

PRIMER NOTE

New microsatellite loci confirm hybrid origin, parthenogenetic inheritance, and mitotic gene conversion in the gynogenetic Amazon molly (*Poecilia formosa*)

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Abstract

We describe the first microsatellite loci for the gynogenetic Amazon molly, *Poecilia formosa*, an all-female species arisen through hybridization of the bisexual species *Poecilia mexicana* and *Poecilia latipinna*. The loci showed one to six alleles and an expected heterozygosity between zero and 0.75. As expected with parthenogenetic inheritance, most loci were either constantly homozygous (five loci) or constantly heterozygous (eight loci). For six loci, both heterozygotes and homozygotes occurred. This and the fact that some loci only showed alleles of one of the ancestral species could indicate genome homogenization through mitotic gene conversion. Our new loci conformed to the hybrid origin of Amazon molly and are also applicable to both ancestral bisexual species.

Keywords: gynogenesis, microsatellites, mitotic gene conversion, *Poecilia formosa*, *Poecilia latipinna*, *Poecilia mexicana*

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The Amazon molly, *Poecilia formosa*, is a North American freshwater poeciliid with obligate parthenogenetic reproduction. This species consists only of females and has arisen from a hybridization of two bisexual poeciliids, *Poecilia mexicana* and *Poecilia latipinna* (Schartl *et al.* 1995). Females produce offspring from unfertilized eggs in a mitotic germ line. For egg development, contact to spermatozoa of either ancestral species is necessary, which – however, – do not contribute to the genotype of the offspring (gynogenesis). Here, we present the first microsatellite primers specifically developed for the Amazon molly. We allocate the origin of each allele to one of the bisexual ancestors, *P. mexicana* or *P. latipinna*, and provide genetic variation estimates for both the gynogenetic and the bisexual species.

Genomic DNA was extracted from dorsal fin clips of 11 Amazon molly specimens ($n = 10$ from several locations in Texas/USA; $n = 1$ from Mexico), using the DNeasy

DNA Extraction Kit (QIAGEN) according to manufacturer's instructions. Additionally, we obtained genomic DNA from seven specimens of each ancestral species, *Poecilia latipinna* ($n = 6$ from Texas/USA; $n = 1$ from Florida/USA) and *P. mexicana* ($n = 7$ from four different locations in Mexico).

Genomic DNA of an Amazon molly sample was simultaneously restricted with *NheI*, *HaeII*, *RsaI*, and *AluI*. A microsatellite-enriched genomic DNA library was constructed in Bluescript plasmids and transformed into competent *Escherichia coli* (XL1-Blue MRF, Stratagene), using 5'-biotin-labelled microsatellite probes [(GA)₁₅ and (GT)₁₅; see Paulus & Tiedemann 2003 for methodical details]. Positive clones were sequenced using the BigDye v1.1 Terminator Cycle Sequencing Kit and analysed on an AB 3100 automatic sequencer (Applied Biosystems). Primers were constructed from flanking regions of microsatellite loci (Table 1). To facilitate cloning of polymerase chain reaction (PCR) products for allele-specific DNA sequencing with the TOPO TA Cloning Kit (Invitrogen), we designed primers starting on guanin (G) at the 5'-end.

About 100 ng of genomic DNA were used as template. PCR was performed in a total volume of 37.5 µL, containing 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl₂,

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Table 1 Continued

Primer name and sequence	GenBank Accession no.	Characteristics in <i>P. formosa</i>					<i>P. latipinna</i>					<i>P. mexicana</i>								
		Repeat sequence	T_m	n	No. of alleles	Allele size; inferred origin	H_E	H_O	n	No. of allele	Allele size	H_E	H_O	P	n	No. of alleles	Allele size	H_E	H_O	P
Loci homo- or heterozygous in <i>P. formosa</i>																				
<i>Pfor_GA-I26_F</i> GCTTCGTCTCTGTTTTCCTGCAA	AJ810456	(GA) ₂₂	52.6	11	4	158 (l)	0.71	0.27	7	2	152, 158	0.26	0.29	1.00	7	8	170–230	0.92	0.57	0.03
<i>Pfor_GA-I26_R</i> GACTATCTGCTTGTTCCTTT						186* 192* 194 (m)														
<i>Pfor_GA-I29B_F</i> GCTTGACTCTTATTCCTGCCTC	AJ810458	(AC) ₂₆	49.3	11	4	231 (l)	0.61	0.82	7	4	229–245	0.66	0.86	0.54	7	7	219–271	0.85	0.43	0.00
<i>Pfor_GA-I29B_R</i> GTGATGTTGATTTCTCTGTGA						259*, 261*, 263* (m)														
<i>Pfor_GA-III28_F</i> GCTGGCAGATATGAAAGCAAAG	AJ810459	(GA) ₂₇ (GT) ₇	52.6	11	6	212* (l) 240* 244* (m)	0.75	0.91	7	8	196–222	0.92	0.86	0.58	7	7	214–254	0.80	0.57	0.12
<i>Pfor_GA-III28_R</i> GCATTCCTGTTGAGGCATCTTTA						218* 220, 222 (l, m)														
<i>Pfor_GA-III29B_F</i> GTGCAGCCAACCACAACATAGA	AJ810460	(GA) ₅ AA	53.6	11	6	224, 254, 258, 260*	0.75	0.09	7	8	216–254	0.89	0.86	0.82	7	2	206–208	0.26	0.29	1.00
<i>Pfor_GA-III29B_R</i> GATCACCCAGGGAACCTTTTA		(GA) ₂ GG(GA) ₂₆				262*, 266* (l)														
<i>Pfor_GT-I13B_F</i> GACACTTGCACGTGATTTCCAA	AJ810463	(GT) ₈ (AT) ₃	52.6	11	2	252 (l) 238 (m)	0.52	0.91	7	2	250, 252	0.36	0.43	1.00	7	2	238, 240	0.54	0.43	1.00
<i>Pfor_GT-I13B_R</i> GCCACCCAAAGACAACTAAAC																				
<i>Pfor_GT-I49_F</i> GTGCCATCACTACTACAACAAG	AJ810473	(TG) ₁₄	45.5	11	3	144 (l)	0.63	0.82	7	2	138, 144	0.49	0.43	1.00	7	5	144–156	0.67	0.43	0.01
<i>Pfor_GT-I49_R</i> GTACCTTATGACTCGACAGCAG						144, 150*, 156 (m)														

T_m (°C), PCR annealing temperature; n , no. of specimens analysed; H_E , expected heterozygosity; H_O , observed heterozygosity; P , P value of Hardy–Weinberg probability test; m, inferred to originate from *P. mexicana*; l, inferred to originate from *P. latipinna*; and *, allele not detected in sample of ancestral species.

0.2 mM of each dNTP, 0.2 mM of both forward and reverse primers (one of them 5'-fluorescence-labelled), and 0.75 U *Taq* polymerase (Qbiogene). Amplifications were performed in a Biometra TGradient thermocycler according to the following reaction profile: one cycle at 94 °C for 5 min, 40 cycles at 94 °C for 30 s, the locus-specific annealing temperature (T_m in Table 1) for 1 min, 72 °C for 45 s; and a final extension at 72 °C for 10 min. Fragment size was determined on an AB 3100 automatic sequencer, using the GENESCAN 2.0 software and an internal size standard (GeneScan-500 LIZ, Applied Biosystems).

In the bisexual species, the new loci had one to eight alleles, an expected heterozygosity (H_E) from 0.00 to 0.96, and an observed heterozygosity (H_O) from 0.00 to 1.00 (Table 1). Heterozygote deficiencies (detected by the Hardy–Weinberg probability test in GENEPOP, Raymond & Rousset 1995) were rare in *P. latipinna*, but frequent in *P. mexicana*. Due to the limited sample size, we could not assess whether this was due to null alleles or geographical population structure. In *P. formosa*, the new loci had one to six alleles, an expected heterozygosity (H_E) from 0.00 to 0.75, and an observed heterozygosity (H_O) from 0.00 to 1.00 (Table 1). The hybrid origin and the subsequent parthenogenetic inheritance mode (with a lack of meiosis) cause both the maternal and the paternal ancestral alleles to remain in the hybrid line. Under the hypothesis of a single ancestral hybridization giving rise to the species *Poecilia formosa* (Schartl *et al.* 1995), we should expect that either all specimens are homozygous (i.e. received the same shared allele from both ancestors) or 'frozen' heterozygous (maintaining an ancestral heterozygous condition from the time of hybridization).

In accordance with this expectation, we found (i) five microsatellites constantly homozygous and (ii) eight loci constantly heterozygous in *P. formosa* (Table 1). At all heterozygous loci, we could identify the putative species of origin for each allele (i.e. *P. mexicana* or *P. latipinna*). At six loci, we identified both homozygous and heterozygous specimens in *P. formosa*. At locus GA-I26, three individuals (27%) were heterozygous (allele 157 from *P. latipinna*, 186 or 194 from *P. mexicana*), whereas the remaining eight were homozygous for *P. mexicana* alleles. At locus GA-I29B, nine individuals (82%) were heterozygous (allele 231 from *P. latipinna*; 259, 261, or 263 from *P. mexicana*), whereas the remaining two were homozygous for *P. latipinna* allele 231. At locus GA-III28, allele size ranges of the two ancestral species show a considerable overlap such that various allelic combinations — all but one heterozygous — appeared in *P. formosa*. At locus GA-III29B, all alleles found in *P. formosa*

apparently originate from *P. latipinna*. Only one specimen was heterozygous. At locus *GT-II3B*, all but one individual were heterozygous, exhibiting one allele of each ancestral species. A single specimen was homozygous for *P. mexicana* allele 238. At locus *GT-I49*, all but two specimens were heterozygous, exhibiting one allele of each ancestral species. A single specimen was homozygous for *P. mexicana* allele 156, another specimen was homozygous for allele 144 shared by both ancestral species.

Some alleles of *P. formosa* were not present in our samples of the two ancestral species (marked * in Table 1). Due to limited sample size (seven specimens per species), we are unable here to distinguish between the two possibilities of (i) mutation after hybridization and (ii) undetected alleles in the ancestral species. The same holds true for homozygous specimens, where we detected allele(s) of only one ancestor. However, the latter could also be a result of mitotic gene conversion, leading to the prevalence of only one ancestral allele in homozygous state (Helleday 2003). Gene conversion could explain the unexpected pattern found at six loci, where we detected both heterozygotes (with one allele from each ancestor) and homozygotes (with alleles of only one ancestor).

Apart from conforming to the hybrid origin and parthenogenetic inheritance of the Amazon molly, these first microsatellite loci specifically developed for this species contain loci with varying degree of polymorphism and hence comprise a set of molecular markers for various applications, which are also usable for the two bisexual species *P. latipinna* and *P. mexicana*.

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