

**CHARACTERIZATION OF CHANNEL CATFISH, *ICTALURUS PUNCTATUS*,
POPULATIONS USING MICROSATELLITE LOCI**

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Introduction

Commercial production of channel catfish, *Ictalurus punctatus*, exceeds that of other cultured food fish in the U.S. Over 445 million pounds of fish were processed in 1995 (USDA, 1996). Increased commercial production has been accomplished mainly by increased pond acreage and improved pond management. Few, if any, production gains due to selective breeding programs have been realized by the industry. The USDA-ARS selective breeding program utilizes selection of families with superior performance in economically important traits such as growth rate, disease resistance, carcass yield, and spawning success. Identification of individual or related fish is necessary for efficient selective breeding and broodstock management, and for measuring traits such as individual spawning success, but the physical characteristics of channel catfish hinder identification. Catfish genetic selection will benefit from the development of genetic markers useful for identification and marker assisted selection.

Microsatellites are a class of genetic markers in vertebrates that consist of short tandem repeat sequences flanked by unique DNA sequence (Weber, 1990; Hearne et al., 1992). Microsatellite loci are distributed widely throughout the genome, display high levels of allelic polymorphism, and the co-dominant alleles are easily and rapidly analyzed using the polymerase chain reaction and gel electrophoresis. Polymorphic microsatellite loci can be used for identification of individuals and for development of genetic maps to identify markers linked to loci affecting economically important traits. Microsatellite loci have been identified in several fish species (Goff et al., 1992; Estoup et al., 1993; Slettan et al., 1993; Brooker et al., 1994; Garcia de Leon et al., 1995). Genome maps based on microsatellites are being developed for use in marker assisted selection of domestic animals for agricultural production (Bishop et al., 1994; Rohrer et al., 1994; Cheng et al., 1995).

The present research was designed to characterize microsatellite loci in channel catfish. Microsatellite loci were identified in clones of channel catfish genomic DNA, oligonucleotide primers were designed to amplify each locus using PCR, and length polymorphism of amplified alleles was detected by electrophoretic analysis. Ten loci were characterized in populations of

wild and domestic catfish, and 7 polymorphic loci were used to determine parentage of 6 spawns from a communally stocked pond. These sequence-defined loci will be useful for identification of individuals and families, development of catfish genome maps, and for selection of genetically superior broodstock.

Methods

Short tandem repeat (microsatellite) loci were identified in plasmid clones containing channel catfish genomic DNA. Recombinant clones were enriched for catfish sequences containing tandem repeats using hybridization/selection with 5'-biotinylated ATA₆, GATA₅, CTG₆, or ATAA₅ oligonucleotides (Kijas et al., 1994 ; Waldbieser, 1995). Selected clones were sequenced using an ALF DNA sequencer (Pharmacia), and oligonucleotide primers were selected from sequence flanking the short tandem repeat region for PCR amplification of fragments ranging from 80-350 bp.

Tissue samples were obtained from three populations of channel catfish. For population studies, a reference population of wild channel catfish from the Mississippi River was obtained from commercial sources (Magnolia Fish Market and Fisherman's Wharf, Greenville, MS). Samples of domestic, farm-raised catfish were obtained as pre-hatching eggs from 5 commercial operations. For parentage determination, spawns were collected at the Catfish Genetics Research Unit from a 0.1 acre pond stocked with 17 females and 10 males of the USDA 103 strain. The spawns were collected in May 1995 and offspring were maintained in separate aquaria. Surviving broodstock (16 females, 8 males) were sampled in December 1995, and the offspring were sampled in February 1996. Three families of known parentage, produced by manual spawning of USDA 102 broodstock, were also used to assess Mendelian transmission of alleles.

Pre-hatching embryos and barbel samples from adult fish were processed for use in PCR reactions (Cui et al., 1989) and stored at -20°C. Oligonucleotide primers from selected clones were used in polymerase chain reactions to screen the reference population for allelic polymorphism. After verification of positive PCR amplification of the locus by agarose gel electrophoresis, one of the oligonucleotide primers was resynthesized with a 5' fluorescent label (fluorescein or Cy5).

Amplified DNA fragments were detected by automated laser fluorescence on an ALF[®] or ALFexpress[®] machine (Pharmacia Biotech, Inc., Piscataway, NJ). After characterization of the range of allele size in each locus, multiplex loading of two or three loci was performed on some gels. Each electrophoresis run contained 1 or 2 standard allele wells for comparisons between gels. The allelic fragments obtained from PCR reactions of the reference population were analyzed for each microsatellite locus using Fragment Manager[®] software (Pharmacia). Allele frequencies of ten loci were recorded for >50 wild fish. Mendelian inheritance was analyzed in several families produced by experimental spawning of known parents. Heterozygosity was calculated from the allele frequencies in the wild population (Hearne et al., 1992).

Results

Genomic clones containing tandem repeating nucleotide motifs were identified in the catfish genome. Fifty clones were identified by sequence analysis, 36 contained sufficient flanking DNA to synthesize oligonucleotide primers for PCR amplification. Twenty five clones produced PCR fragments of the expected size as visualized by agarose gel electrophoresis, and annealing

temperature for PCR amplification was estimated by computer analysis and optimized empirically. One of the primers in the pair was resynthesized with a 5' fluorescent label (fluorescein or Cy5) and the primer sets were used to genotype wild and domestic fish. Ten loci were selected for screening of populations of wild and domestic catfish for allelic polymorphism.

Table 1. Microsatellite alleles in channel catfish

Locus	Repeat type	No. alleles	Allele sizes	No. repeats	Het. ^a	No. fish
Ip001	tri	13	206-213	7 - 20	0.860	54
Ip002	tri	10	214-244	11 - 22	0.847	53
Ip003	tetra	8	181-209	7 - 14	0.811	54
Ip004	tetra	9	132-156 ^b	18 - 24	0.691	54
Ip005	tetra	7	105-133	8 - 16	0.611	54
Ip006	tetra	3	266-274	6 - 8	0.573	55
Ip007	tetra	9	299-339	7 - 17	0.850	53
Ip008	tetra	13	122-178	11 - 25	0.868	57
Ip009	tetra	7	125-149	14 - 20	0.624	53
Ip010	tri	8	204-219 ^c	15 - 20	0.786	54

^a Heterozygosity = $1 - (\sum_{i=1}^n p_i^2)$, where n = the total number of alleles and p_i is the population frequency of the i^{th} allele.

^b Other alleles at 194 and 198 bp.

^c Other alleles at 257 and 287 bp.

Analysis of 50 individuals demonstrated loci that contained 3 to 13 alleles (Table 1). Two loci, Ip004 and Ip010, contained alleles that lay outside the major allelic series. Locus Ip004 contained a continual series of 7 alleles from 132 to 156 bp, and two outlying alleles at 194 and 198 bp. Locus Ip010 contained a continual series of 6 alleles from 204 to 219 bp, and two outlying alleles at 257 and 287 bp. Six loci exhibited high levels of polymorphism (heterozygosity > 0.75), and 4 loci exhibited moderate levels (heterozygosity > 0.5) in the wild and farm populations. The farm populations appeared to contain as much heterozygosity as the wild populations (Table 2). Analysis of the two most polymorphic loci demonstrated the selected strain, USDA 103, maintained a high degree of heterozygosity with fewer alleles than the wild population (Table 2).

Table 2. Heterozygosity of catfish populations for selected microsatellite loci.

Locus	Miss.River			Farms ^a			USDA 103		
	Alleles	Het. ^b	No. Fish	Alleles	Het.	No. Fish	Alleles	Het.	No. Fish
Ip001	12	.860	54	13	.888	55	8	.888	98
Ip002	10	.847	53	10	.788	55	-	-	-
Ip003	8	.811	54	8	.842	56	-	-	-
Ip004	9	.691	54	7	.607	56	-	-	-
Ip005	7	.612	54	7	.629	56	-	-	-
Ip006	3	.573	55	3	.564	56	-	-	-
Ip008	13	.868	57	-	-	-	8	.779	104

^a One fish per spawn, avg. 9 spawns per farm.

^b Heterozygosity

Three USDA 102 families with known parentage demonstrated Mendelian-inheritance patterns of alleles from loci Ip001-Ip010. Mendelian inheritance of the microsatellite loci was used to determine parentage of several spawns taken from a communal pond. Barbel samples were obtained from fingerlings of six spawns in pond A4. All surviving broodstock and eight offspring per family from pond A4 were genotyped using the three most polymorphic loci, Ip001, -007, and -008. Offspring from spawns 6 and 7 had been mixed in one aquaria after hatching.

Genotypes of spawns 6 and 7 were differentiated after genotype analysis with Ip008. Parents were identified for spawns 7 and 8 after analysis with Ip008 and Ip001, and for all spawns after analysis with Ip008, Ip001, and Ip007 (Table 3). Further screening with Ip002, -003, -009, and -010 confirmed the parental genotypes. The two deceased males from pond A4 were assumed to be the parents of spawns 4 and 5 because none of the sampled males fit the deduced paternal genotype. Allelic variation in loci Ip001, -002, -003, -007, and -008 from deduced maternal genotypes of the 6 spawns provided 90,000 potential genotypes in the population of female broodfish. Therefore, it is unlikely the female missing from pond A4 had the same genotype as one of the detected parents. Genotype analysis demonstrated multiple parentage by one male in the pond, and his two spawns were collected 7 days apart. A fish with a genotype corresponding to spawn 3 was found in the sample of spawn 4, presumably due to the fish jumping between adjacent aquaria during rearing in the hatchery.

Table 3. Number of broodstock included as possible parents of 1995 spawns from pond A4 after sequential screening with Ip008, Ip001, and Ip007.

Spawn	Ip008		Ip001		Ip007		4 loci ^a	
	F	M	F	M	F	M	F	M
3	6	3	6	1	1	1	1	1
4	7	1	1	0	1	0	1	0
5	3	2	3	1	1	0	1	0
6	6	2	1	2	1	1	1	1
7	1	2	1	1	1	1	1	1
8	3	3	1	1	1	1	1	1
Alleles ^b	7		8		4			

^a Offspring pools and parents were screened with Ip002, 3, 9, 10 for verification.

^b Number of alleles per locus in 9 dams and 15 sires from pond A4.

Discussion

Microsatellite loci were identified in the channel catfish genome and used to characterize levels of allelic heterozygosity in catfish populations and determine spawn parentage in a communal pond. Tri- and tetranucleotide repeat loci were chosen rather than dinucleotide repeat loci, due to better resolution of alleles and lack of stutter bands in the electrophoretic analysis (Hearne et al., 1992). Tissue biopsy and sample preparation were simple and rapid. Outbred and selected catfish populations demonstrated high levels of allelic heterozygosity at several loci. Microsatellite-based genotypes provided characterization of alleles from individual loci that were defined by unique DNA sequence.

The present research demonstrated the utility of the microsatellite markers for determination of spawn parentage in a communal pond. Parentage of spawns within a select population of fish, with presumed narrowing of DNA sequence diversity, was determined by a maximum of three loci and verified with several more. Many spawns were determined with only two loci, and this technique provides a rapid, efficient method for determining parentage and scoring individual fish for spawning success. This analysis demonstrated multiple spawning by a male and also that one or two spawning males did not dominate the pond, thus narrowing the genetic diversity of the population.

The microsatellite-based genotype assay demonstrated much higher levels of allelic polymorphism than isozyme loci in channel catfish (Carmichael et al., 1992). Unlike isozyme characterization, which is limited to gene coding sequences which make up a small proportion of animal genomes, microsatellite genotyping detects DNA sequence variation throughout the genome. The microsatellite assay is rapid, and can be performed with minimal, non-invasive tissue sampling. High levels of microsatellite allelic variation allow rapid identification of individual fish. For example, using only six loci (Ip001,-002,-003,-007,-008, and -010) to screen spawns 3 and 6 of pond A4, we could identify 2,916 and 5,832 potential composite genotypes within each full sib family, respectively. While loci with the most polymorphic alleles were used to identify fish within a strain, loci with less polymorphism may be more useful for identification of fish between strains.

Use of microsatellite loci will be fundamental in efforts to identify and select genetically superior catfish in selective breeding programs, and in efforts to identify and manage genetically improved stocks released to commercial producers. High levels of microsatellite allele heterozygosity in catfish populations will assist efforts toward the establishment of linkage and physical maps of the channel catfish genome. These maps will assist researchers in the identification of quantitative trait loci that affect economically important traits, especially traits that are difficult, costly, or time consuming to measure.

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