of Jeonju and CRAYFISH no. 15 of Jeongup (genetic distance = 0.605).

Key words: *Cambaroides similis*, Crayfish, Genetic variation, Geographic population

Introduction

Crayfish live in North America, Europe, New Zealand, and the Far East Asia and throughout the world. Nearly all live in freshwater, although a few survive in seawater. A joined head and thorax, or midsection, and a segmented body, which is sandy yellow, green, or dark brown in color, characterize crayfish. The head has a sharp snout, two pairs of sensory antennae, and a pair of eyes on movable stalks. The appendages of the thorax include four pairs of walking legs which, as well as walking, are to probe cracks and crevices between rocks looking for feed. For a few days following each molt, crayfish have soft exoskeletons and are more vulnerable to predators.

Korean freshwater crayfish (*Cambaroides similis*) is of one species an ecologically important

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crustacean species, belonging to the family Cambaridae, and the order Decapoda. A pale central zone along the middle of the carapace and abdomen characterizes this rather plain, gray-green crayfish under natural conditions. The color also depends on diet and habitat environment. In the natural ecosystem, crayfish is widely distributed in the entirety of small rocky brooks, streams, swamps, ponds, rivers, and other freshwater habitats of the Korean Peninsula. It is sometimes found at the mouths of springs. It is nocturnal and eats fish, shrimp, water plants, worms, insects, snails, and plankton. In the contrary, trout, bass, eel, otters, herons, snakes and peoples eat crayfish. During the last five decades this crustacean species is also threatened by over-fishing, development projects and various environmental pollutions. After these threats were excluded, this species spread widely again in the 1990's. Basically, the rate at which freshwater crayfish grows depends very much on feed organisms, population density and water temperature. Especially, the water temperature of 15℃ ~ 25℃ is about optimal. Crayfish is ranked highest among the freshwater species in Korea as a pet animal, owing to peculiar morphological characters such as cephalothorax, big claws called chelipeds, antennae and compound eyes. As the necessity of crayfish increases, the understanding of the genetics of this crustacean species becomes necessary. However, little information currently exists regarding the genetics of crayfish in Korea.

Even if reproducibility of RAPD is a little poor and depends upon PCR conditions, until now, polymorphic bands generated by RAPD-PCR using arbitrary primers were considered to be a reliable method for detecting DNA similarity and/or diversity between organisms (Jeffreys and Morton, 1987; McCormack *et al*., 2000; Kim *et al*., 2004; Park *et al*., 2005). As stated above, the potential of RAPD to identify diagnostic markers for breed, stock, species and population identification in teleosts (Partis and Wells, 1996; Callejas and Ochando, 1998; Iyengar *et al*., 2000; Yoon and Kim, 2004; Siti Azizah *et al*., 2005), in shellfish (Huang *et al*., 2000; Yoon and Kim, 2003a; Kim *et al*., 2004), and in crustacean (Klinbunga *et al*., 2000b; Yoon and Kim, 2003b; Park *et al*., 2005) has been demonstrated.

Our study attempts to elucidate the genetic distances and differences within and among crayfish geographical populations. In order to accomplish this, we performed genetic variations and clustering analyses of two populations of crayfish (*C. similis*) in the Jeonju and Jeongup regions of Korea.

Materials and Methods

Sample collection and extraction of genomic DNA

Two geographical populations of crayfish (*C. similis*) were obtained from two different regions in Korea: Jeonju and Jeongup in two inland areas of Korea. Crayfish muscle was collected in sterile tubes, placed on ice immediately, and stored at -40 ℃ until needed. RAPD-PCR analysis was performed on the muscle extract of 22 individuals using six arbitrarily selected primers. The extraction/purification of genomic DNA was performed under the conditions previously described (Yoon and Kim, 2003b). The DNA pellets were incubation-dried for more than 10 hours, held at -40℃ until analysis, and then dissolved in the ultra-pure water produced by a water purification system (JABA KOREA, Korea). The concentration of the extracted genomic DNA was measured by absorbance ratio at 260 nm by a spectrophotometer (Beckman DU 600 series, UK; Shimadzu, Australia).

Decamer primers, molecular markers and amplification stipulations

The arbitrarily chosen primers were purchased from Operon Technologies, USA and Seoulin Biotechnologies, Korea. The G + C content of the primers was between 60 ~ 70%. Six selected primers; OPC-03 (5'-GGGGGTCTTT-3'), OPC-06 (5'-GAACGGACTC-3'), OPC-09 (5'- CTCACCG TCC -3'), URP-02 (20-mer), URP-07 (20-mer) and URP-09 (20-mer) were shown to generate identical, specific and polymorphic fragments, which could be clearly scored. Thus, we used the primers to study the genetic variations, DNA polymorphisms, genetic diversity, and similarity of the crayfish. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers (MJ Research, Inc., USA; Perkin Elmer Cetus, 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 generated via electrophoresis on 1.4% agarose (VentechBio, Korea) gel containing TBE (90 mM Tris, pH 8.5; 90 mM borate; 2.5 mM EDTA). The 100 bp DNA Ladder (Bioneer Co., 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, USA).

The data analysis

Primers that generated minor bands were excluded from our analyses. Only readily visible fragments between 72 bp and 1,400 bp in size were scored for statistical analysis. The bandsharing (BS) value was calculated by the presence/absence of amplified products at specific positions in the same gel from the RAPD profiles. The values were calculated according to the protocols outlined by Nei (1978) and Jeffreys and Morton (1987). Comparing two lanes, BS values were calculated as follows:

BS = 2 (Nab) / (Na+Nb).

Nab: the number of bands shared by the samples b and a

Na: the total number of bands in sample a

Nb: the total number of bands in sample b.

The average of within-population similarity is calculated by pairwise comparison between individuals within a population. The relatedness between different individuals in the JJC population (CRAYFISH 01 ~ CRAYFISH 11) and JUC population (CRAYFISH 12 ~ CRAYFISH 22) was generated according to the bandsharing values and similarity matrix. A hierarchical clustering tree was constructed using similarity matrices to generate a dendrogram, which was facilitated by the PC-package program Systat version 10 (SPSS Inc., Chicago, IL, USA). Genetic differences and Euclidean genetic distances within- and between-populations were also calculated using the hierarchical dendrogram program Systat version 10.

Results and Discussion

RAPD-PCR variations

RAPD-PCR is one of the fast and simple research methods for the identification of genetic differences and polymorphisms in various organisms. In spite of variation in the RAPD profiles and the differences in reproducibility, RAPD and/or RAPD-based techniques have been widely applied to the identification of genetic characteristics of diverse species of teleosts and invertebrates (Smith *et al*., 1997; Callejas and Ochando, 1998; Iyengar *et al*., 2000; Yoon and Kim, 2004; Kim *et al*., 2004). Also, the advantage of RAPD method is that it does not require prior knowledge of the genome to be effective (Welsh *et al*., 1991; Mamuris *et al*., 1999; Iyen-

gar *et al*., 2000; Klinbunga *et al*., 2000a). Many researchers have used RAPD and/or RAPD-based techniques to estimate population structure in invertebrate and fishes, including the black tiger shrimp (Tassanakajon *et al*., 1998), brown trout (Cagigas *et al*., 1999), blacklip abalone (Huang *et al*., 2000), marsh clam (Yoon and Kim, 2003a), and oyster (Kim *et al*., 2004). Polymorphisms are determined by the banding patterns of primer-amplified products at specific positions (Smith *et al*., 1997; Yoon and Kim, 2001; Yoon and Kim, 2003b).

The genomic DNA was isolated from two geographical crayfish populations in Jeonju and Jeongup of Korea. The amplified products were

Table 1. The total, average, common, specific, and polymorphic fragments generated by RAPD-PCR using 6 random primers in crayfish (*C. similis*) from Jeonju and Jeongup.

Item	No. of average fragment per lane		No. of common fragments		No. of specific fragments		No. of polymorphic fragments	
Primer	Jeonju	Jeongup	Jeonju	Jeongup	Jeonju	Jeongup	Jeonju	Jeongup
OPC-03	7.4 (81)	4.4 (48)	22	11	12	18	5	5
OPC-06	10.5 (115)	5.3 (58)	44	22	6	25	5	5
OPC-09	8.5 (93)	5.0 (55)	33	44	11	15	15	0
URP-02	6.1 (67)	8.5 (94)	33	66	14	10	8	0
URP-07	10.1 (111)	3.9 (43)	44	11	15	18	4	2
URP-09	4.9 (54)	5.1 (56)	33	11	2	4	5	6
Total no.	47.5 (521)	32.2 (354)	209	165	60	90	42	18
Average no. per primer	86.8	59.0	34.8	27.5	10.0	15.0	7.0	3.0

The total number of fragments generated by a primer in crayfish obtained Jeonju and Jeongup is shown in parentheses.

Table 2. The inter-population-specific, and -common fragments generated by RAPD-PCR using 6 random primers in crayfish (*C. similis*) from Jeonju and Jeongup.

Item	No. of inter-population-specific fragment		No. of inter-population-common fragments
Primer \ Population	Jeonju	Jeongup	Two locales
OPC-03	22	11	11
OPC-06	44	22	22
OPC-09	33	44	11
URP-02	33	66	22
URP-07	33	11	11
URP-09	33	11	11
Total no.	198	165	77
Average no. per primer	33.0	26.5	12.8

separated by agarose gel electrophoresis with oligonucleotide decamer primers and 20-mer primers, and stained with ethidium bromide. The six arbitrarily selected primers OPC-03, OPC-06, OPC-09, URP-02, URP-07 and URP-09 generated the common, specific, and polymorphic fragments (Tables 1 ~ 3). The complexity of the banding patterns varied dramatically between the primers from the two locations. Accordingly, in the present study, six decamer and 20-mer primers generated a total of 521 fragments in the JJC population, and 354 in the JUC population, with a DNA fragment size ranging from 100 bp to 2,600 bp, as summarized in Fig. 1. Many researchers studied the sizes of DNA frag-

Table 3. Similarity matrix, including bandsharing values and genetic differences, calculated using Nei and Li's index, of the similarity of crayfish (*C. similis*) from Jeonju and Jeongup. Bandsharing values of crayfish from two regions are above the diagonal and genetic differences are below the diagonal.

		Bandsharing values of crayfish from Jeonju											Bandsharing values of crayfish from Jeongup										
		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 crayfish from Jeonju	1		0.680	0.823	0.794	0.817	0.792	0.807	0.822	0.814	0.722	0.757	0.428	0.465	0.395	0.574	0.464	0.490	0.503	0.446	0.436	0.477	0.495
	2	0.320		0.758	0.678	0.667	0.666	0.621	0.632	0.743	0.801	0.766	0.563	0.670	0.485	0.605	0.573	0.647	0.539	0.587	0.531	0.639	0.635
	3	0.177	0.242		0.851	0.906	0.858	0.843	0.857	0.900	0.874	0.837	0.503	0.537	0.480	0.612	0.534	0.595	0.576	0.524	0.512	0.526	0.566
	4	0.206	0.323	0.150		0.913	0.881	0.893	0.815	0.851	0.698	0.718	0.463	0.572	0.485	0.604	0.607	0.611	0.566	0.516	0.526	0.616	0.546
	5	0.183	0.333	0.094	0.087		0.861	0.852	0.749	0.864	0.798	0.745	0.443	0.525	0.404	0.586	0.509	0.571	0.557	0.500	0.520	0.551	0.525
	6	0.208	0.334	0.142	0.119	0.139		0.830	0.735	0.782	0.744	0.690	0.505	0.562	0.508	0.599	0.556	0.583	0.619	0.516	0.580	0.680	0.580
	7	0.194	0.379	0.157	0.107	0.148	0.170		0.826	0.867	0.814	0.796	0.423	0.473	0.355	0.516	0.494	0.481	0.508	0.435	0.430	0.461	0.502
	8	0.178	0.368	0.143	0.186	0.251	0.265	0.174		0.923	0.876	0.870	0.414	0.545	0.353	0.539	0.485	0.478	0.504	0.458	0.433	0.533	0.498
	9	0.186	0.257	0.101	0.149	0.136	0.218	0.133	0.077		0.857	0.912	0.440	0.551	0.352	0.604	0.516	0.489	0.535	0.481	0.443	0.484	0.528
	10	0.278	0.199	0.127	0.302	0.203	0.256	0.187	0.124	0.144		0.871	0.491	0.632	0.499	0.683	0.542	0.537	0.574	0.579	0.626	0.594	0.638
	11	0.243	0.234	0.163	0.282	0.255	0.310	0.204	0.130	0.088	0.130		0.449	0.600	0.486	0.591	0.521	0.496	0.550	0.564	0.595	0.538	0.628
Genetic differences of crayfish from Jeongup	12	0.572	0.437	0.498	0.537	0.557	0.495	0.577	0.586	0.560	0.509	0.551		0.742	0.614	0.669	0.795	0.584	0.638	0.711	0.649	0.643	0.643
	13	0.535	0.330	0.463	0.428	0.475	0.438	0.528	0.456	0.449	0.368	0.400	0.258		0.695	0.688	0.719	0.694	0.802	0.738	0.718	0.764	0.737
	14	0.606	0.515	0.520	0.515	0.596	0.492	0.645	0.647	0.648	0.501	0.514	0.386	0.306		0.619	0.680	0.666	0.658	0.736	0.708	0.714	0.723
	15	0.426	0.395	0.388	0.396	0.414	0.492	0.484	0.461	0.397	0.317	0.409	0.331	0.313	0.382		0.678	0.579	0.676	0.712	0.745	0.653	0.647
	16	0.537	0.427	0.466	0.393	0.491	0.445	0.519	0.515	0.484	0.458	0.479	0.205	0.281	0.320	0.322		0.692	0.663	0.817	0.724	0.762	0.699
	17	0.510	0.353	0.405	0.389	0.430	0.417	0.519	0.522	0.512	0.463	0.504	0.416	0.306	0.334	0.421	0.308		0.777	0.692	0.693	0.751	0.732
	18	0.497	0.461	0.424	0.434	0.443	0.382	0.492	0.496	0.465	0.426	0.450	0.362	0.198	0.342	0.324	0.338	0.224		0.733	0.722	0.811	0.805
	19	0.554	0.413	0.476	0.484	0.500	0.484	0.565	0.542	0.519	0.421	0.554	0.289	0.262	0.264	0.288	0.183	0.308	0.267		0.785	0.752	0.717
	20	0.564	0.469	0.489	0.474	0.480	0.420	0.570	0.567	0.557	0.374	0.405	0.351	0.282	0.292	0.255	0.276	0.307	0.278	0.216		0.771	0.730
	21	0.524	0.362	0.474	0.384	0.449	0.320	0.539	0.467	0.516	0.406	0.462	0.357	0.236	0.286	0.347	0.238	0.249	0.189	0.248	0.229		0.825
	22	0.505	0.365	0.434	0.454	0.475	0.420	0.499	0.502	0.473	0.362	0.372	0.357	0.263	0.277	0.353	0.301	0.268	0.195	0.284	0.270	0.175	

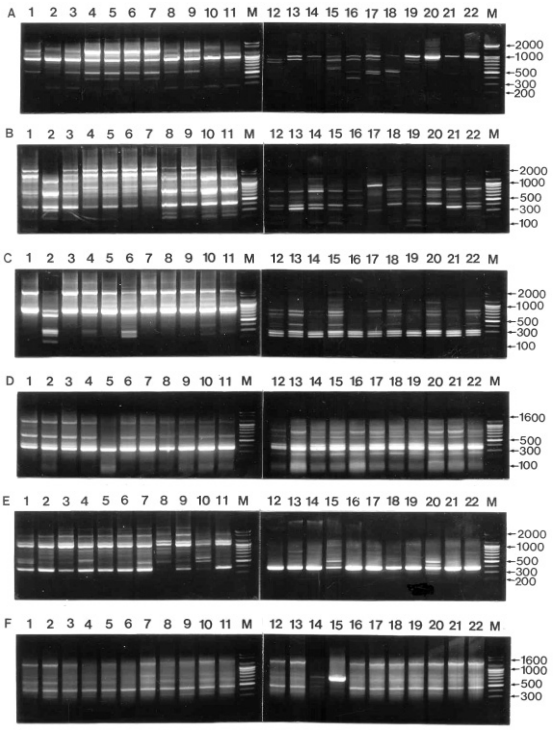

Fig. 1. RAPD-PCR-generated electrophoretic profiles of individual crayfish (*C. similis*). DNA isolated from Jeonju (lane 1 ~ 11) and Jeongup (lane 12 ~ 22) were amplified by random primers OPC-03 (A), OPC-06 (B), OPC-09 (C), URP-02 (D), URP-07 (E) and URP-09 (F). Amplicons were analyzed via electrophoresis on 1.4% agarose gel containing. After electrophoresis, agarose gels were illuminated by ultraviolet rays, and photographed using a Photoman direct copy system. M, 100 bp Ladder DNA marker.

ments in the RAPD-PCR profiles of barramundi (*Lates calcarifer*) (Partis and Wells, 1996), five species of Eastern Pacific abalone (genus *Haliotis*) (Muchmore *et al*., 1998), black tiger shrimp (*Penaeus monodon*) (Tassanakajon *et al*., 1998), brown trout (*Salmo trutta*) (Cagigas *et al*., 1999), four species of the Mullidae family (Mamuris *et al*., 1999), the brittle star (*Amphiura filiformis*) (McCormack *et al*., 2000), crucian carp (Yoon and Park, 2002), marsh clams (*Corbicula* spp) (Yoon and Kim, 2003a) and shrimp population (Yoon and Kim, 2003b). Especially, six primers were used, generating a total of 602 scorable bands in catfish, and 195 in the bullhead population, respectively, ranging in DNA fragment size from less than approximately 100 bp, to more than 2,000 bp (Yoon and Kim, 2004).

We first assessed genetic variation in the JJC population. Primer OPC-03 generated fragments ranging from 300 bp to 2,400 bp (Fig. 1A). This primer detected 22 identical major and/or minor fragments of sizes 500 bp and 1,000 bp, which were identical in all samples. Interestingly, the 11 common fragments that established population identity were 500 bp. The primer generated these minor specific fragments: 400 bp (lanes 4, 5, 6 and 7), 1,600 bp (lanes 2, 3, 10 and 11) and 2,400 bp (lanes 4, 5, 6 and 7). The primer generated a polymorphic RAPD profile with five DNA fragments. This primer generated 81 fragments, compared to the other primers used, with an average of 7.4. The primer OPC-09 generated 33 common fragments in all samples, approximately 800, 1,000, and 2,200 bp in size (Fig. 1C). Interestingly, the 22 common fragments that established population identity were 1,000 and 2,200 bp. This primer detected 11 specific and 15 polymorphic major and/or minor fragments that identified individuals. The primer generated 93 fragments compared to other primers used, with an average of 8.5.

In the present study, on average, a decamer primer generated 86.8 amplified products in the JJC population, as illustrated in Table 1. A RAPD primer generated an average of 7.9 amplified bands per sample, ranging from 4.9 to 10.5 fragments in this population. The oligonucleotide primer URP-02 also generated identical DNA fragments, of approximately 350 bp and 600 bp, in both crayfish populations, as shown in Fig. 1D and Table 2. The other primers also generated identically sized fragments in both crayfish populations, as illustrated in Table 2. It has been reported that the number of fragments generated per primer varied between 17

and 30, with a mean of 24.2 bands per individual and primer, in three endemic Spanish barbel species (*Barbus bocagei, B. graellsii* and *B. sclateri*) (Callejas and Ochando, 1998). It has also been reported that one primer generated 9 to 15 distinct bands in the black tiger shrimp (Tassanakajon *et al*., 1998). The number of scored bands varied from 7 to 12 per primer in four species of the Mullidae family (Mamuris *et al*., 1999). The primers generated 36, 32, and 24 bands in mud crabs from Eastern Thailand (genus *Scylla*) (Klinbunga *et al*., 2000b). 176 common fragments, with an average of 25.1 per primer, were observed in the Buan population, and 99 fragments, with an average of 14.1 per primer, were observed in the Geojedo population (Kim *et al*., 2004).

Moreover, in the JUC population, common banding patterns, corresponding to fragments of 280 bp, 350 bp, 380 bp, 400 bp, 600 bp and 800 bp, were generated by the decamer primer URP-02, as shown in Fig. 1D. The 400 bp and 800 bp bands produced by the primer OPC-06 were identified as being common to two crayfish populations, which were identifying populations and/or species, as shown in Fig. 1B. Especially, the banding patterns generated by the decamer primers OPC-03, OPC-06, and URP-07 of individual JUC population varied widely, as shown in Figs. 1A, 1B, and 1E. The complexity of the banding patterns varied widely between primers and/or geographic locales. It has been reported that the silver dory (*Cyttus australis*) has a major, 460 bp fragment, and that the mirror dory (*Zenopsis nebulosis*) has a major, 422 bp fragment (Partis and Wells, 1996). These major fragments revealed the characteristic profiles of fish species such as the john dory, silver dory and mirror dory. The RAPD-PCR method, using random primers, was applied to the identification of three endemic Spanish barbel species: *Barbus bocagei*, *B. graellsii* and *B. sclateri* (Callejas and Ochando, 1998). Results indicated that *Barbus bocagei* and *B. graellsii* were more closely related to each other than they were to *B. sclateri*. Population-related RAPD fragments were identified in the channel catfish (*Ictalurus punctatus*) and the blue catfish (*I. furcatus*), and also in their F_1, F_2 and backcross hybrids (Liu *et al*., 1998). The frequencies of fragments generated by six primers were calculated in various catfish populations, as described in catfish. Generally, the size and number of fragments generated depends both on the nucleotide sequence of the primer used, and on the source of the template DNA, resulting in a genome-specific DNA fragment (Welsh and McClelland, 1990; Welsh *et al*., 1991).

Generally speaking, using a variety of oligonucleotide primers, RAPD-PCR has been applied to identify polymorphic/specific markers particular to line, breed, species, genus and geographical population, as well as genetic diversity/similarity/ polymorphism in various organisms (Smith *et al*., 1997; Muchmore *et al*., 1998; Kim *et al*., 2000; McCormack *et al*., 2000; Yoon and Kim, 2004; Park *et al*., 2005). In the present study, 6 primers generated 60 specific fragments (60/521 fragment, 11.5%) in the JJC population, and 90 (90/354 fragments, 25.4%) in the JUC population, as illustrated in Table 1. These primers produced 42 polymorphic fragments (8.1%) in the JJC population, and 18 (5.1%) in the JUC population. Especially these results demonstrate that the primers detected numerous specific fragments. It has been reported that the percentage of polymorphic bands obtained from five geographic populations in black tiger shrimp (*Penaeus monodon*) varied from 51.5 to 57.7% (Tassanakajon *et al*., 1998). Two primers yielded the highest levels of polymorphism, which was 88.9%, in the black tiger shrimp. The results of this analysis also illustrated

that 22 out of 80 bands (27.5%) were monomorphic and 58 bands (72.5%) were polymorphic. Of the 46 polymorphic fragments, only 3 allelic markers were private, distinguishing sample 1 from the rest, within and among four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al*., 1999).

Six primers produced 84 polymorphic bands, out of a total of 90 bands in the blacklip abalone (Huang *et al*., 2000). Iyengar *et al*. (2000) used a RAPD-based technique to identify several microsatellite repeats in the turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) and report the characterizations of six novel polymorphic microsatellite markers for Dover sole. McCormack *et al*. (2000) reported that a total of 98 individuals were examined in two populations of *A. filiformis*, using these four primers. They reported that the banding patterns showed a high degree of variation, with individual organisms being clearly distinguishable from one another. All four primers generated 111 polymorphic DNA fragments from 70 individuals. Upon RAPD analysis of genetic differences and characteristics in wild and cultured crucian carp populations, the pattern of polymorphic fragments of fifty individuals in the wild population was reported to be different (Yoon and Park, 2002). Six primers generated 47 polymorphic fragments (24% of 195 fragments) in a bullhead population (Yoon and Kim, 2004). 481 fragments were identified in an oyster population from Buan, and 264 were identified in an oyster population from Geojedo in Korea: 143 polymorphic fragments (29.7%) in the Buan population, and 60 (22.7%) in the Geojedo population (Kim *et al*., 2004). Eight primers generated 27 polymorphic fragments (27/510 fragment, 5.3%) in the Korean lobster (*Ibacus ciliatus*), and 42 (42/526 fragments, 8.0%) in the Indian Ocean lobster (*Puerulus sewelli*) (Park *et al*., 2005).

Here, we have identified two specific fragments of 380 bp (lane 2) and of 500 bp (lane 10) in the JJC population, generated by the decamer primer OPC-06. We have also identified a specific fragment of 100 bp (lane 19) in the JUC population, generated by the same primer. 15 specific fragments generated by the primer URP-15 also exhibited inter-individual-specific characteristics and DNA polymorphisms, as shown in Fig. 1E. The specific primer was found to be useful in the identification of individuals and/or populations, resulting from variations in DNA polymorphisms among individuals/populations (Liu *et al*., 1998; Yoon and Park, 2002; Yoon and Kim, 2003b; Yoon and Kim, 2004). The random RAPD method has been applied to eight fish species: barramundi, Nile perch, john dory, mirror dory, silver dory, spiky oreo, warty oreo and smooth oreo (Partis and Wells, 1996). Generally, polymorphic and/or fragments generated by RAPD-PCR using arbitrary primers were suitable for the detection of genetic similarity/diversity/polymorphisms among organisms (Welsh *et al*., 1991; Liu *et al*., 1998; McCormack *et al*., 2000; Kim *et al*., 2004; Yoon and Kim, 2004). Diagnostic markers in both populations of an eel-loach species (*Pangio* sp.), are considered as species-specific markers, while the other bands are population specific markers (Siti Azizah *et al*., 2005). Three diagnostic markers were observed in *P. piperata* and 14 in *P. shelfoldii* with molecular weights ranging 300 ~ 2000 bp.

The bandsharing values and genetic distances

In this study, based on the average bandsharing values of all samples, the similarity matrix ranged from 0.621 to 0.923 in the JJC population, and from 0.584 to 0.825 in the JUC population (Table 3). The average bandsharing value was 0.802 ± 0.010 within the JJC population, and 0.711 ± 0.008 within the JUC population. The average bandsharing

value between the two geographical crayfish populations 0.528 ± 0.006, ranged from 0.352 to 0.683. Therefore, regarding individual results, individual crayfish from JJC population exhibited higher bandsharing values than did fish from JUC population. The average bandsharing value reported by our study is similar to the value reported for Spanish barbel species (0.71 ~ 0.81) (Callejas and Ochando, 1998) and Indian ocean lobster (0.742 ± 0.009) (Park *et al*., 2005). However, our reported bandsharing values between the two geographical crayfish populations are inconsistent with the previously reported results (Yoon and Park, 2002). Other reports have shown that the average bandsharing value obtained using five random primers was 0.40 ± 0.05 in the wild crucian carp population, and 0.69 ± 0.08 in the cultured crucian carp population. The average bandsharing value recorded in our study is also higher than the average value of the bullhead population (0.504 ± 0.115) (Yoon and Kim, 2004), and also between the two oyster populations (0.282 ± 0.008) (Kim *et al*., 2004). The difference between the two crayfish populations is statistically significant ($P<0.01$). Accordingly, as above-mentioned, RAPD-PCR analysis showed that the JUC population was more genetically diverse than the JJC population. This result implies the genetic similarity due to raising in the same environmental condition or inbreeding within the JJC population. In other words, crayfish may have high levels of genome DNA diversity due to the introduction of the wild population from the other sites to Jeongup even if it may be the geographical diverse distribution of this species.

In this study, based on the similarity matrix generated by bandsharing values and genetic distances, hierarchical clustering analysis was performed in order to obtain a dendrogram, as shown in Fig. 2. The dendrogram, generated by six reliable primers, indicates two genetic clusters. The dendrogram obtained by the six primers indicates two genetic clusters: cluster 1 (CRAYFISH 01 ~ CRAYFISH 11), and cluster 2 (CRAYFISH 12 ~ CRAYFISH 22). The genetic distance between the two geographical populations ranged from 0.053 to 0.605. The shortest genetic distance displaying significant molecular difference was between individuals CRAYFISH no. 04 and CRAYFISH no. 05 from Jeonju (genetic distance = 0.053). Especially, the longest genetic distance displaying significant molecular differences, 0.605, was found to exist between individuals CRAYFISH no. 15 of Jeongup and CRAYFISH no. 02 of Jeonju.

By cluster analysis of genetic similarity values obtained from RAPD data, Callejas and Ochando (1998) indicated that Spanish barbel species (*Barbus bocagei* and *B. graellsii*) were more closely related to each other than to *B. sclateri*. As for inter-specific similarity, the highest value was found to exist between *B. graellsii* and *B. bocagei* (0.4123); *B. sclateri* presented coefficients of inter-specific similarity of 0.3827 with *B. graellsii*, and 0.3981 with *B. bocagei*. The genetic distance ranged from 0.091 to 0.316, with an average of 0.160, within and among four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al*., 1999). The principal aspect of the dendrogram was also a striking separation of sample 1 from the others, which were closely grouped. Nei's genetic distances varied from 0.327 to 0.655 in four species of the Mullidae family (Mamuris *et al*., 1999).

In shellfishes and crustaceans, cluster analysis of the pairwise population matrix, generated from RAPD data, showed that geographically close populations tended to cluster together in the blacklip abalone (Huang *et al*., 2000). A neighbor-joining tree based on the genetic distances between populations, using the RAPD-PCR method, indicates the

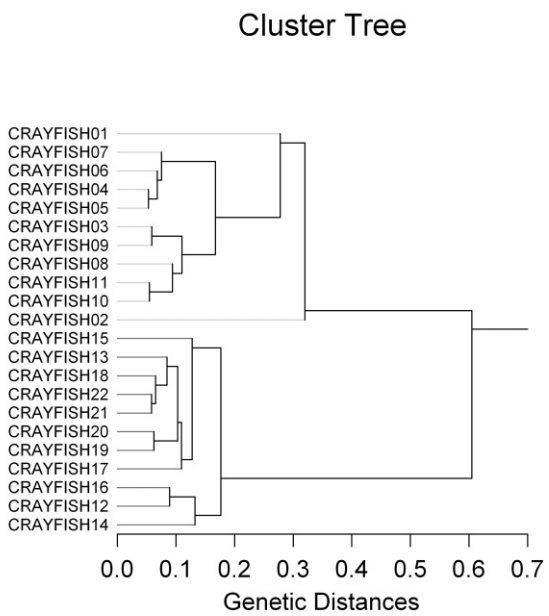

Fig. 2. Hierarchical dendrogram of genetic distances, obtained from two geographical populations of crayfish (*C. similis*). The relatedness between different individuals in the crayfish populations of Jeonju (CRAYFISH 01 ~ CRAYFISH 11) and Jeongup (CRAYFISH 12 ~ CRAYFISH 22) was generated according to the bandsharing values and similarity matrix (see Table 3).

relationships of three mud crab species (Klinbunga *et al*., 2000b). This study showed that large genetic differences could be found between geographical populations within a species, as well as between species. Phylogenetic relationships among 5 *Haliotis* species and one hybrid were conducted by calculation of the distance coefficient and construction of a phylogenetic tree based on RAPD data (Kim *et al*., 2000). Ultimately, they insisted that RAPD analysis constitutes a powerful tool for the elucidation of phylogenetic relationships, based on their analysis of 6 species of *Haliotis*. The dendrogram obtained from the Korean oyster population by the four primers, indicates three genetic clusters (Kim *et al*., 2004). The genetic distance between the two geographic populations ranged from 0.039 to 0.284. The shortest genetic distance displaying significant molecular differences, 0.080, was found to exist between individuals no. 09 and no. 07 from Buan. 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). In their study, the dendrogram obtained with the eight primers also indicates two genetic clusters, designated cluster 1 (SLIPPER 01 ~ SLIPPER 11), and cluster 2 (DEEPSEA 12 ~ DEEPSEA 22).

Cagigas *et al*. (1999) reported that genetic variation within samples is found to be significantly high within and among four natural Spanish populations of brown trout (*Salmo trutta*). The identification of the penaeid shrimp (*Penaeus chinensis*), bullhead (*Pseudobagrus fulvidraco*), and oyster (*Crassostrea gigas*) populations was a necessary step in the inception and development of invertebrate/teleost breeding programs (Yoon and Kim, 2003b; Yoon and Kim, 2004; Kim *et al*., 2004). Molecular genetic markers, including, most notably, quantitative trait loci, and genomic mapping, will be useful in the selection of broodstock for multiple reproductive traits, or health- and production-related traits, in fishery science (Waldbieser and Wolters, 1999). The classification of geographical populations of crayfish is based on morphological variations in cephalothorax, big claws called chelipeds, antennae, compound eyes and body color. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga *et al*., 2004).

In our study, RAPD analysis has revealed a significant genetic distance between two population pairs. RAPD method enabled us to detect the exis-

tence of population discrimination and genetic variation in the crayfish populations of Jeonju and Jeongup. This confirms that the method is a suitable tool for DNA comparisons, both within and between individuals, species, genera, and populations. Furthermore, basic knowledge of the DNA polymorphisms and molecular markers in crayfish (*C. similis*) may contribute significantly to broodstock selection and selective crustacean-breeding programs. The extraordinarily unique gene pools displayed by some samples (especially in case of the photo in Fig. 1B) would require new conservation policies, such that much more wild Korean crayfish populations could be preserved. Accordingly, further analysis with more individuals, primers, and species will be required to fully establish the specificity of loci to particular taxa, and subsequent inter-specific gene flow in the genus *Cambaroides*. Further sampling sites will also be necessary to more precisely determine the area in which the phylogeographic break occurs. In the future, diagnostic RAPD markers will be necessary for characterization of the different geographical crayfish species to correlate with the morphological characters and for clarification of the ambiguity among species and/or geographic populations. Both more time, and a great deal more research, will also be necessary to identify the differentially expressed genes (DEG) between/among populations and species, using an annealing control primer system.

Acknowledgements

The gift of their assistance in sample collection, and their help with the RAPD-PCR techniques, is gratefully acknowledged our laboratory colleagues. The authors also would like to thank the reviewers who assisted us with thorough and profound correction. The authors are grateful to the Fisheries Science Institute of Kunsan National University for providing equipment and funding during program year 2006.

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Manuscript Received : April 3, 2006
Revision Accepted : August 21, 2006
Responsible Editorial Member : Jung-Woo Park (Ulsan Univ.)