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PRIMER NOTE

Polymorphic microsatellite DNA markers from the marine gastropod *Littorina saxatilis*

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Abstract

Seven polymorphic microsatellite loci are described in the marine intertidal gastropod *Littorina saxatilis*. Preliminary data on allelic variation of these loci in a White Sea population of *L. saxatilis* are presented in order to assess their potential utility as high-resolution genetic markers for this model species.

Keywords: genetic population structure, *Littorina*, microsatellite

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A gastropod mollusc *Littorina saxatilis* is a key species in intertidal benthic communities of Atlantic rocky shores and a model subject of numerous ecological and evolutionary studies (review in Reid 1996). Low motility and ovoviviparity of this species may result in significant genetic differentiation of adjacent populations and subpopulational groups (Janson 1987). Microsatellites would be the tool of choice for investigation of such fine-scale metapopulation structure owing to their high levels of polymorphism and presumptive selective neutrality (Goldstein & Schlötterer 1999). Recently, microsatellite markers were developed for *Littorina striata* (Winnepenninckx. & Backeljau 1998) and *L. subrotundata* (Tie *et al*. 2000). However, these two species are only distantly related to *L. saxatilis* (Reid 1996), and cross-species amplification with other *Littorina* species were not successful (Winnepenninckx & Backeljau 1998). In this study, we report the primer sequences and amplification conditions for seven novel microsatellite loci in *L. saxatilis*, and provide the preliminary data on allelic variation of these loci in a White Sea population of *L. saxatilis* in order to assess their potential utility as high-resolution genetic markers for this model species.

Animals were collected in a population from the Ivanov-Navolok (66°22′ N 33°39′ E) in the Kandalaksha Bay of the White Sea. In the laboratory, snails were dissected and checked for trematode infection under the binocular microscope. The foot muscles of uninfected snails (20–50 mg) were cut into small pieces, fixed individually in 1 mL of 20% dimethyl sulfoxide and 0.25 M EDTA in saturated NaCl solution (Seutin *et al*. 1991) and stored at +4 °C for 1–3 months until DNA extraction. DNA was extracted and purified according to the protocol described in Sokolov (2000).

Partial genomic library was constructed as described in Sokolov *et al*. (2001), and the enrichment of the partial library was performed according to Kijas *et al*. (1994). Gel purified fragments from the enriched library were cloned using TOPO TA Cloning Kit for Sequencing (Invitrogen, Groningen, Netherlands). Positive colonies were picked up and plasmid inserts were sequenced manually (T7 Sequenase v.2 Sequencing Kit, Amersham Pharmacia Biotech, Freiburg, Germany). Primer pairs were designed to amplify microsatellites consisting of more than 10 repeats. Optimal temperatures for the amplification of microsatellites were chosen for each primer pair using the gradient thermocycler (TGradient, Whatman Biometra, Göttingen, Germany) and a temperature gradient from 50 to 70 °C across the thermoblock. Only pairs of primers that produced clear product of expected size were used for the subsequent study.

Allelic variation was assessed in 23–82 individuals of *L. saxatilis*. Amplification of microsatellites was performed in a final reaction volume of 15 μL containing 1.5 μL of 10× *Taq* DNA polymerase buffer, 0.5 U of the *Taq* DNA polymerase (both Life Technologies, Karlsruhe, Germany), 1.5 mM $MgCl_2$, 50 μM of each dNTP, 0.2 μM of each primer

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Table 1 Characterization of microsatellites isolated from *L. saxatilis*

Locus*	Repeat motif	Primer sequence (5′ to 3′)	T_a† (°C)	Product length, bp‡	No. of alleles	Sample size, ind.	Allele size range	H_O§	H_E¶
Lsax6CAA	CAA	CGGGTGTTTCATTTCTCAGAAG GTGTSTACGTGAGCAVTGTCATCA	64	200	16	66	173–254	0.576	0.881
Lsax20CAA	CAA	AAAGGTTAATTTGGCAAGGTTTTA CAAGTGACGCGAGTGTTCA	63	287	22	72	149–350, 500**	0.556	0.911
Lx-12	CAA	GTGTACGTGGGAGTGTGAACC ATTATCCAACATCCTAATTTCCTGAA	63	220	22	81	151–220	0.679	0.934
Lx-14	CAA	AAAAAGGGTAAAAACGAAACAGCCAT ACAAACAAGCAAACAGGCAAACAA	63	218	27	75	176–308, 569¶	0.787	0.930
Lx-18	CAA	AGCGTTACAATTTATTGCACTAATGT GGGAAGGAAACAGTATATTTGCTG	60	224	17	82	170–272	0.463	0.614
Lx-20	CA	TTAGAAAGAAGAACATTTGGGATTGT AGGAGTCAACATCATTAAGAGCGT	58	208	9	23	160–234	0.435	0.758
Lx-23	CAA	GACGGGCTGCCCTAACAAT GACCTCAAATCAGTAAGCGATGAA	65	198	22	82	153–225, 750¶	0.805	0.906

*Primer sequences are deposited in GenBank, accession numbers AF393818–AF393824.
†Annealing temperature used in PCR reaction.
‡Based on the sequence product of the cloned fragment.
§Observed heterozygosity.
¶Expected heterozygosity based on the Hardy–Weinberg population model.
**Long alleles (> 380 bp) differing by less then 20 bp were binned to a single 'allele' due to the difficulties with the assessment of their exact length.

and 20–40 ng of genomic DNA. The forward primer was end-labelled with [^{32}P]-ATP. Cycling parameters were: 5 min at 95 °C followed by 35 cycles of 40 s at 95 °C, 1 min at optimal annealing temperature (Table 1) and 35 s at 72 °C and finally, a 5-min extension step at 72 °C. The polymerase chain reaction (PCR) products were resolved on 6% polyacrilamide gels under denaturing conditions and visualized by autoradiography. Allele sizes were determined by comparison to a sequencing size standard obtained from the bacteriophage M13mp18 (Amersham Pharmacia Biotech, USA).

In general, the studied microsatellite loci were moderately to highly polymorphic with 9–27 alleles per locus (Table 1). Three of the seven studied loci (Lsax20CAA, Lx-14 and Lx-23) displayed high variation in the allele length, with the most alleles being 150–300 bp in length and a few being up to 500–750 bp (Table 1). Care must be taken during study of polymorphism in these loci, as neglection of the longest alleles may lead to an underestimation of heterozygosity levels. A considerable deficiency of heterozygotes found at some loci (Table 1) could either be due to the presence of null alleles [although no homozygotes for null alleles (i.e. failed amplifications) were observed] or to the fact that in this preliminary analysis we pooled individuals from several different subpopulations of *L. saxatilis* that may include more than one panmictic unit.

In general, data obtained so far suggest that the described microsatellite loci may serve as a useful tool in the studies of fine-scale population differentiation of *L. saxatilis*. Possibly, they can also be used in the studies of closely related species, as we were able to successfully amplify Lx-18, Lx-12 and Lx-23 loci in *L. littorea*, *L. obtusata* and *L. arcana* from the White and the Barents Seas (author's unpublished data). However, further investigation is necessary to establish the levels of allelic variation, the frequency of null-alleles and thus the potential utility of these markers in other *Littorina* species.

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