Isolation and Characterization of Polymorphic Microsatellite Loci in Selliera radicans (Goodeniaceae)

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PRIMER NOTE

ISOLATION AND CHARACTERIZATION OF POLYMORPHIC MICROSATELLITE LOCI IN *SELLIERA RADICANS* (GOODENIACEAE)[1]

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- *Premise of the study:* Microsatellite markers were developed for species in the genus *Selliera* (Goodeniaceae) for future investigations of population genetic structure and interspecific hybridization within the genus.
- *Methods and Results:* Using 454 pyrosequencing, 15 new markers were developed from microsatellite loci isolated from *S. radicans.* Primers for the new markers amplify di- and trinucleotide repeat loci from the three *Selliera* species screened. Ten of the new markers are polymorphic in *S. radicans* and six of those 10 loci were found to be polymorphic within each congener. For the focal species, *S. radicans*, the average number of alleles per locus is 3.7 (SE = 0.60) and the average observed and expected heterozygosities are 0.23 (SE = 0.07) and 0.47 (SE = 0.08), respectively.
- *Conclusions:* The new markers provide an important resource for future investigations in the genus *Selliera* for both population genetics and research into hybridization between species.

Key words: gene flow; Goodeniaceae; hybridization; microsatellite; *Selliera*.

Three species of *Selliera* Cav. (Goodeniaceae), a small genus of rhizomatous perennial herbs, are currently recognized. The most common, *S. radicans* Cav., was originally described from Australia and also occurs in Chile and New Zealand. Within New Zealand, the species is relatively common along much of the coast and occurs less frequently in inland freshwater habitats. *Selliera radicans* was described by Allan (1961) as "polymorphic, with a considerable range of leaf form and size." Ensuing investigation (Ogden, 1974) into the polymorphic nature of *S. radicans* distinguished an estuarine form and a dune form based on differences in rhizome formation, growth form, and leaf shape, the last of which is the most conspicuous. The estuarine form is typical of *S. radicans*, having elongated spatulate leaves, whereas the dune form has shorter rotund leaves. Ogden (1974) performed common garden studies and determined that the leaf form difference is strongly genetically determined and, on this basis, suggested that the two forms be regarded as distinct ecotypes. Based on further taxonomic investigations, Heenan (1997) later raised the dune ecotype to a distinct species, *S. rotundifolia* Heenan; however, obvious hybrid swarms between *S. radicans* and *S. rotundifolia* have been observed at sites of sympatry.

The third species in the genus, *S. microphylla* Colenso, was described from two regions of New Zealand in 1890 (Colenso, 1890). This species is morphologically similar to but distinguished from *S. radicans* primarily by a smaller form; however, this difference appears to be a plastic developmental response to the environment, as it disappears when field-collected individuals are grown in a common greenhouse (Symonds and Pilkington, pers. obs.). Based on a single individual, *S. microphylla* has a distinct chromosome number ($2n = 56$; Murray and de Lange, 2013) relative to *S. radicans* and *S. rotundifolia* (both $2n = 16$; Dawson, 2000).

Given various degrees of morphological overlap, developmental plasticity, sympatry, and hybridization, genetic distinction among these species and, therefore, taxonomic status warrant further investigation. Here, using 454 pyrosequencing, microsatellite markers were developed for *Selliera* species for use in assessing genetic structure within and investigating hybridization among *Selliera* species in future work.

METHODS AND RESULTS

DNA from *S. radicans* collected from a population at Moana Roa beach (Appendix 1) was chosen for 454 sequencing. Genomic DNA was extracted from silica gel–dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) method with an initial sucrose-Tris-EDTA (STE) wash (Shepherd and McLay, 2011) and an additional RNase step. The resulting DNA was dissolved in 100 µL of TE buffer. The sample had a concentration of 84.8 ng/µL and a 260/280 absorbance reading of 1.99 as measured on a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The DNA was run on a 1% agarose gel to assess DNA quality and ensure that RNA had been removed successfully. Approximately 5 µg of this DNA was used to construct a shotgun genomic DNA library that was sequenced in a full run on a 454 GS FLX system (454 Life Sciences, a Roche Company, Branford, Connecticut, USA) by New Zealand Genomics Ltd.

The 454 sequencing run generated more than 23 Mb of quality data, with 57,561 sequences averaging 407 bp in length. The sequence data were assembled into contigs in Geneious (version 5.6.7; Kearse et al., 2012) to increase the efficiency of microsatellite detection and to prevent locus duplication. The assembly yielded 8101 contigs with an average sequence length of 672 bp. MSATCOMMANDER version 0.8.2 (Faircloth, 2008) was used to search the *Selliera* contigs for di-, tri-, and tetranucleotide repeat motifs with a minimum of seven uninterrupted repeats and with the requirement to design primers at least 50 bp from the repeat region using Primer3 (Rozen and Skaletsky, 1999). Criteria for primer pair

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TABLE 1. Characteristics of 15 microsatellite marker primer pairs developed from *Selliera radicans.*

Locus[a]	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	Pooling group/dye[e]	GenBank accession no.
SR3[b]	F: TGTGCTAGTCACTCTGTGGAG R: TGAGGAACCCACCTTGGTC	$(TC)_7$	196	NA	KU589266
SR4[b]	F: ACCTATGCGCACATCACTTG R: CGCACTGTGTACCTGTCAAC	$(TA)_7$	302	NA	KU589267
SR6	F: GAGCTTGAGCTGGTTCCTC R: CCAGTCTCAGTCACAAGCG	$(GA)_8$	208–214	2/NED	KU522441
SR11[c,d]	F: GCTCTACTTGCACGGCTTC R: GTGCTCACATGGGATCTGC	$(GT)_8$	182–194	2/VIC	KU522442
SR13[c,d]	F: AGGTCCTCACCCTCTTGAAC R: CAGCATGTTTGAAGCTACGTG	$(TC)_7$	313–315	4/FAM	KU522443
SR17	F: AATGGAAAGCAACCAATCCC R: TGTTCCAACGATTTGACCAAC	$(AT)_{12}$	252–258	1/FAM	KU522444
SR24	F: GGCAAGTAGGAAATGTGGGC R: TCTCCTGAACCAGCAACCG	$(AT)_8$	203–209	3/NED	KU522445
SR29[c,d]	F: GAACGGCAGCAAACTACCC R: AGCCTCCAAGAGACTTGACC	$(AG)_7$	397–399	3/VIC	KU522446
SR31	F: CAGCCGAGTGCCTACCTTC R: CGCCCAACTATCAAGCCAC	$(CT)_7$	369–373	1/NED	KU522447
SR33[b]	F: TAAGGAAGGCGGCTTGTGC R: TTGGTCGACTATCGGACGG	$(AT)_9$	235	NA	KU589268
SR35[b]	F: TTGGTATCAGAGCACCCAG R: GCACTTGGTCAGTGCCTTC	$(GT)_9$	177	NA	KU589269
SR37[c]	F: TCAAGCCTTTGGCAAGATAGTC R: ACTCGTGGACGTAGGTTCTG	$(AG)_{10}$	296–302	2/FAM	KU522448
SR41[b]	F: CGATTTCCGGCGAACTAGC R: AAACCCTAGCCGACGAACC	$(AC)_7$	352	NA	KU589270
SR45	F: CTGCGATAGCGTCGATTCC R: GTGGTTGATCCATATTACCAGGC	$(GAT)_9$	271–283	1/VIC	KU522449
SR46[d]	F: AGCAACACGGCCAACAAAG R: CTGCATCGGTGGTTGTACG	$(TA)_{11}$	253–267	4/VIC	KU522450

Note: NA = not applicable.
[a] Annealing temperature used in PCR was 53°C for all loci.
[b] Monomorphic locus in *S. radicans.*
[c] Monomorphic locus in *S. rotundifolia.*
[d] Monomorphic locus in *S. microphylla.*
[e] Marker pooling and dye combinations.

design included PCR product size between 150–350 bp with no long repeats (>4 bp) in the region surrounding the microsatellite (e.g., mononucleotide repeats) and primers optimally with 60% GC content and a GC clamp at the 3′ end.

From the contigs, MSATCOMMANDER (Faircloth, 2008) detected 227 repeat motifs; of these, 196 were dinucleotide repeats (86%), of which there was a high frequency of AT repeats (51%), 30 were trinucleotide repeats (13%), and one was a tetranucleotide repeat (0.4%). Given our criteria, Primer3 (Rozen and Skaletsky, 1999) successfully designed primers for 107 of the 227 repeat regions detected; 90 were designed for dinucleotide repeats, 17 were designed for trinucleotide repeats, and no primer pairs could be designed for the one tetranucleotide repeat.

From the 107 primer pairs, 43 were selected for initial testing based on a refinement of criteria, including a maximum number of uninterrupted repeats (12), primer melting temperatures, and overall maximum repeat length. Selected primer pairs were manufactured by IDT (Coralville, Iowa, USA) and screened initially on 15 individuals representing multiple populations of *S. radicans*, *S. rotundifolia*, and *S. microphylla*. PCR amplification was performed in a volume of 10 µL with 1× buffer BD (Solis BioDyne, Tartu, Estonia), 50 µM of each dNTP, 2.5 µM $MgCl_2$, 0.5 units of FIREPol DNA polymerase (Solis BioDyne), 20 nM of forward primer, 450 nM of reverse primer, and 450 nM M13 tail primer labeled with FAM (see Schuelke, 2000 for M13-tailed PCR). Amplification by PCR was attained by: 95°C for 3 min; 35 cycles of 95°C for 30 s, 53°C

TABLE 2. Results of primer screening in populations of *Selliera radicans.*[a]

	Napier ($N = 20$)			Ohiwa ($N = 20$)			Tauranga ($N = 20$)			Australia ($N = 10$)			Total ($N = 70$)		
Locus	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e
SR6	1	0	0	1	0	0	1	0	0	2	1.00	0.50	3	0.15	0.26
SR11	1	0	0	1	0	0	2	1.00	0.50	2	1.00	0.50	3	0.48	0.39
SR13	1	0	0	1	0	0	1	0	0	1	0	0	2	0	0.23
SR17	1	0	0	1	0	0	2	0	0.19	1	0	0	3	0	0.55
SR24	2	1.00	0.50	2	0.42	0.39	2	0	0.50	1	0	0	4	0.37	0.66
SR29	1	0	0	1	0	0	1	0	0	1	0	0	2	0	0.23
SR31	1	0	0	1	0	0	1	0	0	1	0	0	2	0	0.17
SR37	1	0	0	2	0.70	0.50	4	0.42	0.63	2	1.00	0.50	7	0.46	0.80
SR45	2	0.05	0.14	2	0.10	0.10	3	0.74	0.65	1	0	0	4	0.25	0.68
SR46	2	0.05	0.05	5	0.79	0.63	3	0.79	0.66	2	1.00	0.50	7	0.59	0.76

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity.
[a] Voucher information is provided in Appendix 1.

TABLE 3. Results of primer screening in populations of *Selliera microphylla* and *S. rotundifolia*.[a]

	S. microphylla CVP population ($N = 20$)			*S. rotundifolia* Himatangi population ($N = 20$)		
Locus	*A*	H_o	H_e	*A*	H_o	H_e
SR6	2	0.20	0.18	2	0.20	0.32
SR11	1	0	0	1	0	0
SR13	1	0	0	1	0	0
SR17	2	0	0.26	2	0.05	0.29
SR24	2	0.15	0.14	4	0.16	0.40
SR29	1	0	0	1	0	0
SR31	2	0.10	0.10	3	0.60	0.52
SR37	3	0.11	0.59	1	0	0
SR45	2	0.45	0.40	3	0.80	0.61
SR46	1	0	0	3	0.45	0.50

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

[a]Voucher information is provided in Appendix 1.

for 30 s, and 72°C for 1 min; and a final 72°C hold step for 20 min. PCR products were separated on an ABI 3730 DNA Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) at the Massey Genome Service (Palmerston North, New Zealand) and analyzed in GeneMapper (version 4.0; Applied Biosystems) using CASS size standard (Symonds and Lloyd, 2004). Fifteen loci amplified consistently and generated easily interpretable results (Table 1), 10 of which were polymorphic. The 10 polymorphic loci were further tested across six populations (Table 2) using the methods above, except that for each sample, three markers were pooled together, each labeled with either FAM, NED, or VIC dyes. Twenty individuals were sampled from each population from New Zealand representing all three species, and 10 individuals were sampled from one population of *S. radicans* from Australia (see Appendix 1).

The observed and expected heterozygosity and the number of alleles per locus were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012). All 10 polymorphic markers amplified consistently and yielded two to seven alleles per locus in *S. radicans*. Across *S. radicans* populations and loci, observed heterozygosity ranged from 0 to 1.00, averaging 0.23 (SE = 0.07), and expected heterozygosity ranged from 0 to 0.66, averaging 0.47 (SE = 0.08) (Table 2). The single population of *S. rotundifolia* had observed heterozygosities ranging by locus from 0 to 0.80, averaging 0.23 (SE = 0.09), and expected heterozygosities of 0 to 0.61, averaging 0.26 (SE = 0.08) (Table 3). The *S. microphylla* population had observed heterozygosities ranging by locus from 0 to 0.45, averaging 0.10 (SE = 0.05), and expected heterozygosities of 0 to 0.59, averaging 0.17 (SE = 0.06) (Table 3).

CONCLUSIONS

Here we describe the development of 15 new markers from microsatellite loci isolated from *S. radicans*. Ten of the new markers are polymorphic within *S. radicans* and also amplify from the New Zealand congeners *S. rotundifolia* and *S. microphylla*. These 10 markers will be used in future studies of population structure and hybridization in the genus.

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APPENDIX 1. Voucher information for *Selliera* populations analyzed in this study.

Species	Population locality	GPS coordinates	*N*	Plant ID/Voucher[a]
Selliera radicans	Napier	–39°28′4.15″S, 176°52′30.14″E	20	MPN 47843
Selliera radicans	Ohiwa	–37°59′14.38″S, 177°9′37.47″E	20	MPN 47844
Selliera radicans	Tauranga	–37°43′27.84″S, 176°11′25.04″E	20	MPN 47840
Selliera radicans	Australia	–37°49′37.99″S, 144°59′2.00″E	10	MPN 48358
Selliera rotundifolia	Himatangi	–40°21′44.53″S, 175°13′57.32″E	20	MPN 47848
Selliera microphylla	Central volcanic plateau (CVP)	–38°54′34.95″S, 176°27′20.8″E	20	MPN 47841

[a]Vouchers deposited at the Dame Ella Campbell Herbarium (MPN), Massey University, Palmerston North, New Zealand.