Molecular Ecology Resources (2009) **9**, 579–581 doi: 10.1111/j.1755-0998.2008.02400.x

PERMANENT GENETIC RESOURCES NOTE

Isolation and characterization of 11 microsatellite markers from *Sagittaria latifolia* (Alismataceae)

SARAH B. YAKIMOWSKI,* PAUL D. RYMER,†‡ HARRIET STONE,† SPENCER C. H. BARRETT* and MARCEL E. DORKEN†§

**Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada M5S 3B2, †Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, UK, ‡Imperial College London, Silwood Park Campus, Ascot SL5 7PY, UK, §Department of Biology, Trent University, Peterborough, Ontario, Canada K9J 7B8*

Abstract

We developed 11 microsatellite loci for *Sagittaria latifolia*, an aquatic plant common to wetlands of North America. From an (AG)-enriched library, we identified 66 unique microsatellite sequences for which primers could be designed. Twenty-two loci reliably amplified a clear single band of expected size, and 11 loci were scoreable and polymorphic. For these 11 loci, we genotyped a monoecious and a dioecious population, yielding four to 14 alleles per locus. Three loci exhibited significant linkage disequilibrium leaving eight independent variable loci. Eight loci also amplified in four other *Sagittaria* species. These microsatellite loci will be useful to compare genetic structure among monoecious and dioecious populations of *S. latifolia*.

Keywords: Alismataceae, dioecy, microsatellite, monoecy, *Sagittaria latifolia*, SSR

Received 29 April 2008; revision accepted 9 June 2008

Sagittaria latifolia (Alismataceae) is a common clonal aquatic plant of North American wetlands, and a popular pond ornamental (commonly known as broadleaf arrowhead). The species exhibits considerable sexual system diversity, with populations ranging from monoecy, through subdioecy to dioecy and frequently contain mixtures of hermaphroditic, female and male plants (Wooten 1971; Sarkissian *et al*. 2001). This variation provides opportunities to investigate the ecological and genetic factors associated with the evolution and maintenance of gender strategies. Monoecious and dioecious populations of *S. latifolia* often occur in close geographical proximity, but although interfertile, are generally reproductively isolated as a result of ecological and life-history differentiation (Dorken & Barrett 2003). Our aim here was to characterize polymorphic microsatellite (simple sequence repeat, SSR) markers to facilitate future work on mating systems and population structure.

We extracted genomic DNA from fresh leaf tissue of a single F_2 individual from a full-sib cross between the F_1 progeny of a cross between plants from a monoecious and a dioecious population (from 'Guelph' and 'Downie's'; Dorken & Barrett 2003) using DNeasy plant mini kit (QIAGEN). The microsatellite-enriched library was constructed using the protocol described by Glenn & Schable (2005) with the following modifications. Approximately 2 μg of genomic DNA was digested with *Rsa*I (New England Biolabs), then immediately ligated with double-stranded SuperSNX linkers. Ligated DNA was hybridized with an $(AG)_{12}$, biotinylated probe with a final wash at 55 °C. The polymerase chain reaction (PCR)-amplified enriched product was cloned using TOPO TA cloning kits (Invitrogen).

We amplified and sequenced 480 colonies from the (AG)-enriched library directly from LB culture using universal M13 primers (following PCR and sequencing from Glenn & Schable 2005). Sequences were visualized using an Applied Biosystems 3730xl capillary electrophoresis system. We identified 92 unique sequences containing an SSR using the TROLL module in Pregap4 (version 1.5 Staden Package; Martins *et al*. 2006), which were sequenced in both directions.

We designed 66 primer pairs of 150–450 bp in length containing an SSR, using Primer3 (Rozen & Skaletsky 2000) and NetPrimer (Premier BioSoft International) to identify

Correspondence: Sarah B. Yakimowski, Fax: +1-416-978-5878; E-mail: sarah.yakimowski@utoronto.ca

Table 1 Variable microsatellite loci in *Sagittaria latifolia*. Locus designations are given along with GenBank Accession numbers, repeat motifs, forward (F) and reverse (R) primer sequence (5′–3′ direction), allele size range and allele number (*A*). For a monoecious and a dioecious population from southern Ontario (Monoecious CTP-ON: 43.55333°N, 80.23548°W; Dioecious RRM-ON: 42.28952°N, 82.47963°W), expected and observed heterozygosity are provided. The optimal annealing temperature for all loci is 58°C. We successfully assayed each primer pair for 30 individuals per population

Locus GenBank no.	Repeat	Primer sequence (5′–3′)	Allele size range (bp)	*A*	Monoecious CTP-ON		Dioecious RRM-ON	
					H_E	H_O	H_E	H_O
SL06 EU744550	$(AG)_{15}$	F: AGACCGATGCTGAAGGCTAA-FAM R: GTTGGTGGTCTATGGGAGCA	410–430	7	0.56	0.33*	0.57	0.80*
SL09 EU744551	$(TC)_{16}$	F: CCCGCTCATGCCTTTAGTAG-HEX R: GGGTTTCAACATGCTTTCCT	272–300	11	0.07	0.07	0.65	0.80
SL21 EU744552	$(CT)_9(CA)_{16}$	F: TGGTCCACTTGATGCCTGT-FAM R: GATGGGCAAGAGTGATTCCA	349–363	5	0.21	0.10	0.54	0.20*
SL27 EU744553	$(CT)_{22}$	F: TATGGCGAAGAGGAGAGAGG-FAM R: CCAACTCACATCACGCTACA	294–348	14	0.60	0.73	0.82	1.0*
SL30 EU744554	$(GAA)_8$	F: CTCATCCGAGGTGGAGATATG-HEX R: TGCTTGTAGTGCTTGCCATC	364–376	4	0	0	0.10	0.03
SL31 EU744555	$(CT)_{12}$	F: TGTGAAGAAACACACGAGCA-HEX R: GAGAGGGAAGGAGGAATAAGG	404–446	8	0.54	0.13*	0.64	0.60
SL65 EU744556	$(TC)_{18}$	F: GCCTTGCCATTGTCATCAT-HEX R: GGTGCGACCATTGCTATAAA	236–270	6	0.29	0.33	0.69	0.90*
SL74 EU744557	$(AG)_{22}$	F: GCTCACGCATACATCTCACC-HEX R: TACAATCCCAATACCGACGA	233–293	14	0.76	0.90*	0.73	0.73*
SL75 EU744558	$(TC)_{19}$	F: CGGGTTACTTGGGAGTTTGA-FAM R: ATCGGGATTCCATTCTGTTT	382–416	8	0.63	0.80	0.63	0.83
SL88 EU744559	$(GA)_{20}$	F: TGTCAACATGGCAACCGTTAT-FAM R: CCATACGACCGATTTACAATA	83–177	12	0.57	0.63	0.45	0.40
SL91 EU744560	$(GA)_{17}$	F: TTTGCATTGGGAACTTGAAT-HEX R: ACTCTATGTGTCACCCGATGG	320–340	8	0.70	0.96*	0.67	0.70

*Significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$).

primers 18–25 bp in length with optimal annealing temperature of 60 °C, 40–60% GC content, < 3 repeats, and minimal hairpins and dimers (< 1 and 7 kcal/mol, respectively). Of these, 45 primer pairs exhibited some amplification when subjected to gradient PCR using 0.2 μM of each forward and reverse primer along with 0.15 mM dNTPs, 1× PCR Buffer (20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl (pH 8.8), and 0.01% Tween 20), 2.0 mM $MgCl_2$, 0.25 ng BSA, 0.5 U *Taq* DNA polymerase (Yorkshire Biosciences) and 10 ng of DNA template. PCR was performed using a PTC-220 DNA Engine Dyad Peltier Thermal Cycler, and amplification conditions were 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 52–62 °C for 30 s, 72 °C for 45 s, followed by 72 °C for 20 min. To test the amplification success and quality, we subjected 16 additional *S. latifolia* DNA samples (four samples from each of two dioecious and two monoecious populations) extracted from silica-dried leaf tissue for the 45 primer pairs using the optimal annealing temperature identified in the gradient PCR. Twenty-two of these primer pairs reliably amplified a clear single band of expected size. For these, we performed PCRs for the same 16 *S. latifolia* DNA samples using fluorescently labelled forward primers (FAM, HEX; Applied Biosystems) and sized PCR products on a 3730xl DNA Analyser (Applied Biosystems) alongside GeneScan 500 LIZ Size Standard using GeneMapper Software (version 4.0). Fourteen loci produced scoreable profiles of which 11 were polymorphic.

We genotyped 30 individuals for each of one monoecious (CTP-ON) and one dioecious (RRM-ON) population using the 11 polymorphic loci. All of the loci exhibited polymorphism within populations, with the exception of SL30, which was monomorphic in CTP-ON but variable in RRM-ON. Across the 11 polymorphic loci, the number of alleles ranged from four to 14 (mean = 8.8). Expected and observed heterozygosity levels were in the range of 0–0.76 (mean = 0.45) and 0–0.96 (mean = 0.45) for CTP-ON, and 0.10–0.82 (mean = 0.59) and 0.03–0.90 (mean = 0.64) for RRM-ON, respectively (Table 1).

We tested for deviations from Hardy-Weinberg equilibrium (HWE) for each locus within each population, and linkage disequilibrium (LD) among all loci within each population, using GenePop (Raymond & Rousset 1995) and a Bonferroni correction for multiple tests (Table 1). Within populations, three loci exhibited significant deviations

Table 2 Amplification of 11 microsatellite loci developed for *Sagittaria latifolia* in monoecious *S. cuneata*, *S. longiloba*, *S. montevidensis* ssp. *montevidensis*, and andromonoecious *S. montevidensis* ssp. *calycina*. All species are widespread in North America except *S. longiloba* which is restricted to the south and western USA. '+' denotes amplification of a band in the same size range as *S. latifolia* and '–' denotes no amplification for two populations per species

Locus	*S. cuneata*	*S. longiloba*	*S. montevidensis* ssp. *montevidensis*	*S. montevidensis* ssp. *calycina*
SL06	+ –	+ –	+ +	+ +
SL09	+ +	+ +	+ +	+ +
SL21	+ +	+ +	+ +	+ +
SL27	– –	– –	– –	– –
SL30	– +	– –	– –	– –
SL31	+ +	– +	+ +	– –
SL65	+ +	+ –	– –	– –
SL74	– –	– –	– –	– –
SL75	+ +	– –	+ +	+ +
SL88	+ +	+ –	– –	+ +
SL91	– –	– –	– –	– –

from HWE involving heterozygote deficiency (–), excess (+), and equal frequency of heterozygotes with a deviation due to excess of one particular heterozygotic genotype (=): CTP-ON SL31(–), SL74(+), and SL91(+), RRM-ON SL21(–), SL65(+), and SL74(=). Observation of both heterozygote deficiency and excess is likely due to population structure, although inbreeding or null alleles may also contribute to heterozygote deficiency. Three loci were found to be in strong LD in both populations (SL06, SL27, and SL75); thus, there are eight unlinked loci. Significant LD was also detected for three pairs of loci for each population (CTP-ON: SL74 & SL88, SL75 & SL91, SL88 & SL91; RRM-ON: SL21 & SL31, SL65 & SL74, SL65 & SL91). However, none of these pairs exhibited significant linkage in both populations; thus, the pattern is likely not due to physical linkage of markers, but rather due to population structure. The detection of population subdivision is consistent with the clonal growth form of *S. latifolia*.

To examine the potential utility of the 11 microsatellite markers in other *Sagittaria* species, we tested the amplification of genomic DNA from two populations, using two samples per population, for each of *S. cuneata*, *S. longiloba*, *S. montevidensis* ssp. *montevidensis*, and *S. montevidensis* ssp. *calycina*, all collected in California except for one *S. cuneata* population from Oregon. Eight of the 11 loci that amplified in *S. latifolia* also amplified a band of similar size in these other *Sagittaria* species (Table 2). Thus, these loci should facilitate the study of population structure and mating-system variation in other *Sagittaria* species.

Acknowledgements

We thank Tiina Sarkinen, Marcelo Simon, and Christina Vinson (Plant Sciences, Oxford University) for technical assistance. This work was funded by an NSERC Discovery Grant to S.C.H. Barrett and a John Fell OUP Research Fund and Career Development Fellowship from the University of Oxford to M.E. Dorken.

References

Dorken ME, Barrett SCH (2003) Life-history differentiation and the maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution*, **57**, 1973–1988.

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

Martins W, de Sousa D, Proite K, Guimaraes P, Moretzsohn M, Bertioli D (2006) New softwares for automated microsatellite marker development. *Nucleic Acids Research*, **34**, e31.

Rozen S, Skaletsky H (2000). Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols in the series Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, NJ.

Raymond M, Rousset F (1995) GenePop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Sarkissian TS, Barrett SCH, Harder LD (2001) Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters? *Ecology*, **82**, 360–373.

Wooten JW (1971) The monoecious and dioecious conditions in *Sagittaria latifolia* L. (Alismataceae). *Evolution*, **25**, 549–553.