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PRIMER NOTE

EST and random genomic nuclear microsatellite markers for *Streptocarpus*

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Abstract

Microsatellite markers have been developed from standard enriched genomic libraries and a cDNA library for the genus *Streptocarpus*. Out of 15 loci derived from ESTs (expressed sequence tags), four gave working primer pairs, with expected heterozygosities (H_E) ranging from 0.42 to 0.86. Out of 89 genomic library derived loci, 6 gave working primer pairs, with H_E ranging from 0.63 to 0.93.

Keywords: cDNA, microsatellite, *Streptocarpus*

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Streptocarpus Lindl. (Gesneriaceae) is a herbaceous genus containing approximately 150 species, of which the vast majority occur in Africa and Madagascar. It exhibits marked vegetative flexibility and includes species with caulescent and acaulescent growth forms; basically the acaulescent forms can be either unifoliate (growing a single leaf derived from an enlarged cotyledon), or rosulate (a rosette-like form with several leaves) (Hilliard & Burtt 1971). The genus also possesses a range of floral types which probably reflect several different pollination syndromes (Harrison *et al.* 1999).

A molecular phylogeny of the genus suggests a north–south migration for the genus in Africa, with southern Africa witnessing a recent burst of speciation (Möller & Cronk 2001). The majority of *Streptocarpus* species are restricted to primary forests, which are currently highly fragmented in many parts of the genus' range, especially in southern Africa. A suite of microsatellite markers has been developed for *Streptocarpus* in order to study the population genetics of selected species to gain insight into the evolutionary effects of forest fragmentation, pollination syndrome changes and hybridization.

Loci were isolated from two types of library; an enriched genomic library and a cDNA library. The cDNA library was used as it is planned to apply the loci to a wide range of species, and it has been reported that EST-derived loci are likely to be more transferable due to greater sequence conservation in the regions flanking the microsatellite (Scott 2001).

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Loci were isolated from enriched genomic DNA libraries of five *Streptocarpus* species following Hughes *et al.* (2002). The libraries averaged an enrichment level of 60%, and 89 pairs of primers were designed using PRIMER 3 (Rozen & Skaletsky 2000). PCRs were set up in 10 μL volumes containing 10 ng genomic DNA, 1 × PCR buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20], 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM of each primer and 0.5 units of *Taq* (Bioline), and were thermocycled as follows: 5 min at 95 °C; 30 cycles of 95 °C (15 s), 55 °C (20 s), 72 °C (15 s); 72 °C for 15 min, and finally cooling to 4 °C. Profiles were checked by including 1 μM FAM or JOE labelled dCTP (PerkinElmer) in the PCR and analysing the products on an ABI 377 DNA sequencer. Primers giving profiles that could be identified as polymorphic single loci were end labelled with either FAM, JOE or TAMRA fluorescent dyes for large scale screening.

Loci derived from cDNA were isolated following the protocol in Woodhead *et al.* (2003), using mRNA extracted from petals and roots of *S. primulifolius*. 687 colonies were successfully sequenced, and 20 of these sequences (3%) contained a unique uninterrupted microsatellite region of at least 5 di- or tri- nucleotide repeats, with $(AT)_n$ being the most common motif. 15 pairs of primers were designed and tested as above, with 11 of these giving a PCR product of the expected size, and four of these giving a single copy scoreable profile. Considering both the genomic and EST-derived loci, a total of 10 working primer pairs have been developed for *Streptocarpus* (Table 1). Locus IB511 was tested on 40 individuals of *S. ibityensis* from Madagascar;

Table 1 Primer sequences and locus characteristics for 10 *Streptocarpus* microsatellite markers

Locus	Repeat in clone	Primer sequences	Size range (bp)	No. alleles	H_E	H_O	homology (Acc. No.)*	Putative location	Putative Cross-transferability	GenBank no.
StrepPR239†	$(AC)_{10}(AT)_{12}$	CATGCTATTGTATGTCGAACT §AAATACATGCATTACATTAACT	163–193	14	0.86	0.43***	Unknown	NA	1,2,3,4,5,6,7	AY425968
StrepPR241†	$(AC)_{10}$	§AAGTCCATTCCACGTGCTTCT GAAAAGGGTTGAAATCTTGACAGG	189–225	21	0.89	0.44***	Unknown	NA	1,2,3,4,5,8,9	AY425969
StrepDN110†	$(CT)_{10}$	TGTGCATCACTTGAAGCTTGT §ACGCACGAGATGCACTTCTAT	211–232	8	0.63	0.30***	Unknown	NA	1,2,3,4,5,6,7,8,10	AY425970
StrepIB511†	$(AC)_{11}$	TATTTACATGGTGTGGCCGAT §AAACACGCGCTATTCATCGT	210–216	3	0.64	0.48***	Unknown	NA	6,7,9	AY425973
StrepJH448†	$(AC)_{10}(AT)_{10}$	AAATCTGCTGTAGTAGTGGGC TTTGAAATGGTCGTTCACACTT	207–244	16	0.87	0.44***	Unknown	NA	1,2,3,4,6	AY425972
StrepJH432†	$(AT)_9(GT)_{11}$	§ACACTCATCAAACGGCCTTC TCCTAGCAAATGGATCCCAA	217–305	34	0.93	0.61***	Unknown	NA	1,2,3,4,5,6,7,9,10	AY425971
StrepCtg16‡	$(AT)_7G(TA)_4$	ATAATTCACGTACATTGGCG §ATCAGCCCTTCCACAGATTG	167–187	6	0.64	0.30***	Lipid transfer protein (AAK01293.1)	3′UTR¶	1,2,3,4,5,7,9,10	AY425977
StrepB22‡	$(TA)_5(AT)_3$	§GAGTTCAACATGAACCCGAGA GGCATCAACTACACATTATCAGGA	226–255	9	0.77	0.26***	Unknown	3′UTR	1,2	AY425975
StrepD14‡	$(AT)_{14}$	GACTCCTTTCGAGAAATTGCC §CTGTGCTTTCCTTGCTTTACG	250–268	17	0.86	0.67***	Unknown	Unknown	1,2,3,4,5,6,7,8,9,10	AY425976
StrepK17‡	$(AT)_6$	§AAGCTTCAATGGCAGTCTCAC CTATACATCGCAATCCACCC	261–268	4	0.42	0.15***	Mitochondrial ATP synthase (CAC81058.1)	3′UTR	1,2,3,4,5,6,7,8,9	AY425974

†Enriched library derived loci.
‡ESTderived loci.
§Labelled primer.
¶3′-untranslated region.
*Homologue searches performed using BLAST (Altschul *et al.* 1997).
***$P < 0.001$.
Cross transferability: 1, *S. primulifolius*; 2, *S. johannis*; 3, *S. polyanthus*; 4, *S. dunnii*; 5, *S. daviesii*; 6, *S. micranthus*; 7, *S. ibityensis*; 8, *S. inflatus*; 9, *S. thompsonii*; 10, *Saintpaulia velutina*.

the remainder of the loci were tested on 96 individuals of *S. primulifolius* from South Africa. There was no clear-cut difference in the number of species the two types of loci transferred to. Out of 36 possible pairwise comparisons between the nine loci applied to *S. primulifolius*, none showed significant linkage disequilibrium. The significant deviations from Hardy–Weinberg equilibrium are likely to be due to a high degree of population structure in these poorly dispersed outcrossing plants.

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