

Genetic Similarity and Variation in the Cultured and Wild Crucian Carp (*Carassius carassius*) Estimated with Random Amplified Polymorphic DNA

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ABSTRACT : Random amplified polymorphic DNA (RAPD) analysis based on numerous polymorphic bands have been used to investigate genetic similarity and diversity among and within two cultured and wild populations represented by the species crucian carp (*Carassius carassius*). From RAPD analysis using five primers, a total of 442 polymorphic bands were obtained in the two populations and 273 were found to be specific to a wild population. 169 polymorphic bands were also produced in wild and cultured population. According to RAPD-based estimates, the average number of polymorphic bands in the wild population was approximately 1.5 times as diverse as that in cultured. The average number of polymorphic bands in each population was found to be different and was higher in the wild than in the cultured population. Comparison of banding patterns in the cultured and wild populations revealed substantial differences supporting a previous assessment that the populations may have been subjected to a long period of geographical isolation from each other. The values in wild population altered from 0.21 to 0.51 as calculated by bandsharing analysis. Also, the average level of bandsharing values was 0.40 ± 0.05 in the wild population, compared to 0.69 ± 0.08 in the cultured. With reference to bandsharing values and banding patterns, the wild population was considerably more diverse than the cultured. Knowledge of the genetic diversity of crucian carp could help in formulating more effective strategies for managing this aquacultural fish species and also in evaluating the potential genetic effects induced by hatchery operations. (*Asian-Aust. J. Anim. Sci.* 2002. Vol 15, No. 4 : 470-476)

Key Words : Crucian Carp, Cultured, Wild, Genetic Similarity, Variation, Bandsharing

INTRODUCTION

Isozyme electrophoresis (Smith et al., 1997; Cagigas et al., 1999), restriction fragment length polymorphism (RFLP) (Hallerman and Bekmann, 1988) and microsatellites (Huang et al., 2000) etc. have been so far used to analyze genetic similarity and diversity in genetics and breeding research of fish/invertebrates. Also, molecular markers from random amplified polymorphic DNA (RAPD) have recently been used to evaluate genetic diversity and/or similarity in several organisms (Cagigas et al., 1999; Bartish et al., 2000; Huang et al., 2000; Lumaret et al., 2000; Mohd-Azmi et al., 2000; Hwang et al., 2001). Particularly, markers from 1,357 individuals from 47 populations collected at three sampling dates for 16 sites were used to measure genetic diversity within and among populations (Mengistu et al., 2000). In addition, microsatellite DNA variation was investigated in wild populations and farmed strains of turbot (Coughlan et al., 1998) or Chinese pig (Li et al., 2000) from various sites. Except for a few studies using RAPD methods (Coughlan et al., 1998; Cagigas et al., 1999), most of these studies were investigated by techniques other than RAPD. Also, many genetic techniques have been applied in fish species other

than crucian carp.

Crucian carp is a commercially important warmwater fish species that occurs throughout the northeastern Asia region in countries such as Taiwan, Japan, China and Korea. The individuals of this fish species have decreased significantly owing mainly to imprudent development and reckless fishing during the last two decades. Also, for the last ten years there have operated projects for stocking stream, lake or sea with various species of fishes/invertebrates. In spite of the economic and scientific importance, little information is available on the beneficial or deleterious effects of the stocking projects. Accordingly, as a consequence of the rapid increase in hatchery-reared crucian carp production, there is a need to understand the genetic composition of natural crucian carp populations in order to evaluate the potential genetic effects induced by hatchery operations.

In spite of their economic and scientific importance, little information is available on the genetic relationships among the few crucian carp populations in Korea. RAPDs are among the most frequently used molecular markers for taxonomic and systematic analyses of organisms (Bartish et al., 2000). Therefore, the purpose of this study was to determine the genetic population structure based on genetic similarity and diversity by RAPD, and to look into the utility of random amplified polymorphic DNA in further studies of two crucian carp populations.

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MATERIALS AND METHODS

Blood samples

One-year-old crucian carp were obtained from an aquaculture facility (n=50) and a lake (n=50) in the periphery of Kunsan. RAPD analysis was performed with DNA samples from a total of 100 crucian carp using twelve different random primers. Fishes ranged in size from 50 to 90 g (average 75 g) body weight. Blood samples from fish anaesthetized with MS 222 (100 ppm) were taken from the caudal vein into heparinized vials. The blood obtained was refrigerated at 4°C until use.

Genomic DNA extraction

In order to achieve reproducible results, DNA extraction should be performed with highest quality reagents (Vierling et al., 1994) and according to standard procedures with only minor modification.

Samples of whole blood were taken into 10 ml heparinized vials, to which 4 volumes of lysis buffer I (155 mM NH₄Cl, 10 mM KHCO₃, 1 mM EDTA) was added and mixed gently by inverting the tube several times. The samples were incubated on ice for 30 min and centrifuged at 1,750×g for 10 min at 4°C to get a pellet. The supernatants were decanted and the pellets were resuspended with lysis buffer. The mixtures were transferred to 1.5 ml Eppendorf tubes, and centrifuged at 22,388×g for 1 min. The precipitates were diffused with 0.8 ml lysis buffer II (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS). The aqueous phase were transferred to fresh 1.5 ml Eppendorf tubes, 15 µl proteinase K solution (10 mg/ml) added, and again incubated at 37°C for overnight or 65°C for 4 h.

The mixtures were homogenized properly with the tip of micropipette after 0.3 ml of 6 M NaCl and then 0.6 ml of chloroform (no phenol) were added. Samples were spun down at 22,388×g for 5 min. The cleared lysates were extracted with 2 volume of chilled 70% ethanol, centrifuged at 22,388×g for 5 min, and then precipitated. The DNA pellets were air-dried for 30 min, and dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The purity and concentration of DNA was measured with the absorbance ratio by a spectrophotometer (Beckman DU 600 series).

Primer, marker and amplification conditions

The primers, designed and chosen arbitrarily for these experiments, were obtained from Operon Technologies, USA (table 1). All of these decamer random primers had G+C content in the range 60-70%. The genomic DNA were amplified using PCR with twelve 10-base primers (5' to 3') in a DNA thermal cycler (Perkin Elmer Cetus, USA). Experiments were conducted using varying concentration of template DNA, MgCl₂, dNTP and Taq DNA polymerase to get reproducible amplification and discernible band patterns. The amplification reaction was undertaken in a 20 µl volume of reaction tube containing 10 ng of template DNA, 20 µl AccuPower premix (Bioneer Co., Korea) and 1.0 unit primer (Operon Technologies, USA). The mixture was followed by pre-denaturation at 94°C for 5 min. The thermal cycler programmed for 45 cycles at 94°C for 1 min for denaturation, 36°C for 1 min for annealing, 72°C for 1 min for extension and 72°C for 5 min for post-extension, was used with fastest available transition between each temperature. Amplified products were separated by electrophoresis with X174 DNA/Hae III marker (Promega Co., USA) and lambda DNA marker (Bioneer Co., Korea) in 1.4% agarose gels with ethidium bromide and TBE (0.09 M Tris, pH 8.5, 0.09 M borate, 2.5 mM EDTA) and photographed by photoman direct copy system (Seoulin Co., Korea) under UV illumination.

Analytical method

Bandsharing (BS) scores of DNA products generated were calculated according to the procedures of Jeffreys and Morton (1987) and Mohd-Azmi et al. (2000) with minor modification. If the comparison was between the three lanes, the formula equation would be: $BS=3(N_{abc})/(N_a+N_b+N_c)$ and so on, where: N_{abc} is the number of bands shared by individuals a, b and c, N_a is the total number of bands for individual a, N_b is the total number of bands for individual b and N_c is the total number of bands for individual c. Bands which were readily visible were scored.

RESULTS

Intra-population and inter-population variations

A few DNA changes observed by PCR-RAPD method

Table 1. Basic sequences of 12 selected random primers used in the RAPD analysis

Primer	Sequence (5' to 3')	GC content (%)	Primer	Sequence (5' to 3')	GC content (%)
OPA-01	CAGGCCCTTC	70	OPA-07	GAAACGGGTG	60
OPA-02	TGCCGAGCTG	70	OPA-08	GTGACGTAGG	60
OPA-03	AGTCAGCCAC	60	OPA-09	GGGTAACGCC	70
OPA-04	AATCGGGCTG	60	OPA-10	GTGATCGCAG	60
OPA-05	AGGGGTCTTG	60	OPA-11	CAATCGCCGT	60
OPA-06	GGTCCCTGAC	70	OPA-12	TCGGCGATAG	60

were detected in both cultured and wild crucian carp. There were shown genomic DNA polymorphic bands generated using five informative and reliable primers to amplify DNA. For RAPD analysis, five primers were used generating a total of 1084 scorable bands in wild and cultured populations, ranging in size from 120 to 4,270 base pairs (bp).

The specific minor band patterns produced by random primer OPA-02, which showed DNA polymorphism, were in the range of 0.19 to 0.28 kb (figure 1). Adversely, the

identical band patterns of PCR-RAPD products were observed from 0.23 to less than 0.60 kb. The identical bands from 0.28 to less than 0.60 kb generated by random primer OPA-03 were also observed in figure 2. Especially, although this primer showed a few dense bands in comparison with other primers, there were shown a great variety of specific minor bands. The similar minor band patterns of PCR-RAPD products generated by random primer OPA-05 that were observed ranged from 0.23 to 1.35 kb in the cultured population (figure 3). This primer

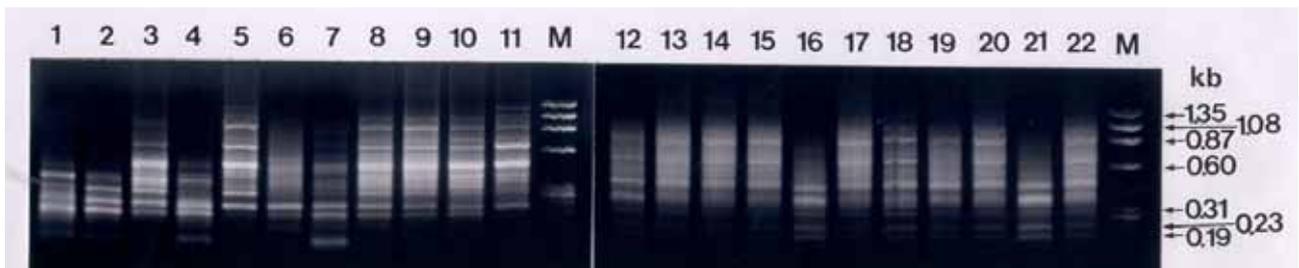


Figure 1. RAPD profiles of crucian carp (*Carassius carassius*) amplified by arbitrary primer OPA-02 (TGCCGAGCTG). Amplification products were electrophoresed on a 1.4% agarose gel with TBE (0.09 M Tris, pH 8.5, 0.09 M boric acid, 2.5 mM EDTA) and detected by staining with ethidium bromide. The gels were illuminated with UV light and taken photographs by photoman direct copy system. Each lane shows different individual DNA samples consisting of wild population (No. 1-11) and cultured (No. 12-22). M: Molecular size standard (X174 DNA marker digested with *Hae* III).



Figure 2. Specific RAPD patterns of crucian carp generated by arbitrary primer OPA-03 (AGTCAGCCAC). Each lane shows different individual DNA samples containing wild population (No. 1-11) and cultured (No. 12-22). M: X174 DNA marker digested with *Hae* III.

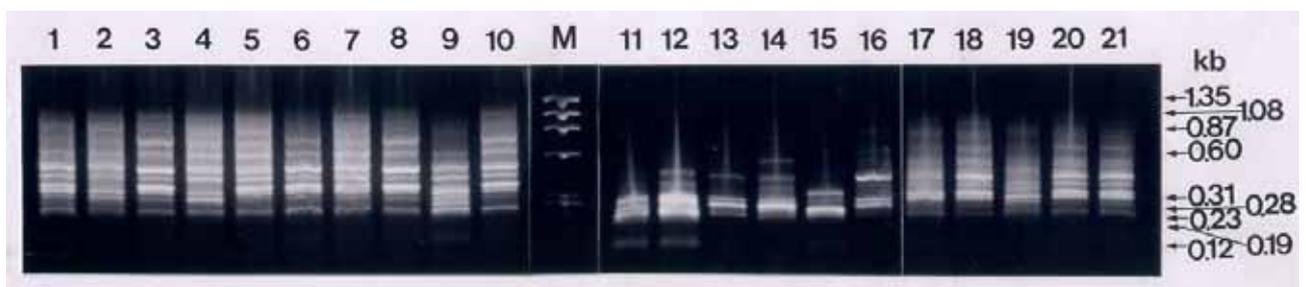


Figure 3. Individual specific RAPD patterns in crucian carp amplified by arbitrary OPA-05 (AGGGGTCTTG). Each lane shows different individual DNA samples including cultured population (No. 1-10) and wild (No. 11-21). M: Molecular size marker (X174 DNA marker digested with *Hae* III).

detected two specific RAPD minor bands in 0.12 kb, which were polymorphic.

The between-population variations were revealed in the band patterns generated by random primer OPA-07 in the range of 0.28 to 4.27 kb (figure 4). Five single patterns, which were polymorphic (lane 12, 13, 15, 17 and 19), were obtained for primer OPA-07. The RAPD profiles obtained by a primer with pooled DNAs of individuals were somewhat different, which exhibited the inter-population-specific characteristic. The common band in the molecular weight in approximately 0.23 kb was also observed. The common bands from 0.31 to less than 0.60 kb were present in every individual (figure 5). These banding patterns showed a species characteristic of this fish species compared with the other fish species.

In the wild population, five primers yielded a total of 467 different amplified products, out of a total of 1084 bands in two populations (table 2). An average of 8.4 bands per lane were observed in the wild population. There was shown the most fragments for OPA-02 than any other primers, even if there was observed the least for OPA-07. Overall, primer OPA-02, OPA-03, OPA-05, OPA-07 and OPA-12 generated an average of 14.1, 7.6, 9.3, 4.8 and 6.2

fragments, respectively, as summarized in table 2. In comparison with the banding patterns observed in the wild population, an average of 11.5 bands per lane (range 9.0 to 15.4) was observed in the cultured population, giving a total of 617 different fragments (table 2).

Bandsharing values

The average level of bandsharing obtained by the five random primers used was 0.40 ± 0.05 in the wild population (table 3); values ranged from 0.21 to 0.51 and were lowest (0.21) in the primer OPA-07. In the cultured population average bandsharing scores of the primers OPA-02, OPA-03, OPA-05, OPA-07 and OPA-12 were 0.92, 0.67, 0.81, 0.43 and 0.64 respectively (table 3). Also in the cultured carp the average of the bandsharing values, $\pm SE$, was 0.69 ± 0.08 , with a total of 3.47. These primers yielded 442 polymorphic bands, with 273 and 169 bands in the wild and cultured populations respectively (table 3).

The RAPD profiles obtained from individuals of the two populations were found to differ in bandsharing value. The average percentage of poly-morphic markers was much higher in the wild population, compared to the cultured population, although the total and average of band numbers



Figure 4. PCR-RAPD products of crucian carp generated by arbitrary OPA-07 (GAAACGGGTG). Each lane shows different individual DNA samples consisting of cultured population (No. 1-11) and wild (No. 12-23). M: Molecular size standard (X174 DNA marker digested with *Hae* III).

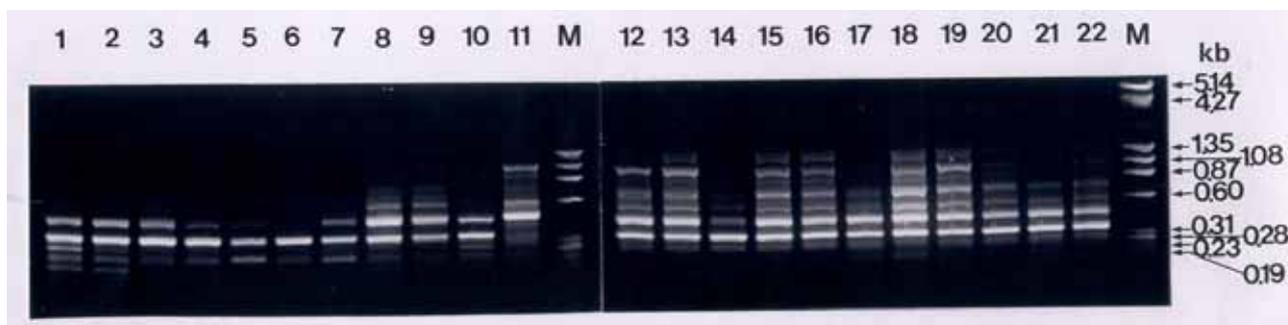


Figure 5. RAPD profiles amplified by arbitrary primer OPA-12 (TCGGCGATAG) in crucian carp. Each lane shows different individual DNA samples containing wild population (No. 1-11) and cultured (No. 12-22). M: Molecular size standard markers (X174 DNA marker digested with *Hae* and lambda DNA marker digested with *Eco*RI + *Hind* III).

Table 2. Number of RAPD bands obtained with five decamer primers from populations of wild and cultured crucian carp

Primers	Number of bands		Total bands		Average	
	Wild	Cultured	Wild	Cultured	Wild	Cultured
OPA-02	10-21	14-18	155	169	14.1	15.4
OPA-03	5-10	9-12	84	104	7.6	9.5
OPA-05	5-13	13-14	102	139	9.3	13.9
OPA-07	1-15	4-12	58	99	4.8	9.0
OPA-12	2-12	6-11	68	106	6.2	9.6
Total			467	617	42	57.4
Average			93.4	123.4	8.4	11.5

Table 3. Bandsharing (BS) within the crucian carp from wild and cultured populations (mean or mean±S.E)

Primers	Total of polymorphic bands		Average number of polymorphic bands		BS values	
	Wild	Cultured	Wild	Cultured	Wild	Cultured
OPA-02	86	14	8.8	1.3	0.45	0.92
OPA-03	41	34	3.7	3.1	0.51	0.67
OPA-05	63	26	5.7	2.6	0.38	0.81
OPA-07	46	57	3.8	5.2	0.21	0.43
OPA-12	37	38	3.4	3.5	0.46	0.64
Total	273	169	25.4	15.7	2.01	3.47
Average	54.6	33.8	5.1	3.1	0.40±0.05	0.69±0.08

were generally much more in the cultured. According to RAPD-based estimates, the average number of polymorphic bands in the wild population was approximately 1.5 times as diverse as that in cultured.

DISCUSSION

In this study, the genetic similarity and diversity of crucian carp for cultured and wild populations were analysed by random amplified polymorphic DNA. Efficient methods to clarify the taxonomic status of both the wild population and the cultivated have been applied to various organisms (Coughlan et al., 1998; Cagigas et al., 1999; Bartish et al., 2000; Lumaret et al., 2000). These works identified the difference in the degree of genetic variability between the cultured and wild population; significant genetic heterogeneity was found between wild and farmed samples in turbot (*Scophthalmus maximus*) from each country (Coughlan et al., 1998). The phylogenetic relationships and differentiation among and within plant populations (*Chaenomeles*) were estimated with RAPDs and isozymes (Bartish et al., 2000). It was reported that populations of two species were considerably more diverse than other populations. Chloroplast DNA variations were studied in the cultivated and wild olive taxa of the genus *Olea* L. (Lumaret et al., 2000). They reported that five length polymorphisms were identified from restriction analysis. The tropical species from central-western Africa also showed a chlorotype that differed substantially from those of the other four *Olea* taxa growing in southern Africa.

From all the banding patterns obtained in crucian carp

with primers, the banding pattern of the wild population were distinguished from that of cultured and there were identified a variety of the patterns. In this study, as summarized in table 2, five primers yielded a total of 1084 reliable bands in wild and cultured populations, which ranged in size from approximately 120 to 4,270 base pairs (bp). This indicated that the genome size of crucian carp was similar to that of blue catfish analysed by Liu et al. (1998). Generally, the size and number of the fragments produced strictly depended upon the nucleotide sequence of the primer used and upon the source of the template DNA.

An average of 8.4 bands per lane were observed, giving a total of 467 different fragments in the wild population (table 2). Additionally, as compared to banding patterns observed in the wild population, an average of 11.5 bands per lane in cultured carp were observed, giving a total of 617 different fragments with a range of 9.0 to 15.4 (table 2). The most fragments were for OPA-02 primer and the least number was for OPA-07.

The RAPD analysis using five primers showed a total of 442 polymorphic bands in the two populations of which 273 were specific to the wild population (table 3). Over all, the average number of polymorphic bands differed between the populations and was higher in the wild than in the cultured carp. Contrasting genetic diversity estimates were obtained for the wild population on one hand and for the cultured on the other hand. In general, polymorphisms were scored from the presence/absence of the band pattern of amplified products at specific positions expressed by various decamer primers (Smith et al., 1997).

Most of the total variability could be attributed to the

between-population differentiation in view of the bandsharing values and banding patterns. On the other hand, the common bands generated by specific random primers, which were present in every individual, seem to show species characteristics of this fish species compared with the other fish species. In this study, as shown in table 3, the average level of bandsharing values with SE was 0.40 ± 0.05 in the wild population and 0.69 ± 0.08 in the cultured. The latter value, with 1.5 times higher relative differences than in the cultured population, may indicate higher variability in the wild population. Accordingly, wild population was considerably more diverse than cultured population. Bandsharing analysis based on RAPD data thus showed that the wild population was distinctly differentiated from the cultured population.

Point Cool population was clearly separated from the two other central populations by analysis method derived from RAPD data (Huang et al., 2000). In that study, all three types of DNA markers generated by RAPD, minisatellite and microsatellite, revealed significant subdivision in the blacklip abalone populations along the coastline in Australia. Also, reduced variability in cultured strains has been described in other cultivated fish species (Bouza et al., 1997). However, no statistically significant reductions were found in mean heterozygosity in farmed strains compared with wild populations (Coughlan et al., 1998). They reported that genetic differentiation between the farmed strains was presumed to be caused by drift in the hatcheries. It has also been suggested that reduced genetic variation can result in reduced performance in fish species or strains (Gjedrem, 1992) and that loss of genetic diversity can be correlated with time elapsed since strains are founded (Coughlan et al., 1998). In this study, the genomic DNA variation in crucian carp, *Carassius carassius*, is similar or rather low, as compared to the genomic DNA variation observed using RAPDs in other fish species with wide geographical distributions, e.g. in turbot (Coughlan et al., 1998) or in brown trout (Cagigas et al., 1999) or blacklip abalone (Huang et al., 2000), as above-mentioned.

As mentioned, the bandsharing values and banding patterns of wild population was considerably more diverse than cultured population. This study is timely for a growing crucian carp aquaculture industry because knowledge of the genetic diversity of crucian carp could help in formulating effective strategies for managing this aquacultural fish species and also in evaluating the potential genetic effects induced by hatchery operations. In future, additional methods such as RFLP, AFLP, minisatellite, microsatellite, and sequence techniques etc. are likely to maximize this research in various fields of interest to the aquaculture industry, especially with regard to the genetic and breeding programs of crucian carp. Additional studies on many populations, sampling sites and individuals will be

necessary to obtain still more exact results.

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