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PRIMER NOTE

Isolation and characterization of microsatellite loci in *Lesquerella fendleri* (Brassicaceae) and cross-species amplification

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Abstract

Fifteen novel microsatellite primer pairs are presented for *Lesquerella fendleri*, which were developed from seven dinucleotide, five trinucleotide and three tetranucleotide microsatellite DNA loci. These loci were characterized for 40 individuals from 24 localities throughout the species range. The number of alleles observed per locus ranged from three to 16, the observed heterozygosity ranged from 0.175 to 0.750, and the polymorphic information content ranged from 0.218 to 0.889. Cross-species transferability tested on nine species of *Lesquerella* and one species of the related genus *Physaria* indicates that these primer pairs may be useful for population genetic studies of other species in *Lesquerella* and possibly other closely related genera.

Keywords: Brassicaceae, *Lesquerella*, microsatellite, *Physaria*

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Lesquerella fendleri (Gray) Wats. (Brassicaceae) is a diploid ($2n = 12$), out-crossing, annual to short-lived perennial herb native to the Chihuahuan Desert of southwestern USA and north-central Mexico (Rollins & Shaw 1973; Rollins 1993). The seed oil of *L. fendleri* is rich in the hydroxy fatty acid (HFA), lesquerolic acid (C20 : 1-OH). Hydroxy fatty acids are used as a chemical feedstock in the production of a wide variety of industrial products including plastics, nylon-11, lubricants, drying agents, protective coatings, surfactants, cosmetics and pharmaceuticals (Roetheli *et al*. 1991). *Lesquerella fendleri* is currently being bred and commercialized by our laboratory in collaboration with other government agencies, universities and private industries as a new industrial oilseed crop for the arid southwestern USA.

Assessment of the genetic diversity in wild germplasm and breeding populations of this species is vital to sustain improvements in our breeding program. Diverse germplasm collections of *L. fendleri* and related species have been conducted (Dierig *et al*. 1996; Salywon *et al*. 2005). To date, only allozymes have been used to investigate the population genetics of *L. fendleri* (Cabin *et al*. 1998). As part of our ongoing breeding program and commercialization efforts for *L. fendleri*, the development of codominant markers is needed in order to: (i) investigate genetic diversity within and among populations of diverse germplasm collections; (ii) develop genetic linkage maps for marker assisted selection; and (iii) reliably identify diverse breeding lines. We report on the development and characterization of microsatellite primer pairs for 15 loci isolated from *L. fendleri*. Additionally, we investigated the potential of these primer pairs for cross-species amplification in 10 related species.

Genomic DNA was extracted from young leaves of a single *L. fendleri* individual using a DNeasy Plant Mini Kit[1] (QIAGEN), and a voucher specimen (Salywon *sn*.) was deposited at Arizona State University (ASU) Herbarium. A microsatellite library enriched for $(TG)_{12}$ $(AG)_{12}$ $(AAG)_8$ $(ATC)_8$ $(AAC)_8$ $(AAT)_{12}$ $(ACT)_{12}$ $(AAAC)_6$ $(AAAG)_6$ $(AATC)_6$ $(AATG)_6$ $(ACCT)_6$ $(ACAG)_6$ $(ACTC)_6$ $(ACTG)_6$ $(AAAT)_8$ $(AACT)_8$ $(AAGT)_8$ $(ACAT)_8$, and $(AGAT)_8$ repeat motifs was developed following the protocol of Glenn & Schable (2005). The products were cloned, and positive colonies were amplified via polymerase chain reaction

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[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture, and does not imply its approval to the exclusion of other products that may be suitable.

(PCR) as described in Glenn & Schable (2005). PCR products were sequenced with M13 forward and reverse primers using CEQ Quick Start Kit chemistry and a CEQ 8000 Genetic Analysis System (Beckman Coulter). PCR primers were developed using PRIMER DESIGNER 5 (Scientific & Educational Software) and an M13-forward (5′-CACGACGTTGTAAAACGAC-3′) tail was added to the 5′ end of the forward primer. The addition of the 5′-tag allows use of a third primer in the PCR (M13F) that is fluorescently labelled for detection on the CEQ 8000 (Boutin-Ganache *et al*. 2001).

PCR evaluation of microsatellite primers was performed in a final volume of 10 μL containing 10× HotMaster *Taq* Reaction Buffer (10 mM Tris-HCl pH 8.3, 45 mM KCl, 2.5 mM $MgCl_2$ and proprietary reagents), 2.5% glycerol, 0.1 mM dNTPs, 0.15 μM tailed forward primer, 0.15 μM reverse primer, 0.15 μM M13F dye labelled primer — labelled with WellRED D3 or D4 florescent dye (Proligo), 0.25 U of Hotmaster *Taq* polymerase (Eppendorf), and 5–10 ng of DNA template. The reaction mixture was subjected to amplification using a PTC-100 (MJ Research). Touchdown thermal cycling conditions were as follows: 2 min at 94 °C for the initial denaturation, followed by 20 cycles of denaturation at 94 °C for 30 s, 30 s primer annealing at 60 °C with a subsequent decrease in temperature of 1 °C every second cycle, and 45 s extension at 70 °C; followed by 15 cycles at 94 °C for 30 s, 50 °C for 30 s and 70 °C for 45 s, with a final extension of 70 °C for 4 min. Amplified products were analysed using a CEQ 8000 Genetic Analysis System and CEQ Fragment Analysis software. Polymorphic information content (PIC) and observed and expected heterozygosities for each locus were calculated using CERVUS 2.0 (Marshall *et al*. 1998).

Table 1 presents results of the 15 microsatellite primer pairs tested on a panel of 40 individuals of *L. fendleri* taken from 24 localities throughout its natural range. Three loci

Table 1 Characteristics of 15 microsatellite loci identified in *Lesquerella fendleri*. Locus name, oligonucleotide primer sequence (forward primer sequences do not include the M13-forward 5′-tail), GenBank Accession no., repeat motif, allele size range (includes additional 19 bp due to the M13-forward 5′-tail), number of alleles observed, polymorphic information content (PIC), observed heterozygosity (H_O) and expected heterozygosity (H_E)

Locus	Primer sequence (5′–3′)	GenBank Accession no.	Repeat motif (bp)	Size range	No. of alleles	PIC	H_O	H_E
Lf-1	F: CATTTGCTTACCTTAATGC R: CATGGCTAACTTAATAGTGC	DQ026793	$(TG)_8$	111–139	10	0.723	0.24	0.77
Lf-3	F: AGTGGATGTATGGGAACAAG R: GCAATCTGAAATGTCCAAAC	DQ026794	$(CATA)_7$	114–126	4	0.218	0.23	0.23
Lf-9	F: ACCGAGCTGCGTTAAGAC R: ACATGTATCTTCTCATTCCG	DQ026795	$(AAG)_{15}$	275–369	16	0.889	0.75	0.91
Lf-10	F: CACTCTTCAACAAGCATACAG R: ACCTTGGCACCAGTGATTAC	DQ026796	$(CT)_{11}$	218–254	13	0.822	0.50	0.85
Lf-14	F: GACCGTACATCGGAAACTGC R: CGTATGGTGAATTTCAAGAC	DQ026797	$(AG)_9$	204–226	10	0.752	0.58	0.79
Lf-15	F: ATGTCAGCTTCATTGATAGG R: ATCTCAGGTAACATGTGATC	DQ026798	$(TC)_{10}$	190–206	7	0.684	0.43	0.73
Lf-17	F: GGCCATGATTTCTGAATCC R: TTGCAGGCATCTGTATAGG	DQ026799	$(ATG)_{10}$	171–210	10	0.817	0.50	0.85
Lf-18	F: CGGTCTTCATCTTCTTGATTG R: TGCCTATTTGACTTCCCATT	DQ026800	$(AG)_{12}$	119–135	8	0.642	0.43	0.69
Lf-20	F: TTCCTTCCTTACTCACATCC R: TGAAGGTCCTAAGCATATC	DQ026801	$(TATG)_5$	152–186	8	0.744	0.73	0.78
Lf-24	F: TGTAATGGGATGACATGTGG R: CTTGCGATGAAAAGAGCTGC	DQ026802	$(TG)_{12}$	257–291	13	0.854	0.50	0.88
Lf-30	F: GAAGCAGCATCGTTCTTTC R: CGACCCCACACGTTCAAGTC	DQ026803	$(CAT)_7$	327–351	7	0.610	0.18	0.68
Lf-31	F: AGAATCACGCTGTGCTTATC R: GGAGACCCAAATGCCAAAA	DQ026804	$(CT)_{11}$	213–229	9	0.739	0.45	0.78
Lf-32	F: ATTAGACCCCATGTCTTG R: CCATGAGAAGCATCCAAATC	DQ026805	$(AAG)_3/(AAG)_7/(AAG)_3/(AAG)_9$	267–327	13	0.805	0.65	0.83
Lf-33	F: ACGAAGAACACGGCAAACTTAAC R: CTCATCGTTAGTGAGATCAG	DQ026806	$(AAC)_2CAC(AAC)_5$	181–208	7	0.451	0.40	0.49
Lf-34	F: TTACCGAGCAAGAACACAG R: CCTATCTAGCCAATGCTATC	DQ026807	$(TTAG)_6$	147–167	6	0.581	0.35	0.66

Table 2 Cross-species amplification of microsatellite primers designed for *Lesquerella fendleri* in nine species of *Lesquerella* and one species of the closely related genus *Physaria*. Assays amplifying homologous PCR products are denoted by '+', no amplification is denoted by '–'. One individual for each species was tested per primer pair

Taxon	Locus														
	Lf-1	Lf-3	Lf-9	Lf-10	Lf-14	Lf-15	Lf-17	Lf-18	Lf-20	Lf-24	Lf-30	Lf-31	Lf-32	Lf-33	Lf-34
L. angustifolia	+	+	–	+	+	+	–	–	+	+	+	+	+	+	+
L. cinerea	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
L. gordonii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. gracilis	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. lindheimeri	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
L. lyrata	+	–	–	+	–	+	–	–	+	+	–	+	+	+	+
L. mexicana	–	–	–	+	+	+	–	–	+	+	+	+	+	+	+
L. pallida	–	–	–	+	–	+	–	+	+	–	+	+	+	+	+
L. tuplashensis	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. acutifolia	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+

(Lf-1, Lf-30 and Lf-34) deviated significantly ($P < 0.001$) from Hardy–Weinberg equilibrium, and the observed heterozygosity for each locus is lower than the expected heterozygosity, suggesting the presence of null alleles and/or a Wahlund effect. Observed heterozygosity and PIC values are generally high (ranging from 0.175 to 0.750 and from 0.218 to 0.889, respectively). Additionally, the number of alleles per locus ranged from three to 16, so the primer pairs should be useful in population genetics studies in this species. The microsatellite PCR primers were also tested for cross-species amplification in nine species of *Lesquerella* and in one species of the closely related genus *Physaria* (Table 2). For all of these amplifications, when the primers amplified products, only one locus per sample was observed and the resulting loci were always near the expected size range. The high percentage of cross-species amplification of these primer pairs suggests that they might be suitable in assessing genetic diversity among the roughly 90 species of *Lesquerella* and 25 species of *Physaria* in North America (Rollins 1993).

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