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

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Abstract Eight primer combinations were used to investigate the application of amplified fragment length polymorphism (AFLP) markers in catfish for genetic analysis. Intraspecific polymorphism was low among channel catfish or blue catfish strains. Interspecific AFLP polymorphism was high between the channel catfish and blue catfish. Each primer combination generated from 70 to more than 200 bands, of which 38.6–75.7% were polymorphic between channel catfish and blue catfish. On average, more than 20 polymorphic bands per primer combination were produced as quality markers suitable for genetic analysis. All AFLP markers were transmitted into channel catfish × blue catfish F1 hybrids, except rare markers that were heterozygous in the parents and therefore were segregating in F1 hybrids. The two reciprocal channel catfish × blue catfish F1 hybrids (channel catfish female × blue catfish male; blue catfish female × channel catfish male) produced identical AFLP profiles. The AFLP markers were inherited and segregated in expected Mendelian ratios. At two loci, E8-b9 and E8-b2, markers were found at significantly lower frequencies than expected with F2 and backcross hybrids which had been selected for increased growth rates. The reproducibility of AFLP was excellent. These characteristics of the catfish AFLP markers make them highly useful for genetic analysis of catfish, especially for construction of genetic linkage and quantitative trait loci maps, and for marker-assisted selection.

Key words DNA polymorphisms · AFLP marker · Catfish · Genome mapping · PCR

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Introduction

Large numbers of molecular markers are needed to construct genetic linkage maps useful for marker-assisted selection (MAS), and for eventual cloning of beneficial genes from various organisms. Three DNA marker systems are currently available, including restriction fragment length polymorphisms (RFLP) (Miller and Tanksley 1990; Vaiman et al. 1996; Smith et al. 1997; Yang and Womack 1997), microsatellites (Hughes and Queller 1993; Queller et al. 1993), and methods based on the polymerase chain reaction (PCR) (Vosberg 1989) such as random amplification of polymorphic DNA (RAPD) (Welsh and McClelland 1990; Williams et al. 1990) and amplified fragment length polymorphism (AFLP) (Vos et al. 1995).

RAPD markers are particularly adapted for efficient DNA fingerprinting of genotypes for genetic variation and construction of linkage maps (Neale and Sederoff 1991; Reiter et al. 1992; Tingey and Deltufo 1992; Grattapaglia and Sederoff 1994; Johnson et al. 1994; Foo et al. 1995; Liu et al. 1997), but the usefulness of the technique is limited by low reproducibility because of low stringency PCR.

AFLP combines the strengths of RFLP and RAPD. It requires a small amount of DNA; it does not require any known sequence information or probes; it overcomes the problem of low reproducibility of RAPD. AFLP is capable of producing large numbers of polymorphic bands in a single analysis, significantly reducing the cost and making genetic analysis of closely related populations possible (Qi and Lindhout 1997). The utilization of AFLP markers in genetic linkage mapping (Ballvora et al. 1995; Becker et al. 1995; Meksem et al. 1995; Cho et al. 1996; Mackill et al. 1996; Otsen et al. 1996) and analyses of genetic resource pools (Folkertsma et al. 1996; Travis et al. 1996; Keim et al. 1997) has facilitated progress that would otherwise take much longer.

Channel catfish (*Ictalurus punctatus)* is the most important cultured fish species in the United States,

accounting for over 50% of all aquacultural production. A genetic map is needed to facilitate breeding programs with MAS. Large numbers of eggs produced from each spawn make catfish a useful system for mapping quantitative trait loci (QTL) using selective genotyping (Darvasi and Soller 1992).

To test the feasibility of using AFLP for genetic analysis including linkage and QTL mapping and genetic resource studies of catfish, we studied the intraspecific and interspecific polymorphism of AFLP markers in channel catfish and blue catfish, and inheritance of these markers in F1, F2, and backcross hybrids of channel catfish × blue catfish. We report here the usefulness of AFLP markers in catfish genetic analysis, including genetic linkage mapping for different mating schemes, and inheritance of AFLP markers in catfish.

Materials and methods

Animals

Experimental fish were raised at the fish genetics facility at Auburn University. Channel catfish and blue catfish were used to make the interspecific F1 hybrids. Sexually mature F1 hybrids were used to make the F2 hybrids and backcross hybrids. All the reciprocal backcrosses to the parents were made. Blood samples were collected from male and female channel catfish and blue catfish. Blood samples were collected also from several F2 hybrids and from several backcross hybrids to analyze inheritance of AFLP markers. Backcross and F2 individuals sampled had been selected for fast growth rate. The two pairs of the parent samples were used to determine any differences between the two reciprocal F1 hybrids because dramatic phenotypic differences have been observed between reciprocal F1 hybrids (Dunham et al. 1982). The F1 individuals used in this study were derived from the parental individuals from this study. F2 and backcross hybrids were obtained from F1 individuals other than those used in this study.

Blood samples were also collected from three fish of each strain to compare intraspecific AFLP polymorphism. Kansas strain, Auburn strain, and Marion strain were used for channel catfish. D & B strain and Rio Grande strain were used for blue catfish.

Blood sample collection and isolation of DNA

Blood samples (0.5–1 ml) were collected in 1-ml syringes and immediately expelled into a 50-ml tube containing 20 ml of DNA extraction buffer (100 mM NaCl, 10 mM TRIS, pH 8, 25 mM EDTA, 0.5% SDS, and freshly added 0.1 mg/ml proteinase K), and DNA was isolated as previously described using standard protocols (Strauss 1989; Liu et al. 1997). Blood was expelled into lysis buffer quickly to disperse the blood cells. The lysates were incubated at 55° C overnight. DNA was extracted twice with phenol and once with chloroform. DNA was precipitated by adding half the original blood volume of 7.5 M ammonium acetate and two volumes of ethanol. DNA was collected by brief centrifugation and washed twice with 70% ethanol, air-dried, and redissolved in water. The concentration was measured with a spectrophotometer for absorption at 260 nm or estimated from intensities of ethidium bromide staining after agarose gel electrophoresis using a 1-kb ladder DNA as reference (Gibco BRL, Md.).

Adaptors and primers

All AFLP adaptors and primers were purchased from Life Technologies (Gibco BRL) included in the AFLP Analysis System I. For long-term records, AFLP primers were identified as indicated in Table 1. *Eco*RI primers were designated with a letter, from A to H, and *Mse*I primers were designated by a number, from 1 to 8. Primer combinations were designated by a letter plus a number with *Eco*RI primer first (e.g., E-AAG/M-CAC primer combination was identified as B2). This type of matrix identification is required for long-term records of molecular markers since many types of markers and their combinations can make nomenclature difficult and confusing (Vaneechoutte 1996). The eight primer combinations used in this study were B2, B6, B8, C2, C6, C8, E2, E6, and E8 (Table 1).

DNA restriction and ligation to adaptors

Genomic DNA isolated from channel catfish, blue catfish, and their hybrids was digested completely with *Eco*RI and *Mse*I as described by the supplier (Gibco BRL). Briefly, 250 ng genomic DNA was digested with 2.5 U of each restriction endonuclease at 37° C for 2 h. Following digestion, adaptors for *Eco*RI and *Mse*I were added to the reaction and ligated to the restriction fragments using T4 DNA ligase for 2 h at 20° C.

Table 1 Nomenclature by matrix identification of amplified fragment length polymorphism (AFLP) primer combinations in catfish. Each *Eco*RI primer was given a letter (A–H) and each *MseI* primer was given a number (1–8). Thus, for instance, the B2 primer combination represents AFLP using primer combination E-AAG/M-CAC

	M-CAA 1	M-CAC 2	M-CAG 3	M-CAT 4	M-CTA 5	M-CTC 6	M-CTG 7	M-CTT 8
E-AAC (A)	A1	A2	A3	A4	A5	A6	A7	A8
E-AAG (B)	B1	B2	B3	B4	B5	B6	B7	B8
E-ACA (C)	C1	C2	C3	C4	C5	C6	C7	C8
E-ACC (D)	D1	D2	D3	D4	D5	D6	D7	D8
E-ACG (E)	E1	E2	E3	E4	E5	E6	E7	E8
E-ACT (F)	F1	F2	F3	F4	F5	F6	F7	F8
E-AGC (G)	G1	G2	G3	G4	G5	G6	G7	G8
E-AGG (H)	H1	H2	H3	H4	H5	H6	H7	H8

Preamplification and selective amplification

AFLP amplifications were performed following instructions of the AFLP kit supplier (Gibco BRL). PCR was performed in two consecutive reactions. The preamplification used AFLP primers, each having one selective nucleotide. The PCR products of the preamplification reaction were diluted (1:50) and used as templates for the selective amplification using two AFLP primers, each containing three selective nucleotides, with the *Eco*RI selective primer being ^{32}P- or ^{33}P-labeled before amplification. Preamplification was performed with 20 cycles with temperature profiles of 94° C for 30 s, 65° C for 30 s, 72° C for 60 s with a 0.7° C decrease of annealing temperature each cycle, followed by 23 cycles of amplification at 94° C for 30 s, 56° C for 30 s, and 72° C for 60 s.

AFLP product analysis

After PCR, an equal volume of formamide dye was added to each reaction. The samples were heated to 90° C to denature for 3 min and immediately placed on ice. A 6% polyacrylamide denaturing gel (19:1 acrylamide:bis; 7.5 M urea, 1 × TBE buffer) with 0.4-mm spacers was prepared and electrophoresed until the bromphenol blue migrated off the gel. The gel was fixed using 20% ethanol and exposed to X-ray film BioMax (Kodak) for 2–3 days.

Data analysis

Blue catfish AFLP markers were pooled for channel backcrosses or F2 hybrids, and channel catfish AFLP markers were pooled for blue catfish backcrosses or F2 hybrids and examined by chi-square analysis to determine if the markers were segregating in expected Mendelian ratios. For locus and allele nomenclature, each locus is designated with one letter plus one number (Table 1) to describe the primer combination used to generate the marker, followed by a dash, a number, and a small letter, either c or b. The dash separates the primer designation from the marker designation. The number following the dash designates the position of the band, with smaller numbers representing fragments of smaller sizes (numbered from the bottom of the gel upwards). The small letter c was used for channel catfish markers and b was used for blue catfish markers. For instance, B2-1c represents an AFLP marker that was generated with the primer combination E-AAG/M-CAC (indicated by B2, Table 1); the marker is a channel catfish marker (indicated by the letter c); it was small in size (indicated by the number 1). Segregation of markers in F2 or backcross hybrids was analyzed by reference to the banding patterns of the parents and that of the F1.

DNA fingerprints were evaluated visually by inspection of autoradiographs on a bench viewer and also by evaluation of computer scans with zoom-in to assist inspection. Only DNA fragments consistently present or absent were evaluated. Among the polymorphic DNA fragments, two subsets can be distinguished: presence/absence and band intensity polymorphisms. Presence/absence polymorphisms result from: (1) the gain or loss of a restriction site; (2) insertions, deletions or reversions between restriction sites; or (3) non-complementarity of the selective nucleotides of the primer used in the AFLP procedure and the sequences adjacent to the restriction site. The intensities of the amplified band vary according to the genetic variation of individuals within a specific strain at that fragment locus. The intensity of the polymorphism is very difficult to score, therefore, only the presence or absence of fragments was scored.

Results

Identification of intraspecific AFLP markers in catfish

To evaluate the usefulness of AFLP markers in gene mapping analysis using intraspecific reference mapping populations, AFLP analyses were performed using genomic DNA isolated from different strains of blue catfish and channel catfish as templates to determine polymorphic levels. Two strains of blue catfish, D&B and Rio Grande, known to be distantly related, were tested with seven primer combinations: B2, C2, C6, C8, E6, E2, and E8 (Fig. 1). Three strains of channel catfish, Kansas, Auburn select, and Marion, were tested with the same primer combinations, with results from three primer combinations (B2, C2, and E8) shown. As shown in Fig. 1 and summarized in Table 2, low intraspecific polymorphisms were detected by the AFLP procedure between both blue catfish and channel catfish strains. From 2 to 12 polymorphic bands were observed between D&B and Rio Grande strains of blue catfish. On average, the seven primer combinations produced 6.7 polymorphic bands in the blue catfish strains per primer combination. Lower intraspecific polymorphisms were observed among strains of channel catfish than in the blue catfish (Fig. 1, Table 2), although not tested statistically. From 1 to 10 polymorphic bands were produced using the three primer combinations (Fig. 1). The other four primer combinations generated similar levels of polymorphism (not shown). On average, 1.7–3.3 polymorphic bands were produced among channel catfish strains (Table 2). In both blue catfish and channel catfish, not all polymorphic bands were quality bands for linkage mapping analysis in catfish since some bands were either weak in intensity or were too close to other bands. Reproducibility of weak bands was low. Bands that were too close to other bands were difficult to score in a segregating population. On average, 3.4 bands per primer combination in blue catfish were quality bands, and 1.3–2.3 bands per primer combination in channel catfish were suitable for use in linkage mapping analysis.

Interspecific AFLP polymorphisms

The low level of intraspecific AFLP polymorphism excludes AFLP as an efficient means of generating molecular markers for gene mapping in an intraspecific mating plan. However, because hybrids can be produced by interspecific mating of channel catfish with blue catfish, we determined levels of polymorphism between channel catfish and blue catfish for potential use in gene mapping analysis. To test the polymorphic level of AFLP bands between channel catfish and blue catfish, AFLP was performed with various primer combinations using channel catfish and blue catfish genomic DNA as templates. As shown in Fig. 2 and summarized in Table 3, higher frequencies of AFLP bands were polymorphic between channel catfish and blue catfish. Over 30% of the bands were polymorphic in initial studies (Fig. 1). We further examined several more primer combinations. From 70 to over 200 distinguishing bands were produced (sum of the channel catfish bands and blue catfish bands minus common bands) using nine primer combinations. The nine primer combinations

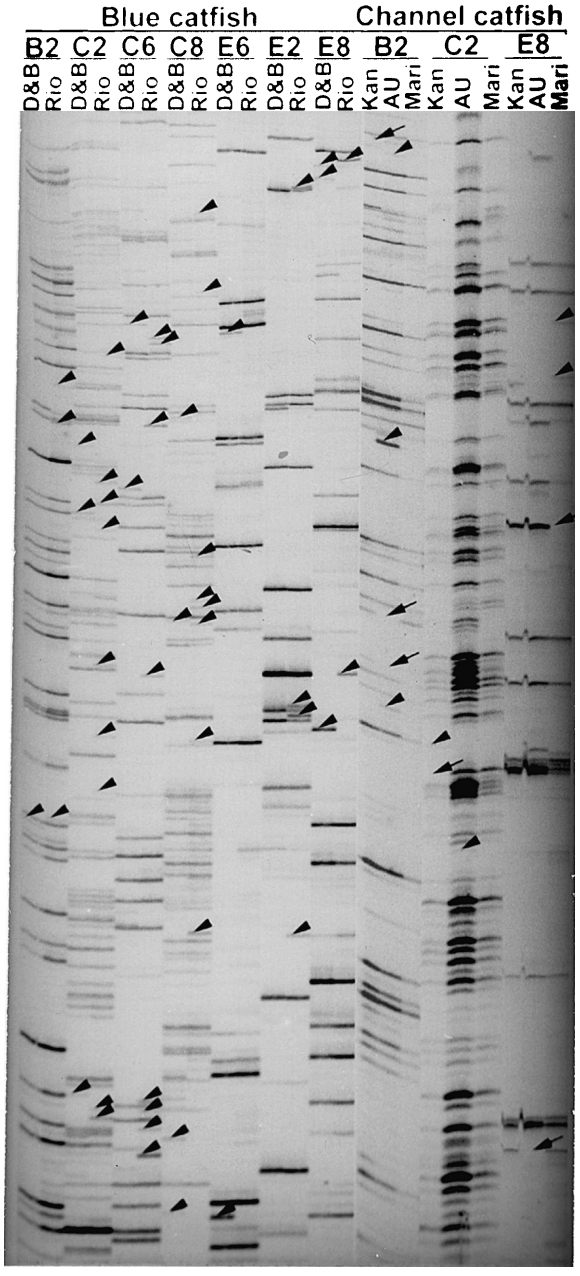

Fig. 1 Intraspecific polymorphisms of channel catfish and of blue catfish. Two distantly related strains of blue catfish, D&B (*D&B*) and Rio Grande (*Rio*), and three distantly related strains of channel catfish, Kansas (*Kan*), Auburn (*AU*), and Marion (*Mari*), were tested for polymorphisms with amplified fragment length polymorphisms (AFLF) using primer combinations B2, C2, C6, C8, E6, E2 and E8 for blue catfish, and primer combinations B2, C2, and E8 for channel catfish. PCR products of AFLP reactions were analyzed in 6% denaturing polyacrylamide gels. Polymorphic bands of blue catfish strains are labeled with arrowheads. For channel catfish, polymorphic bands are labeled with either an arrowhead (for bands existing in only one of the three strains tested), or an *arrow* (for bands absent from one of the three strains tested). Note the generally higher polymorphism between the two blue catfish strains than between any two channel catfish strains tested

exhibited 38–75% polymorphic bands. On average, over 30–50% of all AFLP bands were polymorphic. Again, polymorphic bands that were strong in intensity and separated well from adjacent bands were designated as bands suitable for mapping (Figs. 2, 3). About 50–70% of the polymorphic bands were of a quality suitable for mapping analysis. In general, each AFLP primer combination amplified 50–120 bands from either channel catfish or blue catfish. About 47% of total amplified AFLP bands were either specific to channel catfish or to blue catfish. Each primer combination generated 20 or more quality polymorphic markers.

Table 2 Intraspecific polymorphisms of AFLP bands of channel catfish (Kansas, Auburn, and Marion strains) and blue catfish (D&B and Rio Grande strains) (*nd* not determined)

Primers	Polymorphic bands between strains			
	D&B/Rio Grande	Kansas/ Auburn	Kansas/ Marion	Auburn/ Marion
B2	4	6	0	5
C2	12	3	2	1
C6	10	nd	nd	nd
C8	12	nd	nd	nd
E2	4	nd	nd	nd
E6	2	nd	nd	nd
E8	3	1	3	4
Total	47	10	5	10
Average	6.7	3.3	1.7	3.3

Transmission of AFLP markers to F1 interspecific hybrids

We first examined transmission of AFLP markers to F1 hybrids to determine their suitability for gene mapping analysis. As expected for dominant markers, a sum of all AFLP bands from both parents was generally observed to be present in AFLP profiles of F1 hybrids (Fig. 2), indicating full penetrance and the dominant nature of AFLP markers. The F1 hybrids should have one set of chromosomes from the channel catfish and the other from blue catfish, thus allowing amplification of all AFLP bands from both channel catfish and from blue catfish. The polymorphic paternal and maternal bands showed a dominant Mendelian pattern of inheritance in the F1 progeny. Few segregations of polymorphic AFLP markers were observed in F1 individuals (data not shown), thus indicating that the majority of the polymorphic loci were homozygous in the parental species. The majority of the AFLP markers, therefore, represent species-specific markers. Those markers segregating in F1 individuals represented allele polymorphism within a species. They were heterozygous in the paternal or maternal parents. Since low levels of polymorphisms were detected within either channel catfish or blue catfish, homozygosity at most AFLP loci was expected. The results also indicated that chromosomes of channel catfish and blue catfish paired properly and their AFLP markers followed Mendelian inheritance.

Phenotypically, the reciprocal F1 hybrids (channel catfish female × blue catfish male and blue catfish fe-

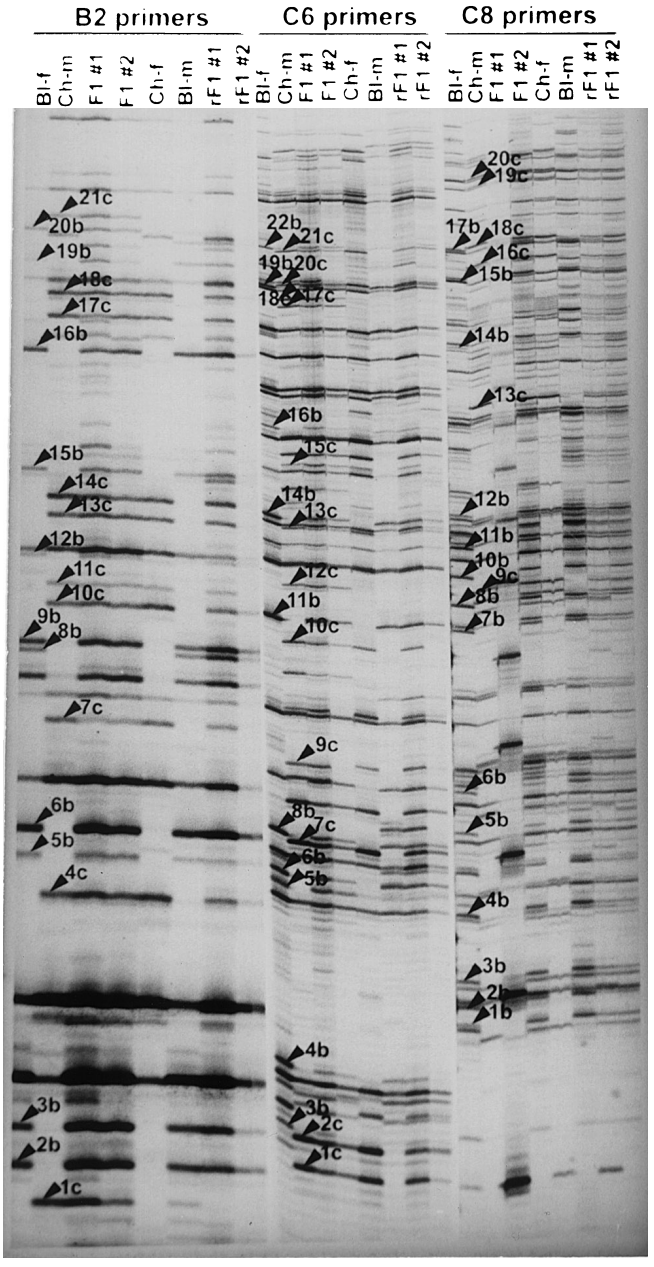

Fig. 2 Analysis of transmission of AFLP markers into reciprocal F1 hybrids. Three primer combinations are shown (B2, C6, and C8). Eight lanes in each of the three panels using three primers are: blue catfish female (*Bl-f*), channel catfish male (*Ch-m*), F1 individual 1 (*F1 #1*), F1 individual 2 (*F1 #2*), chanel catfish female (*Ch-f*), blue catfish male (*Bl-m*), reciprocal F1 hybrid individual 1 (*rF1 #1*), and reciprocal F1 hybrid individual 2 (*rF1 #2*). Each *arrow* and a *number* indicates an AFLP marker, with *b* for blue catfish marker and *c* for channel catfish marker

male × channel catfish male) are different due to paternal predominance (Dunham et al. 1982). The channel catfish × blue catfish F1 hybrids typically exhibit morphometric and meristic phenotypes more similar to the paternal parent. Given that AFLP is capable of displaying many loci in a single analysis, it probably offers an opportunity for examining possible distortions in transmission and segregation of molecular markers. We tested this by making two reciprocal hybrids to determine whether the AFLP markers were transmitted into the reciprocal F1 hybrids in a similar fashion. A set of reciprocal hybrids was tested with each AFLP primer combination. As shown in Fig. 2, in either hybrid, AFLP bands were transmitted into F1 hybrids in a similar fashion, independent of the parental origin of the AFLP markers. This indicated that the phenotypic differences in performance traits were not due to the proportion of the genomic composition. In the context of genetic linkage mapping analysis, these results indicate that both reciprocal hybrids can be used for establishing mapping populations.

Table 3 Interspecific polymorphism of AFLP markers between channel catfish, *Ictalurus punctatus* and blue catfish, *I. furcatus*. Primer combinations were designated as in Table 1. Polymorphism (%) was derived from polymorphic bands divided by all visible bands (*nd* not determined)

Primers	Visible bands	Polymorphic bands	Polymorphism (%)
B2	70	53	75.7
B6	> 200	nd	nd
B8	> 200	nd	nd
C2	132	51	38.6
C6	75	33	44.0
C8	84	48	57.1
E2	64	41	64.0
E6	96	58	60.4
E8	170	74	43.5

Segregation of AFLP markers in the F2 and backcross hybrids

F2 and backcross hybrids derived from channel × blue hybrids were observed to segregate for various morphological, meristic, and production traits (Argue 1996). To test segregation of AFLP markers, F2 and two backcross hybrids were analyzed. Since the AFLP markers are dominant markers, the backcross hybrid from F1 × channel catfish is required for analysis of blue catfish markers; and the backcross hybrid from F1 × blue catfish is required for analysis of channel catfish markers. As shown in Fig. 3 and summarized in Table 4, segregation of AFLP markers was observed from all polymorphic AFLP loci for 69 markers. Channel catfish markers were observed to segregate in F2 and F1 × blue (or reciprocal) backcross hybrids. Blue catfish markers were observed to segregate in F2 and F1 × channel (or reciprocal) backcross hybrids. Because limited numbers of individuals were analyzed in this study, linkage analysis was not attempted. Instead, segregation data for all blue catfish or channel catfish AFLP markers were pooled to determine overall segregation ratios (Table 4). Chi-square analysis indicated that the markers were inherited in expected Mendelian ratios despite the fact that all the individuals sampled had been selected for increased body weight. As shown

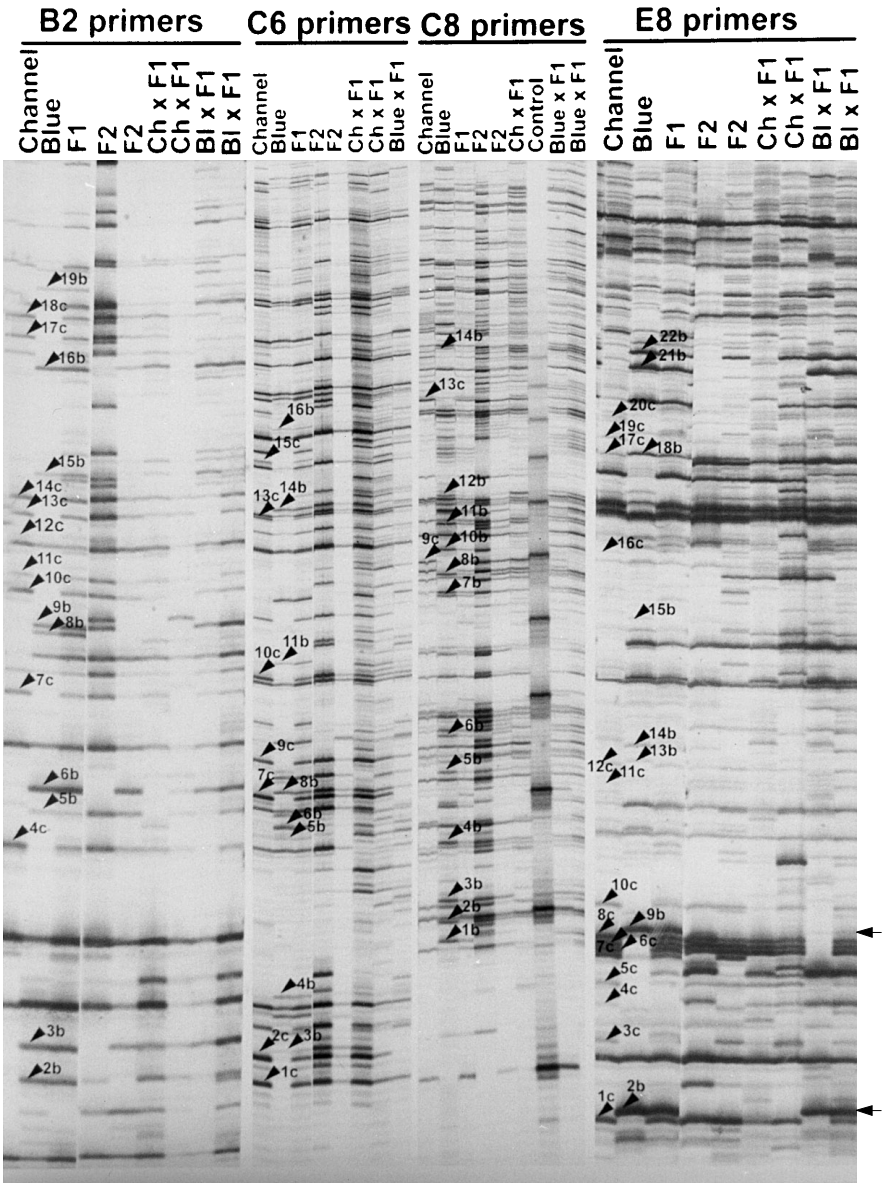

Fig. 3 Genetic segregation of AFLP markers. Four primer combinations (B2, C6, C8, and E8) are shown. In each of the four panels using the four primer combinations, the parental channel catfish female (*Channel*) and blue catfish male (*Blue*), and their F1 hybrid (*F1*) are shown in the first three lanes. F2 hybrid (*F2*), channel catfishs × F1 backcross hybrid (*Ch* × *F1*), and blue catfish × F1 backcross hybrid (*Bl* × *F1*) were analyzed, with each lane representing a different individual. AFLP markers were labeled as in Fig. 2. Two *arrows* at the right of the figure indicate two blue catfish markers (E8-2b and E8-9b) that showed possible segregation distortions

Table 4 Inheritance of AFLP markers in interspecific hybrids of channel catfish × blue catfish: expected and observed values for inheritance of AFLP markers was not different (chi-square analysis). Markers were pooled with each primer combination for blue catfish markers (*b*) or channel catfish markers (*c*). The number after the colon is the number of markers analyzed for that primer. For instance, B2-b: 9 means that the primer combination used was B2 and nine pooled blue catfish markers were under consideration

Markers	F2		F1 × channel		F1 × blue	
	Expected	Observed	Expected	Observed	Expected	Observed
B2-b: 9	13.5/18	10/18	9/18	10/18		
C6-b: 8	12/16	10/16	8/16	10/16		
C8-b: 12	18/24	18/24	6/12	8/12		
E8-b: 8	12/16	8/16	8/16	3/16		
B2-c: 9	13.5/18	17/18			9/18	10/18
C6-c: 7	10.5/14	11/14			3.5/7	4/7
C8-c: 2	3/4	4/4			2/4	3/4
E8-c: 14	21/28	25/28			14/28	16/28
Total: 69	103.5/138	103/138	31/62	31/62	28.5/57	33/57

in Fig. 3 and summarized in Table 4, the observed segregation ratios of markers pooled with every primer combination also fit the expected Mendelian segregation ratios. Channel catfish and blue catfish markers were observed in 75% F2s, and they were observed in 50% appropriate backcross hybrids.

Most of the AFLP markers were species-specific markers. Thus, most of the AFLP markers only differentiate channel catfish from blue catfish whereas individual differences within a species are usually not discernible. However, as shown in Fig. 1, some AFLP markers were polymorphic between individuals of different strains. These AFLP bands could be markers for different strains or represent individual differences. These individual-specific markers are also illustrated in Fig. 3. If the original fish (or the original strain) used to make F1 hybrids are not available for backcrossing, the AFLP profiles of the parents used to make backcross hybrids must be analyzed for marker segregation. If indeed certain AFLP markers are heterozygous in the parents of F1, their frequency in F2 or backcross hybrids would be significantly lower than expected. In a mapping population of large individuals (more than 100 fish), such deviation from expectation should be readily detected. Markers that represent individual (or strain) differences can be omitted from gene mapping analysis using the hybrid system because the majority of markers are species specific.

Putative AFLP markers related to selection for enhanced growth performance

Although every AFLP marker segregated in the F2 or backcross hybrids and overall segregation almost perfectly fit the expected Mendelian ratios, two markers were observed with a strong possibility of segregation distortion. Two blue catfish markers, E8-9b and E8-2b, were detected at a frequency significantly lower than expected (Fig. 3). E8-9b were not observed in three F2 individuals (with two shown in Fig. 3) or in two F1 × channel catfish backcross individuals. Similarly, E8-2b was only observed in one of the three F2 individuals, but not in two backcross hybrids. Because the calculated probability of not observing E8-9b in the samples was less than 0.4%, it was likely that the reduced frequency may have resulted from F2 and backcross hybrids that had been selected for enhanced growth rate. Further analyses of these markers and characterization of the nature of these markers are underway.

Reproducibility of AFLP

To make AFLP an efficient means for gene mapping analysis in catfish, high reproducibility is required. The reproducibility was tested by using DNA templates from different individual fish isolated at different times. High levels of reproducibility were observed. We tested various individuals of channel catfish and blue catfish whose DNA was isolated at different times. All ten channel catfish individuals had identical AFLP profiles. Different numbers of bands may be observed as a function of the different radioisotopes being used in the experiments. We tested ^{32}P and ^{33}P with several pairs of primer combinations. Generally, the same pattern of AFLP profiles was obtained with either ^{32}P or ^{33}P.

However, the use of ^{33}P allowed detection of more AFLP bands (data not shown). This is probably because the low energy radioisotope, ^{33}P, allowed longer exposures than with the high-energy ^{32}P. For instance, with a 1-week exposure of ^{33}P it is possible to obtain reasonably clean autoradiograms, while a 1-week exposure with ^{32}P generated overexposed dark autoradiograms. Longer exposure allowed some weaker bands to be detected which otherwise were not visible or were just too weak. While detection of more bands assisted robust analysis of many loci simultaneously, too many bands could make analysis extremely difficult, especially for adjacent markers all segregating. Another factor for reproducibility of AFLP bands is the size of the amplified products. Generally, amplified products with large sizes, migrating at the top of sequencing gels, have lower reproducibility. Often the large fragments were not efficiently amplified to generate strong bands (Figs. 1–3). AFLP bands of 50–500 bp exhibited the highest reproducibility. Large variation in the number of bands amplified was observed when different primer combinations were used (Figs. 1–3). This is not a reproducibility issue, but is relevant for primer selection for genetic linkage analysis.

Discussion

To evaluate the applicability of AFLP markers for gene mapping analysis of catfish, nine primer combinations were tested to identify intraspecific and interspecific variation of channel catfish and blue catfish. Intraspecific polymorphisms were low and less than 5% of the bands were suitable for gene mapping analysis. Interspecific AFLP polymorphisms were high between channel catfish and blue catfish. Over 50% of the interspecific bands were suitable for use in mapping analysis using the interspecific hybrid system. Transmission of AFLP markers to F1 was nearly complete except for heterozygous marker loci in the parents. The two reciprocal F1 hybrids of channel catfish × blue catfish exhibited identical AFLP profiles. AFLP markers were inherited in expected Mendelian ratios in F2, or backcross hybrids.

High levels of interspecific polymorphism make AFLP an efficient system for construction of a high resolution genetic linkage map of catfish using an interspecific hybrid system (Liu and Dunham 1997; Poompuang and Hallerman 1997). Low levels of intraspecific polymorphism make AFLP an inefficient marker system for mapping analysis using an intraspecific mat-

ing plan, but it may be highly useful for gene pool analysis of catfish strains (Liu et al. 1997).

One of the most important factors determining the applicability of AFLP for gene mapping analysis in catfish is its reproducibility. Concerns are greater with dominant markers because absence of the bands could potentially mean failure in amplification and thus be incorrectly scored. The present results indicated that the reproducibility with catfish genomic DNA as a template was excellent. A useful AFLP marker should be polymorphic and segregational, highly reproducible between samples, prominent in band intensity, well separated from the other bands so that it is easy to score, and between 50 bp and 500 bp in size. Obviously, not all AFLP polymorphic bands were good markers suitable for linkage analysis. Of the interspecific, polymorphic bands observed, 50–70% were quality markers (Figs. 2, 3).

We examined AFLP profiles of the reciprocal hybrids (channel catfish female × blue catfish male, and blue catfish female × channel catfish male) to explore reasons for the strong paternal predominance observed in catfish (Dunham et al. 1982). AFLP technology allows simultaneous examination of many marker loci. Our results did not reveal any difference in AFLP profiles of the two reciprocal hybrids, suggesting proper pairing and segregation of interspecific chromosomes, consistent with karyology studies (LeGrande et al. 1984).

Overall segregation of catfish AFLP markers followed expected ratios. Strong segregation distortion was observed with two blue catfish markers, E8-9b and E8-2b, in the F2 and backcross hybrids selected for increased growth rates. The probability of not detecting E8-9b in our samples was less than 0.4%, and to observe E8-2b in only one F2 individual out of five F2 and backcross hybrids was about 1%. Since the F2 and backcross hybrids were selected for increased growth rate, any distortion in marker detection frequency may be associated with linkage to growth loci. Further analysis and characterization of these markers are underway. If indeed these loci were selected against when the fish were selected for enhanced growth, these marker loci may be linked to genes in blue catfish that negatively regulate growth (Sari-Gorla et al. 1996). Alternatively, if some genes have a negative effect on survival, their absence would favor increased survival and, therefore, they should be detected at a reduced frequency among viable fish. Indeed, segregation distortion due to "selected survival" is common in aquacultural species (e.g., in oysters, P. Gaffney, personal communication). In either case, if the segregation distortion is confirmed, these AFLP markers should be useful for MAS programs in catfish.

In conclusion, AFLP permits rapid generation of large numbers of markers needed for genetic analysis including construction of genetic linkage and QTL mapping of catfish in an interspecific hybrid system. The amplification products are highly reproducible. AFLP markers were transmitted in the same fashion in reciprocal F1 hybrids and segregated in F2 or backcross hybrids with Mendelian expectations. AFLP is a valuable tool to study catfish molecular genetics such as identification of strain markers, analysis of genetic resources of catfish, and for the identification and eventual cloning of important catfish genes involved in the determination of economic traits.

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