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PRIMER NOTES

Polymorphic microsatellite markers in the western mosquitofish, *Gambusia affinis*

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The western mosquitofish, *Gambusia affinis* (Poeciliidae), is native to the south-central United States. Together with the eastern mosquitofish, *G. holbrooki*, this hardy fish has been used in a diverse array of experimental studies (Meffe & Snelson 1989). Although allozyme variation in *Gambusia* is among the highest reported for vertebrates (Wooten *et al.* 1988), this variation may not be sufficient to provide a basis for detecting severe population bottlenecks (Leberg 1992; Richards & Leberg 1996). Here we present seven *G. affinis* microsatellite loci and evaluate their usefulness in related poeciliids.

We isolated *G. affinis* genomic DNA from muscle tissue samples using a CTAB (cetyltrimethylammonium bromide) protocol modified for animal tissue from Rogers & Bendich (1988). We have since supplanted this protocol with Gentra's Puregene DNA Isolation protocol with 7.5 M ammonium acetate for DNA precipitation and resuspension in water.

Five of the loci were located using a library-enrichment protocol modified from Ostrander (1992), in which a genomic library was constructed in pBluescript-KS±. The remaining two loci were located using a *G. affinis* genomic DNA library in pZErO–2.1, and recombinant clones obtained from both methods were screened by hybridization probes for $(CA)_n$ and $(GA)_n$ dinucleotide repeats. Twenty-one hybridization positives were identified for sequencing on both DNA strands. Forward and reverse sequences were aligned with the Eyeball Sequence Editor (Cabot 1991). For 12 of 21 sequences, we developed primers to amplify regions containing dinucleotide repeats using the primer design program OLIGO version 2.0 (NAR; Rychlik & Rhoads 1989). We varied annealing temperature to optimize PCR conditions (Table 1). Upon optimization of PCR conditions, 1 mM of each forward primer was γ^{32}P end-labelled with 12.5 mCi/mL isotope using T4 polynucleotide kinase. Loci were visualized and scored by autoradiography of ^{32}P-labelled PCR products on denaturing 8% polyacrylamide electrophoresed for 4–4.5 h at 1600 V. Analysis using fluorescent-labelled primers in an ABI Prism 310 Genetic Analyser (Perkin-Elmer) produced comparable results.

Loci were amplified by PCR using a Perkin-Elmer 480 thermal cycler. The cycling conditions for all loci began with a 12-min initial denaturation at 94 °C followed by 25 cycles of 40 s denaturation at 94 °C, 40 s at an optimum annealing temperature for each primer pair (Table 1), 1 min ramp to 72 °C, and 1 min extension at 72 °C, and concluded with a 10-min final extension at 72 °C.

PCR amplifications were performed in a 15-μL final reaction volume containing at least 100 ng of genomic DNA tem-

Table 1 Primer sequences (5′ to 3′) and characteristics of the seven *Gambusia affinis* microsatellite loci. Number of alleles and observed heterozygosity were calculated for 20 individuals.

Locus	Repeat motif	Primer Sequence	Annealing temp. (°C)	Expected size (bp)	No. of alleles	H_O	H_E	GenBank Accession no.
Gafμ1†	$[GA]_{11}$	TCTGTTAGTGCTCAGCTGCAA ATCAACAGCAGCCTCCTTCT	52	107 (105–107)	2	0.18	0.16	AF060919
Gafμ2	$[CA]_{17}$	CTCCAAACACACGTCCAATAATC AGTTTCCCCAGCCGTTCAT	65	147 (125–210)	21	0.71	0.80	AF060920
Gafμ3	$[GT]_{33}$	CTCAGCCGTCATTTAGTCTCAT GCACATAACATGGAAACAGTAAAC	65	262 (229–280)	19	0.75	0.93	AF060915
Gafμ4	$[CT]_{27}$	ACAACGGAGACCTGCTGGAGTGG CGCGAACCGTCCGTTATCCGTA	65	218 (171–259)	21	0.60	0.95	AF060914
Gafμ5	$[GA]_7A_2[GA]_{11}$	TGGGCCTTGTCTTGCTTT AAGCCGCGGATATTCATG	52	264 (243–291)	10	0.90	0.81	AF060916
Gafμ6	$[GA]_{10}$	ACGAAGAGAGCAGCGGAGTTTTGG CGCCGGACAGACCAGCCTCA	65	196 (178–202)	8	0.70	0.76	AF060917
Gafμ7	$[AG]_{22}$	CACAGAACAACACAGAAACTGGAGG TGCCGATGGATGTTCCTGTTAG	65	188 (162–197)	20	0.95	0.91	AF060918

†Assays with additional bands outside of the expected size range.

Table 2 Number of alleles from cross-species amplification within the family Poeciliidae using *Gambusia affinis* microsatellite primers. Amplification was attempted on *N* individuals. Numbers in parentheses are the number of individuals that amplified for each species/locus combination.

		Locus						
Species	*N*	Gafμ1	Gafμ2	Gafμ3	Gafμ4	Gafμ5	Gafμ6	Gafμ7
Gambusia holbrooki	5	2 (5) † $H = 1.00$	5 (4) $H = 0.50$	5 (5) † $H = 0.60$	5 (4) $H = 0.75$	2 (5) † $H = 0.40$	3 (5) $H = 0$	3 (5) $H = 0.40$
Poecilia latipinna	4	1 (1) † $H = 0$	–	–	1 (3) $H = 0$	1 (1) † $H = 0$	–	–
Poecilia reticulata	4	1 (4) † $H = 0$	–	1 (3) $H = 0$	1 (4) † $H = 0$	2 (2) † $H = 1.00$	–	–
Heterandria formosa	4	2 (4) † $H = 0.25$	–	4 (4) † $H = 0.50$	1 (4) $H = 0$	3 (4) † $H = 0.50$	–	2(4) $H = 0.25$

– indicates no amplification; † indicates banding outside of expected size range for locus. See Table 1 for expected ranges.

plate, 5.6 pmol forward primer and 0.6 pmol end-labelled forward primer, 6.0 pmol reverse primer, 100 μM each dNTP, 0.6 U *Amplitaq* Gold DNA polymerase (Perkin-Elmer), and PCR buffer (Perkin-Elmer Amplitaq Gold Buffer) overlaid with mineral oil.

Variation at each locus was assessed in a population of *G. affinis* from Lafayette Parish, Louisiana, USA (30°12.3'N, 92°55'W). This population had high levels of allozyme heterozygosity ($\bar{H}_O = 0.11$ for 17 loci) relative to other populations in the area (Rogowski 1997). In our initial survey of 20 individuals, we detected considerably more polymorphism for microsatellite loci than for allozyme loci.

All seven microsatellite loci were polymorphic, with observed heterozygosities ranging from 0.60 to 0.95 ($\bar{H}_O = 0.79$, Table 1). All loci exhibited patterns expected under Mendelian inheritance. Each of six offspring from each of three different females possessed one or two microsatellite alleles, one of which the offspring shared with the female parent. With the addition of three loci designed for *G. holbrooki* (Zane *et al.* 1999), these microsatellite loci provide an excellent basis for assaying genetic diversity in *Gambusia*.

We tested four additional poeciliid species using PCR conditions optimized for *G. affinis* (Table 2). For *G. holbrooki*, every locus amplified, and heterozygosities ranged from 0.50 to 1.00 ($N = 4$). The *Poecilia* species did not amplify at locus Gafμ2 or Gafμ7, and heterozygotes were rare in this genus (Table 2). *Heterandria formosa* also did not amplify at Gafμ2 and had low levels of heterozygosity across the remaining loci when compared to *G. affinis* and *G. holbrooki*. It may be possible to improve the success of amplification in other species by reoptimizing PCR conditions.

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