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CHARACTERIZATION OF 14 MICROSATELLITE MARKERS FOR *SILENE ACAULIS* (CARYOPHYLLACEAE)¹

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- **Premise of the study:** Fifty candidate microsatellite markers, generated using 454 shotgun sequencing, were tested for the widespread arctic/alpine herb *Silene acaulis* (Caryophyllaceae).
- **Methods and Results:** Fourteen out of 50 markers resulted in polymorphic products with profiles that enabled interpretation. The numbers of alleles per locus ranged from two to six, and the expected heterozygosity per locus ranged from 0.06 to 0.68. Analysis of F₀ and F₁ samples proved that one allele was always inherited maternally. Four multiplex mixes have been developed.
- **Conclusions:** Microsatellite markers for this species will be a valuable tool to study detailed small-scale genetic patterns in an arctic/alpine herb and to relate them to demographic parameters.

Key words: Arctic/alpine; Caryophyllaceae; gynodioecious; moss campion; *Silene acaulis*.

Silene L. (Caryophyllaceae) has been recognized as an important model system for plant ecology and evolution, and has been a study system for different sexual and mating systems (Bernasconi et al., 2009). *Silene acaulis* (L.) Jacq., an arctic/alpine species, exhibits features that make it ideal as a model species (e.g., diploid, two genders, usually single individuals, almost linear size increase with age, widespread distribution). The development of molecular markers for this species is therefore of particular interest.

Silene acaulis is a long-lived perennial cushion plant, widely distributed in arctic/alpine tundra ecosystems in Europe, northern Asia, and North America (Elven et al., 2012). It commonly forms dense circular cushions with a central taproot (Jones and Richards, 1962), a feature that facilitates identification of single individuals in dense populations. Individuals of *S. acaulis* are sexually polymorphic because the species is gynodioecious; however, flowers are variable and populations are functionally dioecious or trioecious (Shykoff, 1988; Delph and Carroll, 2001). *Silene acaulis* is diploid ($2n = 24$) throughout its distribution range (Elven et al., 2012) and reproduces solely through sexual reproduction. Data from crossing experiments indicate that sex is inherited by a nuclear-cytoplasmic interaction (Delph et al., 1999). The gender proportions in natural populations are variable (Klaas and Olson, 2006), and in harsher conditions the sex ratio is typically biased toward females (Hermanutz and Innes, 1994; Alatalo and Molau, 1995). The well-defined, nearly circular growth form has been used for demographic studies

(Jones and Richards, 1962; Benedict, 1989; Morris and Doak, 1998). To combine demographic information with fine-scale genetic investigations, high-resolution microsatellite markers have been developed. We present 40 primer pairs for simple sequence repeat (SSR) regions in *S. acaulis*, 14 of which resulted in polymorphic products and were included in a multiplex PCR system. This setup has been tested on 304 individuals collected in the high arctic archipelago of Svalbard, Norway.

METHODS AND RESULTS

Fresh, frozen, and silica gel-dried leaf material collected in Svalbard, Norway, was used for DNA extraction with the QIAGEN Plant Mini Kit according to the manufacturer's protocol (QIAGEN GmbH, Hilden, Nordrhein-Westfalen, Germany). The only minor deviation to the standard protocol was that elution buffer volume was adjusted to 25 μ L or 100 μ L depending on the amount of leaf material used. The DNA amount was measured with a spectrophotometer (NanoDrop, ND-2000; Thermo Scientific, Wilmington, Delaware, USA) and adjusted to 5 ng/ μ L by adding deionized Milli-Q water (Merck Millipore, Darmstadt, Hessen, Germany).

The microsatellite regions were identified using next-generation high-throughput genome sequencing (Abdelkrim et al., 2009). All sequences were isolated by ecogenics GmbH (Zürich-Schlieren, Switzerland). Extracted DNA and plant material from one plant collected in Endalen (Svalbard, Norway; 78°11'12"N, 15°45'39"E; voucher from the breeding population deposited at Tromsø Museum [TROM], University of Tromsø, Tromsø, Norway [voucher no. TROM-V-135413]) were sent to ecogenics GmbH, and 13 μ g of genomic DNA was analyzed on a Roche 454 GS-FLX platform (Roche, Basel, Basel-Stadt, Switzerland) using a 1/16th run and the GS-FLX titanium reagents. The total 38,453 reads had an average length of 357 bp, and 106 of these reads contained a suitable microsatellite insert that was a tetra- or trinucleotide of at least five repeat units or a dinucleotide of at least nine repeat units. Primers for 50 microsatellite inserts were designed, and all of them were tested for amplification.

Out of 50 primer pairs, 10 did not result in a satisfactory amplification using another plant from Endalen (F₀). The remaining 40 primers were tested for

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TABLE 1. Characterization of 40 microsatellite loci isolated from *Silene acaulis*, of which 14 were included in a multiplex PCR approach.^{a,b}

Locus	Primer sequences (5'–3')	Repeat motif ^c	Allele size range (bp) ^d	Evaluation ^e	Mix	Fluorescent label	Forward primer (μL) ^f	GenBank accession no.
Silaca 1	F: TCTTATCATTTCCAACCTAGACGG R: TCGAACCAAGGCAACCCAAC	(CAT) ₇	105–190 (202)	NV	—	—	—	KP722109
Silaca 3	F: GCGGATCTTGCTTGTGACG R: TTCTACTAGTGCCCGCAG	(GTT) _{6,8,5,6}	236–245 (233)	P	1	6-FAM	0.24	KP722111
Silaca 4	F: GGTTGAAAAGGAAATCAAGAGCC R: GTCGCCAGTAAGATCAATCGAC	(AAT) ₉	203–224 (220)	S, NV	—	—	—	KP722112
Silaca 5	F: AAAACGCGAACATTCCGCC R: ATACGCACCATGGCCACTC	(TTA) ₈	— (207)	PA	—	—	—	KP722113
Silaca 6	F: CCGACACATCTTGACGCAC R: AGGTTTGTTCATACGTTAGGGTC	(AAT) ₈	260 (242)	NV	—	—	—	KP722114
Silaca 7	F: TGACTGGAAGTTAAGTGTGGTTC R: AGAGAGTATGGTAGGTGGGG	(TAA) ₈	205–226 (217)	P	2	6-FAM	0.24	KP722115
Silaca 8	F: CACTACTCAGAAAAGGTCAATTGTG R: GGAATCCAAGAAAGGTGCG	(TTA) ₆	215–239 (234)	P	3	NED	0.16	KP722116
Silaca 9	F: CCGAGTCGGGATGAGTCG R: ACTAACCCGAGTAACAATCTCC	(TAT) ₆	154 (183)	S	—	—	—	KP722117
Silaca 10	F: GTTGGTTCGTCGATGGGTC R: ACTCGTCTCACAAACCC	(TTA) ₇	256 (240)	S, NV	—	—	—	KP722118
Silaca 11	F: GGGGAGTATGAGGTGGAGG R: GGACCAGTCTTGTGTTGATTG	(ATT) ₆	214–231 (213)	NV	—	—	—	KP722119
Silaca 13	F: GGGGTCAATGTCATCAACATGAG R: GAAGTAAGTCCATGTCGCC	(ATC) ₆	223 (179)	S, NV	—	—	—	KP722121
Silaca 15	F: CACATTCAACTCATCCACATTCG R: AAAACGCCGTCTCCTGTTG	(TGA) ₆	187–200 (183)	NV	—	—	—	KP722123
Silaca 16	F: TCATACATTAGCAACATCTGAACG R: GGGACATGGTTGAGTCTGTG	(AT) ₁₁	176–180 (173)	S, NV	—	—	—	KP722124
Silaca 17	F: TCCTCATCTTTTATCCCATATTACCC R: AAAGGACGAGAGTAATGGTAGAG	(CTT) ₈	170–215 (165)	S	—	—	—	KP722125
Silaca 18	F: ACAAGTCGGATCAAGTGTGG R: GCTCAACAGACCGGAATGC	(AAAT) ₆	165–173 (171)	P	2	PET	0.24	KP722126
Silaca 20	F: CAGCAACACCAACGGCTAC R: CAACCGAACCAACTCC	(AAT) _{5,6}	266–269 (247)	NV	—	—	—	KP722128
Silaca 21	F: GTGTCCAGCTCTTTACTTGGC R: GATTAAACTGAATACATCAACCCC	(TTA) ₈	306–309 (296)	NV	—	—	—	KP722129
Silaca 22	F: GATTTAGGTGGCGCGTGAG R: AGCCCGTCTACTAACATCCC	(TTA) ₁₄	247 (247)	S, NV	—	—	—	KP722130
Silaca 23	F: CCAGCAACACCAGCAGAAG R: CCATGGAAACATGTGTATGGAGC	(ATG) ₆	236–242 (245)	P	3	6-FAM	0.24	KP722131
Silaca 24	F: CCTCGATCGGAGAGCAGTC R: GCACCATGCCAAGATTCCG	(GAA) ₆	216–223 (206)	S	—	—	—	KP722132
Silaca 25	F: AGCACAACACACACACAG R: TGGCGCATACCTTCATTCC	(ATT) ₈	172–184 (181)	P	1	VIC	0.16	KP722133
Silaca 26	F: TGATGATTGTAACACCCGC R: TGTGACGGTCTTTACTAGATGG	(AAT) ₇	253–263 (238)	S	—	—	—	KP722134
Silaca 28	F: TGACATCCCGGGTTTGTGAG R: CACTTAGCGTCGCATGAAAAG	(GTT) ₆	256–309 (248)	S	—	—	—	KP722136
Silaca 29	F: GCCAAAACACGAAAACCCG R: TGGTGGTCTGTGGTGGAG	(ATT) ₆	200–206 (216)	P	2	VIC	0.16	KP722137
Silaca 30	F: GAGGAGTAAGGCGAGAGGC R: TGGTTTTGTTCATGTTTTGTGCG	(GGA) ₈	198–213 (188)	S	—	—	—	KP722138
Silaca 31	F: CTAAGGCAACACGCCCTTC R: CACGCATCATTAGCCGACC	(ACA) ₇	156–167 (150)	NV	—	—	—	KP722139
Silaca 32	F: GATTCATGTTAGCCGACCCC R: TGCTGCAGTATTAGTGTGTTGTG	(TGT) ₈	144–177 (171)	P	2	NED	0.16	KP722140
Silaca 34	F: TCACCGATGGTCGTCAAGG R: AGGCTCTCAACTAGGATTCGG	(CAA) ₇	171–196 (174)	P	3	PET	0.24	KP722142
Silaca 36	F: ACCCTCCTTACGTTCTTAATTC R: ATGTAGCGGTGACGAAAGC	(TTC) ₈	165–180 (166)	P	4	VIC	0.16	KP722144
Silaca 37	F: GTGGTTGGTCAGTTCGCTG R: ACTCAAGCGGGTCAATCCC	(GTT) ₁₀	218–235 (232)	NV	—	—	—	KP722145
Silaca 38	F: CTTAGGCTTGTAAACCGGAG R: CCCATGGACGGTCTTAAGG	(AAC) ₈	144–147 (150)	P	1	NED	0.16	KP722146
Silaca 39	F: GGCTGAGGATCCCTTGCTG R: GTGTTGTCTCGTCTAATGTTTG	(ATT) ₅ /(ACT) ₉	240–243 (221)	S	—	—	—	KP722147
Silaca 40	F: ACCAGCATGCAATATGAATGGG R: AACCAACCGCTTCTCAG	(ATT) ₁₂	156–183 (168)	P	1	PET	0.24	KP722148
Silaca 41	F: TCAGGTAGTCGGTCACCCC R: TCTAAAATCGCCAGAAATTCAC	(ATT) ₁₀	256–274 (248)	P	—	—	—	KP722149

TABLE 1. Continued.

Locus	Primer sequences (5'–3')	Repeat motif ^c	Allele size range (bp) ^d	Evaluation ^e	Mix	Fluorescent label	Forward primer (μL) ^f	GenBank accession no.
Silaca 42	F: AGCAATTGGAACACATAAAATCGAC R: AAGAGGTATCAATCGCTCTCC	(AAC) ₇ / (CAA) ₈	340–400 (351)	S	—	—	—	KP722150
Silaca 43	F: GCATTGAAAAGGGGAAATGCG R: TCGGATTAGGGTACACGGC	(ATC) ₆	227–244 (225)	NV	—	—	—	KP722151
Silaca 44	F: AGTAGTTATACAGTGGTGGTGG R: TCCTCTATGAACCTCGCTGCC	(AAT) ₁₀	210–216 (225)	P	3	VIC	0.16	KP722152
Silaca 47	F: CCTCCTCCGTTACTACTACTTG R: TGAAGCCGACTCAACAACAAC	(CCT) ₇	249–266 (245)	NV	—	—	—	KP722155
Silaca 49	F: TCTCCATTTTGTCCAAGAGTCAG R: GTTGCTGAAAATGCGTTGGG	(ATC) ₉	249–260 (241)	NV	—	—	—	KP722157
Silaca 50	F: AAGACTCGGGAGAAACCAC R: CTCTTGACTCTCTACCTCCCC	(ATC) ₆	233–239 (238)	P	4	6-FAM	0.24	KP722158

Note: — = data not available.

^aEvaluation of primer pairs included in the multiplexing are based on two subpopulations (Endalen: 78°11'12"N, 15°45'39"E; Hotellneset: 78°14'57"N, 15°30'18"E).

^bAnnealing temperature was 56°C for all reactions.

^cSubscript numbers are amounts of the repeated motif; multiple numbers separated by commas signify that the motif is interrupted by other base pairs.

^dObserved size range with the size of the sequenced fragment given in parentheses.

^eAbbreviations: NV = not variable on the tested spatial scale; P = polymorphic; PA = poor amplification; S = large amount of stutter bands.

^fAmount in a 10-μL mix.

polymorphism using two additional individuals, one from Polheim (79°53'33"N, 16°01'24"E) and one from Kvarstittsletta (77°03'33"N, 15°07'38"E). Further tests were made with 27 primer pairs using two additional individuals from Polheim, one individual collected on Edgeøya (78°04'54"N, 20°48'38"E), and three individuals germinated from seeds (F₁) collected from the F₀ individual; the individual from Kvarstittsletta was dropped because of insufficient DNA quantity. For cost-effective testing of the selected primer pairs, the M13 tail approach was chosen with a 6-FAM (Integrated DNA Technologies, Coralville, Iowa, USA) fluorescent color tail to visualize and estimate the length of the amplification product on a capillary sequencer (ABI-PRISM-3100; Applied Biosystems, Foster City, California, USA) (Schuelke, 2000). All PCR reactions for the amplification of the microsatellite primers had a 10-μL volume. Single substances in the PCR mix were 1.0 μL PCR buffer (QIAGEN), 1.0 μL dNTPs (2.0 mM each, QIAGEN), 0.1 μL HotStarTaq DNA polymerase (QIAGEN), 0.2 μL 5 μM forward primer with M13 tail (biomers.net, Ulm, Baden-Württemberg, Germany), 0.6 μL 5 μM reverse primer, 0.6 μL 5 μM 6-FAM tail (biomers.net), 4.5 μL H₂O, and 2.0 μL 5 ng/μL template DNA. The PCR conditions were the same as in Vik et al. (2012), except that the annealing temperature was set to 55°C. For fragment length determination of the amplification products, the PCR product was diluted 10× before each was mixed with 0.2 μL GeneScan 500 LIZ and 8.8 μL HiDi (both Applied Biosystems). Fragment length was determined on an ABI PRISM 3100 (Applied Biosystems) at the University of Tromsø. The fragments were visually inspected using PeakScanner 1.0 (Applied Biosystems). Fourteen of the 40 markers (Tables 1 and 2) showed promising profiles and polymorphism within the eight tested plant individuals in the collection region of the Svalbard archipelago (Norway). The three included F₁ generation seedlings (EN S F₁) inherited one allele from their mother (EN M F₀) except for primer pair 3 (no profile for EN M F₀ available). Of 14 amplified regions, seven were polymorphic between siblings and 11 were polymorphic between mother and offspring (Table 3).

The 14 polymorphic markers (Table 1) were selected for further testing using a multiplex PCR set-up with 304 *S. acaulis* individuals collected in Endalen and Hotellneset (Svalbard, Norway). The M13-tailed forward primers were exchanged with primers that were labeled with the four different fluorescent colors 6-FAM, VIC, NED, and PET (Applied Biosystems). This allowed multiplexing of up to four primer pairs with a different color-labeled forward primer (Table 1). For calculating mean error rates per allele (Table 2; Pompanon et al., 2005), 20 to 24 individuals were replicated per multiplex PCR; in addition, negative controls were run through the entire procedure to monitor contamination. The fragments were scored using Geneious 7.1.3 (Biomatters Ltd., Auckland, New Zealand).

The multiplexing in PCR mix 1 and mix 4 was satisfactory. However, despite reliable amplifications in nonmultiplexed PCRs, we initially had a high number of allelic drop-outs or samples that did not amplify, especially in PCR mix 2 and mix 3 (see high error rates in Table 2). Nevertheless, reducing the multiplexing to only two or three primers (resulting in the following primer mixes: Silaca 3 and 8; Silaca 7 and 8; Silaca 23, 44, and 34; and Silaca

TABLE 2. Genetic properties of 40 microsatellite loci isolated from *Silene acaulis*.

Locus	A	N	H _o ± SD	H _e ± SD	Mean error rate per allele
Silaca 1	2	8	—	—	—
Silaca 3	4	227	0.07 ± 0.07	0.42 ± 0.22	0.043
Silaca 4	—	8	—	—	—
Silaca 5	—	6	—	—	—
Silaca 6	1	8	—	—	—
Silaca 7	6	227	0.42 ± 0.06	0.51 ± 0.06	0
Silaca 8	6	227	0.17 ± 0.07	0.42 ± 0.015	0.708
Silaca 9	1	8	—	—	—
Silaca 10	1	8	—	—	—
Silaca 11	2	3	—	—	—
Silaca 13	1	3	—	—	—
Silaca 15	2	3	—	—	—
Silaca 16	2	3	—	—	—
Silaca 17	—	8	—	—	—
Silaca 18	3	227	0.24 ± 0.13	0.31 ± 0.17	0.416
Silaca 20	2	3	—	—	—
Silaca 21	2	3	—	—	—
Silaca 22	1	8	—	—	—
Silaca 23	4	227	0.19 ± 0.08	0.39 ± 0.14	0.750
Silaca 24	—	8	—	—	—
Silaca 25	3	227	0.04 ± 0.04	0.06 ± 0.05	0.043
Silaca 26	2	8	—	—	—
Silaca 28	—	8	—	—	—
Silaca 29	2	227	0.16 ± 0.06	0.39 ± 0.09	0.458
Silaca 30	2	8	—	—	—
Silaca 31	2	3	—	—	—
Silaca 32	4	227	0.21 ± 0.09	0.22 ± 0.09	0.125
Silaca 34	6	227	0.58 ± 0.08	0.68 ± 0.04	0.750
Silaca 36	6	227	0.47 ± 0.06	0.57 ± 0.05	0.075
Silaca 37	2	8	—	—	—
Silaca 38	3	227	0.11 ± 0.05	0.15 ± 0.07	0
Silaca 39	2	3	—	—	—
Silaca 40	6	227	0.16 ± 0.05	0.21 ± 0.03	0.217
Silaca 41	3	3	—	—	—
Silaca 42	—	8	—	—	—
Silaca 43	2	3	—	—	—
Silaca 44	3	227	0.32 ± 0.08	0.33 ± 0.04	0.750
Silaca 47	2	3	—	—	—
Silaca 49	2	3	—	—	—
Silaca 50	3	227	0.24 ± 0.12	0.45 ± 0.06	0.200

Note: — = data not available; A = observed number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of individuals.

TABLE 3. Allele distribution of a female *Silene acaulis* individual (EN M F₀) and three of its seedlings (EN S F₁).

Locus	EN M F ₀	EN S 4 F ₁	EN S 5 F ₁	EN S 10 F ₁
Silaca 3	NA	259	259	259
Silaca 7	240	240	240; 243	240; 243
Silaca 8	250	250; 253	250; 253	250; 253
Silaca 18	184; 188	184; 188	184; 188	184
Silaca 23	247; 262	259; 262	259; 262	259; 262
Silaca 25	186; 199	199	199	199
Silaca 29	218; 227	218; 225	218; 225	218
Silaca 32	177; 189	189	189	189
Silaca 34	177; 189	177; 189	189; 200	189; 210
Silaca 36	184; 194	194	194; 196	184
Silaca 38	163; 166	163; 166	163; 166	163; 166
Silaca 40	178; 187	187	178; 187	178; 187
Silaca 44	232; 236	232; 236	NA	232; 236
Silaca 50	257	254; 257	254; 257	257

Note: NA = profiles not available because of poor amplification.

29, 32, and 18) improved the results. Thus, we are confident that the reason for this was not fragmented or low-quality DNA material, but competing primers in the PCR multiplex mix.

CONCLUSIONS

The arctic/alpine species *S. acaulis* has strong potential as a model species for population genetic studies, as genotyping can easily be combined with demographic parameters. To date, the microsatellites have been used for a small-scale genetic study in different size and gender cohorts on the arctic archipelago of Svalbard. The observed heterozygosity was found to be lower (0.04–0.58) than the expected heterozygosity, and no marked differences were found between genders (Svoen et al., in prep.).

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