Genetic Variation and Differences within and between Populations of Cultured and Wild Bullhead (*Pseudobagrus fulvidraco*) Revealed by RAPD-PCR

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ABSTRACT

We used nine decamer primers to generate DNA fragment sizes ranging from 100 bp to 1,600 bp from two bullhead (*Pseudobagrus fulvidraco*) populations of Dangjin in Korea. 376 fragments were identified in the cultured bullhead population, and 454 in the population of wild bullhead from Dangjin: 287 specific fragments (76.3%) in the cultured bullhead population and 207 (45.6%) in the wild bullhead population. On average, a decamer primer was used to generate 34.2 amplified products in a cultured bullhead. A RAPD primer was used to generate an average of 3.1 amplified bands per sample, ranging between 2.5 and 6.0 fragments in this population. Nine primers also generated 24 polymorphic fragments (24/376 fragment, 6.4%) in the cultured bullhead population, and 24 (24/454 fragments, 5.2%) in the wild bullhead population. The OPA-16 primer, notably, produced which 11 out of 11 bands (100%) were monomorphic in the wild bullhead population. 110 intra-population-specific fragments, with an average of 12.2 per primer, were observed in the cultured bullhead population. 99 fragments, with an average of 11.0 per primer, were identified in the wild bullhead. Especially, 55 inter-population-common fragments, with an average of 6.1 per primer, were observed in the two bullhead populations. The bandsharing value (BS value) of individuals within the wild bullhead population was substantially higher than was determined in the cultured bullhead population. The average bandsharing value was 0.596 ± 0.010 within the cultured bullhead population, and 0.657 ± 0.010 within the wild bullhead population. The dendrogram obtained with the nine primers indicates two genetic clusters, designated cluster 1 (CULTURED 01~CULTURED 11), and cluster 2 (WILD 12~WILD 22). Ultimately, the longest genetic distance displaying significant molecular differences was determined to exist between individuals in the two bullhead populations, namely between individuals WILD no. 19 of the wild bullhead population and CULTURED no. 03 of the cultured bullhead population (genetic distance = 0.714). RAPD-PCR allowed us to detect the existence of population discrimination and genetic variation in Korean population of bullhead. This finding indicates that this method constitutes a suitable tool for DNA comparison, both within and between individuals, populations, species, and genera.

(Key words : Bullhead, *Pseudobagrus fulvidraco*, Cultured, Wild, Bandsharing value, Genetic cluster, Genetic distance)

INTRODUCTION

Bullhead (*Pseudobagrus fulvidraco*), economically important aquacultural species, belonging to order Siluriformes including family Bagridae, respectively, widely inhabit the slow river and stream that flows to the West Sea and South Sea in the Korean Peninsula (Kim, 1997). Especially, bullhead is widely distributed in the river and stream in North Korea, Japan China and Siberia. Bullhead is one of the most intensively studied and reared fish species for real income enhancement of farmers because of its importance as a food and sport fish. For that reason, this species of bullhead was successfully spread into many freshwater areas in Southern Korea in 2000's. As the bullhead culture industry is increasing considerably, the understanding of the genetics of this fish species to evaluate exactly the potential genetic effects induced by bullhead production operations. However, little information is kno-

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wn about the genetics of bullhead in Korea. Particularly, the clustering analysis of the genetic distance between populations/species/genera of various invertebrates and fishes from the different geographic sites has been performed using RAPD-PCR is of small number (Cagigas *et al.*, 1999; Kim *et al.*, 2000; Klinbunga *et al.*, 2000; Yoon and Park, 2002). Additionally, the genetic variation, the species-specific markers and the region-specific markers in catfish and oyster have been assessed by molecular methods (Yoon and Kim, 2001; Kim *et al.*, 2004).

Many molecular/genetic researches have used this technique, because RAPD-PCR is a simple, reliable, easy, relatively cheaper and rapid method for the investigation of numerous genomic DNAs with respect to genetic diversity in a population (Liu *et al.*, 1998; McCormack *et al.*, 2000). The greatest merit of this technique is that it does not require prior knowledge of the genome sequence (Williams *et al.*, 1990; Welsh *et al.*, 1991). One of the disadvantages of RAPD-PCR technique is the influence of different factors in band formation, which will affect bandsharing values. The effect of gel on BS value points to the need of adjustments of gel factor to calculate of BS values (Suprabha *et al.*, 2005). 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; Gwakisa *et al.*, 1994; Liu *et al.*, 1998; McCormack *et al.*, 2000; Yoon and Kim, 2001). RAPD have also proven to be useful genetic markers because of their high levels of polymorphisms (Welsh and McClelland, 1990; Welsh *et al.*, 1991). As mentioned above, the potential of RAPD to identify diagnostic markers for breed, stock, species and population identification in teleosts (Partis and Wells, 1996; Yoon and Kim, 2001; Diaz-Jaimes and Uribe-Alcocer, 2003; Kim *et al.*, 2005a; Siti Azizah *et al.*, 2005), and in shellfish (Klinbunga *et al.*, 2000; Yoon and Kim, 2003b) has been demonstrated.

There is a need to understand the genetic characteristics and composition of two bullhead populations in order to evaluate exactly the latent genetic effects induced by seed production operations. Here, to elucidate the genetic distances and the differences within- and between-population, we performed the clustering analysis of two populations of Korean bullhead (*P. fulvidraco*) inhabiting in the Dangjin. We also analyzed the genetic variation and DNA polymorphism of these bullhead populations in Korea.

MATERIALS AND METHODS

Blood Collection and Sources of Genomic DNA

Blood samples were obtained from Korean bullhead (*P. fulvidraco*) from Dangjin of Korea. RAPD-PCR analysis was performed on DNA samples from a total of 22 individuals using nine of decamer primers of two decades of different random 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 (Shimadzu, Australia).

Decamer Primers, Molecular Markers and Amplification Conditions

The arbitrarily chosen primers were purchased from Operon Technologies, USA. The G + C content of the primers was between 60~70%. Nine selected primers; OPA-01 (5'-CAGGCCCTTC-3'), OPA-04 (5'-AATCGGGCTG-3'), OPA-06 (5'-GGTCCCTGAC-3'), OPA-09 (5'-GGGTAACGCC-3'), OPA-11 (5'-CAATCGCCGT-3'), OPA-13 (5'-CAGCACCCAC-3'), OPA-16 (5'-AGCCAGCGAA-3'), OPA-17 (5'-GACCGCTTGT-3') and OPA-18 (5'- AGGTGACCGT -3') 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 bullhead. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers (MJ Research, Inc., USA; Perkin Elmer Cetus, USA). 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) and ethidium bromide. The 100 bp DNA Ladder (Bioneer Co., Korea) was used as a DNA molecular weight marker. The banding patterns were visualized under a UV transilluminator, 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 100 bp and 1,600 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 population of cultured bullhead (*P. fulvidraco*) (CULTURED 01~CULTURED 11) and wild bullhead (WILD 12~WILD 22) was generated according to the bandsharing values and similarity matrix. Using similarity matrices to generate a dendrogram, facilitated by the PC-package program Systat version 10 (SPSS Inc., USA), produced the hierarchical clustering tree. Genetic differences and Euclidean genetic distances within- and between-population were also calculated using the hierarchical dendrogram program Systat version 10. Program Systat version 10 was used to obtain other statistical results such as mean, standard error and *t*-test.

RESULTS AND DISCUSSION

The Variation within and between Species

Despite variations in RAPD profiles and differences in reproducibility, RAPD and/or RAPD-based techniques have been extensively applied to the identification of the genetic characteristics of a variety of finfishes and shellfishes (Smith *et al.*, 1997; Tassanakajon *et al.*, 1998; Huang *et al.*, 2000; Yoon and Kim, 2004; Kim *et al.*, 2004). Polymorphisms can be determined according to the banding patterns of primer-amplified products at a specific set of positions (Williams *et al.*, 1990; Smith *et al.*, 1997; Yoon and Kim, 2003b; Suprabha *et al.*, 2005). In the present study, we used nine decamer primers to generate a total of 376 fragments in a population of cultured bullhead from Dangjin in Korea, and 454 in a population of wild bullhead, with DNA fragment sizes ranging from 100 bp to 1,600 bp, as is summarized in Tables 1 and 2. A host of researchers have studied the sizes of DNA fragments in the RAPD-PCR profiles of barramundi (*Lates calcarifer*) (Partis and Wells 1996), four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al.*, 1999), four species of the Mullidae family (Mamuris *et al.*, 1999), wild and cultured populations of crucian carp (Yoon and Park, 2002), and Korean catfish and bullhead (Yoon and Kim, 2004). The decamer primer OPF-10 produced 11 amplified fragments, with sizes from 200 to 600 bp in the eastern Pacific yellowfin tuna (*Thunnus albacares*) (Diaz-Jaimes and Uribe-Alcocer, 2003). Especially, six primers were used, generating a total of 602 scorable bands in the catfish population, and 195 in the bullhead population, respectively, with DNA fragment sizes ranging from less than approximately 100 bp, to over 2,000 bp (Yoon and Kim, 2004). In livestock, all the 141 primers generated ranging from 270 to 1,350 bp in zebu cattle breeds (Gwakisa *et al.*, 1994). Geng *et al.*, (2002) also reported that 8 random primers generated the fragments, 176 bp to 2,937 bp, in 3-goat populations from China Chaidamu Basin.

In the present study, on average, a decamer primer was used to generate 34.2 amplified products in a cultured bullhead, as is illustrated in Table 1. A RAPD primer was used to generate an average of 3.1 amplified bands per sample, ranging between 2.5 and 6.0 fragments in this population. 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 the number of scored bands varied from 7 to 12 per primer in four species of the Mullidae family (Mamuris *et al.*, 1999).

Table 1. Number of total, average, specific and polymorphic bands generated from RAPD patterns for 9 arbitrary primers in cultured and wild bullhead populations

Item	No. of average fragments per lane		No. of specific fragments		No. of polymorphic fragments	
Primer\ Population	Cultured bullhead	Wild bullhead	Cultured bullhead	Wild bullhead	Cultured bullhead	Wild bullhead
OPA-01	3.0 (33)	1.9 (21)	11	10	0	0
OPA-04	6.0 (66)	8.9 (98)	31	55	6	7
OPA-06	4.2 (46)	2.2 (24)	14	13	5	0
OPA-09	3.2 (35)	4.6 (51)	26	7	0	1
OPA-11	3.8 (42)	4.3 (47)	25	27	2	5
OPA-13	4.8 (53)	8.7 (96)	34	49	9	7
OPA-16	2.5 (28)	1.0 (11)	17	0	0	0
OPA-17	3.5 (39)	1.5 (17)	25	6	2	0
OPA-18	3.1 (34)	8.1 (89)	4	40	0	4
Total no.	34.2(376)	41.3(454)	287	207	24	24
Average no. per primer	41.8	50.4	31.9	23.0	2.7	2.7

The total number of fragments generated by a primer in bullhead population obtained from Dangjin is shown in parentheses.

These primers produced 36, 32, and 24 bands in mud crabs from Eastern Thailand (genus *Scylla*) (Klinbunga *et al.*, 2000). 176 common fragments, with an average of 25.1 per primer, were observed in a population of oysters obtained from Buan in Korea, and 99 fragments, with an average of 14.1 per primer, were observed in a Geojedo population (Kim *et al.*, 2004).

Oligonucleotide primers generated identically-sized fragments in both of the bullhead populations, as was illustrated in Table 2. Intra-population-specific, -common or inter-population-specific, and -common fragments were generated by RAPD-PCR, using 9 random primers in a cultured bullhead the wild bullhead, as is illustrated in Table 2 and Fig. 1. 110 intra-population-specific fragments, with an average of 12.2 per primer, were observed in the cultured bullhead population. 99 fragments, with an average of 11.0 per primer, were identified in the wild bullhead. Especially, 55 inter-population-common fragments, with an average of 6.1 per primer, were observed in the two bullhead populations. Especially, the OPA-11 decamer primer generated inter-population-common DNA fragments, with size of approximately 650 bp, in both the cultured and wild bullhead populations (Fig. 1E). The OPA-17 decamer primer also generated inter-species-common DNA fragments, with size of approximately 300 bp, between the two bullhead populations (Fig. 1H). The complexity of these banding patterns was observed to vary widely between primers and/or geographic locales. Interestingly, inter-population-common fragments that identified the same species were not generated by OPA-13 (Fig. 1F). Population-related RAPD fragments have also been 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). Among the 12 universal rice primers (URP) surveyed, 3 (URP4, URP5 and URP6) were shown to yield specific RAPD markers for the differentiation of red and black seabreams (Kim *et al.*, 2005a). It was also reported that species-specific RAPD markers (or fragments) were generated from 0.5kb to 3.2kb in red or black seabream, and also in their hybrids.

In general, the size and number of fragments generated is dependent on both the nucleotide sequence of the primer used, and on the source of the template DNA, resulting in genome-specific DNA fragments (Welsh and McClelland, 1990; Welsh *et al.*, 1991). The effects of the GC content of primer, the genetic group, and the gel on the bandsharing values were all found to be significant (Suprabha *et al.*, 2005). Thus, adjustments for the GC content of the primer and gel are prerequisites in the evaluation of BS values in RAPD-PCR analysis. Generally speaking, using a variety of oligonucleotide primers, several researchers have utilized RAPD-PCR to identify polymorphic/specific markers particular to a line, breed, species, genus, and geographic population, as well as genetic similarity/polymorphism /diversity in a variety of organisms (Welsh *et al.*, 1991; Smith *et al.*, 1997; Kim *et al.*, 2000; McCormack *et al.*, 2000; Geng *et al.*, 2002; Suprabha *et al.*, 2005).

In the present study, 376 fragments were identified in the cultured bullhead population, and 454 in the population of wild bullhead from Dangjin: 287 specific fragments (76.3%) in the cultured bullhead population and 207 (45.6%) in the wild bullhead population, as is illustrated in Table 1. Nine primers also generated 24 polymorphic fragments (24/376 fragment, 6.4%) in the cultured bullhead population, and 24 (24/454 fragments, 5.2%) in the wild bullhead population, as was illustrated in Table 1. The OPA-16 primer, notably, produced which 11 out of 11 bands (100%) were monomorphic in the wild bullhead population (Fig. 1G). Interestingly, specific and/or polymorphic fragments were not generated by OPA-13. These results in particular, show that the primers had detected inter-population- common fragments that identified the same species. The OPA-13

Table 2. The intra-population-specific and -common or inter-population-specific and -common fragments generated by RAPD-PCR using 9 random primers in cultured and wild bullhead (*P. fulvidraco*) from Dangjin in Korea, respectively

Item	No. of intra-population fragments		No. of inter-population fragments
Primer \ Population	Cultured bullhead	Wild bullhead	Two bullhead populations
OPA-01	11	0	0
OPA-04	0	11	0
OPA-06	22	11	11
OPA-09	0	33	0
OPA-11	11	11	11
OPA-13	22	0	0
OPA-16	11	11	11
OPA-17	11	11	11
OPA-18	22	11	11
Total no.	110	99	55
Average no. per primer	12.2	11.0	6.1

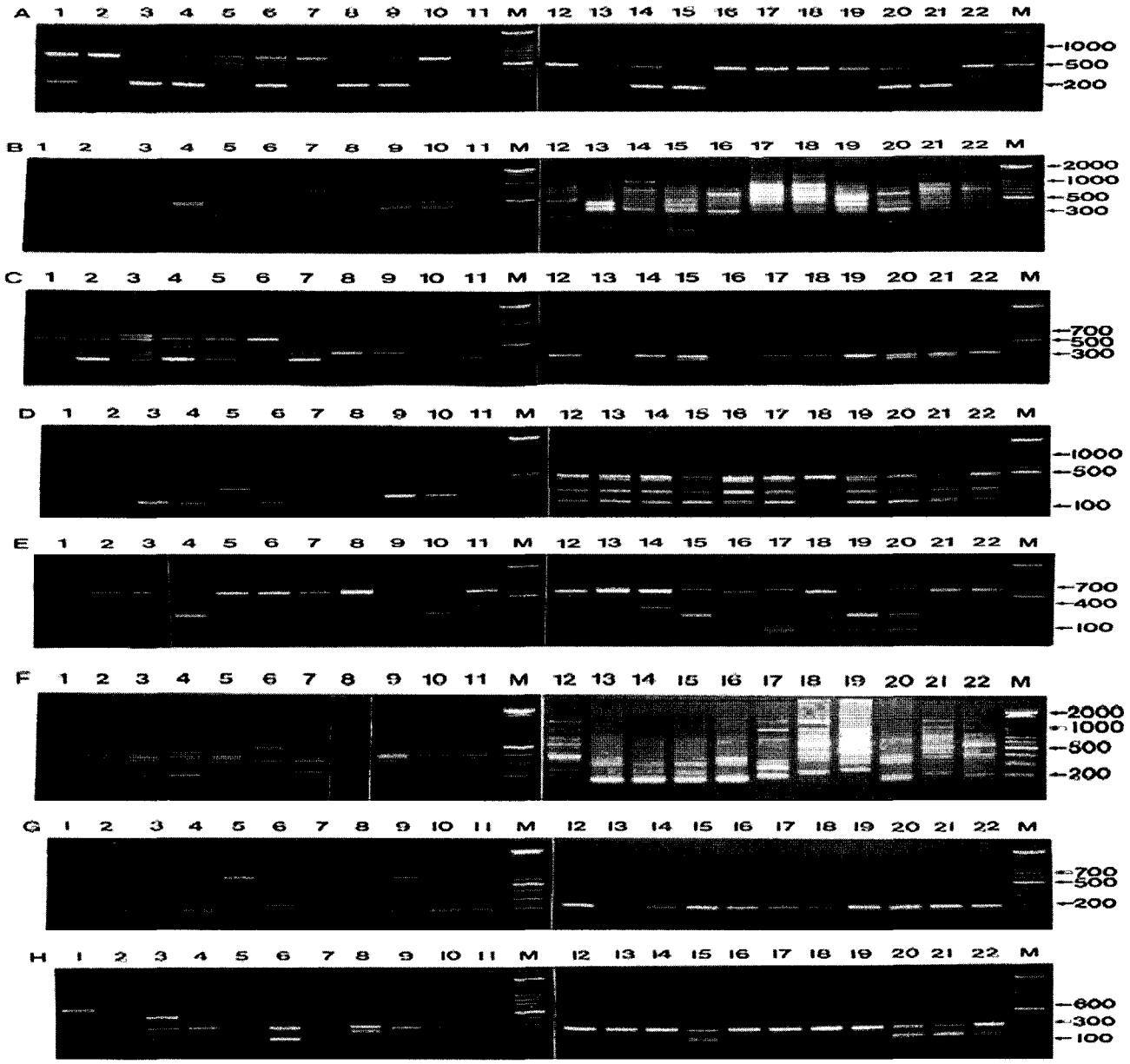

Fig. 1. Individual specific RAPD patterns of cultured bullhead (lane 1~11) and wild bullhead (lane 12~22) amplified by arbitrary primer OPA-01 (A), OPA-04 (B), OPA-06 (C), OPA-09 (D) and OPA-11 (E), OPA-13 (F), OPA-16 (G) and OPA-17 (H), respectively. Amplification products were electrophoresed on a 1.4% agarose gel with TBE (90 mM Tris, pH 8.5; 90 mM boric acid; 2.5 mM EDTA) and detected by staining with ethidium bromide. Each lane shows different individual DNA samples. Molecular size marker: M, 100 bp DNA ladder.

primer, on the contrary, produced a number of specific and polymorphic fragments (Fig. 1F).

It has also been reported that the percentage of polymorphic bands obtained from five geographic populations of black tiger shrimp (*Penaeus monodon*) varied, ranging between 51.5 to 57.7% (Tassanakajon *et al.*, 1998). Two primers yielded the highest levels of polymorphism in the black tiger shrimp, 88.9%. The results of this analysis also showed that 22 out of 80 bands (27.5%) were monomorphic, and 58 (72.5%) were polymorphic. Six primers generated 84 polymorphic bands, out of a total of 90 bands detected in the blacklip abalone (Huang *et al.*, 2000). Upon the RAPD analysis of genetic differences and characteristics in geographic oyster populations, six primers were shown to generate 47 polymorphic fragments (24% of 195 fragments) in a population of bullhead (Yoon and Kim, 2004). 481 fragments were detected in an oyster population from Buan, and 264 were identified in an oyster population from Geojedo, Korea: 143 polymorphic fragments (29.7%) were found in the Buan population and 60 (22.7%) were

found in the Geojedo population (Kim *et al.*, 2004).

Here, we have determined that the OPA-09 primer generated these major/minor specific fragments: 100 bp (lanes 15 and 21), 250 bp (lane 15) and 350 bp (lanes 12, 15, 16 and 21) in the wild bullhead population (Fig. 1D). Moreover, the 11 fragments generated by the decamer primer OPA-06, which were approximately 320 bp in size, were also observed in all samples of cultured bullhead population (Fig. 1C). 11 identical fragments in the two bullhead populations were identified by decamer OPA-11, which established identifications for species/population /genus (Fig. 1E). Interestingly, these common fragments that established species identity were 650 bp. High degrees of RAPD variation were observed in the banding patterns generated by decamer primer OPA-04 in the cultured bullhead population, ranging from 230 bp to 800 bp (Fig. 1B). A high degree of RAPD variation was also observed in the banding patterns of the wild bullhead population, ranging from 100 bp to 1,600 bp, and was generated by the same decamer primer. This primer, as illustrated in Table 1, observed 31 specific bands and 6 polymorphic bands in the cultured bullhead population. This primer also observed 55 specific bands and 7 polymorphic bands in the wild bullhead population. Interestingly, the 11 common fragments that established population identity were 350 bp in the cultured bullhead population. The OPA-16 decamer primer generated inter-population-common DNA fragments, of approximately 200 bp in size in both the cultured and wild bullhead population (Fig. 1G). The OPA-17 decamer primer was also shown to generate inter-population-common DNA fragments, of approximately 300 bp, between the two bullhead populations (Fig. 1H).

McCormack *et al.*, (2000) reported that the banding patterns were characterized by a high degree of variation, with individual organisms being clearly distinguishable from one another, in *A. filiformis*. They also reported that all four of the primers generated 111 polymorphic DNA fragments in 70 individuals. The sum of the average polymorphic products, 73.7, was identified in a combination of the common carp and the Israeli carp (Yoon, 2001). This specific primer also proved useful in the identification of individuals and/or populations, as the result of variations in DNA polymorphisms among individuals/populations (Liu *et al.*, 1998; Yoon and Kim, 2003b; Yoon and Kim, 2004). The random RAPD method has been applied to eight fish species such as barramundi, Nile perch, john dory, mirror dory, silver dory, spiky oreo, warty oreo and smooth oreo (Partis and Wells, 1996). Diagnostic markers which are found to be present in two populations of an eel-loach species (*Pangio* sp.) are considered to be species-specific markers, whereas the other bands were considered to be 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 from 300 bp~2,000 bp.

The Bandsharing Values and Genetic Distances

In this study, the bandsharing value of individuals within the wild bullhead population was substantially higher than was determined in the cultured bullhead population. Based on the average bandsharing values of all samples, the similarity matrix ranged from 0.474 to 0.779 in the cultured bullhead population, and from 0.464 to 0.818 in the wild bullhead population (Table 3). The average bandsharing value was 0.596 ± 0.010 within the cultured bullhead population, and 0.657 ± 0.010 within the wild bullhead population. The bandsharing value between individuals no. 01 and no. 03 was 0.779, which was the highest value identified within the cultured bullhead population. The bandsharing value between individuals no. 01 and no. 04 was 0.474, which was the lowest. The bandsharing value between no. 14 and no. 17 was also 0.818, which was the highest value within the wild bullhead population. The average bandsharing value between the two bullhead populations, 0.415 ± 0.006, ranged between 0.284 and 0.559. Other reports have shown that the average bandsharing value shown in our study was also higher than the average value between the common carp and the Israeli carp species (0.57 ± 0.03) (Yoon, 2001), the bullhead population (0.504 ± 0.115) (Yoon and Kim, 2004), and also between two oyster populations (0.282 ± 0.008) (Kim *et al.*, 2004). The average genetic difference within the cultured bullhead population was 0.404 ± 0.010, and was 0.343 ± 0.010 within the wild bullhead population. This would appear to suggest that the genetic variation in the cultured bullhead population is greater than in the wild bullhead population. The difference between these two bullhead populations was found to be statistically significant ($p<0.01$). Particularly, our RAPD-PCR analysis indicated that the cultured bullhead population obtained from Dangjin of Korea was more genetically diverse than was the wild bullhead population. When trying to think generally, other reports have shown that the genetic diversity of the cultured fish population was higher than that of wild fish population. Accordingly, diagnostic RAPD markers will be required in order to characterize the different geographic bullhead populations, to correlate the markers with morphological characteristics, and to clarify some of the ambiguity among population/species. Moreover, Suprabha *et al.*, (2005) insisted that adjustments for the GC content of primer and gel are a prerequisite to the accurate assessment of BS values in RAPD-PCR analyses.

To obtain the dendrogram, a hierarchical clustering analysis was performed, employing the similarity matrix based on the bandsharing values and genetic differences (Fig. 2). In this study, the dendrogram obtained with the nine primers indicates two genetic clusters, designated cluster 1 (CULTURED 01~CULTURED 11), and cluster 2 (WILD 12~WILD 22). The genetic distance between the two bullhead populations ranged between 0.080 and 0.714.

Table 3. Similarity matrix calculated using Nei and Li's index of similarity for cultured and wild bullhead (*P. fulvidraco*)

		Bandsharing values of cultured bullhead											Bandsharing values of wild bullhead										
		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 cultured bullhead	1		0.622	0.779	0.474	0.605	0.659	0.595	0.500	0.562	0.515	0.533	0.472	0.424	0.471	0.468	0.468	0.425	0.433	0.507	0.554	0.539	0.541
	2	0.378		0.677	0.613	0.672	0.619	0.599	0.677	0.722	0.558	0.589	0.352	0.497	0.395	0.402	0.431	0.300	0.352	0.346	0.435	0.449	0.350
	3	0.221	0.323		0.554	0.698	0.705	0.663	0.623	0.600	0.528	0.516	0.390	0.372	0.384	0.407	0.413	0.345	0.309	0.391	0.442	0.494	0.451
	4	0.526	0.387	0.446		0.569	0.496	0.501	0.671	0.589	0.614	0.486	0.312	0.369	0.369	0.457	0.338	0.378	0.357	0.386	0.463	0.374	0.315
	5	0.395	0.328	0.302	0.431		0.725	0.669	0.596	0.632	0.697	0.550	0.544	0.559	0.526	0.405	0.488	0.453	0.454	0.497	0.465	0.416	0.443
	6	0.341	0.381	0.295	0.504	0.275		0.606	0.539	0.541	0.558	0.583	0.422	0.497	0.484	0.410	0.440	0.422	0.417	0.366	0.458	0.388	0.412
	7	0.405	0.401	0.337	0.499	0.331	0.394		0.613	0.505	0.509	0.497	0.429	0.479	0.479	0.426	0.439	0.398	0.467	0.515	0.434	0.471	0.441
	8	0.500	0.323	0.377	0.329	0.404	0.461	0.387		0.587	0.545	0.546	0.307	0.327	0.348	0.370	0.371	0.334	0.339	0.351	0.400	0.425	0.311
	9	0.438	0.278	0.400	0.411	0.368	0.459	0.495	0.413		0.696	0.597	0.284	0.318	0.328	0.445	0.396	0.347	0.348	0.415	0.496	0.437	0.286
	10	0.485	0.442	0.472	0.386	0.303	0.442	0.491	0.455	0.304		0.580	0.372	0.454	0.483	0.455	0.428	0.489	0.476	0.490	0.477	0.374	0.376
	11	0.467	0.411	0.484	0.514	0.450	0.417	0.503	0.454	0.403	0.420		0.394	0.423	0.377	0.368	0.391	0.358	0.440	0.382	0.374	0.365	0.390
Genetic differences of wild bullhead	12	0.528	0.648	0.610	0.688	0.456	0.578	0.571	0.693	0.716	0.628	0.606		0.689	0.722	0.531	0.686	0.764	0.680	0.676	0.620	0.642	0.788
	13	0.576	0.503	0.628	0.631	0.441	0.503	0.521	0.673	0.682	0.546	0.577	0.311		0.798	0.661	0.686	0.723	0.673	0.697	0.643	0.464	0.653
	14	0.529	0.605	0.616	0.631	0.474	0.516	0.521	0.652	0.672	0.517	0.623	0.278	0.202		0.778	0.628	0.818	0.723	0.683	0.723	0.620	0.641
	15	0.532	0.598	0.593	0.543	0.595	0.516	0.574	0.630	0.555	0.545	0.632	0.469	0.339	0.222		0.547	0.657	0.577	0.662	0.756	0.657	0.581
	16	0.532	0.569	0.587	0.662	0.512	0.560	0.602	0.629	0.604	0.572	0.609	0.314	0.314	0.372	0.453		0.629	0.566	0.662	0.646	0.538	0.615
	17	0.575	0.700	0.655	0.622	0.547	0.578	0.602	0.666	0.653	0.511	0.642	0.236	0.277	0.182	0.343	0.371		0.714	0.700	0.699	0.553	0.635
	18	0.567	0.648	0.691	0.643	0.546	0.583	0.533	0.661	0.652	0.524	0.560	0.320	0.327	0.277	0.423	0.434	0.286		0.610	0.567	0.551	0.691
	19	0.493	0.654	0.609	0.614	0.503	0.634	0.485	0.649	0.585	0.510	0.493	0.324	0.303	0.317	0.338	0.338	0.300	0.390		0.705	0.554	0.641
	20	0.446	0.565	0.558	0.537	0.535	0.542	0.566	0.600	0.504	0.523	0.626	0.380	0.357	0.277	0.244	0.354	0.301	0.433	0.295		0.668	0.603
	21	0.461	0.551	0.506	0.626	0.584	0.612	0.529	0.575	0.563	0.626	0.635	0.358	0.536	0.380	0.343	0.462	0.447	0.449	0.446	0.332		0.720
	22	0.459	0.650	0.549	0.685	0.557	0.588	0.559	0.689	0.714	0.624	0.610	0.212	0.347	0.359	0.419	0.385	0.365	0.309	0.359	0.397	0.280	

In particular, individual WILD no. 17 from the cultured bullhead population was determined to be most closely genetically related to WILD no. 14 (genetic distance = 0.080). On the other hand, individual CULTURED no. 03 of the wild bullhead population was more distantly related to CULTURED no. 08 (genetic distance = 0.174). Ultimately, the longest genetic distance displaying significant molecular differences was determined to exist between individuals in the two bullhead populations, namely between individuals WILD no. 19 of the wild bullhead population and CULTURED no. 03 of the cultured bullhead population (genetic distance = 0.714).

Our cluster analyses revealed patterns similar to the ones posited by Kim *et al.*, (2005b). They reported that both linkage cluster analysis and a dendrogram revealed close and/or distant relationships between individual identities within two geographic populations and/or species. In shellfish, cluster analysis of the pairwise population matrix, which was generated from the RAPD data, demonstrated that geographically close populations showed a

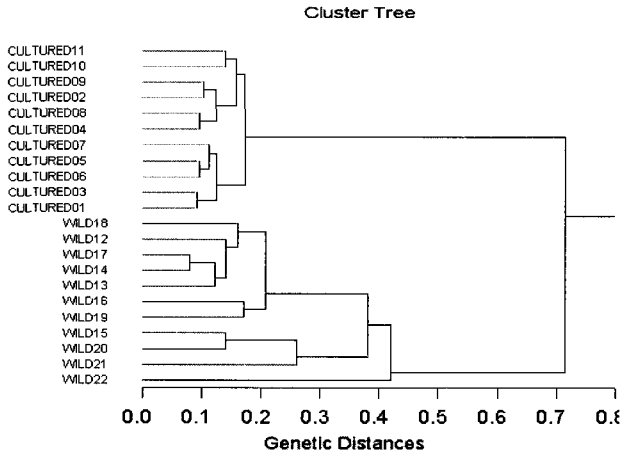

Fig. 2. Dendrogram of genetic distances showing the relatedness among different individuals of cultured bullhead (CULTURED 01 ~ CULTURED 11) and wild bullhead (WILD 12 ~ WILD 22) generated according to the bandsharing values and genetic differences matrix in Table 3.

tendency to cluster together in the blacklip abalone (Huang *et al.*, 2000). Additional principal component analysis, which was also conducted on the basis of the RAPD data, indicated that the Point Cook population was clearly separated from the two other central populations. A neighbor-joining tree based on the genetic distances between populations, constructed via RAPD-PCR, indicates the relationships of three mud crab species (Klinbunga *et al.*, 2000). This study indicated that large genetic differences could be detected between geographic populations within a species, as well as between species. The dendrogram constructed for the Korean oyster population using the four primers indicated three genetic clusters (Kim *et al.*, 2004). The genetic distance between the two geographic populations was shown to range between 0.039 and 0.284.

The identification of the penaeid shrimp (*Penaeus chinensis*), catfish (*Silurus asotus*), and pond-smelt (*Hypomesus olidus*) populations was a prerequisite step in the inception and development of invertebrate/teleost breeding programs (Yoon and Kim, 2003b; Yoon and Kim, 2004; Kim *et al.*, 2005b). Molecular genetic markers, including, most notably, microsatellite loci, quantitative trait loci, and genomic mapping, will almost certainly prove useful in the selection of broodstock for multiple reproductive traits, or health- and production-related traits, in the field of fishery science (Waldbieser and Wolters, 1999). Differences in morphological traits are assumed to reflect the distinct origins or genetic identity of the species (Chenyambuga *et al.*, 2004). There appears, for example, to be a clear association between particular goat types in sub-regions of sub-Saharan Africa.

In our study, RAPD-PCR analysis was used, and revealed a significant genetic distance existing between two population pairs. RAPD-PCR allowed us to detect the existence of population identification and genetic variation in Korean population of bullhead. This finding indicates that this method constitutes a suitable tool for DNA comparison, both within and between individuals, populations, species, and genera. Furthermore, basic knowledge of DNA polymorphisms and molecular markers in bullhead species may contribute significantly to the process of broodstock selection and the efficacy of selective fish-breeding programs. The extraordinarily unique gene pools exhibited by some samples (especially in the case of the photo in Fig. 1B) would require new conservation policies, such that many wild Korean bullhead populations might be preserved. Accordingly, further analysis using a greater number of individuals, primers, and species will be required in order to fully establish the specificity of loci to particular taxa, and subsequent inter-specific gene flow in the genera *Pseudobagrus*. It will also be necessary to conduct a study with more sampling sites, in order to more precisely determine the area in which the phylogeographic break occurs. Both more time, and a great deal more research, will also be necessary to identify the differentially expressed genes (DEG) between/among species, using an annealing control primer system.

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