발생과 생식 제 10권 제 1호, 19~32(2006)
Dev. Reprod. Vol. 10, No. 1, 19~32(2006)

Genetic Differences and Variations in Freshwater Crab(*Eriocheir sinensis*) and Swimming Crab(*Portunus trituberculatus*)

Jong-Man Yoon†

Department of Aquatic Life Medicine, College of Ocean Science and Technology, Kunsan National University, Kunsan 573-701, Korea

참게(*Eriocheir sinensis*)와 꽃게(*Portunus trituberculatus*)의 유전적 차이와 변이

윤 종 만†

국립군산대학교 해양과학대학 수산생명의학과

ABSTRACT : Genomic DNA isolated from two species of Korean freshwater crab(*Eriocheir sinensis*) and swimming crab(*Portunus trituberculatus*) was amplified several times by PCR reactions. The seven arbitrarily selected primers OPA-05, OPA-13, OPA-16, OPB-06, OPB-15, OPB-17 and OPD-10 were used to generate the identical, polymorphic, and specific fragments. 505 fragments were identified in the freshwater crab species, and 513 in the swimming crab from Buan: 81 specific fragments(16.0%) in the freshwater crab species and 100(19.5%) in the swimming crab. 165 identical fragments, with an average of 23.6 per primer, were observed in the freshwater crab species. 66 fragments, with an average of 9.4 per primer, were identified in the swimming crab species. The numbers of polymorphic fragments in the freshwater crab and swimming crab were 50 and 14, respectively. The oligonucleotides decamer primer OPB-17 generated identical DNA fragments, approximately 300 bp, in both the freshwater crab and swimming crab species. Compared separately, the average genetic difference was higher in the swimming crab than in the freshwater crab species. The average genetic difference was 0.726±0.004 between the freshwater crab and swimming crab species. The dendrogram obtained by the seven primers indicates four genetic clusters: cluster 1(FRESHWATER 01), cluster 2(FRESHWATER 02, 03, 04, 05 and 06), cluster 3(FRESHWATER 07, 08, 09, 10 and 11), and cluster 4(SWIMMING 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22). The shortest genetic distance displaying significant molecular difference was between individuals SWIMMING no. 18 and SWIMMING no. 17 from swimming crab(0.096). Ultimately, individual no. 02 of the freshwater crab was most distantly related to freshwater crab no. 03(genetic distance = 0.770). As stated above, the potential of RAPD-PCR to identify diagnostic markers for the identification of two crab species has been demonstrated.

Key words : Bandsharing value, *Eriocheir sinensis*, Freshwater crab, Genetic distance, *Portunus trituberculatus*, Similarity matrix, Swimming crab.

요 약 : 참게(*Eriocheir sinensis*)와 꽃게(*Portunus trituberculatus*)의 2종으로부터 genomic DNA를 분리 추출하였다. 선택된 7개의 OPA-05, OPA-13, OPA-16, OPB-06, OPB-15, OPB-17 and OPD-10의 RAPD primer를 이용하여 identical, polymorphic 그리고 specific fragment를 얻어냈다. 본 연구에서 부안산 참게 집단에서는 505개의 fragment가 나타났고, 꽃게 집단에서는 513개의 fragment가 확인되었다. 참게 집단에서는 165개의 identical fragment가 나타났으며, 이는 primer당 평균적으로 23.6개의 fragment로 확인되었다. 또한 꽃게 집단에서는 66개로서 평균해서 primer당 9.4개의 identical fragment가 나타났다. 참게 집단과 꽃게 집단의 polymorphic fragment는 각각 50개와 14개로 나타났고, 참게 집단과 꽃게 집단의 경우 OPB-17에서 identical fragment가 300 bp의 크기에서 확인되었다. 각각을 비교해 보았을 때 유전적 차이는 참게 집단에서보다 꽃게 집단에서 더 높은 수치를 나타내었고, 2종 사이에서 0.726±0.004의 수치를 나타내었다. 7개의 primer를 사용하여 얻어진 dendrogram은 cluster 1(FRESHWATER 01), cluster 2(FRESHWATER 02, 03, 04, 05 및 06), cluster 3(FRESHWATER 07, 08, 09, 10 및 11) 및 cluster 4(SWIMMING 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22)와 같이 4개의 유전적 클러스터로 나뉘어졌다. 꽃게 집단에서 18번째 개체(SWIMMING no. 18)와 17번째 개체 (SWIMMING no. 17) 사이가 가장 가까운 유전적 관계(genetic distance 0.096)를 나타내었다. 궁극적으로 볼 때 참게 집단의 2번째(FRESHWATER no. 02)와 참게 집단의 3번째(FRESHWATER no. 03) 개체 사이가 가장 먼 유전적 거리(genetic distance=0.770)를 나타내었다. 위에서 언급했던 것처럼 RAPD-

This work was supported by the 2006 Sea Grant Program of Honam District University.

† Correspondence: Department of Aquatic Life Medicine, College of Ocean Science and Technology, Kunsan National University, Kunsan 573-701, Korea. Tel: 82-63-469-1887, Fax: 82-63-463-9493, E-mail: jmyoon@kunsan.ac.kr

PCR 방법은 참게 및 꽃게 2종의 종 판별을 하기 위한 진단적 표지(diagnostic marker) 로 이용할 수 있는 잠재력을 가지고 있는 것으로 확인되었다.

INTRODUCTION

A great deal of analytical and/or molecular techniques have been applied in the analysis of the heredity of organisms, such as the allozyme genetic variation method (Maltagliati, 1998), flow cytometry(Nozaki *et al.,* 2000), genomic *in situ* hybridization(GISH)(Friesen *et al.,* 1997), fluorescence *in situ* hybridization(FISH)(Calderini *et al.,* 1997), and various PCR-based molecular techniques, including restriction fragment length polymorphisms(RFLPs) (Lee & Kim, 2003), amplified fragment length polymorphisms(AFLPs) (Barki *et al.,* 2000), random amplified polymorphic DNAs(RAPD)(Jeffreys & Morton, 1987; Kim *et al.,* 2000; Nozaki *et al.,* 2000; Yoon & Park, 2002) and microsatellites(Waldbieser & Wolters 1999; Supungul *et al.,* 2000; Chenyambuga *et al.,* 2004). Most notably, amplification of the mitochondrial DNA control region (D-loop) was accomplished using universal primers(Bernardi & Talley, 2000). The genetic variation and molecular discrimination of the *Scapharca* species in Korea were also investigated via mitochondrial COI gene sequences and PCR-RFLP analyses(Lee & Kim, 2003).

The polymorphic and/or specific markers specific to line, breed, species, genus or geographical population have been employed in the identification of individuals, species and populations, hybrid parentages and genetic diagnostics(Callejas & Ochando, 1998; Muchmore *et al.,* 1998; Huang *et al.,* 2000; Kim *et al.,* 2000; Yoon & Kim, 2003a). The RAPD method was used to generate fingerprint patterns for 10 meat species: wild boar, pig, horse, buffalo, beef, venison, dog, cat, rabbit and kangaroo(Koh *et al.,* 1998). 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 & Morton, 1987; Liu *et al.,* 1998; McCormack *et al.,* 2000; Kim *et al.,* 2004).

Korean freshwater crab(*Eriocheir sinensis*) is one of economically important aquacultural species, belonging to the family Grapsidae, and the order Decapoda. In the natural ecosystem, freshwater crab is 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. During the last five decades the estuarine fisheries are threatened by over-fishing, migration interception by dam construction and various environmental pollutions, and in the 2000s farmers were engaged in aquaculture of freshwater crabs. In recent years the feasibility of commercial culture investigated in a recently begun project at a few of inland farming culture centers and national fisheries experimental stations.

As with other crustaceans, the chief problem in culturing freshwater crabs is cannibalism. Metamorphosis to the crab stage usually occurs within 25 days of hatching and only 80% of the juveniles survive because of cannibalism during the metamorphosis period. Especially, the survival rate depends not only on the individual density of this crab but also on the population density of the feed organisms; the more dense the zoea population, the better the growth attained. The expense of holding and feeding this crab to avert cannibalism virtually precludes commercial culture. However, this species of crab was successfully spread into many freshwater areas in Southern Korea in 2000's. Freshwater crab is one of the most delicious freshwater species available by gourmets in Korea during the late autumn, owing to the sweet taste.

Basically, the rate at which freshwater crab grows depends very much on feed organisms, population density and water temperature. Especially, the water temperature of 20 ℃~25℃ is about optimal and the transparency of the water river is 20cm to 40cm at a spawning time. Accordingly, the growth period of this crab is from June to August. Like other crabs, this species commonly hatches in seawater and then grows in freshwater, which it attains a marketable size. When they are fully grown among the mountains and

valley, they migrate to the brackish water or mouth of the river and then descend the seawater regions of the spawning ground from September to October. Since crabs are nocturnal creatures, young crabs burrow under the pebbles, sands and rocks in the riverbed during the day and ascend the river at night. During migration, the body color of young crabs gradually becomes darker. Dorsal shell is covered with hard surface without scales. The shape of crab dorsal shell is basically rectangular, embossed pattern on a dorsal shell and four protuberances exist in the brow surface of this shell. Especially, a number of hairs are located in the anterior and basic parts of the claws.

In general, the color, size and type of the crab in this species varies according to habitat, such as river, marsh, or seawater area, the depth of the water, nutrition, and other common factors. The environmental requirements and tolerances of the crab species from different geographic areas are currently unknown, as is crab species discrimination. As the crab culture industry increases, the understanding of the genetics of this crab species becomes more necessary; to evaluate the potential genetic effects induced by crab production operations.

Perhaps swimming crab(*Portunus trituberculatus*) is the most famous of the portunid crabs of the West Sea. The crab is one of economically important crab species, belonging to the family Portunidae, and the order Decapoda. In the natural ecosystem, swimming crab is widely distributed in the seawater areas of the Korean Peninsula, as well as in several areas in China, Japan, Taiwan and the Americas. Morphologically, the shape of crab dorsal shell is basically long rhombus, embossed pattern on a dorsal shell and three protuberances exist in the brow surface of this shell. The crab has very large and long both claws and the body color of this crab is dark purple with blue patterns. Since crabs are nocturnal creatures, they burrow under the pebbles, sands and rocks in the riverbed during the day and migrate at night. Multiple researches have shown that water temperature, population density, water depth and salinity factors are critical in the early larval development of this portunid crab(Ryu, 1970). Swimming crab is the most commonly eaten portunid crab in Korea all the year round, owing to the sweet taste.

Accordingly, these crabs have become a popular crab in various types of traditional restaurants(including restaurants specializing in soy sauce and other seasonings in which crabs are preserved, or "gejangjip and a pepper-pot soup", or "maeuntangjip") for a long while. The consumption of these crabs species have also increased considerably as restaurants specialize in various preparations of freshwater crab and swimming crab such as steamed stuffed freshwater crab in a pan with red pepper, boiled with vegetables, fried, etc. The Korean names for these dishes, regardless of preparation, are "jjim" and "maeuntang".

However, in spite of their economic and scientific consequences, a little information currently exist regarding the genetics and early development of these two crab species in Korea(Ryu & Kim, 1969). Moreover, in recent years over-fishing and environmental pollution has also threatened the fishery for swimming crab in the West Sea of Korea and attention has been given to the possibility of its preservation.

Most especially, clustering analyses of the genetic distances between populations/species/genera of diverse invertebrates and fishes from different geographic sites using RAPD-PCR would be useful. However, only a small number of such studies have been undertaken(Cagigas *et al.*, 1999; Kim *et al.*, 2000; Klinbunga *et al.*, 2000b; McCormack *et al.*, 2000; Yoon & Kim, 2004a). However, genetic variation, species-specific markers, and region-specific markers in catfish, penaeid shrimp and oyster have already been assessed by molecular methods(Yoon & Kim, 2001; Yoon & Kim, 2003b; Kim *et al.*, 2004), proving the feasibility of such a study on other invertebrate and fish species.

Our study attempts to elucidate the genetic distances and differences within and among freshwater crab and swimming crab species. In order to accomplish this, we performed clustering analyses of two species of freshwater crab (*E. sinensis*) and swimming crab(*P. trituberculatus*) in the Buan region of Korea. We also analyzed DNA polymorphisms and genetic variations of these crabs in Korea.

MATERIALS AND METHODS

1. Sample Collection and Extraction of Genomic DNA

Two species of freshwater crab(*E. sinensis*) and swimming crab(*P. trituberculatus*) were obtained from Buan district in the vicinity of the West Sea of Korea. Two species of crab muscle was collected in sterile tubes, immediately placed on ice, and stored at −40℃ until needed. RAPD-PCR analysis was performed on the genomic DNAs from 22 individuals, using seven arbitrarily selected primers of two decades of different decamer primers. The extraction/purification of genomic DNA was performed under the conditions described previously(Yoon & Kim, 2003b). After several washings, lysis buffer Ⅰ(155mM NH_4Cl; 10 mM $KHCO_3$; 1mM EDTA) was added to the samples, and the mixture tubes were gently inverted. The precipitates obtained were then centrifuged and resuspended in lysis buffer Ⅱ(10mM Tris-HCl, pH 8.0; 10mM EDTA; 100 mM NaCl; 0.5% SDS), and 15 μL of proteinase K solution(10 mg/mL) was added. After incubation, we added 300 μL of 3 M NaCl, and gently pipetted for a few minutes. 600 μL of chloroform was then added to the mixture and inverted(no phenol). Ice-cold 70% ethanol was added, and then the samples were centrifuged at 19,621 g for 5 minutes to extract the DNA from the lysates.

The DNA pellets were incubator-dried for 2 h and maintained at −40℃ until analysis, at which time the samples were dissolved in TE buffer [10mM Tris-HCl(pH 8.0), 1 mM EDTA]. The concentration of the extracted genomic DNA was measured by its absorbance ratio at 260nm, with a spectrophotometer(Beckman Coulter, Buckinghamshire, UK).

2. Decamer Primers, Molecular Markers and Amplification Procedures

The arbitrarily chosen primers were purchased from Operon Technologies, USA. The G+C content of the primers was between 60~70%. Among the 20 primers selected, seven primers, OPA-05(5'-AGGGGTCTTG-3'), OPA-13(5'-CAGCACCCAC-3'), OPA-16(5'-AGCCAGCG AA-3'), OPB- 06(5'-GCTCTGCCC-3'), OPB-15(5'-GGAGGGTGTT -3'), OPB-17(5'-AGGGAACGAG-3') and OPD-10(5'-GG TCTA CACC-3') were shown to generate identical, specific and polymorphic fragments which could be clearly scored. We used these primers to determine the genetic variations, DNA polymorphisms, genetic diversity, and similarity of the pond-smelt. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers(Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA). DNA amplification was performed in 25 μL samples, which contained 10 ng of template DNA, 20 μL of premix(Super-Bio Co., Korea), and 1 unit of primer. Amplification products were generated via electrophoresis on 1.4% agarose (VentechBio, Korea; SeaKem LE; FMC BioProducts, Rockland, ME, USA) gel containing TBE [90 mM Tris(pH 8.5), 90 mM borate, 2.5mM EDTA]. The 100 bp Ladder DNA marker(Bioneer Corp., Daejeon, Korea) was used as a DNA molecular weight marker. Bands were detected by ethidium bromide staining. The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a Photoman direct copy system(PECA Products, Beloit, WI, USA) and image analysis system (Syngene Corp., Cambridge, UK).

3. The Data Analysis

Primers that generated minor bands were excluded from our analyses. Only fragments between 100 and 2,400 bp in size, and readily visible, 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), Jeffreys & Morton(1987) and Yoon & Kim (2003b). Comparing the 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-species similarity is calculated by pairwise comparison between individuals within a crab species, respectively. Using similarity matrices to generate a dendrogram, facilitated by the Systat ver. 10 software (SPSS Inc., Chicago, IL, USA), a hierarchical clustering tree was constructed. Genetic differences and Euclidean

genetic distances within and between species were also calculated by complete linkage method using the Systat hierarchical dendrogram program version 10. Systat version 10 was also used to obtain other statistical results, including means, standard errors, and *t*-test scores.

RESULTS AND DISCUSSION

1. PCR Variations

The genomic DNAs isolated from two crab species in Buan, were amplified several times by PCR reaction. The amplified products were separated by agarose gel electrophoresis with an oligonucleotide decamer primer, and stained with ethidium bromide. Out of 20 decamer primers, the seven arbitrarily selected primers OPA-05, OPA-13, OPA-16, OPB-06, OPB-15, OPB-17 and OPD-10 generated the identical, specific, and polymorphic fragments(Tables 1~3).

Despite of genetic variation in the RAPD profiles and the difference in reproducibility, RAPD and/or RAPD-based techniques have been widely applied to the identification of genetically distinctive features of numerous teleosts and invertebrates(Callejas & Ochando, 1998 ; Cagigas *et al.*, 1999; Iyengar *et al.*, 2000 ; Yoon & Kim, 2004b). Generally, RAPD-PCR is one of the fast and easy methods for the identification of genetic differences and DNA polymorphisms in a variety of organisms. Especially, Mamuris *et al.* (1999) illustrated that the main advantage of the RAPD technique over the two other methods was its largely superior discriminative ability. It has been reported that RAPD-PCR analysis does not also require a prior knowledge of the genomic DNA(Welsh *et al.*, 1991 ; Mamuris *et al.*, 1999 ; Iyengar *et al.*, 2000 ; Klinbunga *et al.*, 2000a). Polymorphisms are determined by primers or/and by the banding patterns of amplified products at specific positions (Tassanakajon *et al.*, 1998 ; Nozaki *et al.*, 2000 ; Yoon & Kim, 2001). Accordingly, in this study, seven oligonucleotide primers generated a total of 505 fragments in the freshwater crab, and 513 in the swimming crab species, with a DNA fragment size ranging from 100 to 2,400bp, as summarized in Tables 1 and 2.

As made mention of shellfishes and crustaceans, for RAPD analysis, Yoon & Kim(2003a) reported that 7 decamer primers generated 585 major and minor RAPD bands from three geographic sites, producing approximately 6.6 average products per primer in marsh clams(*Corbicula* spp.) from Gochang. McCormack *et al.*(2000) insisted that DNA

Table 1. The total, average, identical, specific, and polymorphic fragments generated by RAPD analysis using 7 random primers in freshwater crab(*E. sinensis*) and swimming crab(*P. trituberculatus*) from Buan in Korea

Item / Primer	No. of average fragment per lane		No. of identical fragment		No. of specific fragments		No. of polymorphic fragments	
	Freshwater crab	Swimming crab	Freshwater crab	Swimming crab	Freshwater crab	Swimming crab	Freshwater crab	Swimming crab
OPA-05	7.2(79)	3.8(42)	22	0	15	15	12	4
OPA-13	11.7(129)	6.7(74)	66	33	14	15	4	1
OPA-16	6.3(70)	11.5(126)	22	0	6	24	10	6
OPB-06	4.3(48)	7.1(78)	11	11	15	9	4	1
OPB-15	7.5(82)	6.3(69)	22	0	16	8	4	0
OPB-17	3.0(33)	6.6(73)	11	22	6	10	15	1
OPD-10	5.8(64)	4.6(51)	11	0	9	19	1	1
Total no.	45.8(505)	46.6(513)	165	66	81	100	50	14
Average no. per primer	72.1	73.3	23.6	9.4	11.6	14.3	7.1	2.0

The total number of fragments generated by a primer in freshwater crab and swimming crab is shown in parentheses.

Table 2. Similarity matrix, including bandsharing values and genetic differences, calculated using Nei and Li's index, of the similarity of freshwater crab(*E. sinensis*) and swimming crab(*P. trituberculatus*) from Buan in Korea

		Bandsharing values of freshwater crab											Bandsharing values of swimming crab										
		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 freshwater crab	1		0.553	0.530	0.630	0.704	0.627	0.560	0.563	0.631	0.590	0.421	0.230	0.261	0.263	0.366	0.250	0.299	0.321	0.231	0.285	0.242	0.232
	2	0.447		0.480	0.598	0.621	0.571	0.528	0.502	0.538	0.489	0.363	0.278	0.324	0.261	0.325	0.224	0.232	0.321	0.256	0.246	0.260	0.192
	3	0.470	0.520		0.584	0.631	0.604	0.560	0.545	0.591	0.579	0.422	0.240	0.342	0.329	0.237	0.225	0.269	0.306	0.301	0.289	0.226	0.250
	4	0.370	0.402	0.416		0.695	0.659	0.579	0.574	0.606	0.499	0.395	0.285	0.333	0.387	0.336	0.224	0.252	0.240	0.274	0.312	0.212	0.278
	5	0.296	0.379	0.369	0.305		0.717	0.625	0.624	0.671	0.605	0.468	0.335	0.389	0.332	0.190	0.300	0.198	0.312	0.285	0.286	0.268	0.291
	6	0.373	0.429	0.396	0.341	0.283		0.693	0.704	0.675	0.636	0.467	0.222	0.273	0.264	0.249	0.244	0.300	0.233	0.314	0.293	0.213	0.285
	7	0.440	0.472	0.440	0.421	0.375	0.307		0.662	0.637	0.652	0.504	0.235	0.243	0.291	0.210	0.229	0.243	0.275	0.316	0.251	0.255	0.279
	8	0.437	0.498	0.455	0.426	0.376	0.296	0.338		0.739	0.675	0.587	0.290	0.390	0.262	0.332	0.244	0.351	0.302	0.304	0.260	0.242	0.261
	9	0.369	0.462	0.409	0.394	0.329	0.325	0.363	0.261		0.748	0.643	0.304	0.312	0.281	0.249	0.289	0.255	0.263	0.344	0.269	0.141	0.257
	10	0.410	0.511	0.421	0.501	0.395	0.364	0.348	0.325	0.252		0.513	0.303	0.255	0.291	0.215	0.190	0.184	0.361	0.353	0.255	0.311	0.277
	11	0.579	0.637	0.578	0.605	0.532	0.533	0.496	0.413	0.357	0.487		0.225	0.245	0.231	0.320	0.268	0.295	0.266	0.283	0.271	0.353	0.258
Genetic differences of swimming crab	12	0.770	0.722	0.760	0.715	0.665	0.778	0.765	0.710	0.696	0.697	0.775		0.596	0.486	0.472	0.476	0.532	0.580	0.420	0.330	0.418	0.446
	13	0.739	0.676	0.658	0.667	0.611	0.727	0.757	0.610	0.688	0.745	0.755	0.404		0.645	0.604	0.666	0.671	0.635	0.482	0.454	0.585	0.382
	14	0.737	0.739	0.671	0.613	0.668	0.736	0.709	0.738	0.719	0.709	0.769	0.514	0.355		0.559	0.593	0.606	0.619	0.521	0.368	0.506	0.304
	15	0.634	0.675	0.763	0.664	0.810	0.736	0.790	0.668	0.751	0.785	0.680	0.528	0.396	0.441		0.599	0.673	0.685	0.498	0.350	0.549	0.408
	16	0.750	0.776	0.775	0.776	0.700	0.756	0.757	0.756	0.711	0.810	0.732	0.524	0.334	0.407	0.401		0.606	0.669	0.410	0.368	0.620	0.294
	17	0.701	0.768	0.731	0.748	0.802	0.700	0.757	0.649	0.745	0.816	0.705	0.468	0.329	0.394	0.327	0.394		0.706	0.496	0.302	0.654	0.348
	18	0.679	0.679	0.694	0.760	0.688	0.767	0.725	0.698	0.737	0.639	0.734	0.420	0.365	0.381	0.315	0.331	0.294		0.547	0.417	0.627	0.422
	19	0.769	0.744	0.699	0.726	0.715	0.686	0.684	0.696	0.656	0.647	0.769	0.580	0.518	0.479	0.502	0.590	0.504	0.453		0.449	0.493	0.415
	20	0.715	0.754	0.711	0.688	0.714	0.707	0.749	0.740	0.731	0.745	0.729	0.670	0.546	0.632	0.650	0.632	0.698	0.583	0.551		0.357	0.568
	21	0.758	0.740	0.774	0.788	0.732	0.787	0.745	0.758	0.859	0.689	0.647	0.582	0.415	0.494	0.451	0.380	0.346	0.373	0.507	0.643		0.429
	22	0.768	0.808	0.750	0.722	0.709	0.715	0.721	0.739	0.743	0.723	0.742	0.554	0.618	0.696	0.592	0.706	0.652	0.578	0.585	0.432	0.571	

fragments obtained by four primers ranged from 100 to 2,300bp in the brittle star(*Amphiura filiformis*). 481 fragments were identified in the oyster population of Buan, and 264 in the oyster population from Geojedo: 143 polymorphic fragments(29.7%) in the Buan population, and 60(22.7%) in the Geojedo population(Kim *et al.*, 2004). DNA fragment sizes varied from 50bp to 1,600bp. It has been reported that 7 primers generated 317 bands in a cultured shrimp population, and 385 in the wild population, ranging from 100 to 1,800 base pairs(Yoon & Kim, 2003b).

With respect to various fishes, Partis & Wells(1996) reported that DNA fragments ranging from 350 to 700 bp were detected in the RAPD-PCR profiles of barramundi (*Lates calcarifer*). They used nine primers, generating a total of 65 reliable bands, which ranged in size from approximately 200 to 1,000bp, within and among four natural Spanish populations of brown trout(*Salmo trutta*)(Cagigas *et al.*, 1999). Mamuris *et al.*(1999) reported that fragment sizes varied from 220 bp to 1,700 bp in four species of the Mullidae family. It has been reported that five primers generated 1,084 distinct fragments, which ranged in size from 120, to larger than 4,270bp in wild and cultured populations of crucian carp(Yoon & Park, 2002). Six primers were used, generating a total of 602 scoreable bands in cat-

fish, and 195 in the bullhead population, respectively, ranging in DNA fragment size from less than approximately 100, to more than 2,000 base pairs(Yoon & Kim, 2004b).

By other molecular methods, a tandemly repeated satellite DNA of 290~291 base pairs was identified by *Sal*I digestion of the genomic DNA of five species of Eastern Pacific abalone(genus *Haliotis*)(Muchmore *et al.*, 1998). Tassanakajon *et al.*(1998) insisted that eighty fragments, ranging from 200 to 2,200bp, were unambiguously counted in black tiger shrimp(*Penaeus monodon*).

The complexity of the banding patterns varied dramatically among the primers from the two crab species(Fig. 1). In the present study, on average, a decamer primer generated 72.1 amplified products per primer in the freshwater crab species. A RAPD primer generated an average of 6.5 amplified bands per sample, ranging from 3.0 to 11.7 fragments in this species. In the freshwater crab species, the primer OPB-15 generated fragments of a great many sizes, ranged from 100 to 2,200bp, as shown in Fig. 1. In freshwater crab, the oligonucleotide primer OPA-13 generated 66 identical major and/or minor fragments, of 280bp, 400bp, 500bp, 650bp, 800bp and 1,100bp, respectively, as shown in Fig. 1B. This decamer primer also generated identical DNA fragments, of approximately 400bp, in both crab species. The decamer primer OPB-17 also generated identical DNA fragments, of approximately 300bp, in both crab species, as shown in Fig. 1F. The other primers, however, did not generate identically sized fragments in both crab species, as shown in Fig. 1A, C and G. These results demonstrate that the freshwater crab species is genetically different from the swimming crab. These results, in point of fact, indicate that the genome sizes of freshwater crab and/or swimming crab are similar to those of the blue catfish(Liu *et al.*, 1998), black tiger shrimp(Tassanakajon *et al.*, 1998), catfish(Yoon and Kim 2001), penaeid shrimp (Yoon & Kim, 2003b), bullhead(Yoon & Kim, 2004b) and oyster(Kim *et al.*, 2004). Klinbunga *et al.*(2000b) insisted that the primers generated 36, 32, and 24 bands in different mud crab species(genus *Scylla*) from Eastern Thailand, respectively. It has also been reported that one primer generated 9 to 15 distinct bands in the black tiger shrimp(Tassanakajon *et al.*, 1998).

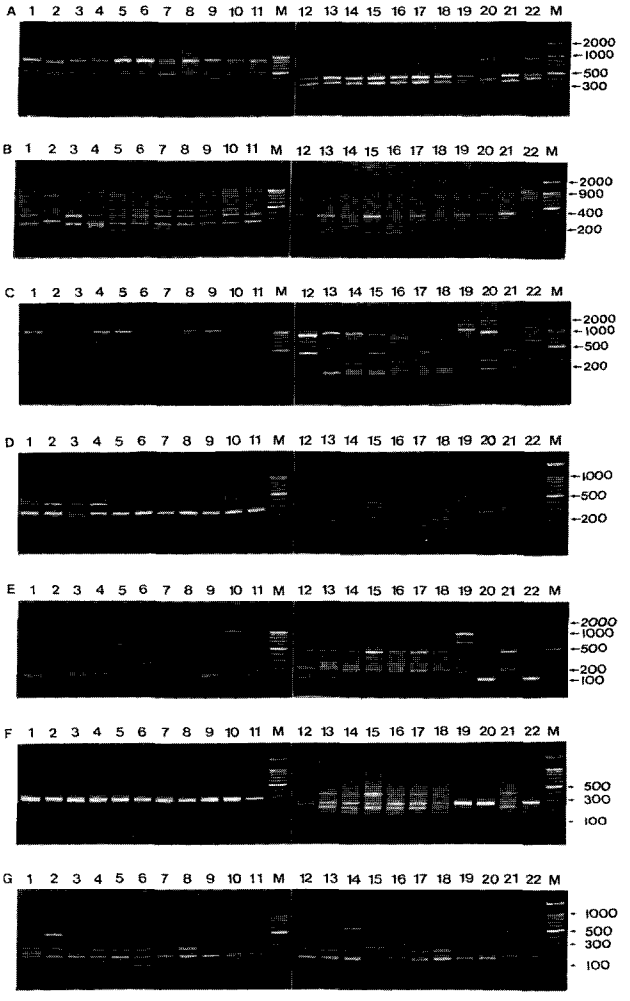

Fig. 1. RAPD-PCR-generated electrophoretic profiles of different individuals in freshwater crab(*E. sinensis*) and swimming crab(*P. trituberculatus*) species. DNA isolated from freshwater crab(lane 1~11) and swimming crab(lane 12~22) were amplified by random primers OPA-05(A), OPA-13(B), OPA-16(C), OPB-06(D), OPB-15(E), OPB-17(F) and OPD-10(G). Amplified products were electrophoresed on 1.4% agarose gel and detected by staining with ethidium bromide. Each lane shows DNA samples from 22 individuals(44 individuals are specimen numbers for duplicate experiments). M, 100 bp Ladder DNA marker.

Callejas & Ochando(1998) 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*). Mamuris *et al.*(1999) reported that the number of scored bands varied from 7 to 12 per primer in four species of the Mullidae family. 176 common

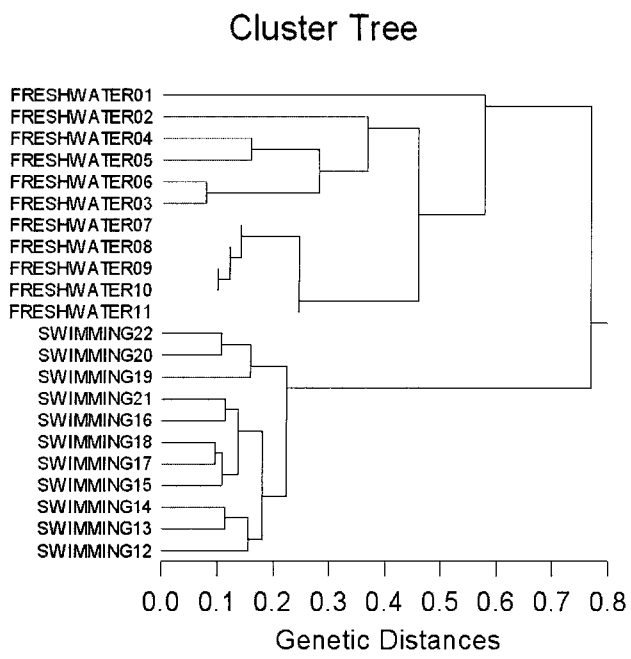

Fig. 2. Hierarchical dendrogram of genetic distances obtained from two geographical species of freshwater crab(No. 01~11) and swimming crab(No. 12~22). The relatedness between different individuals in the two crab species was generated according to the bandsharing values and similarity matrix(see Table 2).

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 swimming crab species, identical banding patterns, corresponding to fragments of 250bp and 300bp, were generated by the decamer primer OPB-17, as shown in Fig. 1F. The banding patterns generated by the decamer primers OPA-16 of individual swimming crab varied widely, as shown in Fig. 1C. The complexity of the banding patterns varied widely between primers and/or geographic locales. 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 & McClelland, 1990; Welsh *et al.*, 1991). Generally speaking, using a variety of arbitrary primers, RAPD-PCR has been applied to identify polymorphic/specific markers particular to breed, line, species and geographical population, as well as genetic diversity/polymorphism/similarity in diverse species of organisms(Partis & Wells, 1996; Callejas & Ochando, 1998; Huang *et al.*, 2000; McCormack *et al.*, 2000; Klinbunga *et al.*, 2000a; Yoon & Kim, 2004a).

Here, 505 fragments were identified in the freshwater crab species, and 513 in the swimming crab from Buan: 81 specific fragments(16.0%) in the freshwater crab species and 100(19.5%) in the swimming crab(Table 1). 165 identical fragments, with an average of 23.6 per primer, were observed in the freshwater crab species. 66 fragments, with an average of 9.4 per primer, were identified in the swimming crab species. In the present study, 7 primers generated 50 polymorphic fragments(50/505 fragment, 9.90%) in the freshwater crab species, and 14(14/513 fragments, 2.73%) in the swimming crab, as illustrated in Table 1. These results demonstrate that the primers detected a good deal of polymorphic 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. Huang *et al.* (2000) reported that six primers produced 84 polymorphic bands, out of a total of 90 bands in the blacklip abalone.

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 & Wells, 1996). These major fragments revealed the characteristic profiles of fish species such as the john dory, silver dory and mirror dory. 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 multiple catfish populations, as described in catfish. 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). It has been reported that the sum of the average polymorphic

products, 73.7, was identified in the combination of the common carp and the Israeli carp(Yoon, 2001). 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 & Park, 2002). Yoon & Kim(2004b) illustrated that six primers generated 47 polymorphic fragments(24% of 195 fragments) in a bullhead population. 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).

With respect to other molecular methods, 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 insisted 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.

Here, we have identified 200bp RAPD-PCR-amplified specific fragment(lane 11) and 1,100bp specific fragments (lanes 3, 4, 9, 10 and 11) in the freshwater crab species, generated by the decamer primer OPD-10. 19 specific fragments generated by the decamer primer OPD-10 also exhibited inter-individual-specific characteristics and DNA polymorphisms, as shown in Fig. 1G. The 400bp bands produced by the primer OPA-13 were identified as being common to two populations, which were identifying populations and/or species, as shown in Fig. 1B. It has been reported that the specific primer was useful in the identification of individuals and/or populations, resulting from variations in DNA polymorphisms among individuals/populations(Liu *et al.*, 1998 ; Yoon & Park, 2002 ; Yoon & Kim, 2003a ; Yoon & Kim, 2004b). Generally, it has been reported that polymorphic fragments generated by RAPD-PCR were suitable for the detection of genetic diversity/polymorphisms/similarity among a large number of organisms(Welsh *et al.*, 1991; Liu *et al.*, 1998; McCormack *et al.*, 2000; Yoon & Park, 2002; Kim *et al.*, 2004).

2. The Variation within and between Populations, and Genetic Distances

In the present study, the oligonucleotide decamer primer OPA-13 generated identical DNA fragments, approximately 400bp, in the freshwater crab and swimming crab species(Fig. 1B). The decamer primer OPB-17 also generated identical DNA fragments, approximately 300bp, in both the freshwater crab and swimming crab species(Fig. 1F).

In this study, the bandsharing value, which is based on the presence or absence of amplified fragments, was utilized to calculate similarity indices, as illustrated in Table 2. The similarity matrix, which was based on the average bandsharing values obtained from all samples, ranged from 0.363 to 0.748 in the freshwater crab species, and ranged 0.294~0.706 in the swimming crab. The average bandsharing value within the freshwater crab species was found to be 0.587±0.012, and, in the swimming crab, was found to be 0.508±0.016. The bandsharing value between the two crab species ranged from 0.141 to 0.390, with an average of 0.274±0.004. The bandsharing value between individuals no. 09 and no. 10 was 0.748, which was the highest value identified within the freshwater crab species. The value between no. 16 and no. 22 was 0.294, which was the lowest within the swimming crab species. The bandsharing value between individuals no. 08 and no. 13 was 0.390, which was the highest between the two crab species. The value between individuals no. 09 and no. 21 was 0.141, which was the lowest between the two crab species. Regarding individual results, individuals within freshwater crab exhibited higher bandsharing values than did fishes within swimming crab.

Our reported bandsharing values between the two crab species are inconsistent with the previously reported results (Callejas & Ochando, 1998; Yoon & Park, 2002). To illustrate, reports have shown that the average bandsharing value obtained using five random primers was 0.40±0.05 in the wild crucian carp population, 0.69±0.08 in the cultured crucian carp population(Yoon & Park, 2002), and 0.282±

0.008 between the two geographical oyster populations (Kim *et al.*, 2004). The average bandsharing value illustrated by our study is also lower than the value reported for Spanish barbel species(0.71~0.81)(Callejas & Ochando, 1998). However, the average bandsharing value illustrated in our study is similar to that obtained between the common carp and Israeli carp species(0.57±0.03)(Yoon, 2001), and the bullhead population(0.504±0.115)(Yoon & Kim, 2004b).

The average genetic difference was 0.413±0.012 within the freshwater crab, and 0.492±0.016 within the swimming crab species. Comparatively speaking, the average genetic difference was higher in the swimming crab than in the freshwater crab species. This suggests that the genetic variation in the swimming crab species is higher than in the freshwater crab. The average genetic difference was 0.726 ±0.004 between the freshwater crab and swimming crab species. It has been reported that the average genetic difference level between the two shrimp(*Penaeus chinensis*) populations was approximately 0.327±0.072(Yoon & Kim, 2003b). Kim *et al.*(2004) illustrated that the average genetic difference was approximately 0.710±0.009 between the two oyster(*Crassostrea gigas*) populations.

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 seven reliable primers, indicates four genetic clusters: cluster 1(FRESHWATER 01), cluster 2(FRESHWATER 02, 03, 04, 05 and 06), cluster 3(FRESHWATER 07, 08, 09, 10 and 11), and cluster 4(SWIMMING 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22). The shortest genetic distance displaying significant molecular difference was between individuals SWIMMING no. 18 and SWIMMING no. 17 from swimming crab(0.096). On the other hand, the longest genetic distance displaying significant molecular differences was found to exist between individuals in the two crab species, between individuals no. 03 of freshwater crab and no. 14 of swimming crab(0.507). Finally, individual no. 02 of the freshwater crab was most distantly related to freshwater crab no. 03(genetic distance=0.770). Our cluster analysis revealed a pattern similar to the one posited by Yoon & Kim(2004b). They reported that single linkage cluster analysis, which indicated four genetic groupings, and a dendrogram revealed close relationships between individual identities within two geographical populations.

In invertebrates, by cluster analysis of the pairwise population matrix, generated from RAPD data, Huang *et al.*(2000) illustrated that geographically close populations tended to cluster together in the blacklip abalone. Additional principal component analysis, again based on RAPD data, showed that the Point Cook population was clearly separated from the two other central populations(Huang *et al.*, 2000). A neighbor-joining tree based on the genetic distances between populations, using the RAPD-PCR method, indicates the 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. Kim *et al.*(2000) reported that phylogenetic relationships among 5 abalone(*Haliotis* spp.) species and one hybrid were conducted by calculation of the distance coefficient and construction of a phylogenetic tree based on RAPD data. Decisively, 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(*Crassostrea gigas*) 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.

In teleosts, with regard to cluster analysis of genetic similarity values obtained from RAPD data, Callejas & Ochando(1998) indicated that Spanish barbel species(*Barbus bocagei* and *B. graellsii*) were more closely related to each other than to *B. sclateri*. Nei's genetic distances varied from 0.327 to 0.655 in four species of the Mullidae family (Mamuris *et al.*, 1999). 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.

By phylogenetic tree resulting from neighbor joining and parsimony analyses, Mamuris *et al.*(1999) reported that the two phylogenetic trees showed the same topology in distinguishing Mullidae species. Bernardi & Talley(2000) illustrated that phylogenetic relationships were assessed using the neighbor-joining and maximum parsimony methods in killifish, *Fundulus parvipinnis*. At a higher level, samples were partitioned into two major clades. These two clades were very robust(99 to 100% of bootstrap replicates) and genetically distant(average sequence divergence 5.8%).

According to microsatellite analysis, Supungul *et al.* (2000) insisted that genetic variations and differentiation within Thai *Penaeus monodon* from five geographic locations were investigated using five microsatellite loci. The greatest genetic distance was observed to exist between Trad and Satun populations(genetic distance=0.030), whereas the shortest distance was between Satun and Trang populations(genetic distance=0.024). A neighbor-joining tree showed a close clustering of Satun, Trang, and Phangnga at the Andaman Sea, indicating the closeness of their genetic relations, whereas the populations of Chumphon, in the South, and Trad, on the coast of the Gulf of Thailand, should be regarded as different populations. Additionally, Lee & Kim(2003) illustrated that the genetic variation and molecular discrimination of Korean arkshell *Scapharca* species were investigated via mitochondrial COI gene sequences and PCR-RFLP analyses and two distinct fragments were produced in *S. broughtonii* and *S. subcrenatta*. It was supposed that this difference in phylogenetic grouping might result from differences in the analyzed regions of the COI genes and two species were clearly distinguished, especially by their morphological characters. Cagigas *et al.*(1999) reported that genetic variation within samples was also found to be significantly higher by microsatellite and RAPD analysis, than by enzyme loci within and among four natural Spanish populations of brown trout(*Salmo trutta*).

The identification of the black tiger shrimp(*Penaeus monodon*), penaeid shrimp(*Penaeus chinensis*), shortnecked clam(*Ruditapes philippinarum*), bullhead(*Pseudobagrus fulvidraco*), and oyster(*Crassostrea gigas*) populations was a necessary step in the inception and development of invertebrate/shellfish/finfish breeding programs(Tassanakajon *et al.*, 1998 ; Yoon & Kim, 2003a and b ; Yoon & Kim, 2004b; Kim *et al.*, 2004). Waldbieser & Wolters(1999) illustrated that molecular genetic markers, including, most notably, microsatellite loci, quantitative trait loci, qualitative trait loci, and genomic mapping will be useful in the selection of broodstock for multiple reproductive traits or hygiene- and production-related traits, in fishery science and aquaculture industry.

As stated above, the potential of RAPD to identify diagnostic markers for breed, stock, species and population identification in teleosts(Mamuris *et al.*, 1999; Iyengar *et al.*, 2000; Yoon & Kim, 2001; Yoon & Park, 2002), and in shellfish(Tassanakajon *et al.*, 1998; Klinbunga *et al.*, 2000b; McCormack *et al.*, 2000; Yoon & Kim, 2003a; Kim *et al.*, 2004) has also been demonstrated.

In our study, RAPD-PCR analysis has revealed a significant genetic distance between two species pairs. RAPD-PCR enabled us to detect the existence of species identification and genetic variation in the crab species. This confirms that the method is a suitable tool for DNA comparisons, both within and between individuals, species, and populations. Fundamentally, the classification of crab species is based on morphological variations in body size, body type, body color, claw type, claw size, carapace size, and carapace type. It is assumed by Chenyambuga *et al.*(2004) that differences in such traits reflect distinct origins or genetic identity. Furthermore, basic knowledge of the DNA polymorphisms and molecular markers in two crab species may contribute significantly to broodstock selection and selective invertebrate-breeding programs. The extraordinarily unique gene pools displayed by some samples(especially in case of the photo in Fig. 1F) would require new conservation policies, such that much more wild Korean crab species 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 family Grapsidae and the family Portunidae. Further sampling sites will also be necessary to more precisely determine the area in which the phylogeographic break occurs.

Especially, further assessments of other research methods, such as microsatellite, AFLP, FISH, GISH, NMR, single strand conformational polymorphism(SSCP), short tandem repeats(STR), variable number of tandem repeats(VNTR), expressed sequence tags(EST), heteroduplex mobility assay, single nucleotide polymorphism(SNP), and 2 or more different PCR-founded molecular methods may be required for further investigations. Additional statistical analytical techniques, such as AMOVA, Wright's *F*-statistics, Hardy-Weinberg equilibrium, bootstrapping, and principal components based on RAPD-PCR data will also be necessary for the possession of more profound and combined assessments of genetic traits and relationships among multiple species. In the future, we need to develop multiple single nucleotide polymorphism, bandmapping and genetic linkage maps for portunid crabs based on morphological characters, RAPD, AFLP, microsatellite, VNTR, EST and SNP marker data.

ACKNOWLEDGEMENTS

Particular thanks go to our laboratory colleagues, for their assistance in sample collection, their help with the PCR techniques, and their collaboration with the statistical analysis. The authors also would like to thank the reviewers who assisted us with thorough and far-reaching criticisms.

REFERENCES

Barki Y, Douek J, Graur D, Gateño D, Rinkevich B (2001) Polymorphism in soft coral larvae revealed by amplified fragment-length polymorphism(AFLP) markers. Mar Biol 136:37-41.

Bernardi G, Talley D(2000) Genetic evidence for limited dispersal in the coastal California killifish, *Fundulus parvipinnis*. J Exper Mar Biol Ecol 255:187-199.

Cagigas ME, Vazquez E, Blanco G, Sánchez JA(1999) Combined assessment of genetic variability in populations of brown trout(*Salmo trutta* L.) based on allozymes, microsatellites, and RAPD markers. Mar Biotech 1:286-296.

Calderini O, Pupilli F, Paolocci F, Arcioni S(1997) A repetitive and species-specific sequence as a tool for detecting the genome contribution in somatic hybrids of the genus *Medicago*. Theor Appl Genet 95:734-740.

Callejas C, Ochando MD(1998) Identification of Spanish barbel species using the RAPD technique. J Fish Biol 53:208-215.

Chenyambuga SW, Hanotte O, Hirbo J, Watts PC, Kemp SJ, Kifaro GC, Gwakisa PS, Petersen PH, Rege JEO (2004) Genetic characterization of indigenous goats of sub-Saharan Africa using microsatellite DNA markers. Asian-Aust J Anim Sci 17:445-452.

Friesen N, Fritsch R, Bachmann K (1997) Hybrid origin of some ornamentals of *Allium* subgenus *melanocrommyum* verified with GISH and RAPD. Theor Appl Genet 95:1229-1238.

Huang BX, Peakall R, Hanna PJ (2000) Analysis of genetic structure of blacklip abalone(*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. Mar Biol 136:207-216.

Iyengar A, Piyapattanakorn S, Dtone DM, Heipel DA, Howell BR, Baynes SM, Maclean N (2000) Identification of microsatellite repeats in turbot(*Scophthalmus maximus*) and dover sole(*Solea solea*) using a RAPD-based technique: Characterization of microsatellite markers in dover sole. Mar Biotech 2:49-56.

Jeffreys AJ, Morton DB (1987) DNA fingerprints of dogs and cats. Anim Genet 18:1-15.

Kim JY, Park CY, Yoon JM (2004) Genetic differences and DNA polymorphism in oyster(*Crassostrea* spp.) analysed by RAPD-PCR. Korean J Genet 26:123-134.

Kim SK., Jung YH, Han SH, Oh YS, Ko MH, Oh MY (2000) Phylogenetic relationship among *Haliotis* spp. distributed in Korea by the RAPD analysis. Korean J Genet 22:43-49.

Klinbunga S, Ampayup P, Tassanakajon A, Jarayabhand P, Yoosukh W (2000a) Development of species-specific markers of the tropical oyster(*Crassostrea belcheri*) in Thailand. Mar Biotech 2:476-484.

Klinbunga S, Boonyapakdee A, Pratoomchat B (2000b) Genetic diversity and species-diagnostic markers of mud crabs(Genus *Scylla*) in Eastern Thailand determined by

RAPD analysis. Mar Biotech 2:180-187.

Koh MC, Lim CH, Chua SB, Chew ST, Phang ST (1998) Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci 48:275-285.

Lee SY, Kim SH (2003) Genetic variation and discrimination of Korean arkshell *Scapharca* species(Bivalvia, Arcoida) based on mitochondrial COI gene sequences and PCR-RFLP. Korean J Genet 25:309-315.

Liu Z, Li P, Argue BJ, Dunham RA (1998) Inheritance of RAPD markers in channel catfish(*Ictalurus punctatus*), blue catfish(*I. furcatus*) and their F_1, F_2 and backcross hybrids. Anim Genet 29:58-62.

Maltagliati F (1998) A preliminary investigation of allozyme genetic variation and population geographical structure in *Aphanius fasciatus* from Italian brackish-water habitats. J Fish Biol 52:1130-1140.

Mamuris Z, Stamatis C, Bani M, Triantaphyllidis C (1999) Taxonomic relationships between four species of the Mullidae family revealed by three genetic methods: allozymes, random amplified polymorphic DNA and mitochondrial DNA. J Fish Biol 55:572-587.

McCormack GC, Powell R, Keegan B (2000) Comparative analysis of two populations of the brittle star *Amphiura filiformis*(Echinodermata: Ophiuroidae) with different life history strategies using RAPD markers. Mar Biotech 2:100-106.

Muchmore ME, Moy GW, Swanson WJ, Vacquier VD (1998) Direct sequencing of genomic DNA for characterization of a satellite DNA in five species of Eastern Pacific abalone. Mol Mar Biol Biotech 7:1-6.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nozaki T, Mishiba K, Mii M, Koba T (2000) Construction of synteny groups od *Brassica alboglabra* by RAPD markers and detection of chromosome aberrations and distorted transmission under the genetic background of *B. campestris*. Theor Appl Genet 101:538-546.

Partis L, Wells RJ (1996) Identification of fish species using random amplified polymorphic DNA (RAPD). Mol Cell Probes 10:435-441.

Ryu BS (1970) Studies on the biomass of a crab, *Eriocheir sinensis* and environmental factors in its habitat. J Korean Limnol 3:1-9.

Ryu BS, Kim CR (1969) Ecological studies on the planktonic larvae of some *Eriocheir sinensis* found in Man Kyeong River. J Korean Limnol 2:23-28.

Supungul P, Sootanam P, Klinbunga S, Kamonrat W, Jarayabhand P, Tassanakajon A (2000) Microsatellite polymorphism and the population structure of the black tiger shrimp(*Penaeus monodon*) in Thailand. Mar Biotech 2: 339-347.

Tassanakajon A, Pongsomboon S, Jarayabhand P, Klinbunga S, Boonsaeng V (1998) Genetic structure in wild populations of black tiger shrimp(*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. J Mar Biotech 6:249-254.

Waldbieser GC, Wolters WR (1999) Application of polymorphic microsatellite loci in a channel catfish *Ictalurus punctatus* breeding program. J World Aqua Soc 30:256-262.

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213-7218.

Welsh J, Petersen C, McClelland M (1991) Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res 19:303-306.

Yoon JM (2001) Genetic similarity and difference between common carp and Israeli carp(*Cyprinus carpio*) based on random amplified polymorphic DNAs analyses. Korean J Biol Sci 5:333-339.

Yoon JM, Kim GW (2001) Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish *Silurus asotus*. J Biosci 26:641-647.

Yoon JM, Kim GW (2003b) Genetic differences between cultured and wild penaeid shrimp(*Penaeus chinensis*) populations analysed by RAPD-PCR. Korean J Genet 25:21-32.

Yoon JM, Kim JY (2004b) Genetic differences within and between populations of Korean catfish(*S. asotus*) and bullhead(*P. fulvidraco*) analysed by RAPD-PCR. Asian-

Aust J Anim Sci 17:1053-1061.

Yoon JM, Kim YH (2003a) Wide marsh clam(*Corbicula* spp.) populations from three sites analysed by RAPD-PCR-AGE. Bull Electrochem 19:337-348.

Yoon JM, Kim YH (2004a) 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. J Aqua 17:12-23.

Yoon JM, Park HY (2002) Genetic similarity and variation in the cultured and wild crucian carp(*Carassius carassius*) estimated with random amplified polymorphic DNA. Asian-Aust J Anim Sci 15:470-476.