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Microsatellite loci in tench: isolation and variability in a test population

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Abstract. Because of their high variability and rapid evolution, microsatellites became increasingly important in genetic research, e.g. population structure and differentiation studies, gene mapping and parentage analysis. However, such loci have not been isolated in tench so far. Applying a PCR based method of generating microsatellite enriched DNA fragment libraries we were able to identify nine loci (*MTT-1* to *MTT-9*). The variability of these microsatellite loci was determined in 50 tench individuals originating from a wild population of Lake Döllnsee, Germany. Three loci were found to be monomorphic. The remaining six loci segregated for two to nine alleles. The observed heterozygosities at polymorphic loci were high (0.500–0.959) with only one exception: locus *MTT-8* (0.167). These polymorphic microsatellite loci showed a much higher level of genetic variability than the allozyme loci previously studied in the same individuals. Thus, they seem to be more suitable for genetic studies of tench. On the other hand, it remains to be checked in other populations if the three loci that did not show any variation in this population are generally monomorphic in this species.

Introduction

Microsatellites or simple sequence repeats (SSRs) consist of stretches of tandemly repeated nucleotide motifs of the DNA that are usually two to six base pairs (bp) long. They often express high levels of polymorphism as a result of variation in the number of these repeat units. Microsatellite loci have been estimated to have mutation rates of about 10^{-4} per generation (Weber and Wong 1993). Moreover, they appear to be frequent and randomly distributed throughout eukaryotic genomes. Because of these favourable characteristics (in particular high variability and rapid evolution), microsatellites became increasingly important in genetic research, e.g. population structure and differentiation studies, gene mapping and parentage analysis. However, such loci have not been isolated in tench so far. We identified nine loci and describe their variability in a test population.

Materials and methods

Isolation of microsatellite loci

Genomic DNA of a single tench individual caught in lake Müggelsee, Berlin, was isolated using the E.Z.N.A. Tissue DNA Kit II (Peqlab Biotechnologie) and digested by the restriction enzyme *Mbo*I (MBI-Fermentas). Size selected fragments of 400–900 bp were enriched for $(CA)_n$ repeats as described by Hammond et al. (1998) using a $(TG)_{10}$ probe labelled with biotin at the 3′ end. PCR products were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen) and plasmids from positive colonies were recovered using the E.Z.N.A. Plasmid Miniprep Kit I (Peqlab Biotechnologie). Inserts of purified plasmids were sequenced on a CEQ 8000 (Beckman Coulter) using the CEQ DTCS – Quick Start Kit. Sequence information of regions flanking the repeat motifs was used to construct locus specific primers (primer3 software).

Analysis of microsatellite variability in a test population

The genetic variability of newly identified microsatellite loci was examined in 50 tench individuals originating from a wild population of Lake Döllnsee, Germany (approx. 100 km north of Berlin). Nineteen individuals were directly caught in the lake and examined. The remaining 31 individuals were one year old progeny from five pairs of spawners also caught in the lake but transferred into a small experimental pond at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin where they reproduced spontaneously. This pond was not inhabited by any other tench. All of these 50 individuals had already been studied for their enzyme variability (Kohlmann and Kersten 1998).

Microsatellite loci were amplified by PCR. Each PCR reaction mix was composed of 1.5 µl of 10× PCR buffer with $(NH_4)_2SO_4$ (MBI-Fermentas), 1.2 µl of 25 mM $MgCl_2$, 1.2 µl of 1.25 mM dNTPs, 0.3 µl of each primer (10 pmol/µl), 3 µl genomic DNA, 0.1 µl of *Taq* DNA-polymerase (5 units/µl; MBI-Fermentas) and sterile water up to a final volume of 15 µl. The forward primer of each pair was labelled with one of the WellRed fluorescence dyes D2-PA, D3-PA, and D4-PA, respectively (Proligo). For all loci the PCR reaction amplification consisted of an initial denaturation at 95 °C for 5 min, followed by 5 cycles consisting of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and another 35 cycles consisting of denaturation at 90 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min. The final extension step at 72 °C was 7 min. Allele sizes were determined on a CEQ 8000 (Beckman Coulter) using the 400 bp internal size standard. Alleles were designated according to their size (bp). Genotypes were recorded and used as input data for the GENEPOP software package (Raymond and Rousset 1995) to calculate allele and genotype frequencies, observed and expected heterozygosities and to test for deviations from Hardy–Weinberg

equilibrium (probability test: estimation of exact *p*-values by the Markov chain method).

Results

Among sequenced clones nine were found to contain microsatellite motifs with flanking regions suitable for PCR primer construction (Table 1). The derived loci were designated as *MTT-1* to *MTT-9* (for: Microsatellite Tinca Tinca). As expected, most of the microsatellite motifs were composed of $(CA)_n$ repeats. However, loci *MTT-3* and *MTT-5* contained $(GA)_n$ and locus *MTT-7* a low number of $(CAA)_n$ repeats.

Examination of variability of these nine loci in the test population revealed that three loci (*MTT-3*, *MTT-4* and *MTT-7*) did not show any polymorphism (Table 2). The remaining six loci expressed two (*MTT-2*) to nine alleles (*MTT-9*). Observed heterozygosities of polymorphic loci were high, ranging from 0.500 at locus *MTT-5* to 0.959 at locus *MTT-9*. The only exception was locus *MTT-8* with a low value of 0.167. If all 50 tench individuals were considered, three out of the six polymorphic loci showed significant deviations from Hardy–Weinberg expectations (Table 2). However, if only the 19 directly wild caught tench were considered, only locus *MTT-1* showed significant deviation. The restriction of the Hardy–Weinberg test to these 19 individuals did not change the number of alleles at all but one locus: at *MTT-5* one allele was missing now.

Discussion

There seems to be some kind of relationship between the number of repeat units in the sequenced clone (Table 1) and the number of alleles that can be expected later on during routine population screening (Table 2). If this should be true, efforts can be reduced in further attempts to isolate microsatellites by a 'pre-selection' giving priority to those clones with high numbers of repeat units (> 10). On the other hand, it remains to be checked in other populations if the three loci that did not show any variation in this population are indeed generally monomorphic in tench.

The genetic variability of the newly identified microsatellite loci was much higher than the enzyme variability observed in the same 50 individuals: Kohlmann and Kersten (1998) examined 11 enzymatic systems representing 24 loci and found 30.4% polymorphic loci, an average number of 1.3 alleles per locus, a mean observed heterozygosity of 0.081, and a mean expected heterozygosity of 0.078.

The discrepancy in results of the two series of Hardy–Weinberg probability tests indicates that some sampling error had been introduced when adding the cultured progeny from the small number of wild caught spawners to the sample

Table 1. Tench microsatellite locus information.

Locus	GenBank acc. no.	Repeat motif	Forward primer	Reverse primer
MTT-1	DQ080084	$(CA)_{11}$	GTCCTCGCAATGCAAGAAAT	TTGGCTCATATTGGGTGTGA
MTT-2	DQ080085	$(AC)_{8}$	CTGGTCTCCTCCTTGTGCTC	TGGGTGAAGGATTGGTTGTT
MTT-3	DQ080086	$(AGAC)_{3}(AG)_{12}$	CCAGCAGAGCCCTACACTTC	AGGACGTGACCATCAACACA
MTT-4	DQ080087	$(CA)_{6}$	TTAAAACCGCCACACTTTCC	ACGTGCGGCTGTGAGATTAT
MTT-5	DQ080088	$(GA)_{4}GG(GA)_{13}$	GGGAGCCAGTTCACACTCAT	GACATGAAAACGGTGCTGTG
MTT-6	DQ080089	$(CA)_{16}$	TGTGTGAGGTGGCACAGAAT	ATGTGAGCAATGGCTGTGAG
MTT-7	DQ080090	$(CAA)_{3}CA(CAA)_{1}$	ACCTCGCCATGTATGCTTTT	GTTGACCTGTGCATGCATTT
MTT-8	DQ080091	$(CA)_{12}N_{6}(CA)_{3}(GACA)_{2}\ (CA)_{8}$	GAAATGTCCCCACAAACCAC	GACACCGCTATCACCATCAG
MTT-9	DQ080092	$(AC)_{28}$	CAATCTGGTGGAAGTGAGCA	ACGCGTCAGTGACAGAGAGA

Table 2 Variability of nine microsatellite loci in tench originating from a wild population.

Locus	Alleles		All examined fish (n = 50)			Wild caught fish only (n = 19)		
	Total number	Size range	H_O	H_E	P_{HW}	H_O	H_E	P_{HW}
MTT-1	5	167–177	0.700	0.683	0.000	0.632	0.740	0.043
MTT-2	2	236–240	0.540	0.416	0.040	0.579	0.462	0.345
MTT-3	1	160	–	–	–	–	–	–
MTT-4	1	211	–	–	–	–	–	–
MTT-5	5	207–217	0.500	0.651	0.053	0.474	0.661	0.089
MTT-6	6	160–174	0.659	0.548	0.199	0.722	0.624	0.385
MTT-7	1	213	–	–	–	–	–	–
MTT-8	3	230–236	0.167	0.173	0.106	0.118	0.169	0.091
MTT-9	9	130–178	0.959	0.844	0.020	0.889	0.859	0.542

H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HW}, exact *p*-value of Hardy–Weinberg probability test.

set. Analysing solely the wild caught tench might reflect the genetic structure of the population from Lake Döllnsee more accurately. On the other hand, enzyme data for the same 50 tench showed that the population was in Hardy–Weinberg equilibrium (Kohlmann and Kersten 1998).

To summarize, our results demonstrate that in tench microsatellite loci seem to be more suitable markers for genetic research than enzyme loci. Another, rather methodical advantage of using microsatellites is that only small amounts of tissue (about 30 mg) are needed for DNA extraction and that the individuals do not have to be sacrificed.

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