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PRIMER NOTE

Isolation and characterization of highly polymorphic microsatellite loci in the bladder campion, *Silene vulgaris* (Caryophyllaceae)

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Abstract

This study reports the isolation and characterization of seven highly polymorphic microsatellite loci in *Silene vulgaris* (Caryophyllaceae). The loci were isolated from two libraries constructed from genomic DNA enriched for CA and GA repeats. These markers yielded nine to 40 alleles per locus (mean 22.1) in a survey of 45 individuals from a single population located in the western Swiss Alps. Average observed heterozygosity ranged from 16.2 to 77.4%. These microsatellite loci should be valuable tools for studying fine-scale genetic structure.

Keywords: bladder campion, microsatellite, *Silene vulgaris*

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Silene vulgaris, the bladder campion, is a perennial weed common in Europe and Northern America. The species is found mostly in disturbed habitats such as roadsides, but it is also found on mountains in open meadows up to 2500 m. In *S. vulgaris*, as in many other Caryophyllaceae, hermaphrodite and male sterile plants coexist in the same populations (gynodioecy), and this plant has become a widely used model for studying the evolution of gynodioecy (e.g. Charlesworth & Laporte 1998; McCauley *et al*. 2000). Previous studies led with allozyme markers have investigated how population genetic structure can influence the evolution of this breeding system. Here, we describe the development of microsatellite markers which may be useful for characterizing gene exchange at a macrogeographical scale.

Based on 100 μg of nondegraded DNA extracted from a single plant with a DNeasy Plant Maxi Kit (Qiagen), two microsatellite-enriched libraries were constructed by Genetic Identification Services, Inc. (GIS, Chatsworth, CA, USA, http://www.genetic-id-services.com/; see Peacock *et al*. 2002 for a detailed protocol). Microsatellite-enriched plasmid (pUC 19) libraries were transformed into *E. coli* (strain JM 109, Invitrogen) following a heat-shock protocol (Sambrook *et al*. 1989). Transformed bacteria were spread on X-gal/IPTG/ampicillin plates (Qbiogen) and incubated overnight. About 800 bacterial clones were taken for polymerase chain reaction (PCR) sizing using heat-lysed bacterial clones as template and M13 universal primers. Bacterial clones containing inserts of between 500 and 700 bp were chosen for further analysis.

Plasmid DNA was isolated from overnight bacterial cultures of selected clones using a QIAprep miniprep kit (Qiagen) and sequenced in both directions, starting from M13 primers, with the ABI Prism Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) on an ABI 377 automated sequencer (PE Applied Biosytems).

Of the 100 sequenced clones, 26 were unique sequences containing a microsatellite of a length of at least 10 repeats and possessing suitable flanking sequences for primer design.

Twenty-six primer pairs were then designed using the software PRIMER 3 (Rozen & Skaletsky 1998). Fifteen individuals were used for a preliminary test of polymorphism following optimization of PCR conditions for each locus. Among these primer sets, five did not produce an amplified product, four were monomorphic and 10 were not scorable (i.e. gave more than two alleles or unspecific products).

DNA was extracted from dried leaves using a column-based protocol (DNeasy Plant Mini Kit, Qiagen) following to the supplier's instructions. Amplification reactions were

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Table 1 Characteristics of seven highly polymorphic loci in *Silene vulgaris*. Number of alleles (N_a), and average expected (H_E) and observed (H_O) heterozygosities, are based on a sample of 45 individuals from Mont d'Or population

Locus	Primer sequence 5′–3′	Repeat motif	T_a (°C)	*n*	Size range	N_a	H_O	H_E	GenBank accession no.
A2	GGTAAGCAACACCAACGAGG 6-FAM-GAGACGGTATCCCTCCAAGG	$(CA)_{14}TA(CA)_3$	60	35	136–156	9	0.774	0.673	AY225968
A5	HEX-GCAGGGTTGCCTAATTCGTA AATCCCACCGAGTCACAAAC	$(CA)_{21}CT(CA)_2$	60	35	150–245	35	0.71	0.937	AY225969
A11	6-FAM-GTTACAACAATGCTTGACCCG AATGGCATTTATGGCTGAGG	$(CA)_2A(CA)_{24}$	60	35	156–219	25	0.773	0.868	AY225970
B12	6-FAM-ACTCCTTCCAATCTCTAAATCTCC AGAGATGGTCGTGGGTTGG	$(CTT)_2C(CTT)_2T(CTT)_2(CATT)_5$	58	35	247–293	17	0.368	0.877	AY225971
B17	NED-CGCATGAGACTGAGTTCCAG GGACCAGAAAGATGTGAGGC	$(GA)_{22}AA(GA)_2AA(GA)_9$	60	35	112–155	16	0.162	0.818	AY225972
B29	NED-GATTGATGGATTTGTGTGAAGG CCAACCCGACTTTTCCCTAT	$(GA)_{16}$	65–55*	20 + 15	133–177	13	0.669	0.736	AY225973
G3	HEX-CGAACCAAAGACACAATCTCG TTGGGTTGGCATAGTTCCTC	$(GA)_{20}$	65–55*	20 + 15	136–223	40	0.613	0.96	AY225974
Mean						22.1	0.55	0.816	

T_a, hybridization temperature; *n* number of cycles. *Starting from 65 °C, the hybridization temperature is decreasing by 0.5 °C per cycle for the first 20 cycles, then is stabilized at 55 °C for the 15 remaining cycles.

prepared in a 10 µL volume containing approximately 5–10 ng template DNA, 1 × Q-solution (Qiagen), 1 × PCR buffer (Qiagen, containing 1.5 mM $MgCl_2$), 0.25 mM of each dNTP (Fermentas), 0.5 µM of each designed primer and 0.5 U of *Taq* polymerase (Qiagen). Amplifications were performed in a Biometra T thermal cycler (Perkin Elmer) with the following cycling conditions: 3 min at 94 °C, *n* cycles composed of 40 s denaturing at 94 °C, 50 s annealing at T_a, 40 s extension at 72 °C, plus an extra 7 min final extension at 72 °C (see Table 1 for T_a and *n*). PCR products were amplified with one primer of each primer pair end–labelled with a fluorescent dye, either 6-FAM, NED or HEX. Three to four PCR products of different loci were multiplexed together and added to a loading buffer (containing formamide and GENSCAN 350 ROX size standard; PE Applied Biosystems). After 3 min of denaturing at 93 °C, fragments were loaded on 4.75% polyacrylamide gel (Sequagel, National Diagnostic) and separated by electrophoresis for 2 h on an automated sequencer ABI 377 (Perkin Elmer). Microsatellite allele sizes were determined with GENSCAN 3.1.2 Analysis software (Perkin Elmer).

Variability of these microsatellite loci was tested on 45 individuals from a population located in Mont-d'Or in the western Swiss Alps (46°23′ N, 7°03′ E, altitude 1900 m). The number of alleles per locus (*n*), and observed (H_o) and expected (H_E) heterozygosities, were calculated with the program FSTAT (Goudet 1995; Table 1). The seven loci are highly polymorphic, with the number of alleles ranging from nine to 40 (mean 22.1); H_O ranged from 0.162 to 0.774 and H_E from 0.673 to 0.960. B12 and B17 loci showed a lower observed heterozygosity than other loci, suggesting that null alleles might be present.

The loci described here should be useful for assessing genetic structure at a fine geographical scale and, particularly, for parentage analysis.

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