Molecular Ecology Notes (2006) **6**, 530–532 doi: 10.1111/j.1471-8286.2006.01308.x

PRIMER NOTE

Isolation and characterization of microsatellites in *Sparganium emersum* and cross-species amplification in the related species *S. erectum*

B. J. A. POLLUX*† and N. J. OUBORG*

*Section of Molecular Ecology, Department of Aquatic Ecology and Environmental Biology, Institute for Wetland and Water Research, Radboud University Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen, The Netherlands, †Department of Plant–Animal Interaction, Centre for Limnology, Netherlands Institute of Ecology (NIOO-KNAW), Maarssen, The Netherlands

Abstract

We developed seven novel polymorphic microsatellite loci for the aquatic macrophyte *Sparganium emersum* (Sparganiaceae). These were characterized on 62 individuals collected from nine different populations. In this set of individuals, seven to 20 alleles per locus were detected and observed heterozygosity ranged between 0.16 and 0.95. Cross-species amplification was tested in the related species *Sparganium erectum*, and was successful for five of the seven microsatellite loci.

Keywords: dispersal, gene flow, microsatellites, null alleles, population genetics

Received 04 December 2005; revision accepted 09 January 2006

Rivers offer special environments for plant dispersal, because of their unidirectional water flow and linear arrangement of suitable habitats. Gaining insight in the functioning of riparian plant populations requires studying the rate and direction of dispersal, as well as the contribution of seed dispersal compared to the dispersal of clonal structures (Ouborg *et al*. 1999).

Unbranched bur-reed, *Sparganium emersum* Rehmann, 1871 (Sparganiaceae), is a monoecious aquatic macrophyte that is commonly found in rivers and streams throughout Eurasia and North America. *Sparganium emersum* is a wind-pollinated species, whose seeds are mainly dispersed by water currents (Boedeltje *et al*. 2004) and, less frequently, by waterfowl species (Pollux *et al*. 2005). The plant reproduces vegetatively via the production of stolons from which new ramets emerge. Occasionally, plant parts may break off, be dispersed by water currents and become successfully established in new locations (Barrat-Segretain & Bornette 2000). These characteristics, common to many other riparian species, make *S. emersum* a useful species for studying the functioning of riparian plant populations in rivers. Here we present the characterization of seven polymorphic microsatellite loci, suitable for the study of gene flow and the genetic structure of *S. emersum* populations in rivers.

Correspondence: B. J. A. Pollux, Fax: +31 (0)24 3653047; E-mail: b.pollux@science.ru.nl

Di-, tri- and tetranucleotide repeat enriched libraries of *S. emersum* genomic DNA were constructed by a selective hybridization procedure (Karagyozov *et al*. 1993), using the method described by Arens *et al*. (2000, 2004) with minor modifications. Genomic DNA of five individuals was isolated using the DNeasy Plant Mini Kit (QIAGEN). Genomic DNA was restricted with *Mbo*I (MBI Fermentas) and size-fractionated by agarose gel electrophoresis. DNA fragments from 300 to 1250 bp in size were extracted from the agarose gels using the QIAEx II Gel Extraction Kit (QIAGEN), and enriched by hybridization to either single di- or trinucleotide repeats $(GA)_{12}$, $(GT)_{12}$, $(AGT)_9$, $(TGA)_9$, $(TGT)_{10}$, $(TCT)_{10}$, a pool of trinucleotides [$(GAG)_8$, $(GTG)_8$, $(CGT)_8$, $(GCC)_7$, $(GCT)_8$, $(TAA)_{12}$)], or a pool of tetranucleotides [$(TGTT)_8$, $(CTAT)_8$, $(GATA)_8$, $(GACA)_8$, $(GGAT)_7$, $(TCTT)_8$]. Enriched fragments were ligated into the pGEM-T Easy Vector System I (Promega) and transformed into *Escherichia coli* DH5α competent cells. Colonies were transferred onto Hybond N+ membranes (Amersham-Pharmacia). Phage filters were probed with a set of ^{32}P-labelled synthetic repeat polynucleotides, and positive clones were identified by autoradiography. Sixty-four positive clones (1.3% of the total number) were obtained, which were all sequenced on a model 4000 L DNA Sequencer (LI-COR) using the SequiTherm EXEL™ II DNA Sequencing Kit-LC (Epicentre Technologies) and IR-800 dye-labelled M13 primers (Biolegio).

Table 1 Characterization of seven microsatellite loci in *Sparganium emersum*, tested on 62 individuals from nine populations in the Netherlands. Primer sequences (F, forward; R, reverse), $MgCl_2$ concentration, annealing temperature (T_a), repeat motif, allele size range, number of alleles per locus (A), observed heterozygosity (H_O) and expected heterozygosity (H_E).

Locus	GenBank Accession no.	Primer sequence (5′–3′)	$MgCl_2$ (mM)	T (°C)	Repeat motif	Size range (bp)	A	H_O	H_E	Cross-species amplification in *Sparganium erectum*
SEM01	DQ304634	F: GTCGGAGCCCTCTGCCTTCA R: TTCATGTAAATTGGTTGTCTTCA	2.5	53	$(GA)_n$	153–171	9	0.754	0.659	+*
SEM05	DQ304636	F: TACACTTTCCTATCCCCATTCA R: CCAAAGCCAAAACAAGATACC	1.25	52	$(GA)_n$	303–349	17	0.952	0.791	++†
SEM08	DQ304638	F: GTGGTGGCGATGGCAATAAT R: CAAGGTAGTGGCGACAAG	2.5	56.4	$(GA)_n$	151–183	14	0.873	0.861	++
SEM12	DQ304640	F: CAGGCCGGTTGGACAGGTAGTT R: GGGAAGAGCAGCCAAGACGAAGTA	1.25	60	$(CTAT)_n(CT)_n$	215–265	9	0.495	0.517	–
SEM14	DQ304642	F: TTCATGTAAATTGGTTGTCTTC R: TCCCCTACTTCCCCTAATCGTTGTC	2.5	60	$(GA)_n$	225–239	7	0.823	0.674	+†
SEM15	DQ304643	F: GGCGTGGACGTGGGTGGTGT R: CATTGGGCTAGTAGGCTTGTTTAT	2.5	61.6	$(GA)_n$	234–274	13	0.794	0.767	++
SEM17	DQ304644	F: CACATACGCCACTGCTTTTT R: TTTTTCCCGCGCTCTCAAC	2.5	60	$(GA)_n$	201–285	20	0.159	0.845	–

Amplification test on three individuals of *S. erectum*: ++, good amplification; +, weak amplification; –, no amplification.
*Indicates polymorphic bands between individuals.
†Indicates individuals with a homozygote band pattern.

Thirteen sequences were selected for microsatellite primer development, eight containing a $(GA)_n$ repeat and the remaining five containing either a $(CA)_n$, $(TCC)_n$, $(GTT)_n$, $(AGT)_n$ or a $(CTAT)_n(CT)_n$ repeat. Primer pairs were designed using PRIMERSELECT (DNAstar) and synthesized by Biolegio. The markers were tested and optimized using different $MgCl_2$ concentrations and annealing temperatures (50–70 °C on a T-Gradient, Biometra). The amplification reactions were performed in a 25 µl volume containing 20 ng template DNA, 1.25 × NH_4 Bioline buffer, 0.25 mM dNTPs, $MgCl_2$ as in Table 1, and 0.05 µM of each primer (forward primers were IR-800 dye-labelled) and 1 U Bio*Taq* Red DNA Polymerase (Bioline). Amplification was carried out in a T3 Thermocycler (Biometra) with the following thermal profile: 1 initial cycle of 5 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 45 s at annealing temperature as in Table 1, and 1 min at 72 °C, followed by a final extension step of 10 min at 72 °C. Fragments were analysed on a model 4200 IR2 DNA Analyser (LI-COR) using the SAGA automated microsatellite analysis software version 2.1 (LI-COR).

Out of 13 tested markers, seven primer pairs gave reproducible well-scorable polymerase chain reaction (PCR) products of the expected size (Table 1). The seven primer pairs were tested on 62 plants collected from nine populations in the Swalm and Rur rivers (the Netherlands). Heterozygosity values for each locus were calculated using FSTAT version 2.9.3 (Goudet 1995). Observed heterozygosities were generally high and deviations from expected heterozygosities small, with the exception of SEM17 (Table 1). The relatively large deviation in SEM17, indicating an excess of homozygotes, could be due to inbreeding, the Wahlunds' effect or the presence of null alleles. However, since the deviations in the other loci are generally small, inbreeding and the Wahlunds' effect are unlikely, suggesting that the locus SEM17 may potentially contain null alleles. The Fisher's exact test for Hardy–Weinberg equilibrium (HWE) using GENEPOP version 3.4 (Raymond & Rousset 1995), showed that three out of eight loci were not in HWE (SEM07, SEM08 and SEM17; $P < 0.05$). A significant genotypic association was found for two out of the 28 pairs of loci: SEM1–SEM12 ($P = 0.001$) and SEM01–SEM08 ($P = 0.003$). Results show that the microsatellites will be a useful tool for studying dispersal and the genetic structure of *S. emersum* populations. Finally, the primers were tested for cross-species amplification on the related species *S. erectum*, using three individuals from two different populations. Successful amplification was observed for five of the seven loci (Table 1).

Acknowledgements

We wish to thank Ramses Rengelink, Maaike de Jong, Erik Verbruggen and Anneke Steegh for their assistance in the laboratory. Paul Arens provided helpful comments to improve the manuscript. This is publication 3755 of the Netherlands Institute of Ecology (NIOO-KNAW).

References

Arens P, Van't Westende W, Bugter R, Smulders MJM, Vosman B (2000) Microsatellite markers for the European tree frog *Hyla arborea*. *Molecular Ecology*, **9**, 1944–1946.

Arens P, Durka W, Wernke-Lenting JH, Smulders MJM (2004) Isolation and characterization of microsatellite loci in *Geum urbanum* (Rosaceae) and their transferability within the genus *Geum*. *Molecular Ecology Notes*, **4**, 209–212.

Barrat-Segretain MH, Bornette G (2000) Regeneration and colonization abilities of aquatic plant fragments: effect of disturbance seasonality. *Hydrobiologia*, **421**, 31–39.

Boedeltje G, Bakker JP, Ten Brinke A, Van Groenendael JM, Soesbergen M (2004) Dispersal phenology of hydrochorous plants in relation to discharge, seed release time and buoyancy of seeds: the flood pulse concept supported. *Journal of Ecology*, **92**, 786–796.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.

Karagyozov L, Kalcheva ID, Chapman M (1993) Construction of random small-insert genomic libraries highly enriched for simple sequence repeats. *Nucleic Acid Research*, **21**, 3911–3912.

Ouborg NJ, Piquot Y, Van Groenendael JM (1999) Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology*, **87**, 551–568.

Pollux BJA, Santamaria L, Ouborg NJ (2005) Differences in endozoochorous dispersal between aquatic plant species, with reference to plant population persistence in rivers. *Freshwater Biology*, **50**, 232–242.

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