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Genetic variation in three Chinese peat mosses (*Sphagnum*) based on microsatellite markers, with primer information and analysis of ascertainment bias

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Abstract. Primer sequences are provided for amplification of 21 microsatellite-containing loci in *Sphagnum*. Although these primers were developed for species in *Sphagnum* section *Subsecunda*, they amplify microsatellite loci in most species that have been tested across the genus *Sphagnum*. Results are described from a survey of genetic variation in three species

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of *Sphagnum* collected in China: *S. junghuhnianum* in section *Acutifolia*, and *S. palustre* and *S. imbricatum* in section *Sphagnum*. Six and eight multilocus genotypes were detected within one population each of *S. junghuhnianum* and *S. palustre*, respectively. Four populations of *S. imbricatum* were sampled; they vary substantially in allele frequencies and in the amount of genetic diversity detected; overall, approximately 40% of the genetic variation sampled within *S. imbricatum* could be attributed to differentiation among populations. Microsatellite profiles indicate that *S. palustre* gametophytes are diploid whereas those of *S. junghuhnianum* and *S. imbricatum* are haploid.

Keywords. Ascertainment bias, DNA fingerprinting, microsatellites, Sphagnaceae, *Sphagnum*, *S. imbricatum*, *S. junghuhnianum*, *S. palustre*.

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Population genetic structure can now be quantified and described using a broad range of molecular markers. Isozymes were introduced for population-based studies by Lewontin and Hubby (1966) and Harris (1966) and were first applied to bryophytes in the 1970s (e.g., Krzakowa 1977; Meyer et al. 1974; Szweykowski & Krzakowa 1979). More recent DNA-based methods applied to bryophyte populations include nucleotide sequencing and a variety of so-called ''fingerprinting'' methods.

Nucleotide sequencing sometimes but not always reveals infra-specific genetic variation, depending on the taxon and the locus being sequenced (e.g., Vanderpoorten et al. 2006). Few studies have investigated within-population variation at the nucleotide sequence level (see however Gunnarsson et al. 2007), but typically modest mutation rates suggest that sequencing is not the tool of choice for studies at the level of individuals within populations. Nucleotide sequencing is relatively costly as well, and is not a practical tool for studies where large sample sizes are required.

Isozymes are used less often these days than they were 10–20 years ago, but have several advantageous features. These include relatively low costs for marker development, the fact that markers developed for one species generally work with little or no modification on related or even distant species, and their co-dominant expression such that heterozygotes can be distinguished from homozygotes. Disadvantages include the need for fresh, living plant material, and relatively low levels of polymorphism that miss a substantial amount of underlying genetic variation at the DNA level.

DNA fingerprinting methods utilize hypervariable markers that in theory at least, are polymorphic enough to identify and distinguish individual clones. Fingerprinting methods that have been applied to bryophyte populations include Random Amplified Polymorphic DNA (RAPDs) (e.g., Boisselier-Dubayle et al. 1995; So & Grolle 2000), Amplified Fragment Length Polymorphism (AFLPs) (e.g., Zartman et al. 2006), Inter Simple Sequence Repeats (ISSRs) (e.g., Werner et al. 2003) and microsatellites (e.g., van der Velde & Bijlsma 2001). RAPDs have been criticized because of problems with reproducibility related to non-specificity of polymerase chain reactions (PCR) that can lead to the amplification of artifacts resulting from contamination of samples by other organisms, often microscopic or even endophytic (Stevens et al. 2007). AFLP markers were developed as an alternative to RAPDs to get around the problem of reproducibility, and ISSR banding patterns are also said to be more consistent than RAPDs. Unlike proteins (including isozymes), DNA does not break down rapidly in dried material, and either freshly collected or dried specimens can be utilized for RAPD, AFLP and ISSR analyses. However, scoring of results from these methods is generally based on the presence/absence of bands and some DNA degradation could lead to the artifactual absence of bands, so freshly collected material is desirable. Unlike isozymes and microsatellites, RAPD, AFLP

and ISSR markers are dominant, so heterozygotes cannot be distinguished from homozygotes. They are still valuable for many studies of genetic variation and structure but are less ideally suited for detailed work on mating patterns where identification of heterozygotes is very useful.

Microsatellite markers, also known as simple sequence repeats (SSRs) and short tandem repeats (STRs), are more expensive and/or time consuming (which translates into expense) to develop but have the advantage of being highly polymorphic, and are codominant such that heterozygotes can be distinguished from homozygotes. Amplification of microsatellites is based on taxon-specific primers so artifactual bands from contaminating microorganisms are unlikely. In seed plants, microsatellite primers developed for one species often have limited utility for even related congenerics, which is a major disadvantage if species are to be compared. Very high mutation rates can result in size homoplasy between taxa as well (Estoup et al. 2002), but the potential for erroneously comparing nonhomologous alleles (bands) is a feature shared with other highly polymorphic fingerprinting methods and to some extent, isozymes as well.

Korpelainen et al. (2007) described general protocols for developing microsatellite markers in mosses, and primer sequences for nine loci that contain microsatellite repeats in *Sphagnum capillifolium* were provided by Provan and Wilson (2007). Van der Velde and Bijlsma (2001, 2004) and van der Velde et al. (2001) used microsatellites for work on mating patterns and interspecific hybridization in *Polytrichum*. In this paper, we provide primer sequences for 21 microsatellite-containing loci in *Sphagnum*. Two additional primer sequences are provided which target two loci from among the 21, but which result in amplified fragments which are longer or shorter than those produced by the original primer pair. All these markers were developed for on-going research on *Sphagnum* section *Subsecunda*, but to our surprise they also amplify highly polymorphic microsatellite loci in many other species of *Sphagnum* in other sections as well. We describe several small data sets derived from sampling three Chinese species representing two sections of *Sphagnum*, both outside sect. *Subsecunda*. We compare our results to unpublished data for eight other species of *Sphagnum* representing all four major sections of the genus in order to evaluate whether ascertainment bias ensues when primers designed for species in sect. *Subsecunda* are used with other distantly related in the genus.

Materials and Methods

Study populations and vouchering. Virtually nothing is known about the genetic variation in species of *Sphagnum* outside of Europe and North America. Collections of three species recently collected in China were subjected to microsatellite analyses: *S. imbricatum* and *S. palustre* in sect. *Sphagnum* and *S. junghuhnianum* in sect. *Acutifolia*. Twenty-eight collections of *S. junghuhnianum* and 30 of *S. palustre* were made in south-central China: Yunnan Province, Liuku Co., Caojian Village, Zibenshan Mtn., at approximately 2490 m elevation. The site is a narrow, wet valley with marshy soil and shrubs along a small stream. Both species occurred as more or less discrete cushions and plants were sampled haphazardly along an informal transect going up the valley. Samples were separated by one to several meters to reduce the likelihood of repeatedly collecting ramets of the same clone. An additional specimen of *S. junghuhnianum* was sampled from a second Yunnan site: DaLi Co., west slope of Cang Shan Mtn., ca. 5.5 km W of Old DaLi. This site was a moist north-facing rock outcrop on a disturbed road bank surrounded by *Pinus armandii-P. yunnanensis-Abies* sp. forest, with just a few scattered cushions of *S. junghuhnianum*.

Both *Sphagnum junghuhnianum* and S. *palustre* have unisexual gametophytes; sporophytes were fairly abundant on *S. junghuhnianum* at the collecting site where most of the specimens were collected but sporophytes were not observed on *S. palustre*. Sporophytes observed in the former were consistently buried within the gametophyte colonies and were only detected when hummocks were opened to look for them, despite the fact that pseudopodia were elongated and the capsules appeared mature. Collections of *S. junghuhnianum* and *S. palustre* were made on 21–22 Sep 2006.

One hundred and eight samples of *S. imbricatum* were collected at four sites in northeastern China. The first two sites were in Jilin Province: Antun Xian, Changbai Mtns. Site 1 was around the margins of Yunchi Lake on peaty, medium to rich fen soils surrounded by *Betula-Larix* forest. The second site was a medium-rich fen along the road to Yunchi Lake but about 4 km from the lake, ca. 45 km. southeast of Erdaobaihe. These collections were made on 10 Sep 2006. The other two collecting sites for *S. imbricatum* were in Heilongjiang Province. One site was along Yijia Road, ca. 2 km south of Wuyilin in a poor fen surrounded by a richer shrub fen with abundant *Tomentypnum nitens* (collected 14 Sep 2006). The second site was: Wuying area, 48 km NW of Wuying, along forest road 10.5 km SE of Ciubei (collected 15 Sep 2006).

The individual stems from which DNA was extracted were placed in small envelopes after a portion of the capitulum was sampled, and returned to the herbarium packet containing the rest of the collection. These vouchered specimens are deposited in DUKE, with duplicates deposited in KUN. Duplicates of many but not all specimens from these sites are also deposited in NY.

DNA extraction, microsatellite amplification and marker scoring. DNA was extracted according to protocols described in Shaw et al. (2003). For microsatellite development we combined genomic DNA from six species of *Sphagnum* sect. *Subsecunda*: *S. auriculatum*, *S. contortum*, *S. inundatum*, *S. lescurii*, *S. platyphyllum* and *S. subsecundum*. Genetic Identification Services (GIS), of Chatsworth, California, "enriched" the genomic DNA for microsatellite motifs. Their methods for doing so employ a proprietary process that involves selection from among fragments of genomic DNA, of regions that contain microsatellites of a particular motif (such as –AC). The enriched product is then packaged into a plasmid library for screening and design of primers targeting known microsatellite-containing regions. Loci are isolated from the entire genome, and libraries are constructed for various motifs (see **Table 1**). Primer sequences, microsatellite motifs and the approximate sizes of flanking regions are described in **Table 1**.

Microsatellites were amplified in 8 µl multiplexed reactions, each targeting a set of three loci. Primer sets were arrayed for multiplexing according to expected fragment sizes (for non-overlapping amplification products) and alternating fluorophores. Each primer pair included a forward primer fluorescently labeled with HEX or 6-FAM (Integrated DNA Technologies, Coralville, IA). Multiplexing was accomplished using a Qiagen Multiplex PCR kit (Valencia, CA), scaled for smaller reactions, but otherwise used according to the manufacturer's recommendations. Five to 20 ng of genomic DNA in 3 µl dH_20 served as template in each reaction. A standard thermocycling regime was implemented for all primer sets, with no additional optimization. This consisted of an initial denaturation and hot-start activation at 95°C for 15 min, then 30 cycles of 94°C for 30 sec, 54°C for 90 sec and 72°C for 60 seconds. A final extension at 60°C for 30 min was performed. PCR products were diluted in sterile water, and 1.2 µl of the dilution was mixed with GS500 size standard and Hi-Di™ Formamide (Applied Biosystems, Foster City, CA) for electrophoresis on an ABI 3730 sequencer. Size determinations and genotype assignments were made using GeneMarker 1.30 software (Softgenetics, State College, PA).

Statistical Analyses. Analyses of the data were accomplished using GenAlEx (Peakall & Smouse 2006). Fragment sizes were coded as "standard data" and microsatellite repeat numbers were not calculated, as it was clear from sequencing selected fragments that some of the allelic variation could be attributed to indels in flanking regions rather than to variation in repeat number alone (data not shown).

Results and Discussion

Genetic diversity statistics for each of the three species are provided in **Table 2**. All samples of *Sphagnum palustre* were collected from a single population; the diversity estimates for *S. junghuhnianum* are based on plants from the same site, where all but one sample of that species was also collected. Populations of *S. imbricatum* were divergent so diversity statistics are presented separately for the three populations from which sample sizes are sufficient to permit meaningful estimates.

Table 1. Microsatellite primer sequences, repeat motif at each locus, and the approximate size of the flanking regions upstream and downstream from each microsatellite. Flanking sequence sizes are approximate because of length mutations in those regions among samples.

Locus	5′–3′ Forward primer (label)	5′–3′ Reverse primer	Motif	Forward flank (bp)	Reverse flank (bp)
1	AACCACAAGTGAGCATTACC (FAM)	ACCTCTCCTCTCTGATTTCTG	CA-	124	112
3	CCATTGGGTCATCATAGTAGTG (HEX)	GCTTTTCTTTGAGGATTTTGAG	CA-	104	50
4	TTGTGAGGAAGTGGTGTTG (HEX)	TCAGCAAGAGATTTGTGACC	CA-	140	23
4long	CAGTCATTTAAACAAACAGTTGTGAG (FAM)	TCAGCAAGAGATTTGTGACC	CA-	204	23
5	CTCAAGCCAATCTCTCACATTC (HEX)	TCCGCTGTAACACCAACTACTC	GT-	34	143
6	TAGCCACCTTTCATAACCATAG (HEX)	TGGGCACATAATCCACAG	GT-	37	127
6short	TAGCCACCTTTCATAACCATAG (HEX)	CAAAACATTCACAAATTGGTTTCT	GT-	37	39
7	TCCAATGACGGTAGGAAAC (HEX)	TCCAAGTGTCTTACAATGTCTG	GA-	30	128
9	GCATTTGATTACGAACAAGAG (FAM)	CGGATGAGCAGAAACAAC	CT-	54	98
10	GGGTTAGGGGATGATCCTG (FAM)	CTTCAGCCACGAATCCATT	GA-	164	34
12	TGGAATGAATTGGAGCAAC (FAM)	TTGGTATGTATGCGAAGAAAG	AC-	86	23
14	TCCCCCACTCCTCTACTTG (HEX)	CTTGGATTCCTTTGCTTCTG	AG-	68	109
15	GGAGTGAGTAGGTAGGTGGTAA (FAM)	GGATGTATGAGGTCCATTTTAG	CT-	75	33
16	GCTTTCTCCCGTCTGTTG (FAM)	AAAAACTCGTCTGTTGCTGTC	CTT-	73	163
17	CTTCCCCCTTGAAACAAAC (FAM)	TGGGTGCTCTCCAGAATAG	AAG-	95	43
18	CTCCTATTGGCGACAGATTTC (FAM)	CCTCGTTCTCTTCCTTCCAC	AAG-	60	48
19	GCAAACCCTAAAACACAGTG (FAM)	ATCGGCGTATCTTGATGTC	AAG-	170	72
20	ACCCAACGGACTCTACGG (FAM)	AACGCTGAAACAGACCTCG	TTC-	119	145
22	TCCCCAACACAAACCTTC (FAM)	GCTTTGAAGAAAGTTCCAGTG	GAT-	32	34
26	CCAGATAACTTCCCCACAAC (HEX)	CTTGCTACCGCAGACACTG	ATC-	80	70
28	CCGAAAAGGGAACAAACAAA (FAM)	TCCTTCTCCTCTGCGTGTTT	AC-	44	178
29	CTCATCAGCCCAGTCAGTCA (HEX)	ACCCACGCATCAAAAGAAAC	AAG-	134	41
30	ACCACCCTCCTCTCAATCCT (FAM)	AGTGTTTGCCAGTGCCTCTT	GAT-	88	29

Table 2. Genetic diversity statistics for *Sphagnum junghuhnianum*, *S. palustre* and *S. imbricatum* in China. Integers after *S. imbricatum* refer to collection site numbers, and correspond to Site numbers in Fig. 1. Only two samples of *S. imbricatum* were collected at Site 2, so diversity statistics are not included here.

	No. loci	Expected heterozygosity	Percentage polymorphic loci	Mean no. alleles/ locus	Information content (I)	No. multilocus genotypes
S. junghuhnianum (29)	14	0.174 ± 0.053	50	2.143 ± 0.345	0.330 ± 0.101	6
S. palustre (30)	17	0.367 ± 0.070	65	2.294 ± 0.306	0.593 ± 0.118	8
*S. imbricatum*1(44)	16	0.204 ± 0.062	56	2.313 ± 0.481	0.375 ± 0.120	15
*S. imbricatum*2 (37)	16	0.076 ± 0.026	50	1.875 ± 0.328	0.159 ± 0.054	8
*S. imbricatum*3 (25)	16	0.298 ± 0.073	63	2.938 ± 0.739	0.573 ± 0.161	21

The percentages of polymorphic loci within populations were 50–65% and all populations of the three species contained multiple genotypes (**Table 2**). The populations of *Sphagnum junghuhnianum* contained one very common genotype with five additional rare genotypes, whereas *S. palustre* at the same site had a more even distribution of genotypes. That difference in diversity and evenness is reflected in the much higher estimate of "I" for *S. palustre*, the Information Index, which is a measure of diversity that takes into account both the number of alleles and the relative dominance or evenness of particular genetic types within the population (comparable to the Shannon-Weaver index of ecology).

As noted, populations of *Sphagnum imbricatum* are divergent, and in fact, approximately 40% of the total variation detected in this species is partitioned among rather than within populations (**Table 3**). The genetic pattern in Site 2 is rather like that of *S. junghuhnianum* in that a single genotype dominates, with seven additional genotypes that were represented by one or two individuals each (**Fig. 1**). In contrast, no single genotype out of 21 that were resolved by microsatellite markers was especially common at Site 3 (**Fig. 1**). This population was also high in both the mean number of alleles per locus, and the Information Index of genetic diversity (**Table 2**). The genetic structure of *S. imbricatum* at Site 1 was roughly intermediate between that at Sites 2 and 3; there was one relatively common genotype, several of moderate frequency, and nine additional genotypes represented by one or two sampled stems.

Microsatellite primers amplify a single allele at each locus for all plants of *Sphagnum junghuhnianum*, consistent with the interpretation that this species is gametophytically haploid. In *S. imbricatum*, one allele amplified for each of 14 out of the 15 loci, but one locus consistently yielded two alleles of different sizes. Because it was just one locus, we interpreted this locus as having undergone a duplication, and consider it likely that *S. imbricatum* is also gametophytically haploid. This is consistent with a similar conclusion by Thingsgaard (2002) based on isozyme data from a single population of *S. imbricatum* from Japan. For purposes of genetic diversity estimates, the duplicated locus was treated as two separate loci.

Amplifications of *Sphagnum palustre*, in contrast, yielded two alleles at eight out of 17 loci. This pattern is consistent with an interpretation that *S. palustre* gametophytes are diploid, in agreement with flow cytometric estimates of genome size for this species (Temsch et al. 1998). Moreover, all sampled

Table 3. Analysis of molecular variance for microsatellite data from four populations of *Sphagnum imbricatum* in northeastern China. Source = main effects in the model; df = degrees of freedom; SS = sums of squares; estimated variance (%) = percentages of the total molecular variance in the data sets attributable to each of the two main effects.

Source	df	SS	MS	Estimated Variance (%)
Among populations	3	77.743	25.914	1.024	41 ($P < 0.001$)
Within populations	104	154.239	1.483	1.483	59
Total	107	231.981	27.397	2.507	

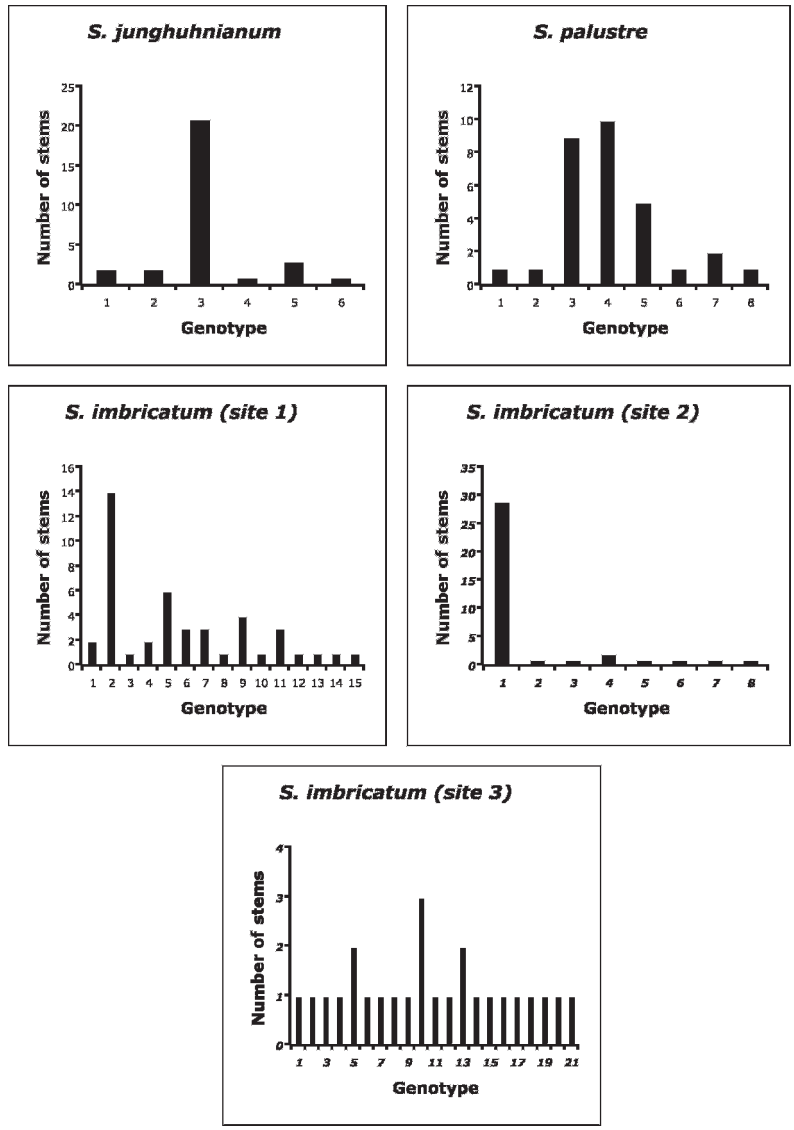

Figure 1. Numbers of samples representing different multilocus genotypes in populations of *Sphagnum imbricatum*, *S. junghuhnianum* and *S. palustre*.

individuals were heterozygous; the fact that no homozygotes were observed strongly suggests that this species is characterized by fixed heterozygosity. Fixed heterozygosity reflects non-segregation of alleles at meiosis, strongly supporting an allopolyploid origin for this species.

In many plants and animals, microsatellite loci are more or less species-specific such that varying percentages of loci developed for one species fail to amplify in related species (e.g., Prasad et al. 2005). In addition, ascertainment bias can complicate cross-species comparisons of genetic diversity (e.g., Annos et al. 2003). The evidence for ascertainment bias is that the species or population from which DNA was used for microsatellite primer development often shows greater allele sizes on average, and higher genetic diversity, in relation to other species for which the same primers are used. Sometimes ascertainment bias can be detected when transferring microsatellite primers among genetically divergent conspecific populations (Wright et al. 2004). Some studies have failed to detect ascertainment bias (Crawford et al. 1998; Shepperd et al. 2002). Prudent use of microsatellite primers includes inconsideration

Table 4. Number of alleles detected, expected heterozygosity, and allele size ranges in four species of *Sphagnum* sect. *Subsecunda* for which the microsatellite primers were developed. *Sphagnum subsecundum*, *S. contortum* and *S. platyphyllum* were sampled from Newfoundland and Norway, and all *S. lescurii* collections were made in Newfoundland.

	Sect. Subsecunda											
	S. subsecundum(43)			**S. contortum(69)**			**S. platyphyllum(22)**			**S. lescurii(47)**		
Locus	**Na**	**He**	**Size**	**Na**	**He**	**Size**	**Na**	**He**	**Size**	**Na**	**He**	**Size**
1	4	0.613	256–264	4	0.264	250–258	1	0.000	256	4	0.548	256–262
3	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
4	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
5	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
6	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
7	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
9	2	0.169	190–191	16	0.915	171–211	4	0.678	167–196	11	0.793	167–192
10	9	0.855	222–248	7	0.719	230–244	2	0.087	228–230	13	0.885	215–240
12	4	0.531	117–126	1	0.000	118	1	0.000	118	7	0.718	117–129
14	9	0.796	210–226	7	0.764	200–224	10	0.855	208–236	20	0.924	196–242
17	5	0.597	155–167	5	0.599	155–173	3	0.541	164–170	9	0.775	155–176
18	6	0.789	149–170	2	0.268	149–158	4	0.602	132–141	11	0.886	132–170
19	4	0.485	261–277	2	0.487	239–253	1	0.000	255	6	0.653	253–277
20	11	0.821	290–317	9	0.720	292–308	3	0.616	293–297	9	0.763	281–302
22	3	0.495	86–102	2	0.427	86–102	2	0.397	108–111	4	0.615	90–102
26	4	0.646	172–181	4	0.570	166–178	1	0.000	172	5	0.627	163–178
28	6	0.734	237–253	4	0.747	243–249	2	0.496	235–237	7	0.718	235–258
29	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
30	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
Mean	**5.6**	**0.628**		**5.3**	**0.540**		**2.8**	**0.356**		**8.8**	**0.742**	

Table 5. Number of alleles detected, expected heterozygosity, and allele size ranges in seven species representing three sections of *Sphagnum*. Sampling ranges for each species was as follows. *Sphagnum imbricatum*: northeastern China (see Methods); *S. magellanicum*: western and eastern North America, Europe and Asia; *S. palustre* and *S. junghuhnianum*: one site in China (see Methods); *S. subfulvum*: Alaska and Norway; *S. torreyanum*: Newfoundland to Georgia; *S. cuspidatum*: eastern North America, South America, Europe.

	sect. Sphagnum									sect. Acutifolia							sect. Cuspidata					
	S.imbricatum(110)			S. magellanicum(58)			S. palustre(30)			S. junghuhnianum(29)			S. subfulvum(25)				S. torreyanum(96)			S. cuspidatum(43)		
Locus	Na	He	Size	Na	He	Size	Na	He	Size	Na	He	Size	Na	He	Size range	Size	Na	He	Size	Na	He	Size
1	2	0.255	255–257	7	0.593	250–274	2	0.500	246–253	1	0.000	248	2	0.083	248–254	248–254	7	0.710	248–269	13	0.873	243–274
3	1	0.000	169	Na	Na	Na	1	0.000	168	1	0.000	182	6	0.753	175–238	175–238	Na	Na	Na	Na	Na	Na
4	6	0.402	179–197	5	0.177	182–187	1	0.000	199	4	0.449	179–193	5	0.431	175–191	175–191	8	0.676	175–193	13	0.773	175–204
5	4	0.628	192–198	7	0.773	188–200	4	0.486	192–204	Na	Na	Na	Na	Na	Na	Na	9	0.815	192–206	12	0.889	158–201
6	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	1	0.000	181	Na	Na	Na
7	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	16	0.797	184–205	Na	Na	Na
9	1	0.000	172	24	0.933	172–222	2	0.480	179–183	3	0.416	176–182	9	0.836	168–191	168–191	19	0.918	172–206	13	0.853	168–199
10	5	0.311	218–232	17	0.881	216–241	5	0.713	233–248	4	0.445	233–243	15	0.897	215–257	215–257	42	0.956	229–334	20	0.919	214–269
12	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	1	0.000	117	Na	Na	Na
14	18	0.806	191–233	16	0.900	191–237	4	0.673	223–235	3	0.245	203–219	14	0.900	199–241	199–241	34	0.957	195–264	19	0.907	201–247
17	2	0.018	156–158	Na	Na	Na	1	0.000	162	1	0.000	149	7	0.683	155–170	155–170	13	0.676	158–189	9	0.834	146–173
18	6	0.609	121–148	8	0.686	121–144	3	0.620	124–135	3	0.300	141–147	7	0.764	123–148	123–148	3	0.510	118–133	4	0.233	117–132
19	2	0.018	264–272	7	0.586	261–272	1	0.000	260	2	0.128	258–264	7	0.668	261–278	261–278	5	0.695	258–270	10	0.842	258–277
20	4	0.185	275–293	9	0.683	275–295	3	0.524	278–296	1	0.000	280	6	0.681	277–298	277–298	11	0.817	278–305	10	0.490	277–302
22	3	0.018	96–108	7	0.620	96–111	2	0.500	99–105	1	0.000	102	7	0.719	86–111	86–111	3	0.505	83–93	5	0.254	83–105
26	Na	Na	Na	Na	Na	Na	1	0.000	168	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
28	9	0.737	226–250	6	0.235	226–249	3	0.620	226–240	4	0.447	242–270	9	0.840	224–246	224–246	5	0.373	233–259			
29	1	0.000	195	5	0.603	190–201	2	0.500	192–195	1	0.000	201	9	0.861	189–207	189–207						
30	4	0.482	137–149	8	0.643	133–151	3	0.620	140–146	1	0.000	143	6	0.530	127–150	127–150	4	0.202	117–141	8	0.595	128–147
Mean	**4.8**	**0.298**		**10.3**	**0.642**		**2.4**	**0.390**		**2.1**	**0.174**		**7.8**	**0.688**			**11.8**	**0.627**		**11.6**	**0.715**	

of any such potential bias, especially when making interspecific comparisons.

In *Sphagnum*, there is little evidence of ascertainment bias across species from all four major sections of the genus (**Tables 4, 5**). The microsatellite loci presented in this paper were developed using combined DNA that included the four species of sect. *Subsecunda* included in **Table 4**. This was a strategy for identifying microsatellite loci that would be applicable to all species in the complex. We currently have (unpublished) data from seven additional species representing the sections *Acutifolia, Cuspidata* and *Sphagnum* (**Table 5**). Neither the average numbers of alleles per locus (Na) nor expected heterozygosity (He) (both averaged across loci) differ significantly between species of the sect. *Subsecunda* used for microsatellite development, and species from the other three sections ($P > 0.44$ and $P > 0.59$, respectively). Average allele sizes were also computed for each of 11 loci for which sufficient data were available; non-sect. *Subsecunda* species had significantly smaller average allele sizes for two loci (locus 18, $P < 0.03$; locus 20, $P < 0.02$). It thus appears that minimal ascertainment bias is associated with transferring microsatellite primers developed for species of the *S. subsecundum* complex to other sphagna, even those quite distantly related.

There appear to be species-specific patterns of allelic variation at different microsatellite loci, although locus 14 is highly variable in all taxa and locus 10 is extremely variable in some (**Tables 4**, **5**). We detected 42 alleles for locus 10 in eastern North American plants of *S. torreyanum*, a species of sect. *Cuspidata*. Our data are insufficient to make species comparisons because sampling was not undertaken for that purpose and is very much heterogenous across species. For example, all samples of *S. palustre* and *S. junghuhnianum* came from a single site, whereas *S. torreyanum* was sampled across its whole range from Newfoundland to the Gulf of Mexico. Section *Subsecunda* species were sampled from Newfoundland and Norway (expect *S. lescurii*, which does not occur in Europe). Additional information about the samples included in **Tables 4 and 5** is provided in the legends for those tables. While sample sizes and sampling intensity differed widely among species, there was no systematic difference in sampling between species of the sect. *Subsecunda* and other *Sphagnum* taxa that would likely "hide" any strong ascertainment bias associated with transferring primers to divergent congeneric species.

Conclusions

Microsatellite markers have been used infrequently in mosses to-date, but offer much potential for evolutionary research. The co-dominant genetics of microsatellites offers a major advantage over other fingerprinting approaches such as RAPDs, AFLPs and ISSRs, especially for studies of hybridization and mating patterns since both parental genomes can be detected directly by PCR amplification. The present results demonstrate that microsatellite primers developed for species in *Sphagnum* sect. *Subsecunda* also work well with species in other sections across the genus, and there seems to be little if any ascertainment bias introduced by interspecific transfer of markers.

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