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PRIMER NOTE

Microsatellite DNA markers for parental assignment in hybrid striped bass (*Morone saxatilis* × *Morone chrysops*)

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Abstract

Development of nine polymorphic microsatellites from a genomic library of hybrid striped bass (female *Morone chrysops* × male *Morone saxatilus*) DNA is described. Breeding of hybrid striped bass for aquaculture is based largely on breeding wild fish. Molecular markers such as microsatellites will be useful tools for developing broodstock, estimating heritability for production traits, and selective breeding via marker-assisted selection. The nine polymorphic microsatellites include six dinucleotide and three complex repeat motifs. The number of alleles detected among a sample of 10 individuals of each species was relatively low. All polymerase chain reaction primer pairs also amplified products in the sea bass *Dicentrarchus labrax*.

Keywords: genome library, hybrid striped bass, microsatellites, polymerase chain reaction primers

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Aquaculture of hybrid striped bass (female *Morone chrysops* × male *M. saxatilis*) is one of the fastest growing industries in the United States (Kohler 2000). However, a major constraint currently limiting expanded production of hybrid striped bass is suboptimal production efficiency stemming from uncontrolled variation in performance of fish derived from undomesticated broodstock. Through crossing of selected parental fish, we plan to implement a 10 × 10 factorial animal-breeding design to estimate heritability of traits of importance to hybrid striped bass aquaculture. Offspring will be raised in a 'common-garden' environment and parents of offspring will be determined via genotypes at polymorphic nuclear-encoded genetic markers (microsatellites). Molecular markers such as microsatellites are useful tools for this purpose as they allow determination of pedigree and quantification of inbreeding. Available polymerase chain reaction (PCR) primers for microsatellites or sequences developed by other authors for the same or related species (i.e. striped bass: Han *et al*. 2000, Roy *et al*. 2000, Westerman *et al*., unpublished; and sea bass, *Dicentrarchus labrax*: Garcia de Leon *et al*. 1995, Castilho & McAndrew 1998) were investigated initially. However, we found that these markers were limited in their ability to identify parents in controlled crosses because of low levels of allelic polymorphism. Consequently, additional microsatellites were developed from a hybrid striped bass individual obtained from Keo Fish Farms, Inc, in Lonoke, Arkansas. In this note, we describe the development and characterization of 10 (nine polymorphic) microsatellites isolated from the genomic library of hybrid striped bass DNA.

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DNA was extracted using a standard phenol–chloroform procedure and digested with *Sau*3A1 (Promega). DNA fragments in the range 500–1200 base pairs (bp) were size-selected, gel purified (QIAEX II gel extraction system, Qiagen), ligated into a *Bam*HI (Promega) digested and dephosphorylated (Shrimp Alkaline Phosphatase, Promega) pBluescript vector, and transformed into DH10-Beta electro-competent cells via the Gibco BRL™ Cell-Porator® Electroporation System I. Transformation inoculation included 1 mL SOC medium (2% Bacto tryptone, 0.5% Bacto yeast, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose, pH 7.0) incubated at 37 °C for 45 min. Transformed cells were plated on X-Gal — IPTG Luria-Bertani (LB) agar with 50 μg/mL of ampicillin and grown

overnight at 37 °C. Recombinant colonies were picked using a Genetix QBOT and inoculated into 384-well plates containing 50 μL LB freezing media [36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM sodium citrate, 0.4 mM $MgSO_4$, 6.8 mM $(NH_4)_2SO_4$, 4.4% (v/v) glycerol, 50 μg/mL ampicillin, LB] and incubated at 37 °C overnight before freezing at −80 °C.

A total of 14 500 clones from the genomic library were spotted onto 22.5 cm × 22.5 cm membranes (Amersham). Membranes were placed on LB agar plates with 50 μg/mL ampicillin and incubated at 37 °C for 24 h. Colonies were fixed to membranes by placing the filters on chromatography paper (3M) soaked in the following solutions: 10% sodium dodecyl sulfate for 3 min, denaturing solution (1.5 mM NaCl, 1.5 M NaOH) for 5 min, neutralizing solution (1.5 mM NaCl, 1.5 mM Tris) for 5 min, and 2 × saline sodium citrate for 5 min. Filters were incubated for 5 h at 65 °C.

The resulting colonies were probed separately with the ^{32}P-labelled dinucleotides $(CA)_{15}$ and $(GT)_{15}$. Approximately 200 positive clones were amplified by PCR by using 'universal' M13 primers. PCR products were cleaned with QIAquick PCR purification Kit (Qiagen) and sequenced using M13 primers and ABI PRISM BigDye™ Terminator version 3.0 Cycle Sequencing Ready Reaction Kit. Sequences were run on an ABI Prism® 377 DNA sequencer (Applied Biosystem) and aligned in Sequencher version 4.1.2 (Gene Codes Coporation Inc.). Twenty-nine complete sequences containing microsatellite arrays were identified. All 29 sequences are deposited in GenBank (Accession nos AY453795–AY453823). Of these, PCR primer pairs were designed for 21 microsatellite arrays by using Netprimer®. Oligonucleotide primers were synthesized by Invitrogen and then tested for reliable PCR amplification by screening DNA from 10 white bass (*M. chrysops*), 10 striped bass (*M. saxatilis*) and 10 sea bass (*D. labrax*). PCR reactions were performed in a 10 μL reaction volume containing 1 μL (100 ng) DNA, 1 μL 10 × reaction buffer (500 mM KCl, 200 mM Tris-HCl, pH 8.4), 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 5 pmols of each primer, and 0.5 U *Taq* DNA polymerase (Gibco-BRL). Amplifications were performed in an Omni-E thermal cycler (Hybaid) and consisted of an initial denaturation at 94 °C for 3 min, followed by 25–35 cycles of denaturation at 94 °C for 30 s, annealing at 47–57 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were electrophoresed in a 3% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light, using an Eagle *Eye*II video system (Stratagene). Initially, the annealing temperature generated by Netprimer® was used; amplification conditions, including annealing temperature, were then optimized based on results from agarose electrophoresis. One primer of each of the 10 primer pairs ultimately optimized (Table 1) was fluorescently labelled and primer sets screened in a 6% denaturing polyacrylamide gel, using an ABI 377 DNA sequencer in GENESCAN® mode, version 3.1.2. Estimates of allele diversity for each microsatellite were obtained by genotyping 10 white bass, 10 striped bass, and 10 sea bass. Fragment analysis was conducted using the ABI 377, and allele scoring was performed with GENOTYPER® software, version 2.5. Alleles were scored by length in base pairs.

Summary data for the 10 microsatellites are presented in Table 1. Data for each microsatellite include forward and reverse PCR primers, repeat sequence(s) of the cloned allele, annealing temperature, and number and size range of alleles detected in the three species (white bass, striped bass, and sea bass). The 10 microsatellites included six 'perfect' dinucleotide repeats and four imperfect repeats. Lengths of the cloned alleles ranged from 190 to > 400 base pairs (the maximum size of the ladder used in genotyping was 400 bp). Optimal annealing temperatures ranged from 47 to 57 °C. Eight of the microsatellites were polymorphic in all three species. Only one allele at microsatellite *Hsb7*E was detected in striped bass, and microsatellite *Hsb9*A was monomorphic among all individuals surveyed. The level of polymorphism in terms of the average number of alleles detected per microsatellite (3.1 in white bass, 3.6 in striped bass and 4.9 in sea bass) was very low when compared to that found in other species of fish (averages of 7.5 for freshwater fish and 11.3 for anadromous fish — DeWoody & Avise 2000). This is possibly a result of a small sample size or perhaps sampling bias resulting from possibly having related individuals in the sample. However, comparable findings of low allelic diversity in striped bass have been reported previously (Han *et al.* 2000). The low allelic diversity in hybrid striped bass suggests that additional microsatellite markers may need to be developed to assess heritability of production traits or to carry out marker-assisted selection.

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Table 1 Summary data for microsatellites developed from hybrid striped bass (*Morone saxatilus* × *M. chrysops*). PCR primer sequences are forward (F) and reverse (R). The fluorescently labelled primer is in boldface. Repeat sequence indicates the repeat motif. AY # is the GenBank Accession no. of the sequence of the cloned allele. T_a is the annealing temperature. No. alleles is the number of alleles detected (in 10 individuals from each species)

				White bass		Striped bass		Sea bass	
Microsatellite	Primer sequences (5′–3′)	Repeat sequence/AY #	T_a (°C)	No. alleles/ size range	H_E/H_O	No. alleles/ size range	H_E/H_O	No. alleles/ size range	H_E/H_O
*Hsb*1B	F: GCAGCAGAAGTTGGGACTGGT	$(GT)_{60}$/AY453797	54	6	0.74/0.78	7	0.85/0.89	5	0.49/0.52
	R: **GGCACCAAACAAGACATATAGTGA**			(230–280)		(228–248)		(227–243)	
*Hsb*1C	F: **GTACGGTGTTCCTGCCTCA**	$(GT)_{22}$/AY453798	47	4	0.74/0.77	2	0.48/0.51	7	0.81/0.85
	R: GGAGTGTCCATAGATACAGTAAGTG			(263–273)		(253–257)		(279–303)	
*Hsb*2C	F: **GCTGCCATTGTCGAATTACCCATT**	$(GT)_{26}$/AY453802	57	1	0/0	3	0.60/0.63	3	0.47/0.49
	R: GCTACGCAGGAACACCTCGCTC			(242)		(246–252)		(238–244)	
*Hsb*5B	F: GGGACCCACTGCCTGATTTC	$(CT)_8ATT(CA)_{13}$/AY453810	52	3	0.34/0.35	2	0.38/0.39	10	0.85/0.89
	R: **CCACACTCGCCCACTCCTTA**			(230–238)		(224–228)		(300–364)	
*Hsb*6C	F: **CAGAAACACTCGCTTCGCATCA**	$(CT)_{24}(N)_{105}(CA)_{19}$/AY453811	57	8	0.80/0.84	≥ 5	0.63/0.66	≥ 1	0/0
	R: GGAGCGTTCTTCAATGTTCTCTCAA			(299–317)		(371–400*)		(400*)	
*Hsb*7C	F: **GGCTGAGGGCAGTAGTCAGA**	$(AC)_{35}$/AY453815	52	2	0.32/0.34	6	0.73/0.76	≥ 3	0.19/0.19
	R: GGTGATACTGGTGGGTTTCAA			(325–330)		(263–313)		(386–400+)	
*Hsb*7E	F: **GTCATCTCCATATCAGTATAGGT**	$(AC)_{16}(N)_{23}(AC)_4$/AY453816	49	2	0.18/0.19	NA	NA	8	0.80/0.84
	R: GCTCACACAAACAAATACAAA			(290–292)				(350–389)	
*Hsb*9A	F: **CACCCCTCAACATGCTCATCTA**	$(CA)_4(N)_{10}(CA)_4AA(CA)_7$/AY453818	52	1	0/0	1	0/0	1	0/0
	R: GCAACCACGACAGAATACGAA			(260)		(260)		(260)	
*Hsb*14C	F: **GAAAAAGGCATTTGTATGTGTAC**	$(GT)_{22}$/AY453800	50	2	0.50/0.52	4	0.48/0.50	7	0.83/0.87
	R: GCCATCACATTATTCAGTCTAC			(180–182)		(191–238)		(179–199)	
*Hsb*14F	F: GCACACAGCATTGTTTTACAA	$(AC)_{22}$/AY453823	50	2	0.42/0.44	2	0.09/0.10	4	0.34/0.36
	R: **GCTAACACAGGCAGGAATACA**			(213–226)		(209–219)		(205–225)	

*Fragments > 400 bp were observed but could not be sized as the ladder employed had a maximum size of 400 base pairs (bp).
NA indicates region not able to be amplified.

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