Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish *Silurus asotus*

JONG-MAN YOON* and GYE-WOONG KIM†

Department of Marine Biomedical Science, College of Ocean Science and Technology, Kunsan National University, Kunsan City, Jeollabuk-do, 573-702, the Republic of Korea

†*Department of Animal Science, College of Industry Science, Kongju National University, Yesan-kun, 340-800, the Republic of Korea*

**Corresponding author (Fax, 82-63-463-9493; Email, jmyoon@kunsan.ac.kr).*

Genetic similarity and diversity of cultured catfish *Silurus asotus* populations collected from two areas in western Korea were examined using randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Out of 20 random primers tested, 5 produced 1344 RAPD bands ranging from 8·2 to 13·6 polymorphic bands per primer. The polymorphic bands in these populations ranged from 56·4% to 59·6%. Polymorphic bands per lane within populations ranged from 4·9% to 5·3%. The similarity within the Kunsan population varied from 0·39 to 0·82 with a mean (± SD) of 0·56 ± 0·08. The level of bandsharing values was 0·59 ± 0·07 within the catfish population from Yesan. The genetic similarity in cultured catfish populations may have been caused because individuals from two populations were reared in the same environmental conditions or by inbreeding during several generations. However, in view of bandsharing values, polymorphic bands and also the specific major bands that were inter-population-specific, significant genetic differentiation between these populations were present even if bandsharing (BS) values were somewhat numerically different. Therefore, the number of RAPD polymorphisms identified in this study may be sufficient to permit estimating genetic similarity and diversity. However, in future, additional populations, sampling sites and individuals will be necessary to make up for these weak points.

1. Introduction

The polymorphic DNA markers that were shown to genetically link to a trait of interest could be used for individual and pedigree identification, pathogenic diagnostics, and trait improvement in genetics and breeding programmes. Morphological criteria (Bernardi and Talley 2000), biochemical data (Jensen 2000), isozyme electrophoresis (Smith *et al* 1997; Begg *et al* 1998; Cagigas *et al* 1999), restriction fragment length polymorphism (RFLP) (Hallerman and Bekmann 1988; Garcia-Mas *et al* 2000), minisatellites (Taggart and Ferguson 1990), microsatellites (Li *et al* 2000), randomly amplified polymorphic DNAs (RAPD) (Welsh *et al* 1991; Bernardi and Talley 2000; Fischer *et al* 2000; Garcia-Mas *et al* 2000; Lehmann *et al* 2000; Mohd-Azmi *et al* 2000) and fluorescence *in situ* hybridization (FISH) (Menke *et al* 1998; Brutovská *et al* 2000) have been so far used to analyse genetic similarity and diversity in genetics and breeding research of animal/plant/microbes.

The polymerase chain reaction-randomly amplified polymorphic DNAs (PCR-RAPD) has been particularly used for genetic and molecular studies as it is a simple and rapid method for determining genetic diversity and

Keywords. Bandsharing; cultured catfish; DNA diversity; polymorphic bands; RAPD-PCR

Abbreviations used: BS, bandsharing; PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

similarity in various organisms. It also has the advantage that no prior knowledge of the genome under research is necessary (Fischer *et al* 2000; Klinbunga *et al* 2000).

Catfish is a commercially important warmwater fish species, which is distributed all over the world. As in warmwater fish species, the catfish, population/ density of this fish species is decreased significantly owing mainly to imprudent development and reckless fishing during the last two decades. Consequent to the rapid increase in hatchery-reared catfish production, there is a need to understand the genetic composition of natural catfish populations in order to evaluate the exact the latent genetic effects induced by hatchery operations.

In spite of its economic and scientific importance, little information is available on the phylogenetic relationships among the few catfish populations in Korea. RAPD technique is one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Bartish *et al* 2000). Therefore, the objective of the present work was to identify genetic similarity and diversity within and between populations from two geographical areas by RAPD-PCR and by means of the analysis of bandsharing values in cultured catfish (*Silurus asotus*).

2. Materials and methods

2.1 *Blood collection*

Blood samples of one-year-old Korean catfish (*Silurus asotus*) were obtained from two aquaculture facilities (50 fish per aquaculture, a total of 100 fish) in the periphery of Kunsan and Yesan in western area of Korea. The geographical distance between the two aquaculture facilities was approximately 200 km. Catfish were caught by fish traps, brought to the laboratory alive and anaesthetized immediately with MS 222 (tricaine methane sulphonate) (100 ppm). Blood samples of anaesthetized fish were taken from the caudal vein into heparinized glass tubes and refrigerated at 4° until use. All glasswares, micropipette tips, centrifuge tubes, glass pipettes and buffer solutions were autoclaved to avoid DNA contamination.

2.2 *Sources of genomic DNA*

Genomic DNA was extracted with highest quality reagents (Vierling *et al* 1994) and according to the following standard separation and extraction procedures (without phenol).

Samples of whole blood were placed into 10 ml heparinized vials, to which 4 volumes of lysis buffer I was added, and the mixture tubes were gently inverted several times. The samples were incubated on ice for 30 min and centrifuged at 1,750 *g* for 10 min at 4°C and the pellets were resuspended with lysis buffer I. The pellets were transferred to 1·5 ml Eppendorf tubes with lysis buffer I, and were centrifuged at 22,380 *g* for 1 min. The precipitates were diffused with 0·8 ml lysis buffer II.

Samples were transferred to 1·5 ml Eppendorf tubes and 15 µl proteinase K solution (10 mg/ml) was additionally added to it. The mixtures were gently inverted and incubated at 37°C for overnight or 65°C for 4 h. After incubation, 0·3 ml of 6 M NaCl was added and gently pipetted for a few min. To the mixture 0·6 ml of chloroform was added and then inverted. Samples were spun down at 22,380 *g* for 5 min. The cleared lysates were extracted with 2 volume of ice-cold 70% ethanol, then centrifuged at 22,380 *g* for 5 min and then precipitated. The DNA pellets were air-dried for 30 min and then dissolved TE buffer (10 mM Tris-HCl, pH 8·0, 1 mm EDTA).

Purity and concentration of genomic DNA was estimated by calculating the ratio of the optical density measured at 260–280 nm with a spectrophotometer (Beckman DU 600 series).

2.3 *Primers, markers and amplification conditions*

Out of 20 decamer primers (Operon Technologies, USA) screened, 5 random primers were used to analyse genetic similarity and diversity within and between populations by RAPD-PCR and bandsharing analysis (table 1).

Amplification reactions were undertaken in volumes of 20 µl. This contained 10 ng of template DNA, 20 µl AccuPower premix (Bioneer Co., Korea) and 1·0 unit primer (Operon Technologies, USA). Amplification was performed in a DNA Thermal Cycler (GeneAmp PCR System 2400, Perkin Elmer, USA). The mixture was followed by pre-denaturation at 94°C for 5 min. The PCR conditions are as follows: 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.

Amplified products were analysed by electrophoresis with *F* X174 DNA/*Hae*III marker (Promega Co., USA) in 1·4% agarose gels with TBE and detected by staining with ethidium bromide. The gels were viewed and photographed by photoman direct copy system (Seoulin Co., Korea).

2.4 *Analytical method*

To calculate bandsharing (BS) values the formula of Jeffreys and Morton (1987) and Mohd-Azmi *et al* (2000): $BS = N\ (Ba\ldots.n)/(Ba + Bb + \ldots.. + Bn)$ was modified to $BS = 2\ (Bab)/(Ba + Bb)$, where *Bab* is the number of

bands shared by individuals *a* and *b*, *Ba* is the total number of bands for individual *a*, and *Bb* is the total number of bands for individual *b*. If the comparison is between the three lanes, the formula would be: $BS = 3(Nabc)/(Na + Nb + Nc)$ and so on. Only bands that were surely visible were scored. BS values were scored by the presence or absence of an amplified product at specific positions in the same gel from the RAPD profiles. PCR amplification and BS experiments on the same DNA sample were carried out to examine the efficiency and reproducibility and then the data obtained were used in this experiment and data analyses mentioned above.

3. Results

3.1 *Within-population variations (from Kunsan)*

A total of 100 catfish individuals were investigated using five arbitrarily selected primers. Major and minor RAPD fragments ranging from 0·19 to 0·31 kilobase pairs (kb) were polymorphic in Kunsan catfish population (figure 1). Various primers revealed varying degrees of polymorphism. The various bands in the molecular weight ranging from 0·19 to 1·35 kb generated by random primer OPA-17 were analysed (figure 4). Among them, the identical band patterns were observed from less than 0·60 kb to larger than 0·60 kb.

A total of 652 amplified bands were produced of which 298 were polymorphic (45·7%) to Kunsan catfish population (tables 2 and 3). The average number of polymorphic bands varied from 1·5 to 7·5 (table 3). The average value (59·6) of total polymorphic bands (298) were approximately 20% and also specific to Kunsan catfish population. In particular, primer OPA-6 produced highest number of fragments among the primers used, with an average of 7·5. On the other hand, primer OPA-19 produced the lowest number of fragments with an average of 1·5.

3.2 *Within-population variations (from Yesan)*

In five primers used, the number of bands produced per primer within Yesan catfish population varied from 3 to 18 with an average of 11·9, as shown in table 2. Of them, five primers amplified fragments that were consistently polymorphic between individuals (figures 1–5). The specific polymorphic patterns showing DNA polymorphism were observed not only from 0·60 kb to larger than 1·35 kb but also from less than 0·19 kb to 0·31 kb in Yesan catfish population (figure 1). The identical band

Table 1. Primers and primer sequences used for the detection of polymorphism in catfish (*S. asotus*).

Primer	Sequence (5′ to 3′)	GC content (%)	Primer	Sequence (5′ to 3′)	GC content (%)
OPA-01	CAGGCCCTTC	70	OPA-11	CAATCGCCGT	60
OPA-02	TGCCGAGCTG	70	OPA-12	TCGGCGATAG	60
OPA-03	AGTCAGCCAC	60	OPA-13	CAGCACCCAC	70
OPA-04	AATCGGGCTG	60	OPA-14	TCTGTGCTGG	60
OPA-05	AGGGGTCTTG	60	OPA-15	TTCCGAACCC	60
OPA-06	GGTCCCTGAC	70	OPA-16	AGCCAGCGAA	60
OPA-07	GAAACGGGTG	60	OPA-17	GACCGCTTGT	60
OPA-08	GTGACGTAGG	60	OPA-18	AGGTGACCGT	60
OPA-09	GGGTAACGCC	70	OPA-19	CAAACGTCGG	60
OPA-10	GTGATCGCAG	60	OPA-20	GTTGCGATCC	60

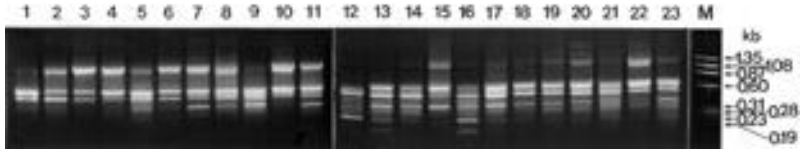

Figure 1. Amplified products were electrophoresed on a 1.4% agarose gel with TBE and detected by staining with ethidium bromide. Individual specific RAPD patterns of catfish amplified by arbitrary OPA-6 (GGTCCCTGAC). Each lane shows different individual DNA samples from Kunsan (lane No. 1–11) and Yesan (lane No. 12–23). [M, molecular size standard (*F*X174 DNA marker digested with *Hae*III)].

from 0·60 kb to 1·08 kb generated by random primer OPA-9 was analysed (figure 2). This primer produced specific RAPD profiles of polymorphic bands from 1·08 kb to 1·35 kb.

A total of 692 amplified bands were generated, 282 (40·8%) of which were polymorphic to Yesan catfish population (tables 2 and 3). An average of 4·9 amplified products were polymorphic to Yesan catfish population (table 3). Primer OPA-14 in particular, generated the highest number of fragments among the primers used, with an average of 6·9. However, OPA-19 generated the lowest number of fragments, with an average of 2·7.

3.3 *Between-populations variations and bandsharing values*

The identical band patterns of RAPD-PCR products produced by the primer OPA-6 were observed from less than 0·60 to 0·60 kb in two populations (figure 1). Especially, specific bands corresponding to 0·31 kb differentiating two populations were identified in the Yesan population. Another primer OPA-9 generated the common DNA bands of approximately 0·23 kb present in every individuals of two populations except for three individuals (figure 2). Also, the specific major band (1·35 kb) that were inter-population-specific were identified. Various band patterns ranging from 0·19 kb to 1·35 kb generated by primer OPA-14 (figure 3) were also seen. In this range (from approximately 0·19 kb to 1·35 kb), the band patterns were highly reproducible and polymorphic. DNA bands ranging from 1·35 kb to larger then 1·35 kb and from 0·19 kb to 0·31 kb, generated by primer OPA-17 were also very different between two populations (figure 4). Especially, population-specific DNA bands, ranging from 1·35 kb to greater 1·35 kb, can also be differentiated in the two populations. The same pattern of major band generated by random primer OPA-19 was identified at 0·28 kb and observed in all individuals of the two populations (figure 5). Also, the specific major band corresponding to 0·23 kb exhibited inter-population-specific characteristics. Numerical differences in total or average bands between Kunsan and Yesan catfish population were observed (table 2). Therefore, one population was significantly different from the other.

Table 2. Number of RAPD bands for each primer using agarose gel in catfish (*S. asotus*) population from two area.

Primer (No.)	Range of band (No.)	Total No. of bands		No. of average bands	
		Kunsan	Yesan	Kunsan	Yesan
OPA-06	8–17	135	149	12·3	12·4
OPA-09	10–17	153	146	12·8	12·2
OPA-14	6–16	142	138	11·8	12·5
OPA-17	9–18	132	163	12·0	13·6
OPA-19	3–12	90	96	8·2	8·7
Subtotal		652	692	57·1	59·4
Total		1344			
Average		130·4	138·4	11·4	11·9

Table 3. Bandsharing (BS) comparisons of the RAPD profiles of two catfish populations from Kunsan and Yesan obtained with five arbitrary primers (mean or mean ± SE).

Primer (No.)	Total of polymorphic bands (No.)		Average number of polymorphic bands		Number of specific bands		BS values	
	Kunsan	Yesan	Kunsan	Yesan	Kunsan	Yesan	Kunsan	Yesan
OPA-06	82	62	7·5	5·2	0	12	0·39	0·58
OPA-09	57	80	4·8	6·7	0	6	0·63	0·45
OPA-14	74	76	6·2	6·9	0	0	0·48	0·45
OPA-17	69	34	6·3	2·8	0	9	0·48	0·79
OPA-19	16	30	1·5	2·7	0	9	0·82	0·69
Subtotal	298	282	26·3	24·3	0	36	2·79	2·96
Total	580				36			
Average	59·6	56·4	5·3	4·9	0	7·2	0·56 ± 0·08*	0.59 ± 0.07*

*Standard error.

The BS values of catfish population from Kunsan varied from 0·39 to 0·82 with an average of 0·56 (table 3). In Kunsan catfish population, the average level of BS generated by primer OPA-6 was lower than any other primer, which was 0·39. Also, the degree of similarity frequency (BS value) generated using random primer OPA-19 in Kunsan catfish population showed the highest level (0·82).

The degree of similarity varied from 0·45 to 0·79 as calculated by BS analysis in Yesan catfish population. Also, the average level of BS was 0·59 ± 0·07 within this population. In view of BS value, RAPD-PCR profiles obtained between two different populations were numerically different. Therefore, there was a middle level of significant difference in BS value between two catfish populations.

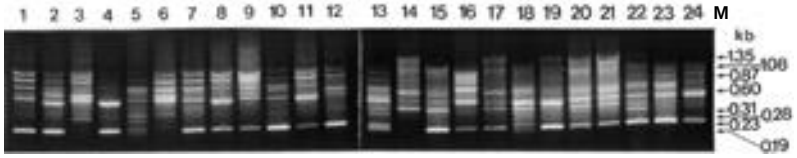

Figure 2. Specific RAPD patterns of catfish (*S. asotus*) amplified by arbitrary primer OPA-9 (GGGTAACGCC). Each lane (1–24) shows different individual DNA samples from Kunsan (lane No. 1–12) and Yesan (lane No. 13–24) (M, *F*X174 DNA marker digested with *Hae*III).

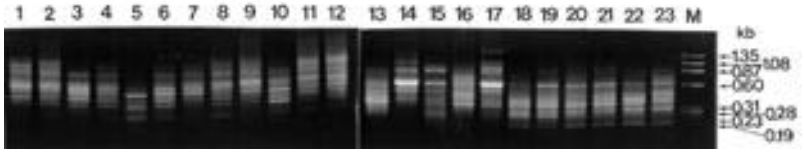

Figure 3. Individual specific RAPD patterns in catfish amplified by arbitrary OPA-14 (TCTGTGCTGG). Each lane (1–23) shows different individual DNA samples from Kunsan (lane No. 1–12) and Yesan (lane No. 13–23) [M, Molecular size marker (*F*X174 DNA marker digested with *Hae*III)].

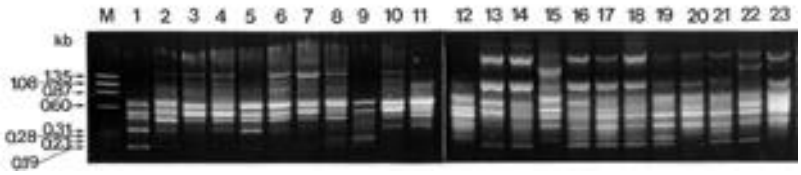

Figure 4. Amplified products were electrophoresed on a 1.4% agarose gel with TBE and detected by staining with ethidium bromide. Individual specific RAPD patterns in catfish amplified by arbitrary OPA-17 (GACCGCTTGT). Each lane shows different individual DNA samples from Kunsan (lane No. 1–11) and Yesan (lane No. 12–23) [M, Molecular size marker (*F*X174 DNA marker digested with *Hae*III)].

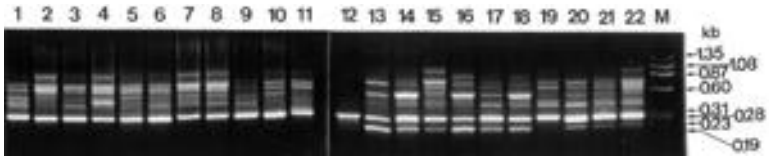

Figure 5. Specific RAPD fingerprints generated in catfish by arbitrary primer OPA-19 (CAAACGTCGG). Each lane (1–23) shows different individual DNA samples (lanes 1–11 from Kunsan and lanes 12–22 from Yesan) [M, Standard markers (*F*X174 DNA marker digested with *Hae*III)].

4. Discussion

4.1 *Within-population variations*

The RAPD-PCR method can be applied to identify genetic similarity and diversity in catfish (*S. asotus*) using five out of twenty primers. In this study, genomic DNA bands were produced by amplifying DNAs isolated from the blood of individuals using primers as shown in figures 1–5. In this study, DNA fragments with a genome size of 100 bp to 1,500 bp were used. Five primers generated a total of 298 highly reproducible RAPD polymorphic fragments, producing an average of 5·3 polymorphic bands per primer in Kunsan population as summarized in table 3. Especially, on average, 4·9 of a total of 282 amplified polymorphic bands were found to be polymorphic in Yesan population as summarized in table 3. For these results, there were population-specific RAPD fragments in catfish and there were differences in frequencies of five primer fragments, as has been reported in other fish (Cagigas *et al* 1999) and in plants (Vierling *et al* 1994; Fischer *et al* 2000). Also, Liu *et al* (1998) reported that some intra-specific RAPD variations were observed for different strains or individuals. They screened a collection of RAPD markers in catfish and identified 22 primers that revealed 171 strain-specific genetic markers.

The bands in the molecular weight range from 0·31 kb to 1·35 kb generated by OPA-6 (GGTCCCTGAC) were observed as shown in figure 1 (lane No. 1–11). Also, the RAPD polymorphism generated by random primer OPA-19 (CAAACGTCGG) showed polymorphic bands as shown in figure 5. In this manner, the identical or specific band patterns of RAPD-PCR products were observed in figures 1–5. Generally, the number and size of the fragments generated, strictly depend upon the nucleotide sequence of the primer used and upon the source of the template DNA, resulting in the genome-specific fingerprints of random DNA fragments (Welsh *et al* 1991). Many authors could detect genetic polymorphisms in a wide variety of organisms by RAPD-PCR using a large number of different primers (Welsh *et al* 1991). Especially, polymorphisms were scored by the presence or absence of amplified products at specific positions expressed by various primers (Smith *et al* 1997). Also, inter-specific, intra-specific, inter-strain or between-population variations in the pattern were observed for each primer and such data should be of value not only in the discrimination of the correlation with the economic traits but also in the construction of genetic relationships (Welsh *et al* 1991; Vierling *et al* 1994; Smith *et al* 1997; Bartish *et al* 2000; Bernardi and Talley 2000; Lehmann *et al* 2000). The RAPD-PCR method can be applied to detect genetic diversity and similarity in numerous organisms using the various primers (Welsh *et al* 1991; Levin *et al* 1993; Cagigas *et al* 1999; Bernardi and Talley 2000).

4.2 *Between-populations variations and bandsharing values*

Among the primers that generated polymorphic amplification products, each primer in Kunsan catfish population generated on an average 5·3 polymorphic bands compared with a mean of 4·9 for each primer in Yesan catfish population, as summarized in table 3. The proportion of polymorphic bands per total number of bands detected was also lower for Yesan catfish population (40·8 versus 45·7% for Kunsan catfish population), as shown in tables 2 and 3.

In view of BS, BS values were somewhat different numerically. However, because of the specific major bands which were the inter-population-specific as shown in figures 1, 2, 4 and 5, we could find significant genetic differentiation between the two populations. These substantial differences in the number of polymorphic bands and BS values suggest that the level of genetic diversity between two catfish populations may be sufficient for developing an intra-specific population. Accordingly, the number of RAPD polymorphisms identified in this study may be sufficient to permit an estimation of genetic diversity and similarity. However, there were a few weak points in the study, such as limited distance of populations and a possible migration of one population to the other, as they were only 200 km apart during the performance of this study. Additionally, within-population variation is significantly less than between-population variation. The observed small-scale differentiation in RAPD variation within population indicates that gene flow would not be sufficient to counteract effects of selection (Fischer *et al* 2000). Therefore, in future, additional populations, sampling sites and individuals will be necessary to counter these weak points and increase the efficiency of this study.

In future, additional method such as RFLP, microsatellite, amplified fragment length polymorphism (AFLP), minisatellite and sequence techniques etc. are likely to maximize the efficiency of efforts in various fields of interest in the aquaculture industry, especially with regard to the genetic and breeding programmes of catfish.

Acknowledgments

The authors are grateful to the Fisheries Science Institute of Kunsan National University for equipment and funding in the programme year of 2001 and wish to thank Mr T S Kim and Ms S H Moon for their assistance in fish collection and their help with the method of RAPD-PCR. Also, in particular, thanks go to referees who assisted in the thorough correction of the manuscript.

References

Bartish I V, Garkava L P, Rumpunen K and Nybom H 2000 Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozymes; *Theor. Appl. Genet.* **101** 554–561

Begg G A, Keenan C P and Sellin M J 1998 Genetic variation and stock structure of school mackerel and spotted mackerel in northern Australian waters; *J. Fish Biol.* **53** 543–559

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

Brutovská R, Čellárová E and Schubert I 2000 Cytogenetic characterization of three *Hypericum* species by *in situ* hybridization; *Theor. Appl. Genet.* **101** 46–50

Cagigas M E, Vazquez E, Blanco G and Sánchez J A 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

Fischer M, Husi R, Prati D, Peintinger M, Kleunen M V and Schmid B 2000 RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae); *Am. J. Bot.* **87** 1128–1137

Garcia-Mas J, Oliver M, Gomez-Paniagua H and Vicente M C 2000 Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon; *Theor. Appl. Genet.* **101** 860–864

Hallerman E M and Beckmann J S 1988 DNA-level polymorphism as a tool in fisheries science; *Can. J. Fish. Aquat. Sci.* **45** 1075–1087

Jeffreys A J and Morton D B 1987 DNA fingerprints of dogs and cats; *Anim. Genet.* **18** 1–15

Jensen S R 2000 Chemical relationships of *Polypremum procumbens*, *Tetrachondra hamiltonii* and *Peltanthera floribunda; Biochem. Syst. Ecol.* **28** 45–51

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

Lehmann D, Hettwer H and Taraschewski H 2000 RAPD-PCR investigations of systematic relationships among four species of eels (Teleostei: Anguillidae), particularly *Anguilla anguilla* and *A. rostrata*; *Mar. Biol.* **137** 195–204

Levin I, Crittenden L B and Dodgson J B 1993 Genetic map of the chicken Z-chromosome using random amplified polymorphic DNA (RAPD) markers; *Genomics* **16** 224–230

Li X, Li K, Fan B, Gong Y, Zhao S, Peng Z and Liu B 2000 The genetic diversity of seven pig breeds in China, estimated by means of microsatellites; *Asian-Aus. J. Anim. Sci.* **13** 1193–1195

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

Menke M, Fuchs J and Schubert I 1998 A comparison of sequence resolution on plant chromosomes: PRINS versus FISH; *Theor. Appl. Genet.* **97** 1314–1320

Mohd-Azmi M, Ali A S and Kheng W K 2000 DNA fingerprinting of red jungle fowl, village chicken and broilers; *Asian–Aus. J. Anim. Sci.* **13** 1040–1043

Smith P J, Benson P G and McVeagh S M 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

Taggart J B and Ferguson A 1990 Minisatellite DNA fingerprints of salmonid fishes; *Anim. Genet.* **21** 377–389

Vierling R A, Xiang Z, Joshi C P, Gilbert M L and Nguyen H T 1994 Genetic diversity among elite *Sorghum* lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs; *Theor. Appl. Genet.* **87** 816–820

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

MS received 31 July 2001; accepted 18 October 2001

Corresponding editor: VIDYANAND NANJUNDIAH