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TECHNICAL NOTE

Microsatellite markers for the endangered razorback sucker, *Xyrauchen texanus*, are widely applicable to genetic studies of other catostomine fishes

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Abstract We developed 10 polymorphic microsatellite markers for the federally endangered razorback sucker, *Xyrauchen texanus*. PCR optimization and cross-species amplification experiments indicated that these markers will be useful for analysis of fine-scale population structure in razorback and two other sucker species; the white sucker, *Catostomus commersonii* and the Rio Grande sucker, *C. plebeius*. Alleles at locus Xte4 did not overlap when compared between razorback and bluehead (*C. discobolus*) suckers and permits detection of F1 hybrids. These microsatellite markers appear widely applicable for identifying genetic consequences of population decline, hatchery rearing and release, and hybridization in razorback and other castostomine suckers.

Keywords *Catostomus* · Microsatellite · Hybridization

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The freshwater fish family Catostomidae is characterized by ecologically and morphologically diverse species known commonly as suckers. Species richness is highest in North America where 74 of the 76 described species reside mostly in temperate streams, rivers and lakes (Harris and Mayden 2001). Along with minnows (family Cyprinidae), suckers were once dominant in river communities of the southwestern United States. However, some species have suffered alarming declines in numerical abundance as a result of regulation and development of surface waters over the last few decades. The razorback sucker (*Xyrauchen texanus*), a large-bodied and charismatically hump-backed fish, was among the first harbingers of ecosystem decline in the Colorado River (Minckley and Deacon 1968), and the species was listed as federally endangered in 1991. To assist in conservation and recovery of razorback sucker, we developed a set of microsatellite markers that can be applied to genetic monitoring of natural populations and to hatchery brood stock development and maintenance. We tested and optimized these markers for razorback and two relatively distantly-related sucker species within the tribe Catostomini to assess utility of these loci in the most species-rich monophyletic group in the family Catostomidae (Smith 1992).

Microsatellite DNA sequences were isolated from a partial genomic library constructed for the razorback sucker. Tissues from a single individual were obtained during routine monitoring of the remnant population in Lake Mohave, Arizona, USA (Minckley et al 1991). Nucleic acids were isolated and purified through density-gradient ultracentrifugation and then digested with the restriction endonuclease *Dpn*II (New England Biolabs). Resulting fragments were size-selected in the range of approximately 200–800 base pairs (bp) by electrophoresis through a low-melting point agarose gel and purified using

Prep-A-Gene (Bio-Rad) DNA purification kits. Size-selected fragments were ligated into pUC18 vector, and heat-shock transformed into *E. coli* strain DH5α following Sambrook et al. (1989). The resulting partial genomic library consisted of 864 clones that were transferred to nylon membranes. Membranes were screened for three classes of synthetic oligonucleotide probes: di-($[CA]_{15}$, $[GA]_{15}$); tri-($[CCT]_7$, $[ATT]_7$); and tetranucleotide ($[GATA]_5$, $[GAGC]_5$, $[GTCA]_5$, $[CTCA]_5$, $[GACT]_5$, $[CTAG]_5$, $[GCAT]_5$, $[GCAC]_5$) repeats (Sigma-GenoSys). Dinucleotide screening yielded 39 positive clones, and tri- and tetranucleotide screening identified six positive clones. Positive clones were sequenced in forward and reverse directions with M13 sequencing primers and an ABI 377 automated nucleotide sequencer. Twelve positive clones contained repeated DNA sequences and sufficient unique flanking sequence to facilitate primer design and were analyzed further.

Primers for DNA amplification via polymerase chain reaction (PCR) were designed from unique nucleotide sequence regions flanking microsatellites using the computer progam OLIGO™ (Macintosh vers. 4.0, National Biosciences). Annealing temperatures and $MgCl_2$ conditions were optimized for 10 primer pairs (Table 1) by characterizing gene products from 16 razorback sucker template DNAs isolated from fin clips supplied by the US Fish and Wildlife Service. A microsatellite locus was considered optimized when PCR produced one or two strongly amplified bands with minimal stutter bands and other ancillary products. PCR amplification was carried out in 10 µl volumes containing 1 µl (50–200 ng) sample DNA, 1 µl 10X reaction buffer (500 mM KCl, 100 mM Tris [pH 9.0], 10% Triton-X 100), 200 µM of each dNTP, 2–3 mM $MgCl_2$, 5 pmol of each PCR primer, and 0.375 units *Taq* DNA polymerase (Promega GoTaq® Flexi). Thermal cycling consisted of 30 cycles of denaturation at 90°C for 30 s, annealing at 48–62°C for 30 s, and extension at 72°C for 30 s, preceded by an initial denaturation step at 90°C for 2 min. PCR amplification was conducted with forward primers labeled with fluoroscein dyes (Table 1). Fragment lengths (in base pairs, *bp*) of PCR products were characterized on an ABI-3100 capillary automated sequencer equipped with Genemapper software. We detected no evidence of linkage disequilibrium between any pair of loci reported in Table 1.

Following optimization in razorback sucker, we expanded our test panel to include two other catostomine fish

Table 1 Primer sequences and PCR conditions for 10 microsatellite loci developed for razorback sucker, *Xyrauchen texanus* where F, forward primer; R, reverse primer; T_a, annealing temperature

Locus[c]	Genbank Accession No.	Repeat motif	Dye label	Primer sequence (5′–3′)	Fragment length (bp)	$MgCl_2$ (mM)	T_a (°C)
Xte1	EU490505	$AG_{[13]}GA_{[6]}$	NED	F: GGT TTC TAT GCT GCT GTA AG	135	2.5	53
				R: ATA GCC AAA ATC TCC CAC TT			
Xte2	EU490506	$TC_{[6]}$	HEX	F: GGA ATG AAA GAG GGC TGA AAT A	76	2.5	49
				R: GCC ATC CGA GGG TCT GA			
Xte4[b]	EU490513	$AC_{[11]}$	FAM	F: GGA TTG CCT TTA TGG TGT CT	320	2.5	48
				R: TTC TCT TCA ACT GGT CTA AAT			
Xte5	EU490508	$TG_{[25]}$[a]	NED	F: ACC CAA GCC TCC GTT ACT	292	2.0	57
				R: GCC GCA TTA TTG TTC ATC			
Xte6	EU490503	$GA_{[39]}$	NED	F: CCA CAT TGC TCT TAC CAC ATC T	338	3.0	57
				R: CCC TTA CCC TTC ATC CAT CTA C			
Xte7[b]	EU490504	$TC_{[12]}$	FAM	F: GGA ATA ATG GTA GAG AAG AAC G	143	2.5	50
				R: TAA TAA TGG AAA GAG GGT GAG G			
Xte8	EU490510	$AC_{[12]}$	HEX	F: ATT GCC ACA GAG TCA GAT TG	412	2.5	48
				R: AAG GGT GAC TGG TTG ATT TT			
Xte10	EU490509	$TC_{[13]}$	HEX	F: CTA AGA TGT TTG GCA GAA TGT T	331	2.5	50
				R: ATG GGA AGT CTG TGG TTA GG			
Xte11	EU490511	$TG_{[8]}$	FAM	F: CCA CTA TAG GGA TTA CAA AA	296	2.5	49
				R: CAC CTG AGC AAC ACA CCT T			
Xte12	EU490512	$TC_{[35]}$[a]	HEX	F: GCA AGC GGC AAT AGT CA	198	2.0	52
				R: GAA AGA GGG ATG CCA ATA C			

[a] Indicates imperfect repeat

[b] Primer sequences previously reported in McPhee and Turner (2004)

[c] Two loci, Xte3 and Xte9, failed to amplify in the razorback sucker test panel (n = 16) and are not included in Table 1

Table 2 Summary of test results for genetic screening and cross-amplification of microsatellite loci in razorback and two other catostomine species, including number of alleles (n_a), size range of alleles in base pairs (bp), observed (H_O) and expected (H_E) heterozygosities

Locus	*Xyrauchen texanus* (n = 16)				*Catostomus plebeius* (n = 14)				*C. commersonii* (n = 10)			
	n_a	size range (bp)	H_O	H_E	n_a	size range (bp)	H_O	H_E	n_a	size range (bp)	H_O	H_E
Xte1	2	132–134	0.39	0.32	–	–	–	–	2	137–139	0.13	0.13
Xte2	4	60–98	0.56	0.54	–	–	–	–	–	–	–	–
Xte3[a]	–	–	–	–	3	141–147	0.23	0.34	5	138–150	0.40	0.43
Xte4	4	314–322	0.30	0.57	2	286–294	0.00	0.19	2	298–306	0.14	0.14
Xte5	23	365–443	0.88	0.97	5	115–131	0.39	0.61	–	–	–	–
Xte6	19	372–488	0.79	0.97	6	370–402	0.46	0.84	15	340–404	0.90	0.80
Xte7	9	141–193	0.56	0.73	11	143–165	0.54	0.47	2	137–139	0.27	0.44
Xte8	9	406–434	0.64	0.84	6	384–408	0.83	0.70	13	416–464	0.60	0.83
Xte10	9	316–336	0.77	0.82	18	316–354	0.57	0.77	–	–	–	–
Xte11	9	296–318	0.85	0.80	10	264–292	0.77	0.73	3	284–294	0.50	0.40
Xte12	9	160–214	1.00	0.83	–	–	–	–	9	288–294	0.30	0.57

[a] Locus Xte3 failed to amplify in *X. texanus*, but did amplify in other sucker species. Xte2 and Xte3 are genetically linked in razorback sucker. Locus Xte3 repeat motif: $GAG_{[30]}$; Dye Marker HEX, Primer sequences F: 5′-GAG ACT TGT GTT GCT GTG GT-3′; R: 5′-ATT TCA GCC CTC TTT ATT CC-3′; clone length: 193 bp; Genbank accession no EU490507. (–) scorable products were not obtained

species: white sucker, *Catostomus commersonii*; and Rio Grande sucker, *C. plebeius*. PCR was conducted as described above except annealing temperature and $MgCl_2$ concentration were held constant at 50°C and 2.0 mM per reaction, respectively, for all amplifications. Two sets of eight primer pairs produced well-resolved, polymorphic, and scorable products (Table 2). Each locus was tested for deviation of Hardy-Weinberg expected and observed heterozygosities with goodness-of-fit tests. No locus deviated significantly from expected values for any species tested.

Allele size ranges for Xte4 and Xte7 exhibited no overlap when compared between *C. commersonii* and *C. plebeius* (Table 2). This result suggested that these loci may be useful for hybridization studies of other catostomine fishes. To test this, we examined genotypes at these loci for razorback (n = 10) and co-occurring bluehead, *C. discobolus* (n = 59), and flannelmouth, *C. latipinnis* (n = 32), suckers. Larval and adult fishes for this test were collected in the San Juan River (Colorado Drainage) in New Mexico. Products from locus Xte7 exhibited substantial overlap of allele sizes among all three species. For locus Xte4, *C. discobolus* was fixed for an allele size 228 bp whereas *X. texanus* and *C. latipinnis* exhibited overlap in alleles that ranged in size from 302 to 320 bp. The allelic size difference between species appears to result from loss of the microsatellite repeats in *C. discobolus*. Nevertheless, it appears that locus Xte4 will be useful for studies of hybridization in suckers of the Colorado River and other rivers of the southwestern US (e.g., McPhee and Turner 2004).

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