Ploidy level, genetic diversity, and differentiation in two closely related mosses, *Scorpidium cossonii* and *S. revolvens* (Calliergonaceae)

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As ploidy level and mating system can affect genetic diversity and differentiation, we conducted population genetic analyses of two closely related mosses, *Scorpidium cossonii* (Schimp.) Hedénäs, and *S. revolvens* (Sw. ex Anonymo) Rubers which differ in ploidy level and sexual system. We collected 315 specimens in total from five populations of *S. cossonii* and four populations of *S. revolvens* in the Swiss Alps. Ploidy level, genetic diversity within populations, and genetic differentiation between populations and species were estimated using nine microsatellite markers. In each *S. cossonii* sample, each locus bore only one allele, while in *S. revolvens*, seven out of the nine loci were fixed or nearly fixed for two alleles per locus per individual. These findings are consistent with a gametophytic haploid *S. cossonii* and allodiploid *S. revolvens*. The haploid and dioicous *S. cossonii* was genetically more diverse than the (allo)diploid and monoicous *S. revolvens*. Differences in genetic diversity between the two species may be explained by different mating systems, different population sizes, and different population histories. Genetic differentiation among populations of *S. cossonii* was higher than among those of *S. revolvens*. The low genetic differentiation among populations of the monoicous species was not unexpected, since monoicous species frequently produce sporophytes, long-distance spore dispersal is more likely and leads to low differentiation.

Keywords: Allodiploidy, Calcareous fen, Dioicous, Haploid, Monoicous

Introduction

In common with many wetland mosses, the species of the genus *Scorpidium* show large morphological plasticity challenging the circumscription of these species. *Scorpidium cossonii* (Schimp.) Hedénäs and *S. revolvens* (Sw. ex Anonymo) Rubers are very similar in morphology and ecology and were not separated in some earlier floras (e.g. Smith, 1978). In contrast, in the revision of Hedénäs (1989), the two taxa are considered as separate species. Since the two species look very different from the third species of the genus, *S. scropioides* (Hedw.) Limpr., some authors have placed *S. cossonii* and *S. revolvens* into the genus *Limprichtia* (e.g. Corley & Crundwell, 1991), which was described by Loeske (1907). Using chloroplast genes and nuclear internal transcribed spacer (ITS) sequences, Hedénäs & Eldenäs (2008) found *S. cossonii* to be more closely related to *S. scropioides* than to *S. revolvens*. This finding clearly contradicts morphological analyses (Hedénäs, 1989, 2003): *S. scropioides* is a robust species with no leaf costa, while the two other species are more slender with costate leaves.

While *S. cossonii* is dioicous and seems to be a gametophytic haploid, *S. revolvens* is monoicous and is considered to be a gametophytic diploid. However, the estimation of ploidy is based on only two specimens, one from each species (Smith & Newton, 1968). *Scorpidium cossonii* is commonly found in wet and calcium-rich habitats, such as fens, wet meadows, springs and shores, and occurs from sea level up to the alpine region (Hedénäs, 1989, 2003). It is widely distributed in the Holarctic and also in South America and New Zealand. *Scorpidium revolvens* grows in similar habitats to *S. cossonii* but is more restricted to sites with lower pH and conductivity, however, the two species grow in an overlapping range of habitats (Kooijman & Hedénäs, 1991). *Scorpidium revolvens* is also very widely distributed.
in temperate to sub-polar areas of both hemispheres (Hedenäs, 2003).

Until the beginning of the 1980s, it was assumed that haploid gametophytes retain low levels of genetic variation due to natural selection operating directly on all alleles, i.e. there is no retention of deleterious alleles, as in diploid organisms, so that those alleles are eliminated quickly from the genomes of haploid bryophytes (Anderson, 1963). Nevertheless, high levels of genetic diversity in haploid gametophytes have been found as revealed by several kinds of genetic markers, such as RAPDs and isozymes (Cummins & Wyatt, 1981; Wyatt et al., 1989, 2005; Stenoien & Sästad, 1999). ISSRs (Spagnuolo et al., 2007; Buczewska et al., 2010), and microsatellites (Wilson & Provan, 2003; Shaw et al., 2008; Hutsemekers et al., 2010). It is suggested that genetic diversity in haploid bryophytes could be maintained by multiple-niche selections (Wyatt et al., 1989), interlocus interaction, i.e. epistasis (Shaw & Beer, 1999), sexual reproduction (Wyatt et al., 2005), and somatic mutations (Skotnicki et al., 2005). Furthermore, Taylor et al. (2007) found that predominance of haploids does not purge all deleterious alleles.

There is increasing evidence that gametophytic diploidy is a common phenomena in liverworts such as Pellia, Calypogeia, and Porella (for a review, see Shaw, 2009). However, chromosome counts imply that in mosses also, diploid or even polyploid species are probably quite common (Fritsch, 1991; Sästad, 2005). Molecular studies in some moss families, mainly Sphagnaceae, Polytrichaceae, and Mniaceae corroborate this view (Shaw, 2009). Diploids have also been found in the Brachytheciaceae (Platynhynidium riparioiides (Hedw.) Dixon; Hutsemekers et al., 2010) and Calliergonaceae (Warnstorfia, Scopridium: Nagl & Ullmann, 1973; Hedenäs, 1989; Fritsch, 1991). Near or total fixation for two alleles per locus per individual leads to the assumption that polyploids in bryophytes are mainly of allopolyploid origin (Sästad, 2005; Shaw, 2009; Karlin et al., 2010a). However, as Shaw (2009) has pointed out, ‘Sästad’s (2005) rejection of autoploidy as an unimportant feature of bryophyte evolution may have been premature’. Indeed, Hutsemekers et al. (2010) found evidence for autoploidy in Platynhynidium (Rhynchohystegium) riparioiides.

In monoicous mosses, levels of selfing are usually much higher than in dioicous mosses (Epplsey et al., 2007). Populations of outbreeding species are generally expected to contain higher genetic diversity than inbred populations (Frankham et al., 2004). The breeding system thus affects the genetic diversity of species. In monoicous, allopolyploid species, however, the effect of selfing is reduced due to fixation for two alleles per locus individual, i.e. heterozygosity is maintained through generations (Comai, 2005) and inbreeding depression may thus be low (Vandepitte et al., 2011). The mating system may also influence genetic differentiation between bryophyte populations. Selfing is reported as frequent in monoicous bryophytes (Wyatt, 1994; Roads & Longton, 2003; Epplsey et al., 2007; Karlin et al., 2011a) and sporophytes are commonly produced in such species (Longton & Miles, 1982). In contrast, sporophytes are more rarely produced in dioicous species (Longton & Miles, 1982) because distances between male and female gametangia in these species are often too large for the transfer of spermatozoids, which have very limited dispersal distances (centimetres up to a few decimetres; Rydin, 2009). As spores may be dispersed over long distances by wind, little genetic differentiation is found between populations of monoicous, sporophyte-producing species (e.g. Wyatt et al., 1992, 1993b; Stenoien & Sästad, 1999; Szövényi et al., 2008; Karlin et al., 2011a).

In this study, we compared the genetic diversity and differentiation between populations and species of the haploid, dioicous S. cossonii and the diploid, monoicous S. revolvens using microsatellite markers to obtain deeper insights into the genetic structure of bryophyte populations. We did not include S. scoprioides in this study because its populations are too small and patchily distributed in Switzerland for our study design. We asked the following specific questions:

1. Is there evidence for diploidy in S. revolvens?
2. Is there a difference in genetic diversity between S. cossonii and S. revolvens?
3. How strong is the genetic differentiation among populations within species and between the two species?

Materials and Methods

Sampling

We sampled 315 specimens in total from five populations of S. cossonii and four populations of S. revolvens from the Alps in the Canton of Graubünden, Switzerland (Figure 1 and Table 1). In each site, five circular plots with 2 m radius were set up within an area of approx. 1 hectare (circle with radius of 56.4 m). In each plot, seven specimens consisting of two to five shoots were collected. The first specimen was always collected in the centre of the plot. The other six specimens were sampled at 60° intervals along the circumference of the plot. If there was no Scopridium at these predefined positions, then the search strategy was to move slowly closer to the centre until a specimen could be found. Owing to the small population size of S. revolvens, these plots could not be set up in two populations (R.Alb and R.San). In these cases, we collected the 35 specimens in the one hectare plot while trying to
disperse the sampling positions as much as possible within the hectare. In total, each population was represented by 35 samples (but see results).

**Confirmation of the species identity by nuclear ITS sequence analysis**

One single shoot from each population as well as morphologically ambiguous samples (total 19 specimens examined) were sequenced for the nrITS region (Rydin et al., 2004). PCR reactions were performed in a volume of 10 \( \mu l \) containing 0.4 \( \mu g \) genomic DNA, 0.25 \( \mu M \) of each primer, and 3.6 \( \mu l \) JumpStart REDTaq ReadyMix Reaction Mix for PCR (Sigma-Aldrich, St Louis, MO, USA). PCR cycles were started with 2 min at 94°C for initial activation, followed by 30 cycles of 30 s at 94°C, 1 min at 54°C, and 90 s at 72°C, ending with a final extension step of 10 min at 72°C. PCR products were purified using ExoSAP-IT PCR Cleaning Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer’s protocols. Sequencing reactions were accomplished using the BigDye Terminator v3.1 Cycle Sequencing Kit and products were cleaned up using the BigDye XTerminator Purification Kit (Life Technologies, Carlsbad, CA, USA) following the manufacturer’s protocol. DNA was sequenced on an ABI3130 automated sequencer (Life Technologies). Subsequently, the sequences were submitted to BLASTn (GenBank) to identify similarity to known sequences. For each species and sample (JN681177-85, JX070656-65), the polymorphic sites were recorded (Table 2).

**Microsatellite analysis**

Microsatellite sequences and protocols for DNA extraction, PCR amplification and allele sizing are described in Kophimai et al. (2011). For *S. revolvens*,...
all annealing temperatures were optimized: for marker Sc01, we used Ta=54°C; for Sc02, Sc04, Sc07, and Sc09, Ta=58°C; finally for all other markers, Ta=56°C.

All 14 microsatellite markers developed for S. cossonii amplified successfully with S. revolvens and these products were sequenced to verify the microsatellite character of the sequences. In fact, 13 loci consisted of the same type of microsatellite as in S. cossonii, but one locus, Sc22, did not contain a microsatellite. Therefore, this locus was excluded. After analysing the 13 loci for all samples, four loci were not polymorphic in S. revolvens and, thus, were omitted from further analyses. Finally, nine polymorphic loci (Sc02, Sc03, Sc04, Sc07, Sc09, Sc16, Sc17, Sc18, and Sc21) were applied in this study. As microsatellites were developed for S. cossonii, the presence of one allele per locus per individual in S. revolvens may result from null alleles (Peakall et al., 1998). To eliminate effects of potential null alleles, data were also analysed using only seven loci that had two alleles per locus in all samples of S. revolvens (Sc03, Sc04, Sc09, Sc16, Sc17, Sc18, and Sc21).

Ploidy determination
The two species studied are reported to have different numbers of chromosomes; the gametophyte of S. cossonii has n=10+1 m chromosomes, whereas S. revolvens has n=20 chromosomes (Smith & Newton, 1968). Gametophytes are thus being interpreted as haploid for S. cossonii but diploid for S. revolvens. According to Ricca et al. (2008) and Karlin et al. (2011b) the ploidy level can be estimated based on the zygosity character of microsatellite markers. Samples with more than 50% heterozygous loci are defined as diploid and with less than 15% as haploid. If heterozygosity is fixed or nearly fixed at most of the studied loci, a species can be considered to be of allopolyploid origin (Karlin et al., 2010a,b).

Data analysis
Microsatellite alleles were coded as allele lengths. Since the two species differed in their ploidy levels, they were analysed separately except for comparison between species (principal coordinates analyses and cluster analyses) and allelic richness. For the purpose of these analyses, the genetic data of the haploid species were coded as homozygous, diploid data.

Genetic diversity
Arlequin ver. 3.5 (Excoffier & Schneider, 2005) was used to calculate the number of multilocus genotypes (for S. cossonii), the mean number of alleles and Nei’s gene diversity (Nei, 1987). The number of multilocus genotypes of S. revolvens was calculated using GenClone ver. 2.0 (Arnaud-Haond & Belkhir, 2007). The effective number of alleles was estimated by GenAlEx 6.5 (Peakall & Smouse, 2012). In S. revolvens, observed heterozygosity was computed manually. Owing to the different numbers of samples in populations (see the section on ‘Results’), allelic richness was corrected for unequal sample size using rarefaction. Rarefaction of allelic richness was performed using the programme Microsatellite Analyzer ver. 4.05 (Dieringer & Schlötterer, 2003). The genetic diversity of seven and nine loci was compared between species using Wilcoxon’s test implemented in the software R version 2.15.1 (R Development Core Team, 2012).

Multilocus linkage disequilibrium, r_d, was analysed using MultiLocus 1.3 (Agapow & Burt, 2001) to estimate the extent of clonality and recombination. Since clonality can mask effects of recombination, data for this analysis were clone corrected. The significance was tested under the null hypothesis of no linkage disequilibrium, i.e. infinite recombination, by shuffling alleles across individuals in the same population (1000 randomizations). However, the data were not analysed when the number of multilocus genotypes in a population was less than four (Brown, 1975).

Genetic differentiation between populations and species
As rare alleles are indicative of genetic isolation, the number of private alleles (alleles that are observed in only one of the studied populations of each species) was quantified using GenAlEx 6.5 (Peakall & Smouse, 2012). The same program was used to calculate the number of shared alleles between species and to perform principal coordinates analysis.
Although these results demonstrate that both species can co-exist at rather small spatial scales (distances between specimens < 2 m). The nrITS DNA sequence analysis of one sample from each population corresponded to the morphologically defined species as detected by haplotype matching to known sequences. The final number of samples of *S. revolvens* was thus 130 and six loci in this species had a heterozygosity of 1.0 (Table 3).

### Genetic diversity

The number of alleles observed at each locus varied between 3 and 27 for *S. cossonii* and between 2 and 8 for *S. revolvens* (Table 3). Over all samples, *S. cossonii* yielded 85 alleles and 62 multilocus genotypes. *Scorpidium revolvens* was considerably less diverse with only 31 alleles and 13 multilocus genotypes. At the population level, *S. cossonii* showed more genetic diversity within populations than *S. revolvens* (Table 1). A comparison of genetic diversity of nine loci between species showed that there were marginally significant differences in mean gene diversity (Wilcoxon test: $W=18$, $P=0.063$) and the effective number of alleles ($W=18$, $P=0.065$), although there were strong differences in the mean number of alleles ($W=20$, $P=0.016$) and in the number of multilocus genotypes ($W=19.5$, $P=0.027$) with *S. cossonii* always having higher values for all of these measures.

As microsatellite markers were developed for *S. cossonii*, there is some chance of having null alleles (non-amplified alleles; Peakall *et al.*, 1998) in *S. revolvens*. Comparisons of genetic diversity between the two species were thus re-analysed using only the seven loci that had two alleles per locus in all samples of *S. revolvens*. Using only these seven loci, mean gene diversity and effective number of alleles were not significantly different between species ($W=9$, $P=0.905$ and $W=11$, $P=0.905$, respectively). However, mean number of alleles and number of multilocus genotypes were still significantly higher in *S. cossonii* ($W=20$, $P=0.016$ for both comparisons).

Mean allelic richness, based on a rarefaction of the data to 28 samples per population, of seven and nine loci were 3.976 and 4.540 for *S. cossonii* and 2.422 and 2.190 for *S. revolvens*, respectively. Significant differences in mean allelic richness between species were found for both marker sets ($W=20$, $P=0.016$).

![Image of Scorpidium sp](image-url)
A high incidence of multilocus linkage disequilibrium was found in *S. cossonii* (Table 1). In *S. revolvens*, multilocus linkage disequilibrium was calculated for only two populations which had at least four multilocus genotypes. For both of these populations, the measures of linkage disequilibrium were as high as in *S. cossonii* but not significant. Because the number of multilocus genotypes in *S. revolvens* was low, the non-significance of the multilocus linkage disequilibrium should be interpreted with caution.

**Genetic differentiation between populations and species**

In general, the populations of *S. cossonii* tended to have more private alleles than the populations of *S. revolvens* (1–16 versus 0–8; Table 1). However, the differences were not significant. When considering nine loci, seven alleles at five loci were shared between the two species. However, only one allele had a high frequency in both species (0.49 in *S. cossonii*, 0.50 in *S. revolvens*).

Based on the $F_{ST}$ values, all populations of *S. cossonii* were different from each other ($P < 0.05$; Table 4). In contrast, the populations of *S. revolvens* revealed a lower degree of differentiation (Table 5). Overall means of pairwise $F_{ST}$ values of seven and nine loci were 0.218 and 0.200 for *S. cossonii*, and 0.091 and 0.125 for *S. revolvens*, respectively. $D_{est}$ showed similar trend to $F_{ST}$ (Tables 4 and 5). The genetic differentiation between populations of *S. cossonii* was also higher than between those of *S. revolvens*. The means of $D_{est}$ for seven and nine loci were 0.076 and 0.080 for *S. cossonii*, and 0.048 and 0.066 for *S. revolvens*, respectively. Furthermore, the genetic variation among the populations of *S. cossonii* was higher than among those of *S. revolvens* (approximately 20 and 22% versus 15 and 10%; Table 6).

**Discussion**

**Ploidy level**

Each sample of *S. cossonii* in the five sampled populations had only one allele at each locus, which is highly indicative of a haploid genome. This is in accordance with published chromosome numbers where *S. cossonii* is reported to have $n = 10 + 1$ in chromosomes and *S. revolvens* $n = 20$ (Smith & Newton, 1968).

As several loci in *S. revolvens* were fixed or nearly fixed for two alleles per locus per individual, we conclude that this species is most likely allohexaploid (Karlin et al., 2010a,b). Occurrences of one allele per locus per individual may be caused either by identical alleles at those loci in the haploid parents, by the non-amplification of one allele (null allele) or by the

<table>
<thead>
<tr>
<th>Population</th>
<th>C.Pun</th>
<th>C.Mue</th>
<th>C.Tar</th>
<th>C.Scu</th>
<th>C.Biv</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.Pun</td>
<td>0.035 (0.017)</td>
<td>...</td>
<td>0.083 (0.059)</td>
<td>0.056 (0.044)</td>
<td>0.116 (0.124)</td>
</tr>
<tr>
<td>C.Mue</td>
<td>0.100 (0.079)</td>
<td>...</td>
<td>0.093 (0.105)</td>
<td>0.010 (0.008)</td>
<td>0.107 (0.126)</td>
</tr>
<tr>
<td>C.Tar</td>
<td>0.195 (0.184)</td>
<td>0.211 (0.228)</td>
<td>...</td>
<td>0.057 (0.052)</td>
<td>0.122 (0.101)</td>
</tr>
<tr>
<td>C.Scu</td>
<td>0.145 (0.142)</td>
<td>0.082 (0.079)</td>
<td>0.209 (0.221)</td>
<td>...</td>
<td>0.117 (0.128)</td>
</tr>
<tr>
<td>C.Biv</td>
<td>0.226 (0.266)</td>
<td>0.257 (0.317)</td>
<td>0.270 (0.293)</td>
<td>0.307 (0.369)</td>
<td>...</td>
</tr>
</tbody>
</table>

**Note:** $F_{ST}$ values significantly ($P < 0.05$) different from zero are indicated in bold.

Table 4 Pairwise $F_{ST}$ (lower diagonal) and $D_{est}$ (upper diagonal) of *Scorpidium cossonii* populations estimated from nine loci and seven loci (in brackets)

Table 5 Pairwise $F_{ST}$ (lower diagonal) and $D_{est}$ (upper diagonal) of *Scorpidium revolvens* populations estimated from nine loci and seven loci (in brackets)

<table>
<thead>
<tr>
<th>Population</th>
<th>R.Alb</th>
<th>R.Ma</th>
<th>R.Ro</th>
<th>R.San</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.Alb</td>
<td>...</td>
<td>0.010 (0.015)</td>
<td>0.002 (0.003)</td>
<td>0.164 (0.124)</td>
</tr>
<tr>
<td>R.Ma</td>
<td>0.055 (0.054)</td>
<td>...</td>
<td>0.001 (0.001)</td>
<td>0.105 (0.072)</td>
</tr>
<tr>
<td>R.Ro</td>
<td>0.027 (0.027)</td>
<td>0.023 (0.021)</td>
<td>...</td>
<td>0.113 (0.071)</td>
</tr>
<tr>
<td>R.San</td>
<td>0.242 (0.176)</td>
<td>0.185 (0.127)</td>
<td>0.219 (0.140)</td>
<td>...</td>
</tr>
</tbody>
</table>

**Note:** $F_{ST}$ values significantly ($P < 0.05$) different from zero are indicated in bold.
pating of a homeologous chromosome (Ricca et al., 2008). According to molecular evidence, it is apparent that polyploid bryophytes are mostly of allopolyploid origin (Sástad, 2005), but there may be an investigation bias. For example, Hutsemekers et al. (2010) found no populations fixed for two alleles per locus per individual in the diploid aquatic moss Platyhypnidium (Rhynchostegium) riparioiodes and interpreted this species as autopolyploid.

Of the ten S. cossonii specimens intermingled in the S. revolvens populations, one locus had two alleles per individual in nine samples but all other loci had one allele per locus per individual in all 10 samples indicating gene duplication, aneuploidy or genetic mosaics rather than diploidy as hypothesized for S. revolvens populations, one locus had two alleles and interpreted this species as autopolyploid.

An open question is what the possible parental species of S. revolvens are. Very likely, S. cossonii is one of the parents as it is morphologically very similar to S. revolvens (Hedenäs, 1989), all 14 primers of the microsatellites developed for S. cossonii successfully amplified in S. revolvens (Kophimai et al., 2011), and 13 microsatellite loci contain the same repeat type in the two species. Moreover, some alleles are shared between the two species while one allele had a high frequency in both species. Scorpidium scorpioides is a further possibility. Twelve microsatellites were amplified in S. scorpioides (Kophimai et al., 2011) and morphologically it is even closer to S. revolvens (Hedenäs, 1989), all 14 primers of the microsatellites developed for S. cossonii as was shown by Hedenäs and Eldenäs (2008). Hamatocaulis vernicosus (Mitt.) Hedenäs could also be a candidate. H. vernicosus is the closest relative to the genus Scorpidium (Hedenäs, 1989). For this species nine out of the 14 original microsatellite loci amplified successfully (Y. Kophimai, unpubl. results).

**Genetic diversity**

At the species level, average gene diversities over all populations and nine loci of S. cossonii and S. revolvens were 0.594 and 0.457, respectively. When corrected for possible null alleles, mean gene diversity changed to 0.561 for S. cossonii and 0.545 for S. revolvens. The levels of gene diversity in both species are intermediate to high when compared to other mosses which were also analysed by microsatellites and similar numbers of samples per population (25–44 samples). For example, in the moss genus Polytrichum, gene diversities of 0.815 have been reported for P. commune Hedw. (Wilson & Provan, 2003) and 0.252 for P. formosum Hedw. (van der Velde et al., 2001). In three Chinese Sphagnum L. species, values ranged from 0.174 to 0.367 (Shaw et al., 2008) and in Rhytidadelphus subpinnatus (Lindb.) T.J.Kop. and R. squarrosus (Hedw.) Warnst., gene diversities were 0.28 and 0.20 respectively (Korpelainen et al., 2008).

The data in Table 1 show that genetic diversity in S. cossonii tends to be higher than that of S. revolvens, especially in the number of alleles and multilocus genotypes. The higher level of genetic diversity in S. cossonii relative to that of S. revolvens may be due to the more frequent occurrence of high ploidy levels in the former species. This is supported by the finding that the highest genetic diversity was found in S. cossonii populations.

### Table 6 Analysis of molecular variance (AMOVA) for microsatellite variation of Scorpidium cossonii and S. revolvens estimated from nine and seven loci (in brackets)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>SS (in brackets)</th>
<th>Variance%</th>
<th>Fixation index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. cossonii</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>4</td>
<td>106.7 (87.5)</td>
<td>20.4 (22.4)</td>
<td>F_{ST}=0.204***</td>
</tr>
<tr>
<td>Within populations</td>
<td>170</td>
<td>454.6 (333.9)</td>
<td>79.6 (77.6)</td>
<td>F_{ST}=0.224***</td>
</tr>
<tr>
<td><strong>S. revolvens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>3</td>
<td>75.0 (46.4)</td>
<td>14.7 (9.9 )</td>
<td>F_{ST}=0.147***</td>
</tr>
<tr>
<td>Within populations</td>
<td>256</td>
<td>592.2 (488.3)</td>
<td>85.3 (90.1)</td>
<td>F_{ST}=0.099***</td>
</tr>
</tbody>
</table>

Note: ***P < 0.001.
was unexpected because the first species is considered to be a haploid and the second an (allo)diploid. Diploids have a higher effective population size and twice the number of gene copies are sampled in a diploid species in comparison to a haploid species. Moreover, if microsatellite markers are linked to a gene that is negatively selected, genetic diversity in haploid species could be further reduced due to elimination of deleterious alleles. Shaw et al. (2008) and Wyatt et al. (1993a) found that diploid and polyploid species had higher genetic diversity than haploid species. Therefore, the pattern found for S. cossonii and S. revolvens suggests that allopolyploidy per se might not be a good predictor for the expected level of genetic diversity in bryophytes.

How can the rather high genetic diversity in S. cossonii be explained? Scorpidium cossonii is dioicous and thus, sporophytes are either produced by outcrossing or by inter-gametophytic selfing. However, sporophytes have never been found in the study area and there is no recent finding of their occurrence in Switzerland (NISM, 2004–2013). During field work, only a few gametophytes expressing female gametangia were found (personal observations). These observations may imply a female-biased sex ratio in this species, as is commonly detected in other dioicous bryophytes (Bisang & Hedénäs, 2005; Hock et al., 2009). Owing to the lack of sporophytes and high incidences of multilocus linkage disequilibrium even in clone-corrected data ($r_{D}$; Table 1), recent genetic recombination is unlikely. Moreover, there is evidence that two or more samples from the same plot differ at only one locus, especially the highly polymorphic one, Sc02. Somatic mutations are a possible explanation of the detected genetic diversity as has been assumed for other bryophytes (e.g. Skotnicki et al., 2004, 2005; Buczkowska et al., 2010; Karlin et al., 2012).

Long-distance dispersal might be a further cause of the high genetic diversity found within S. cossonii. There is ample evidence of long distance dispersal in bryophytes (e.g. Muñoz et al., 2004; Vanderpoorten et al., 2008; Karlin et al., 2011a, 2012). It is reported that 19.7% of Swedish herbarium specimens of S. cossonii bear sporophytes (Bisang & Hedénäs, 2005) and spore size is rather small (14–20 μm; Hedénäs, 1989). Given this small spore size and much more frequent sporophyte production at least in some areas, long-distance spore dispersal might have occurred and a high number of founders could contribute to the genetic diversity within populations of this species. If long-distance dispersal is rather frequent, then isolated sites may also show high genetic diversity due to frequent founders from the ‘spore cloud’ (inverse isolation hypothesis, Szövenyi et al., 2012).

What could be the causes of lower genetic diversity in S. revolvens, despite it having two alleles per locus per individual? Because this species is monoicous, sporophytes may be produced by outcrossing, inter-gametophytic selfing, and/or intra-gametophytic selfing. So far, all monoicous bryophyte species tested have been reported to be capable of intra-gametophytic selfing (Shaw, 2000). Since the successful fertilization depends on the distance between male and female gametangia, monoicous species are more effective in sexual reproduction than dioicous ones (Longton & Miles, 1982; Mishler, 1988). Unsurprisingly, high rates of intra-gametophytic selfing were reported in monoicous mosses (Anderson, 1963; Stark, 1983; Mishler, 2001; Eppley et al., 2007; Karlin et al., 2011a). Therefore, the genetic variation of monoicous mosses is expected to be low (Roads & Longton, 2003) and lower than that of outcrossing species (Cronberg, 1996; Appelgren & Cronberg, 1999). However, cross-fertilization is not excluded in monoicous species and has been demonstrated for example in a monoicous liverwort (Mishler, 1988). In line with selfing, we found a low number of multilocus genotypes in S. revolvens.

The rarity of S. revolvens may further contribute to its low levels of genetic diversity. In Switzerland, S. revolvens is considerably rarer than S. cossonii (NISM, 2004–2013). As random genetic drift plays an important role in small populations, rare alleles may be lost by chance and common alleles may become fixed, resulting in low genetic diversity, but probably high differentiation between populations (Frankham et al., 2004). Lower genetic diversity in rare species compared to common species is a well-known phenomenon in flowering plants (Cole, 2003) and has also been reported in the moss genus Plagiomnium section Rosulata (Wyatt, 1992).

Besides the mating system and the regional frequency, different population histories may account for the differences in genetic diversity found in the two species. For example, a recent origin of S. revolvens (Baumel et al., 2002; Jakobsson et al., 2006) or a population bottleneck after inter-species hybridization (Hazzouri et al., 2008) could cause the observed low genetic diversity of S. revolvens. A population bottleneck during the last glacial period is a further possibility. Scorpidium revolvens prefers more acidic substrates than S. cossonii (Kooijman & Hedénäs, 1991). In Switzerland, S. revolvens thus occurs mainly in the Central Alps (NISM, 2004–2013, pers. observation) where acidic rocks predominate. These areas were heavily glaciated during the last glacial period and refugial areas with acidic bedrock are less common than those with calcareous bedrock, and are mainly located in the Southern Alps.
(Schönswetter et al., 2005). It thus seems not unlikely that the lower genetic diversity of *S. revolvens* reflects a bottleneck during the last glaciation.

For the analysis of genetic variation in diploid species, it may be useful to distinguish within individual and among individual genetic variation. However, for *S. revolvens*, a hierarchical AMOVA including within individual variation revealed anomalous results (AMOVA not shown). Percentage of variance among individuals was strongly negative (−57.9%) as were the *F*~ST~ and *F*~IS~ values, −0.42 and −0.69, respectively). This indicates that this type of analysis is not appropriate for *S. revolvens*, probably due to small numbers of genotypes, and fixed for two alleles per locus per individual.

Differences in genetic diversity between the two moss species hold true for the particular set of markers used in this study. As the microsatellite markers were developed for *S. cossonii*, we have to consider the possibility of an ascertainment bias when applying these markers to other species (Ellegren et al., 1995). If ascertainment bias played a role in this study, the true level of genetic diversity of *S. revolvens* would be higher than our estimates. However, the data in Table 3 imply that genetic diversity in *S. revolvens* tends to be low because seven out of nine loci showed a rather low number of alleles and even the most polymorphic locus contained only eight alleles in 130 individuals. Moreover, there are some other lines of evidence supporting our conclusion of lower genetic diversity in *S. revolvens* as mentioned above such as the possibility of intra-gametophytic selfing, different population histories, much smaller population size of the species in Switzerland compared to that of *S. cossonii*. However, this study covers only a small part of the species distribution range and thus an extension of the study region and an increase in population sampling may provide more accurate estimations of the genetic diversity and differentiation of the two mosses.

**Genetic differentiations among populations**

*Scorpidium cossonii* populations differ more from one another than those of *S. revolvens*, as indicated by larger numbers of private alleles, higher *F*~ST~ and *D*~est~ values and greater genetic variation among populations as quantified by AMOVA. The stronger differentiation among populations of *S. cossonii* than among *S. revolvens* could be explained by predominantly asexual reproduction (e.g. by stem fragments) in *S. cossonii*. Dispersal distances for asexual propagules are usually short, as due to their weight they are less suitable for long distance dispersal (Kimmerer, 1991; Laaka-Lindberg et al., 2003; Löbel et al., 2006). Therefore, genetic differentiation among populations of *S. cossonii* is rather high.

Sporophytes are more common in *S. revolvens* than in *S. cossonii* in Switzerland (Amann et al., 1918) and spore size is rather small, (12–16 μm; Smith, 2004). Spores of this size are usually considered to be easily dispersed by wind over long distances (Frahm, 2008). If such long-distance transport of spores together with successful establishment occurs occasionally, then the genetic differentiation between populations is low (Karlin et al., 2011a) and this is likely to be the case for *S. revolvens*. Indeed some studies have shown that long distance dispersal is highly likely in mosses. For example, no significant genetic differentiation was observed between *Polytrichum formosum* populations in Denmark and the Netherlands which are 450 km apart (Van der Velde et al., 2001) and little genetic differentiation was found between European and North American populations of *Sphagnum* species (Szövényi et al., 2008). Low genetic differentiation between populations of monoecious mosses was also detected in *Plagiomnium medium* (Bruch & Schimp.) T.J.Kop. (Wyatt et al., 1992), *Rhizomnium pseudopunctatum* (Bruch & Schimp.) T.J.Kop. (Wyatt et al., 1993b), and *Sphagnum lindbergii* Schimp. (Stenoien & Sästad, 1999).

One population of *S. revolvens* (R.san) appeared to be more differentiated from the other populations. R.San is the most western population of *S. revolvens* in our study area (Figure 1) and is located in the Southern Alps, although very close to the border with the Central Alps. The presence of a distinct multilocus genotype in R.San (Figure 2A) suggests that this population has had a different history and that gene flow from R.San to other populations is rather limited as indicated by the high and significant *F*~ST~ values in Table 6.

**Genetic differentiation between species**

In contrast to their morphological similarity, there is no molecular evidence for treating *S. cossonii* and *S. revolvens* as a single species. Our nuclear microsatellites and internal transcribed spacer data are consistent with the nrITS and cDNA phylogenetic studies of Hedénäs and Eldénäs (2008) in demonstrating that *S. cossonii* is more closely related to *S. scorpioides* than to *S. revolvens*.

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References


