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TECHNICAL NOTE

Ten novel microsatellite markers for the western mosquitofish *Gambusia affinis*

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Abstract We isolated and characterized 10 microsatellite loci from the western mosquitofish, *Gambusia affinis*. Loci were screened in 30 individuals from a single location from their native range in coastal Texas. The number of alleles per locus ranged from 4 to 25, observed heterozygosity ranged from 0.40 to 0.93, and the probability of identity values from 6.0×10^{-3} to 2.7×10^{-1}, with an overall PI for all loci of 1.3×10^{-13}. These new loci provide tools for examining the genetic diversity, structure, and pedigree *G. affinis* populations.

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Gambusia affinis (Baird and Girard 1853) is a small (max SL 40–50 mm), sexually dimorphic, Poecilliid species that is native to the south central United States, which has been widely introduced for its presumed value in controlling mosquito populations (Pyke 2008; Stockwell and Henkanaththegedara in press). This species has been used as a model to examine a wide variety of conservation and evolutionary questions. Currently, there are only seven microsatellite loci developed from *G. affinis*, but two of these have low variation (Spencer et al. 1999). To increase the number of available loci we developed an additional 10 loci for this species.

We extracted total DNA from one individual *G. affinis*, using the DNeasy tissue kit protocol (Qiagen, Valencia, CA). DNA was then serially enriched twice for microsatellites using 3 probe mixes following Glenn and Schable (2005) with the changes described in Lance et al. (2010). There were two primary changes to the Glenn and Schable (2005) protocol. First, a different linker was used (SimpleX-4 Forward 5′-AAAAGCAGCAGCGGAATC and SimpleX-4 Reverse 5′- pGATTCCGCTGCTGC). Second, the enriched libraries were sequenced on a 454 using titanium chemistry following standard Roche 454 library protocols (454 Life Sciences, a Roche company, Branford CT). All methods for sequencing, microsatellite identification, primer design, and primer screening are as described in Lance et al. (2010).

Twenty-four primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals of *G. affinis*. PCR amplifications were performed in a 12.5 µl volume (10 mM Tris pH 8.4, 50 mM KCl,

Table 1 Details for 10 polymorphic microsatellite loci developed for *Gambusia affinis*

Locus	Primer sequence 5′ → 3′	Repeat motif	Size range	*N*	*k*	H_o	H_e	PI
Gaaf7	F: TCCATCCCATTATGACCACAG	$(AATC)_{31}$	148–276	30	16	0.90	0.82	5.1×10^{-2}
	R: *GCACTTAGAAATGCCTCGC							
Gaaf9	F: GGTGCAAATCCGCAGCTTG	$(ACAG)_{14}$	235–275	29	9	0.62	0.76	8.0×10^{-2}
	R: *GGGAAATACTCCTGGACTCG							
Gaaf10	F: GAACTGAACCACCCAAAGGC	$(ATCC)_{12}$	252–386	27	25	0.92	0.94	6.0×10^{-3}
	R: *TCCATCTGGAGACAGGTGTG							
Gaaf11	F: ACTCAAGGCTGCCATACTGC	$(ACAG)_{16}$	104–148	29	10	0.93	0.76	8.4×10^{-2}
	R: *GGACTTAAGAGTGCCATCTGTC							
Gaaf13	F: ACTTGGTGGCAGATTTCAGG	$(GATT)_{18}$	131–255	29	20	0.92	0.90	1.5×10^{-2}
	R: *AAGGAAACAACATGCTGGC							
Gaaf14†	F: ATCCTTGCCAGATAGAACGTC	$(GGAT)_9$	253–339	28	25	0.85	0.94	6.0×10^{-3}
	R: *TGGATCCTAACACAACCTGGG							
Gaaf15	F: TGCATGTGTGTTTGGTAAGG	$(AATG)_8$	148–166	29	7	0.79	0.75	1.0×10^{-1}
	R: *GATCCCTGTTACACTGCTGG							
Gaaf16	F: *GTAGGTCATCTTCAATCTGGG	$(ATCC)_7$	234–278	30	8	0.40	0.64	1.7×10^{-1}
	R: CTCCAGAGGCAGAATGTGTG							
Gaaf22	F: ATGCGACCTGAAACTTCTGC	$(ATC)_{19}$	249–285	30	8	0.80	0.69	1.4×10^{-1}
	R: *CCGAGGTCCTTGAGGTTTATAG							
Gaaf23	F: *TCCTCTTGGCATTGCTGAAAC	$(GAT)_8$	170–179	29	4	0.27	0.51	2.7×10^{-1}
	R: GGCTGCTCCATCCAATGTG							

The number of individuals genotyped is *N*; size range indicates the range of observed alleles in base pairs and includes the length of the CAG tag; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus

* Indicates CAG tag (5′-CAGTCGGGCGTCATCA-3′) label; † indicates significant deviations from Hardy–Weinberg expectations

25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04 μM tag labeled primer, 0.36 μM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units JumpStart Taq DNA Polymerase (Sigma), and ~20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures ranging between 65–55°C were used for all loci (Table 1). Touchdown cycling parameters consisted of 20 cycles of 96°C for 30 s, highest annealing temperature of 65°C (decreased 0.5°C per cycle) for 30 s, and 72°C for 30 s; and 20 cycles of 96°C for 30 s, 55°C for 30 s, and 72°C for 30 s. PCR products were run on an ABI-3130xl sequencer and sized with a Naurox size standard (DeWoody et al. 2004). Results were analyzed using GeneMapper version 3.7 (Applied Biosystems). Ten of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of 10 polymorphic loci in 30 specimens sampled from Clear Lake, TX (29°33′49″N, 95°04′09″W). Fish were sacrificed in a lethal dose of MS-222 (North Dakota State University IACUC #A-0902). Conditions and characteristics of the 10 loci are given in Table 1. We estimated number of alleles per locus (*k*), observed and expected heterozygosity (H_o and H_e) and probability of identity (PI) using GenAlEx v6.0 (Peakall and Smouse 2006). Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Sequential Bonferroni correction for multiple comparisons (Rice 1989) a single locus showed significant deviations from expectations under HWE and no linkage disequilibrium was detected for any of the 45 paired loci comparisons. The number of alleles per locus ranged from 4 to 25, observed heterozygosity ranged from 0.40 to 0.93, and the probability of identity values ranged from 6.0×10^{-3} to 2.7×10^{-1}, with an overall PI for all loci of 1.3×10^{-13}. In general, these new markers show higher variation than those reported by Spencer et al. (1999). They reported number of alleles per locus as 2–21 and observed heterozygosity values ranging from 0.18 to 0.95, but with 4 of the 7 loci having heterozygosity values below 0.75 (Spencer et al. 1999). The addition of 10 loci will effectively double the current battery of microsatellites for this species and increase the ability to discern genetic structure, patterns of diversity, and increase the resolution of pedigree analysis with this species.

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