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PRIMER NOTE

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

J. SECKINGER, H. BRINKMANN and A. MEYER
*Department of Biology, University of Konstanz, Universitätsstrasse 10, LS Evolutionsbiologie, Fach M617, 78457 Konstanz, Germany*

**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*.

Correspondence: Axel Meyer. Fax: 0049 7531 88 3018; E-mail: Axel.Meyer@uni-konstanz.de

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 dd$H_2O$. 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

**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<br>R: TGAATTTGAATACAAGTATG | 46 | $(GT)_{11}$ | 423 | 423 | x | x | x |
| KonD6 | F: CTTTAATACCCAATCAGTGG<br>R: CAACTGGAAGAGGAGTTGTC | 53 | $(CA)_7$ | 205 | 205–209 | 3 | 0.133 | 0.131 |
| KonD8 | F: CCAAGGATATTGCTGACTCC<br>R: TTCACTGAGCTTAAAGGCAG | 51 | $(CA)_7$ | 360 | 360–362 | 2 | 0.267 | 0.248 |
| KonD10 | F: CAGGACTTAGAATTAACAGG<br>R: AGAACCAGTTGGACTGACAG | 52 | $(CA)_{13}$ | 283 | 283 | x | x | x |
| KonD15 | F: CATCCAGCCTGCTTAGTGAG<br>R: TGTTCGTCATTAATTTGCAG | 52 | $(CA)_{17}$ | 275 | 273–275 | 6 | 0.600 | 0.777 |
| KonD21 | F: TCATCTGGAGCAGGCACATG<br>R: GCGTTTGGTTTCCTACTGAC | 57 | $(CA)_{15}$ | 273 | 265–285 | 10 | 1.000 | 0.784 |
| KonD26 | F: CTTCTCCAACCAAAGAACTG<br>R: TTGCAGACTGCTTTGTTCTG | 54 | $(GT)_{13}$ | 343 | 341–345 | 3 | 0.533 | 0.481 |
| KonD29 | F: CAGAACGATGAAACAGAATC<br>R: TGACCATGTCTACAGAGTGG | 52 | $(GT)_{12}$ | 290 | 290–296 | 4 | 0.600 | 0.476 |
| KonT30 | F: CCCAGTTTTATTATTATCAT<br>R: GGAAGAGATTTTATTATTAT | 48 | $(TAA)_{10}$ | 144 | 135–177 | 9 | 0.867 | 0.667 |
| KonT38 | F: CGACGTGTAGAAACTGAGTA<br>R: CTCTATTCCTGGTTTGACAT | 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.

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

| Locus name | *X. multilineatus* | *X. cortezi* | *X. birchmanni* | *X. maculatus* |
|---|---|---|---|---|
| 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 |

?, 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 redisolved in 4 μL of a mixture of formamide-blue dextran (5:1) and 0.25 μL 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 μL 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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