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., 2012; 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:**

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 F_{st} and F_{i} 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.

**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 μL or 100 μL 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/μL by adding denoized 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 μg 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 (F_{0}). The remaining 40 primers were tested for...
### 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: TCTTATCATTTCCACACCTAGCCG | (CAT)\(_7\) | 105–190 (202) | NV | — | — | KP722109 |
| Silaca 3 | F: TGGAACCAAGCACCACACACCAC | (GTA)\(_{1,3,5}\) | 236–245 (233) | P | 1 | 6-FAM | 0.24 | KP722111 |
| Silaca 4 | F: GGTGAgAAGAACTTCAAGGACC | (AAT)\(_9\) | 203–224 (220) | S, NV | — | — | KP722112 |
| Silaca 5 | F: AAACGCAGACATCAGTGGACC | (TAA)\(_3\) | — | 207 | PA | — | KP722113 |
| Silaca 6 | F: CGCAACATCTGACCCAC | (AAT)\(_8\) | 260 (242) | NV | — | — | KP722114 |
| Silaca 7 | F: TGACTGGAAGTTAAGTGTGGTTC | (TAA)\(_8\) | 205–226 (217) | P | 2 | 6-FAM | 0.24 | KP722115 |
| Silaca 8 | F: CACTACTCAGAAAAGGTCATTGTC | (TTA)\(_6\) | 215–239 (234) | P | 3 | NED | 0.16 | KP722116 |
| Silaca 9 | F: AAAACGCGAACATTCCGCC | (TTA)\(_8\) | — | 207 | PA | — | KP722117 |
| Silaca 10 | F: CCGACACATCTGACCCAC | (AAT)\(_8\) | 260 (242) | NV | — | — | KP722114 |
| Silaca 11 | F: GGGAGTAGGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722119 |
| Silaca 12 | F: GGCGTGGAGAGATGAGTGGAG | (ATT)\(_6\) | 223 (179) | S, NV | — | — | KP722120 |
| Silaca 13 | F: CACGCGACATCTGACCCAC | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 14 | F: GGGATGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 15 | F: GGGTGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 16 | F: CACGCGACATCTGACCCAC | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 17 | F: CACGCGACATCTGACCCAC | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 18 | F: GGGATGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 19 | F: GGGTGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 20 | F: GGGTGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 21 | F: GGGTGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 22 | F: GGGTGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 23 | F: GGGTGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |
| Silaca 24 | F: GGGTGGAGATGTTGGAGAG | (ATT)\(_6\) | 214–231 (213) | NV | — | — | KP722120 |

a,b Indicates loci included in a multiplex PCR approach.
polymorphism using two additional individuals, one from Polheim (79°57′33″N, 16°01′24″E) and one from Kvartsittletta (77°03′33″, 15°07′38″E). Further tests were made with 27 primer pairs using two additional individuals from Polheim, one individual collected on Edgeyta (78°04′54″N, 20°48′38″E), and three individuals germinated from F1 seed from the F0 individual; the individual from Kvartsittletta 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, Uml, 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 analyses were performed 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 F0 generation seedlings (EN S F0) inherited one allele from their mother (EN M F0) and one from their father (EN M F1) except for primer pair 3 (no profile for EN M F0 available). Of 14 amplified polymorphic loci, 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 29, 32, 34, and 37) was promising (Table 2).

| Locus | Primer sequences (5′–3′) | Repeat motif† | Allele size range (bp)§ | Evaluation‡ | Fluorescent label | Forward primer (μL)¶ | GenBank accession no. |
|-------|-------------------------|---------------|-------------------------|-------------|------------------|----------------------|---------------------|
| Silaca 42 | F: AGCAATTGGAACATACATATGCAC | (AAC)6 | 340–400 (351) | S | — | — | — |
| Silaca 43 | R: AAGGGTTATCAACTGCTCTCC | (CAA)6 | 227–244 (225) | NV | — | — | — |
| Silaca 44 | F: GCAATGAAAGGGAGAAATGCG | (ATC)6 | 210–216 (225) | P | 3 | VIC | 0.16 |
| Silaca 47 | F: TCTCTTAACTGCTCCTGACC | (CCT)6 | 249–266 (245) | NV | — | — | — |
| Silaca 49 | F: TTCCTATTGGTCGAAGTACGAC | (ATC)6 | 249–260 (241) | NV | — | — | — |
| Silaca 50 | R: TGGCTTGAATATGCTTGGG | (ATC)6 | 233–239 (238) | P | 4 | 6-FAM | 0.24 |

Note: — = data not available.

†Evaluation of primer pairs included in the multiplexing are based on two subpopulations (Endalen: 78°11′12″N, 15°45′39″E; Hotellineset: 78°14′57″N, 15°30′18″E).
‡Annealing temperature was 56°C for all reactions.
§Subscript numbers are amounts of the repeated motif; multiple numbers separated by commas signify that the motif is interrupted by other base pairs.
¶Observed size range with the size of the sequenced fragment given in parentheses.
Designations: NV = not variable on the tested spatial scale; P = polymorphic; PA = poor amplification; S = large amount of stutter bands.
| Locus | A | N | Hs ± SD | He ± 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 ± 0.15 | 0.708 |
| Silaca 9 | 1 | 8 | — | — | — |
| Silaca 10 | 1 | 8 | — | — | — |
| Silaca 11 | 2 | 3 | — | — | — |
| Silaca 12 | 3 | 1 | — | — | — |
| 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 | 227 | 0.45 ± 0.06 | 0.45 ± 0.06 | 0.200 |

Note: — = data not available; A = observed number of alleles; Hs = expected heterozygosity; He = 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 F0 | EN S 4 F1 | EN S 5 F1 | EN S 10 F1 |
|--------|---------|-----------|-----------|------------|
| Silaca 3 | NA      | 259       | 259       | 259        |
| Silaca 7 | 240     | 240       | 240       | 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  | 254; 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.).

LITERATURE CITED

ABDELKRIM, J., B. ROBERTSON, J.-A. STANTON, AND N. GEMMELL. 2009. Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing. BioTechniques 46: 185–192.

ALATALO, J. M., AND U. MOLAU. 1995. Effect of altitude on the sex ratio in populations of Silene acaulis (Caryophyllaceae). Nordic Journal of Botany 15: 251–256.

BENEDICT, J. B. 1989. Use of Silene acaulis for dating: The relationship of cushion diameter to age. Arctic, Antarctic, and Alpine Research 21: 91–96.

BERNASCONI, G., J. ANTONOVICS, A. BERE, D. CHARLESWORTH, L. F. DELPH, D. FILATOV, T. GIRAUD, ET AL. 2009. Silene as a model system in ecology and evolution. Heredity 103: 5–14.

DELPH, L. F., M. F. BAILEY, AND D. L. MARR. 1999. Seed provisioning in gynodioecious Silene acaulis (Caryophyllaceae). American Journal of Botany 86: 140–144.

DELPH, L. F., AND S. B. CARROLL. 2001. Factors affecting relative seed fitness and female frequency in a gynodioecious species, Silene acaulis. Evolutionary Ecology Research 3: 487–505.

ELVEN, R., D. F. MURRAY, V. Y. RAZZHIVIN, AND B. A. YURTSEV. 2012. Annotated checklist of the panarctic flora (PAF) vascular plants [online]. Website http://nhm2.uio.no/paf/ [accessed 7 April 2015].

HERMANUTZ, L., AND D. INNES. 1994. Gender variation in Silene acaulis (Caryophyllaceae). Plant Systematics and Evolution 191: 69–81.

JONES, V., AND P. W. RICHARDS. 1962. Silene acaulis (L.) Jacq. Journal of Ecology 50: 475–487.

KLAAS, A. L., AND M. S. OLSON. 2006. Spatial distributions of cytoplasmic types and sex expression in Alaskan populations of Silene acaulis. International Journal of Plant Sciences 167: 179–189.

MORRIS, W. F., AND D. F. DOAK. 1998. Life history of the long-lived gynodioecious cushion plant Silene acaulis (Caryophyllaceae), inferred from size-based population projection matrices. American Journal of Botany 85: 784–793.

POMPANOON, F., A. BONIN, E. BELLEMAIN, AND P. TABERLET. 2005. Genotyping errors: Causes, consequences and solutions. Nature Reviews Genetics 6: 847–846.

SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233–234.

SHYKOFF, J. A. 1988. Maintenance of gynodioecy in Silene acaulis (Caryophyllaceae): Stage-specific fecundity and viability selection. American Journal of Botany 75: 844–850.

VIR, U., T. CARLSEN, P. B. EIDESEN, A. K. BRYSTING, AND H. KAUSERUD. 2012. Microsatellite markers for Bistorta vivipara (Polygonaceae). American Journal of Botany 99: e226–e229.