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PRIMER NOTE

Characterization of microsatellite loci in Sacramento perch (*Archoplites interruptus*)

RACHEL S. SCHWARTZ and BERNIE MAY
Department of Animal Science, University of California — Davis, Davis, CA 95616, USA

Abstract

We characterized 23 polymorphic tetranucleotide microsatellite loci for Sacramento perch (*Archoplites interruptus*). This species is extirpated in its native range, the Sacramento–San Joaquin Delta (California, USA), and is therefore targeted for recovery. A concerted effort is currently underway to re-establish self-sustaining populations of Sacramento perch in its native range. These microsatellites will be used to analyse the population structure of the species and, in conjunction with life history and physiological data, develop a comprehensive recovery plan.

Keywords: Centrarchidae, microsatellites, primers, Sacramento perch

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The Sacramento perch (*Archoplites interruptus*) is the only sunfish (family Centrarchidae) native to the region west of the Rocky Mountains. Today it is extirpated from its native range, the Sacramento–San Joaquin watershed of California, although it was previously the dominant piscivore in the region (Moyle 2002). A few transplanted populations remain in other locations, including Pyramid Lake and Stillwater National Wildlife Refuge in Nevada, and Clear Lake Reservoir and Abbott's Lagoon in California. Another population may remain in Clear Lake, California, although no fish were caught in two attempts in 2003. Much of the potential habitat in the native range of Sacramento perch is now dominated by non-native centrarchids, such as largemouth bass and bluegill, or has been altered by changing water regimes in the region (Moyle 1995); these changes limit potential restoration sites for Sacramento perch. As a result, the Sacramento perch is listed as a species of special concern by the California Department of Fish and Game (Moyle *et al*. 1995) and is targeted for recovery in the Delta Native Fishes Recovery Plan (Moyle *et al*. 1996).

The microsatellite primers described here will be used to analyse population structure and the history of transplantation of Sacramento perch in order to design a recovery plan that retains the genetic diversity of the species. Primers designed for other species in the family Centrarchidae did not successfully amplify Sacramento perch DNA, possibly due to the historic isolation of this species. Microsatellite data will be used to characterize the amount of genetic variation within each sampled population and the genetic distinctiveness among them. By combining the genetic data with the historical records of transplants we hope to identify several source populations with sufficient genetic variation for use in restoration efforts on behalf of this species.

Correspondence: Rachel S. Schwartz. Fax: +1 530 752 0175; E-mail: rsschwartz@ucdavis.edu

Whole genomic DNA was extracted from caudal fins of juvenile Sacramento perch preserved in 95% ethanol using the DNeasy™ Tissue Kit (Qiagen). Four libraries enriched for tetranucleotide repeat motifs $(TAGA)_n$, $(CAGA)_n$, $(TACA)_n$ and $(CATC)_n$ were constructed from this DNA by Genetic Identification Services (Chatsworth, CA, USA) according to the protocol described in Meredith & May (2002). Following construction, libraries were screened for DNA fragments between 300 and 700 bp according to the following protocol. Recombinant clones were plated onto Luria–Bertani broth containing bluo-gal/IPTG/ampicillin and incubated overnight at 37 °C. Cells from isolated white colonies were heated to 100 °C for 10 min in a 10 μL polymerase chain reaction (PCR) mixture containing 3.4 μL water, 1× PCR buffer, 3.0 mM $MgCl_2$, 1.5 μL sucrose/cresol red loading buffer (10 mM cresol red in 20% sucrose 400), 1.5 mM each dNTP, 1.5 μM each primer and 0.42 mg/mL RNAse A, overlaid with 22 μL of mineral oil. Polymerase 'hot start solution' containing 4.4 μL water, 1× PCR buffer (0.5 μL of 10× buffer) and 0.94 U BIOTAQ DNA polymerase (Bioline USA) was added and the insert was amplified for 21 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for

30 s, followed by 72 °C for 2 min and held at 5 °C. The PCR product was separated by electrophoresis on a 3% agarose gel and sized by comparison with Phi-X/*Hae*III standard. Colonies containing inserts in the desired size range were sequenced using the DYEnamic ET terminator Cycle Sequencing Kit (Amersham) on an ABI 377 sequencer (Applied Biosystems). Screening and sequencing were carried out by Genetic Identification Services. We eliminated duplicate sequences and designed primers using PRIMERSELECT 4.0 (DNAStar Inc.).

Primers were initially tested on a total of six individuals from three populations to determine whether the locus was polymorphic. Polymorphic loci were screened on a total of 34 individuals, including 27 from Stillwater National Wildlife Refuge (Fallon, NV, USA), two each from Pyramid Lake (NV, USA), Clear Lake Reservoir (CA, USA) and Lagoon Valley Reservoir (CA, USA), and one from Abbott's Lagoon (CA, USA). PCR was performed using 1× *Taq* DNA polymerase buffer B (Promega), 2.0 mM $MgCl_2$, 0.2 mM each dNTP (Promega), 1 μM of each primer and 0.38 U *Taq* DNA polymerase (Promega), with a total volume of 10 μL. PCR was carried out in an MJ Research PTC-100 under the following conditions: 94 °C for 2 min 30 s, 30 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 5 min, and held at 4 °C.

Amplification products were mixed 1 : 1 with 98% formamide loading dye, denatured for 3 min at 95 °C, and cooled on ice. The amplification product/dye mixture was run on 5% denaturing acrylamide gels at 1900 V for 70 min. Products were detected using an agarose overlay and SYBR Green nucleic acid stain (Cambrex BioScience) following the procedure of Rodzen *et al*. (1998) and scanned with a Molecular Dynamics 595 fluorimager. Product sizes were estimated by comparison with a standard 400 bp ladder (The Gel Company).

Details of the 23 polymorphic loci are summarized in Table 1. All loci were tested for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium using the 27 individuals caught in Stillwater National Wildlife Refuge. Tests were performed using GDA (Lewis & Zaykin 2001) and the Fisher's exact test; missing data were discarded and 3200 shufflings were used. All loci were found to be in HWE for this population following a sequential Bonferroni correction ($P = 0.0023$) (Rice 1989) except locus *AinA120* ($P = 0.0003$); the deficiency in observed heterozygosity may reflect the presence of null alleles at this locus. Significant pairwise linkage disequilibrium was found only between loci *AinA106* and *AinC202* ($P < 0.001$) following a Bonferroni correction ($P = 0.000198$); genotypes were used for this test to prevent within locus disequilibrium from affecting the significance of disequilibrium between loci. The primers discussed here will be useful in analysing the population structure of Sacramento perch and collecting data to develop a restoration plan for this species.

Table 1 Summary data for microsatellites developed for Sacramento perch (*Archoplites interruptus*), including the repeat motif, size range of the alleles, number of alleles, expected and observed heterozygosities (H_E and H_O), number of individuals (of 27) that successfully amplified, and probability of Hardy–Weinberg equilibrium for each locus in the population in Stillwater National Wildlife Refuge

Locus	Genbank Accession no.	Primer sequence	Repeat motif	Allele size range*	Allele size range†	No. of alleles*	No. of allele†	H_E*	H_O*	No. of individuals amplified	*P* (HWE)
AinA2	AY643770	F: ATACCGGCAGATTGTGT R: TTAGGGTGGGAAGAAAG	$(TCTA)_{24}$	188–244	188–244	4	5	0.75	0.77	26	0.563
AinA6	AY643771	F: GTCAAAGCCTTTACTCACT R: ACACATAGATGCCCAGAC	$(ATAG)_{26}$	170–202	158–202	6	11	0.56	0.67	24	0.833
AinA11	AY643772	F: AGAATACACTGCAATAAAAA R: GTCCTGATCCTGAACA	$(TAGA)_{24}$	196–272	186–272	6	10	0.76	0.70	23	0.004
AinA106	AY643773	F: CCTACGGCTAATGTGAA R: TACTGAATATGAGAAATGTC	$(ATCT)_{17}$	276–296	276–320	3	6	0.64	0.85	20	0.067
AinA108	AY643774	F: TGCTGCATTAACCAAAACTGT R: GGCGATGAAGACCGTGTG	$(ATCT)_{14}$	204–240	204–252	6	9	0.73	0.58	24	0.173
AinA117	AY643775	F: TGTTCCATTTAGCTGTTTTTACCTG R: CACTGATGCTCCTGATTTCTATGA	$(GATA)_{22}$	156–196	152–196	4	10	0.67	0.60	25	0.631

Table 1 *Continued*

Locus	Genbank Accession no.	Primer sequence	Repeat motif	Allele size range*	Allele size range†	No. of alleles*	No. of alleles†	H_E*	H_O*	No. of individuals amplified	*P* (HWE)
AinA218	AY643776	F: CTCTGCCCAATCTACCAACAC R: AAAACAGAGCAGCAGACTATGAAT	$(TAGA)_9$	270	262–278	1	3	0.00	0.00	27	1.000
AinA120	AY643777	F: GTGCAACTTAAGACAAAACAA R: GTAAGAGCGCACGACAAA	$(TCTA)_{12}(TCCA)(TCTA)_9(TA)(CTA)_5$	276–400	276–400	5	8	0.56	0.22	27	0.000‡
AinA203	AY643778	F: CTGCCTTTCACCCAATA R: CTCAGTTCAGCTCAGTTCC	$(TC)_3(TATC)_4(TATA)(TATC)_{26}$	280–316	280–316	5	6	0.70	0.65	26	0.699
AinA207b	AY643779	F: CTCTGTGCTGTGACGGGACTGA R: ATGGCTTTTTATTGGGGGTTTTCT	$(TAGA)_{20}$	336–380	336–380	5	5	0.74	0.54	24	0.224
AinA212	AY643780	F: AGGCGAGCTTGACATTTTACC R: TCAGAAGGATTTGTTGGACTAT	$(TCTA)_{11}$ … $(TGTC)_3$	296–340	296–340	8	10	0.73	0.81	26	0.318
AinA216	AY643781	F: ATCAAAGCAGACTCAAGACAG R: GTGCAGTAAAGGAAAAATAGAC	$(ATCT)_{14}$	154–190	154–190	4	5	0.59	0.56	27	0.862
AinC11	AY643782	F: GATGGGGCGACCTCAAAT R: CTAGTCCTCCCCCTCATCAGTCT	$(ATAC)_7(CATC)_9$	280–300	280–308	3	7	0.54	0.48	27	0.715
AinC202	AY643783	F: TGAGGGGACACAGTTTAC R: GATTCAGGTTTCTCGTTCAT	$(ATAC)_{14}$	228–316	220–316	3	8	0.63	0.65	26	0.403
AinD101	AY643784	F: CCCCCGCGACCCTGTATG R: CACTGTTGCCCTGATGATGAAATG	$(GATG)_{15}$	122–134	122–162	4	8	0.67	0.81	27	0.472
AinD106	AY643785	F: TTAAACAAACCCCCTGAAGAAACC R: TGCGATGGACTGGCGACCTG	$(CATC)_{12}$	226–238	226–242	3	4	0.45	0.41	27	0.548
AinD119	AY643786	F: TGTACACAGGATAAGCGGTTGAC R: CCCCTCTGGCCTGTGGAATC	$(GATG)_{11}$	178–194	178–202	4	6	0.56	0.48	27	0.500
AinD212	AY643787	F: GTGAGGGGCATTTTGGACA R: CATTTTGCACAGGCTACATT	$(TCCA)_{16}$	296–318	276–370	4	8	0.68	0.65	26	0.522
AinC105	AY643788	F: ACGCTAGACGGCTGTTTTCAC R: CATAGGGGAGATTTCGGTCAA	$(CATA)_7$	272–280	272–280	3	3	0.60	0.38	26	0.018
AinC212	AY643789	F: CTCGCTTTCATCTTCTGCTCTG R: ACAACCACGCTCCATTTCACT	$(GTAT)_8$	130–146	130–150	2	3	0.33	0.33	26	1.000
AinD202	AY643790	F: TGTATGGAGTGGAGTGGTTTATTG R: TAGGTTGATGAGTGGTTGTTTGTC	$(CATC)_8$	248–252	248–252	2	2	0.28	0.33	27	1.000
AinC203	AY643791	F: GTGCTGGATTTTACCTGTGTCTGT R: TAGGGTGATGATGGATGGATGAAG	$(ATGT)_{10}$	172–180	172–180	3	3	0.48	0.41	27	0.539
AinB202	AY643792	F: GACACCTGCCCGCCTCCTC R: GACTCCGCCCACCACATCCT	$(CTGT)_6$	216–220	216–224	2	3	0.18	0.19	26	1.000

*Stillwater population only.
†Total for the Stillwater population and selected individuals from other populations.
‡This locus shows departure from Hardy–Weinberg equilibrium after Bonferroni correction.

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