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SHORT COMMUNICATION

Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), and their F1, F2 and backcross hybrids

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

Summary

The channel catfish (*Ictalurus punctatus*) has become the most important aquaculture species in the USA. A genetic linkage map in catfish is needed to improve efficiency of breeding by marker-assisted selection (MAS) and for identification of economically important genes such as disease resistance genes. To identify DNA-based genetic polymorphism, the present authors tested 42 randomly amplified polymorphic DNA (RAPD) primers for their utility in identifying genetic polymorphism in catfish. Out of these primers, 22 generated 171 highly reproducible RAPD markers, producing almost eight polymorphic bands per primer. The remaining 20 primers produced an additional 20 polymorphic bands. The RAPD markers were highly reproducible, transmitted to F1 hybrids, and segregated in F2 or backcross progeny in ratios that did not differ from Mendelian expectations. Because the interspecific hybrids of channel catfish and blue catfish are fertile, RAPD markers using the interspecific hybrid system will be useful for rapid construction of genetic linkage maps of catfish and for analysis of important quantitative trait loci.

Keywords: DNA polymorphism, random primers, PCR, catfish, marker, RAPD

The channel catfish (*Ictalurus punctatus*) is the most important aquaculture species in the USA, accounting for over 50% of all aquaculture production. Hybrids can be made between channel catfish with blue catfish (*I. furcatus*) by artificial spawning. Backcross or F2 hybrids can be produced from the F1 hybrids (Argue 1996; Argue & Dunham 1998), making this interspecific hybrid system an ideal system for gene mapping analysis. Gene mapping analysis using the hybrid system has several advantages: maximizing polymorphism for identifying molecular markers, constructing genetic linkage maps of the two species simultaneously and allowing mapping of quantitative trait loci (QTL) from both species. Several important economic traits are inherited from the blue catfish. For instance, resistance to the major bacterial disease, enteric septicaemia of catfish caused by *Edwardsiella ictaluri*, is inherited from the blue catfish (Dunham *et al.* 1993; Wolters *et al.* 1996). Additionally, research to date indicates high levels of similarity in genomic organization of the two species (LeGrande *et al.* 1984; Dunham *et al.* 1993; Argue 1996). Genetic linkage maps of the two species should be similar and developed markers can probably be applied to a marker-assisted selection (MAS) programme for either species.

The specific objectives of the present study were to establish the methodology and applicability of random amplified polymorphic DNA (RAPD) markers (Williams *et al.* 1990; Welsh & McClelland 1990; Foo *et al.* 1995) for catfish gene mapping analysis, and to examine the inheritance of RAPD markers in catfish. The present results demonstrate that RAPD markers are abundant, reproducible and are inherited in Mendelian expectations using the channel × blue catfish hybrid system, and thus, provide a basis for future gene mapping and MAS in these important aquaculture species using RAPD polymorphic markers.

Experimental fish were raised at the fish genetics facility at Auburn University, Auburn, AL. Two channel catfish (Kansas strain) and two blue catfish (Rio Grande strain and D & B strain) were used to make the interspecific F1 hybrids. At sexual maturity, the F1 hybrids (channel × blue) were used to make the F2 hybrids and backcross hybrids. Blood samples were collected for preparation of DNA (Strauss 1989) from channel and blue catfish parents, F1 hybrids, and individuals of F2 or backcross hybrids. PCR was performed using standard buffer, 100 μM dNTPs, 4·5 μM primer, 25 ng DNA template and 0·75 units of *Taq* DNA polymerase (Promega Corporation, Madison, WI). After an initial heat denaturation of 5 min

Z Liu
P Li
B J Argue
R A Dunham
The Fish Molecular Genetics and Biotechnology Laboratory, Department of Fisheries and Allied Aquacultures, 203 Swingle Hall, Auburn University, Auburn, AL 36849, USA

Correspondence: Zhanjiang Liu.

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at 94°C, the DNA was amplified for 45 cycles consisting of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. Amplified fragments were resolved in a 1·4% agarose gels (Sambrook *et al.* 1989).

RAPD markers were named by using the primer followed by a dash, a small letter 'c' or 'b' for channel catfish markers or blue catfish markers, respectively. The number following the small c or b represents the fragment size in base pairs (bp). For instance A3-b300 indicates a RAPD marker of about 300 bp that is amplified with primer A3 in blue catfish.

To determine the incidence of RAPD between channel catfish and blue catfish, the RAPD analysis was performed with various primers (Table 1) using channel catfish and blue catfish genomic DNA as templates. As shown in Fig. 1 and summarized in Table 1, high levels of polymorphism were found between the two species. On average, each random RAPD primer (Operon Technologies, Inc., Alameda, CA) amplified 6·8 bands from channel catfish and 6·6 bands from blue catfish. About 47% of total amplified bands from channel catfish and blue catfish were either specific to channel catfish or to blue catfish (no band-sharing). Therefore, these bands are potentially polymorphic markers.

Johnson *et al.* (1994) screened a collection of RAPD markers in zebrafish and identified 116 primers that revealed 721 strain-specific genetic markers. With the hope that these primers would also work well in catfish, the present authors performed RAPD with 12 of their 'X' and 'Y' primers (designated after nomenclature of Operon Technologies, Inc.; see Johnson *et al.* 1994). These selected RAPD primers generated similar total amplified bands as the previously tested primers, but with lower levels of polymorphism (Table 1). Only 26% of the total amplified bands with the selected RAPD primers were polymorphic, as compared to 47% with random RAPD primers.

High-GC primers were reported to amplify an increased number of RAPD bands in a broad range of fungi (Kubelik & Szabo 1995). To test whether or not the high-GC primers also amplify more RAPD bands in catfish and are more useful for gene mapping analysis than random RAPD primers, the present authors tested 10 high-GC primers containing 80–100% GC, designated as CRL primers in Table 1 (provided by Dr Les Szabo at the USDA ARS Cereal Rust Laboratory, University of Minnesota, St. Paul, MN). Overall, the high-GC primers amplified more total bands than the random RAPD primers. More than 13 bands were amplified on average from each of the high-GC primer as compared to less than seven bands with random RAPD primers. Several high-GC primers amplified too many bands (greater than 20) to be further characterized (Table 1). Polymorphism levels were much lower with the high-GC primers than with random RAPD primers and only 10% of the total amplified bands with high-GC primers were polymorphic. Thus, high-GC primers appeared to be less useful in catfish than

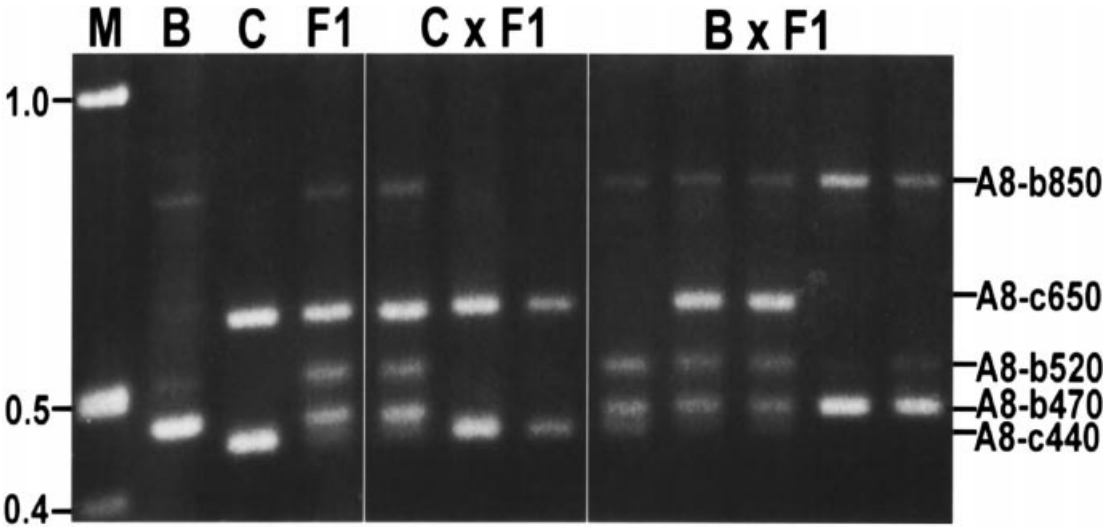

Fig. 1. Inheritance of RAPD markers in catfish using primer A8: (M) 1-kb molecular weight markers with bands shown from the bottom (396, 506/517, 1·0 kb); (B) blue catfish parent; (C) channel catfish parent; (F1) the interspecific F1 hybrid; (C × F1) backcross progeny from F1 × channel catfish; (B × F1) backcross progeny from F1 × blue catfish. For these crosses, the first individual is the female parent and the second individual is the male parent. Each lane under each category represents a single individual. The segregating markers were: A8-c440, A8-b470, A8-b520, A8-c650 and A8-b850.

Table 1. Summary of RAPD primers and amplification for channel catfish (*Ictalurus punctatus*), and blue catfish (*I. furcatus*)[a]

Primer number	Sequence (5′–3′)	Total bands Channel catfish	Total bands Blue catfish	Unique bands Channel catfish	Unique bands Blue catfish
Random RAPD primers					
A1**	CAGGCCCTTC	8	9	3	4
A2	TGCCGAGCTG	–	–	–	–
A3*	AGTCAGCCAC	9	10	2	2
A4*	AATCGGGCTG	19	21	4	3
A5	AGGGGTCTTG	12	10	2	1
A6**	GGTCCCTGAC	8	6	8	6
A7**	GAAACGGGTG	8	8	3	3
A8**	GTGACGTAGG	7	6	5	4
A9*	GGGTAACGCC	6	4	4	1
A10*/*	GTGATCGCAG	7	4	5	2
A11*	CAATCGCCGT	7	8	4	5
A12	TCGGCGATAG	3	3	2	2
A13**	CAGCACCCAC	3	3	2	2
A14	TCTGTGCTGG	–	–	–	–
A15*	TTCCGAACCC	5	5	5	5
A16**	AGCCAGCGAA	11	10	5	4
A17**	GACCGCTTGT	2	6	2	6
A18	AGGTGACCGT	9	6	2	0
A19**	CAAACGTCGG	7	9	5	7
A20**	GTTGCGATCC	5	4	4	3
Selected primers					
X4**	CCGCTACCGA	5	4	5	4
X13	ACGGGAGCAA	–	–	–	–
X16**	CTCTGTTCGG	1	1	1	1
X17*	GACACGGACC	10	10	1	1
X19	TGGCAAGGCA	10	10	0	0
Y1	GTGGCATCTC	9	9	0	0
Y3	ACAGCCTGCT	4	4	2	2
Y4**	GGCTGCAATG	8	8	2	2
Y8	AGGCAGAGCA	>20	>20	–	–
Y13**	GGGTCTCGGT	4	8	3	7
Y17	GACGTGGTGA	9	9	0	0
Y20	AGCCGTGGAA	6	2	5	1
High-GC (GC-rich) primers					
CRL5**	CCAGCGTCCC	7	5	4	2
CRL6	CCCGCGCCC	>20	>20	–	–
CRL7	GCCCGCCGCC	10	9	1	1
CRL8	CTGCCGCCAC	13	15	–	–
CRL9	CAGCCGCCCC	10	12	1	2
CRL30	CCGCGCCCGC	>20	>20	–	–
CRL31**	CGTGGCCCCG	3	8	2	6
CRL32**	CGTGCCCGGC	9	5	5	3
CRL33	CTCGCGCCCC	>20	>20	–	–
CRL34	GACCGCGCCC	>20	>20	–	–
Total	42 primers	354	351	99	92

[a]Forty-two primers were tested to identify RAPD markers; 20 of the primers were random RAPD primers (A1–A20), 12 were selected RAPD primers designated by X or Y (Johnson *et al.* 1994), and 10 were high-GC primers designated by CRL (Kubelik & Szabo 1995): (**) excellent primers; (*) good primers; (*/*) between excellent and good primers; and (–) the primer under testing failed to generate scorable bands.

random RAPD primers for generating polymorphic markers.

Channel catfish and blue catfish have equal number of chromosomes (2n = 58; Wolters *et al.* 1981; LeGrande *et al.* 1984). Studies of the karyotypes revealed that the morphology of chromosomal arms is indistinguishable between the two species (LeGrande *et al.* 1984). The resulting F1 hybrids are fertile (Argue 1996), and F2 and F3 hybrids have been produced (Argue 1996; unpublished results). Available genetic information indicates that the interspecific chromosomes pair appropriately. In this study, the present authors examined the transmission of RAPD markers in F1 hybrids. As expected, all RAPD bands from both parents were observed in RAPD profiles of F1 hybrids (Fig. 1), indicating full penetrance and the dominant nature of RAPD markers. There were rare cases of segregation of polymorphic RAPD markers in F1 individuals (data not shown), indicating that some of the parents were heterozygous at a few loci.

To test segregation of RAPD markers, F2 and backcross hybrids were analysed for RAPD profiles. As shown in Fig. 1, segregation of RAPD markers was observed for polymorphic RAPD loci. As expected from dominant markers, channel catfish markers were observed in all individuals of the backcross progeny of channel catfish × F1 (A8-c440 and A8-c650; Fig. 1), but these segregated in the F2 (data not shown) and the backcross progeny from blue catfish × F1 hybrids. Similarly, blue catfish markers were observed in all individuals of the backcross progeny from blue catfish × F1 hybrids (A8-b470, A8-b520 and A8-b850; Fig. 1), but these segregated in the F2 (not shown) and the backcross progeny from channel catfish × F1 hybrids. Data from all RAPD loci were pooled for χ^2 analysis to determine if the markers were segregating in expected Mendelian ratios. The pooled segregation data again confirmed the dominant nature of the RAPD markers. Segregation ratios were not different from Mendelian expectations; channel catfish markers segregated in a 1:1 ratio in blue catfish backcross progeny and in a 3:1 ratio in F2 progeny, and the expected ratios were also found with blue catfish markers in channel catfish backcross progeny and F2 hybrids.

Most of the RAPD markers were species-specific markers (data not shown) and differentiated channel catfish from blue catfish. However, some intraspecific RAPD variation was observed for different strains or for individuals. For genetic mapping analysis using the channel × blue catfish hybrid system, markers that represent individual (or strain) differences can be omitted because the majority of markers should be species markers.

Reproducibility of high molecular weight bands may be lower than low molecular weight bands. The present authors have observed some bands that appeared to be 'new' in some of F2 hybrids (bands greater than 1·6 kb, data not shown), but actually existed in the parents. These were not highly visible in the parents because of lower quantities of the PCR amplification products. Caution should be excised when using RAPD markers of large size (greater than 2 kb in size).

In conclusion, RAPD permits very rapid generation of large numbers of markers needed for gene mapping studies of catfish in an interspecific hybrid system. The RAPD markers followed Mendelian inheritance and were highly reproducible with size ranges of 400–1500 bp. With the availability of large numbers of RAPD markers, as rapid progress can be expected as that achieved in the gene mapping of other species (Reiter *et al.* 1992; Grattapaglia & Sederoff 1994).

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