Conservation Genetics **3:** 79–81, 2002.
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Microsatellite markers for the three-spined stickleback (*Gasterosteus aculeatus* L.) and their applicability in a freshwater and an anadromous population

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Received 2 May 2001; accepted 3 July 2001

Key words: Gasterosteus aculeatus, genetic markers, microsatellites, short tandem repeats, three-spined stickleback

The three-spined stickleback, *Gasterosteus aculeatus*, is a small freshwater fish species, which plays a prominent role as a model organism in evolutionary biology. It has a wide geographical distribution and is actually a large complex of biological species and differentiated populations (Bell and Foster 1994; Taylor and McPhail 2000). In the context of conservation genetics, the numerous fragmented and endemic populations in this species provide an excellent model system to study the effects of restricted gene flow and environmental factors on populations of different sizes (e.g. Bergstrom and Reimchen 2000). Microsatellites can be used both to estimate the extent of gene flow and the effective sizes of populations. Especially the accuracy of the latter application depends strongly on the number of loci employed. When previously described microsatellite primers are applied to new populations, a large set of loci may be necessary for the selection of suitable markers since technical problems with the amplification of microsatellite loci in genetically differentiated populations may occur due to mutations in primer regions. In this study, we describe the isolation of ten new microsatellite loci of *G. aculeatus* and their applicability to a freshwater and an anadromous population.

Microsatellites were isolated as described in Estoup et al. (1993) and Largiadèr et al. (1999). A genomic library was constructed with DNA ($\approx$10 μg) isolated from muscle tissue of a *G. aculeatus* individual from a population near Roche, Switzerland (46°26′ N, 6°55′ E). A mixture of the six probes $(TC)_{10}$, $(TG)_{10}$, $(CAC)_5CA$, $CT(CCT)_5$, $CT(ATCT)_6$, and $(TGTA)_6TG$ was used to screen for microsatellite sequences. 113 of approximately 1600 colonies contained positively hybridizing clones. Plasmid DNA of positive clones was extracted with the QIAprep Spin Miniprep Kit™ (Qiagen). Both strands of the stickleback DNA inserts were sequenced with the Thermo sequenase cycle sequencing kit™ (Amersham) and M13 primers. Primers were end-labelled with fluorescent dye (IRD800™; LI-COR) to allow for detection of sequence reaction products on an automated DNA sequencer (model 4200™; LI-COR).

DNA from muscle tissue was extracted using a phenol-chloroform method (Sambrook et al. 1989) and samples were stored in dH_2O at −20 °C until amplification. PCR-amplifications (10 μl) were carried out in a PTC100™-system (MJ Research, USA). Reactions contained about 20 ng of genomic template DNA, $MgCl_2$ (concentrations in Table 1), 0.06 mm of each dNTP, 0.25 units *Taq* polymerase (Qiagen), 1× buffer supplied with the polymerase and 2 pmol of each primer, one of which was end-labelled with a fluorescent dye (IRD800™; LI-COR). Reaction conditions were as follows: an initial denaturation step of 5 min at 95 °C, 5 cycles consisting of 30 s at 95 °C, 30 s at annealing temperature (see Table 1), and 75 s at 72 °C, 25 cycles consisting of 30 s at 94 °C, 30 s at annealing temperature, and 75 s at 72 °C, followed by a final 5 min extension at 72 °C. PCR products were

Table 1. Characteristics and PCR conditions of ten microsatellite loci of *G. aculeatus*. Repeat motifs and fragment sizes are given for the cloned allele

Locus	GenBank accession no.	Repeat motif	Primer sequences (5′→3′)	T (°C)	$MgCl_2$ (mM)	Fragment size (bp)
Gac2142PBBE	AJ311857	$(GAA)_7$	*TGC ATG GCT TAA AGA TGA GA TTG CTG AGG CTC TGA GTG TA	54	1.2	106
Gac4115PBBE	AJ311858	$(CA)_{14}$	*CCA CCG TGG TCT AGA GCT CCG CAC TCG TCA GGT GTC AGC GC	61	1.2	113
Gac4160PBBE	AJ311859	$(CA)_{14}$	*GAT CTC TAG CCA TGT TCT CC TAC AAT GTA GGC AGA TAC ACC	56	1.2	117
Gac5017PBBE	AJ311860	$(TG)_{26}$	*TGG CTC CAT ATA GTC ATC TG AGA ACA AAG ACA TCC CTC AT	60	0.8	110
Gac5161PBBE	AJ311861	$(AC)_{12}$	*CCT CCA ACT TGA ACT CAT TC TCT TTG CAA GTG ACA GGC	62	0.5	93
Gac6053PBBE	AJ311862	$(CA)_{20}$	*TCT GTC GGC CAT CTC CGT G AGC ACA TCG TCA GCA GAA TGC	58	1.2	163
Gac7010PBBE	AJ311863	$(GT)_8GCCCG(GT)_2$ $ATGTCT(GT)_{12}$	*CGA GTA AAG ACA CGG AGT AG CTG TAG GGA GGG TTG ACT	56	1.2	180
Gac7080PBBE	AJ311864	$(TG)_{12}$	*ACG TCT CAC TTC TAA CC TGT CCG GGA ATC TGT CTT CAG	55	1.2	161
Gac7148PBBE	AJ311865	$(CA)_{12}$	*CAG TCG GCA TGG TAG AGT CG GGG AAC ATT GTG CGC CTC	56	1.2	166
Gac8110PBBE	AJ311866	$(CA)_7AA(CA)_{14}$	*ACA TCA CAG CAT CTG AAG TA ACG TGA GTG GGT TTG TT	53	1.2	180

Table 2. Number of alleles, size range and heterozygosities for ten microsatellite loci of *G. aculeatus*. Data are based on 50 individuals from the Roche population and 15 individuals from the Texel population

Locus	Roche				Texel			
	N	Size range (bp)	H_O	H_E	*N*	Size range (bp)	H_O	H_E
Gac2142PBBE	2	106–108	0.30	0.46	5	106–118	0.53	0.62
Gac4115PBBE	4	113–135	0.42	0.47	14	111–147	0.87	0.94
Gac4160PBBE	2	117–125	0.40	0.32	14	109–148	0.67	0.90
Gac5017PBBE	6	86–110	0.62	0.57	12	78–112	0.85	0.90
Gac5161PBBE	2	85–93	0.02	0.02	10	93–131	0.71	0.79
Gac6053PBBE	6	161–183	0.76	0.71	12	151–217	0.60	0.92
Gac7010PBBE	5	174–180	0.64	0.66	8	170–212	0.92	0.83
Gac7080PBBE	2	161–163	0.32	0.32	7	161–197	0.60	0.71
Gac7148PBBE	2	166–168	0.02	0.02	7	154–172	0.46	0.82
Gac8110PBBE	3	180–184	0.28	0.27	5	180–190	0.67	0.71

N, Number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity

analysed on an automated DNA sequencer (model 4200^{TM}; LI-COR) and alleles were sized by loading the amplification product of the respective clone on the gels.

Variability of the loci was tested with samples from two *G. aculeatus* populations from Roche, Switzerland (46°26′ N, 6°55′ E) and the island of Texel, The Netherlands (53°03′ N, 4°48′ E). The samples from Roche comprised the same 50 individuals that were used by Largiadèr et al. (1999) for the characterization of different microsatellite loci. The samples from Texel (N = 15) were obtained from an anadromous population. The numbers of alleles per locus, fragment sizes, and observed and expected heterozygosities are listed for each population in Table 2. All loci were polymorphic in both populations but the variability was consistently higher in the Texel population. Allele numbers per locus ranged between two and six in the Roche population with observed heterozygosities between 0.02 and 0.76. Between five and 14 different alleles per locus were detected in the Texel sample and heterozygosities ranged from 0.46 to 0.92. Tests for linkage disequilibria between all pairs of loci and for departures from Hardy-Weinberg expectations were performed with the program GENEPOP (version 3.2a; Raymond and Rousset 1995). Significance levels were adjusted using the Bonferroni correction for multiple testing. No significant deviations from expectations for unlinked loci were found for either population. Heterozygote deficiencies at the loci were not significant for the Roche sample but significant deviations from the expected number of heterozygotes were found for the Texel sample at the loci Gac4160PBBE, Gac6053PBBE and Gac7148PBBE. Therefore "null" alleles may be present at these loci in the Texel population.

Acknowledgements

Thanks go to Irene Keller for laboratory assistance. We thank the Swiss National Science Foundation for financial support.

References

Bell MA, Foster SA (eds.) (1994) *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press, Oxford.

Bergstrom CA, Reimchen TE (2000) Functional implications of fluctuating asymmetry among endemic populations of *Gasterosteus aculeatus*. *Behaviour*, **137**, 1097–1112.

Estoup A, Solignac M, Harry M, Cornuet JM (1993) Characterisation of $(GT)_n$ and $(CT)_n$ microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. *Nucleic Acids Research*, **21**, 1427–1431.

Largiadèr CR, Fries V, Kobler B, Bakker TCM (1999) Isolation and characterization of microsatellite loci from the three-spined stickleback (*Gasterosteus aculeatus* L.). *Mol. Ecol.*, **8**, 342–344.

Raymond M, Rousset F (1995) GENEPOP: A population genetic software for exact tests and eumenicism. *Journal of Heredity*, **86**, 248–249.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Taylor EB and McPhail JD (2000) Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proc. R. Soc. Lond. B Biol. Sci.*, **267**, 2375–2384.