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Identification and characterization of microsatellite loci in the rush *Juncus effusus* (Juncaceae)[1]

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- *Premise of the study:* Microsatellite markers were developed for the common wetland plant *Juncus effusus* to assess levels of within- and among-population genetic diversity.
- *Methods and Results:* Using a next-generation sequencing approach we identified new microsatellite loci, from which 23 were further characterized in a large population of *J. effusus*. Expected heterozygosity ranged from 0 to 0.64 with a mean of 0.407. Inbreeding coefficients (mean = 0.984) were very high, confirming earlier suggestions of an inbred mating system. Cross-amplification was tested in *J. conglomeratus*, *J. inflexus*, *J. drummondii*, and *J. filiformis*, with best results in the former.
- *Conclusions:* The described microsatellite markers will be helpful in assisting studies on, e.g., reproduction, taxonomy, and phylogeography in *J. effusus* and related species.

Key words: 454 sequencing; inbreeding; Juncaceae; *Juncus effusus*; wetland.

Species with a wide distributional range covering a great variety of ecological and climatic conditions are particularly suited as model species in eco-evolutionary research. The study of quantitative, functional traits in such species can highly benefit from the assessment of neutral genetic variation within and across natural populations because it allows for inference of individual relatedness or comparison between the divergence in neutral and quantitative traits.

The common or soft rush, *Juncus effusus* L. (Juncaceae), is a cosmopolitan species, occurring mainly in temperate wetland habitats. It varies greatly in quantitative traits, leading to the description of numerous subspecies (Kirschner et al., 2002). *Juncus effusus* is a perennial herb, growing in dense tufts and able to reproduce vegetatively by short rhizomes. Generative reproduction is accomplished by the production of tiny seeds with high potential for long-distance dispersal. *Juncus effusus* is self-compatible and, like most other *Juncus* species, putatively predominantly selfing (Buchenau, 1892). It is well characterized ecologically and frequently used in phytoremediation approaches (e.g., Tanner, 1996).

Additionally, *J. effusus* shows a number of characteristics that make the species an ideal model for future studies concerning functional traits in wetland plants. First, *J. effusus* is diploid ($2n = 42$) and hence fits into basic assumptions of theoretical models on population and quantitative genetics. Second, its relatively small habit will simplify laboratory and greenhouse work on a larger sample. Lastly, the relatively small genome size of *J. effusus* (C-value of 1C = 0.30 pg; Bennett and Leitch, 2010) and an available draft plastome (Givnish et al., 2010) may provide additional support for marker-assisted studies. However, studies on intraspecific variability in *J. effusus* are lacking, particularly on the molecular level. Here we report the characterization of 23 nuclear microsatellite loci for *J. effusus*, developed primarily using next-generation sequencing, that can be used to assess neutral genetic variation within and among populations and that will help to establish *J. effusus* as a model species in wetland ecosystems.

METHODS AND RESULTS

Total genomic DNA was extracted from young leaves of single individuals from a large population in central Germany (51.510°N, 11.927°E) using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. DNA quality was checked by agarose gel electrophoresis and a NanoDrop Spectrophotometer (Nanodrop Technologies, Wilmington, North Carolina, USA). DNA was shotgun sequenced (1/8th run) using a Roche 454 Genome Sequencer FLX Titanium (Roche Diagnostics, Pleasanton, California, USA) at the Duke Institute for Genome Science and Policy (Durham, North Carolina, USA). The 454 sequencing technique is described in Margulies et al. (2005). We obtained 124 864 reads with a median length of 404 bp. After vector trimming, putative chloroplast sequences were eliminated by assembling fragments against the chloroplast genome of *Typha latifolia* L. (GenBank accession number GU195652). All remaining fragments were assembled without reference and are assumed to be nuclear sequences. Assembling yielded 14 024 contigs and 4351 unique sequences for further analysis. Consensus sequences of contigs and unique sequences were screened for microsatellite motives, i.e., di- and trinucleotide motifs with at least seven repeats using MSATCOMMANDER version 1.03 for Mac OS X (Faircloth, 2008). For 53 out of 810 sequences containing the desired microsatellite motifs, primer pairs could be successfully generated with Primer3 (Rozen et al., 2000) as implemented in MSATCOMMANDER version 1.03. To the 5′ end of either the forward or the reverse primer we added a GTTT tail to promote adenylation.

Additionally, we designed primers for five loci available for *J. effusus* from GenBank (AY493569, AY493568, AY493567, AY493566, and AY493565). Screening of chosen microsatellites was done using directly fluorescent-labeled primers or using a CAG or M13R tag following the method of Schuelke (2000).

Amplification and variability of products was assessed for 31 individuals from one population of *J. effusus* (51.510°N, 11.927°E). Cross-amplification was tested for four other species of section *Juncotypus*, each represented by

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individuals from one population, namely *J. conglomeratus* L. (N = 16; 51.510°N, 11.927°E), *J. inflexus* L. (N = 8; 51.488°N, 11.926°E.), *J. drummondii* E. Mey. (N = 8; 52.686°N, 118.056°W), and *J. filiformis* L. (N = 4; 51.835°N, 12.260°E).

Amplification with directly fluorescent-labeled primers was carried out in multiplex reactions in a final volume of 8 μL containing 0.2 μM of each forward and reverse primer, 4 μL of QIAGEN Multiplex PCR kit, ~20 ng genomic DNA (1 μL) and 0.8 μL 5× Q-solution (QIAGEN) and 1.2 μL (or 2.0 μL RNase-free water only) for primer-mix A and B, or C, respectively (Table 1). For primer-mix A and B, the following PCR program was performed: 95°C for 15 min, followed by 30 cycles of 95°C for 25 s, 60°C for 60 s, 72°C for 60 s, followed by 72°C for 10 min. For primer-mix C, the annealing temperature was 56°C.

Amplification with CAG- or M13R-tailed primers was accomplished in single reactions of 5 μL containing 0.25 μM of forward and reverse primer, from which one was either CAG- or M13R-tailed, 0.25 μM of the fluorescent-labeled CAG or M13R primer, respectively, ~20 ng genomic DNA (1 μL), and 0.5 μL RNase-free water. Touchdown PCR was run with the following conditions: 95°C for 15 min; followed by 20 cycles of 94°C for 30 s, 60°C for 60 s with an increment of −0.5°C per cycle, 72°C for 90 s; followed by 20 cycles of 94°C for 30 s, 50°C for 60 s, 72°C for 90 s; and a final elongation step of 10 min at 72°C.

Fragments were separated on an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, California, USA) with internal size standard Genescan 500 LIZ (Applied Biosystems). Individuals were genotyped using GeneMapper version 4.0 (Applied Biosystems).

For *J. effusus*, scoreable products within the expected size range were obtained for 23 out of 58 tested loci, from which three were monomorphic in the investigated population (Table 2). The number of alleles (*A*), observed heterozygosity (H_o), and expected heterozygosity (H_e) for each locus were computed using MSA version 3.12 (Dieringer and Schlötterer, 2003). The number of alleles ranged from one to six with an average of 3.1 alleles per locus. H_o was low, ranging from 0 to 0.074, with a mean of $H_o = 0.008$. H_e varied between 0 and 0.64 with a mean of $H_e = 0.407$. All F_{IS} values as computed for polymorphic

TABLE 1. Characteristics for 23 microsatellite loci in *Juncus effusus*. Shown for each locus are the repeat motif, the forward (F) and reverse (R) primer sequence, the fluorescent label attached to either the forward or the reverse primer, the reaction mix in which the locus was amplified (in single or multiplex reaction A, B, and C), the allelic size range for 31 individuals of one population, and the GenBank accession number.

Locus	Repeat motif	Primer sequence (5′–3′)	Label	Reaction mix	Size range (bp)	GenBank Accession No.
Jeff01	$(AAT)_{17}$	F: CTCCACTCCTTCCACCAC R: GTTTCGTTCCTCCATACATCATC	VIC—F	C	117–147	JN230524
Jeff02	$(AAT)_{20}$	F: CCAAACCCTGTTCTTGTTAC R: GTTTCGTCCACAACAAATAACTAAAC	VIC—F	C	265	JN230525
Jeff03	$(AG)_{12}$	F: GGAAACAGCTATGACCATGTGTTGAGGCTTTCTGATTC R: GTTTCACCACGATACTAACCCTAAAC	FAM (M13R—F)	Single	238–252	JN230526
Jeff04	$(AG)_{12}$	F: GTTTATGAGGCGATCTAAACACTG R: ACGTGTCAATCTCCGTTATG	PET—R	A	233–235	JN230527
Jeff06	$(AG)_{11}$	F: GCAGCCCTAACTTCATC R: GTTTAGGCAGTGAGAGAGGTCATC	VIC—F	A	186–194	JN230528
Jeff07	$(AG)_{10}$	F: GTTTGGTTCCTTTGTTGGTAATC R: CGTAAACCTACTGCCAGATC	NED—R	C	186–188	JN230529
Jeff09	$(AAG)_{10}$	F: TCGATTTCACCACGTACTC R: GTTTATCTGTGCGTCTCTTTGTTC	NED—F	C	321–345	JN230530
Jeff10	$(AAG)_{8}$	F: CTCTCTGCCATATCCACAG R: GTTTACAGAGAACCAACGAGAGG	FAM—F	B	290–293	JN230531
Jeff11	$(AG)_{14}$	F: GTTTGGGAGAGATATTAATTGGTGTG R: CAGTCGGGCGTCATCAATACAACCTCGCACAAC	NED (CAG—R)	Single	143–161	JN230532
Jeff12	$(AAG)_{8}$	F: GTTTCAGGTGTAAATTTGTGTTG R: GGAAACAGCTATGACCATCGATCTTCCTTTGGTATTG	VIC (M13R—R)	Single	453–493	JN230533
Jeff15	$(AC)_{14}$	F: GTTTGGAAGCCAACATATCAGGTAC R: AAGAAGAGTCAGCGAAATTC	PET—R	B	335	JN230534
Jeff16	$(AAG)_{8}$	F: GTTTAGTTCGAGCTGGACTTC R: GCTCATCTCTACCCTTTCAG	NED—R	B	422–425	JN230535
Jeff29	$(AT)_{10}$	F: CCCACATAAAGAAAGGACAG R: GTTTCAACTCCATCACAGAAGAGC	VIC—F	B	279–283	JN230536
Jeff31	$(AAG)_{12}$	F: GTTTGAAGGAAGAGATGGAGGATC R: CCCTTCTTTCCCTCTTAAC	FAM—R	C	303–327	JN230537
Jeff36	$(AAG)_{11}$	F: GTTTCCTAAACTTACTGCCACCTC R: CTTACTGCGAACCAAACTC	FAM—R	A	388–403	JN230538
Jeff37	$(AAG)_{12}$	F: CCAACTTCTCCGAGACTC R: GTTTGAATGCAAAGAGAGCTCAAC	FAM—F	A	248–257	JN230539
Jeff42	$(AG)_{11}$	F: GTTTCTATTGTTTGGGAGGTATGC R: AAATCCTGAACTCCATAACG	PET—R	C	186–200	JN230540
Jeff43	$(AAT)_{9}$	F: GTTTCAACAAACCCAACTGAATAAC R: GAAATGAAGGTGAGCTC	NED—R	C	113–119	JN230541
Jeff46	$(AAT)_{9}$	F: GGAAACAGCTATGACCATGCATACAAACCAATTAATTCAC R: GTTTGAACGATGTGGAGGTAATTG	PET (M13R—F)	Single	421–430	JN230542
Jeff52	$(AG)_{14}$	F: GTTTAGTGTGTGCATGGAGATGAG R: AATACGATTGCACTCTCCTC	PET—R	A	128–132	JN230543
AY493569	$(AG)_{10}…(AG)_{7}$	F: TGGAATTGGGGATCAATGTT R: CGCCTTCTCAAATTCTCCTG	NED—F	B	191	AY493569
AY493568	$(AG)_{20}$	F: GCAGAAACAAGCCAACAAGA R: CGATAACACAAGCCATGACG	FAM—F	A	154–162	AY493568
AY493565	$(AG)_{22}$	F: ATTGGCATCCGAAATGGTAT R: ATGAGCGTCCGTTTCTCACT	FAM—F	C	222–248	AY493565

Table 2. Results of primer screening in one population of *Juncus effusus* and cross-amplification in four other *Juncus* species. Shown for each locus in *J. effusus* and *J. conglomeratus* are the observed heterozygosity, the expected heterozygosity, the number of alleles, and the inbreeding coefficient. For *J. inflexus*, *J. drummondii*, and *J. filiformis*, the allelic size range is given for loci with successful amplification.

	J. effusus (N = 31)				*J. conglomeratus* (N = 16)				*J. inflexus* (N = 8)	*J. drummondii* (N = 8)	*J. filiformis* (N = 4)
Locus name	H_o	H_e	A	F_{IS}	H_o	H_e	A	F_{IS}	Allelic size range	Allelic size range	Allelic size range
Jeff01	0.000	0.566	6	1.000	0.000	0.233	2	1.000	–	–	–
Jeff02	0.000	0.000	1*	–	0.000	0.000	1*	–	–	–	–
Jeff03	0.074	0.610	4*	0.878	0.000	0.642	4	1.000	242	–	–
Jeff04	0.000	0.129	2	1.000	0.000	0.000	1	–	–	227	–
Jeff06	0.000	0.480	3	1.000	0.000	0.692	4	1.000	188	176–180	188
Jeff07	0.000	0.287	2	1.000	0.000	0.000	1	–	–	–	–
Jeff09	0.000	0.490	5	1.000	0.000	0.717	4	1.000	319	243–247	316
Jeff10	0.000	0.452	2	1.000	0.125	0.552	3	0.772	307–310	284–295	–
Jeff11	0.000	0.594	6	1.000	0.000	0.695	4	1.000	137	–	–
Jeff12	0.040	0.514	4*	0.922	0.063	0.448	3	0.860	–	–	–
Jeff15	0.000	0.000	1*	–	0.000	0.000	1*	–	322	–	–
Jeff16	0.000	0.476	2*	1.000	0.167	0.572	5*	0.705	–	–	–
Jeff29	0.000	0.480	3	1.000	0.000	0.648	3	1.000	271	278	251
Jeff31	0.000	0.453	3	1.000	0.000	0.458	2	1.000	–	–	–
Jeff36	0.032	0.485	4	0.933	0.000	0.484	3	1.000	–	–	–
Jeff37	0.000	0.287	2	1.000	0.000	0.536	2*	1.000	223	223	223
Jeff42	0.000	0.514	4	1.000	0.063	0.281	2	0.776	–	188	–
Jeff43	0.000	0.443	3	1.000	0.000	0.000	1*	–	–	–	–
Jeff46	0.000	0.443	2	1.000	0.063	0.448	3	0.860	378	–	–
Jeff52	0.000	0.640	3*	1.000	0.000	0.242	3	1.000	–	–	–
AY493569	0.000	0.000	1	–	–	–	–	–	199	232–238	234
AY493568	0.000	0.426	2	1.000	0.000	0.433	3	1.000	140	–	152
AY493565	0.032	0.583	6	0.945	0.063	0.531	2	0.882	–	–	–

Note: A = number of alleles; F_{IS} = inbreeding coefficient; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of individuals investigated.

* Signifies null alleles present.

loci using FSTAT version 2.932 (Goudet, 1995) indicated significant deviations from Hardy–Weinberg equilibrium (mean F_{IS} = 0.984), suggesting a highly inbred mating system. Individuals with missing products at a locus despite successful amplification at other loci were regarded to exhibit null alleles at this locus. Six of 23 loci showed evidence of null alleles in *J. effusus* (Table 2). Cross-amplification yielded the best results in *J. conglomeratus*, for which descriptive parameters at 22 loci are displayed in Table 2. In *J. inflexus*, *J. drummondii*, and *J. filiformis*, interpretable products could be obtained for 11, eight, and six microsatellite loci, respectively.

CONCLUSIONS

Assessing the genetic variation at the described set of nuclear microsatellite loci for *J. effusus* and related *Juncus* species makes possible significant contributions to a wide range of scientific issues. In addition to their use in research on functional traits, they can support taxonomic and systematic work and studies on interspecific hybridization, reproduction, and phylogeography.

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