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PRIMER NOTE

Microsatellite loci in the Lahontan tui chub, *Gila bicolor obesa*, and their utilization in other chub species

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Abstract

Primers were developed for 17 microsatellite loci from an enriched $(GATA)_n$ library from DNA from *Gila bicolor obesa*. These primers were tested for cross-species amplification in *Gila bicolor snyderi*, *Gila orcutti* and *Gila coerulea*.

Keywords: chub, Cyprinidae, *Gila bicolor*, *Gila*, microsatellites, primers

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The family Cyprinidae consists of over 330 species of freshwater minnow and carp, of which the genus *Gila* comprises nearly 50 species and subspecies of chub living in parts of the western United States (Mayden *et al*. 1992). Some of these species, including the Owens tui chub (*Gila bicolor snyderi*) and the Mohave tui chub (*G. bicolor mohavensis*), are currently listed as either threatened or endangered under the U.S. Endangered Species Act. Hybridization between species is a prime cause for the near elimination of several species (Moyle 1976). However, in both California and Nevada, recovery efforts to preserve or restore rare chub species have been occurring through the use of planned releases and intentional stockings. To better study the gene flow occurring in chub populations, we produced a tetranucleotide microsatellite library for *G. bicolor obesa*, from which 17 variable microsatellite markers were developed. Each of these loci was tested for use in individuals from two other *Gila* species and one other *Gila bicolor* subspecies.

Genomic DNA was extracted from dried fin samples of *G. bicolor obesa* using a TNES–urea procedure (Belfiore & May 2000). Two subgenomic libraries were constructed by Genetic Identification Services (Chatsworth, CA) by partially digesting genomic DNA with *Bsr*BR1, *Eco*RV, *Hae*III, *Pvu*II, *Sca*I and *Stu*I. An oligonucleotide linker containing a *Hin*dIII site was ligated to fragments in the range of 400–700 base pairs. These fragments were enriched by magnetic bead capture (Jones *et al*. 2002) to create a library of $(GATA)_n$ repeats. The captured fragments were digested and ligated into the H*ind*III site of pUC19, and the products were electroporated into competent *Escherichia coli* DH5-α. Transformed DH5-α cells were plated on LB ampicillin plates. Recombinant clones (sampled with a toothpick) from the $(GATA)_n$ library were amplified directly in 15 μL reactions containing: 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 5 mM $MgCl_2$, 1.5 mM dNTPs, 0.5 μm pUC19 forward and reverse sequencing primers, and 0.5 units *Taq* DNA polymerase (Gibco). Polymerase chain reactions (PCR) were amplified using an M. J. Research PTC-100 96 V thermocycler with the following cycling profile: 94 °C for 3 min, 25 cycles of 94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s, and then 72 °C for 5 min. To identify inserts of 300–800 base pairs, approximately 1 : 1 of PCR product and formamide loading buffer was run on a 3% TAE-agarose gel stained with 0.01× ™Vista Green nucleic acid stain (Amersham Pharmacia Biotech, Piscataway, NJ). Colonies containing the desired inserts were grown overnight in LB, and plasmids were purified using the QIAprep Spin Miniprep Kit (Qiagen). A total of 113 clones from the $(GATA)_n$ library were sequenced (Davis Sequencing, Davis, CA) using the ABI Big Dye™ Terminator cycle sequencing protocol and visualized on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA).

PCR primers were developed for 37 tetranucleotide loci using PRIMERSELECT software (Lasergene 5.1, DNASTAR Inc.). For all samples screened, a total of 10 ng of genomic DNA was amplified in 20 μL reactions containing: 20 mM Tris–HCl (pH 8.4) 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 μm primers, and 0.5 units *Taq* DNA polymerase (Gibco). Reaction mixtures were amplified using the following PCR conditions: 95 °C for 1 min 30 s, 40 cycles of (95 °C for 1 min, 52 °C for 30 s, 72 °C for 1 min) and 72 °C for 10 min (MJ Research PTC-100 96 V thermocycler). Amplification products were mixed 1 : 1 with 98% formamide loading dye, denatured for 3 min at 95 °C, and

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Table 1 Characterization of 17 microsatellite loci in the Lahontan Tui Chub (*Gila bicolor obesa*) based on seven individuals

Locus ID	GenBank accession no.	Primer sequence (5′–3′)	Repeat motif*	No. of alleles	Gel estimated allele size range (bp)	Sequenced clone size (bp)	H_O	H_E	*Gila orcutti* (*n* = 7)	*Gila bicolor snyderi* (*n* = 7)	*Gila coerulea* (*n* = 6)
Gbi-G2	AF393659	F: CTCCTCTCCCACCCACAAG	1.	6	260–320	283	0.71	0.80	—	4	2
		R: GACAGACAAACAGACAGACATACA								(300–340)	(215–235)
Gbi-G3	AF393660	F: GCACTTAGCGATGAATGGAC	$(GATA)_{23}$	7	250–310	302	0.86	0.79	5	7	0
		R: TTAGACACAAACATGGAGTGACAG							(345–400)	(270–330)	
Gbi-G10	AF393661	F: GTGTGAGGGCGGGACAGATT	$(GATA)_{10}$	9	260–310	260	0.71	0.87	—	5	0
		R: TTCATTGGGAGTCGCAGTTATTAC								(230–320)	
Gbi-G12	AF393662	F: AGCGATGCCATTTTCTTCTG	$(GATA)_{10}$	10	230–280	249	1.00	0.74	—	8	—
		R: CTGCTTCGCTGCCCTACG								(225–285)	
Gbi-G13	AF393663	F: TCAAAAGATAAACAATAGAAACC	$(GATA)_{12}$	3	210–230	237	0.43	0.57	7	3	8
		R: CTGTCAGAAATTGTCAAGTGTAAC							(200–270)	(200–225)	(220–280)
Gbi-G19	AF393664	F: CAGTCTGAGTGAGTGTGAAGC	$(GATA)_{19}$	3	280–320	297	1.00	0.62	—	5	7
		R: GATAGATTGATACATGGGTGGATA								(290–360)	(225–315)
Gbi-G27	AF393665	F: GAACTTTCATGACACGACCTAC	2.	9	160–240	275	0.86	0.85	—	6	8
		R: GAACAAAACACAGTGAAAACCA								(170–220)	(210–320)
Gbi-G34	AF393666	F: GTCTCCGGGTCTCCAACTCC	$(GATA)_{14}$	6	200–270	202	0.71	0.74	8	6	8
		R: GCTCGCCCCTGTCACCA							(210–270)	(195–245)	(200–260)
Gbi-G38	AF393667	F: CAAACAGAGGCGACCTTCA	$(GATA)_{26}$	7	260–340	317	0.80	0.82	4	6	6
		R: TTTGTTCCCACATATAAATCACTA							(370–400)	(280–380)	(370–430)
Gbi-G39	AF393668	F: GAGCGGGTGGATTTTTACTATTAT	$(GATA)_{11}$	6	185–225	199	0.86	0.83	—	8	—
		R: ATTCATTATCCGGGGTCTCAT								(180–240)	
Gbi-G79	AF393669	F: GGGGAAACACCCATACTGACT	$(GATA)_{11}$	7	190–240	226	1.00	0.83	0	8	8
		R: TTGCATTTGCATGTTACGACA								(190–240)	(210–350)
Gbi-G87	AF393670	F: TGTGGCTTTAAGTAAATGATGACC	$(GATA)_{10}$	6	180–240	179	0.86	0.68	—	5	6
		R: TCGGGTGTTATAGAAAATGTTCC								(180–215)	(200–290)
Gbi-G99	AF393671	F: CCTTCTGGATGCCCTGTGT	$(GATA)_{24}$	6	250–330	333	0.71	0.79	2	6	3
		R: GAATGGATGGATGGATGGATAGA							(280–295)	(275–330)	(280–295)
Gbi-G211	AF393672	F: ACCCCCTTTCTCTGTATCCCTCTC	$(GATA)_{17}$	6	150–190	182	1.00	0.77	—	7	—
		R: ATCCTGCTCAGTGTTGTGCTTCC								(150–220)	
Gbi-G243	AF393673	F: AACATTACTGGTCGGTCGGTCTAT	$(GATA)_{10}$	6	250–360	230	0.00	0.83	0	6	0
		R: TTCGCAAACCATTCCTCTGG								(245–360)	
Gbi-G291	AF393674	F: ACTGAGCCCTGCATAATGAGAAT	3.	4	280–320	282	0.43	0.70	—	6	—
		R: GAATGCCGAAAAAGTTTGAGAAC								(280–340)	
Gbi-G294	AF393675	F: TGTTCCCCTCATCATCATAG	$(GATA)_7$	4	200–230	209	0.57	0.46	5	3	7
		R: AGAACAATAGAACAATACACAGA							(215–265)	(200–210)	(210–255)

GenBank accession numbers, primer sequences, repeat motif, number of alleles, allele size range, clone size and observed and expected heterozygosity (H_O and H_E from GENES IN POPULATIONS version 2.2; designed by B. May and C. C. Kreuger, written in C by W. Eng and E. Paul). Cross-species amplification with the 17 microsatellite loci in *Gila orcutti*, *Gila bicolor snyderi*, and *Gila coerulea* is also shown. *n* indicates the number of individuals tested, numbers in each cell indicate the number of presumptive alleles, '—' indicates unclear amplification, and '0' indicates no amplification. The numbers in parentheses below the number of presumed alleles represent the gel estimated allele size range (bp) of the cross-species amplification products.
*1., $(GATA)_{11}TATA(GATA)_5(GGTAGATA)_3$; 2., $(GATA)_{26}(GACA)_4GATA$; 3., $(GAGA)_7(GATG)_5(GATA)_6$ … $(GATA)_7$.

then cooled on ice before running on 5% denaturing acrylamide gels at 35 W for 1 h 10 min. Products were detected by fluorescence using the agarose and ™Vista Green overlay procedure of Rodzen *et al*. (1998) and scanned with a Molecular Dynamics 595 fluorimager.

Seventeen of the 37 tetranucleotide primer pairs amplified clearly resolved PCR products in *G. bicolor obesa*, as shown in Table 1. Using identical reagent and cycling conditions, these 17 primer pairs were tested in *G. orcutti* (Arroyo chub), *G. bicolor snyderi* (Owens tui chub), and *G. coerulea* (Blue chub). Due to the high amplification success in the three other chub species, the microsatellite markers presented here have the potential to be of great use for further genetic investigations in other *Gila* species.

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