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PRIMER NOTE

Microsatellite markers isolated from polyploid wood-sorrel *Oxalis alpina* (Oxalidaceae)

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Abstract

Twelve polymorphic microsatellite loci were isolated from polyploid alpine wood-sorrel, *Oxalis alpina* (Oxalidaceae), and optimized for future studies of its breeding system. The loci were screened for variability among 72 individuals from Pinos Altos, New Mexico. The primers amplified loci with allele number ranging from two to 17 per locus and with estimates of Nei's genetic diversity varying from 0.10 to 0.99. These primers provide an opportunity to use polymorphic DNA markers to study the causes of breeding system variability in this species.

Keywords: breeding system, microsatellites, *Oxalis alpina*, PCR, polymorphism, polyploidy, primer

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Oxalis alpina (Oxalidaceae) is a heterostylous plant species occurring in a series of isolated mountain ranges in Mexico, adjacent Arizona and New Mexico. Populations of *O. alpina* show many intermediate stages in transition from tristyly to distyly, and provide an excellent opportunity to investigate how changes in incompatibility may result in the evolution of a new breeding system (Weller & Denton 1976; Weller *et al.* in press). We report on the characterization of 12 microsatellite loci which will be used for analysing selfing rates and characterizing gene flow within *O. alpina* populations to further clarify its breeding system.

Leaf samples were collected and dried on silica gel, and genomic DNA was extracted using QIAGEN DNeasy Kit. DNA from one individual (from Animas Mountains, New Mexico) was enriched twice for microsatellites using three probe mixes [mix 2 = $(AG)_{12}$ $(TG)_{12}$ $(AAC)_6$ $(AAG)_8$ $(AAT)_{12}$ $(ACT)_{12}$ $(ATC)_8$; mix 3 = $(AAAC)_6$ $(AAAG)_6$ $(AATC)_6$ $(AATG)_6$ $(ACAG)_6$ $(ACCT)_6$ $(ACTC)_6$ $(ACTG)_6$; mix 4 = $(AAAT)_8$ $(AACT)_8$ $(AAGT)_8$ $(ACAT)_8$ $(AGAT)_8$] following Glenn & Schable (2005). Briefly, the DNA was digested with restriction enzyme *Rsa*I (New England Biolabs) and simultaneously ligated to double-stranded SuperSNX linkers (SuperSNX24 forward 5′-GTTTAAGGCCTAGCTAGCAGCAGAATC and SuperSNX24 reverse 5′-GATTCTGCTAGCTAGGCCT-TAAACAAAA). Linker-ligated DNA was denatured and hybridized to biotinylated microsatellite oligonucleotide mixes, which were then captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was washed away and the remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SuperSNX24 as a primer, and cloned with TOPO TA Cloning Kits (Invitrogen). β-galactosidase gene was used to detect clones with inserts. A total of 384 positive clones were isolated and the inserts were amplified with M13 forward and reverse primers. A total of 288 plasmids were sequenced using the BigDye Terminators version 3.1 (Applied Biosystems) and an ABI PRISM 3130xl capillary sequencer. Sequences from both strands were assembled in SEQUENCHER 4.1 (Genecodes) and exported to EPHEMERIS 1.0 (available at www.uga.edu/srel/DNA_Lab/programs.htm) for microsatellite searching. Two hundred and sixty-nine of the 288 sequenced clones contained microsatellites. Sixty-three PCR primer pairs were designed using OLIGO 6.67 (Molecular Biology Insights), and one primer in each pair was modified on the 5′ end with an engineered sequence (CAG tag 5′-CAGTCGGGCGTCATCA-3′ or M13R tag 5′- GGAAACAGCTATGACCAT-3′; see www.uga.edu/srel/DNA_Lab/protocols.htm) to allow use in the PCR of a third primer that is fluorescently labelled for detection on the ABI 3130xl.

The 63 PCR primer pairs were tested for amplification using one individual from each of eight different populations: one from Arizona (Ancha Mountains), two from

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Table 1 Characterization of 12 polymorphic microsatellite loci for *Oxalis alpina*. Size indicates the range of observed alleles in bp; k is total number of alleles and k_i is number of alleles per individual; n is the number of individuals successfully genotyped; 72 individuals from one population were screened; T_D is initial annealing temperature for amplifications; $MgCl_2$ is an optimized concentration for magnesium chloride

Locus accession no	Primer sequence 5′–3′	Repeat motif	Size (bp)	T_D	k	k_i	n	Nei's genetc diversity	$MgCl_2$ mM
Oxa6 EF534294	U: FAMGGGGGAAATGGTTTATGA L: ATTCTGGCTGGGCATTAG	$(GAA)_{17}$	304–383	60	21	1–4	72	0.99	4.5
Oxa9 EF534295	U: AGGCCTAGCTAGCAGAAT L: VICTCTCCCCCTCACAGAC	$(CT)_{14}$	225–263	60	3	1–2	72	0.38	4.5
Oxa17 EF534296	U: VICTTCTGCGTCTCTGTAATTAC L: AGAAAAGCAGCACCTTGAGAG	$(GTTA)_7$	190–206	60	4	2–3	72	0.48	3.0
Oxa22 EF534297	U: FAMCTGTGGAGGAAGTTTTTAGA L: AGGATCACACTGGTGGTAGA	$(CT)_{12}$	237–300	65	11	2–4	72	0.97	2.0
Oxa25 EF534298	U: FAMAACCATCACCAACTCAAGTA L: AATTGGCGATGATGAATAG	$(AAAC)_4$ ... $(AAAC)_5$	237–264	65	5	1–4	72	0.81	4.5
Oxa41 EF534299	U: FAMTGTTCCTTAGAAAGTCATACT L: GTTTGAAAGGAAGAGAACACTTAC	$(GTT)_5$	218–276	55	4	1–4	72	0.76	4.5
Oxa43 EF534300	U: TAAGCGCCTGTTAGTG L: FAMTCATCTCAATGGGCATTATT	$(AACT)_5$	127–302	60	5	1–3	71	0.68	4.5
Oxa46 EF534301	U: FAMAGATTGAAACCCTTTAC L: TTAGGTGGTCACATAAGA	$(ATC)_5$	255–258	55	2	1–2	72	0.10	4.5
Oxa62a EF534302	U: FAMCGTTCACGACCACTGGAAC L: CTCCCTTTGACGCTGTTGG	$(ATGT)_5$	164–176	55	4	2–4	72	0.79	3.0
Oxa81 EF534303	U: TTATTTTGGTTGCATACA L: FAMAAATAAGAAGGCCTAGTG	$(ATGT)_6$	195–235	55	5	1–5	72	0.79	3.0
Oxa84 EF534304	U: TTTCCCTTTCCTCTCATTAGT L: FAMGTAGGGAAGAGCAAGTTGTAT	$(ACAT)_6$	225–237	55	3	1–3	72	0.57	3.0
Oxa88 EF534305	U: FAMGTAAACAGGTTTTCATCTAGT L: TTCTGCTTCTCCATCTTA	$(AGG)_8$	208–246	55	8	1–3	72	0.92	3.0

Primers with CAG (5′- CAGTCGGGCGTCATCA-3′) or M13R (5′-GGAAACAGCTATGACCAT-3′) tag are indicated with superscript FAM or VIC, which was used as the fluorescent dye for genotyping. There are two primers (Oxa62a and Oxa81) with M13 tag.

New Mexico (Pinos Altos and Animas Mountains), and five from Sonora, Mexico (San Luis, San Jose, Azul, Los Ajos and La Purica). The PCR amplifications were performed in a 11.5-μL volume (1× PCR buffer, 25.0 μg/mL BSA, 0.4 μM unlabelled primer, 0.04 μM tag-labelled primer, 0.36 μM universal dye-labelled primer, 2–4.5 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 U JumpStart *Taq* DNA Polymerase (Sigma), and 5–50 ng DNA template) using an Applied Biosystems thermal cycler (GeneAmp PCR System 9700). Touchdown thermal cycling programs (Don *et al*. 1991) encompassing a 10° span of annealing temperatures ranging between 65 °C and 55 °C, 60–50 °C or 55–45 °C were used for the amplification. Cycling parameters were 21 cycles of 96 °C for 20 s, highest annealing temperature (decreased 0.5 °C per cycle) for 20 s, and 72 °C for 30 s; and 15 cycles of 96 °C for 20 s, lowest annealing temperature for 20 s, and 72 °C for 30 s. PCR products were run on an ABI PRISM 3130xl sequencer and sized with Naurox size standard. Results were analysed using GENEMAPPER version 4.0 (Applied Biosystems). Twelve of the tested primer pairs successfully amplified PCR product of high quality.

Samples from 72 individuals (Pinos Altos, New Mexico) were used to test for microsatellite variability across the 12 polymorphic loci. Characteristics of the 12 working primer pairs and their optimal amplification conditions are given in Table 1. The number of alleles per locus ranged from two to 17 per locus and up to five alleles per individual indicating the polyploid nature of this species as previously reported by Weller & Denton (1976) where the authors described uniform tetraploidy (n = 14) in *O. alpina* plants from this area. The presence of five alleles at the locus Oxa81 is likely due to gene duplication, which is common (Taylor & Raes 2005). Nei's genetic diversity was estimated using GENOTYPE and GENODIVE software allowing analysis of polyploid data (Meirmans & Van Tienderen 2004).

Because *O. alpina* is polyploid and the exact copy number for each locus is currently unknown, standard tests for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were not conducted. BLAST searches of *O. alpina* sequences indicate partial homology of locus Oxa6

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with *Arabidopsis thaliana* (102 bp matched partially to accession AC067753).

The microsatellite markers described here will be used to examine gene flow patterns among populations and analyse selfing rates within populations, information useful for understanding factors underlying the transition from tristyly to distyly in *O. alpina*.

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References

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research*, **19**, 4008.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, **4**, 792–794.

Taylor JS, Raes J (2005) Small-scale gene duplications. In: *The Evolution of the Genome* (ed. Gregory TR), pp. 289–327. Elsevier Academic Press, San Diego.

Weller SG, Denton MF (1976) Cytogeographic evidence for the evolution of distyly from tristyly in the North American species of *Oxalis* section *Ionoxalis*. *American Journal of Botany*, **63**, 120–125.

Weller SG, Dominguez CA, Molina-Freaner FE, Fornoni J, LeBuhn G, The evolution of distyly from tristyly in populations of *Oxalis alpina* in the Sky Islands of the Sonoran Desert. *American Journal of Botany* (in press).

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