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Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish

Z.J. Liu *, P. Li, B.J. Argue [1], R.A. Dunham

The Fish Molecular Genetics and Biotechnology Laboratory, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849, USA

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Abstract

Catfish are the most important aquacultural species in the United States. A genetic linkage map is needed to improve efficiency of breeding by marker-assisted selection (MAS), and for identification, isolation and eventual cloning of commercially important genes. To identify DNA-based genetic polymorphism for constructing a genetic linkage map of catfish, we tested 100 random amplification of polymorphic DNA (RAPD) primers for their utility in identifying genetic polymorphism in catfish. The overall polymorphism was low among strains within a species for both channel catfish (*Ictalurus punctatus)* and blue catfish (*Ictalurus furcatus)*. However, considerably higher levels of polymorphism were detected between channel catfish and blue catfish. Among the 100 primers tested, 42 produced highly clean and reproducible RAPD profiles; 33 produced medium quality RAPD profiles; and 25 produced poorly reproducible RAPD profiles or non-polymorphic RAPD profiles. The 75 high and medium quality primers generated 462 polymorphic bands, an average of 6.1 bands per primer. The RAPD markers were highly reproducible in a size range from 200 to 1500 base pairs (bp). They were transmitted to F_1 hybrids as dominant markers. There was no difference in RAPD profiles between channel catfish $\times$ blue catfish F_1 hybrids or their reciprocal hybrids. The markers segregated in F_2 or backcross progeny with ratios as expected from Mendelian inheritance. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Polymorphism; Primer; PCR; Catfish; RAPD; Marker; *Ictalurus*; Gene mapping

* Corresponding author. Tel.: +1-334-844-4054; Fax: +1-334-844-9208; E-mail: zliu@acesag.auburn.edu
[1] Current address: The Oceanic Institute, 41-202 Kalanianaole Hwy, Waimanalo, HI 96795.

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1. Introduction

Animal stocks may be improved by gene transfer or by use of marker-assisted selection (MAS). However, progress is limited by availability of useful genes or identified markers that are linked to important traits. Thus, identification of sufficient numbers of molecular markers is critically important for gene mapping, for marker-assisted selection (MAS) and for eventual cloning of beneficial genes from channel catfish.

Random amplification of polymorphic DNA (RAPD) markers are polymorphic DNA separated by gel electrophoresis after PCR using short random oligonucleotide primers (Welsh and McClelland, 1990; Williams et al., 1990). Genetic analysis with RAPD markers is relatively easy, fast and efficient. However, RAPD markers are dominant alleles. This is a disadvantage for RAPD analysis because dominant markers fail to distinguish between heterozygous and homozygous individuals possessing a specific allele. Despite the disadvantages of dominance and lower reproducibility due to low stringent PCR with RAPD, it has been used for detection of genetic variation in various fish species (Dinesh et al., 1993; Johnson et al., 1994; Foo et al., 1995; Bielawski and Pumo, 1997; Caccone et al., 1997; Cunningham and Mo, 1997). RAPD has also been used for phylogenetic studies for species and subspecies identification of fish (Bardakci and Skibinski, 1994; Borowsky et al., 1995; Sultmann et al., 1995; Partis and Wells, 1996), for gynogenetic fish identification (Chen and Leibenguth, 1995; Corley-Smith et al., 1996) and for gene mapping studies in fish (Postlethwait et al., 1994; Kazianis et al., 1996).

Channel catfish is the most important cultured fish in the United States, accounting for over 50% of all aquacultural production. It is also one of the important sporting fish in many southern states. To construct a genetic linkage map for use in MAS, we exploited the channel catfish × blue catfish hybrid system, from which greater numbers of polymorphic markers are available. We previously studied the segregation of RAPD markers in the F_2 hybrid and backcross hybrids (Liu et al., 1998b). In this paper, we evaluated feasibility of using RAPD markers for both intraspecific mating plans and interspecific hybrid mating plans. An additional 100 RAPD primers were evaluated for their usefulness in catfish. Of the 100 primers, 42 were good; 33 were medium quality; and 25 were poor. A total of 462 new polymorphic RAPD markers were identified using the 75 good and medium quality primers. Additionally, no differences were detected in RAPD profiles between the two reciprocal F_1 hybrids despite dramatic phenotypic differences between the two reciprocal hybrids due to paternal predominance (Dunham et al., 1982).

2. Materials and methods

2.1. Animals

Experimental fish were raised at the Fish Genetics Research Unit at Auburn University. Six channel catfish and three blue catfish were used to make the interspecific F_1

hybrids. All the reciprocal backcrosses to the parent species were made (Argue, 1996). Blood samples were collected from male and female parental channel catfish and blue catfish. Blood samples were also collected from 20 F_2 hybrids and 14 backcross hybrids (six backcrosses to channel catfish and eight backcrosses to blue catfish). Both of these backcrosses were needed for genetic analysis of dominant marker types. The reciprocal F_1 hybrids (female channel catfish × male blue catfish and female blue catfish × male channel catfish) were examined to determine if paternal predominance (Dunham et al., 1982) could be related to differences in RAPD profiles. To estimate RAPD variations within a particular strain, 20 individuals were examined using three primers. To estimate RAPD variations among strains of catfish, 10 individuals each of channel catfish were examined from Auburn strain, Kansas strain, Marion strain and Stuttgart strain, and 10 each of blue catfish were examined from Rio Grande strain, Craft strain, and D and B strain, using three primers: A1, A6 and A19. To compare RAPD profiles of reciprocal hybrids, five individuals of each hybrid were examined using three primers: A1, A8 and A20 (for sequences of these A-primers, see Liu et al., 1998b).

2.2. DNA preparation and RAPD procedures

Blood samples (0.1–0.2 ml) were collected using a 1-ml syringe, immediately transferred to a microcentrifuge tube, and centrifuged in a microcentrifuge for 20 s at maximum speed. Serum was removed with a 1-ml pipette. Blood cells were dispersed by pipetting 300 μl distilled water up and down. The blood cells were expelled into lysis buffer quickly to disperse the blood cells. DNA was isolated using standard protocol (Strauss, 1989; Liu et al., 1998a). RAPD was conducted and RAPD markers named as previously described (Liu et al., 1998b). Primer sequences are available from Operon Technologies (Alameda, CA).

3. Results

3.1. Low levels of RAPD polymorphism among strains of channel catfish or blue catfish

Low levels of polymorphism exist among strains of channel catfish (Fig. 1), even though distinct phenotypes were observed and inherited for each of these strains (Dunham et al., 1993). Thus, four strains of channel catfish (Kansas, Auburn, Marion and Stuttgart) exhibited similar RAPD profiles (Fig. 1). Similarly, three strains of blue catfish (Rio Grande, Craft and D and B) also exhibited a low level of intraspecific polymorphism (data not shown). Overall, only 5–10% of bands were polymorphic between strains within species.

3.2. High rates of RAPD polymorphism between channel catfish and blue catfish

Polymorphic rates of RAPD bands were much higher between channel catfish and blue catfish than those found among strains within each species (Fig. 2). The 100 RAPD

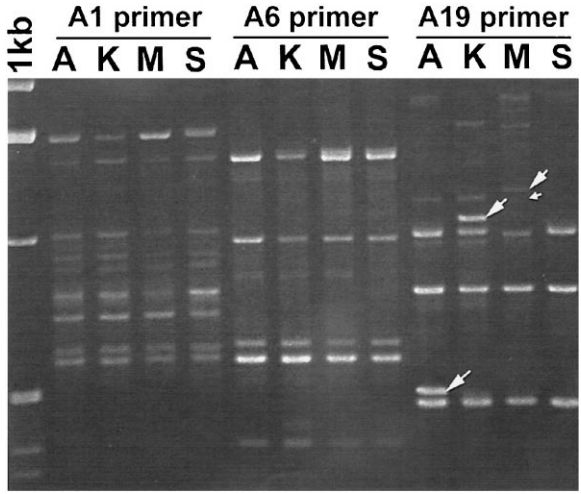

Fig. 1. Low levels of polymorphism among strains of channel catfish (*Ictalurus punctatus)*. RAPD amplification products of three primers are shown. (A1) primer A1; (A6) primer A6; (A19) primer A19. Designations for the lanes are: (1 kb) 1 kb molecular weight markers; (A) Auburn strain of channel catfish; (K) Kansas strain of channel catfish; (M) Marion strain of channel catfish; (S) Stuttgart strain of channel catfish. Large white arrows indicate strain-specific bands. Small white arrows indicate absence of a specific band from that strain.

primers amplified an average of 5.3 bands from channel catfish and 5.5 bands from blue catfish. About 47% of the amplified bands from channel catfish and blue catfish were

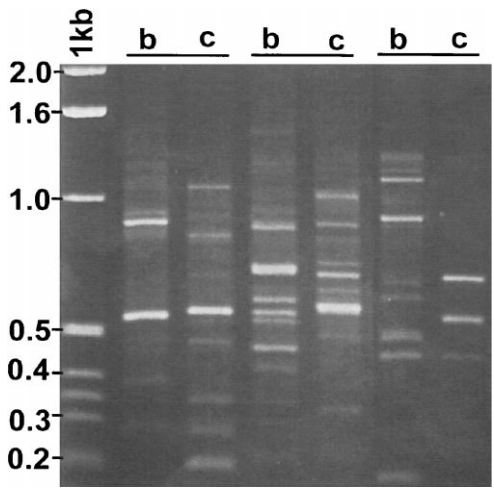

Fig. 2. High levels of polymorphism observed from channel catfish (*I. punctatus*) and blue catfish (*Ictalurus furcatus)*. RAPD amplification products of three primers are shown, with products from each primer grouped together by underlining. ('b'), blue catfish; ('c'), channel catfish; ('1 kb'), 1 kb molecular weight markers with sizes marked on the left margin.

either specific to channel catfish or blue catfish. A total of 1082 bands were amplified from the 100 RAPD primers, of which 244 bands were specific to channel catfish and 268 were specific to blue catfish. On average, each RAPD primer produced about 2.5 polymorphic bands between channel catfish and blue catfish.

3.3. Transmission of RAPD markers to interspecific F_1 hybrids

Almost all RAPD bands from both parents were found in RAPD profiles of F_1 hybrids (Fig. 3), indicating high penetrance and the dominant nature of RAPD markers. Very low levels of segregation of polymorphic RAPD markers were observed in F_1 individuals (data not shown), indicating the heterozygous nature of some individuals at the RAPD marker loci in the parents, consistent with low levels of intraspecific polymorphism.

The reciprocal F_1 hybrids (female channel catfish × male blue catfish and female blue catfish × male channel catfish) exhibited the same RAPD profiles. They are phenotypically different due to paternal predominance (Dunham et al., 1982). To test whether the RAPD markers were transmitted into the reciprocal F_1 hybrids similarly, reciprocal hybrids were tested with RAPD primers. All RAPD bands were transmitted into the reciprocal F_1 hybrids, regardless of the sex of the parents (Fig. 3). Similar results were found in our recent studies using AFLP markers (Liu et al., 1998a). RAPD markers was observed to segregate properly in the F_2 hybrids or appropriate backcross catfish hybrids as previously reported (Liu et al., 1998b).

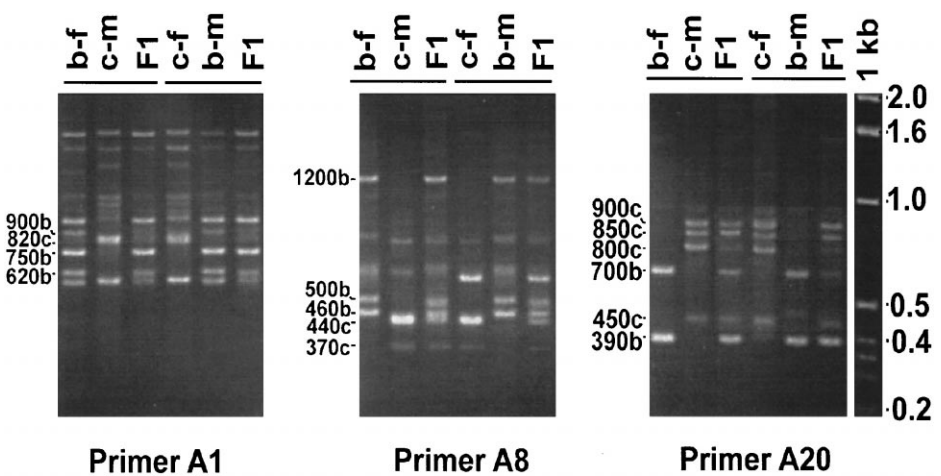

Fig. 3. Comparison of RAPD profiles of reciprocal F_1 hybrids of channel catfish × blue catfish. Amplification products of three primers (A1, A8 and A20) are shown. Each primer was tested with two reciprocal pairs of parents and their F_1 hybrids. (b-f) Blue catfish female; (c-m), channel catfish male; (F_1) F_1 hybrids; (c-f) channel catfish female; (b-m) blue catfish male. (1 kb) 1 kb molecular markers with sizes marked on the right margin. Markers are labeled on the left margins of each panel.

3.4. Useful RAPD primers and markers for gene mapping analysis in catfish

Among the 100 tested primers, 42 primers produced highly reproducible and clean RAPD profiles (Table 1); 33 primers produced reproducible and medium clean RAPD

Table 1
RAPD profiles of excellent primers for channel catfish *I. punctatus* and blue catfish *I. furcatus*

Primer	Shared bands	Channel catfish bands	Blue catfish bands
B1	5	4	3
B4	3	3	3
B5	5	3	2
B7	3	5	4
B9	2	3	3
B11	1	6	7
B12	2	2	1
B13	5	2	2
B14	2	4	7
B15	4	3	6
B19	1	2	1
B20	0	2	10
C2	5	7	7
C4	2	10	6
C5	2	6	9
C12	6	3	4
C13	3	6	7
C14	4	4	2
C19	6	3	2
D3	3	2	2
D7	4	3	3
D8	6	2	2
D9	1	1	1
D10	2	2	2
D11	1	2	5
D13	1	4	3
D15	3	3	4
E1	3	6	2
E2	4	3	2
E6	3	5	4
E10	3	2	4
E11	2	3	2
E12	0	2	2
E14	0	2	2
E17	1	4	3
F1	2	4	6
F5	3	1	5
F8	0	2	3
F12	2	2	4
F15	0	4	5
F16	2	3	5
F19	1	1	0
Total: 42	108	141	159

Primer designations are the same as supplied by Operon Technologies.

Table 2
RAPD profiles of medium quality primers for channel catfish *I. punctatus* and blue catfish *I. furcatus*

Primer	Shared bands	Channel catfish bands	Blue catfish bands
B3	5	4	1
B6	3	4	3
B8	3	2	2
B10	2	2	3
B16	1	2	3
B17	5	3	5
C1	2	0	5
C6	3	4	3
C9	6	6	8
C15	5	2	1
C16	6	0	2
C17	3	3	4
C20	7	1	0
D1	2	5	2
D2	6	1	0
D4	1	2	3
D5	6	2	3
D14	1	2	2
D16	2	7	4
D19	1	3	2
D20	3	3	4
E3	2	2	1
E5	2	3	2
E8	1	3	4
E9	1	0	1
E18	3	1	0
F2	2	2	2
F6	0	1	8
F7	2	1	2
F9	4	1	2
F11	1	1	1
F17	1	2	2
F18	1	1	1
Total: 33	97	76	86

Primer designations are the same as supplied by Operon Technologies.

profiles (Table 2). The remaining 25 primers produced poorly reproducible RAPD profiles or produced profiles with no polymorphism (Table 3). The poor primers either

Table 3
RAPD primers of poor reproducibility and RAPD primers producing no polymorphic bands (*) for channel catfish *I. punctatus* and blue catfish *I. furcatus*

B-kit	C-kit	D-kit	E-kit	F-kit	Total
B2, B18	C3, C7, C8, C10, C11, C18	D6, D12, D17, D18	E4, E7, E13, E15, E16, E19, E20	F3*, F4, F10*, F13, F14, F20	25 primers

Primer and kit designations are the same as supplied by Operon Technologies.

generated too many bands to evaluate, were very difficult to use, or exhibited no polymorphism. These primers should be eliminated from future use in channel catfish and blue catfish.

A total of 462 RAPD markers were identified using the 75 high and medium quality primers (Tables 1 and 2). The most useful markers are those produced by the excellent primers. The 42 excellent primers produced a total of 300 polymorphic bands (Table 1). The 33 medium quality primers produced a total of 162 polymorphic bands (Table 2). Polymorphic bands produced from poor primers will have little use because of their low reproducibility.

Primers that showed high levels of reproducibility also produced more polymorphic bands. Over seven polymorphic bands per primer were produced from the 42 excellent primers on average. The 33 medium quality primers produced an average of 4.9 polymorphic bands per primer. The 24 poor primers produced only 63 polymorphic bands or 2.6 polymorphic bands per primer (data not shown).

3.5. Factors affecting reproducibility of RAPD for individuals

Reproducibility was tested by using DNA templates from several fish isolated at different times. Exact reproducibility was observed when similar concentrations of DNA template were utilized. We tested 10 individuals of channel catfish whose DNA was isolated at different times. All 10 individuals of channel catfish had identical RAPD profiles (data not shown). Variation in numbers of amplified bands was observed with drastic changes (greater than 1000-fold) in DNA template concentration. Similarly, different numbers of bands were amplified with drastic changes (greater than 10-fold) in primer concentrations. Generally, higher DNA template concentration and primer concentration led to amplification of more bands, making scoring more difficult. However, when consistent quantities of DNA and primers were used, consistent and reproducible results were obtained.

Another factor for reproducibility of RAPD bands is the size of amplified products. Generally, amplified products with large sizes (more than 2 kb) showed low reproducibility. Good reproducibility was obtained with bands between 200–1500 bp.

4. Discussion

RAPD generated a large number of polymorphic DNA bands between channel catfish and blue catfish without requiring any previous knowledge of the catfish genomes, thus making it one of the most efficient systems for generating DNA markers using the interspecific hybrid system. However, low levels of intraspecific variation were found in RAPD profiles among strains of channel catfish or blue catfish, which excludes RAPD as an efficient system to generate molecular markers for gene mapping in an intraspecific mating plan. This partially explains why RAPD is more popular in research of plants and microorganisms, where interspecific hybrids are often available for use in mapping analysis.

One of the most important factors determining the applicability of RAPD for gene mapping analysis is its reproducibility (reviewed by Hadrys et al., 1992; Hedrik, 1992; Riedy et al., 1992; Scott et al., 1992; Powell et al., 1995). The present results indicate that good reproducibility can be achieved using high quality primers and for PCR products of 200–1500 bp. Caution must be exercised for medium quality primers. Low quality primers should be avoided. Bands greater than 2000 bp should not be scored unless one is sure about the reproducibility of bands with higher molecular weight.

RAPD is perhaps the most appropriate and economical means for hybrid identification because of its dominant inheritance. It may be useful for genetic resource analysis of domestic and wild catfish populations and perhaps for other fish species as well (Bielawski and Pumo, 1997; Caccone et al., 1997; Cunningham and Mo, 1997). This is particularly important because RAPD does not require any known genetic information. Its capacity for genetic analysis in catfish is greater than isozymes and expressed sequence tag (EST) markers (Karsi et al., 1998; Liu et al., 1999a), but is much lower than AFLP markers (Liu et al., 1998a) and microsatellite markers (Liu et al., 1999b), although sequence information is required for microsatellite analysis.

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