Genetic Comparison Between Crucian Carp (*Carassius auratus* Linnaeus) and Crucian Carp (*C. cuvieri* Temminck and Schlegel)

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붕어(*Carassius auratus* Linnaeus)와 떡붕어(*C. cuvieri* Temminck and Schlegel)의 유전적 비교

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요 약

한국의 예산과 당진에서 각각 채취된 붕어 (*Carassius auratus*)와 떡붕어 (*Carassius cuvieri*)로부터 genomic DNA를 분리 추출하여 반복해서 PCR로 증폭시켰다. 선택된 7개의 RAPD primer를 이용하여 primer 당 total loci, shared loci by each species, polymorphic 및 specific loci를 얻어냈다. 2 종의 붕어로부터 primer와 2지역간에 banding patterns의 복잡성이 두드러지게 나타났다. DNA fragment 의 분자적 크기는 150 bp에서부터 1,600 bp까지 커다란 차이를 나타내었다. 본 연구에서 CCY 붕어 종에서는 458개의 loci가 나타났고, CCD 떡붕어 종에서는 358개의 loci가 확인되었다. 또한 CCY 붕어 종에서는 84개의 polymorphic loci (18.3%)가 확인되었고, CCD떡붕어 종에서는 48개의 polymorphic loci (13.4%)가 확인되었다. CCY 붕어 종에서는 154개의 shared loci 가 나타났으며, 이는 primer당 평균적으로 22개의 loci로 확인되었다. 또한 CCD떡붕어 종에서는 187개의 shared loci 가 확인되었고, 평균해서 primer 당 26.7개의 loci 가 나타났다. CCY붕어 종과 CCD 떡붕어 종의 polymorphic loci는 각각 84개와 48개로 확인되었다. 모든 붕어와 떡붕어 시료의 평균적인 BS value를 기초로 해서 CCY 붕어 종의 similarity matrix를 조사해 본 결과 0.434로부터 0.868까지 나타났고, CCD 떡붕어 종의 값은 0.449로부터 0.924까지 확인되었다. CCY 붕어 종내의 평균적인 BS value는 0.641 ± 0.013 이고, CCD 떡붕어 종내의 BS value의 평균값은 0.684 ± 0.013을 나타내었다. 결과적으로 CCD 떡붕어 종내의 개체의 BS value 평균값이 CCY 붕어 종내의 평균값보다 높게 나타났다. 2 붕어와 떡붕어간의 평균적인 BS value은 0.484 ± 0.007 (0.307～0.682)를 나타내었다. 7개의 primer 를 사용하여 얻어진 dendrogram은 cluster 1 (AURATUS no. 01～AURATUS no. 11), cluster 2 (CUVIERI no. 12～CUVIERI no. 21) 및cluster 3 (CUVIERI no. 22)와 같이 3개의 유전적 클러스터로 나뉘어졌다. CCY 붕어 종내의 8번째 개체 (AURATUS no. 08)와 9번째 개체 (AURATUS no. 09) 사이가 가장 가까운 유전적 관계 (0.064)를 나타내었다. 또한 CCY붕어 종의 11번째(AURATUS no. 11)와 CCD떡붕어 종의 17번째 (CUVIERI no. 17) 사이가 가장 먼 유전적 거리 (0.477)를 나타내었다. 결과적으로 볼 때 한국 및 대서양산 lobster (0.612), 갈치 (0.708), 동자개(0.714)에 비해서 상대적으로 낮은 유전적 거리를 나타내었다.

(**Key words** : *Carassius auratus*, *Carassius cuvieri*, Crucian carp, DNA Polymorphism, Genetic distance)

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Ⅰ. INTRODUCTION

Molecular research methods are efficient for evolutionary, ecological and population genetics studies. PCR-based molecular techniques including restriction fragment length polymorphisms (RFLPs) (Jaiswal et al., 1998), random amplified polymorphic DNAs (RAPD) (Callejas and Ochando, 1998; Kim et al., 2000; Zhou et al., 2000; Yoon and Kim, 2003a; Sharma et al., 2004) and microsatellite of mitochondrial or genomic DNA (Muchmore et al., 1998; Chenyambuga et al., 2004) have been applied to analyze the genetic character of various organisms.

RAPD results also showed that the RAPD technique could be used to identify markers for different cytoplasms used in cytoplasmic male-sterile of sorghum (Jaiswal et al., 1998). Particularly, the polymorphic and/or specific markers specific to the breed, the species, the genus or the geographical populations have been applied for the of individuals and species, hybrid parentage and for the screening of DNA markers for the marker-assisted selection (MAS) and the genotype-assisted selection (GAS) (Liu et al., 1998; Muchmore et al., 1998; Tassanakajon et al., 1998; Huang et al., 2000; Ramesha et al., 2002 Siti Azizah et al., 2005). Until now, polymorphic bands generated by RAPD-PCR using arbitrary primers had good merits for detecting DNA similarity and diversity between life organisms (Gwakisa et al., 1994; Liu et al., 1998; McCormack et al., 2000). Generally, RAPD-PCR is one of fastest and simplest research methods to identify genetic difference and the polymorphism in various organisms that does not require the prior knowledge of the genomic DNA (Welsh et al., 1991; Bommineni et al., 1997; Mamuris et al., 1999; Iyengar et al., 2000 Klinbunga et al., 2000a). The polymorphisms are determined by the banding patterns of amplified products at the specific positions by primers (Tassanakajon et al., 1998; Yoon and Kim, 2001). Thus, RAPD and/or RAPD-based techniques have been applied to the identification of the genetic characteristic of diverse species of teleost and invertebrates (Iyengar et al., 2000; Yoon and Kim, 2003a; Yoon and Kim, 2004; Islam et al., 2005; Siti Azizah et al., 2005).

Under the natural ecosystem, crucian carp is widely distributed in the entire lakes, marshes and rivers in the Korean Peninsula as well as in the several areas in Japan, China, Taiwan, Siberia and the European Continent. In particular, one species of crucian carp (*Carassius auratus*) is an economically important aquacultural species belonging to the family Cyprinidae. The common name, crucian carp in Korea was identified, *Carassius auratus*, by means of morphology and electrophoretic analysis (Nam et al., 1989). The genus *Carassius* can be categorized as a species complex because morphological identification among some member species is problematical and due to genetic variation or morphological differences resulting from environmental influences (Nam et al., 1989). Another species of crucian carp (*Carassius cuvieri*) is native of the Yodogawa River and the Biwakko Lake region, on the west coast of Japan. This fish species has been successfully introduced into the many waters in Korea in the 1970's. Crucian carp is ranked highest among the freshwater fishes in Korea as a game fish attracting millions of anglers owing to the quake of fingertips.

The color of the crucian carp is brownish-blue and yellowish-brown but varies widely according to their environment (Nam et al., 1989). The color, size and the type of the crucian carp varies according to their habitat such as lakes, marshes, rivers, the depth of the water, nutrition etc. As the crucian carp culture industry is increasing considerably, the understanding of the genetics of this fish species to evaluate exactly the patent genetic effects induced by crucian carp production operations. However, little information is known

about the genetics of crucian carp in Korea. Particularly, the clustering analysis of the genetic distance between genera/species/populations of various fishes and invertebrates from the different geographic sites has been performed using RAPD-PCR (Kim et al., 2000; Klinbunga et al., 2000b McCormack et al., 2000; Yoon and Park, 2002). In addition, the genetic variation, the species-specific markers and the region-specific markers in catfish have been assessed by molecular biological methods (Yoon and Kim, 2001). The genetic variation and relatedness among three major river populations of the Indian major carp (*Catla catla*) (Islam et al., 2005), and genetic heterogeneity among five gynogenetic clones of silver crucian carp (*Carassius auratus gibelio* Block) (Zhou et al., 2000), respectively, were analyzed by RAPD markers.

To elucidate the genetic distances and the differences in crucian carp, we performed the clustering analysis of two species of crucian carp (*Carassius auratus* and *Carassius cuvieri*) growing in the Yesan and in the Dangjin. We also analyzed the genetic diversity of these crucian carp species in Korea.

Ⅱ. MATERIALS & METHODS

1. Sample collection and extraction of genomic DNA

Genomic DNA samples were isolated from *Carassius auratus* (crucian carp from Yesan CCY) and *Carassius cuvieri* (crucian carp from Dangjin CCD) collected at Yesan and Dangjin, Korea, respectively. The blood of crucian carp was collected in sterile heparinized vials, placed the tubes on ice immediately, and stored in the refrigerator until use. The RAPD analysis was performed on the blood extract of 22 individuals using seven primers selected from two decades of different decamer primers. The extraction of genomic DNA was performed under conditions as described (Yoon and Park, 2002; Yoon and Kim, 2004). After washing several times, samples of muscle tissues were placed into 10 ㎖ test tubes, to which an 3 volumes of lysis buffer Ⅰ (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA) was added, and the mixture tubes were gently inverted. The precipitates obtained were diffused with lysis buffer Ⅱ (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS). Samples were added 15 μl proteinase K solution (10 mg/ml). After incubation, there was added 300 μl of 6 M NaCl and gently pipetted for a few of min. 600 μl of chloroform was added to the mixture and then inverted (no phenol). The cleared lysates were extracted with 2 volume of ice-cold 70% ethanol, then centrifuged at 19,621g for 5 min, then precipitated. The DNA pellets were incubation-dried for 2 hrs, held at −40℃ until analysis and then dissolved in the TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). The concentration of the extracted genomic DNA was measured with the absorbance ratio at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

2. Decamer primers and PCR amplification

Among 20 primers selected, the seven arbitrarily selected primers (Operon Technologies, Alameda, CA, USA) OPC-11 (5'-AAAGCTGCGG-3'), OPC-14 (5'-TGCGTGCTTG-3'), OPC-18 (5'-TGAGTGGGTG-3'), OPD-02 (5'-GGACCCAACC-3'), OPD-11 (5'-AGCGCCATTG-3'), OPD-15 (5'-CATCCGTGCT-3') and OPD-20 (5'-ACCCGGTCAC-3') generated the total loci observed, shared loci by each species, specific loci and polymorphic loci that can be scored clearly and reproducibly (Tables 1～2). We thus used the primers to identify the genetic polymorphism, diversity and similarity of crucian carp. PCR was performed using two Programmable DNA Thermal Cyclers (Perkin Elmer Cetus, USA; MJ Research, Inc., USA). RAPD-PCR amplification

Table 1. The number of loci observed, number of shared loci by each species, number of specific loci and number of polymorphic loci generated by RAPD analysis using 7 decamer primers in CCY and CCD species

Item	No. of loci observed per primer		No. of shared loci by each species		No. of specific loci		No. of polymorphic loci	
Primer	*C. auratus*	*C. cuvieri*	*C. auratus*	*C. cuvieri*	*C. auratus*	*C. cuvieri*	*C. auratus*	*C. cuvieri*
OPC-11	3.8 (42)	3.4 (37)	22	33	2	4	12	0
OPC-14	7.2 (79)	5.1 (56)	33	22	11	27	25	7
OPC-18	2.1 (23)	2.7 (30)	11	11	7	6	10	13
OPD-02	8.0 (88)	6.2 (68)	11	55	13	9	10	3
OPD-11	8.4 (92)	5.1 (56)	22	11	24	9	12	9
OPD-15	5.7 (63)	5.5 (60)	22	22	32	10	7	8
OPD-20	6.5 (71)	4.6 (51)	33	33	6	2	8	8
Total no.	41.7(458)	32.6(358)	154	187	95	68	84	48
Average no. per primer	65.4	51.1	22	26.7	13.6	9.7	12	6.9

The total number of fragments generated by a primer in crucian carp obtained from Yesan and Dangjin is shown in parentheses.

reactions were performed in volumes of 25 μl contained 10 ng of template DNA, 20 μl premix (Super-Bio Co., Korea) and 1.0 unit primer. Amplification products were separated by electrophoresis in 1.4% agarose gels (SeaKem LE, FMC Bioproducts) with TBE (0.09 M Tris, pH 8.5; 0.09 M borate; 2.5 mM EDTA), using 100 bp DNA Ladder (Bioneer Co., Korea) as DNA molecular weight marker and detected by staining with ethidium bromide. After electrophoresis, gels were stained with ethidium bromide, illuminated with ultraviolet ray, and then photographed by photoman direct copy system (PECA Products, Beloit, WI, USA).

3. Data analysis

The BS value was calculated by the presence/absence of amplified products at the specific positions in the same gel from the RAPD profiles. The values were calculated according to Nei (1987) and Jeffreys and Morton (1987). Comparing the two lanes, the BS was 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 the sample a

Nb : the total number of bands in the sample b.

The average of within-species similarity was calculated by pairwise comparison between individuals within a species. The relatedness among different individuals of CCY species (AURATUS 01～AURATUS 11) and the CCD species (CUVIERI 12～CUVIERI 22) was generated according to the BS values and similarity matrix. A hierarchical clustering tree was constructed using similarity matrices to generate a dendrogram, which was facilitated by the PC-package program Systat version 10 (SPSS Inc., Chicago, IL, USA). Euclidean genetic distances within and between species were also calculated using the Systat hierarchical dendrogram program version 10.

Ⅲ. RESULTS & DISCUSSION

1. Genetic variation of amplified products

Genomic DNA isolated from two crucian carp species obtained from Yesan and Dangjin in the vicinity of the West Sea were amplified at several times by PCR reactions. The amplified products were separated by agarose gel electrophoresis with oligonucleotides decamer primer and stained with ethidium bromide. The complexity of the banding patterns varied dramatically between the primers and two locations (Fig. 1). In this study, seven decamer primers were used to generate a total 458 fragments in the CCY species and 358 fragments in CCD species with the DNA fragment size ranging from 150 to 1,600 bp, as shown in Table 1 and Fig. 1. In particular, the OPD-11 primer gave DNA profiles with more fragments than the other six primers in the CCY species and the OPD-02 from CCD species (Table 1). DNA fragments ranging from 350 to 700 bp were also detected in the RAPD-PCR profile of barramundi (*Lates calcarifer*) (Partis and Wells, 1996). A tandemly repeated satellite DNA containing 290 − 291 base pairs was identified by the *Sal*I digestion of the genomic DNA from five species of Eastern Pacific abalone using another molecular method (genus *Haliotis*) (Muchmore et al., 1998). Eighty fragments ranging from 200 bp to 2,200 bp were unambiguously counted in the black tiger shrimp (*Penaeus monodon*) (Tassanakajon et al., 1998). The size of the fragments varied from 220 bp to 1,700 bp in four species of the Mullidae family (Mamuris et al., 1999). The DNA fragments obtained using the four primers ranged from 100 to 2,300 bp in the brittle star (*Amphiura filiformis*) (McCormack et al., 2000). In wild and cultured species of crucian carp, five primers generated a total of 1,084 distinct fragments ranging in size from 120 to > 4,270 bp (Yoon and Park, 2002). In the marsh clam from Gochang (*Corbicula* spp), 7 out of 20 primers generated 585 major and minor RAPD bands from three geographic sites, producing approximately 6.6 products per primer on average (Yoon and Kim, 2003a). Seven primers generated 317 bands in the cultured shrimp population and 385 in the wild population, ranging 100 to 1,800 base pairs (Yoon and Kim, 2003b). The primer OPF-10 produced 11 amplified fragments in the eastern Pacific yellowfin tuna (*Thunnus albacares*), with sizes ranging from 200 to 600 bp (Diaz-Jaimes and Uribe-Alcocer, 2003). Six primers were used to generate a total of 602 and 195 scorable bands in the catfish and bullhead populations, respectively, with DNA fragments ranging in size from < 100 to > 2,000 base pairs(Yoon and Kim, 2004). In livestock and insects, all 141 primers generated from Zebu cattle breeds ranged from 270 to 1,350 bp (Gwakisa et al., 1994). Geng et al. (2002) also reported that 8 random primers generated 176 to 2,937 bp fragments in 3-goat populations from the China Chaidamu Basin. Finally, twenty-six primers produced a total of 137 polymorphic RAPD markers from the silkworm, ranging from 200 bp to 4,000 bp (*Bombyx mori*) (Hwang et al., 1995). In sorghum, 17 primers amplified from 4 to 12 DNA fragments ranging in size from 500 bp to 2,500 bp (Jaiswal et al., 1998).

In this study, a decamer primer generated an average of 65.4 amplified products in CCY species. A decamer primer generated an average of 3.7 amplified bands per sample, ranging from 2.1 to 8.4 fragments in this species. In the CCY species, the primer OPD-02 generated various sized fragments ranging in size from 200 to 1,600 bp, as shown in Fig. 1D. The oligonucleotide primer OPC-11 generated identical DNA fragments, approximately 300 bp, in the CCY and CCD species, as shown in Fig. 1A. The decamer primer, OPC-18, also generated of total loci observed per primer, approximately 400 bp in size, from the CCY and CCD species, as shown in Fig. 1C. In addition, the other primers did not generate identical sized fragments from the CCY and CCD species. This demonstrates

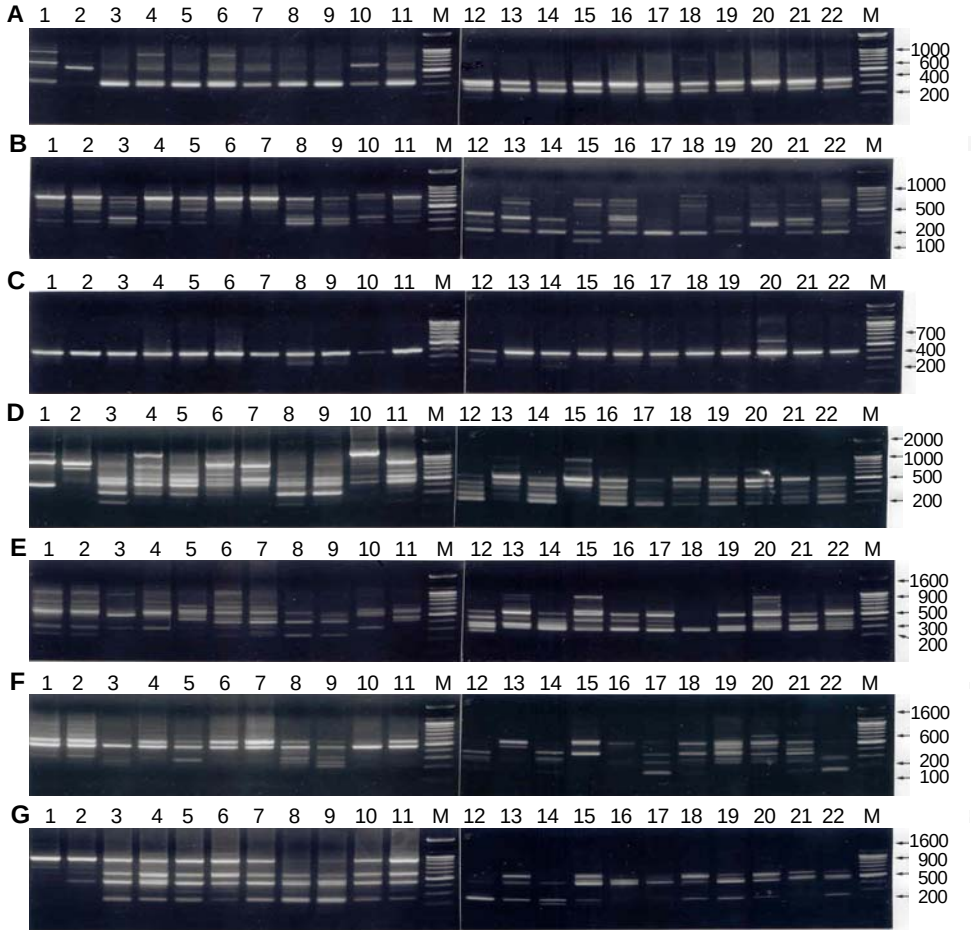

Fig. 1. RAPD-PCR-generated electrophoretic profiles. Each lane shows DNA sample of 22 individuals. DNA isolated from Yesan (lane 1～11) and Dangjin (lane 12～22) were amplified by decamer primer OPC-11 (A), OPC-14 (B), OPC-18 (C), OPD-02 (D), OPD-11 (E), OPD-15 (F) and OPD-20 (G). A 100 bp DNA Ladder (M) was used as size standard.

that the CCY species is genetically different from the CCD species. In the CCY species, the oligonucleotide decamer primer, OPC-14, generated 33 shared loci, approximately 300 bp, 500 bp and 700 bp in size, respectively, as shown in Fig. 1B. This indicates that the genome sizes of the CCY species were similar to the blue catfish (Liu et al., 1998), the black tiger shrimp (Tassanakajon et al., 1998), catfish (Yoon and Kim, 2001), the penaeid shrimp (Yoon and Kim, 2003b), and the bullhead (Yoon and Kim, 2004). The number of fragments generated per primer in three endemic Spanish barbel species (*Barbus bocagei*, *B. graellsii* and *B. sclateri*) varied from 17 to 30, with a mean of 24.2 bands per individual and primer (Callejas and Ochando, 1998). It was reported that a primer generates 9 to 15 distinct bands in the black tiger shrimp (Tassanakajon et al., 1998). The number of scored bands varied from 7 to 12 per primer in four species of the Mullidae family (Mamuris et al., 1999). The primers generated 36, 32 and 24 bands in mud crabs from

Eastern Thailand (genus *Scylla*) (Klinbunga et al., 2000b). Fifty-three RAPD markers obtained from 7 primers were common to all breeds, 22 were specific to individuals, and 18 were polymorphic in the different breeds of zebu cattle (Ramesha et al., 2002). Four of the fragments were polymorphic for all samples in eastern Pacific yellowfin tuna (*Thunnus albacares*) (Diaz-Jaimes and Uribe-Alcocer, 2003).

Moreover, in the CCD species, the common banding patterns, 280 bp, 300 bp and 350 bp fragments, were generated using the decamer primer, OPC-11, as shown in Fig. 1A. The banding patterns generated by the decamer primers, OPC-14 and OPD-15, of individual CCD species varied widely, as shown in Figs. 1B and 1F. The complexity of the banding pattern showed large variations between primers and/or the geographically locale. Generally, the size and the number of fragments generated depends on the nucleotide sequence of the primer used as well as on the source of the template DNA, resulting in a genome-specific DNA fragment (Welsh and McClelland, 1990; Welsh et al., 1991).

Here, 458 fragments were identified in the CCY species and 358 in the CCD species: 84 polymorphic fragments (18.3%) in the CCY species and 48 (13.4%) in the CCD species (Table 1). 154 shared loci by each species, the average 22 per primer, were observed in the CCY species and 187 loci observed, the average 26.7 per primer, in the CCD species. The number of specific loci in the CCY and CCD species was 95 and 68, respectively. The oligonucleotide decamer primer OPC-18 generated the identical DNA fragments, approximately 400 bp, in the *C. auratus* species as well as in the CCD species (Fig. 1C). These results demonstrate that the primer detected a large amount of polymorphic fragments, suggesting that the genetic variation in the CCY is higher than in the CCD species. Islam et al. (2005) reported that 32 fragments were yielded from four primers in the Indian major carp (*Catla catla*) of which 24 (75%) were polymorphic. A total of 88 polymorphic fragments were scored from 24 primers after excluding the bands that were monomorphic for the five clones of silver crucian carp, *Carassius auratus gibelio* Block (Zhou et al., 2000).

This study identified 700 RAPD-PCR-amplified specific fragments (lanes 13, 15, 16, 18, 19 and 20) and 1,000 bp fragments (lanes 13, 15 and 19) in the CCD species. Twenty-seven specific fragments generated by the decamer primer, OPC-14, also showed inter-individual-specific characteristics and DNA polymorphisms, as shown in Fig. 1B. In particular, the 400 bp bands produced by the primer, OPC-18, were identified in two species, which were used to identify the populations and/or species, as shown in Fig. 1C. The specific primer was found to be useful for identifying individuals and/or populations, by determining the different DNA polymorphisms among the individuals/ population (Liu et al., 1998; Yoon and Park, 2002 Yoon and Kim, 2003b Yoon and Kim, 2004; Siti Azizah et al., 2005). 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). Zhou et al. (2000) reported that the RAPD markers identified will likely benefit evolutionary genetics and selective breeding studies. The RAPD method was used to generate fingerprint patterns for 10 meat species: wild boar, pig, horse, buffalo, beef, venison, dog, cat, rabbit and kangaroo (Koh et al., 1998). The advantages and disadvantages of using RAPD-PCR for identifying red meat species were also discussed. RAPD for species identification is likely to be used as a rapid and qualitative way for meat speciation.

Geng et al. (2002) reported that a RAPD marker

should be more effective in analyzing the genetic relationship of 3 goat populations. Although the main disadvantage of the RAPD method is its reproducibility, this method was considered to be suitable for species identification. Three out of 40 nanomer primers, in which the amplified DNA fragments specific to a grass species or to a durum cultivar, were identified (Bommineni et al., 1997). Similarly, the primer PR23 amplified the fragments specific to a grass species. The amplified fragments ranging in size from 1,000 to 1,200 bp were found to be specific to *Thinopyrum junceiforme*. The DNA fragment, approximately 1.5kb in size, was specific to *Lophopyrum elongatum*. Generally, the polymorphic fragments generated by RAPD-PCR using arbitrary primers are suitable for detecting genetic similarity/ diversity/polymorphisms among various organisms (Welsh et al., 1991; Bommineni et al., 1997; McCormack et al., 2000; Ramesha et al., 2002 Islam et al., 2005).

2. Variation within and between species, bandsharing values,and genetic distances

In this study, the BS value based on the presence or absence of amplified fragments was used to calculate the similarity indices, as illustrated in Table 2. The similarity matrix, which was based on the average BS value of all the samples, ranged from 0.434 to 0.868 in the CCY species and 0.449～0.924 to the CCD species. The average BS value within the CCY species and CCD species was 0.641 ± 0.013 and 0.684 ± 0.013, respectively. The BS value between the two crucian carp species ranged from 0.307 to 0.682 with an average of 0.484 ± 0.007. The BS value between individuals No. 09 and No. 16 was the highest (0.682) between the two crucian carp species. The value between individuals No. 03 and No. 18 was the lowest (0.307). Compared separately, the BS values of the individual CCD species were higher than those from CCY species. These BS values between the two crucian carp species are different from those reported previously (Yoon and Park, 2002). The average BS value obtained using the five random primers was 0.40 ± 0.05 in the wild crucian carp species and 0.69 ± 0.08 in the cultured crucian carp species. The average BS value of this study is lower than that the between the species common carp and the Israeli carp (0.57 ± 0.03) (Yoon, 2001), bullhead population (0.504 ± 0.115) (Yoon and Kim, 2004), Spanish barbel species (0.71～0.81) (Callejas and Ochando, 1998), zebu cattle breeds (0.73～0.79) (Gwakisa et al., 1994), and indigenous cattle breeds (0.92) (Sharma et al., 2004). In contrast, the average BS value of this study is higher than that of turkey lines (0.202～0.230) (Ye et al., 1998).

The average genetic difference in the CCY and CCD species was approximately 0.359 ± 0.013 and 0.316 ± 0.013, respectively. The average genetic difference of the CCY species was higher than that of CCD species. The average genetic difference between the two crucian carp species was approximately 0.516 ± 0.007. The difference between the two crucian carp species was statistically significant. Accordingly, as stated above, RAPD-PCR analysis showed that the CCY species was more separated from the CCD species.

Hierarchical clustering analysis was performed to obtain the dendrogram based on the similarity matrix generated by the BS values and genetic distances, as shown in Fig. 2. The dendrogram obtained using the seven primers, indicated three genetic clusters: cluster 1 (AURATUS no. 01, 02, 03, 04, 05, 06, 07, 08, 09, 10 and 11), cluster 2 (CUVIERI no. 12, 13, 14, 15, 16, 17, 18, 19, 20, 21) and cluster 3 (CUVIERI no. 22). The genetic distance between the two crucian carp species ranged from 0.064 to 0.596, as shown

in Fig. 2. The individual AURATUS no. 02 from the CCY species was genetically similar to AURATUS no. 01 from the CCY species (genetic distance = 0.068). The shortest genetic distance displaying a significant molecular difference was between the individuals, CUVIERI no. 21 and CUVIERI no. 13, from the CCD species (0.068). The longest genetic distance showing significant molecular differences between two crucian carp species was observed in individuals CUVIERI no. 17 and AURATUS no. 11 (0.477). The individual, CUVIERI no. 17, of the Dangjin species was distantly related to CUVIERI no. 22 from Dangjin (genetic distance = 0.596). The genetic distances between breeds confirmed the existence of a high degree of genetic similarity between two breeds of indigenous cattle (Rathi and Tharparkar) (Sharma et al., 2004). Cluster analysis showed a similar pattern to that illustrated by Yoon and Kim (2004). They reported that single linkage cluster analysis, which indicated four genetic groupings, and the dendrogram revealed a close relationship between the individual identities within two geographical populations. The values of the pairwise comparisons of unbiased genetic distance between the populations of the Indian major carp (*Catla catla*) from the combined data for the four primers, ranged from 0.025 to 0.052 (Islam et al., 2005). They reported that the Padma and the Jamuna populations were separated from each other with the lowest genetic distance (D = 0.025).

In other fish and invertebrates, cluster analysis of the pairwise species matrix generated from RAPD data showed that geographically close populations of blacklip abalone tended to cluster together (Huang et al., 2000). A phylogenetic tree was constructed using UPGMA cluster analysis based on a total of 3,744 distinguishable fragments in gynogenetic clones from the silver crucian carp, *Carassius auratus gibelio* Block (Zhou et al., 2000). Clones A and P were the most closely related, whereas the most divergence

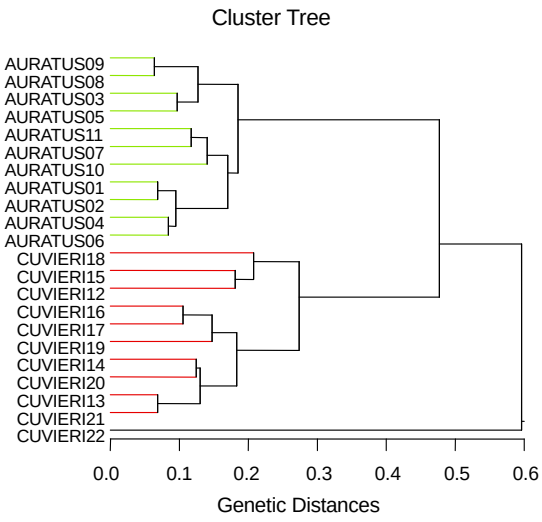

Fig. 2. Hierarchical dendrogram of genetic distances obtained from two crucian carp species. The relatedness among different individuals of CCY species (AURATUS 01～AURATUS 11) and the CCD species (CUVIERI 12～CUVIERI 22) was generated according to the bandsharing values and similarity matrix as in table 2. A hierarchical clustering tree was constructed using similarity matrices to generate a dendrogram, which was facilitated by the PC-package program Systat version 10.

was observed between clone D and clones E or F. The inter-population similarity indices and genetic distance values indicated that the Jamuna-Padma population pair of the Indian major carp (*Catla catla*) was genetically closer than the Halda-Jamuna and the Halda-Padma population pairs, which agreed with the geographical distances between them (Islam et al., 2005). They suggested that the RAPD technique could be used to discriminate between different river populations of major carp. A neighbor-joining tree based on the genetic distance between populations using the RAPD-PCR method indicates the relationships between three

mud crab species (Klinbunga et al., 2000b). They showed that there were large genetic differences between geographically separated populations within a species and between species. The two phylogenetic trees resulting from the neighbor-joining and parsimony analyses showed the same topology in distinguishing the Mullidae species (Mamuris et al., 1999).

Phylogenetic relationships were assessed using the neighbor-joining and maximum parsimony methods in killifish, *Fundulus parvipinnis* (Bernardi and Talley, 2000). The samples partitioned in two major clades at a higher level. These two clades were quite robust (99 to 100% of the bootstrap replicates) and were genetically distant (average sequence divergence 5.8%). The phylogenetic relationships among the 5 *Haliotis* species and one hybrid were carried out by calculating the distance coefficient and constructing a phylogenetic tree based on the RAPD data (Kim et al., 2000). Consequently, they reported that RAPD analysis is a powerful tool for determining the phylogenetic relationship between 6 *Haliotis* species.

In this study, RAPD analysis revealed a significant genetic distance between the two crucian carp species pairs. This shows that this method is an adequate tool for comparing the DNA in individuals, species and populations. Furthermore, the basic knowledge of DNA polymorphisms and the molecular markers of the crucian carp (genus *Carassius*) might make a significant contribution to a broodstock selection and selective fish-breeding program.

The identification of the black tiger shrimp (*Penaeus monodon*), bullhead (*Pseudobagrus fulvidraco*), and eel-loach (*Pangio* spp.) populations is essential for invertebrate/teleost breeding programs (Tassanakajon et al., 1998; Yoon and Kim, 2004; Siti Azizah et al., 2005). The classification of the crucian carp species is based on the morphological variation in the head type, body size, body type, body color, fin type and eye type. Siti Azizah et al. (2005) identified the RAPD markers using themorphology and external features in wild and cultured populations of eel-loach (*Pangio* sp.). As stated above, the potential of RAPD in determining the diagnostic markers for the breed, line, stock, species and geographic population identification in teleost (Mamuris et al., 1999; Diaz-Jaimes and Uribe-Alcocer, 2003), in shellfish (Tassanakajon et al., 1998; McCormack et al., 2000; Yoon and Kim, 2003b), in silkworm (Hwang et al., 1995), in livestock (Jeffreys and Morton, 1987; Gwakisa et al., 1994; Koh et al., 1998; Sharma et al., 2004) andin poultry (Ye et al., 1998) has also been demonstrated.

Nevertheless, further analysis using more individuals, primers and species will be needed to fully establish the specificity of the loci to particular taxa and the subsequent inter-specific gene flow in the genus *Carassius*. Further sampling sites will also be necessary to determine the precise area where the phylogeographic break occurs. Further studies involving a large number of samples and primers need to be performed in order to obtain more precise information about the genetic structure of the two species of crucian carp. The ploidy levels of the samples will also be necessary in the native Korean species of genus *Carassius*. Future work will need to obtain a more profound and further assessment of the genetic relationships between species for the crucian carp based on the various morphological traits, RFLP, AFLP and microsatellite markers.

Ⅳ. ABSTRACT

Genomic DNA isolated from two crucian carp species obtained from Yesan (*Carassius auratus*) and Dangjin (*Carassius cuvieri*) in Korea were amplified at several times by polymerase chain reaction (PCR). The amplified products were separated by agarose gel electrophoresis (AGE) with oligonucleotides decamer primer and stained

with ethidium bromide. The seven arbitrarily selected primers OPC-11, OPC-14, OPC-18, OPD-02, OPD-11, OPD-15 and OPD-20 generated the shared loci by each species, the polymorphic and specific loci. The seven primers generated the total 458 loci that can be scored from the crucian carp obtained in *C. auratus* species. 358 fragments were generated from the species obtained in *C. cuvieri* species. The size of DNA fragments varies from 150 to 1,600 bp. The complexity of the banding patterns varies dramatically between the primers and two locations. In this study, 458 loci were identified in the crucian carp species from Yesan and 358 in the crucian carp species from Dangjin: 84 polymorphic loci (18.3%) in the *C. auratus* species and 48 (13.4%) in the *C. cuvieri* species. 154 shared loci by each species, the average 22 per primer, were observed in the *C. auratus* species and 187 loci, the average 26.7 per primer, in the Dangjin species. Based on the average bandsharing (BS) values of all samples, the similarity matrix ranged from 0.434 to 0.868 in the *C. auratus* species and from 0.449 to 0.924 in the *C. cuvieri* species. The average BS value was 0.641 ± 0.013 within the *C. auratus* species and 0.684 ± 0.013 within the *C. cuvieri* species. The average BS value between two crucian carp species 0.484 ± 0.007, ranged from 0.307 to 0.682. The BS value between the individual No. 09 and No. 16 was 0.682, which was the highest between two crucian carp species. Compared separately, the BS value of individuals within the *C. cuvieri* species was higher than the *C. auratus* species. The dendrogram obtained by the seven primers, indicates three genetic clusters: cluster 1 (AURATUS No. 01, 02, 03, 04, 05, 06, 07, 08, 09, 10 and 11), cluster 2 (CUVIERI No. 12, 13, 14, 15, 16, 17, 18, 19, 20 and 21) and cluster 3 (CUVIERI no. 22). The shortest genetic distance displaying significant molecular difference was between the individual AURATUS No. 09 and AURATUS No. 08 from Yesan (genetic distance = 0.064). The longest genetic distance displaying significant molecular differences was between the individual CUVIERI No. 17 and AURATUS No. 11 between two crucian carp species (0.477). RAPD-PCR analysis has revealed the significant genetic distance between two crucian carp species pairs.

(Key words : *Carassius auratus*, *Carassius cuvieri*, Crucian Carp, DNA Polymorphism, Genetic Distance)

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