Molecular Ecology Notes (2002) **2**, 554–556 doi: 10.1046/j.1471-8278 .2002.00316.x

PRIMER NOTE

Microsatellite loci for studies of population differentiation and range expansion in *Solidago sempervirens* L. (Asteraceae)

A. M. WIECZOREK* and M. A. GEBER
Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY — 14853—2701, USA

Abstract

For studies of population differentiation and range expansion in the seaside goldenrod, *Solidago sempervirens*, we identified hypervariable molecular markers by screening genomic libraries enriched for microsatellite motifs. We designed primers that reliably amplified nine polymorphic loci. High polymorphism in a population from Delaware Bay, USA suggests that the loci will be useful in population studies. The success of cross-amplifications in 11 species of Asteraceae varied among loci and did not appear to reflect phylogenetic relationships within *Solidago*.

Keywords: cross-species amplification, Goldenrod, polymorphism

Received 9 May 2002; revision received 12 Aug 2002; accepted 12 Aug 2002

Solidago sempervirens is an Atlantic coastal species with a distribution from Nova Scotia to the Carribean. Over the last 40 years, its range has expanded inland in the northern USA (Swink 1974; Catling & McKay 1980; Innes & Hermanutz 1988; Brauer & Geber 2002). In order to reconstruct the routes of range expansion and to study the effects of expansion on genetic population structure, we identified microsatellite loci in *S. sempervirens*.

An enriched genomic library was prepared according to the protocol of Kijas *et al*. (1994). Genomic DNA from plants from an inland site in Watkins Glen, New York (Brauer & Geber 2002) was digested with SAU 3A1, and size-selected by excision from agarose gels (300–1000 base pairs). All loci were cloned following methods of Castleberry *et al*. (2000). Magnagraph nylon membranes (Micron Separation, Inc.) containing lifted colonies with inserts were probed with a series of di-, tri-, and tetranucleotide repeat probes. Positive clones were detected by chemiluminescence (Lifecodes Corp), amplified with vector-specific primers and sequenced directly with dGTP BigDye terminator cycle sequencing components on an ABI Prism® 377 or 310 (Applied Biosystems). Primers were designed with PRIMER 3.0 program (Rozen & Skaletsky 1998) for flanking regions of 32 microsatellites. Following optimization, 20 loci reliably yielded specific polymerase chain reaction (PCR) product of good concentration.

We screened 96 individuals from a population in Woodland Beach, Delaware Bay, DE, for microsatellite variability. Total genomic DNA was isolated from frozen samples using Qiagen DNeasy Plant Mini and Maxi Kits. PCR amplifications were performed in 10 μL volumes on an MJ Research PCR machine (model PTC-100), as follows: 5 min denaturation at 95 °C; 34 cycles of 30 s at 95 °C, 30 s at specific annealing temperatures, and 45 s at 72 °C; and a final extension of 72 °C for 30 min (Table 1). Reactions contained ~100 ng of DNA, 0.25 pmoles of each primer, 0.25 mM dNTPs, 10 mM Tris-HCL pH 8.3, 50 mM KCL, 1.0–1.5 mM $MgCl_2$, and 0.5 U *Taq* polymerase (Roche Diagnostic) (specific $MgCl_2$ concentrations an Ta are shown in Table 1). Forward primers were 5′-labelled with a fluorescent dye (HEX, NED or 6-FAM) for analysis on the automated sequencer. Genescan500ROX was used for fragment sizing with GENESCAN vs. 3.1 and GENOTYPER vs. 2.1 (PE Biosystems).

Nine of the 20 loci were polymorphic. Allele number ranged from six to 25 (average = 13.5); observed heterozygosity (H_O) ranged from 0.309 to 0.671 (average = 0.577), and expected heterozygosity (H_E) ranged from 0.389 to 0.864 (average = 0.762) (Table 1). The high levels of

Correspondence: A. M. Wieczorek. *Present address: Department of Tropical Plant and Soil Science, University of Hawaii at Manoa, Honolulu, HI 96744, USA. E-mail: ania@hawaii.edu

© 2002 Blackwell Science Ltd

Table 1 Primers, amplification conditions, and measures of polymorphism for *Solidago sempervirens* microsatellite loci

Locus name	Primer sequence (5′–3′)	Core motif	Size range (bp)	T_a (°C)	[$MgCl_2$]	No. of alleles	H_O	H_E	GenBank
SS24F	F-AGCTTTTCTTCGCCATTTCCTTCC R-AATTTGGTTACTGGGTTTTCTTGA	$(CAT)_8$	156–222	59	1.5	16	0.478	0.791	AF506908
SS1B	F-TTCCTGAAGAAGCTTCGCATA R-CAGCAGCATGCATTCCATAA	$(GTA)_8$	156–210	58	1.5	16	0.440	0.804	AF506907
SS5A	F-GACGGTCCCATGCCTATCC R-TGGTTTACACTTTCTTTTCTTTCTTTATTT	$(GAA)_7$	258–348	58	1.5	25	0.549	0.864	AF506905
SS19D	F-CATTTGCCTTCAAACCATGA R-CAATTGACACATCATTCGCC	$(CA)_2(GA)(CA)(GA)(CA)_8$	125–183	58	1.5	9	0.373	0.511	AF506911
SS4G	F-TGTGACAGCTTGTTAACTTTATACTGA R-CACCCCCTTTCCAAATATGA	$(CT)_{10}$	171–227	46	1.5	10	0.309	0.389	AF506906
SS19C	F-TTAATTGAAAACCCAGATG R-ACAAACCGATAGTGATACG	$(GAT)_{11}(GAC)(GAT)_4$	252–285	46	1.5	7	0.587	0.718	AF506909
SS20E	F-CACACAGACACTCAAAGCTTCA R-ACCCGCCCTAAAAATAAAGA	$(TA)_4(TG)_{12}$	273–299	50	1.0	12	0.671	0.843	AF506912
SS4F	F-ACACGTGGACCAGGTAAAGC R-CGCGAAGAACAGCAATACAA	$(CTT)_7$	168–192	50	1.0	6	0.613	0.575	AF506913
SS3C	F-CATTTGTCCCCCACTCTTATTTTC R-TTGTTTTGAGGAGGTGGGTTTTAT	$(CT)_{12}$	200–256	50	1.0	7	0.598	0.602	AF506910

T_a: annealing temperature; H_O: Total observed heterozygosity; H_E: Total expected heterozygosity.

Table 2 Cross–species amplification of nine *Solidago sempervirens* microsatellites. Sectional, subsectional and series affiliations of *Solidago* species are from (Zhang 1996)

Species	Section	Subsection	Series	SS5A	SS4G	SS1B	SS24F	SS19C	SS3C	SS19D	SS20E	SS4F
Congeners												
S. sempervirens	Solidago	Maritimae	Maritimae	+	+	+	+	+	+	+	+	+
S. rugosa	Solidago	Maritimae	Venosae	–	–	+	–	+	+	+	+	+
S. altissima	Solidago	Triplinerviae	Trinerves	–	–	–	–	+	+	+	+	+
S. juncea	Solidago	Triplinerviae	Junceae	–	–	–	+	–	+	+	+	+
S. bicolor	Solidago	Triplinerviae	Erectae	–	–	–	–	–	+	–	+	+
S. patula	Solidago	Triplinerviae	Erectae	–	–	+	+	–	–	+	+	+
S. squarrosa	Solidago	Triplinerviae	Erectae	–	–	+	–	–	–	+	+	+
S. caesia	Glomeruliflorae	Glomeruliflorae		–	–	+	+	+	+	+	+	+
S. flexicaulis	Glomeruliflorae	Glomeruliflorae		–	–	–	–	+	+	+	+	+
S. nemoralis	Nemorales		Nemorales	–	–	–	+	–	–	–	–	+
Non-congeners												
Euthamia graminifolia				–	–	–	–	+	–	–	–	–
Aster sp.				–	–	–	–	–	–	–	–	–

'+': Amplification worked; '–': Amplification did not work.

polymorphism suggest that these markers will be valuable for population genetic studies.

Primmer *et al.* (1996) reported that cross-species amplification of microsatellites is possible, especially for closely related species. We attempted to amplify the nine loci in 11 species (four individuals for each species were tested) in the Asteraceae (Table 2). Loci SS5A and SS4G amplified only in *S. sempervirens*, but cross-amplification was more successful for other loci. Based on a recent molecular systematic analysis of the genus *Solidago* (Zhang 1996), the degree of cross-amplification does not appear to reflect phylogenetic relationship.

Acknowledgements

We thank Stephen Bonser for his assistance in developing of the microsatellite markers. This work was funded by a grant from the Andrew Mellon Foundation to MAG.

References

Brauer J, Geber MA (2002) Population differentiation in the range expansion of a native maritime plant, *Solidago sempervirens* L. *International Journal of Plant Science*, **163**, 141–150.

Castleberry SB, King TL, Wood PB, Ford WM (2000) Microsatellite DNA markers for the study of Allegheny woodrat (*Neotoma magister*) populations and cross-species amplification in the genus *Neotoma*. *Molecular Ecology*, **9**, 824–826.

Catling PM, McKay SM (1980) Halophytic plants in Southern Ontario. *Canadian Field Naturalist*, **94**, 248–258.

Innes DJ, Hermanutz LA (1988) The mating system and genetic structure in a disjunct population of the seaside goldenrod *Solidago sempervirens* L. (Asteraceae). *Heredity*, **61**, 447–454.

Kijas JMH, Fowler JCS, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from the citrus genome using biotynilated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *Biotechniques*, **16**, 187–193.

Primmer CR, Moller AP, Ellegren H (1996) A wide-range survey of cross-species microsatellite amplification in birds. *Molecular Ecology*, **5**, 365–378.

Rozen S, Skaletsky HJ (1998) *PRIMER 3*. Code available at *http://www-genome.wi.mit.edu/genome_software/other/primer3.html*.

Swink F (1974) *Plants of the Chicago Region*, 2nd edn. Morton Arboretum, Lisle, Illinois, USA.

Zhang J (1996) A molecular biosystematic study of North American *Solidago* and related genera (Asteraceae: Astereae) based on chloroplast DNA RFLP Analysis. PhD Dissertation, University of Waterloo, Canada.