

PRIMER NOTE

Microsatellites in the genus *Xiphophorus*, developed in *Xiphophorus montezumae*

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Abstract

Species of the genus *Xiphophorus* (swordtails and platies) are of great interest for the study of evolution of sexually selected traits like the sword, which is an elongation of ventral fin rays of the male caudal fin, that has evolved in several species within this genus. The detection of 10 microsatellites within the genus *Xiphophorus* will enable studies about the correlation of this trait with sexual reproductive success of males possessing swords of different lengths. These microsatellites will also be useful in determining population structure and enable paternity analysis in these species, where sperm storage is widespread.

Keywords: microsatellites, multiple paternity, Poeciliidae, sexually selected traits, sword, *Xiphophorus*

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The 22 species of platies and swordtails belong to the genus *Xiphophorus*. This genus is one of the 22 genera composing the family Poeciliidae, a widespread and diverse group of small-sized fishes that are endemic to the New World. Poeciliids have internal fertilization and bear live young (except for one species, which is a facultative livebearer), hence their common name, the livebearers. Xiphophorids are model organisms in several biological subdisciplines, such as research on sexual selection, particularly mating preference (Basolo 1990, 1991, 1995), and cancer research (Schartl *et al.* 1999). Phylogenetic studies have largely resolved evolutionary relationships among species within *Xiphophorus* (Meyer *et al.* 1994; Meyer 1997), but no genetic work at the population level has been conducted.

In this note we present 10 microsatellite loci that will aid further genetic studies of these fish and their relatives. Up to now, there is no information concerning the population structure or mating systems in *Xiphophorus*. Female choice experiments demonstrate that females spend more time in courtship behaviour with males possessing longer swords (Basolo 1995). Moreover, platy females seem to prefer heterospecific males with swords over their conspecific swordless males (Basolo 1990). Paternity analysis using microsatellites could resolve remaining questions about the fitness of swords in males. Microsatellites can also provide

information about population structure, variability in natural populations and even about mating systems. Microsatellite studies offer many opportunities for future behavioural and ecological studies of species of the genus *Xiphophorus*.

Genomic DNA from *Xiphophorus montezumae* was used to screen for microsatellites. DNA from a single fish was extracted, following a general phenol–chloroform extraction protocol and stored in ddH₂O. DNA was digested with the restriction enzyme *Sau3AI* and fragments of a size of 300–1000 bp were ligated into the bluescript vector pBS (Stratagene), and the vectors transfected into electrocompetent bacteria. Microsatellites were screened with dioxygenin-labeled di-/tri- and tetranucleotide-repeats. Plasmids from positive clones were extracted with a plasmid extraction kit (Quiagen), and the inserts were sequenced with universal primers M13F and M13R.

The primers for the amplification of *Xiphophorus* DNA were designed with the program OLIGO 4.0-s, and placed approximately 100–150 bp away from the microsatellite in an effort to standardize amplification products to a range between 200 and 400 bp. Amplification was carried out in a GeneAmp PCR System 9700 Thermocycler (ABI). The polymerase chain reaction (PCR) conditions were 3 min denaturing at 94 °C, then 35 cycles of 35 s denaturing (94 °C), 35 s primer annealing (between 46 and 52 °C (Table 1), and 75 s for elongation at 72 °C, and a final elongation of 7 min at 72 °C. Two µL of the PCR product were precipitated with 95% EtOH, washed with 70% EtOH

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Table 1 Ten microsatellites in overview

Locus name	Primer sequence (5'-3')	T_a (°C)	Repeat of cloned allele	Size in bp of cloned allele	Length variation of alleles in bp	No. of alleles	H_O	H_E
KonD3	F: AGTAAGTAATTGACAAGGAG R: TGAATTTGAATACAAGTATG	46	(GT) ₁₁	423	423	x	x	x
KonD6	F: CTTTAATACCCAATCAGTGG R: CAACTGGAGAGGAGTTGTC	53	(CA) ₇	205	205–209	3	0.133	0.131
KonD8	F: CCAAGGATATTGCTGACTCC R: TTCACTGAGCTTAAAGGCAG	51	(CA) ₇	360	360–362	2	0.267	0.248
KonD10	F: CAGGACTTAGAATTAACAGG R: AGAACCCAGTTGGACTGACAG	52	(CA) ₁₃	283	283	x	x	x
KonD15	F: CATCCAGCCTGCTTAGTGAG R: TGTTTCGTCATTAATTTGCAG	52	(CA) ₁₇	275	273–275	6	0.600	0.777
KonD21	F: TCATCTGGAGCAGGCACATG R: GCGTTTGGTTTCTACTGAC	57	(CA) ₁₅	273	265–285	10	1.000	0.784
KonD26	F: CTTCCTCCAAACCAAGAACTG R: TTGCAGACTGCTTTGTTCCTG	54	(GT) ₁₃	343	341–345	3	0.533	0.481
KonD29	F: CAGAACGATGAAACAGAATC R: TGACCATGTCTACAGAGTGG	52	(GT) ₁₂	290	290–296	4	0.600	0.476
KonT30	F: CCCAGTTTTATTATTATCAT R: GGAAGAGATTTTATTATTAT	48	(TAA) ₁₀	144	135–177	9	0.867	0.667
KonT38	F: CGACGTGTAGAAACTGAGTA R: CTCTATTCTGGTTTGACAT	49	(ATT) ₆	169	160–181	8	0.800	0.802

KonD, dinucleotide-repeats; KonT, trinucleotide-repeats. T_a , annealing temperature; H_O , observed heterozygosity; H_E , expected heterozygosity; bp, basepairs; x, no PCR amplification for *X. multilineatus* but for other species of *Xiphophorus*. GenBank accession numbers AF368425–AF368434.

Locus name	<i>X. multilineatus</i>	<i>X. cortezi</i>	<i>X. birchmanni</i>	<i>X. maculatus</i>
KonD3	variable	?	?	?
KonD6	variable	not variable	?	?
KonD8	variable	variable	variable	variable
KonD10	not variable	not variable	not variable	variable
KonD15	variable	?	?	variable
KonD21	variable	variable	variable	variable
KonD26	variable	not variable	?	not variable
KonD29	variable	not variable	?	not variable
KonT30	variable	variable	variable	variable
KonT38	variable	variable	variable	variable

Table 2 Variability of the microsatellite loci in different species of *Xiphophorus*

?, no amplification-product with PCR; not variable, monomorphic locus for that species; variable, two or more alleles at this locus.

and dried in a speed vacuum for 6 min. The DNA-pellet was redissolved in 4 μ L of a mixture of formamide-blue dextran (5:1) and 0.25 μ L of a length standard, GeneScan-500 [ROX] Size Standard (PE Applied Biosystems). The variability of the detected microsatellites was tested on an acrylamide-gel and analysed on an ABI Prism 377 DNA Sequencer. An amount of 1.8 μ L of the mixture was loaded in each lane. Data were analysed with the GENESCAN software (Perkin Elmer).

Variability was tested in several species of the genus *Xiphophorus* (Table 2). All microsatellite loci were also sequenced in at least two individuals of each species to test if the amplification products were the homologous

microsatellite loci, and if the variability is restricted to the microsatellite repeats and not to the flanking regions. Sequencing reactions followed the manufacturer's protocol (ABI) and the products were loaded on a polyacrylamide gel on the ABI Prism 377 DNA Sequencer. We analysed the sequence data with Sequencing Analysis and Sequence Navigator (ABI Biosystems).

In total, we identified 10 variable microsatellites in *X. multilineatus*, *X. cortezi*, *X. birchmanni* and *X. maculatus*, but not all were variable in all species (Table 2). Most of the tests for variability were made in *X. multilineatus*, where 15 different samples from three different locations were tested.

Eight out of 10 tested loci were variable in this species, with up to 10 different alleles per locus (Table 1).

The heterogeneity of the microsatellites varies among species. Some microsatellites that are monomorphic for *X. multilineatus* are variable in other species of *Xiphophorus* (Table 2). Because we sampled data for members of some representative species of *Xiphophorus* (see phylogenetic tree in Meyer *et al.* 1994), these primers should work in the majority of *Xiphophorus* species. These microsatellites can therefore become an important tool for future behavioural and ecological studies within the genus *Xiphophorus*.

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References

- Basolo AL (1990) Female preference predates the evolution of the sword in swordtail fish. *Science*, **250**, 808–810.
- Basolo AL (1991) Male swords and female preferences. *Science*, **253**, 1426–1427.
- Basolo AL (1995) A further examination of a pre-existing bias favouring a sword in the genus *Xiphophorus*. *Journal of Animal Behaviour*, **50**, 365–375.
- Meyer A (1997) The evolution of sexually selected traits in male swordtail fishes (*Xiphophorus*: Poeciliidae). *Heredity*, **79**, 329–337.
- Meyer A, Morrissey JM, Scharl M (1994) Recurrent origin of a sexually trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature*, **168**, 539–542.
- Scharl M, Hornung U, Gutbrod H, Volff JN, Wittbrodt J (1999) Melanoma loss-of-function mutants in *Xiphophorus* caused by Xmrk-oncogene deletion and gene disruption by a transposable element. *Genetics*, **153**, 1385–1394.