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PERMANENT GENETIC RESOURCES NOTE

Isolation of 11 polymorphic tri- and tetranucleotide microsatellite loci in a North American sedge (*Carex scoparia*: Cyperaceae) and cross-species amplification in three additional *Carex* species

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Abstract

We report on the isolation and evaluation of 11 microsatellites from a widespread eastern North American wetland sedge, *Carex scoparia*. Loci exhibit 3–9 alleles over five populations and significant F_{IS} (0.204–0.717) in most populations. All primers cross-amplify in at least two other species, and 10 cross-amplify in the more distantly related *C. stipata*. These markers will be used to examine population genetics and patterns of chromosomal diversification in this ecologically important sedge species and its relatives.

Keywords: *Carex* subgenus *Vignea*, chromosome diversity, Cyperaceae, microsatellite enrichment, sedges

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The sedge genus *Carex* L. numbers approximately 2000 species worldwide, making it one of the largest angiosperm genera (Reznicek 1990). In this study, we report on the development of 11 polymorphic microsatellite markers in the eastern North American wetland species *Carex scoparia* var. *scoparia*. No previous genetic studies have been published in this species (other *Carex* microsatellites have been published in Ohsako & Yamane 2007 and in King & Roalson 2008).

Microsatellite markers were developed using an enrichment protocol (Glenn & Schable 2005). Genomic DNA (gDNA) from one individual was extracted from fresh leaf tissue using the DNeasy Plant Kit (QIAGEN) and digested with *Rsa*I and *Xmn*I. SuperSNX24 linkers (F: GTTTAAGGCCTAGCTAGCAGAATC, R: GATTCTGCTAGCTAGGCCTTAAACAAAA) were ligated onto the ends of gDNA fragments. Biotinylated tetranucleotide probes (AAAT, AACT, AAGT, ACAT, AGAT) and trinucleotide probes (AAC, AAG, AAT, ACT, ATC) were hybridized to gDNA. The biotinylated probe-gDNA complex was added to magnetic beads coated with steptavidin (Dynabeads M-280 Invitrogen). This mixture was washed twice with 2×SSC, 0.1% SDS and four times with 1×SSC, 0.1% SDS at 53 °C. Between washes, a magnetic particle-collecting unit was used to capture the magnetic beads, which are bound to the biotin-gDNA complex. Enriched fragments were removed from the biotinylated probe by denaturing at 95 °C and precipitated with 95% ethanol and 3 M sodium acetate. To increase the amount of enriched fragments, a 'recovery' polymerase chain reaction (PCR) was performed in a 25-μL reaction containing 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 0.16 mM of each dNTP, 10XBSA, 0.52 μM of the SuperSNX24 forward primer, 1 U *Taq* DNA polymerase, and approximately 25 ng of the enriched gDNA fragments. Thermal cycling was performed as follows: 95 °C for 2 min followed by 25 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 90 s, and a final elongation step of 72 °C for 30 min. Subsequent PCR fragments were cloned using the TOPO-TA Cloning kit following the manufacturer's protocol (Invitrogen). Bacterial colonies containing a vector with gDNA were used as a template for subsequent PCR following standard protocols. PCR

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Table 1 Eleven polymorphic microsatellite loci isolated from *Carex scoparia*. Number (NA) and size range of alleles (bp, base pairs), as well as observed (H_O) and expected (H_E) heterozygosities under Hardy–Weinberg expectations, are based on $N = 115$ individuals for five populations ('Global' statistics), or $N = 20$ individuals for a single population ('Powderhorn Lake' statistics). *P* value for the 'global' analysis tests the heterozygote deficit hypothesis, whereas the *P* value for the Powderhorn Lake population tests the heterozygote excess alternate hypothesis. Multiplex groups (A, B, C, D) indicate which primers were amplified together, as well as the annealing temperature (T_a in °C) for multiplex PCR; S087 was amplified in a multiplex reaction with a marker that was subsequently removed from analysis. Allele sizes for loci S102 and S177 include the 18-bp M13 sequence

						Global ($N = 115$)					Powderhorn Lake ($N = 20$)					GenBank Accession no.
Primer	Motif	Primer sequences 5′–3′	T_a (in °C)	Size (bp)	Multiplex	NA	H_O	H_E	F_{IS}	*P*	NA	H_E	H_O	F_{IS}	*P*	
S082	$(GAT)_{11}$	F: TGAGAACCCTAGGCAGATGG R: GGGGAAACAAGGTCGTTTAGA	57	144–164	A56	8	0.205	0.562	0.626	<0.0001	3	0.483	0.650	–0.357	0.0528	EU369640
S180	$(GAT)_8$	F: ACATGATTGTGGACGACAGG R: TCACCAAAGTCCTGAAAATCAA	59	184–196	A56	4	0.138	0.465	0.717	<0.0001	1	—	—	–0.049	0.6277	EU369648
S245	$(CTT)_{11}$	F: GAAACAAAGGTGCCCCACT R: GTTGCAAGCGGGTCTAATTC	59	193–211	A56	5	0.317	0.637	0.486	<0.0001	3	0.668	0.700	–0.126	0.3757	EU369650
S047	$(ATGT)_{10}$	F: AGATCGGTTTGAACGGTTTG R: GGAACTTCTAACAAATCACCCTGT	55	220–265	B54	9	0.211	0.516	0.579	<0.0001	3	0.668	0.750	–0.029	0.5299	EU369639
S128	$(GTT)_{17}$	F: TTGTTGTCATGAGTTCACCAGA R: AATTTGGCCAGACAAGATCC	62	142–180	B54	8	0.252	0.430	0.393	<0.0001	3	0.681	0.700	–0.166	0.4310	EU369644
S175	$(CTT)_8$	F: TATTGGGTGTGCGATTGAGA R: TCAGATCAGCCAAGTCATCG	60	114–121	B54	3	0.130	0.391	0.629	<0.0001	2	0.431	0.500	–0.023	0.6039	EU369646
S087	$(CTT)_{12}$	F: TGAGTCAGGTTGTTGCAACTGT R: TCTGCATGAGCATGAGTGTTC	59	209–233	C54	8	0.248	0.680	0.622	<0.0001	3	0.636	0.650	–0.063	0.3950	EU369641
S119	$(CTT)_{10}$	F: CAGTGCTTTTCTGCTTTTCACA R: CCACTGCAGCCATTAGTCAA	62	162–177	D59	6	0.227	0.543	0.570	<0.0001	4	0.706	0.750	–0.204	0.1656	EU369643
S181	$(AAG)_{12}$	F: CCAACTTGCCCTTGTTCATT R: CGTTTGCACGCTTTGTAGAT	62	228–255	D59	8	0.221	0.616	0.620	<0.0001	3	0.668	0.800	0.000	0.8020	EU369649
S102	$(ACAT)_7$	F: CGGAAAGAGGTAGCACAAGC R: AATCTGCTGATGCAACAATTTA	62	211–232	—	5	0.215	0.512	0.576	<0.0001	3	0.650	0.650	–0.357	0.0528	EU369642
S177	$(AAC)_8$	F: GCATTGTTTTGTTTGCCTCTT R: TTCATTTGTGATTGGGTTTCA	55	172–200	—	5	0.164	0.210	0.204	0.0007	3	0.483	0.650	–0.357	0.0528	EU369647

Table 2 Cross-species amplification in *Carex scoparia* var. *tessellata* and three additional *Carex* species. A single specimen of each taxon other than *C. scoparia* var. *scoparia* was sampled. Sizes of alleles are reported in bp. All sizes reflect amplification using M13-labelled primers, and sizes are consequently 18 bp longer than sizes expected with standard labelled primers (see text and Schuelke 2000)

Section	Species	S047	S082	S087	S102	S119	S128	S175	S177	S180	S181	**S245**
Ovales	*C. scop.* var. *scoparia*	238–283	162–182	227–251	211–232	180–195	160–198	132–139	172–200	202–214	246–273	211–229
Ovales	*C. scop.* var. *tessellata*	249	163	230	205	201	176	138	191	201	250	214
Ovales	*Carex normalis*	237	167	220	211	204	197	138	180	204	247	211
Ovales	*Carex vexans*	—	167	214	213	189	179	135	186	—	262	211
Vulpinae	*Carex stipata*	268	164	214	221	177	142	129	200	—	235/238	217

products were cleaned using MultiScreen-PCR Filter Plates (Millipore) and sequenced using the BigDye Terminator version 3.1 Kit [Applied Biosystems (ABI)]. Sequencing reactions were run on an ABI3730 DNA Analyser. Sequences were edited and duplicates assembled in Sequencher version 4.6 (Gene Codes) and repeats identified using Ephemeris 1.0 (http://www.uga.edu/srel/DNA_Lab/programs.htm). Primers flanking 40 candidate microsatellite repeats were developed using Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Eleven loci consistently amplified clean, unambiguous peaks on gDNA extracted from frozen leaf tissue of 115 individuals representing five populations of *C. scoparia* var. *scoparia* collected in the greater Chicago region (North America; Table 1) as well as a single individual of each of four additional species (Table 2). For each primer pair, trial amplifications were conducted in which the forward primer had an M13 tail attached to the 5′ end to allow for labelling with 6-FAM fluorescent-labelled M13 primers (Schuelke 2000). PCRs (21 μL) consisted of approximately 1–10 ng of genomic DNA, 1× $MgCl_2$-free PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 μg/μL BSA, 0.16 μM-labelled M13 primer, 0.16 μM reverse primer, 0.04 μM forward primer (with M13 tail), and 0.65 U *Taq* DNA

polymerase (GoTAQ, Promega). Thermal cycling profiles were as follows: 94 °C for 4 min; 35 cycles of 94 °C for 30 s, annealing temperature (T_a °C, Table 1) for 30 s, 72 °C for 45 s; followed by eight cycles of 94 °C for 30 s, 53 °C for 30 s, 72 °C for 45 s; and a final extension at 72 °C for 10 min. Multiplex PCR was subsequently used for nine loci (Table 1), with the following PCR modifications: the forward primer for each locus was separately labelled, all primers were diluted to 0.01 μM (0.02 μM for S047), $MgCl_2$ was diluted to 2.0 mM, BSA was not used, and the final round of eight PCR cycles at T_a = 53 °C was not used (Table 1). PCR products and ROX-labelled GeneFlo 625 size standard (CHIMERx) were suspended in formamide before running on an ABI3730 DNA Analyser. Fragment analysis was conducted using GeneMapper version 4.0 (ABI).

Data were analysed in GenePop version 4.0.7 for Windows (Rousset 2008). Loci exhibited strong heterozygote deficiency ($P < 0.001$ using the Hardy–Weinberg exact test) across all populations except the Powderhorn Lake population (Table 1), which is compatible with previous studies in the genus that demonstrate a high rate of selfing as well as molecular genetic evidence of inbreeding (Whitkus 1988; King & Roalson 2008). Linkage disequilibrium (LD) was detected in the Powderhorn Lake population for 23 out of 55 pairwise comparisons among loci (Bonferroni-corrected $P < 0.01$) involving all loci except for S180 and S175. In the other four populations sampled, six out of 220 pairwise comparisons demonstrated significant LD (Bonferroni-corrected $P < 0.01$). None of these pairs are replicated across populations. Sequenced clones were compared for all loci, and no sequence similarity was observed to explain the result. Given the rapid rate of chromosome evolution within this species and close relatives, shifting patterns of LD may be a reasonable expectation due to population-specific chromosome arrangements (Whitkus 1988; Hipp *et al*. in preparation). In spite of strong homozygosity, variability in these loci and the cross-amplification of many markers in the distantly related *C. stipata* (Table 2) will make them useful in population genetic studies throughout much of the genus.

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