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Geographic Variations and DNA Polymorphisms in Gizzard-shad (*Konosirus punctatus*)

Su-Young Park, Jong-Yeon Kim[1] and Jong-Man Yoon*

Department of Aquatic Life Medicine, [1]Department of Marine Aquaculture and Biotechnology, College of Ocean Science and Technology, Kunsan National University, Gunsan 573-701, Korea

Genomic DNA isolated from three geographical gizzard-shad (*Konosirus punctatus*) populations in Seocheon (SC), Busan (BS) and Gochang (GC) collected in the West Sea and the southern sea, respectively, off the Korean Peninsula, were PCR-amplified repeatedly. Eight selected decamer and 20-mer primers generated a total of 713 loci in the SC population, 791 in the BS population, and 732 in the GC population, with a DNA fragment size ranging from 100 bp to 2,800 bp. We identified 50 unique loci for the SC population, 70 unique loci for the BS population and 130 for the GC population: 120 shared loci for the three populations. There were 108 specific loci (15.1%) for the SC population, 74 (9.4%) for the BS population, and 67 (9.2%) for the GC population. Eight primers also generated 48 polymorphic loci (6.7%) for the SC population, 26 (3.3%) for the BS population, and 16 (2.2%) for the GC population. The similarity matrix ranged from 0.756 to 0.936 for the SC population, from 0.800 to 0.938 for the BS population, and from 0.731 to 0.959 for the GC population. The dendrogram obtained by the eight primers indicates three genetic clusters: cluster 1 (SEOCHEON 01～SEOCHEON 10), cluster 2 (BUSAN 11～BUSAN 20 and GOCHANG 23～GOCHANG 24), and cluster 3 (GOCHANG 21, 22, 25, 26, 27, 28, 29 and 30). As stated above, some individuals of the GC population appear to belong in BS population. When seeing this result, it was thought with the fact that some individuals of 2 populations seem to come and go partially. Thus, RAPD-PCR analysis revealed a significant genetic distance between the three geographical gizzard-shad populations. Using various decamer and 20-mer primers, RAPD-PCR may be applied to identify specific/polymorphic markers that are particular to a species and geographic population, and to define genetic diversity, polymorphisms, and similarities among geographical gizzard-shad populations.

Key words : DNA polymorphism, genetic distance, genetic variation, gizzard-shad, *Konosirus punctatus*

Introduction

An advantage of this method is that it does not require prior knowledge of the genome to be effective (Iyengar *et al*., 2000; Klinbunga *et al*., 2000a). Genetic variation within samples was also found to be significantly higher by microsatellites and RAPD than by analysis of enzyme loci within and among four natural Spanish populations of brown trout (Salmo trutta) (Cagigas *et al*., 1999). Even if reproducibility of RAPD is a little poor and depends upon PCR conditions, until now, polymorphic bands generated by RAPD-

*Corresponding author: jmyoon@kunsan.ac.kr

PCR using arbitrary primers were considered to be a reliable method for detecting DNA similarity and/or diversity between organisms (Jeffreys and Morton, 1987; Liu *et al*., 1998; McCormack *et al*., 2000; Kim *et al*., 2004; Kim *et al*., 2006). RAPDs have proven to be useful genetic markers because of their high levels of polymorphisms (Welsh and McClelland, 1990; Welsh *et al*., 1991). As stated above, the potential of RAPD to identify diagnostic markers for breed, stock, species and population identification in teleosts (Mamuris *et al*., 1999; Iyengar *et al*., 2000; Yoon and Kim, 2004; Siti Azizah *et al*., 2005; Yoon and Park, 2006), and in shellfish (Tassanakajon *et al*., 1998; Klinbunga *et al*., 2000b; Yoon and Kim, 2003b; Kim *et al*., 2004) has been demonstrated.

Gizzard-shad (*Konosirus punctatus*) lives in Korea, Japan, the South China Sea, and the Gulf of Pohai and throughout the world. Korean seawater fish, gizzard-shad is one of ecologically important fish species, belonging to the family Clupeidae, and the order Clupeiformes. Nearly all live in seawater, although a few survive in fresh-water. An enlarged elliptic body, and dark yellow caudal fin, which is glossy bluish in dorsal color, or silver-white in ventral color, characterize gizzard-shad. A large black spot is distributed on the surface of the flank of the body. The longitudinal stripes with brownish spots along the back characterize this rather yellowish and light gray gizzard-shad in the natural ecosystem. Its color, however, depends on diet and habitat environment.

In general, gizzard-shad is widely distributed in the West, South and East Sea along the coast of the Korean Peninsula. It is sometimes found at the mouths of springs. It is nocturnal and eats Diatommaceae, copepoda, and small shrimp. In June to September they are in deep waters and move toward the coast from October to May in Korea. They breed from March to June. At that they come in groups and breed in shallow waters. Basically, the rate at which seawater gizzard-shad grows depends very much on feed organisms, population density and water temperature. Especially, the water temperature of 15～25℃ is about optimal.

The sliced raw gizzard-shad dish is very appetizing with its slight vinegar and pepper flavor. The broiled gizzard shad and the salted viscera of gizzard shad are considered delicacies. Particularly in autumn, the gizzard shad gain fat and attain an excellent chewy texture. The Gizzard Shad Festival is aimed to introduce the superior specialties of the region and make an offer the fresh foodstuffs directly to the consumers at low prices, in order to enhance the profitability of the local dwellers and the long-term economical development. As the necessity of gizzard-shad increases, the understanding of the genetics of this fish species becomes necessary. However, there is little information regarding the genetics of gizzard-shad in Korea and foreign countries. Many researchers have used RAPD and/or RAPD -based techniques to estimate population structure in invertebrate and fishes, including the black tiger shrimp (Tassanakajon *et al*., 1998), brown trout (Cagigas *et al*., 1999), marsh clam (Yoon and Kim, 2003a), crayfish (Kim *et al*., 2006), and crucian carp (Yoon and Park, 2006). Polymorphisms are determined by the banding patterns of primer-amplified products at specific positions (Smith *et al*., 1997; Tassanakajon *et al*., 1998; Yoon and Kim, 2001; Yoon and Kim, 2003b).

Accordingly, our study attempts to elucidate the genetic variations and DNA polymorphisms of three gizzard-shad (*K. punctatus*) populations in Seocheon, Busan and Gochang regions of the Korean Peninsula. In order to accomplish them, we analyzed the clustering analyses, genetic distances and differences within and among gizzard -shad geographical populations.

Materials and Methods

1. Sample collection and extraction of genomic DNA

Three geographical populations of gizzard-shad (*K. punctatus*) were obtained from three different regions in Korea: Seocheon, Busan and Gochang in three coastal areas of the Korean Peninsula. RAPD analysis was performed on the muscle extract of 30 individuals using eight arbitrarily selected primers. The extraction/purification of genomic DNA was performed under the conditions previously described (Yoon and Kim, 2004). The DNA pellets were incubation-dried for more than 9 hours, held at −40℃ until analysis, and then dissolved in the ultra-pure water (Kwangmyung Co., Ltd., Korea) produced by a water purification system. The concentration of the purified genomic DNA was calculated with optical density values at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

2. Oligonucleotide primers, markers and amplification stipulations

The oligonucleotide primers were purchased from Bioneer Technologies and Seoulin Biotechnologies, Korea, respectively. Eight selected primers; BION-01 (5′-CAGGCCCTTC-3′), BION-03 (5′-AGGGGTCTTG-3′), BION-06 (5′-AGCCAGCGAA-3′), BION-11 (5′-GTGATCGCAG-3′), BION-13 (5′-GTTTCGCTCC-3′) BION-14 (5′-TGGATTGGTC-3′) BION-19 (5′-GTCCACACGG-3′) and URP-01 (20-mer) were shown to generate the average loci per lane, shared loci by each population, specific and polymorphic loci, which could be clearly scored (Kim *et al*., 2006). Thus, we used the primers to study the genetic diversity, similarity, genetic variations, and DNA polymorphisms of the gizzard-shad. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers (Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA). Optimal DNA concentrations for amplification were determined by testing twice dilutions, one of which was taken as the standard for every subsequent amplification (Park *et al*., 2005). Amplification products were generated by 1.4% agarose gel electrophoresis (AGE) (VentechBio, Korea) with TBE [90 mM Tris (pH 8.5), 90 mM borate, 2.5 mM EDTA]. 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. The data analysis of similarity matrix and dendrogram

The bandsharing values were calculated according to the protocols outlined by Jeffreys and Morton (1987), and Yoon and Park (2002). Comparing two lanes, bandsharing 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 relatedness between different individuals in the gizzard-shad populations of Seocheon (SEOCHEON 01～SEOCHEON 10), Busan (BUSAN 11～BUSAN 20) and Gochang (GOCHANG 21～GOCHANG 30) was generated according to the BS values and similarity matrix (Yoon and Kim, 2003a). The average of within-population similarity is calculated by pairwise comparison between individuals within a population. The complete linkage clustering tree was analyzed by the similarity matrices to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., Chicago, IL, USA) (Park *et al*., 2005). Euclidean genetic distances within- and between-populations were also calculated using the hierarchical dendrogram program Systat version 10. Program Systat version 10 was also utilized to obtain general statistical results such as mean, standard error and t-test.

Results and Discussion

1. Genetic variations of products

The genomic DNA was isolated from three geographical gizzard-shad populations in Seocheon, Busan and Gochang of Korea. The amplified products were separated by AGE with oligonucleotide decamer primers and 20-mer primers, and stained with ethidium bromide. The six arbitrarily selected primers BION-01, BION-03, BION-06, BION-11, BION-13, BION-14, BION-19 and URP-01 generated the number of average loci per lane, shared loci by each population, specific loci and polymorphic loci (Table 1-2).

In the present study, eight decamer and 20-mer primers generated a total of 713 loci in the SC population, 791 in the BS population, and 732 in the GC population, with a DNA fragment size ranging from 100 bp to 2,800 bp, as was summarized in Table 1. Many researchers studied the sizes of DNA fragments in the RAPD-PCR profiles of barramundi (*Lates calcarifer*) (Partis and Wells, 1996), brown trout (*Salmo trutta*) (Cagigas *et al*., 1999), the Mullidae family (Mamuris *et al*., 1999), the brittle star (*Amphiura filiformis*) (McCormack *et al*., 2000), largehead hairtail (Park and Yoon, 2005), and crucian carp (Yoon and Park, 2006). Especially, six primers were used, generating a total of 602 scorable bands in catfish, and 195 in the bullhead population, respectively, ranging in DNA fragment size from less than approximately 100, to more than 2,000 base pairs (Yoon and Kim, 2004).

In the present study, on average, a decamer primer generated 89.1 amplified products in the SC population, as illustrated in Table 1. A RAPD

Table 1. The number of average loci per lane, shared loci by each population, specific loci and polymorphic loci generated by RAPD analysis using 8 primers in gizzard-shad (*K. punctatus*) in Seocheon, Busan and Gochang of Korea

Item	No. of average loci per lane			No. of shared loci by each population			No. of specific loci			No. of polymorphic loci		
Primer	SC	BS	GC	SC	BS	GC	SC	BS	GC	SC	BS	GC
BION-01	12.5 (125)	8.1 (81)	9.0 (90)	70	10	40	13	11	17	4	0	1
BION-03	8.1 (81)	9.1 (91)	8.4 (84)	20	30	30	3	14	9	6	3	1
BION-06	8.6 (86)	10.6 (106)	9.8 (98)	0	60	70	31	7	6	8	0	5
BION-11	6.9 (69)	9.1 (91)	9.7 (97)	10	50	70	22	5	1	6	3	1
BION-13	7.4 (74)	11.3 (113)	10.7 (107)	30	40	40	13	5	4	4	2	1
BION-14	8.8 (88)	8.5 (85)	8.8 (88)	40	40	10	10	12	14	4	3	3
BION-19	7.9 (79)	9.7 (97)	6.4 (64)	20	20	40	9	16	10	0	15	4
URP-01	11.1 (111)	12.7 (127)	10.4 (104)	40	70	70	7	4	6	0	0	0
Total no.	71.3 (713)	79.1 (791)	73.2 (732)	230	320	370	108	74	67	48	26	16
Average no. per primer	89.1	98.9	91.5	28.8	40.0	46.3	15.4	9.3	8.4	6.0	3.3	2.0

The total number of loci generated by a primer in gizzard-shad obtained from Seocheon (SC), Busan (BS) and Gochang (GC) is shown in parentheses.

Table 2. The number of unique loci to each population and number of shared loci by the three populations obtained using 8 primers in gizzard-shad (*K. punctatus*) in Seocheon, Busan and Gochang from the West and South Sea of Korea

Item	No. of unique loci to each population			No. of shared loci by the three populations
Primer \ Population	Seocheon	Busan	Gochang	Three locales
BION-01	20	0	0	10
BION-03	0	0	0	20
BION-06	0	40	50	20
BION-11	0	0	20	10
BION-13	20	0	0	10
BION-14	0	0	0	10
BION-19	10	10	30	10
URP-01	0	20	30	30
Total no.	50	70	130	120
Average no. per primer	6.3	8.8	16.3	15.0

primer generated an average of 71.3 amplified loci per sample, ranging from 6.9 to 12.5 loci in this population. The oligonucleotide primer URP-01 also generated shared loci, of approximately 300 bp, 400 bp and 600 bp, in three gizzard-shad populations, as shown in other primers also generate identically sized loci in three gizzard-shad populations, as illustrated in Table 2. It has been reported that 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 (Callejas and Ochando, 1998). The number of scored bands varied from 7 to 12 per primer in four species of the Mullidae family (Mamuris *et al*., 1999). Various primers generated 36, 32, and 24 bands, respectively, in mud crabs from Eastern Thailand (genus *Scylla*) (Klinbunga *et al*., 2000b). 176 common fragments, with an average of 25.1 per primer, were observed in the Buan population, and 99 fragments, with an average of 14.1 per primer, were observed in the Geojedo population (Kim *et al*., 2004).

We first assessed genetic variation in the SC population. Primer BION-01 generated loci ranging from 200 bp to 2,500 bp (Fig. 1A). This primer detected 70 shared major and/or minor loci of sizes 200 bp, 350 bp, 450 bp, 500 bp, 550 bp, 650 bp and 1,400 bp, which were identical in all samples. Interestingly, the 20 unique loci that established population identity were 350 bp and 500 bp. The primer generated these major and/or minor specific loci: 300 bp (lanes 5 and 7), 900 bp (lanes 1, 2, 3, 7, 8, 9 and 10) and 1,000 bp (lanes 1, 2, 3 and 4). The primer generated a polymorphic RAPD profile with four DNA fragments. This primer, notably, produced the highest number of loci, a total of 125, although the average was 12.5. The primer BION-11 detected 10 shared loci by the three populations of sizes 500 bp, in all samples (Table 1) (Fig. 1D). This primer produced the lowest number of loci (a total of 69), in comparison to the other primers used, with an average of 6.9. Interestingly, the 10 shared loci

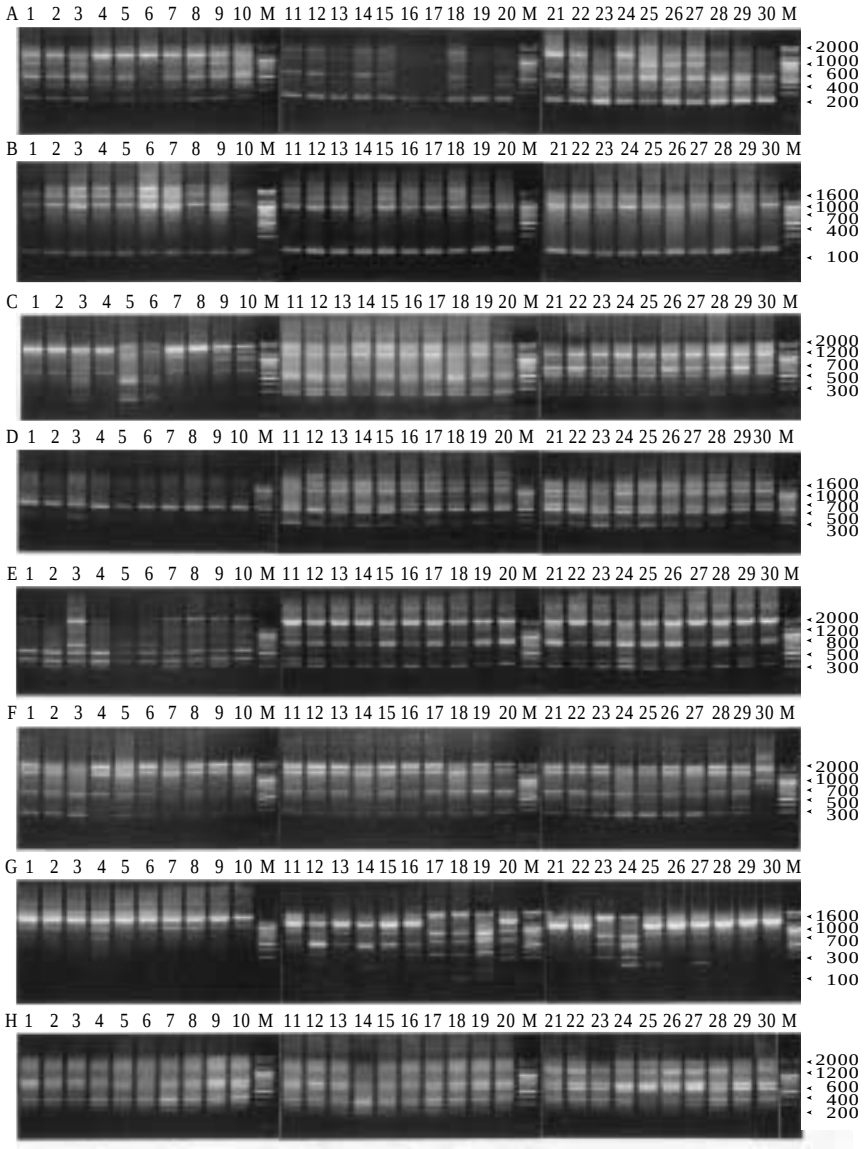

Fig. 1. PCR-generated electrophoretic profiles of individual gizzard-shad (*K. punctatus*) of three geographic populations. Each lane shows DNA samples extracted from 30 individuals. DNA isolated from Seocheon (lane 1~10), Busan (lane 11~20) and Gochang (lane 21~30) were amplified by random primers BION-01 (A), BION-03 (B), BION-06 (C), BION-11 (D), BION-13 (E) BION-14 (F) BION-19 (G) and URP-01 (H). Amplification products were generated via electrophoresis on 1.4% agarose gel containing ethidium bromide. The 100 bp DNA Ladder (M) was used as a DNA molecular weight marker.

that established population identity were 500 bp. This primer detected 22 specific major and/or minor loci, which were approximately 550 bp and larger than 2,400 bp, respectively. This decamer primer generated 5 minor polymorphic bands, approximately 550 bp in size (lanes 1, 2, 3, 8, 9 and 10). This primer, interestingly enough, generated a single monomorphic locus (lane 5).

We secondly assessed genetic variation in the BS population. The genetic variation was identified in the banding pattern produced by decamer BION-01, which ranged from approximately 200 bp to 1,400 bp, as illustrated in Fig. 1A. This primer generated the lowest number of loci, a total of 81 loci, with an average of 8.1 (Table 1). The 10 fragments obtained by this primer, approximately 200 bp in size, were observed in all samples. The primer detected 11 specific major and/or minor loci: 300 bp (lane 14), 500 bp (lanes 12, 14, 15, 18 and 20), 900 bp (lanes 14 and 18) and 1,200

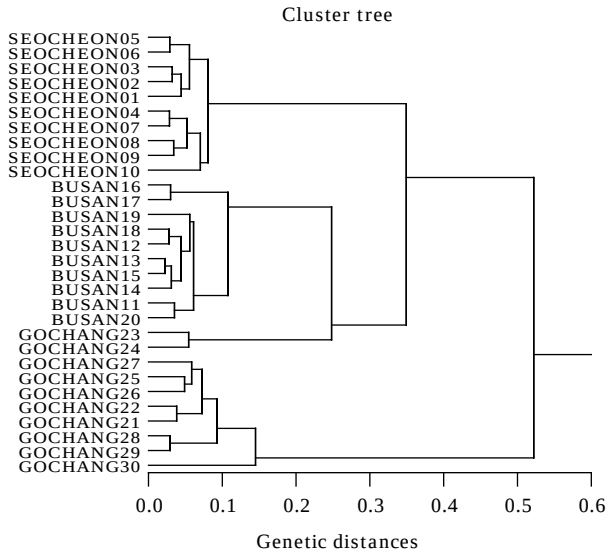

Fig. 2. Hierarchical dendrogram of genetic distances, obtained from three geographical populations of gizzard-shad (*K. punctatus*). The relatedness between different individuals in the gizzard-shad populations of Seocheon (SEOCHEON 01～SEOCHEON 10), Busan (BUSAN 11～BUSAN 20) and Gochang (GOCHANG 21～GOCHANG 30) was generated according to the bandsharing values and similarity matrix (see Table 3).

bp (lanes 14, 15 and 18). The specific loci generated by this primer exhibited inter-individual-specific characteristics, and thus revealed DNA polymorphisms. Polymorphic loci that identified populations and/or species, however, were not identified here. This primer, interestingly enough, generated two single monomorphic loci (lanes 16 and 17). High degrees of RAPD variation were observed in the banding patterns generated by universal primer URP-01, ranging from 250 to 2,000 bp (Fig. 1H). Interestingly, the 70 shared loci that established population identity were 300 bp, 400 bp, 450 bp, 600 bp, 750 bp, 1,200 bp and 1,400 bp. Four specific loci were observed at 500 bp (lane 17) and 900 bp (lanes 12, 13 and 19). This primer, markedly, produced the highest number of loci, a total of 127, although the average was 12.7.

Moreover, in the GC gizzard-shad population, the genetic variation was identified in the banding pattern produced by decamer BION-13, which ranged from approximately 200 bp to 2,600 bp, as illustrated in Fig. 1E. The primer generated the highest number of loci (a total of 107), with an average of 10.7, as illustrated in Table 1. Interestingly, the 40 shared loci that established population identity were 300 bp, 750 bp, 850 bp and 2,000 bp. This primer detected 4 specific and 1 polymorphic major and/or minor loci that identified individuals. Interestingly, the 10 shared loci that established identifications for populations and/or species were 2,000 bp.

The complexity of the banding patterns varied widely between primers and/or geographic locales. It has been reported that the silver dory (*Cyttus australis*) has a major, 460 bp fragment, and that the mirror dory (*Zenopsis nebulosis*) has a major, 422 bp fragment (Partis and Wells, 1996). These major fragments revealed the characteristic profiles of fish species such as the silver dory and mirror dory. The RAPD-PCR method using random primers, was applied to the identification of three endemic Spanish barbel species: *Barbus bocagei, B. graellsii* and *B. sclateri* (Callejas and Ochando, 1998). Results indicated that *B. bocagei* and *B. graellsii* were more closely related to each other than they were to *B. sclateri*. Population-related RAPD fragments were identified in the channel catfish (*Ictalurus punctatus*) and the blue catfish (*I. furcatus*), and also in their F_1, F_2 and backcross hybrids (Liu *et al*., 1998). It has been reported that the two species of crucian carp (*Carassius auratus* and *C. cuvieri*) have the shared and major, 400 bp loci (Yoon and Park, 2006). This major locus revealed the characteristic profiles of crucian carp species such as the *Carassius auratus* and *C. cuvieri*. The frequencies of fragments generated by six primers were calculated in various catfish populations, as described in catfish. Generally, the size and number of fragments generated depends both on the nucleotide sequence of the primer, and on the source of the template DNA, resulting in a genome-specific DNA fragment (Welsh and McClelland 1990; Welsh *et al*., 1991). RAPD-PCR is one of the fast and simplest research methods for the identification of genetic differences and polymorphisms in various organisms (Smith *et al*., 1997; Iyengar *et al*., 2000; Yoon and Kim, 2004; Kim *et al*., 2004; Yoon and Park, 2006).

2. Variation within and between populations, and genetic distances

Here, 50 unique loci were identified for the SC population, 70 unique loci for the BS population, and 130 for the GC population: 120 shared loci for the three populations (Table 2). Especially,

the universal primer URP-01 also generated 30 shared loci, which were identifying populations and/or species, approximately 300 bp, 400 bp and 600 bp, among the three geographical gizzard-shad populations (Fig. 1H). In the present study, 8 primers generated 108 specific loci (108/713 loci, 15.1%) in the SC population, 74 (74/791 loci, 9.4%) in the BS population, and 67 (67/732 loci, 9.2%) in the GC population, as illustrated in Table 1. Especially, these results demonstrate that the primers detected numerous specific loci. These primers generated 48 polymorphic loci (6.7%) in the SC population, 26 (3.3%) in the BS population, and 16 (2.2%) in the GC population. Of the 46 polymorphic loci, only 3 allelic markers were private, distinguishing sample 1 from the rest, within and among four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al*., 1999). Iyengar *et al*. (2000) used a RAPD-based technique to identify several microsatellite repeats in the turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) and report the characterizations of six novel polymorphic microsatellite markers for Dover sole. McCormack *et al*. (2000) reported that a total of 98 individuals were examined in two populations of *A. filiformis*, using these four primers. They reported that the banding patterns showed a high degree of variation, with individual organisms being clearly distinguishable from one another. All four primers generated 111 polymorphic DNA loci from 70 individuals. Upon RAPD analysis of genetic differences and characteristics in wild and cultured crucian carp populations, the pattern of polymorphic fragments of fifty individuals in the wild population was reported to be different (Yoon and Park, 2002). Six primers generated 47 polymorphic fragments (24% of 195 fragments) in a bullhead population (Yoon and Kim, 2004). 148 specific fragments (15.6%) were identified in the Korean largehead hairtail population, and 61 (9.5%) in the Atlantic population (Park and Yoon, 2005).

The specific primers were discovered to be available in the identification of individuals and/or populations, resulting from variations in DNA polymorphisms among individuals/populations (Liu *et al*., 1998; Yoon and Park, 2002; Yoon and Kim, 2004; Siti Azizah *et al*., 2005). The random RAPD method has been applied to eight perch fish species (Partis and Wells, 1996). Diagnostic markers are considered as species-specific markers which are present in both populations of an eel-loach species (*Pangio* sp.), while the other bands are population specific markers (Siti Azizah *et al*., 2005). Three diagnostic markers were observed in *Pangio piperata* and 14 in *P. shelfoldii* with molecular weights ranging 300～2,000 bp. Generally speaking, using a variety of oligonucleotide primers, RAPD-PCR has been applied to identify polymorphic/specific markers particular to species, genus and geographical population, as well as genetic variability/similarity/polymorphism in various organisms (Smith *et al*., 1997; McCormack *et al*., 2000; Klinbunga *et al*., 2000a; Yoon and Kim, 2004; Kim *et al*., 2006).

In this study, the bandsharing value, which is based on the presence or absence of amplified fragments, was utilized to calculate similarity indices, as illustrated in Table 3. Based on the average bandsharing values of all samples, the similarity matrix ranged from 0.756 to 0.936 in the SC population, from 0.800 to 0.938 in the BS population, and from 0.731 to 0.959 in the GC population. The bandsharing value between individuals' no. 04 and no. 06 was 0.621, which was the lowest. The bandsharing value between no. 13 and no. 15 was 0.938, which was the highest value within the BS population. The value between no. 14 and no. 17 was 0.800, which was the lowest. The bandsharing value between individuals' no. 07 of SC population and no. 20 of BS population was 0.681, which was the highest between the two geographical populations. The value between individuals' no. 10 of SC population and no. 16 of BS population was 0.506, which was the lowest between the two geographical populations. The average bandsharing value was 0.832±0.007 within the SC population, 0.877± 0.006 within the BS population, and 0.864±0.006 within the GC population (Table 4). Therefore, regarding individual results, the bandsharing value of individuals within the BS population was much higher than that any other populations. The average bandsharing value between the BS population and the GC population was 0.674±0.004, ranged from 0.579 to 0.755. The bandsharing value between individuals no. 02 and no. 03 was 0.936, which was the highest value identified within the SC population.

The average bandsharing value acquired by our study is similar to the value reported for Spanish barbel species (0.71～0.81) (Callejas and Ochando, 1998). However, our bandsharing values between the three geographical gizzard-shad populations are inconsistent with the previously

Table 3. Similarity matrix, including bandsharing values and genetic differences, of gizzard-shad (*K. punctatus*) from Seocheon, Busan and Gochang. Bandsharing values of gizzard-shad from three regions are above the diagonal and genetic differences are below the diagonal

		Bandsharing values of gizzard-shad from Seocheon										Bandsharing values of gizzard-shad from Busan										Bandsharing values of gizzard-shad from Gochang									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Genetic differences of gizzard-shad from Seocheon	1		0.908	0.864	0.845	0.844	0.805	0.805	0.827	0.862	0.816	0.639	0.621	0.608	0.578	0.605	0.535	0.556	0.635	0.586	0.601	0.531	0.573	0.607	0.574	0.564	0.566	0.534	0.573	0.558	0.553
	2	0.092		0.936	0.896	0.841	0.811	0.864	0.805	0.809	0.826	0.614	0.598	0.604	0.626	0.612	0.542	0.522	0.613	0.565	0.621	0.525	0.565	0.580	0.560	0.552	0.566	0.541	0.565	0.565	0.547
	3	0.136	0.064		0.879	0.847	0.804	0.866	0.780	0.785	0.797	0.676	0.667	0.646	0.665	0.651	0.581	0.575	0.666	0.593	0.647	0.513	0.554	0.576	0.552	0.541	0.573	0.531	0.563	0.561	0.542
	4	0.155	0.104	0.121		0.820	0.756	0.905	0.863	0.816	0.791	0.633	0.630	0.623	0.634	0.630	0.562	0.565	0.627	0.578	0.651	0.502	0.555	0.562	0.548	0.534	0.562	0.540	0.555	0.574	0.531
	5	0.156	0.159	0.153	0.180		0.907	0.841	0.759	0.777	0.767	0.610	0.605	0.599	0.630	0.602	0.535	0.516	0.608	0.561	0.616	0.487	0.524	0.550	0.532	0.511	0.518	0.492	0.527	0.511	0.504
	6	0.195	0.189	0.196	0.244	0.093		0.829	0.775	0.792	0.788	0.596	0.607	0.615	0.642	0.629	0.565	0.558	0.626	0.592	0.645	0.495	0.548	0.587	0.516	0.495	0.528	0.503	0.555	0.541	0.478
	7	0.195	0.136	0.134	0.095	0.159	0.171		0.902	0.842	0.796	0.641	0.634	0.643	0.661	0.661	0.589	0.581	0.652	0.611	0.681	0.524	0.574	0.563	0.554	0.537	0.567	0.550	0.584	0.587	0.551
	8	0.173	0.195	0.220	0.137	0.241	0.225	0.098		0.895	0.856	0.666	0.660	0.652	0.644	0.669	0.601	0.593	0.646	0.624	0.685	0.552	0.583	0.616	0.583	0.554	0.574	0.542	0.600	0.600	0.526
	9	0.138	0.191	0.215	0.184	0.223	0.208	0.158	0.105		0.858	0.618	0.614	0.606	0.626	0.625	0.570	0.565	0.629	0.607	0.654	0.550	0.597	0.642	0.582	0.569	0.587	0.573	0.611	0.614	0.526
	10	0.184	0.174	0.203	0.209	0.233	0.212	0.204	0.144	0.142		0.590	0.577	0.562	0.552	0.560	0.506	0.532	0.595	0.550	0.592	0.533	0.577	0.600	0.539	0.519	0.563	0.526	0.582	0.565	0.526
Genetic differences of gizzard-shad from Busan	11	0.361	0.386	0.324	0.367	0.390	0.404	0.359	0.334	0.382	0.410		0.924	0.922	0.854	0.890	0.825	0.803	0.868	0.839	0.907	0.681	0.733	0.735	0.681	0.701	0.701	0.664	0.721	0.702	0.681
	12	0.379	0.402	0.333	0.370	0.395	0.393	0.366	0.340	0.386	0.423	0.076		0.926	0.893	0.928	0.852	0.833	0.922	0.843	0.930	0.671	0.721	0.741	0.728	0.720	0.662	0.659	0.706	0.697	0.657
	13	0.392	0.396	0.354	0.377	0.401	0.385	0.357	0.348	0.394	0.438	0.078	0.074		0.902	0.938	0.873	0.847	0.911	0.845	0.895	0.658	0.709	0.700	0.688	0.690	0.680	0.644	0.700	0.681	0.661
	14	0.422	0.374	0.335	0.366	0.370	0.358	0.339	0.356	0.374	0.448	0.146	0.107	0.098		0.938	0.879	0.800	0.862	0.845	0.871	0.652	0.688	0.690	0.679	0.695	0.682	0.654	0.702	0.700	0.640
	15	0.395	0.388	0.349	0.370	0.398	0.358	0.339	0.331	0.375	0.440	0.110	0.072	0.062	0.062		0.901	0.846	0.922	0.840	0.893	0.639	0.676	0.707	0.709	0.698	0.658	0.641	0.681	0.679	0.641
	16	0.465	0.458	0.419	0.438	0.465	0.435	0.419	0.399	0.430	0.494	0.175	0.148	0.127	0.121	0.099		0.912	0.838	0.855	0.834	0.596	0.617	0.648	0.635	0.624	0.608	0.604	0.636	0.633	0.579
	17	0.444	0.478	0.425	0.435	0.484	0.442	0.419	0.407	0.435	0.468	0.197	0.167	0.153	0.200	0.154	0.088		0.890	0.870	0.842	0.600	0.632	0.677	0.652	0.619	0.604	0.622	0.642	0.637	0.583
	18	0.365	0.387	0.334	0.373	0.392	0.374	0.348	0.354	0.371	0.405	0.132	0.078	0.089	0.138	0.078	0.162	0.110		0.871	0.902	0.647	0.695	0.736	0.713	0.704	0.663	0.647	0.687	0.683	0.663
	19	0.414	0.435	0.407	0.422	0.439	0.408	0.389	0.376	0.393	0.450	0.414	0.157	0.155	0.155	0.160	0.145	0.130	0.129		0.875	0.644	0.672	0.719	0.682	0.680	0.683	0.634	0.691	0.672	0.634
	20	0.399	0.379	0.353	0.349	0.384	0.355	0.319	0.315	0.346	0.408	0.093	0.070	0.105	0.129	0.107	0.166	0.158	0.098	0.125		0.698	0.737	0.734	0.693	0.703	0.711	0.694	0.755	0.751	0.685
Genetic differences of gizzard-shad from Gochang	21	0.469	0.475	0.487	0.498	0.513	0.505	0.476	0.448	0.450	0.467	0.319	0.329	0.342	0.348	0.361	0.404	0.400	0.353	0.356	0.302		0.922	0.826	0.817	0.877	0.840	0.868	0.893	0.886	0.864
	22	0.427	0.435	0.446	0.445	0.476	0.452	0.426	0.417	0.403	0.423	0.267	0.279	0.291	0.312	0.324	0.383	0.368	0.305	0.328	0.263	0.078		0.889	0.838	0.880	0.882	0.871	0.914	0.906	0.866
	23	0.393	0.420	0.424	0.438	0.450	0.413	0.437	0.384	0.358	0.400	0.265	0.259	0.300	0.310	0.293	0.352	0.323	0.264	0.281	0.266	0.174	0.111		0.893	0.863	0.808	0.799	0.841	0.834	0.731
	24	0.426	0.440	0.448	0.452	0.468	0.484	0.446	0.417	0.418	0.461	0.319	0.272	0.312	0.321	0.291	0.365	0.348	0.287	0.318	0.307	0.183	0.162	0.107		0.915	0.837	0.826	0.818	0.811	0.794
	25	0.436	0.448	0.459	0.466	0.489	0.505	0.463	0.446	0.431	0.481	0.299	0.280	0.310	0.305	0.302	0.376	0.381	0.296	0.320	0.297	0.123	0.120	0.137	0.085		0.898	0.885	0.868	0.881	0.869
	26	0.434	0.434	0.427	0.438	0.482	0.472	0.433	0.426	0.413	0.437	0.299	0.338	0.320	0.318	0.342	0.392	0.396	0.337	0.317	0.289	0.160	0.118	0.192	0.163	0.102		0.889	0.904	0.874	0.855
	27	0.466	0.459	0.469	0.460	0.508	0.497	0.450	0.458	0.427	0.474	0.336	0.341	0.356	0.346	0.359	0.396	0.378	0.353	0.366	0.306	0.132	0.129	0.201	0.174	0.115	0.111		0.868	0.882	0.859
	28	0.427	0.435	0.437	0.445	0.473	0.445	0.416	0.400	0.389	0.418	0.279	0.294	0.300	0.298	0.319	0.364	0.358	0.313	0.309	0.245	0.107	0.086	0.159	0.182	0.132	0.096	0.132		0.959	0.886
	29	0.442	0.435	0.439	0.426	0.489	0.459	0.413	0.400	0.386	0.435	0.298	0.303	0.319	0.300	0.321	0.367	0.363	0.317	0.328	0.249	0.114	0.094	0.166	0.189	0.119	0.126	0.118	0.041		0.892
	30	0.447	0.453	0.458	0.469	0.496	0.522	0.449	0.474	0.474	0.474	0.319	0.343	0.339	0.360	0.359	0.421	0.417	0.337	0.366	0.315	0.136	0.134	0.269	0.206	0.131	0.145	0.141	0.114	0.108	

Table 4. Multiple comparisons of average bandsharing values among gizzard-shad (*K. punctatus*) populations from three regions were generated according to the bandsharing values and similarity matrix (see Table 3)

Population	SC	BS	GC
SC	0.832	0.609	0.553
BS	–	0.877	0.674
GC	–	–	0.864

SC: Seocheon, BS: Busan, GC: Gochang

reported results (Yoon and Park, 2002). Other reports have shown that the average bandsharing value obtained using five random primers was 0.40±0.05 in the wild crucian carp population, and 0.69±0.08 in the cultured crucian carp population. The average bandsharing value recorded in our study is also higher than the average value of the bullhead population (0.504± 0.115) (Yoon and Kim, 2004), and also between the two oyster populations (0.282±0.008) (Kim *et al*., 2004). The bandsharing value of individuals within the Atlantic largehead hairtail population was much higher than in the Korean population (Park and Yoon, 2005). The average bandsharing value was 0.859±0.004 within the Korean largehead hairtail population, and 0.916±0.006 within the Atlantic population.

To obtain the dendrogram, a hierarchical clustering analysis was performed, employing the similarity matrix based on the bandsharing values and genetic differences (Fig. 1). The dendrogram obtained by the eight primers indicates three genetic clusters: cluster 1 (SEOCHEON 01～SEOCHEON 10), cluster 2 (BUSAN 11～BUSAN 20 and GOCHANG 23～GOCHANG 24), and cluster 3 (GOCHANG 21, 22, 25, 26, 27, 28, 29 and 30). As stated above, some individuals of the GC population appear to belong in BS population. When seeing this result, it was thought with the fact that some individuals of 2 populations seem to come and go partially. The genetic distance between the three geographical populations ranged from 0.022 to 0.522. The shortest genetic distance displaying significant molecular difference was between individuals BUSAN no. 15 and BUSAN no. 13 from Busan (genetic distance=0.022). Especially, in SC population, individual SEOCHEON no. 07 from Seocheon was genetically most closely related to SEOCHEON no. 04 from Seocheon (genetic distance=0.028). On the other hand, in GC population, individual GOCHANG no. 29 of the Gochang population was most distantly related to GOCHANG no. 30 of Gochang (genetic distance=0.145). Ultimately, the longest genetic distance displaying significant molecular differences was found to exist between individuals in the three gizzard-shad populations, between individuals SEOCHEON no. 07 of Seocheon and SEOCHEON no. 29 of Seocheon (genetic distance=0.522). Our cluster analysis revealed a pattern similar to the one posited by Park *et al.* (2005). They reported that complete cluster analysis, which indicated two genetic groupings, and a dendrogram revealed close and/or distant relationships between individual identities within each lobster species.

By cluster analysis of genetic similarity values, the genetic distance ranged from 0.091 to 0.316, with an average of 0.160, within and among four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al*., 1999). The principal aspect of the dendrogram was also a striking separation of sample 1 from the others, which were closely grouped. Nei's genetic distances varied from 0.327 to 0.655 in four species of the Mullidae family (Mamuris *et al*., 1999). The population and/or species identification of the bullhead (*Pseudobagrus fulvidraco*), oyster (*Crassostrea gigas*), and slipper lobster (*Ibacus ciliatus*) and deep sea lobster (*Puerulus sewelli*) was a necessary step in the inception and development of invertebrate/teleost breeding programs (Yoon and Kim, 2004; Kim *et al*., 2004; Park *et al*., 2005). Molecular genetic markers, including, most markedly, microsatellite loci, (ML) quantitative trait loci (QTL), and genomic mapping, will be available for the selection and/or breeding of broodstock for multiple reproductive traits (MRT), or hygiene- and production-related traits, in fishery science (Waldbieser and Wolters, 1999). The classification of geographical populations of gizzard-shad (*K. punctatus*) is based on the morphological variations such as enlarged elliptic body, dark yellow caudal fin, glossy bluish in dorsal color, and silver-white in ventral color. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga *et al*., 2004; Kim *et al*., 2006; Yoon and Park, 2006).

In our study, RAPD-PCR analysis has disclosed a significant genetic distance between three population pairs. RAPD-PCR enabled us to clarify the existence of population discrimination and genetic variation in the gizzard-shad populations of Seocheon, Busan and Gochang of Korea. This

confirms that the method is a suitable tool for DNA comparisons, both within and between individuals, species, genera, and populations, if a few of analytical methods are supplemented. In the future, diagnostic RAPD markers will be necessary for characterization of the different geographical gizzard-shad species to correlate with the morphological characters and for clarification of the ambiguity among population and/ or species.

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References

Cagigas, M.E., E. Vazquez, G. Blanco and J.A. Sanchez. 1999. Combined assessment of genetic variability in populations of brown trout (*Salmo trutta* L.) based on allozymes, microsatellites, and RAPD markers. Mar. Biotechnol., 1 : 286～296.

Callejas, C. and M.D. Ochando. 1998. Identification of Spanish barbel species using the RAPD technique. J. Fish Biol., 53 : 208～215.

Chenyambuga, S.W., O. Hanotte, J. Hirbo, P.C. Watts, S.J. Kemp, G.C. Kifaro, P.S. Gwakisa, P.H. Petersen and J.E.O. Rege. 2004. Genetic characterization of indigenous goats of sub-Saharan Africa using microsatellite DNA markers. Asian-Aust. J. Anim. Sci., 17 : 445～452.

Iyengar, A., S. Piyapattanakorn, D.M. Dtone, D.A. Heipel, B.R. Howell, S.M. Baynes and N. Maclean. 2000. Identification of microsatellite repeats in turbot (*Scophthalmus maximus*) and dover sole (*Solea solea*) using a RAPD-based technique: Characterization of microsatellite markers in dover sole. Mar. Biotechnol., 2 : 49～56.

Jeffreys, A.J. and D.B. Morton. 1987. DNA fingerprints of dogs and cats. Anim. Genet., 18 : 1～15.

Kim, J.Y., C.Y. Park and J.M. Yoon. 2004. Genetic differences and DNA polymorphism in oyster (*Crassostrea* spp.) analysed by RAPD-PCR. Korean J. Genet., 26 : 123～134.

Kim, S., Y.H. Kim and J.M. Yoon. 2006. Genetic variation in geographic crayfish (*Cambaroides similis*) populations. J. Fish Pathol., 19(2) : 141～153.

Klinbunga, S., P. Ampayup, A. Tassanakajon, P. Jarayabhand and W. Yoosukh. 2000a. Development of species-specific markers of the tropical oyster (*Crassostrea belcheri*) in Thailand. Mar. Biotechnol., 2 : 476～484.

Klinbunga, S., A. Boonyapakdee and B. Pratoomchat. 2000b. Genetic diversity and species-diagnostic markers of mud crabs (Genus *Scylla*) in Eastern Thailand determined by RAPD analysis. Mar. Biotechnol., 2 : 180～187.

Liu, Z., P. Li, B.J. Argue and R.A. Dunham. 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.

Mamuris, Z., C. Stamatis, M. Bani and C. Triantaphyllidis. 1999. Taxonomic relationships between four species of the Mullidae family revealed by three genetic methods: allozymes, random amplified polymorphic DNA and mitochondrial DNA. J. Fish Biol., 55 : 572～587.

McCormack, G.C., R. Powell and B. Keegan. 2000. Comparative analysis of two populations of the brittle star *Amphiura filiformis* (Echinodermata: Ophiuroidae) with different life history strategies using RAPD markers. Mar. Biotechnol., 2 : 100～106.

Park, C.Y. and J.M. Yoon. 2005. Genetic differences and variation in two largehead hairtail (*Trichiurus lepturus*) populations determined by RAPD-PCR analysis. Korean J. Ichthyol., 17 : 173～186.

Park, S.Y., J.S. Park, J.M. Yoon. 2005. Genetic difference and variation in slipper lobster (*Ibacus ciliatus*) and deep sea lobster (*Puerulus sewelli*) throughout its distribution range determined by RAPD-PCR Analysis. Korean J. Genet., 27 : 307～317.

Partis, L. and R.J. Wells. 1996. Identification of fish species using random amplified polymorphic DNA (RAPD). Mol. Cell. Probes, 10 : 435～441.

Siti Azizah, M.N., A. Ruzainah and I. Patimah. 2005. Development of RAPD markers in the eel-loach (*Pangio* spp.) for genetic discrimination and monitoring of wild and cultured populations. World. Aqua., 36(1) : 37～43.

Smith, P.J., P.G. Benson and S.M. McVeagh. 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.

Tassanakajon, A., S. Pongsomboon, P. Jarayabhand, S. Klinbunga and V. Boonsaeng. 1998. Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. J. Mar. Biotechnol., 6 : 249～254.

Waldbieser, G.C. and W.R. Wolters. 1999. Application of polymorphic microsatellite loci in a channel catfish *Ictalurus punctatus* breeding program. J. World Aquacult. Soc., 30 : 256～262.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18 : 7213～7218.

Welsh, J., C. Petersen and M. McClelland. 1991. Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res., 19 : 303～306.

Yoon, J.M. and G.W. Kim. 2001. Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish

Silurus asotus. J. Biosci., 26 : 641～647.
Yoon, J.M. and H.Y. Park. 2002. Genetic similarity and variation in the cultured and wild crucian carp (*Carassius carassius*) estimated with random amplified polymorphic DNA. Asian-Aust. J. Anim. Sci., 15 : 470～476.
Yoon, J.M. and Y.H. Kim. 2003a. Wide marsh clam (*Corbicula* spp.) populations from three sites analysed by RAPD-PCR-AGE. Bull. Electrochem., 19 : 337～348.
Yoon, J.M. and G.W. Kim. 2003b. Genetic differences between cultured and wild penaeid shrimp (*Penaeus chinensis*) populations analysed by RAPD-PCR. Korean J. Genet., 25 : 21～32.
Yoon, J.M. and J.Y. Kim. 2004. Genetic differences within and between populations of Korean catfish (*S. asotus*) and bullhead (*P. fulvidraco*) analysed by RAPD-PCR. Asian-Aust. J. Anim. Sci., 17 : 1053～1061.
Yoon, J.M. and S.Y. Park. 2006. Genetic comparison between crucian carp (*Carassius auratus* Linnaeus) and crucian carp (*C. cuvieri* Temminck and Schlegel). J. Anim. Sci. & Technol., 48(5) : 637～650.

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전어 (*Konosirus punctatus*)의 지리적 변이와 DNA 다형성

박 수 영 · 김 종 연[1] · 윤 종 만*

국립군산대학교 해양과학대학 수산생명의학과, [1]해양생명과학부

한국 서해안의 서천 및 고창지역과 남해안의 부산지역으로부터 채취한 전어 (*Konosirus punctatus*) 3개 집단의 개체로부터 genomic DNA를 분리 추출하여 PCR로 반복해서 증폭시켰다. 8개의 decamer와 20-mer를 사용하여 전체적으로 서천의 전어집단에서 713개의 loci, 부산집단에서 791개 및 고창 전어집단으로부터 732개의 100 bp에서 2,800 bp의 크기에 해당되는 total loci를 얻어냈다. 우리는 서천 전어집단에서 독특한 50개의 unique loci, 부산 전어집단으로부터 70개의 unique loci 그리고 고창의 전어집단으로부터 130개의 unique loci를 각각 확인하였고, 또한 3개 전어집단 모두에 대해서 공통적으로 가지고 있는 120개의 shared loci도 확인하였다. 특이한 specific loci를 확인한 결과 서천 전어집단에서는 108개 (15.1%), 부산집단에서는 74개 (9.4%) 그리고 고창 전어집단에서는 67개 (9.2%)를 각각 얻어냈다. 또한 8개의 primer를 통해서 서천 전어집단에서 48개 (6.7%), 부산 전어집단에서는 26개 (3.3%) 그리고 고창 전어집단에서 16개 (2.2%)의 polymorphic loci를 얻어냈다. Similarity matrix를 통해서 볼 때 서천 전어집단에서 0.756에서 0.936까지, 부산집단에서 0.800에서 0.938까지 그리고 고창 전어집단에서 0.731에서 0.959까지의 공유가 (bandsharing value)를 확인하였다. 8개의 primer를 이용하여 얻어진 dendrogram을 통해서 볼 때 genetic cluster는 cluster 1 (SEOCHEON 01～SEOCHEON 10), cluster 2 (BUSAN 11～BUSAN 20과 GOCHANG 23～GOCHANG 24) 그리고 cluster 3 (GOCHANG 21, 22, 25, 26, 27, 28, 29 및 30)와 같이 3개의 cluster로 나누어졌다. 위에서와 같이 고창 전어집단의 일부 개체는 부산 전어집단에 속하는 것으로 나타났으며, 따라서 2 전어집단의 일부 개체들은 부분적으로 오고 가는 이주현상을 나타내는 것으로 사려된다. 이러한 결과를 볼 때 RAPD-PCR 분석 방법을 통해서 우리는 지리적으로 떨어져 있는 3개의 전어 집단에 존재하는 유의성이 있는 유전적 거리를 확인할 수 있었다. 여러 가지 decamer와 20-mer를 이용한 RAPD-PCR 분석 방법은 종 및 지리적 집단과 지리적 전어집단에 존재하는 유전적 다양성, 다형성 및 유전적 유사성을 확인하는데 필요로 하는 독특한 specific/polymorphic marker를 확인할 수 있는 이용 가능한 방법이라고 할 수 있다.