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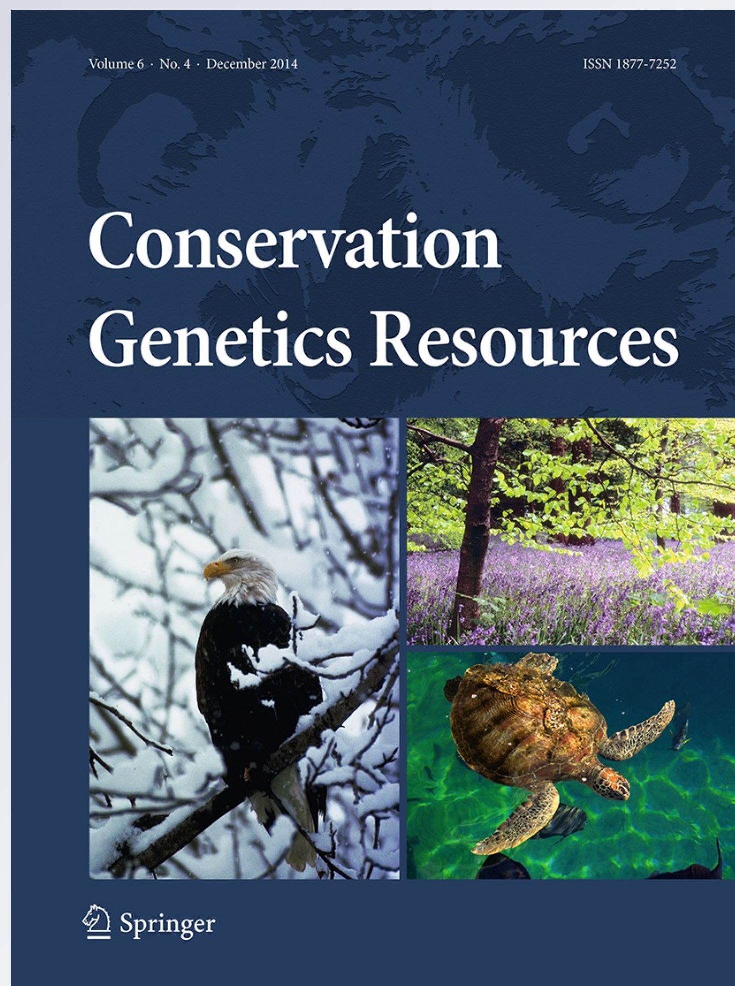

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MICROSATELLITE LETTERS

Characterization of 24 polymorphic microsatellite markers for *Silene nutans*, a gynodioecious–gynomonoecious species, and cross-species amplification in other *Silene* species

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Abstract *Silene nutans* (Caryophyllaceae) is a rare, vulnerable plant species that exhibits gynodioecy, containing both female and hermaphroditic individuals in natural populations. We developed and characterized 24 novel polymorphic microsatellite markers from next-generation sequencing to gain insights into the mating system and population-genetic structure of this species. In 36 individuals from three populations, the number of alleles and expected heterozygosity ranged from 5 to 30 and from 0.156 to 0.903 respectively. Departures from panmixia were found for 58.33 % of the loci with a mean multilocus F_{IS} estimate of 0.232, which is expected in a self-compatible species exhibiting a mixed-mating system. Cross-species amplification was examined among eight additional *Silene* species and was successful for 7–19 loci, depending on the taxa. Overall, these newly developed microsatellite markers exhibited a high level of polymorphism, which will facilitate paternity analyses and fine- and large-scale population-genetic studies.

Keywords *Silene nutans* · Gynodioecy · Microsatellites · Cross-amplification

Silene nutans (Caryophyllaceae) is a long-lived perennial rosette plant growing in dry, open grass communities of hillsides. It is described as gynomonoecious–gynodioecious, with female, gynomonoecious, and hermaphroditic individuals found in natural populations (Dufaÿ et al. 2010). This species is self-compatible, classified as rare and vulnerable, and is included in the regional Red list of endangered species from northern France. A mixed-mating system may differentially impact the reproductive success of sexual phenotypes if inbreeding depression or pollen limitation occurs (Frankham et al. 2010). To analyse the population structure and mating system of this species, we therefore isolated and characterised for polymorphism 24 microsatellite markers and tested them for cross-amplification in eight additional *Silene* species that display a wide-range of reproductive systems.

Total genomic DNA from *S. nutans* was isolated using the NucleoSpin 96 plant II kit (Macherey-Nagel, Duren, Germany) following the manufacturer's protocol and sent to GenoScreen, Lille, France (www.genoscreen.fr). By coupling multiplex microsatellite enrichment and next-generation sequencing, 1 μg was used for the development of microsatellite libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as

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Table 1 Name, primer sequence (5′–3′), repeat motif from the original sequence, annealing temperature (*Tm*), allelic size range (bp), multiplex number, dye used, the total number of allele (A_n), the mean observed (H_o) and expected (H_e) heterozygosity, the mean intra-population fixation index (F_{IS}) estimated over three populations ($N = 36$ individuals) and the accession number for 24 polymorphic microsatellite loci isolated in *S. nutans*

Locus name	Primer sequences (5′–3′)	Repeat motif	*Tm*	Allelic size range	Multiplex number	Dye	A_n	H_o	H_e	F_{IS}	Accession no.
B09	F: AAGGGCACAAAATTGAGAAGG R: CCAAAGGTGAAGCTCATATAAACC	$(AG)_9$	68/47[a]	166–187	1	PET	15	0.463	0.716	0.317***	KJ671551
H07	F: AAGCAAAACCCCTTATAAGCATC R: ACCTTTCCCCTTCCTCCTTT	$(GA)_{24}$	68/47[a]	169–221	1	NED	12	0.667	0.680	0.060	KJ671555
G01	F: CCCTACCTCATAGCAACAAGC R: CCTTCTCCTCCTTCCTTTAACC	$(GA)_9$	68/47[a]	276–331	1	VIC	30	0.810	0.910	0.104**	KJ671554
E08	F: GTTGGTCGTTGGTAGTTCACAG R: AATGCGAATCGGTCAATTTTAC	$(TGTATG)_8$	68/47[a]	224–347	1	6-FAM	13	0.352	0.441	0.267***	KJ671553
D10	F: CGGGCTAAGTTTACAGCATCA R: TGCCGTTATGCCATTCATTA	$(CA)_9$	68/47[a]	175–200	1	VIC	12	0.509	0.689	0.229*	KJ671552
SIL19	F: TTCTGAGAATTTGCACTTGAATC R: ACAAGTAACAATCTTATCCTCCATACT	$(CTT)_{11}$	55	114–156	2	6-FAM	14	0.806	0.818	0.019	KJ671563
SIL24	F: AATGGGTGTTGGAGAGGGA R: AAAGAACGGGAAGAAGGAGG	$(TC)_{12}$	55	137–239	2	VIC	30	0.903	0.920	0.011	KJ671564
SIL36	F: AGCGGAATCTACGATCTAAGGA R: TCACCTAGCAAACAAAGGACAA	$(GTT)_{17}$	55	84–141	2	NED	17	0.676	0.712	0.106	KJ671571
SIL42	F: TTCCATCTTTATTCTACCGATGTG R: TTCCCTTACTGGCAATTTGG	$(CTT)_{21}$	55	97–159	2	PET	18	0.741	0.878	0.246**	KJ671574
SIL16	F: GCCAAAACTAACAAGCAGCC R: TTTTGGGATTAAGGCTGTGA	$(CAA)_{10}$	55	121–147	3	6-FAM	12	0.474	0.715	0.223*	KJ671561
SIL31	F: TTGCCCTATTCTTTTACCCAA R: CGGACTTGTAAGGCCTGAAT	$(TTG)_{15}$	55	155–212	3	VIC	12	0.495	0.586	0.249*	KJ671569
SIL35	F: TCTGTGAATCTGTGATACTAACTGC R: ACCTCTATCCCACCATGTCA	$(AC)_{17}$	55	98–140	3	NED	18	0.778	0.868	0.067	KJ671570
SIL37	F: AAAGATGATTCATGTCAGGCG R: TGATGTTGGCCTGTACATTTC	$(TTG)_{17}$	55	166–231	3	PET	18	0.907	0.898	−0.016	KJ671572
SIL15	F: ATGTGAATCTTATTAGATGGCAGG R: TCCCATTTGAACCTTGCATT	$(GGA)_{10}$	55	194–221	4	6-FAM	9	0.282	0.576	0.423***	KJ671560
SIL08	F: ACAAGTTTGGCTCAACTCGAA R: GGTGTTGATTTGATGGGGTT	$(CTT)_8$	55	292–314	4	VIC	7	0.327	0.598	0.493***	KJ671559
SIL03	F: AAGCTTCATCAAATGAAATCGG R: GGTGGAGGAGAAGACCACAG	$(AG)_8$	55	208–214	4	NED	4	0.375	0.444	0.114	KJ671557
SIL18	F: TGCATAATTGTTCCCCAAATC R: GGGGCATATTTGCCTAAACA	$(TGT)_{11}$	55	116–168	5	FAM	12	0.351	0.569	0.419***	KJ671562

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Table 1 continued

Locus name	Primer sequences (5′–3′)	Repeat motif	*Tm*	Allelic size range	Multiplex number	Dye	A_n	H_o	H_e	F_{IS}	Accession no.
SIL29	F: ACAGCAGACCTGGAAATTAGGA	$(ATGT)_{14}$	55	125–168	5	VIC	7	0.156	0.485	0.821***	KJ671567
	R: TGTGGAATGAGATTTGTGAGG										
SIL01	F: CATAAGGCAGCAAGTTTGGC	$(AC)_8$	55	167–209	5	NED	18	0.819	0.784	−0.008	KJ671556
	R: GCCAATAAAATTCTGGTGATTAGG										
SIL05	F: CATAAGCTCATTGGGCTTC	$(TG)_8$	55	184–191	5	PET	5	0.430	0.577	0.298*	KJ671558
	R: CTAGAAATCCTGGTTCCGCC										
SIL41	F: AACAATCCAAACATTTCTTCCA	$(AAC)_{21}$	55	97–137	5	PET	10	0.741	0.762	0.001	KJ671573
	R: TGAGGCCTTGTTCTTCTTTCA										
SIL26	F: AGCATGTCTTAATCATGGAGCA	$(TTG)_{13}$	55	203–268	6	VIC	8	0.226	0.827	0.737***	KJ671565
	R: CGTGCAGAGGCTGAACTG										
SIL27	F: TGATTGCTTTGTTTCCACCA	$(AAC)_{13}$	55	115–174	6	FAM	14	0.580	0.644	0.122	KJ671566
	R: CAAATTGTATACTACTGTCCATCTCCA										
SIL30	F: TTTTGGGTATTTTAAGAGTTAATGAGT	$(TGT)_{15}$	55	136–253	6	NED	8	0.222	0.346	0.453*	KJ671568
	R: TCAACACTTTCTCTTTTATCATCA										

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] Touchdown PCR

described in Malausa et al. (2011). Briefly, total DNA was mechanically fragmented and enriched for TG, TC, AAC, AAG, AGG, ACG, ACAT and ACTC repeat motifs. Enriched fragments were subsequently amplified. PCR products were purified, quantified and GsFLX librairies were then carried out following manufacturer's protocols and sequenced on a GsFLX PTP. Of 74,387 randomly fragmented sequences, 438 sequences likely to contain suitable markers including microsatellite motif longer than five repeats were then returned.

Forward primers of the selected loci were labelled with 6-FAM, PET, NED, or VIC fluorescent dye (Applied biosystem). PCR reactions were performed separately for each locus in 10 µl volume containing 20 ng of genomic DNA, 1× multiplex PCR master mix (QIAGEN Hilden, Germany), and 0.1 µM of forward and reverse primer. The PCR cycling program had an initial denaturation of 95 °C for 15 min; 32 cycles of 94 °C for 30 s, annealing temperature (see Table 1) for 1 min, and 72 °C for 1 min 15 s; and a final extension at 60 °C for 30 min. For five markers (B09, H07, G01, E08 and D10 loci, see Table 1), we used a touchdown PCR-cycling program according to the conditions described in Godé et al. (2012). The PCR amplicons were subsequently electrophoresed and sized using an ABI PRISM 3130 Sequencer (Applied Biosystems) and the software GeneMapper version 4.0, respectively.

We tested the polymorphism of isolated suitable microsatellite markers on individuals coming from three populations of *S. nutans* located in Germany (N 49.228–E 7.016, $n = 9$ individuals; N 48.971–E 12.018, $n = 9$ individuals, respectively) and southern France (N 42.802–E 0.453, $n = 18$ individuals). Overall, 24 markers were polymorphic, and had easily readable chromatograms with no stutter peaks. Primer pairs were successfully combined into six multiplex ranging from three to five markers (Table 1). Basic parameters of genetic diversity were estimated using FSTAT, version 2.9.3 (Goudet 1995). The number of alleles varied from 5 to 30 among loci (mean = 5.458), for a total of 323 alleles observed. The mean observed (*Ho*) and mean expected heterozygosity (*He*) values ranged from 0.156 to 0.903 and from 0.346 to 0.920, respectively. As expected for a species thought to exhibit a mixed-mating system, the fixation index F_{IS} was significant for 14 out 24 markers, with estimates ranging from −0.016 to 0.821 for a mean multilocus value of 0.232 (±0.050). Out of 276 comparisons and after Bonferroni correction, no linkage disequilibrium was observed for any pairs of loci. Cross-species amplification was further examined among eight *Silene* species and was successful at 7–19 loci depending on the taxa (Table 2).

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Table 2 Results of cross-species amplification of 24 microsatellite loci in eight additional *Silene* species: no amplification (–); successful amplification (+) together with the observed number of alleles

Locus name	Species	*Silene acaulis* (4)	*Silene italica* (16)	*Silene latifolia* (8)	*Silene noctiflora* (1)	*Silene otites* (6)	*Silene paradoxa* (8)	*Silene vulgaris* (8)	*Silene scouleri* (2)
B09		+ (6)	–	–	–	+ (4)	+ (5)	–	–
H07		+ (2)	–	–	+ (1)	+ (1)	+ (2)	+ (3)	–
G01		+ (6)	+ (10)	–	–	+ (6)	+ (1)	–	–
E08		–	+ (3)	–	–	–	+ (2)	–	–
D10		+ (2)	+ (9)	+ (1)	–	–	+ (1)	–	+ (1)
SIL19		+ (6)	+ (11)	–	–	+ (1)	+ (2)	–	–
SIL24		+ (2)	+ (5)	+ (6)	+ (1)	+ (9)	–	+ (3)	–
SIL36		+ (3)	+ (1)	–	–	+ (1)	+ (1)	–	–
SIL42		+ (1)	–	–	+ (1)	–	–	–	–
SIL16		+ (2)	+ (8)	+ (2)	+ (2)	+ (6)	+ (4)	+ (2)	+ (2)
SIL31		+ (2)	+ (3)	+ (1)	+ (1)	+ (2)	+ (3)	+ (2)	+ (2)
SIL35		+ (2)	+ (14)	–	–	+ (1)	+ (5)	+ (1)	+ (1)
SIL37		+ (7)	+ (2)	+ (2)	+ (2)	+ (1)	+ (1)	+ (1)	+ (2)
SIL15		–	–	–	–	–	–	–	–
SIL08		+ (1)	+ (4)	+ (1)	–	+ (2)	+ (3)	–	–
SIL03		+ (2)	+ (10)	+ (11)	+ (1)	+ (8)	+ (1)	+ (4)	+ (1)
SIL18		+ (1)	–	–	–	–	–	–	–
SIL29		–	–	–	–	–	–	+ (6)	–
SIL01		+ (2)	+ (1)	–	+ (1)	+ (1)	+ (1)	+ (2)	+ (1)
SIL05		+ (3)	+ (3)	–	+ (1)	–	–	+ (3)	–
SIL41		+ (3)	+ (1)	–	–	+ (5)	+ (6)	–	–
SIL26		–	+ (2)	–	–	–	–	–	–
SIL27		+ (1)	+ (3)	–	–	–	+ (1)	–	–
SIL30		–	+ (1)	–	–	+ (2)	+ (6)	–	–

The number of tested individuals is indicated next to the species' name

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