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Isolation and characterization of microsatellite loci in the rush *Juncus atratus* (Juncaceae)

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The Black Rush, *Juncus atratus* Krock., is a diploid ($2n = 2x = 40$) member of the Juncaceae, section *Ozophyllum* (Kirschner et al. 2002). It is distributed from subcontinental Europe to western and central Asia. It can be found on seasonal wet or subsaline grasslands and sandy shores (Kirschner et al. 2002). *Juncus atratus* is endangered throughout Central Europe (Schnittler and Günther 1999) where it is reaching the north western range edge. Here, the species occurs mainly on open soils as a so called river corridor plant (Burkart 2001) and grows exclusively along the floodplains of the rivers Elbe and Havel (Burkart 1995). These rare Central European populations are highly dynamic and undergo frequent extinction and colonisation events. Despite *Juncus* being a species rich genus, species specific genetic markers are not available and studies on genetic diversity for *Juncus* species are lacking. Thus, microsatellite markers would be highly desirable tools to study the population genetics of *J. atratus* and related species.

We collected seeds in the field from a *Juncus atratus* population near Havelberg, Gemany and raised plants in the botanical garden of Potsdam. DNA was extracted with the DNeasy extraction kit (QIAGEN) from leaves. After marker development, however, a closer inspection of the adults reared from these seeds revealed some intermediate characters between *J. atratus* and *Juncus articulatus* which grew abundant on the specific site. Also sequencing of the nuclear ribosomal external transcribed spacer (nrETS) region showed intraindividual polymorphisms which may indicate a hybridisation as it is known for the nrITS region (Wissemann 2003). Thus, we can not exclude that markers were developed from hybrid source material.

An enriched library was made by ECOGENICS GmbH (Zurich-Schlieren, Switzerland) from size selected genomic DNA ligated into SAULA/SAULB-linker (5′-GCGGTACCCGGGAAGCTTGG/ 5′-GATCCCAAGCTTCCCGGGTACCGC (Armour et al. 1994) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschi et al. 2000a, b). Of 384 recombinant colonies screened, 144 gave a positive signal after hybridization. Plasmids from 48 positive clones were sequenced. Primer pairs for 28 potentially usable microsatellite loci were designed with the Primer3 web program (Rozen and Skaletsky 2000) and first tested in twelve samples from two different geographic regions (Germany, Ukraine).

Polymorphism for 21 of the 28 potential loci was tested in multiplex PCR, performed in 15 μl

Table 1. Eight polymorphic microsatellite loci from *Juncus atratus* based on 29 individuals genotyped from one population. Annealing temperature for all primers sets was 55 °C except for Loci Ja07 and Ja21b where it was 57 °C

Locus	Repeat motif in sequenced clone	Primer sequence (5′–3′)	No. of alleles	Size range (bp)	H_o	H_e	F_{IS}	Genbank accession no.
Ja01	$CA_{11}GA_{11}$	F: CCTGGGCCCATAAATAAATAAG R: GCGACTGCATTTGTGCTTAC	4	170–184	0.10	0.25	0.59*	AY899200
Ja28	GA_{23}	F: GGTGAGAGAGAGCTAGACGAATG R: AAAAATGTTGCCCAAGGAGAC	10	221–245	0.59	0.83	0.30*	AY899201
Ja29[a]	TC_{19}	F: TGGGCCACTACTTCCGTACT R: ACACGTGCGAGAGAGGGTAT	5	140–150	0.52	0.44	−0.18	AY899202
Ja31	TC_6TC_{31}	F: CCCGTCTCAAAACCCTAACA R: GCGAGAGAGGGAGATTCAAC	9	169–193	0.57	0.80	0.27*	AY899203
Ja42	GA_{18}	F: AGAGGGTGCTCGAGATTGAA R: CTCGTCCCTCTCTGCAAAAC	5	152–168	0.28	0.28	0.02	AY899204
Ja47	$AG_{20}AG_4GA_3$	F: TTACCGACACAAATCAGCGA R: CCCTCTCCCCTTTTCTGTTC	7	240–252	0.71	0.77	0.08	AY899205
Ja21b	$(GT)_{10}(GA)_9$	F: ACTGGGAGAGTATGCTTGGC R: TCTCCCCTCTCTCTTCCCTC	3	214–218	0.52	0.55	0.05	AY899206
Ja07	$(CA)_9(CA)_2(CA)_{20}(CA)_8$	F: CACACACACATAAACACACGC R: TGGACCTTCGAGTCCTTGTC	7	214–230	0.52	0.59	0.13	AY899207

F, forward primer; R, reverse primer; H_o, observed heterozygosity; H_e, expected heterozygosity. [a] In Ja29 additional bands were detected in all samples at 160–176 bp. * Significant deviation from Hardy–Weinberg equilibrium.

reaction volumes containing 10 ng genomic DNA, 7.5 pmol of each forward and reverse primer, 1.5 μl 10 × PCR buffer with $(NH_4)_2SO_4$ (Fermentas GmbH), 0.2 mM dNTPs, 1.875 mM $MgCl_2$, 0.75 μl DMSO and 0.6 units *Taq* DNA polymerase (Fermentas GmbH). A fluorescent label was present on all forward primers. The polymorphic Loci Ja29 (label: FAM), Ja28 (JOE) and Ja31 (TAMRA) were later amplified in one, Loci Ja42 (FAM), Ja01 (JOE) in another reaction and Ja47 (JOE) in a third one. The following PCR program was performed: 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 45 s, 72 °C for 80 s, followed by 72 °C for 10 min.

For reasons of economy the seven remaining of the 28 potential loci were tested for polymorphism using the cheaper M13 primer genotyping protocol (Schuelke 2000), from witch loci Ja07 and Ja21b were usable. The PCR reaction differed from above in that we used 7 pmol of the reverse primer, 5 pmol of the M13 tailed forward primer and 7 pmol of the labelled M13 primer (FAM for Ja21b, JOE for Ja07). The PCR program for this protocol was: 95 °C for 3 min, followed by 30 cycles of 94 °C for 15 s, 57 °C for 45 s, 72 °C for 80 s, 15 cycles of 94 °C for 15 s, 53 °C for 45 s, 72 °C for 80 s completed by 72 °C for 15 min. Fragments were separated on an ABI 310 Genetic Analyzer (Applied Biosystems) with internal size standard MapMarker 400 (BioVentures, Inc., USA).

Only eight out of the 28 tested primer pairs gave polymorphic signals and could be interpreted as diploid codominant markers, others were monomorphic or no clear product could be obtained. Primer sequences and allele sizes are reported in Table 1.

To analyse the allelic variability of a natural population we sampled 29 individuals from one population near Rathenow, Germany. Heterozygosity values were determined using the MSA software (Dieringer and Schlötterer 2003). Departure from Hardy–Weinberg equilibrium was tested with FStat (Goudet 1995). We tested for linkage disequilibrium using GENEPOP version 3.4 (Raymond and Rousset 1995) and the markov chain method with 100 batches and 1000 iterations per batch.

In the 29 individuals analysed, expected heterozygosity among loci ranged from 0.25 to 0.83. Significant departures from Hardy–Weinberg equilibrium were found for three individual markers ($P < 0.01$, Table 1) and over all loci ($F_{IS} = 0.16$, $P < 0.001$). This is not unexpected since Juncaceae are suspected to be highly self-pollinated (Proctor et al. 1996). We found significant linkage disequilibrium ($P < 0.05$) in three

out of 28 pairwise comparisons (Loci Ja29 & Ja31, Ja21b & Ja31 and Ja29 & Ja07).

Trans-species amplification of polymorphic microsatellites was tested in four other *Juncus* species also associated to section *Ozophyllum*. We analysed at least eight individuals of each species. Reaction conditions were as described above and annealing temperature was varied between 43 and 63 °C. However, either only monomorphic, nonexistent, or complex banding patterns prevented interpretation for *Juncus acutiflorus* Ehrh. ex Hoffm, *Juncus alpinoarticulatus* Chaix and *Juncus articulatus* L. (tetraploid with $2n = 4x = 80$). Only in *Juncus bulbosus* L., locus Ja01 gave useful bands. Thus, the markers presented here might be useful for *Juncus atratus* only or for very close relatives, e.g. *Juncus thomasii* Ten., *J. alpigenus* K. Koch or *J. anatolicus* Snogerup, which were not tested here.

The microsatellite markers will be used to analyse the genetic structure of Central European *Juncus atratus* populations in comparison with populations from the centre of the distribution in Eastern Europe. The markers can also be helpful for a better insight in the breeding system of *Juncus atratus*.

References

Armour JA, Neumann R, Gobert S, Jeffreys AJ (1994) Isolation of human simple repeat loci by hybridization selection. *Hum. Mol. Genet*., **3**, 565–599.

Burkart M (1995) *Juncus atratus* KROCKER in Nordostdeutschland. *Verhandlungen des Botanischen Vereins Berlin Brandenburg*, **128**, 83–107.

Burkart M (2001) River corridor plants (Stromtalpflanzen) in Central European lowland: a review of a poorly understood plant distribution pattern. *Global Ecol. Biogeogr*., **10**, 449–468.

Dieringer D, Schlötterer C (2003) A platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes*, **3**, 167–169.

Gautschi B, Tenzer I, Müller JP, Schmid B (2000a) Isolation and characterization of microsatellite loci in the bearded vulture (*Gypaetus barbatus*) and cross-amplification in three Old World vulture species. *Mol. Ecol*., **9**, 2193–2195.

Gautschi B, Widmer A, Koella J (2000b) Isolation and characterization of microsatellite loci in the Dice Snake (*Natrix tessellata*). *Mol. Ecol*., **9**, 2191–2193.

Goudet J (1995) FSTAT v-12: a computer program to calculate *F*-statistics. *J. Hered*., **86**, 485–486.

Kirschner J et al. (2002) Juncaceae 2: *Juncus* subg. *Juncus*. In: *Species Plantarum: Flora of the World Part 7* (ed. Orchard AE), pp. 1–336. Australian Biological Resource Study, Canberra.

Proctor M, Yeo P, Lack A (1996) *The Natural History of Pollination*, HarperCollins, London.

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

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

Schnittler M, Günther KF (1999) Central European vascular plants requiring priority conservation measures – an analysis from national Red Lists and distribution maps. *Biodivers. Conserv*., **8**, 891–925.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments *Nat. Biotech*., **18**, 233–234.

Wissemann V (2003) Hybridization and the evolution of the nrITS spacer region In: Plant Genome, Biodiversity and Evolution Vol I Phanerogams (eds. Ak Sharma et al.), pp. 57–71. Science Publishers, Enfield.