Development of Highly Variable Microsatellite Markers for the Tetraploid Silene stellata (Caryophyllaceae)

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Primer Note

Development of highly variable microsatellite markers for the tetraploid *Silene stellata* (Caryophyllaceae)[1]

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- *Premise of the study:* We designed and tested microsatellite markers for the North American native species *Silene stellata* (Caryophyllaceae) to investigate its population genetic structure and identify selection on floral design through male reproductive success.
- *Methods and Results:* A total of 153 candidate microsatellite loci were isolated based on next-generation sequencing. We identified 18 polymorphic microsatellite loci in three populations of *S. stellata*, with di- or trinucleotide repeats. Genotyping results showed the number of alleles per locus ranged from six to 45 and expected heterozygosity ranged from 0.511 to 0.951. Five of these loci were successfully amplified in *S. virginica* and *S. caroliniana* and were also polymorphic.
- *Conclusions:* The microsatellite markers reported here provide a valuable tool for paternity analysis in *S. stellata.* They will also be useful for investigating the population genetic structures of *S. stellata* and related species.

Key words: Caryophyllaceae; microsatellites; plant–animal interaction; polyploidy; *Silene stellata*.

There are nearly 700 species in the genus *Silene* L. (Caryophyllaceae). *Silene* species exhibit diverse pollination specialization as manifested by their floral diversity (Kephart et al., 2006). Approximately one third of all *Silene* species in the Old and New World exhibit nocturnal pollination syndromes and usually form close interactions with noctuid moths from the genus *Hadena* (Noctuidae) (Kephart et al., 2006). *Silene stellata* (L.) W. T. Aiton is an infrequent native perennial herb that is distributed throughout the eastern part of the United States. The flowers are pollinated by *Hadena ectypa* as well as by a number of generalist nocturnal moths (Reynolds et al., 2009; Kula et al., 2013). In addition to the positive effect of pollination, oviposition by female *H. ectypa* inside the calyx results in strong negative effects through larval predation on the reproductive tissues of *S. stellata* (Kula et al., 2013). Depending on the amount of pollination service provided by the generalist moths, the net outcome of the *Silene–Hadena* interaction can range from mutualism to parasitism (Reynolds et al., 2012), making it a valuable system for understanding the evolutionary dynamics of interspecific interactions (Kephart et al., 2006; Bernasconi et al., 2009).

Ecological and evolutionary studies utilizing genetic markers of North American *Silene* species are relatively few compared with the European species (Moyle, 2006), potentially due to technical complications caused by the prevalent polyploidy: most North American *Silene* species have been shown to be polyploids, including tetra-, hexa-, and octoploids, with tetraploidy being the most common ploidy level (Popp and Oxelman, 2007). Prior to this study, we tested nine microsatellite markers developed for *S. latifolia* Poir. (Magalhaes et al., 2011) in 20 individuals of *S. stellata* and did not identify any polymorphic loci. We report the development of 18 highly variable microsatellite markers for the tetraploid *S. stellata.* These markers are being used to quantify the population genetic structure of *S. stellata*. We will also use these markers to assess individual siring abilities through paternity assignment to investigate selection on floral design through the male function of the hermaphroditic *S. stellata.* We also tested transferability of these markers to two closely related tetraploid *Silene* species: the specialist hummingbird-pollinated *S. virginica* L. and the primarily *Bombus*- and hawk moth–pollinated *S. caroliniana* Walter (Reynolds et al., 2009).

METHODS AND RESULTS

Genomic DNA was extracted from fresh leaf tissue of a *S. stellata* individual collected from a natural population near Mountain Lake Biological Station in Giles County, Virginia, USA (37.348296°N, 80.544301°W, elevation ca. 1100–1300 m; Appendix 1), using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) following the manufacturer's protocol. DNA libraries were prepared using the KAPA Library Preparation Kit version 2.13 (KR0453; Kapa Biosystems, Wilmington, Massachusetts, USA) following the manufacturer's protocol. Libraries were quantified with a Qubit Fluorometric Quantitation instrument (Life Technologies, Carlsbad, California, USA) and then sequenced using an Illumina MiSeq version 3 kit (Illumina, San Diego, California, USA) to produce paired-end reads of ≤301 bases. A total of 8,557,438 reads were imported and paired by name in Geneious 7.0.6 (Biomatters, Auckland,

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TABLE 1. Characteristics of 18 microsatellite loci developed in *Silene stellata*.[a]

Locus	Primer sequences (5′–3′)	Fluorescent dye	Repeat motif	Allele size range (bp)	T_a (°C)	GenBank accession no.
SS1010	F: ACACAGCGGATGGTGTCG	HEX	$(AG)_{26}$	174–248	60	KX712873
	R: CTCTCTTTCAATCCTGGCCC					
SS62979	F: CTTTGGGTTGTGCTCAGAATAG	HEX	$(AG)_8$	155–239	55	KX712874
	R: TTGTGTCTGCCATCCTTGC					
SS63719	F: GTACGCGTCGCTTTGTTAAC	FAM	$(AT)_8$	135–154	58	KX712875
	R: TGATACTGCTCACCGGATGC					
SS64450	F: CAAGTGGTATCAGAGCCAGG	FAM	$(AT)_9$	220–254	60	KX712876
	R: ACCTGTAACTTGTTGGGATGC					
SS64692	F: TGGTACCCTCAAGACTAGGC	HEX	$(AAT)_{14}$	148–265	60	KX712877
	R: CCTCCCTGAACGCCTCTTC					
SS68587	F: TCAGGTCATAAGGCTGTGGG	HEX	$(AAC)_8$	191–281	55	KX712878
	R: CTGGGTGGTTGATGAAATTTGG					
SS71250	F: TAGACGGTTGCGGAATGGG	FAM	$(AG)_{14}$	125–203	58	KX712879
	R: TTTCTCACAACCTCACGCTC					
SS73434	F: GAGAAGGGAAGAGACCGGTG	FAM	$(AG)_{10}$	184–210	60	KX712880
	R: TGACCCGAGACCTACTGATTG					
SS78777	F: ACGTACAAGTAGCATAGGTGGG	HEX	$(AC)_8$	156–248	60	KX712881
	R: TCGAGGTCATAGTAGGCCAC					
SS85007	F: CAGGGTCAACATCAACAGGC	FAM	$(AAT)_{41}$	149–356	60	KX712882
	R: AGATTGTCTCTCGGCTCCC					
SS89178	F: TCACTACCCACCCTTTACGG	FAM	$(AG)_8$	254–374	55	KX712883
	R: ATCCCTAGATGCCCGCAC					
SS100056	F: GCATCTTGTGTTCCCGAGC	FAM	$(AAG)_{37}$	91–205	60	KX712884
	R: GGGCTATCTTGATCTCCACTC					
SS115569	F: CCCAGCTATCCCAACGAATC	HEX	$(AAC)_{15}$	377–399	55	KX712885
	R: ACCCAACCCAACGAATCAAAC					
SS122722	F: ACACAATTGTTTCTGACTTGCG	FAM	$(AAT)_{15}$	176–287	60	KX712886
	R: ATTGTATCTCGGCTGCCCAC					
SS129528	F: AGTGACGGATACCATGGAGC	HEX	$(AAG)_{31}$	96–226	60	KX712887
	R: CATCCAATCCGCCTTTCCTC					
SS131968	F: ACCCAACATACATCAACCTCTC	FAM	$(AAT)_9$	154–205	55	KX712888
	R: GAGCGGTTGAGAATGGTGTG					
SS131990	F: TCAGCTACCCACCAATTTCTTG	HEX	$(AT)_{19}$	186–282	55	KX712889
	R: CCCACCACTTAATACGTACACC					
SS141347	F: GGGAAGAAATGGAGGTGCTC	HEX	$(ACC)_{16}$	132–192	60	KX712890
	R: GAGAGATCAGACCGAGGCTG					

Note: T_a = annealing temperature.
[a]All values are based on 95 samples representing three populations in Virginia.

New Zealand). A de novo assembly was performed on the first 1,000,000 sequences for which both reads of any pair were ≥200 bases.

A total of 99,506 consensus sequences between 200 and 400 bp were extracted and screened for potential microsatellite loci using MSATCOMMANDER 1.0.8 beta (Faircloth, 2008) with default settings. Primers were designed for sequences with perfect di-, tri-, and tetranucleotide repeats in Primer3 software (Rozen and Skaletsky, 1999). We designed primers for 153 out of a total of 946 loci identified as containing microsatellites. One hundred fourteen primer pairs were first tested with seven randomly selected *S. stellata* samples for amplification success and polymorphism. For each primer pair, we modified the forward primer by attaching a CAG tag (5′-CAGTCGGGCGTCATCA-3′) preceding the 5′ end to enable the cost-efficient fluorescent labeling system of PCR products described by Boutin-Ganache et al. (2001) and Glenn (2001). Ten microliter PCR reactions were performed using the QIAGEN Type-it Microsatellite PCR Kit. Each reaction contained the following components: ~10 ng of genomic DNA, 5 µL of the 2× Multiplex PCR Master Mix, 0.02 µM of the modified forward primer, 0.2 µM of reverse primer, and 0.2 µM of the fluorescently labeled CATG primer (5′-6FAM, 5′-HEX). A touchdown PCR protocol was used to test all primer pairs: 5 min of denaturing at 95°C; five cycles of 95°C for 30 s, 60°C for 1.5 min, and 72°C for 30 s; followed by 28 cycles of 95°C for 30 s, 55°C for 1.5 min, and 72°C for 30 s; and a final extension at 60°C for 30 min.

Of the 114 primer pairs tested, 50 produced bands consistently on an agarose gel. Amplicons of these primers were analyzed using an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA), then visualized and scored in Geneious 7.0.6 (Biomatters). Eighteen loci showed clear peak patterns and were polymorphic (Table 1). These 18 polymorphic loci were further characterized using a total of 95 *S. stellata* individuals collected from three local populations near Mountain Lake Biological Station (Meadow, Woodland, and Windrock) within 8 km of one another. To investigate marker transferability, these 18 loci were also tested on six individuals from one population each of *S. virginica* from Newport, Virginia, and *S. caroliniana* from Potomac, Maryland (Appendix 1).

We report the following parameters for the three populations of *S. stellata*: sample size, number of alleles, number of private alleles, observed heterozygosity, and expected heterozygosity. The parameters were estimated using GenoDive version 2.0b27 (Meirmans and Van Tienderen, 2004) while correcting for unknown dosage of alleles for partial heterozygotes. Across the three populations, the number of alleles ranged from six to 45, and expected heterozygosity ranged from 0.511 to 0.951 (Table 2).

Of the 18 loci tested in *S. virginica* and *S. caroliniana*, 10 loci were successfully amplified in both species. Genotyping results showed two loci were monomorphic in both species, one locus was polymorphic in *S. virginica* but monomorphic in *S. caroliniana*, five loci were polymorphic in both species, and two loci showed multiple bands (Table 3). Vouchers for the *Silene* species were deposited at the Norton-Brown Herbarium (University of Maryland, College Park, Maryland, USA; Appendix 1).

CONCLUSIONS

We developed 18 novel microsatellite loci for *S. stellata*. These loci showed high variability in *S. stellata* and therefore are suitable for future paternity analysis. Five of these markers are polymorphic in the related *S. virginica* and *S. caroliniana.* These microsatellites will also be useful for studying the

Table 2. Genetic diversity of the 18 polymorphic microsatellites of *Silene stellata*.[a]

	Total (n = 95)			Meadow (n = 55)				Windrock (n = 20)				Woodland (n = 20)			
Locus	A	H_o	H_e	A	A_p	H_o	H_e	A	A_p	H_o	H_e	A	A_p	H_o	H_e
SS1010	31	0.847	0.894	28	10	0.824	0.903	17	0	0.840	0.912	19	2	0.886	0.876
SS62979	36	0.828	0.913	28	12	0.664	0.925	17	4	0.823	0.918	15	4	0.996	0.894
SS63719	18	0.468	0.877	14	2	0.527	0.858	12	2	0.364	0.891	12	2	0.512	0.885
SS64450	15	0.486	0.712	10	4	0.393	0.626	7	2	0.474	0.724	9	3	0.591	0.782
SS64692	38	0.707	0.906	29	11	0.658	0.909	17	4	0.712	0.879	19	4	0.737	0.915
SS68587	27	0.866	0.932	25	5	0.883	0.931	17	1	0.835	0.932	16	1	0.879	0.933
SS71250	26	0.999	0.918	24	4	0.999	0.921	21	1	0.989	0.912	18	1	1.010	0.922
SS73434	13	0.210	0.875	10	2	0.305	0.890	7	2	0.154	0.842	9	0	0.172	0.889
SS78777	34	0.892	0.919	33	12	0.975	0.931	17	0	0.770	0.911	17	1	0.933	0.915
SS85007	45	0.621	0.921	38	17	0.716	0.922	18	3	0.585	0.926	20	3	0.555	0.902
SS89178	37	0.826	0.887	26	9	0.903	0.866	19	5	0.677	0.866	18	3	0.931	0.951
SS100056	35	0.821	0.951	33	8	0.799	0.961	22	1	0.883	0.943	21	1	0.781	0.948
SS115569	6	0.394	0.511	6	4	0.458	0.551	2	0	0.397	0.538	2	0	0.328	0.442
SS122722	38	0.733	0.944	31	9	0.807	0.957	18	4	0.547	0.916	19	2	0.856	0.959
SS129528	35	0.896	0.937	29	7	0.825	0.924	20	3	0.982	0.933	23	2	0.883	0.954
SS131968	18	0.779	0.761	17	3	0.699	0.730	14	1	0.760	0.704	11	0	0.869	0.844
SS131990	30	0.440	0.939	27	8	0.458	0.937	15	0	0.407	0.943	15	3	0.455	0.937
SS141347	17	0.993	0.891	16	2	0.998	0.884	15	0	0.989	0.895	14	1	0.990	0.891

Note: A = number of alleles; A_p = number of private alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals sampled.

[a]Locality and voucher information are provided in Appendix 1.

population genetics of *S. stellata* and related North American species in this genus.

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Table 3. Genetic diversity of six microsatellite loci developed in *Silene stellata* in two related native *Silene* species.[a]

	S. virginica (n = 6)				*S. caroliniana* (n = 6)			
Locus	Allele size range (bp)	A	H_o	H_e	Allele size range (bp)	A	H_o	H_e
SS64692	154–196	7	1	0.94	151–214	8	1	0.861
SS68587	188–191	2	0.5	0.369	161–191	5	0.667	0.614
SS71250	113–129	5	0.833	0.754	117–165	6	1	0.662
SS78777	198–254	11	0.833	0.969	184–244	12	1	0.939
SS131968[b]	—	—	—	—	157–169	3	0.333	0.489
SS141347	144–180	9	0.5	0.867	147–168	7	1	0.826

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals sampled.

[a]Locality and voucher information are provided in Appendix 1.

[b]Locus SS131968 was monomorphic in *S. virginica*.

Appendix 1. Geographic location and voucher information of *Silene* populations used in this study.

Species	Location	Population	Geographic coordinates	Voucher no.[a]
S. stellata (L.) W. T. Aiton[b]	Newport, VA, USA	Meadow	37.348296°N, 80.544301°W	MARY1012978
S. stellata	Newport, VA, USA	Meadow	37.348296°N, 80.544301°W	MARY1012979
S. stellata	Newport, VA, USA	Windrock	37.413889°N, 80.519444°W	MARY1012974
S. stellata	Newport, VA, USA	Windrock	37.413889°N, 80.519444°W	MARY1012975
S. stellata	Newport, VA, USA	Woodland	37.355415°N, 80.553469°W	MARY1012977
S. stellata	Newport, VA, USA	Woodland	37.355415°N, 80.553469°W	MARY1012978
S. caroliniana Walter	Potomac, MD, USA	Great Falls	38.989511°N, 77.246713°W	MARY1012980
S. caroliniana	Potomac, MD, USA	Great Falls	38.989511°N, 77.246713°W	MARY1012981
S. virginica L.	Newport, VA, USA	Windrock	37.413889°N, 80.519444°W	MARY1012982
S. virginica	Newport, VA, USA	Windrock	37.413889°N, 80.519444°W	MARY1012983

[a] Herbarium vouchers were deposited at the University of Maryland Herbarium (MARY).
[b] Individual used for DNA extraction.