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PRIMER NOTE

Microsatellite loci for the plains topminnow (*Fundulus sciadicus*, Fundulidae)

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Abstract

The plains topminnow (*Fundulus sciadicus*) is a killifish endemic to the Great Plains region. Recent field surveys indicate that populations may be declining in northerly portions of its range and perhaps throughout. Here, we describe the isolation and characterization of 11 microsatellite loci developed for population genetic studies in the species. We also conducted cross-species amplifications to test primer efficacy in five additional taxa, including four killifishes.

Keywords: *Fundulus sciadicus*, microsatellite loci, plains topminnow

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The plains topminnow (*Fundulus sciadicus*) is a freshwater killifish endemic to the Great Plains region with a disjunct distribution covering three major areas. Two occur in Missouri and the other primarily in Nebraska (Shute 1979; Pflieger 1997). Field surveys indicate that populations have declined in Nebraska in recent years (Lynch & Roh 1996; Bessert, Li & Brock, unpublished data). Investigation of population genetic structure in *F. sciadicus* is important for conservation of the species and for understanding the historical and current forces shaping the evolution of fish fauna in the Great Plains region. Preliminary mitochondrial sequence data showed little variation in *F. sciadicus* in either Nebraska or Missouri (Li, Bessert & Orti, unpublished data); thus, we turned to microsatellite loci to determine population dynamics of the species. Here, we describe the isolation and characterization of 11 microsatellite loci developed for this species.

Genomic DNA was isolated from muscle tissue and fin clippings using DNeasy kits (QIAGEN). Genomic DNA was digested using *Sau*3AI at 37 °C over night. The protocol used to enrich microsatellite-containing fragments was modified from Kandpal *et al*. (1994). Enriched DNA was polymerase chain reaction (PCR)-amplified with the following cocktail: 1.25 μL of 2 mM dNTPs, 2.5 μL of 10× PCR buffer, 0.75 μL of 50 mM $MgCl_2$, 1.25 μL of 10 mM *Sau*lA primer, 0.3 μL (0.5 U) of *Taq* DNA polymerase (Invitrogen), 10.95 μL sterile ddH_2O, and 8 μL eluted DNA fragments. Cycling conditions were: 94 °C (3 min), 25 cycles at 94 °C (1 min), 68 °C (1 min), 72 °C (2 min), followed by a final extension at 72 °C (10 min). PCR products were cloned using the pGEM-T Easy Vector System I (Promega). PCR-based screening was performed to determine insert length in approximately 200 positive clones. Inserts were amplified using flanking primers M13F and M13R. Products were electrophoresed on a 2% agarose gel to visualize insert length. Approximately 100 clones with inserts > 400 bp were selected for sequencing.

Clones were sequenced in one direction with a BigDye Terminator (Applied Biosystems) cycle sequencing reaction using the M13F primer. Forty-two clones contained microsatellite sequences (motifs repeated more than five times). These were sequenced in the opposite direction with the M13R primer. Complimentary sequences were aligned and edited with SEQUENCHER 4.2.2 (Gene Codes Corp.).

PRIMER 3 (Rozen & Skaletsky 2000) was used to design primer pairs for 24 candidate loci that possessed adequate flanking regions (at least 50 bp). PCR optimization was conducted using a gradient thermal cycler (MJ Research PTC-200) with an annealing step of 48–66 °C to determine optimum annealing temperature. Reaction mixtures had a total volume of 10 μL and contained 2.0 μL of 1 mM dNTPs, 1.0 μL of 10× PCR buffer, 0.4 μL of 50 mM $MgCl_2$, 0.4 μL 10 mM forward primer, 0.4 μL 10 mM reverse primer,

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Table 1 Primer sequence, repeat motif and allelic diversity for 11 microsatellite markers in *Fundulus sciadicus*, each screened in 20 individuals.

Locus	Primers (5′–3′)	Repeat motif	Size (bp)	T_a (°C)	k	H_O†	H_E
F01	F: ACCTGTCCTGGTTGTGGAGCCT R: CCGAGGACAGCGGTTTAAAATAT	$(CTG)_7(ATG)_{17}$	195–234	58	8	0.750	0.773
F02	F: AGAGGCTGAAAGCTACCCGC R: CCACTGAGACACACACACCAACAT	$(GT)_{15}$	135–153	58	7	0.850	0.668
F04S	F: CCCAGTAGTTAAAGCTCTATCT R: CCACTTCTTCAAAGATAGATAG	$(CTAT)_{22}$	100–146	52	8	0.647*	0.839
F10	F: TCTGACGCCATGTTTGTTAGACCA R: CAGGAGCTCACATCAATCCATCTGT	$(ATGG)_{21}$	178–214	58	10	0.800	0.792
F17	F: GATCAACAGCATGCAAGTAACA R: GCTCAGATGAGCTATATATGTCAAA	$(GATA)_{64}$	218–382	58	21	0.888	0.792
F67	F: ACAGAGATTTCCAGGGCATAAAA R: GCCCGGGATAACACAAACTTC	$(GT)_{50}$	158–220	58	16	1.000	0.924
F82	F: GCAGGGAGACACAAGTACCATATG R: GGGGGGAAACGTGAGAAGGT	$(GT)_{22}$	90–122	58	8	0.750	0.768
F87	F: TGGAGCGGGACGGGTTAAAT R: ACAGCTCATCCAGCACGCAC	$(GT)_{27}$	98–134	58	9	0.785	0.788
F90	F: GGCTGTGAGTGTACCTTAATTGCA R: CCACAAACACACCGATGAACAC	$(GT)_{17}$	96–124	58	5	0.700	0.733
F105	F: ATAGTCTGCCTGACAGAGCCACT R TGGCTCCACATCACCTCTTGAT	$(GT)_{19}(GC)_4$	99–133	58	6	0.750	0.674
B08	F: GGCATCAAGGGCAAAGCGTAGG R: AGTTTGGCCAGGCATATCTGAGTG	$(GT)_{33}$	165–181	62	5	0.800	0.700

T_a, annealing temperature; k, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. †Hardy–Weinberg equilibrium (HWE) for each locus was checked using HWE probability test, GENEPOP 3.4 (Raymond *et al.* 1995). *$P < 0.05$.

0.1 μL (0.5 U) of *Taq* DNA polymerase (Invitrogen), 4.7 μL sterile ddH_2O, and 1.0 μL (~100 ng) DNA. Reactions were denatured at 94 °C for 2 min, then carried out for 30 cycles at 94 °C (30 s), annealing temperature (30 s), 72 °C (40 s), followed by a final extension of 72 °C (2 min). Eleven of these loci amplified cleanly. Sequences of the clones having these 11 loci were deposited in GenBank under accession nos EF105300–EF105310. For these 11 loci, an ABI PRISM 310 Genetic Analyser (Applied Biosystems) was used to score allele lengths in 20 individuals collected from Bone Creek, located 1.5 km West of Ainsworth, Nebraska, USA.

Heterozygosity values for each locus and tests for Hardy–Weinberg equilibrium (HWE) were calculated using GENEPOP 3.1 (Raymond & Rousset 1995). The number of alleles per locus ranged from five to 21 with a mean of 9.4 alleles per locus. One locus (F04S) deviated significantly from HWE (Table 1).

Tests for cross-taxon amplification were carried out in five additional taxa. Reactions were conducted in single individuals along with positive and negative controls. Only three loci amplified cleanly within the expected size ranges in other species (Table 2). All products were verified by genotyping as described previously. In conclusion, these novel microsatellite loci will be useful for studies in *F. sciadicus*, and some loci may have broader utility within the family Fundulidae.

Table 2 Cross-taxon amplification to determine utility of the loci in additional species. Numbers below each locus represent actual genotypes (allele lengths in base pairs) in a single individual of the given taxon. 'X' indicates no successful amplication. All reactions were conducted under conditions optimized for *Fundulus sciadicus*. Loci that did not amplify in any of the taxa tested are not listed here

	Locus		
Taxon	F90	F105	B08
Fundulus zebrinus	X	X	219/212
Fundulus catenatus	X	101/101	X
Fundulus heteroclitus	110/116	87/89	183/187
Gambusia affinis	X	X	X
Cycleptus elongatus	X	X	X

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