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Genetic Similarity and Difference between Common Carp and Israeli Carp (*Cyprinus carpio*) Based on Random Amplified Polymorphic DNAs Analyses

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Common carp (*Cyprinus carpio*) and its aquaculture breed Israeli carp samples were obtained from two separate aquaculture facilities under the similar raising conditions during two years in the Kunsan National University, Korea. Genomic DNA was isolated from the common carp and Israeli carp for identification of genetic characteristics and genomic polymorphisms by polymerase chain reaction amplification of DNA using arbitrary primers. The arbitrary primer No. 21 (ACTTCGCCAC) yielded the highest number of fragments with the average of 15.0 among the primers used in Israeli carp. A total of 294 polymorphic products in common carp and 336 in Israeli carp were observed by random primers. The average number of polymorphic products generated by random RAPD primer No. 2 (GTAGACCCGT) showed 8.0 in Israeli carp. On average, each random RAPD primer produced 5.4 amplified polymorphic products in common carp and 6.2 in Israeli carp. An average genetic similarity (BS value) was 0.44±0.05 within the common carp and 0.32±0.04 within the Israeli carp. The degree of similarity frequency (BS) between two carps was 0.67 as generated by the primer No. 19 (GACGGATCAG). The average level of bandsharing was 0.57 ±0.03 between the two carps. Accordingly, the two carp populations were genetically a little distant. The electrophoretic analysis of PCR-RAPD products showed middle levels of variation between the two carp populations. This result implies that the genetic diversity among intra-population may be higher when compared with that between the two carps. The RAPD polymorphism generated by these random primers might be used as a genetic marker for populations or lines identification in important aquacultural carp.

The selection criteria of animal and plant breeding program/policy have focused mainly on the traits of economic importance. The conventional methods used for artificial selection have been the biometrical approaches combined with general genetic principles. Genetic polymorphisms are playing an increasingly important role as genetic markers in many fields of animal, plant and microorganism breeding. These polymorphic genetic markers proved useful for individual identification, pedigree analysis, genetic relationship, gene cloning, pathological diagnostics, genetic structure of animal and plant populations and marker assisted selection programs (Williams et al., 1990; Welsh et al., 1991; Simpson et al., 1993; Vierling et al., 1994; Smith et al., 1997; Oidtmann et al., 1999). Especially, identification of individual or commercially-important fish species is necessary for efficient selective breeding and broodstock management, and for the measurement of various traits. DNA markers that are shown to be genetically linked to a trait of interest can be used for gene cloning, pathological diagnostics, transgenic studies and for trait estimate in fish breeding programs (Oidtmann et al., 1999; Waldbieser and Wolters, 1999). Also, development of genetic markers in fish is needed to improve the efficiency of breeding by marker-assisted selection and for identification of economically important genes such as disease resistance genes, antifreezing peptide genes and growth hormone genes.

The recent advance in genetic techniques with molecular biological methods showed a great potential to accelerate the biological perspectives. The DNA markers most commonly used are restriction fragment length polymorphisms (RFLP) (Lloyd et al., 1989; Beckenbach et al., 1990). RFLPs were successful in detecting polymorphism in animal and plant.

However, random amplified polymorphic DNA

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(RAPD) based on polymerase chain reaction (PCR) produced a higher percentage of multiple-band outlines than RFLP probes (Williams et al., 1990; Welsh et al., 1991). PCR is a rapid, simple, relatively cheap and sensitive procedure for *in vitro* amplification of specific DNA sequences using appropriate single primers. Genomic fingerprints generated by PCR amplification of DNA using single arbitrary primers have compatibility for detecting DNA polymorphisms between organisms (Williams et al., 1990; Welsh et al., 1991; Dias Neto et al., 1993; Simpson et al., 1993; Orozco-Castillo et al., 1994; Vierling et al., 1994; Mohd-Azmi et al., 2000). Especially, most of application of RAPD's in fisheries has been done with fish and shellfish species (Smith et al., 1997; Liu et al., 1998; Waldbieser and Wolters, 1999; Bernardi and Talley, 2000). More recently PCR using arbitrary and/or specific primers has been applied to inter- and intra-species differentiation (Dias Neto et al., 1993; Simpson et al., 1993; Smith et al., 1997; Waldman et al., 1997; Won et al., 1999; Lee et al., 2000). These random primers can detect polymorphisms in the presence or absence of specific nucleotide sequence information, and the polymorphisms can serve as genetic markers.

Common carp and Israeli carp are commercially important warmwater fish species with the longest history of aquaculture, and are distributed all over the world. Consequent of the long aquaculture in hatchery-reared carp production, there is a need to understand the genetic composition of common carp and/or Israeli carp populations in order to evaluate exactly the latent genetic effects induced by hatchery operations. In spite of its economic and scientific importance, little information is available on the genetic relationships between the two carp populations in Korea. Therefore, in this study, DNAs isolated from common carp and Israeli carp were analyzed by 24 RAPD primers in order to identify the genetic relationship of the two populations with an identical scientific name by investigating their genetic similarity and diversity.

Materials and Methods

Sampling and blood collection

Common carp (*Cyprinus carpio*) and Israeli carp (*C. carpio*) DNA samples were obtained from two separate aquaculture facilities under the similar raising conditions during two years in the Kunsan National University, Jeollabuk-do, Korea. Preliminary RAPD analysis was performed on genomic DNA samples from a total of 12 carps (6 fish individuals/carp) using 24 different random primers (6 fishes/primer). The DNA samples were collected from blood in two-year-old common carp and Israeli carp anaesthetized with MS 222 (100 ppm). Blood samples were taken with sterile test tubes from the caudal vein into heparinized vials. The samples were refrigerated at 4℃ until use.

Sources of genomic DNA

Blood of whole carp was placed into a 1.5 ml microcentrifuge tube, to which an equal volume of Solution I (10 mM Tris HCl (pH 7.6) / 10 mM KCl / 10 mM $MgCl_2$/ Nonidet P-40) was added and mixed gently by inverting the tube several times. The samples were incubated on ice for 5 min and centrifuged at 3,000 rpm for 30 min at 4℃. The supernatant was decanted and nuclei pellet was resuspended in Solution II (10 mM Tris HCl (pH 7.6) / 10 mM KCl / 10 mM $MgCl_2$ / 0.5 mM NaCl / 2 mM EDTA). Samples were transferred to 1.5 ml tube, and mixed with 50 μl of 10% SDS buffer and 20 μl of 20 mg/ml proteinase K solution. The mixture was gently mixed by inverting the tube and incubated at 37℃ for overnight. 400 μl of phenol was added and centrifuged for 1 min at 1,000 rpm. 200 μl of phenol and 200 μl of chloroform : isoamylalcohol were added to the mixture. Samples were spun down for 1 min at 1,000 rpm and mixed with 700 μl of chloroform : isoamylalcohol. The cleared lysates were extracted with 2 volume of ice-cold ethanol, then centrifuged for 5 min at 3,000 rpm. The DNA pellet was air-dried for 30 min, and then dissolved in 200 μl of TE (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) buffer. Purity and concentration of DNA were estimated by calculating the ratio of the absorbances measured at 260 nm and 280 nm with a spectrophotometer (Beckman DU series 60).

Primers

Of the 24 arbitrarily selected primers purchased from Operon Technologies, 9 random primers (No. 2, 6, 8, 10, 12, 19, 21, 23, and 24) were used for the identification of the number and frequency of the polymorphic products. All primers had 60% or 70% G+C content. The oligonucleotide primer sequences (5' to 3') are shown in Table 1.

Amplification conditions

Amplification reactions were performed in volumes of 25 μl containing 20 ng of genomic DNA, 1 × buffer (50 mM KCl, 10 mM Tris-HCl, 2.0 mM $MgCl_2$, 0.01% gelatin, 0.025% Nonidet P-40, and 0.025% Tween 20), 200 μM dNTP (Advanced Biotechnologies), 0.5 unit of Taq polymerase (Perkin Elmer Cetus), and 0.3 μM primer (Operon Technologies). Amplification was performed in a DNA Thermal Cycler (Perkin Elmer Cetus). The mixture was overlaid with mineral oil, and followed by initial predenaturation at 94℃ for 3 min. Thermal Cycler was programmed as follows: 45 cycles at 94℃ for 1 min for denaturation, at 35℃ for 1 min for annealing and 72℃ for 2 min for extension with fastest available transition between each temperature. Amplification products were analyzed by electrophoresis in 1.4% agarose gels with TBE (0.09 M Tris, HCl (pH 8.5), 0.09 M boric acid, and 2.5 mM EDTA) and detected by staining with ethidium bromide. The gels

Table 1. Primers and primer sequences used for the detection of polymorphism in common carp and Israeli carp

Primer	Sequence(5′ to 3′)	GC content (%)
1	TCCGCTCTGG	70
2	GTAGACCCGT	60
3	GGAGGGTGTT	60
4	TTTGCCCGGA	60
5	CTGCTGGGAC	70
6	TGGGGGACTC	70
7	GTCCACACGG	70
8	GGTGACGCAG	70
9	TTCCCCCGCT	70
10	CCTTGACGCA	60
11	GGACCCTTAC	60
12	TGCTCTGCCC	70
13	ACCCCCGAAG	70
14	TGCGCCCTTC	70
15	TGATCCCTGG	60
16	TGTCTGGGTG	60
17	GAACGGACTC	60
18	AAAGCTGCGG	60
19	GACGGATCAG	60
20	TGAGTGGGTG	60
21	ACTTCGCCAC	60
22	TTCGAGCCAG	60
23	GGGGGTCTTT	60
24	GTGAGGCGTC	70

were illuminated with UV light and photographed by UV DNA photographic system (Seoulin Co. Korea).

Analytical method

Only those bands which were readily visible were scored. RAPD outlines were compared only on sample lanes in the same gel. Bandsharing (BS) of DNA sequences was calculated according to the formula of Jeffreys and Morton (1987): BS = 2 (Bab) / (Ba+Bb). Bab is the number of bands shared by individuals a and b, Ba is the total number of bands for individual a, and Bb is the total number of bands for individual b. Percentage difference=Nab / Na+Nb × 100, where Nab is the number of fragments that differed between two individuals for a single primer. Na is the number of fragments resolved in individual a. Nb is the number of fragments resolved in individual b. Number (frequency) of RAPD band was determined from the ratio of the number of animals carrying the band (n) to the total number of animals screened within a population (N).

Results

Intra-population variation (especially, Israeli carp)

When PCR amplification of genomic DNA was performed with 24 short oligonucleotide primers of arbitrary sequence at low annealing temperatures, the amplification products could be generated as DNA polymorphic bands, the patterns being characteristic of both the template DNA and the primers (Table 1). It was used with DNA extracted from common carp and Israeli carp and had yielded band sizes of from 10^2 to 10^3 bp. Genomic DNA fingerprints generated by 10 random primers screened to amplify DNA isolated from common carp and Israeli carp were shown in Figs. 1, 2 and 3. The number of products generated per primer varied with an average of 7.6 in common carp and 7.9 Israeli cap (Table 2). On average, the random RAPD primer No. 6 (TGGGGGACTC) amplified 8.0 bands in common carp and 8.5 in Israeli carp. Especially, the arbitrary primer No. 21 (ACTTCGCCAC) yielded highest number of fragments with an average of 15.0 among the primers used in Israeli carp. The products generated by random primer No. 7 (GTCCACACGG) showed an average of 3.5 bands in Israeli carp (Table 2). The polymorphic products generated by random primer No. 2 (GTAGACCCGT) showed an average of 8.0 bands in Israeli carp, as summarized in Table 3. The degree of similarity in common carp varied from 0.25 to 0.64 and 0.16 to 0.53 in Israeli carp as calculated by bandsharing analysis (Table 3). Especially, the arbitrary primer No. 23 (GGGGGTCTTT) produced the highest bandsharing values with the average of 0.64 while primers No. 6 yielded the lowest values (0.25) in common carp. The average level of bandsharing was 0.44 ± 0.05 within common carp and 0.32 ± 0.04 within Israeli carp. In addition, the RAPD outlines obtained with DNA of different individuals in the same population greatly differed in the common and Israeli carps.

Table 2. Average number of products generated from RAPD patterns for 24 arbitrary primers in common carp and Israeli carp

Primer	Average no. of products	
	common carp	Israeli carp
1	4.5 (27) [a]	5.5 (33)
2	9.0 (54)	9.5 (57)
3	6.0 (36)	9.0 (54)
4	3.0 (18)	3.0 (18)
5	7.0 (42)	6.0 (36)
6	8.0 (48)	8.5 (51)
7	5.5 (33)	3.5 (21)
8	11.5 (69)	8.5 (51)
9	2.0 (12)	5.5 (33)
10	10.5 (63)	6.5 (39)
11	10.5 (63)	11.5 (69)
12	9.5 (57)	10.5 (63)
13	4.5 (27)	7.5 (45)
14	3.0 (18)	11 (66)
15	4.0 (24)	5.0 (30)
16	7.5 (45)	10.0 (60)
17	6.0 (36)	8.0 (48)
18	12.0 (72)	6.5 (39)
19	8.5 (51)	7.0 (42)
20	8.0 (48)	8.5 (51)
21	10.5 (63)	15.0 (90)
22	9.5 (57)	5.0 (30)
23	11.0 (66)	8.0 (48)
24	11.5 (69)	10.5 (63)
Average	7.6	7.9

[a] Parentheses are the total number of products.

Variations between common carp and Israeli carp

In our study, 38% of the random primers appeared to amplify polymorphic bands (Table 4). 9 primers produced amplified fragments which were consistently polymorphic between common carp and Israeli carp. 5 random primers produced polymorphic DNA bands

Table 3. Number and bandsharing (BS) of polymorphic products generated by 9 random primers in common carp and Israeli carp

Primer	Number of polymorphic products		Average number of polymorphic products		BS values	
	Common carp	Israeli carp	Common carp	Israeli carp	Common carp	Israeli carp
2	30	48	5.0	8.0	0.44	0.16
6	36	42	6.0	7.0	0.25	0.18
8	30	36	5.0	6.0	0.57	0.29
10	24	24	4.0	4.0	0.62	0.38
12	36	42	6.0	7.0	0.37	0.33
19	36	36	6.0	6.0	0.29	0.19
21	36	42	6.0	7.0	0.43	0.53
23	24	30	4.0	5.0	0.64	0.38
24	42	36	7.0	6.0	0.39	0.43
Total Average	294 32.7	336 37.3	49 5.4	56 6.2	4.0 0.44±0.05*	2.87 0.32±0.04*

* Standard error

whose sizes ranged from approximately 193 to lower than 603 base pairs within Israeli carp. A total of 73.7 average number of polymorphic products were observed between common carp and Israeli carp as summarized in Table 4. On average, each random RAPD primer produced 8.2 amplified products. The products generated by random RAPD primer No. 8 (GGTGACGCAG) showed an average of 10.4 bands. The average level of polymorphic bands generated by primer No. 19 (GACGGATCAG) was 5.1 which was lower than any other primers. The degree of similarity (BS value) varied from 0.46 to 0.67 as calculated by bandsharing analysis between the common and Israeli carps (Table 4). The average level of bandsharing was approximately 0.57±0.03 between the two carps. The average level of bandsharing generated by primer No. 10 (CCTTGACGCA) was lower than any other primers, which was 0.46. The degree of similarity frequency (BS value) between the two carps generated using random primer No. 12 (TGCTCTGCCC) showed 0.56. The BS value between the two carps generated using random primer No. 19 showed 0.67.

Bands in the molecular weight range from 0.72 to 0.87 kb were generated by 9 random primers (primer no. 16~24) (Fig. 3). The similar band patterns were observed in 1.90 and 3.53 kb. Specific differences between the two carp populations were revealed in the band patterns ranging in 234 and 194 bp, respectively. The RAPD outlines obtained by various primers with DNA pooled from individuals were somewhat different. Another primer, No. 18, detected the DNA bands higher than 603 bp present in every individuals of the two carp populations. The primer No. 24 (GTGAGGCGTC) also generated the similar band patterns ranged from 118 to 194 bp.

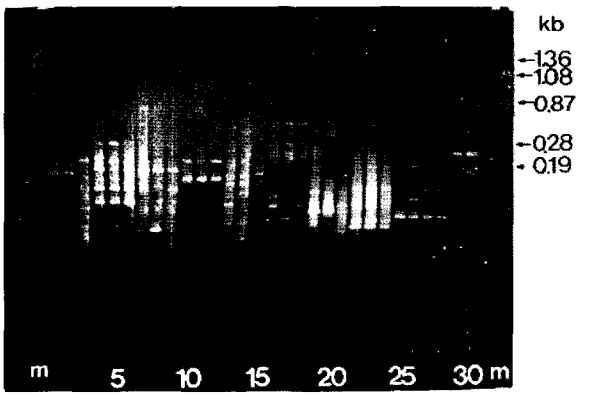

Fig. 1. Individual specific RAPD patterns generated from common carp and Israeli carp by 10 arbitrary random primers (primer no. 1~10, as summarized Table 1). Lanes 1, 4, 7, 10, 13, 16, 19, 22, 25, 26 and 29: common carp. Lanes 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 23, 24, 27, 28, 30 and 31: Israeli carp. Each lane shows different individual DNA samples. lanes 1, 2, 3 (primer no. 1); lanes 4, 5, 6 (primer no. 2); lanes 7, 8, 9 (primer no. 3); lanes 10, 11, 12 (primer no. 4); lanes 13, 14, 15 (primer no. 5); lanes 16, 17, 18 (primer no. 6); lanes 19, 20, 21 (primer no. 7); lanes 22, 23, 24 (primer no. 8); lanes 25, 26, 27, 28 (primer no. 9); lanes 29, 30, 31 (primer no. 10). m: Molecular weight markers (ΦX174 DNA digested with *Hind*III and *Hae*III as molecular size markers).

Discussion

In this study, bands in the molecular weight range of 0.72 to 0.87 kb were generated by 9 random primers (primer no. 16~24) as shown in Fig. 3. Generally, the

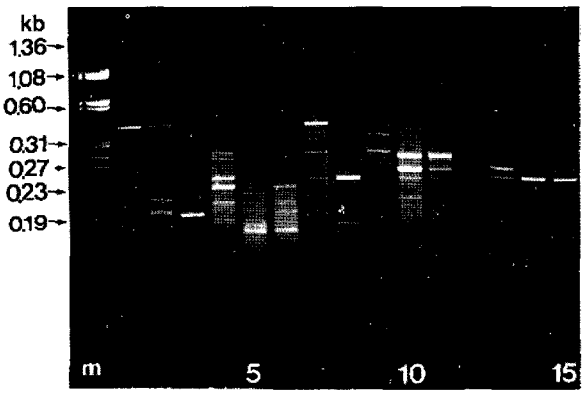

Fig. 2. RAPD patterns generated from common carp and Israeli carp by 5 arbitrary primers (primer no. 11~15). lanes 1, 2, 3 (primer no. 11); lanes 4, 5, 6 (primer no. 12); lanes 7, 8, 9 (primer no. 13); lanes 10, 11, 12 (primer no. 14); lanes 13, 14, 15 (primer no. 15). Each lane shows different individual DNA samples. Lanes 1, 2, 4, 5, 7, 8, 10, 11, 13 and 14: Israeli carp. Lanes 3, 6, 9 and 15: common carp. m: Molecular size markers (ΦX174 DNA digested with *Hind*III and *Hae*III as molecular size markers).

Table 4. Bandsharing (BS) between common carp (6 fishes/primer) and Israeli carp in the products generated by 9 random primers

Primer	Average number of polymorphic products	BS values
2	8.5	0.54
6	5.8	0.65
8	10.4	0.48
10	8.9	0.46
12	8.8	0.56
19	5.1	0.67
21	8.4	0.67
23	9.9	0.48
24	7.9	0.64
Total	73.7	5.15
Average	8.2	0.57 ± 0.03*

* *Standard error*

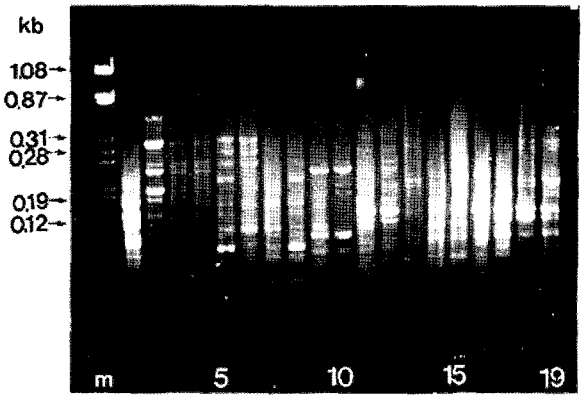

Fig. 3. Common carp- and Israeli carp-specific RAPD patterns generated by 9 arbitrary primers (primer no. 16~24). Each lane shows different individual DNA samples. Lanes 1, 3, 5, 7, 9, 11, 13, 15 and 18: common carp. Lanes 2, 4, 6, 8, 10, 12, 14, 16, 17 and 19: Israeli carp. lanes 1, 2 (primer no. 16); lanes 3, 4 (primer no. 17); lanes 5, 6 (primer no. 18); lanes 7, 8 (primer no. 19); lanes 9, 10 (primer no. 20); lanes 11, 12 (primer no. 21); lanes 13, 14 (primer no. 22); lanes 15, 16, 17 (primer no. 23); lanes 18, 19 (primer no. 24). m: Molecular weight markers were made by digesting ΦX174 DNA to completion with both *Hind*III and *Hae*III enzymes.

number and size of the fragments generated strictly depended upon the nucleotide sequence of the primer used and the source of template DNA, resulting in the genome-specific fingerprints of random DNA fragments (Williams et al., 1990; Welsh et al., 1991). The PCR-RAPD method can be applied to detect genetic diversity in numerous organisms using various primers (Williams et al., 1990; Welsh et al., 1991; Dias Neto et al., 1993; Simpson et al., 1993; Johnson et al., 1994; Orozco-Castillo et al., 1994; Vierling et al., 1994). The potential of RAPDs to identify diagnostic markers for strain, breed or species identification in pathogens (Bishop et al., 1993; Simpson et al., 1993; Dias Neto et al., 1993; Oidtmann et al., 1999), in livestock (Jeffreys and Morton, 1987; Gwakisa et al., 1994; Mohd-Azmi et al., 2000), in plants (Orozco-Castillo et al., 1994; Vierling et al., 1994) and in fish (Lloyd et al., 1989; Johnson et al., 1994; Liu et al., 1998; Yoon, 1999; Bernardi and Talley, 2000) has also been demonstrated.

In this study, 9 primers generated amplified products which were consistently polymorphic between common carp and Israeli carp. With the primer No. 13 (ACCC-CCGAAG), a few of the common bands found in one Israeli carp was not detected in the other. These specific bands were independent of sex and individuals, and may be useful as a DNA marker for identifying the strain (Lloyd et al., 1989; Welsh et al., 1991). Primer No. 21 (ACTTCGCCAC) generated the highest number of fragments among the primers (an average of 15). This specific primer was found to be useful, resulting from different DNA polymorphism among individuals. There were population-specific RAPD fragments between the Israeli and common carps and there were differences in frequencies of 9 primer fragments, as have been reported in catfish (Liu et al., 1998) and sorghum (Vierling et al., 1994). Liu et al.(1998) reported that some intra-specific RAPD variations were observed for different strains or for individuals. They screened a collection of RAPD markers in catfish and identified 22 primers that revealed 171 strain-specific genetic markers. Also, Johnson et al. (1994) screened a collection of RAPD markers in zebrafish and identified 116 primers that revealed 721 strain-specific genetic markers. These workers have found more variation within a strain than between strains considered as a whole. However, the differences in the RAPD profiles between species vary to the extent that it has not been possible to yet undertake analysis of their relatedness (Dias Neto et al., 1993). Accordingly, the worker also insisted that further analysis be required to identify primers that amplified sufficient specific bands shared by the species to permit a quantitative analysis. In this study, the average bandsharing values of amplified total polymorphic products were specific to the common carp (0.44± 0.05) and the Israeli carp (0.32±0.04). Also, an average of bandsharing value between the two carps 0.57±0.03 as shown in Table 4. Even if there were of a small sample size, this author suggested that there must be some differences between the two carps. For reference, these BS values lower than rainbow trout (0.695) (Yoon, 1999) and zebu cattle breeds (0.73~ 0.79) (Gwakisa et al., 1994). This result implies that the genetic variation or diversity within the two carps may be relatively higher when compared with those from rainbow trout. In addition, bandsharing scores were calculated as an index of similarity of RAPD fingerprints of animals from either the same or different breeds (Jeffreys and Morton, 1987; Gwakisa et al., 1994; Liu et al., 1998). The number of intra-strain polymorphisms was greater than that of inter-strain polymorphisms (Welsh et al., 1991). Obviously, in this study, the author obtained the same result in the two carps as summarized in Tables 3 and 4. On the contrary, the intra-specific divergence estimates based on sequence were less than the inter-specific diver-

gence estimated from restriction fragment analysis (Beckenbach et al., 1990). Generally, intra- or inter-specific variations in the genetic pattern or sequence divergence were observed for each primer and such data should be of value not only in the discrimination of the correlation with the economic traits but also in the construction of phylogenetic tree (Welsh et al., 1991; Orozco-Castillo et al., 1994; Vierling et al., 1994). Also, this genetic variation can be used as a potential genetic marker for linkage analysis with economically important traits in fish (Smith et al., 1997; Liu et al., 1998).

Based on the results such as the degree of similarity and diversity calculated by bandsharing analysis, it might be expected that the RAPD profiles obtained with genomic DNA between Israeli carp and common carp from Jeollabuk-do in Korea were somewhat different. Regardless of identical scientific name, PCR-RAPD indicated that one population might be more or less different from the other. That a few of polymorphic bands were identified in these carp populations implies the genetic variation or diversity in inter- and intra-genus. RAPD markers produced by primers were effective in determining genetic polymorphism between lines (Vierling et al., 1994) and inter-specific hybrid (Liu et al., 1998), in discriminating the individuals (Bernardi and Talley, 2000), and in identifying strains (Welsh et al., 1991; Dias Neto et al., 1993). Polymorphisms were scored by the presence or absence of amplification products at specific positions expressed by a large number of primers (Orozco-Castillo et al., 1994; Smith et al., 1997). The present study has shown that the RAPD-PCR technique allows for the isolation of informative molecular probes to be utilized in DNA fingerprinting and genome identification. The identification, isolation and reconstruction of genes responsible for desirable economical traits would make great strides over traditional selection and breeding methods. It is concluded that RAPD polymorphisms are useful as genetic markers not only for carp species but also for important fish species differentiation in the aquaculture industry. However, an average of bandsharing value between the two carp populations was higher than that in intra-population. In due course, this result should be discussed in more genetic researches. Additionally, it seems to be essential to get better data on the genetic distances between a large quantity of carp populations.

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References

Beckenbach AT, Thomas WK, and Sohrabi H (1990) Intraspecific sequence variation in the mitochondrial genome of rainbow trout (*Oncorhynchus mykiss*). *Genome* 33: 13-15.

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

Bishop RB, Sohanpal B, and Morzaria S (1993) *Theileria parva*: detection of genomic polymorphisms by polymerase chain reaction amplification of DNA using arbitrary primers. *Exp Parasitol* 77: 53-61.

Dias Neto E, Steindel M, Passos LKF, Pereira de Souza C, Rollinson D, Katz N, Romanha AJ, Pena SDJ, and Simpson AJG (1993) The use of RAPDs for the study of the genetic diversity of *Schistosoma mansoni* and *Trypanosoma cruzi*. *DNA Fingerprinting:* 339-345.

Gwakisa PS, Kemp SJ, and Teale AJ (1994) Characterization of zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers. *Anim Genet* 25: 89-94.

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

Johnson SL, Midson C, Ballinger EW, and Postlethwait JH (1994) Identification of RAPD primers that reveal extensive polymorphisms between laboratory strains of zebrafish. *Genomics* 19: 152-156.

Lee JE, Yang SY, and Lee HY (2000) Genetic relationships among six Korean *Rana* species (Amphibia; Ranidae) based on the mitochondrial cytochrome b gene. *Korean J Biol Sci* 4: 117-121.

Liu Z, Li P, Argue BJ, and 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.

Lloyd MA, Fields MJ, and Thorgaard GH (1989) BKm minisatellite sequence are not sex associated but reveal DNA fingerprint polymorphisms in rainbow trout. *Genome* 32: 865-868.

Mohd-Azmi M, Ali AS, and Kheng WK (2000) DNA fingerprinting of red jungle fowl, village chicken and broilers. *Asian-Aus J Anim Sci* 13: 1040-1043.

Oidtmann B, Cerenius L, Schmid I, Hoffmann R, and Söderhäll K (1999) Crayfish plague epizootics in Germany: classification of two German isolates of the crayfish plague fungus *Aphanomyces astaci* by random amplification of polymorphic DNA. *Dis Aquat Org* 35: 235-238.

Orozco-Castillo C, Chalmers KJ, Waugh R, and Powell W (1994) Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. *Theor Appl Genet* 87: 934-940.

Simpson AJG, Dias Neto E, Steindel M, Caballero OLSD, Passons LKJ, and Pena SDJ (1993) The use of RAPDs for the analysis of parasites. *DNA Fingerprinting*: 331-337.

Smith PJ, Benson PG, and Margaret McVeagh S (1997) A comparison of three genetic methods used for stock discrimination of orange roughy, *Hoplostethus atlanticus*: allozymes, mitochondrial DNA, and random amplified polymorphic DNA. *Fish Bull* 95: 800-811.

Vierling RA, Xiang Z, Joshi CP, Gilbert ML, and Nguyen HT (1994) Genetic diversity among elite *Sorghum* lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. *Theor Appl Genet* 87: 816-820.

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

Waldman JR, Richards RA, Schill WB, Wirgin I, and Fabrizio MC (1997) An empirical comparison of stock identification techniques applied to stripped bass. *Trans Amer Fish Soc* 126: 369-385.

Welsh J, Petersen C, and 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.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 18: 6531-6535.

Won JH, Rho BJ, and Song JI (1999) Systematic relationships of the urochordates based on partial 18S rDNA sequences. *Korean J Biol Sci* 3: 359-363.

Yoon JM (1999) Genomic polymorphisms of genome DNA by polymerase chain reaction-RAPD analysis using arbitrary primers in rainbow trout. *Korean J Anim Reprod* 23: 303-311.

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