GENUS-WIDE MICROSATELLITE PRIMERS FOR THE GOLDENRODS (Solidago; Asteraceae)  

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The ca. 120 species of goldenrod (Solidago L.; Asteraceae) are largely confined to North America and occupy an impressive array of habitats, including tundra, rock outcrops, bogs, sand dunes, prairies, barrens, rockhouses, and a variety of woodlands (Semple and Cook, 2006). This taxonomic and ecological diversity has led to Solidago’s popularity as a study system in evolution and ecology. Microsatellite, or simple sequence repeat (SSR), markers could represent a valuable tool in many of these instances, for example, allowing for the estimation of kinship, the identification of invasive genotypes, and the estimation of gene flow among populations.

Microsatellite data could also help clarify Solidago species boundaries. The taxonomic complexity of the genus is widely recognized, a problem stemming from sheer species richness, low overall levels of genetic differentiation, occasional interspecific hybridization, and frequent polyploidy (Semple and Cook, 2006). An accurate delimitation of Solidago species would provide a robust account of biodiversity in the genus and enhance the evolutionary and ecological studies noted above. Given the low overall genetic divergence among Solidago species (Schilling et al., 2008), it should be possible to identify SSR loci that amplify in most species, providing a standard comparative genetic toolkit for the genus.

METHODS AND RESULTS

Silica-dried tissue from a diploid individual of S. gigantea Aiton (confirmed by a meiotic chromosome count) was collected in Chester County, Tennessee, USA. A voucher specimen for this collection (Beck 1258) has been deposited at the Wichita State University Herbarium (WICH). Total DNA was extracted with a DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). An Illumina paired-end shotgun library was prepared by shearing 1 μg of DNA using a Covaris S220 ultrasonicator (Covaris, Woburn, Massachusetts, USA) and following the standard Illumina TruSeq DNA Library Kit protocol (Illumina, San Diego, California, USA) using a multiplex identifier adapter index. Sequencing was conducted on the Illumina HiSeq 2000 with 100-bp paired-end reads. Five million of the resulting reads were analyzed with the program PAL_FINDER_v0.02.03 (Castoe et al., 2012) to extract those reads that contained di-, tri-, tetra-, penta-, and hexanucleotide SSRs. Once positive reads were identified in PAL_FINDER_v0.02.03, they were batched to a local installation of Primer3 version 2.0.0 (Rozen and Skaletsky, 2000) for primer design. To avoid targeting multiple-copy loci, only those for which either primer sequence occurred one or two times in the 5 million reads were selected. A total of 1888 loci met this criterion.

To select a set of loci for initial screening, we focused on loci with tetra- and trinucleotide repeat motifs and with primer melting temperatures between 55°C and 65°C. Furthermore, loci were targeted for which only one of the paired-end reads sequenced into the repeat motif to avoid relatively small fragment sizes. Using these criteria, 80 loci were chosen for initial screening using a “CAG-tag” strategy similar to the M13 approach in Schuelke (2000). The forward primer from each locus was 5’ modified with an engineered “CAG-tag” sequence (5’-CAGTCGGGCCGTATCA-3’) to enable use of a third, fluorescently labeled primer (identical to the CAG-tag) in PCR. In addition, the “PIG-tail” primer sequence (5’-PICKY-3’) was used to allow cycling optimization in the multiplex.

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sequence GTTT was added to the 5′ end of the reverse primer to reduce double peaks. Reactions (10 μL) included 1× Promega GoTag Buffer (Promega Corporation, Fitchburg, Wisconsin, USA); 0.2 mM each dNTP; 2.5 mM MgCl₂; 0.025 μg bovine serum albumin (BSA); 0.5 U Promega GoTag; 0.4 μM unlabeled primer; 0.04 μM CAG-labeled primer; 0.4 μM labeled CAG-tag, and ca. 30 ng DNA template. PCR involved the touchdown cycling protocol outlined in Lance et al. (2010). CAG-tag screening included DNA extracted from eight herbarium specimens representing four of the subsections of Solidago sect. Solidago (Solidago subsect. Triplinerviae (Torrey & A. Gray) G. L. Nesom, Solidago subsect. Glomeruliflorae (Torrey & A. Gray) A. Gray, Solidago subsect. Squarrosae A. Gray, and Solidago subsect. Junceae (Ryd.) G. L. Nesom) and a sample of Brintonia disoidea (Elliott) Greene, representing a monotypic genus potentially sister to Solidago (Schilling et al., 2008). Full details for these three species are provided in Appendix 1.

Fourteen loci (Table 1) were identified as variable, interpretable, and broadly amplifiable across the four tested Solidago subsections and outgroup Brintonia Greene. These loci were then further evaluated in a larger set of diploid individuals from Solidago subsect. Triplinerviae (47 samples representing 10 species), Solidago subsect. Squarrosae (47 samples representing 10 species), and Solidago subsect. Junceae (32 samples representing seven species). Full speciﬁmum details are provided in Appendix 1. All 126 samples were extracted from herbarium specimens archived at the University of Waterloo Herbarium (WAT), the University of Tennessee Herbarium (TENN), the Duke University Herbarium (DUKE), or the Missouri Botanical Garden Herbarium (MO) using the modiﬁed cetlytrimethylammonium bromide (CTAB) protocol detailed in Beck et al. (2012). Forward primers (minus the CAG-tag) were dye labeled with either 6-FAM or HEX, while reverse primers remained unlabeled. The primers for the 14 loci were designed based on the observations from microsatellite screening in Beck et al. (2012) and subsequent studies (Zhao et al., 2012; Sakata et al., 2013).

**CONCLUSIONS**

The general transferability, single-copy status, and variability of these loci suggest that primers designed for a single Solidago species should be applicable across the genus. Screening of the 14 SSR loci described here and those previously reported for S. sempervirens L. (Wieczorek and Geber, 2002), S. canadensis L. (Zhao et al., 2012), and S. altissima L. (Sakata et al., 2013) should therefore provide a set of ≥20 informative SSR loci for any goldenrod species. These loci were also readily amplifiable from herbarium specimens of a wide age range (1932–2007, Appendix 1), creating opportunities for the broad inclusion of archived museum material in future studies.

**Table 1. Characteristics of 14 loci broadly amplifiable in Solidago.**

| Locus | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) |
|-------|-------------------------|--------------|-----------------------|
| Sg_1  | F: GCCTAGCTTTATTTATTTGATATTCATTATTAACCNG | (TTGG) | 116–153 |
|       | R: ACGATGGGCTTCCCCTGTCGATG |                 |                       |
| Sg_2  | F: TCTAAACCTGATAGCTTGTGATGAAAACC | (ATG) | 167–248 |
|       | R: GCCGTCCATCTTACTCCAA |                 |                       |
| Sg_3  | F: TTGAAAGATCAATGTTGCTTCCACC | (AAC) | 92–182 |
|       | R: GTTTAACAATCTTGTACCTGACTGCG |                 |                       |
| Sg_4  | F: CAATCTGTGATTTAACTTACCTTCCC | (TTCC) | 104–201 |
|       | R: GTTGCCCTTTAATGGAATTCGATCC |                 |                       |
| Sg_5  | F: TTGTCTCGGATGGAATTCGATGCC | (TTC) | 256–296 |
|       | R: GATTTAACAATGGAATTCGATCC |                 |                       |
| Sg_6  | F: TTTACCCTTGAAAGCTGCCC | (AAAT) | 200–244 |
|       | R: GATTTAAGCATCAATGAACTGATGCC |                 |                       |
| Sg_7  | F: TTGTGATCAGTTGCAAGGCGG | (AAAG) | 360–378 |
|       | R: GTTTACAGCTGCAAATGATCCC |                 |                       |
| Sg_8  | F: TCGTCTTTTCTTTTTCTACCAACCC | (AAAG) | 126–172 |
|       | R: GATTTAACAATCTTGTACCTGACTGCG |                 |                       |
| Sg_9  | F: GACGGTCGCTAATATTAGGTGTAAGC | (AATG) | 170–190 |
|       | R: GTTGCAACAGTCAATGAACTGATGCC |                 |                       |
| Sg_10 | F: CTGGTCCTTTTCTTTCTACCAACCC | (ATCT) | 276–330 |
| Sg_11 | F: GATTTGTATTTTTTTCTACCAACCC | (AAAT) | 119–155 |
| Sg_12 | F: CTAGAAGATTGATGATTCGACCC | (AAAT) | 182–208 |
| Sg_13 | F: TTGAAAGTTGGAATTTAGGTGTAAGC | (AAC) | 153–172 |
| Sg_14 | F: ACCCTGTGTTGTGTGATTTGTAATAGG | (AAC) | 317–355 |

1. Paired-end sequence data are deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.72p7k (Beck et al., 2014).
2. A multiplex amplification protocol incorporating a single annealing temperature (see text) was used for all loci.
3. Nucleotides added to create PIG-tail are noted in boldface for relevant primers.
4. Total repeat motif number is not reported because it could not be determined whether paired-end reads sequenced through the entire repeat region.
5. Full size range across the three Solidago subsections evaluated in the broad analysis.

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TABLE 2. Number of alleles, size range, and amplification success in three Solidago subsections. Loci successfully amplified in all taxa are shown in bold.

| Locus | Allele size range (bp) | Amplification success a |
|-------|-----------------------|------------------------|
| Sg_1  | 121–144               | 10/10                  |
| Sg_2  | 167–226               | 10/10                  |
| Sg_3  | 92–182                | 10/10                  |
| Sg_4  | 127–201               | 10/10                  |
| Sg_5  | 116–153               | 7/7                    |
| Sg_6  | 116–153               | 27/27                  |
| Sg_7  | 168–182               | 3/10                   |
| Sg_8  | 126–172               | 10/10                  |
| Sg_9  | 171–209               | 7/7                    |
| Sg_10 | 200–244               | 27/27                  |
| Sg_11 | 119–143               | 10/10                  |
| Sg_12 | 122–155               | 10/10                  |
| Sg_13 | 122–155               | 10/10                  |
| Sg_14 | 122–155               | 10/10                  |

Note: A = number of alleles. Number of taxa with successful amplification/number of taxa attempted.

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Appendix 1. Sampling information for the eight individuals used in CAG-tag screening followed by the 126 individuals analyzed in the broader survey of locus transferability. Information presented: taxon, sample number, collector and number, herbarium, country: state/province/region, year collected.

**Solidago altissima L.**, S206, Semple 11415, WAT, USA: Nebraska, 2006.

**Solidago caesia L.**, S351, Semple 10778, WAT, USA: Kentucky, 1999.

**Solidago gigantea Aiton.** S208, Cook C-456, WAT, USA: Iowa, 2001. S215, Semple 10165, WAT, USA: Mississippi, 1991. S217, Semple 9620, WAT, USA: Kentucky, 1991.

**Solidago pinetorum Small.** S536, Semple 11625, WAT, USA: North Carolina, 2006.

**Solidago squarrosa Muhl.** S384, Semple 11529, WAT, USA: New Brunswick, 2006.

**Brintonia discoidea (Elliott) Greene.** S298, Semple 11194, WAT, USA: Alabama, 2003.

**Solidago subsect. Junceaes (Ryd. B.) G. L. Nesom**

**Solidago confinis A. Gray.** S506, Semple 8984, WAT, USA: California, 1987. S507, Semple 9632, WAT, USA: California, 1990. S508, Semple 9347, WAT, USA: California, 1990. S510, Semple 8970, WAT, USA: California, 1987. **Solidago gettngerei Chapm. ex A. Gray.** S521, Semple 5208, WAT, USA: Missouri, 1980. S522, Dietrich 49, MO, USA: Missouri, 1994. S524, McNeils 93-1443, TENN, USA: Tennessee, 1993. S525, Nordman s.n., TENN, USA: Tennessee, 2000. S526, Baily s.n., TENN, USA: Tennessee, 2000. **Solidago guiradonis A. Gray.** S502, Semple 9356, WAT, USA: California, 1990. S503, Semple 9351, WAT, USA: California, 1990. S504, Semple 9355, WAT, USA: California, 1990. S505, Semple 9352, WAT, USA: California, 1990. **Solidago juncea Aiton.** S540, Semple 10677, WAT, USA: Pennsylvania, 1999. S542, Semple 4897, WAT, Canada: Nova Scotia, 1980. S543, Semple 2757, WAT, USA: Missouri, 1977. S544, Semple 2759, WAT, USA: Michigan, 1977. **Solidago missouriensis Nutt.** S527, Semple 7699, WAT, USA: Colorado, 1985. S528, Semple 9195, WAT, USA: Nebraska, 1990. S530, Semple 9267, WAT, USA: Utah, 1990. S531, Semple 8844, WAT, USA: Wisconsin, 1987. S532, Semple 9387, WAT, USA: New Mexico, 1990. S534, Semple 2669, WAT, Canada: Manitoba, 1977. **Solidago pinetorum Small.** S535, Semple 11122, WAT, USA: North Carolina, 2003. S536, Semple 11625, WAT, USA: North Carolina, 2006. S537, Semple 11599, WAT, USA: North Carolina, 2006. S538, Semple 9734, WAT, USA: North Carolina, 1991. **Solidago spectabilis (D. C. Eaton) A. Gray.** S511, Semple 8717, WAT, USA: California, 1986. S512, Semple 9301, WAT, USA: California, 1990. S513, Semple 9299, WAT, USA: California, 1990. S516, Semple 8401, WAT, USA: California, 1986.

**Solidago subsect. Squarrose A. Gray**

**Solidago bicolor L.** S385, Semple 10681, WAT, USA: West Virginia, 1999. S389, Semple 5927, WAT, USA: Virginia, 1981. S390, Semple 3487, WAT, USA: Vermont, 1978. S391, Semple 9487, WAT, USA: Pennsylvania, 1991. S393, Semple 6002, WAT, USA: North Carolina, 1981. S398, Semple 3614, WAT, USA: Connecticut, 1978. S400, Semple 4708, WAT, Canada: New Brunswick, 1980. S406, Semple 11472, WAT, Canada: Prince Edward Island, 2006. **Solidago erecta Banks ex Pursh.** S424, Semple 5984, WAT, USA: Virginia, 1981. S425, Semple 11189, WAT, USA: Tennessee, 2003. S428, Semple 9501, WAT, USA: New Jersey, 1991. S429, Semple 9454, WAT, USA: Kentucky, 1990. S433, Semple 6098, WAT, USA: South Carolina, 1981. S434, Semple 10175, WAT, USA: Mississippi, 1991. **Solidago hispida Muhl.** S408, Semple 3638, WAT, USA: New York, 1978. S411, Semple 4634, WAT, USA: Maine, 1990. S418, Semple 11065, WAT, Canada: Ontario, 2001. S419, Morton 12474, WAT, Canada: Newfoundland, 1978. S420, Semple 8298, WAT, USA: Arkansas, 1985. **Solidago pallida (Porter) Rydb.** S465, Semple 8082, WAT, USA: New Mexico, 1985. S462, Semple 11304, WAT, USA: South Dakota, 2004. S464, Semple 11401, WAT, USA: Wyoming, 2006. **Solidago pubera Fruct.** S437, Semple 11625, WAT, USA: North Carolina, 2006. S440, Kral 44276, WAT, USA: Alabama, 1971. S441, Semple 10137, WAT, USA: Florida, 1991. S442, Semple 9813, WAT, USA: South Carolina, 1991. S445, Cook C-118, WAT, Canada: Quebec, 2000. S448, Semple 7628, WAT, USA: Maryland, 1984. S451, Semple 6867, WAT, USA: Massachusetts, 1982. S452, Semple 10815, WAT, USA: North Carolina, 1999. **Solidago rigidula (Torr. & A. Gray) Porter.** S466, Semple 10062, WAT, Canada: Ontario, 1997. S467, Semple 4532, WAT, USA: Indiana, 1979. S468, Semple 9121, WAT, USA: Tennessee, 1986. S469, Semple 5063, WAT, USA: Wisconsin, 1980. **Solidago roanensis Porter.** S455, Cook C-332, WAT, USA: Tennessee, 2000. S457, Cook C-537, WAT, USA: North Carolina, 2001. S458, Semple 9658, WAT, USA: North Carolina, 1991. S459, Poindexter 05-1580, WAT, USA: North Carolina, 2005. **Solidago scoposess Nutt.** S470, Semple 6180, WAT, USA: South Carolina, 1981. S471, Semple 11613, WAT, USA: Virginia, 2006. **Solidago squarrosa Muhl.** S371, Semple 2426, WAT, Canada: Ontario, 1976. S375, Semple 4660, WAT, USA: Maine, 1980. S379, Semple 3692, WAT, Canada: Ontario, 1978. S383, Cook C-125, WAT, Canada: Quebec, 2000. S384, Semple 11529, WAT, Canada: New Brunswick, 2006. **Solidago villosicaarpa LeBlond.** S460, Semple 11645, WAT, USA: North Carolina, 2006. S461, Semple 11637, WAT, USA: North Carolina, 2006.