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Geographic Variation in Pond Smelt (*Hypomesus nipponensis*) by RAPD Analysis

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Genomic DNA isolated from two geographical populations of pond-smelt (*Hypomesus nipponensis*) was amplified for RAPD (randomly amplified polymorphic DNA) analysis. The populations were obtained from Chungju (CJ), in the inland area, and Dangjin (DJ), in the vicinity of the West Sea in Korea. Seven arbitrarily selected primers, OPB-06, OPB-10, OPB-13, OPB-17, OPC-09, OPC-17 and OPC-20, were used to generate the shared loci, polymorphic, and specific loci. Three hundred and eighty-three loci observed per primer were identified in the CJ population, and 287 were identified in the DJ population. Among them, 91 polymorphic loci or 23.8% were polymorphic in the CJ population, and 47 (16.4%) in the DJ population. The number of shared loci observed was 198 in the CJ population and 176 in the DJ population. Forty-four and 75 specific loci were detected in the CJ and DJ populations, respectively. Especially, 99 numbers of shared loci by the two populations, with an average of 14.1 per primer, were observed in the two pond-smelt populations. The average bandsharing value between the two geographical pond-smelt populations was 0.700±0.008, ranging from 0.600 to 0.846. Compared separately, the bandsharing value of individuals within the CJ population was higher than that of the DJ population. The dendrogram obtained using the data from the seven primers indicated three genetic clusters: cluster 1, CJ 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, and 11; cluster 2, DJ 01, 02, 03, 04, 05, 06, 07, 08, and 09; and cluster 3, DJ 10 and 11. The genetic distance between the two geographical populations ranged from 0.040 to 0.545. Thus, RAPD-PCR analysis revealed a significant genetic distance between the two pond-smelt populations.

Key words : Genetic cluster, genetic variation, geographical population, *Hypomesus nipponensis*, pond-smelt, RAPD analysis

Introduction

Genetic markers have been employed in the identification and discrimination of individuals, species and populations, hybrid parentages and genetic diagnostics (Smith *et al*., 1997; Callejas and Ochando, 1998; Huang *et al*., 2000; Yoon and Kim, 2001; Yoon and Kim, 2004; Park and Yoon, 2005). The RAPD method was used to generate fingerprint patterns for 10 meat species: wild horse, buffalo, beef, venison, boar, pig, dog, cat, kangaroo and rabbit (Koh *et al*., 1998). Genetic variation revealed by microsatellites and RAPD methods is significantly higher than that by analysis of enzyme loci within and among four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al*., 1999). Even though the reproducibility of RAPD is somewhat poor and depends upon PCR conditions, polymorphic bands generated by RAPD-PCR using arbitrary primers have been considered as an acceptable

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method for the detection of DNA similarity and/or diversity between organisms (Jeffreys and Morton, 1987; Liu *et al*., 1998; McCormack *et al*., 2000; Kim *et al*., 2004; Park and Yoon, 2005). Many molecular/genetic studies have employed this technique, as RAPD-PCR is an easy, and relatively speedy method for the investigation of numerous genomic DNAs. The major advantage of this method is that it requires no prior knowledge of the genome (Welsh and McClelland, 1990; Welsh *et al*., 1991; Koh *et al*., 1998; Iyengar *et al*., 2000).

The pond-smelt (*Hypomesus nipponensis* McAllister) (Kim *et al*., 2005), which belongs to the family Osmeridae, is an economically important aquacultural species. In the natural ecosystem, pond-smelt is distributed widely throughout the entirety of lakes, marshes, rivers, brackish-water habitats and seawater areas of the Korean Peninsula, as well as in several regions of China, Japan, Russia, and the Americas. This species of fish was successfully introduced into many freshwater areas in Southern Korea from the Yongheung River in Hamgyeongnam-do in 1925, and then spread widely again in the 1980's. Pond-smelt is ranked the highest among freshwater fishes in Korea as a game fish, and attracts millions of anglers during the winter. Pond-smelt is also one of the most liked freshwater fish species available during the Korean winter. Accordingly, pond-smelt has become a popular fish in a variety of restaurants (including restaurants specializing in sliced raw fish, or "hoejip") over the last two decades. The consumption of this species has also seen a considerable upshift as restaurants have begun to specialize in various pond-smelt recipes. The Korean name for this fish, regardless of its preparation, is "jjim".

The growth rate of pond-smelt depends on water temperature. Water temperature of 5～12°C is considered optimal. The fish are silvery white in the lateral and abdominal regions under natural conditions. The tips of the dorsal and caudal fins are brownish-yellow or yellowish-white. They also possess median dorsal fins, and a small adipose fin. In general, the color, size, and type of fish from this species vary according to habitat, water depth, nutrition, and other environmental factors. The environmental requirements and tolerances of pond-smelt from different geographic areas remain unknown, as does its population structure. As the pond-smelt culture industry grows, so does interest in the genetics of this species. However, little information exists regarding the population genetics of Korean pond-smelt.

In this study, we analyzed genetic variations within and between two pond-smelt populations from Chungju and Dangjin regions of Korea using RAPD.

Materials and Methods

Sample collection and extraction of genomic DNA

Two geographical populations of pond-smelt (*H. nipponensis*) were obtained from two different regions in Korea: Chungju, an inland area, and Dangjin, in the vicinity of the West Sea. Pond-smelt muscle was collected in sterile tubes, immediately placed on ice, and stored at −40°C until needed. RAPD-PCR analysis was performed on 22 individuals (11 per population). The extraction/purification of genomic DNA was performed under the conditions described previously (Yoon and Kim, 2003b). After several washings, lysis buffer I (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA) was added to the samples. The precipitates obtained were then centrifuged and resuspended in lysis buffer II [10 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 0.5% SDS], and 15 μL of proteinase K solution (10 mg/mL) was added. Six hundred μL of chloroform was then added to the mixture and mixed (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. DNA pellets were then incubation-dried for more than 10 hours, maintained at −40°C, and dissolved in the ultra-pure water produced by a water purification system (JABA KOREA, Korea) before analysis. The concentration of the extracted genomic DNA was measured by its absorbance ratio at 260 nm, with a spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

Decamer primers, molecular markers and amplification stipulations

The arbitrarily chosen primers were purchased from Operon Technologies, Alameda, CA, USA. The G+C content of the primers was between 60～70%. Among the 20 selected primers, seven primers, OPB-06 (5′-TGCTCTGCCC-3′), OPB-10 (5′-CTGCTGGGAC-3′), OPB-13 (5′-TTCCCCCG-

CT-3′), OPB-17 (5′-AGGGAACGAG-3′), OPC-09 (5′-CTCACCGTCC-3′), OPC-17 (5′-TTCCCCCC-AG-3′) and OPC-20 (5′-ACTTCGCCAC-3′) were shown to generate the shared loci, specific and polymorphic loci which could be clearly scored. We used these seven primers to determine the genetic variations in 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 volume, which contained 10 ng of template DNA, 20 μL of premix (Super-Bio Co., Korea), and 1 unit of primer. Amplification products were separated 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 ϕX174 DNA, digested with *Hae*III (Bioneer Corp., Daejeon, Korea), was utilized as a DNA molecular weight marker. Bands were detected with 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).

Data analysis

Primers that generated minor bands were excluded from our analyses. Only readily visible bands between 72 bp and 1,400 bp in size, were scored for statistical analysis. Bandsharing (BS) values were calculated according to the presence or absence of amplified band at specific positions in the same gel of RAPD band profiles. The values were calculated according to the protocols developed by 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 within-population similarity was calculated by pairwise comparison between individuals within a population. The levels of relatedness among different individuals of the CJ population (lane 01～11) and DJ population (lane 12～22) were 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 Systat version 10 software (SPSS Inc., Chicago, IL, USA). The Systat software was also used to calculate genetic differences, Euclidean genetic distances within and between populations, means, standard errors, and *t*-test scores.

Results

Variations within and between populations, and genetic distances

Out of 20 decamer primers, seven arbitrarily selected primers OPB-06, OPB-10, OPB-13, OPB -17, OPC-09, OPC-17 and OPC-20 were found to generate the shared, specific, and polymorphic loci (Table 1-2). The complexity of the banding patterns varied dramatically among primers and between the two locations (Fig. 1). The size of the DNA loci also varied greatly, from 72 bp to 1,400 bp (Fig. 1).

Here, 383 loci were identified in the CJ population, and 287 in the DJ population: 91 polymorphic loci (23.8%) in the CJ population, and 47 (16.4%) in the DJ population (Table 1). One hundred and ninety-eight shared loci, with an average of 28.3 per primer, were observed in the CJ population. 176-shared loci, with an average of 25.1 per primer, were identified in the DJ population. The numbers of specific loci in the CJ and DJ population were 44 and 75, respectively. The decamer primer, OPB-06, generated the shared loci, of approximately 190 and 230 bp, respectively, in both the CJ and DJ population (Fig. 1A). The oligonucleotide decamer primer, OPB-17, also generated the shared loci, of approximately 280 bp each, in the CJ and DJ population (Fig. 1D).

The number of unique loci to each population and number of shared loci by the two populations generated by RAPD-PCR using 7 random primers in DJ population (Table 2). 242 numbers of unique loci to each population, with an average of 15.7 per primer, were observed in the CJ population. 77 unique loci, with an average of 11.0 per primer, were identified in the DJ population. Especially, 99 numbers of shared loci by the two populations, with an average of 14.1 per primer, were observed in the two pond-smelt populations. The decamer primer OPB-10 generated the shared loci by the two populations, approximately 150 bp and 600 bp, respectively, in both

Table 1. The number of loci observed, number of shared loci by each population, number of specific loci and number of polymorphic loci generated by RAPD-PCR using 7 random primers in pond-smelt (*H. nipponensis*) from Chungju and Dangjin of Korea

Item	No. of loci observed per primer		No. of shared loci by each population		No. of specific loci		No. of polymorphic loci	
Primer	Chungju	Dangjin	Chungju	Dangjin	Chungju	Dangjin	Chungju	Dangjin
OPB-06	37 (3.4)	22 (2.0)	22	22	1	1	2	0
OPB-10	86 (7.8)	48 (4.4)	55	44	3	2	18	11
OPB-13	34 (3.1)	18 (1.6)	33	11	0	0	1	6
OPB-17	59 (5.4)	19 (1.7)	22	11	8	10	18	0
OPC-09	55 (5.0)	38 (3.5)	33	11	1	27	17	0
OPC-17	38 (3.5)	27 (2.5)	11	0	12	20	17	7
OPC-20	74 (6.7)	115 (10.5)	22	77	19	15	18	23
Total no.	383 (34.8)	287 (26.1)	198	176	44	75	91	47
Average no. per primer	54.7	41.0	28.3	25.1	6.3	10.7	13.0	6.7

The average number of loci per lane generated by a primer in pond-smelt obtained Chungju and Dangjin is shown in parentheses.

Table 2. The number of unique loci to each population and number of shared loci by the two populations generated by RAPD-PCR using 7 random primers in pond-smelt (*H. nipponensis*) from Chungju and Dangjin of Korea

Item	No. of unique loci to each population		No. of shared loci by the two populations
Primer \ Population	Chungju	Dangjin	Two populations
OPB-06	0	0	22
OPB-10	33	22	22
OPB-13	22	0	11
OPB-17	11	0	11
OPC-09	22	0	11
OPC-17	11	0	0
OPC-20	11	55	22
Total no.	110	77	99
Average no. per primer	15.7	11.0	14.1

CJ and DJ population (Table 2) (Fig. 1B). The decamer primer OPC-20 also generated the shared loci by the two populations, approximately 400 bp and 500 bp, between the two populations (Fig. 1G).

Based on bandsharing values of all samples, the similarity matrix were derived with values ranged from 0.662 to 0.971 in the CJ population, and from 0.600 to 0.846 in the DJ population (Table 3). The average bandsharing value was 0.809 ± 0.010 within the CJ population, and 0.550 ± 0.006 within the DJ population. The average bandsharing value between the two geographical populations was 0.700 ± 0.008, ranging from 0.600 to 0.846. The bandsharing value between individuals no. 03 and no. 11 was 0.971, which was the highest value identified within the CJ population. The bandsharing value between individuals no. 08 and no. 09 was 0.662, which was the lowest observed. The bandsharing value between no. 15 and no. 20 was 0.846, which was the highest value observed within the DJ population. The value between individuals no. 12 and no. 14 was 0.600, which was the lowest measured. Therefore, the bandsharing value of individuals within the CJ population was much higher than in the DJ population. The bandsharing value between individuals no. 04 and no. 13 was 0.704, which was the highest measured between the two geographical populations. The value between individuals no. 02 and no. 17 was 0.318, which was the lowest seen between the two geographical populations.

The genetic difference derived from the bandsharing values varied from 0.029 to 0.338 in the CJ population, and from 0.154 to 0.400 in the DJ population (Table 3). The genetic difference varied from 0.296 to 0.682 in the CJ and DJ populations. The average genetic difference was 0.191 ± 0.010 within the CJ population, and 0.315 ± 0.008 within the DJ population. Compared separately, the average genetic difference was greater in the DJ population than in the CJ population. The average genetic difference was 0.462 ± 0.006 between the two geographical populations.

The dendrogram obtained hierarchical analysis revealed three clusters: cluster 1, CJ 01, 02, 03, 04, 05, 06, 07, 08, 09, 10, and 11; cluster 2, DJ

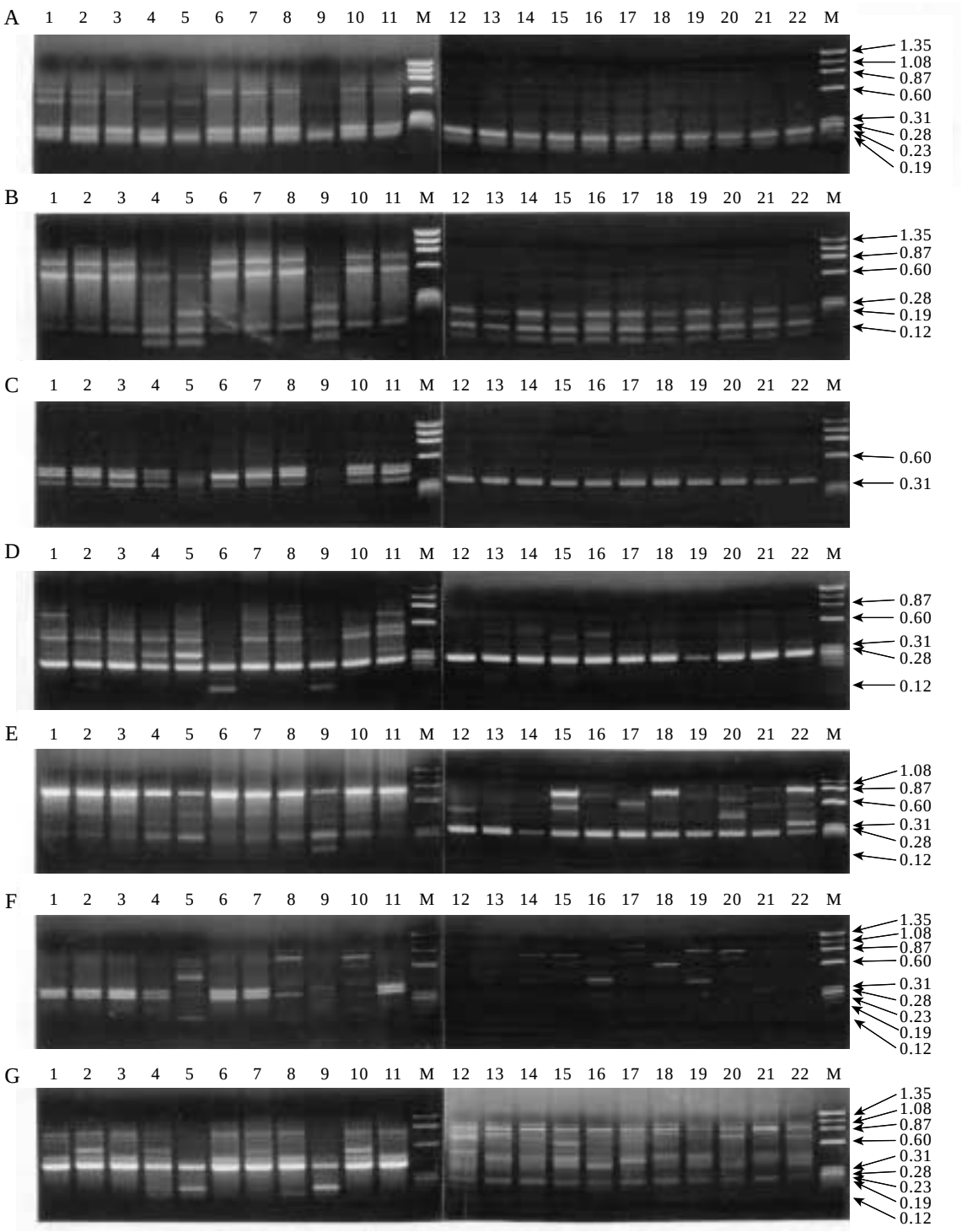

Fig. 1. RAPD-PCR-generated electrophoretic profiles of individual pond-smelt (*H. nipponensis*). DNA isolated from Chungju (lane 1-11) and Dangjin (lane 12-22) were amplified by random primers OPB-06 (A), OPB-10 (B), OPB-13 (C), OPB-17 (D), OPC-09 (E), OPC-17 (F) and OPC-20 (G). Amplified products were electrophoresed on 1.4% agarose gel and detected by staining with ethidium bromide. M, ϕX174 DNA marker digested with *Hae*III.

01, 02, 03, 04, 05, 06, 07, 08, and 09; and cluster 3, DJ 10 and 11 (Fig. 2). The genetic distance between the two geographical populations ranged from 0.040 to 0.545. The shortest genetic distan-

Table 3. Similarity matrix, including bandsharing values and genetic differences, calculated using Nei and Li's index, of the similarity of pond-smelt (*H. nipponensis*) from Chungju and Dangjin

		Genetic differences of pond-smelt from Chungju											Genetic differences of pond-smelt from Dangjin										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Bandsharing values of pond-smelt from Chungju	1	–	0.052	0.064	0.173	0.263	0.227	0.228	0.189	0.274	0.165	0.073	0.512	0.422	0.596	0.518	0.469	0.535	0.562	0.595	0.529	0.526	0.591
	2	0.948	–	0.054	0.142	0.256	0.190	0.087	0.176	0.257	0.134	0.062	0.392	0.367	0.568	0.451	0.460	0.682	0.476	0.574	0.518	0.489	0.399
	3	0.936	0.946	–	0.173	0.290	0.193	0.083	0.151	0.238	0.160	0.029	0.402	0.379	0.537	0.456	0.446	0.504	0.447	0.564	0.490	0.479	0.384
	4	0.827	0.858	0.827	–	0.159	0.270	0.156	0.235	0.234	0.198	0.149	0.342	0.296	0.507	0.459	0.382	0.471	0.397	0.518	0.489	0.405	0.327
	5	0.737	0.744	0.710	0.841	–	0.316	0.210	0.280	0.231	0.194	0.254	0.455	0.400	0.484	0.468	0.441	0.473	0.385	0.521	0.423	0.481	0.403
	6	0.773	0.810	0.807	0.730	0.684	–	0.190	0.318	0.224	0.269	0.168	0.411	0.365	0.517	0.357	0.409	0.463	0.416	0.446	0.435	0.418	0.403
	7	0.772	0.913	0.917	0.844	0.790	0.810	–	0.247	0.223	0.210	0.085	0.417	0.353	0.510	0.438	0.477	0.467	0.384	0.469	0.480	0.438	0.376
	8	0.811	0.824	0.849	0.765	0.720	0.682	0.753	–	0.338	0.144	0.173	0.487	0.467	0.531	0.455	0.384	0.447	0.440	0.491	0.447	0.534	0.545
	9	0.726	0.743	0.762	0.766	0.769	0.776	0.777	0.662	–	0.289	0.189	0.446	0.337	0.510	0.397	0.343	0.465	0.364	0.441	0.442	0.413	0.345
	10	0.835	0.866	0.840	0.802	0.806	0.731	0.790	0.856	0.711	–	0.150	0.469	0.400	0.438	0.401	0.404	0.381	0.413	0.485	0.429	0.517	0.458
	11	0.927	0.938	0.971	0.851	0.746	0.832	0.915	0.827	0.811	0.850	–	0.410	0.329	0.519	0.420	0.378	0.463	0.406	0.460	0.478	0.444	0.365
Bandsharing values of pond-smelt from Dangjin	12	0.488	0.608	0.598	0.658	0.545	0.589	0.583	0.513	0.544	0.531	0.590	–	0.393	0.400	0.302	0.306	0.304	0.298	0.269	0.342	0.214	0.211
	13	0.578	0.633	0.621	0.704	0.600	0.635	0.647	0.533	0.663	0.600	0.671	0.607	–	0.320	0.328	0.357	0.327	0.399	0.365	0.367	0.267	0.268
	14	0.404	0.432	0.463	0.493	0.516	0.483	0.490	0.469	0.490	0.562	0.481	0.600	0.680	–	0.291	0.364	0.323	0.349	0.212	0.251	0.336	0.341
	15	0.492	0.549	0.544	0.541	0.532	0.643	0.562	0.545	0.603	0.599	0.580	0.698	0.672	0.709	–	0.374	0.256	0.208	0.242	0.154	0.316	0.329
	16	0.531	0.540	0.554	0.618	0.559	0.591	0.523	0.616	0.657	0.596	0.622	0.694	0.643	0.636	0.626	–	0.346	0.345	0.227	0.220	0.312	0.350
	17	0.465	0.318	0.496	0.529	0.527	0.537	0.533	0.553	0.535	0.619	0.537	0.696	0.673	0.677	0.744	0.654	–	0.317	0.246	0.283	0.264	0.354
	18	0.438	0.524	0.553	0.603	0.615	0.584	0.616	0.560	0.636	0.587	0.594	0.702	0.601	0.651	0.792	0.655	0.683	–	0.301	0.336	0.319	0.353
	19	0.405	0.426	0.436	0.482	0.479	0.554	0.531	0.509	0.559	0.515	0.540	0.731	0.635	0.788	0.758	0.773	0.754	0.699	-	0.261	0.220	0.273
	20	0.471	0.482	0.510	0.511	0.577	0.565	0.520	0.553	0.558	0.571	0.522	0.658	0.633	0.749	0.846	0.780	0.717	0.664	0.739	–	0.281	0.309
	21	0.474	0.511	0.521	0.595	0.519	0.582	0.562	0.466	0.587	0.483	0.556	0.786	0.733	0.664	0.684	0.688	0.736	0.681	0.780	0.719	–	0.182
	22	0.509	0.601	0.616	0.673	0.597	0.597	0.624	0.455	0.655	0.542	0.635	0.789	0.732	0.659	0.671	0.650	0.646	0.647	0.727	0.691	0.818	–

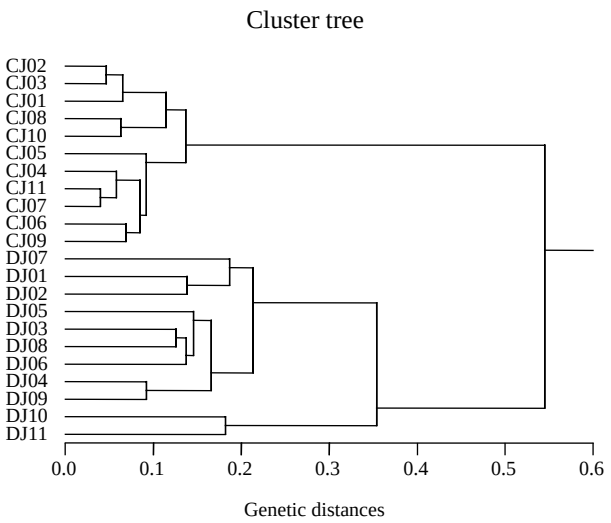

Fig. 2. Hierarchical dendrogram of genetic distances, obtained from two geographical populations of pond-smelt (*H. nipponensis*). The relatedness between different individuals in the pond-smelt populations of Chungju (CJ) and Dangjin (DJ) were generated according to the bandsharing values and similarity matrix (see Table 3).

ce representing a significant molecular difference was between individuals no. 11 and no. 07 from Chungju (0.040). The longest genetic distance was between individuals no. 04 and no. 09 in CJ population (0.137). On the other hand, individual no. 09 of the DJ population was most closely related to DJ no. 04 (genetic distance=0.092). Ultimately, the longest genetic distance was found to exist between individuals in the two populations, between individuals no. 05 of Chungju and no. 11 of Dangjin (0.545).

Discussion

In spite of variation in RAPD profiles and 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; Cagigas *et al*., 1999; Mamuris *et al*., 1999; Iyengar *et al*., 2000; Yoon and Kim, 2003a; Yoon and Kim, 2004; Kim *et al*., 2004).

In this study, on average, a decamer primer generated an average of 5.0 amplified loci per fish, ranging from 3.1 to 7.8 loci in this population. The number of bands 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). Primers generated 36, 32, and 24 bands in mud crabs from Eastern Thailand (genus *Scylla*) (Klinbunga *et al*., 2000). 176 common fragments, at an average of 25.1 per primer, were observed in the Buan population, and 99 fragments, at an average of 14.1 per primer, were observed in the Geojedo population (Kim *et al*., 2004). Moreover, in the DJ population, banding patterns of the shared loci by the two populations, corresponding to loci of 190 bp and 230 bp, were generated by the decamer primer, OPB-06, as shown in Fig. 1A. The banding patterns generated by the decamer primers OPC -09, OPC-17, and OPC-20 of the individual Dangjin pond-smelt varied widely, as shown in Figs. 1E, 1F, and 1G. The complexity of the banding patterns varied widely between primers and/or geographic locales. Generally, the size and number of loci 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 band (Welsh and McClelland, 1990; Welsh *et al*., 1991).

These results demonstrate that the primers detected a great deal of polymorphic loci. It has been reported that the silver dory (*Cyttus australis*) has a major 460-bp band, and that the mirror dory (*Zenopsis nebulosis*) has a major 422 -bp band (Partis and Wells, 1996). These major bands 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 bands 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 bands generated by six primers were calculated in a variety of catfish populations, as

previously described. It has also 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). 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 bands from 70 individuals. 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 patterns of polymorphic fragments of 50 individuals in the wild population were 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). Park and Yoon (2005) have also observed 148 RAPD-PCR-amplified specific bands and 76 polymorphic bands generated by eight decamer primers for Korean largehead hairtail (*Trichiurus lepturus*) and 61 specific bands and 27 polymorphic bands for largehead hairtail population from the Atlantic Ocean.

Here, we have identified two specific loci (lanes 18 and 20) of 500 bp in the DJ population, generated by the decamer primer OPB-10. This specific primer proved 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). In general, the polymorphic bands generated by RAPD-PCR using arbitrary primers were suitable for the detection of genetic similarity/diversity/polymorphisms among various organisms (Welsh *et al*., 1991; Liu *et al*., 1998; McCormack *et al*., 2000; Kim *et al*., 2004).

Individual pond-smelt from Dangjin exhibited higher bandsharing values than did fish from Chungju. Our reported bandsharing values between the two geographical populations are inconsistent with previously reported results (Yoon and Park, 2002). Other reports have indicated 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 of the two populations recorded in our study is higher than the average value between the common carp and Israeli carp species (0.57 ± 0.03) (Yoon, 2001), the bullhead population (0.504 ± 0.115) (Yoon and Kim, 2004), between the two oyster populations (0.282 ± 0.008) (Kim *et al*., 2004) and also between the two largehead hairtail populations (0.340) (Park and Yoon, 2005). However, the average bandsharing value between the two pond-smelt populations reported by our study is similar to the value reported for Spanish barbel species ($0.71 \sim 0.81$) (Callejas and Ochando, 1998).

The genetic difference observed here, 0.191 in the CJ population and was 0.315 in the DJ population, suggests that genetic variation in the DJ population is more pronounced than in the CJ population. The average genetic difference level between the two populations was approximately 0.462. The difference between the two populations is statistically significant ($P < 0.001$).

The dendrogram from hierarchical analysis indicates three clusters. In particular, the longest genetic distance representing significant molecular differences, 0.545, was found to exist between individuals no. 05 of Chungju and no. 11 of Dangjin. Our cluster analysis revealed a pattern similar to the one posited by Yoon and Kim (2004). They reported that single linkage cluster analysis, which indicated four genetic groupings,

and the dendrogram revealed close relationships between individual identities within two geographical populations.

In fish and invertebrates, 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 relationships of three mud crab species (Klinbunga *et al*., 2000). This study showed that large genetic differences could be found between geographical populations within a species, as well as between species. The two phylogenetic trees resulting from neighbor-joining and parsimony analyses both showed the same topology in distinguishing Mullidae species (Mamuris *et al*., 1999). The main advantage of the RAPD technique over the two other methods was its largely superior discriminative ability.

Genetic variation within samples was also found to be significantly higher by microsatellite and/or RAPD analysis, than by enzyme loci within and among four natural Spanish brown trout (*Salmo trutta*) populations (Cagigas *et al*., 1999). The identification of the penaeid shrimp (*Penaeus chinensis*), bullhead (*Pseudobagrus fulvidraco*), and oyster (*Crassostrea gigas*) populations constituted 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, microsatellite loci, quantitative trait loci, and genomic mapping, will ultimately prove useful in the selection of broodstocks for multiple reproductive traits, or health- and production-related traits, in fishery science (Waldbieser and Wolters, 1999). The classification of geographical populations of pond-smelt is based on morphological variations in head type, body size, body type, body color, fin type, and eye type. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga *et al*., 2004). 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; Mamuris *et al*., 1999; Iyengar *et al*., 2000; Yoon and Kim, 2004; Park and Yoon, 2005), and in shellfish (Tassanakajon *et al*., 1998; Klinbunga *et al*., 2000; McCormack *et al*., 2000; Yoon and Kim, 2003b; Kim *et al*., 2004) has also been well established.

In this study, RAPD-PCR analysis has revealed a significant genetic distance between two pond-smelt populations. The existence of population differentiation and DNA polymorphisms between two CJ and DJ populations were detected by RAPD-PCR. Compared the genetic distances of two geographic populations, the possibility of the flow and/or migration almost is thought with the fact that it is not. This confirms that the method is a suitable tool for DNA comparisons, both within and between individuals, and populations. Furthermore, the basic knowledge acquired regarding DNA polymorphisms and molecular markers in pond-smelt species may significantly contribute to broodstock selection and the selective fish-breeding program. This study is also timely for a growing pond-smelt aquaculture industry because knowledge of the genetic diversity of pond-smelt could help in formulating effective strategies for managing this aquacultural fish species. In future, increased sample sizes, increased sample sites, additional PCR-based techniques, such as microsatellites, genetic sequencing, annealing control primer (ACP) technology, gene fishing technology, and gene chip analysis may be required to advance studies in this research.

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RAPD 분석에 의한 빙어 (*Hypomesus nipponensis*)의 지리적 변이

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RAPD 분석을 하기 위해서 우리나라 내륙의 충주지역과 서해에 인접한 당진지역에서 빙어 (*Hypomesus nipponensis*)의 두 지리적 집단으로부터 genomic DNA를 분리 추출하였다. OPB-06, OPB-10, OPB-13, OPB-17, OPC-09, OPC-17 및 OPC-20의 7개 primer를 사용하여 shared loci, polymorphic 및 specific loci를 확인하였다. 충주 빙어집단에서는 한 primer 당 383개의 loci가 관찰되었고, 당진 집단에서는 287개의 loci가 확인되었다. 관찰된 loci 중에 23.8%에 해당되는 91개의 polymorphic loci가 충주 집단에서 확인되었고, 당진 집단에서는 47 (16.4%)개가 확인되었다. 각 집단에서 공유하는 loci의 수는 각각 충주 빙어집단에서 198개 그리고 당진 집단에서는 176개로 관찰되었다. 충주 빙어집단과 당진 집단에서는 각각 44개와 75개의 specific loci가 나타났다. 특히 두 집단이 공유하는 loci의 수는 99개로서 primer 당 평균 14.1개로 확인되었다. 두 빙어 집단의 bandsharing value의 평균값은 0.700±0.008로서 0.600에서 0.846의 범위를 나타내었다. 각각을 비교해 보면, 충주 집단에 속한 개체의 bandsharing value의 평균값이 당진 집단에서의 값보다 높게 나타났다. 7개의 primer를 사용하여 얻어진 dendrogram은 cluster 1 (CJ 01, 02, 03, 04, 05, 06, 07, 08, 09, 10 및 11), cluster 2 (DJ 01, 02, 03, 04, 05, 06, 07, 08 및 09) 및 cluster 3 (DJ 10 및 11)와 같이 3개의 유전적 클러스터로 나뉘어졌다. 두 집단의 유전적 거리는 0.040에서 0.545 사이로 나타났다. 따라서 RAPD-PCR 분석을 통해서 빙어 두 집단의 유의성이 있는 유전적 거리를 확인하였다.