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PRIMER NOTE

Isolation of seven polymorphic microsatellite loci, using an enrichment protocol, in the high Andean *Asteraceous Chaetanthera pusilla*

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Abstract

We report the development of seven microsatellite markers in the high Andean Asteraceae *Chaetanthera pusilla*. An enrichment protocol was used to isolate microsatellite loci, and polymorphism was explored with samples from two natural populations collected in the high Andes at La Parva and Valle Nevado (Chile). We found a high level of polymorphism, heterozygote deficiency and strong differentiation among populations. Four of the seven loci successfully cross-amplified in other *Chaetanthera* species.

Keywords: endemic, enriched library, plants, selfing rate, South America

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While some studies suggest that the frequency of self-compatibility and self-pollination increases with elevation on high mountains (Medan *et al*. 2002), others have found an increase in outcrossing systems (e.g. Arroyo & Squeo 1990). Detailed knowledge of selfing rates over elevational gradients is critical for understanding breeding systems and patterns of genetic variation in high mountains. *Chaetanthera* (Asteraceae), endemic to South America, provides excellent biological material for studying such patterns. Breeding systems range from strong selfing to self-incompatibility and gynodioecy. Here we describe microsatellites in alpine *C. pusilla* from central Chile, which were developed for studying patterns of genetic variation and selfing rates.

Two microsatellite enriched-libraries were built according to Giraud *et al*. (2002) using biotin-labelled microsatellite oligoprobes and streptavidin-coated magnetic beads. Genomic DNA was extracted from one plant of *C. pusilla* using a Qiagen™ plant kit. The two libraries were built using the oligoprobes $(TG)_{10}$ and $(TC)_{10}$. In the first enriched library, 400 clones were screened and 76 gave positive responses (19%). In the second enriched library, 150 clones were screened and 93 gave positive responses (62%). In the first library, 49 clones were sequenced. Five (10%) of these were found to be redundant, always corresponding to the same clone, which turned out to be a contaminant from a previous enrichment (Giraud *et al*. 2002). In the second library, all 93 clones were sequenced, of which 20 were analysed.

PCR primers were designed for 30 loci (21 from the first library and nine from the second library), using the software PRIMER 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Each locus was screened for variation using 25 individuals from each of two populations collected in the high Andes at La Parva (3170 m.a.s.l) and Valle Nevado (3320 m.a.s.l.) (4 km linear distance) Chile, and was tested for cross-amplification using one individual of each of six other *Chaetanthera* species (see Table 1). DNA was extracted using Qiagen™ plant kits. Amplification reactions were prepared in 25 μL volumes containing approximately 6 ng DNA, 0.2 mM of each dNTP, 1 μM of each primer (one of which was fluorescent, see Table 1, Applied Biosystems or Proligo), *x* mM $MgCl_2$ (see Table 1 for $[Mg^{2+}]$), 1 U Gold *Taq* polymerase (Perkin Elmer) and 1x *Taq* buffer (Perkin Elmer). Polymerase chain reaction (PCR) amplifications were performed using a Perkin Elmer thermal cycler, with the following cycling conditions: 10 min

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Table 1 Characteristics of the seven microsatellite loci isolated in *Chaetanthera pusilla*: repeat motif, GenBank accession number, primer sequences, amplification conditions (annealing temperature, T_a, and $MgCl_2$ concentration, x), amplification size for the sequenced clone, number of alleles in the two analysed populations of *C. pusilla* (N = 25 individuals each), expected heterozygosity (H_E), observed heterozygosity (H_O) and species in which cross amplification was successful

Locus	Repeat motif$	GenBank accession number	Primer sequences (5′–3′)	T_a (°C)	x (mM)	Size (bp)	Nb of alleles†	H_E%‡	H_O%‡	Cross amplification§
CHA2	$(CA)_7$	AY566631	CGAGGGTCGAACGTATCATT GAGAGTTATTTGGGAGGAGGTG¶	60	2.5	223	11 (11)	80.6 82.9	14.3* 22.7*	Cli, Cpl, Cre, Ceu, Cly
CHA8	$(CT)_{15}$	AY566632	GAATTCCTACTCCCGCCTTC¶ AGCGACTCTACAAGAGCTTCG	60	2	183	12 (12)	86.8 87.2	68* 76	
CHB5	$(CA)_8$	AY566633	CACTTGTGATCTTAGCTCTAGTCAA TCCCAATGTTTTTGTCATGC¶	60	1	130	2 (1)	11.5 4	12 4	Ceu
CHO4	$(AC)_4N_{18}(AC)_4$ $N_2(CA)_6N_{40}(CA)_4$	AY566634	TTCTTGTCAATACATCCAGTTGAA TTCCTCTCCTGATCCAACGA¶	62	2	221	12 (12)	80.4 87.2	45* 33.3*	Cpl, Ceu
CHP2	$(CA)_9$	AY566635	AGCTTCTCGTATTATAACATCTGACTG TGGATTGTGTGTGTGAATGG¶	60	2	157	5 (4)	68.9 66.1	76 64	
CHP6	$(CT)_9$	AY566636	AACCAATGGCACCAAATAGC ATCTGGTTCCACGTCATTCC¶	60	2	155	5 (8)	73.8 72.8	60 36*	Cre
CHP8	$(CA)_8$	AY566637	TCACTCACGCACTTTCTTGC¶ ACTATTCACGCGGATGTGC	60	1	188	6 (7)	71.2 62.1	36* 62.5*	

$N_y indicates that two microsatellite motifs were separated by y base pairs in the clone.

†Within brackets: maximal difference in number of repeats among alleles.

‡In each column, the first value is for the La Parva population, the value below is for Valle Nevado. *: significant heterozygote deficiency ($P < 0.05$).

§Species for which amplification products were obtained are indicated by their initials (Cli = *C. linearis*, Cpl = *C. planiseta*, Cre = *C. renifolia*, Ceu = *C. euphrasioides*, Cly = *C. lycopodioides*, Cap = *C. apiculata*).

¶fluorescent primer.

at 95 °C; 40 cycles composed of 30 s at 95 °C, 30 s at annealing temperature (T_a); and 30 s at 72 °C, and 10 min at 72 °C to complete extension (see Table 1 for T_a). Amplified fragments were then electrophoresed on an ABI Prism® 3100 Genetic Analyser (Applied Biosystems). Microsatellite patterns were visualized with ABI Prism GENEMAPPER™ version 3.0. Genetic data were analysed using the software GENEPOP version 3.4 (Raymond & Rousset 1995). For the six other species, the amplification conditions were the same as above, with an annealing temperature of 50 °C and 2 mM [Mg^{2+}]. The PCR products were visualized on an agarose gel, and only PCR products between 100 and 700 bp were considered.

Of the 30 primer pairs tested, 15 successfully amplified fragments of the expected size in *C. pusilla* and seven were found to be polymorphic (Table 1). Expected heterozygosity was generally high (Table 1), indicating high genetic diversity, and significantly higher than observed heterozygosity (Table 1), possibly indicating selfing. Heterozygote deficiency is not likely to stem from the presence of null alleles, because all individuals gave amplification product for all loci. The two populations were significantly differentiated (Genic differentiation test, $P < 10^{-6}$). No linkage disequilibrium was detected among loci in either population.

Given their high level of polymorphism, the microsatellite loci characterized here will allow the characterization of genetic diversity and selfing rates in populations of *C. pusilla* distributed over an elevational gradient from 2300 and 3500 m.a.s.l. in the central Andes. Among the seven polymorphic loci, four cross-amplified in other *Chaetanthera* species (Table 1) and may therefore also be useful to compare genetic diversity and selfing rates among species.

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References

Arroyo MTK, Squeo F (1990) Relationship between plant breeding systems and pollination. In: *Biology Approaches and Evolutionary Trends in Plants* (ed. Kawano S), pp. 205–227. Academic Press, London.

Giraud T, Fournier E, Vautrin D, Solignac M, Shykoff JA (2002) Isolation of 44 polymorphic microsatellite loci in three host races of the phytopathogenic fungus *Microbotryum violaceum*. *Molecular Ecology Notes*, **2**, 142–146.

Medan D, Montaldo NH, Mantese A, Vasellati V, Roitman GG, Bartolini NH (2002) Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. *Arctic, Antarctic, and Alpine Research*, **34**, 233–241.

Raymond M, Rousset F (1995) GENEPOP (V. 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.