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# Microsatellite markers for the high Andean species *Schizanthus hookeri* and *S. grahamii* (Solanaceae)[1]

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- *Premise of the study:* Seven microsatellite loci were developed for the two closely related high Andean species *Schizanthus hookeri* and *S. grahamii*. These species are annual to biannual herbs with zygomorphic and showy flowers that differ in floral morphology, autonomous selfing capacity, and in the identities of major flower visitors.
- *Methods and Results:* Polymorphisms were evaluated in a total of 45 plants, including individuals from two populations of *S. grahamii* and one population of *S. hookeri*. The number of alleles per locus ranged from two to nine in each population. We also tested these loci for cross-amplification in another seven species of the genus. Four primer pairs amplified in these seven species.
- *Conclusions:* Characterized microsatellites are conserved in the closely related species *S. hookeri* and *S. grahamii,* and they have enough polymorphism to be used in future studies of their mating systems and genetic structure.

**Key words:** autonomous selfing; cross-species amplification; high Andes; mating system; simple sequence repeats.

The genus *Schizanthus* Ruiz & Pavon is a southern South American genus that diverged early from the rest of the Solanaceae and constitutes the monogeneric tribe Schizanthoideae (Olmstead and Palmer, 1992). Unlike the other members of the family, *Schizanthus* species have zygomorphic and bilabiate, papillionaceous flowers. The genus comprises 12 species of annual to biannual herbs that grow in areas with Mediterranean-type climates, winter rainfall deserts, and above-treeline habitats in Chile and Argentina (Grau and Grönbach, 1984). Strong differences in floral morphology, pollination systems (Pérez et al., 2006), and mating systems (Pérez et al., 2009) characterize these species.

The high Andean species *S. hookeri* Gill. ex Graham and *S. grahamii* Gill. ex Hook. evolved in isolation from the remaining species of the genus (Pérez et al., 2006). Both species are autocompatible, but while *S. hookeri* is strongly dependent on pollinators for seed set, *S. grahamii* exhibits late autonomous selfing (Pérez et al., 2009). These species also differ in floral morphology and the identity of major pollinators (Pérez et al., 2006). Here we describe the isolation and characterization of seven microsatellite markers for *S. hookeri* and *S. grahamii* and their cross-amplification in another seven species of the genus. Microsatellite markers would be useful for future ecological and evolutionary studies with *Schizanthus*, particularly for estimating outcrossing rates, genetic diversity, and gene flow among populations.

## METHODS AND RESULTS

To develop primers to amplify microsatellite markers, genomic DNA was extracted from dried foliar samples of a single individual of *S. grahamii* using CTAB (Doyle and Doyle, 1987). An enriched library was compiled by ATG Genetics (Vancouver, Canada) from genomic DNA digested with the restriction enzymes HaeIII and PsHA1, which were ligated into M28/29-linker and enriched by biotin capture of dinucleotides $TC_n$ and $AC_n$ repeats. Plasmid clones containing a dinucleotide motif were purified and amplified by PCR. Twenty unique microsatellites with sufficient flanking regions were chosen to design prime pairs using the software Primer 3 (Rozen and Skaletsky, 2000).To test for amplification consistency and specificity of the primer pairs, we conducted PCR amplification with two individuals of *S. grahamii* and three individuals of *S. hookeri.* Seven of the 20 primers gave reliable amplifications for the two species (Table 1). Amplifications were carried out using forward primers with a fluorescently labeled M13 tail (Schuelke, 2000). PCRs were 10 μL and contained 5 ng of template DNA, 1.6 pmol of the reverse primer, 0.8 pmol of M13-tailed forward primer, 1.6 pmol of fluorescently labeled (FAM, VICor NED) M13 universal primer, 5 μL of 2× GoTaq Master Mix (containing TaqDNA polymerase, dNTPs, $MgCl_2$and reaction buffers).Cycling conditions consisted of an initial denaturing step of 5 min at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s at 52°C, 45 s at 72°C, and a final elongation step at 72°C for 10 min. For genotyping, 1 μL of the PCR product was added to 22 μL formamide and 0.5 μL LIZ-400 size standard. The mixture was run on the ABI PRISM 310 (Applied Biosystems, Foster City, California, USA), and analyzed using Peak Scanner Software version 1.0 (Applied Biosystems).

Polymorphism was evaluated on 15 individuals of *S. hookeri* and 15 individuals of *S. grahamii* from a single locality in the high Andes of central Chile, Laguna Los Cristales (34°34′S, 70°31′W), where both species grow sympatrically. An additional 15 individuals of *S. grahamii* from a second population located 36 km further south in Termas del Flaco (34°57′S, 70°25′W) were also genotyped. All markers were highly polymorphic in the three studied populations (Table 2). The number of alleles per locus ranged from two to nine. Ob-

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TABLE 1. Characteristics of seven microsatellite primers developed in *Schizanthus hookeri* and *S. grahamii*. Shown for each primer pair are the forward and reverse sequence, repeat type, size of the original fragment (bp), annealing temperature when run individually (Ta) and the GenBank accession number

| Locus | Primer sequence (5′–3′) | Repeat | Size | *Ta* | GenBank Accession No. |
|---|---|---|---|---|---|
| P1 | F: ATTCATTCATTCATCAAACTCTCC | (GA)9 | 146 | 52 | HQ593728 |
| | R: GGTTTTGTAGAGACGACGAAGCC | | | | |
| P3 | F: CCTTGCAGATATGAGTGCACT | (AG)8 | 217 | 52 | HQ593729 |
| | R: GTTTCGGTGCAATAAAAGTAGCT | | | | |
| P5 | F: GGAGTAGATACTGCAGCCA | (CT)8 | 253 | 52 | HQ593730 |
| | R: GGTCATATTTTCCGATGTC | | | | |
| P6 | F: CCCATATAATGAGCTTCCTGAT | (CT)3CC(CT)3CC (CT)4 | 234 | 52 | HQ593731 |
| | R: TATATCTCTCTTAGCTTTGTAC | | | | |
| P7 | F: GGAAATACTGACCATCAAAATCC | (TC)11 | 215 | 52 | HQ593732 |
| | R: AGGTCAATTTTGTTTGCTACC | | | | |
| P8 | F: GAAGAAGAGAGGTTATGGATCT | (TC)9 | 231 | 52 | HQ593733 |
| | R: CCGTAGTGGGAATTGGGCT | | | | |
| P10 | F: GTCGGCTCTTCAATCTCTATAA | (GA)13 | 218 | 52 | HQ593734 |
| | R: CTCGATCGCTCGAACCAAGA | | | | |

TABLE 2. Number of alleles (*A*), observed ($H_o$) and expected ($H_e$) heterozygosity for one population of *Schizanthus hookeri* and two populations of *Schizanthus grahamii*. The sample size for each population is shown in parentheses. Asterisks indicate a significant deviation from Hardy–Weinberg Equilibrium (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

| | *S. hookeri* Laguna Los Cristales ($N = 15$) | | | *S. grahamii* Laguna Los Cristales ($N = 15$) | | | *S. grahamii* Termas del Flaco ($N = 15$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Locus | A | $H_O$ | $H_E$ | A | $H_O$ | $H_E$ | A | $H_O$ | $H_E$ |
| P1 | 9 | 0.53* | 0.87 | 6 | 0.53 | 0.79 | 4 | 0.53 | 0.53 |
| P3 | 7 | 0.80 | 0.83 | 5 | 0.38 | 0.60 | 7 | 0.36** | 0.79 |
| P5 | 6 | 0.60 | 0.71 | 3 | 0.53 | 0.51 | 3 | 0.14*** | 0.59 |
| P6 | 4 | 0.57 | 0.63 | 3 | 0.43 | 0.54 | 3 | 0.73 | 0.69 |
| P7 | 5 | 0.33** | 0.77 | 4 | 0.20*** | 0.71 | 7 | 0.67 | 0.63 |
| P8 | 6 | 0.60* | 0.78 | 4 | 0.36* | 0.60 | 2 | 0.33 | 0.37 |
| P10 | 7 | 0.92 | 0.76 | 4 | 0.40 | 0.43 | 3 | 0.27 | 0.25 |

TABLE 3. Cross-amplification of microsatellite primers in seven species of *Schizanthus*. Two individuals per species were tested. PCR products resolved on agarose gel indicated +, amplification; or —, no amplification. The number of alleles is shown in parentheses.

| | P1 | P3 | P5 | P6 | P7 | P8 | P10 |
|---|---|---|---|---|---|---|---|
| *S. candidus* | +(3) | +(1) | +(1) | — | +(2) | — | — |
| *S. integrifolius* | +(2) | +(2) | +(1) | — | +(2) | — | — |
| *S. laetus* | +(1) | +(3) | +(2) | — | +(2) | — | — |
| *S. litoralis* | +(4) | +(3) | +(1) | — | +(4) | — | — |
| *S. pinnatus* | +(2) | +(3) | +(3) | — | +(3) | — | — |
| *S. porrigens* | +(1) | +(2) | +(4) | — | +(3) | — | — |
| *S. tricolor* | +(2) | +(3) | +(1) | — | +(1) | — | — |

served ($H_O$) and expected ($H_E$) heterozygosity and deviations from Hardy–Weinberg Equilibrium were calculated with Arlequin 3.1 (Excoffier et al., 2005). Three loci in *S. hookeri*, two loci in *S. grahamii* from Laguna los Cristales, and two loci in *S. grahamii* from Termas del Flaco exhibited significant heterozygote deficiency (Table 1).

The cross-species amplification success of microsatellite loci were tested in another seven *Schizanthus* species to determine if these markers were conserved across the genus. We assayed two individuals per species. Data on geographic location of sampled populations is shown in Appendix 1. Samples of *S. grahamii* were included as positive controls. The primer pairs P1, P3, P5, and P7 amplified in the seven tested species of *Schizanthus* and were polymorphic in most of them (Table 3). The other three loci failed to amplify in any species.

## CONCLUSIONS

Our results suggest that the characterized microsatellites are conserved in the closely related species *S. hookeri* and *S. grahamii* and that they have enough polymorphism to be used in future studies of their mating systems and genetic structure. These microsatellites would also be a valuable tool for detecting ongoing hybridization between these species within the area of sympatry. Our results also show that four of the seven characterized microsatellite are conserved within the genus and would greatly contribute to the understanding of the genetic

relationships between *Schizanthus* species, as well as to the understanding of the evolution of mating systems.

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APPENDIX 1. Locations and voucher number of studied populations of *Schizanthus* species. Data include political region in Chile, name of study site, elevation, latitude, and longitude.

| Species | Location |
|---|---|
| *S. candidus* Lindl. | II, Arrayán, 40 m a.s.l., 28°15′S, 70°09′W, HULS 6264 |
| *S. grahamii* Gill. ex Hook | VI, Laguna Los Cristales,2190 m a.s.l., 34°34′S, 70°31′W, CONC 171985 |
| | VI, Termas del Flaco, 1969 m.a. s. l., 34°57′S, 70°25′W, HULS 6265 |
| *S. hookeri* Gill. ex Graham | VI, Laguna Los Cristales, 2190 m a.s.l., 34°34′S, 70°31′W,CONC 171986 |
| *S. integrifolius* Phil. | III, Alto de Carmen, 710 m a.s.l., 28°45′S, 70°29′ W, HULS 6267 |
| *S. lacteus* Phil. | II, Paposo, 144 m a.s.l., 25°06′S, 70°27′W, HULS 6268 |
| *S. laeteus* Phil. | II, Paposo, 455 m a.s.l., 25°00′S, 70°26′W, HULS 6269 |
| *S. pinnatus* Ruiz & Pav. | RM, R. N. Río Clarillo, 800 m a.s.l., 33°43′S, 70°56′W, HULS 6272 |
| *S. porrigens* Graham | IV, Juan Soldado, 150 m a.s.l., 29°38′S, 71°17′W, HULS 6273 |
| *S. tricolor* Grau & Gronb. | V, Papudo, 130 m a.s.l., 32°31′S, 71°28′′W, HULS 6274 |