Amount and structure of intra- and interspecific genetic variation in the moss genus *Polytrichum*

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Allozyme electrophoresis was used to determine amount and structure of genetic variation within and between five congeneric haploid moss species: *Polytrichum formosum*, *P. commune*, *P. uliginosum*, *P. piliferum* and *P. juniperinum*. For the different species, gene diversity within populations (H_S) ranged from very low (0.025) to moderate values (0.127), being, on average, lower than those observed for vascular plants and many other moss species. *Polytrichum piliferum* and *P. juniperinum* showed significantly higher levels of H_S than the other species examined, which possibly might be explained by sexual reproduction being more prevalent in these two species, that often live in more dynamic habitats, where turnover of individuals is more frequent. Genetic variability was observed to be structured in contrasting ways at different levels. At the intraspecific level genetic differentiation among populations of most *Polytrichum* species was low, $F_{ST} \leq 0.1$, indicating a considerable degree of gene flow by wind-dispersed spores over substantial distances. At the interspecific level strong divergence, genetic identities being on average $I = 0.222$, was observed between most species studied, despite low levels of divergence at the morphological level. These *I*-values are significantly lower than observed for congeneric vascular plant species and most congeneric bryophyte species. This suggests that the morphological conservatism is not due to lack of genetic variability and evolutionary potential.

Keywords: allozymes, bryophytes, genetic differentiation, genetic variation, *Polytrichum*, species differentiation.

Introduction

In contrast to a large number of investigations that have determined the amount and structure of genetic variation within and among vascular plant populations (Hamrick & Godt, 1990), bryophytes have received relatively little attention in this respect (Ennos, 1990). This seems undeserved as they are the second largest group of land plants and can be found in almost every terrestrial habitat and in many freshwater habitats, where they can reach high levels of abundance (During & Van Tooren, 1987). Moreover, bryophytes show various modes of reproduction: asexual reproduction via clonal growth and vegetative propagules, and sexual reproduction via spores produced by monoecious and dioecious species (During & Van Tooren, 1987; During, 1990). Because of this versatility in reproductive characteristics, they offer a unique opportunity to study the

importance of different life histories on the level and structure of genetic variation (Ennos, 1990).

For vascular plants, it has been shown that breeding system, mode of reproduction, dispersal ability and spatial population structure significantly affect the structure and amount of genetic variation (Hamrick & Godt, 1990). Among the factors that are thought to shape the level and structure of genetic variation in bryophytes, many are related to reproductive biology (Longton, 1976; During, 1990; Ennos, 1990). Most of these reproductive characteristics of bryophytes are expected to decrease levels of genetic variation: predominance of asexual reproduction (e.g. During, 1990), high levels of self-fertilization in monoecious species (Longton, 1976; During, 1990; Ennos, 1990), rarity of sexual reproduction in dioecious species due to spatial separation of the sexes (Longton, 1976), and low regeneration probabilities of spores produced by sexual reproduction (e.g. Miles & Longton, 1987). In addition, most bryophytes show a predominantly haploid life cycle and consequently new recessive (slightly)

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deleterious mutations will not be concealed in heterozygotes but will be directly selected against (Longton, 1976; Ennos, 1990). Moreover, many forms of balancing selection, such as (marginal) overdominance, are not effective in haploids (Hoekstra, 1978). This possibly could explain the slow evolutionary change in morphology that has been observed for many bryophyte species, on the basis of which it has been assumed that since the Permian era speciation in bryophytes has occurred at a much lower rate than in vascular plants (Longton, 1976).

The few existing studies on allozyme variation in bryophytes (e.g. De Vries *et al*., 1983, 1989; Wyatt *et al*., 1989a; Derda & Wyatt, 1990; Hofman, 1991; Cronberg *et al*., 1997; Cronberg, 1998) do not particularly suggest low levels of genetic variation. On average, the level of genetic variation observed seemed not much lower than generally observed for vascular plants (Wyatt *et al*., 1989b). However, conspicuous differences in the amount of genetic variation have been observed between congeneric moss species, necessitating further investigations (Ennos, 1990).

The amount of genetic differentiation between populations is the result of the opposing forces of genetic drift and gene flow, respectively, promoting and constraining genetic differentiation. The degree of differentiation therefore can be a useful indicator of the amount of gene flow between (sub)populations (Wright, 1951). In mosses, gene flow is mediated by male gametes, spores, and asexual propagules or gametophyte fragments. Male gamete dispersal is very restricted and probably limited to a few centimetres (Longton, 1976), while spore dispersal appears to be strongly leptokurtic, most spores being deposited within a few metres from the source (e.g. Söderström & Herben, 1997). This would promote strong differentiation between populations. However, spores are produced in vast quantities, commonly exceeding a million spores per sporophyte in Polytrichaceae (Longton, 1997). Consequently, the small fraction of spores that disperses beyond the population borders, could still represent a considerable number. Moreover, spores possibly may disperse over very large distances, resulting in gene flow between geographically quite distant populations (Van Zanten & Pócs, 1981). However, as recruitment of new individuals from spores has been observed to be rare (e.g. Miles & Longton, 1987), effective gene flow by spores may still be very limited, notwithstanding substantial spore dispersal. Because many bryophyte species show strong clonal reproduction (During, 1990), this situation becomes even more complicated, making clear-cut predictions about expected levels of genetic differentiation rather difficult. Not surprisingly therefore both low (Cronberg *et al*. (1997) for *Hylocomium splendens*)

and very high values (Wyatt (1992) for *Plagiomnium ellipticum*) of genetic differentiation among populations within species have been reported.

From a phylogenetic perspective, genetic differentiation between congeneric moss species has been observed to be large: genetic identities between vascular plant species have been found to be considerably higher than between bryophyte species (Wyatt *et al*., 1989b). This may indicate that the evolutionary rate is not constrained in bryophytes, but that morphological characters evolve at a different rate from allozymes.

To gain additional insights into the population biology of bryophytes and to investigate the effect of life history characteristics on genetic structure, we address in this paper several of these issues for five taxa of the moss genus *Polytrichum*: *P. formosum*, *P. commune*, *P. uliginosum*, *P. piliferum* and *P. juniperinum*. We use protein electrophoresis to estimate levels of genetic variation both within and among populations of these *Polytrichum* species from two different geographical origins. We discuss whether the differences in levels of genetic variation and genetic differentiation that we observed can be ascribed to differences in ecology or life history characteristics of the species. Finally, the level of genetic differentiation between the species is investigated and phylogenetic relationships are evaluated.

Materials and methods

The species

Five taxa of the moss genus *Polytrichum* (Hair-cap moss), *P. formosum*, *P. piliferum*, *P. juniperinum*, *P. commune* and *P. uliginosum*, have been investigated. The last two are often regarded as two infra-specific taxa of *P. commune* s.l. (*P. commune* var. *commune* and *P. commune* var. *uliginosum* Wallr.), but in this study we have treated them as two distinct species, because of the high level of genetic differentiation that has been observed between these two taxa (Bijlsma *et al*., 2000).

The five species are medium to large sized mosses and are all commonly found throughout Western Europe, and the Northern Hemisphere in general (Touw & Rubers, 1989), but prefer quite distinct habitats: *P. piliferum* and *P. juniperinum* are usually found in more disturbed and exposed, sandy habitats, whereas *P. formosum*, *P. commune* and *P. uliginosum* are generally found in more stable habitats, like acidic, humus-rich forest soils for *P. formosum*, wet peat bogs for *P. uliginosum* and relatively dry peat soils for *P. commune* (Touw & Rubers, 1989).

Phylogenetic relationships among *Polytrichum* species are still a matter of debate. Some authors have argued

that *P. formosum* should be ascribed to a different genus *Polytrichastrum* instead of *Polytrichum* (Smith, 1971). Although generally haploid ($n = 7$), for all species, in particular for *P. formosum* (Crum & Anderson, 1981), diploid specimens ($n = 14$) have been observed (Fritsch, 1982), but in our study all populations of all species investigated seemed haploid and dioecious.

Sampling

Several Danish and Dutch populations were sampled for each of the five different species (see Table 1). To ensure that different individuals (genets) were sampled, samples were taken from clearly separated moss cushions at least two metres apart, and from each cushion one sample was taken as a small clump of neighbouring

Table 1 Location, sample size and estimates of genetic variation of 12 Danish (DK) and 17 Dutch (NL) populations for five different *Polytrichum* species

Species/Population	Location	N	P	A	H_S	H_T
P. uliginosum						
1 Grena Å (DK)	55°43′N, 09°05′E	66	12.5	1.1	0.028	
2 Hallund (DK)	56°01′N, 09°07′E	67	18.8	1.2	0.029	
3 Anloo (NL)	53°03′N, 06°42′E	32	12.5	1.1	0.008	
4 Kniphorstbos (NL)	53°03′N, 06°36′E	54.7	6.3	1.1	0.014	
5 Grolloo (NL)	52°56′N, 06°35′E	33	18.8	1.2	0.034	
Mean			**13.7**	**1.1**	**0.023**	**0.026**
P. commune						
6 Norlund-1 (DK)	56°02′N, 09°07′E	38	6.3	1.1	0.014	
7 Norlund-2 (DK)	56°02′N, 09°07′E	22	6.3	1.1	0.015	
8 Gjerrild (DK)	56°32′N, 10°44′E	77	6.3	1.1	0.023	
9 N.-Willemskanaal (NL)	53°09′N, 06°33′E	52	18.8	1.3	0.039	
10 Foxhol (NL)	53°11′N, 06°40′E	42	18.8	1.2	0.028	
11 Kniphorstbos (NL)	53°03′N, 06°36′E	42	12.5	1.1	0.024	
Mean			**11.6**	**1.2**	**0.025**	**0.025**
P. formosum						
12 Moesgård (DK)	56°06′N, 10°13′E	25	12.5	1.2	0.038	
13 Skov Mølle (DK)	56°07′N, 10°13′E	24.9	12.5	1.2	0.059	
14 Voerlade (DK)	56°02′N, 09°37′E	40	12.5	1.1	0.028	
15 Grolloo (NL)	52°56′N, 06°35′E	29	12.5	1.1	0.028	
16 Kniphorstbos (NL)	53°03′N, 06°36′E	52	12.5	1.1	0.050	
17 Appelbergen (NL)	53°08′N, 06°34′E	71	12.5	1.1	0.019	
Mean			**12.5**	**1.1**	**0.034**	**0.038**
P. juniperinum						
18 Fjellerup (DK)	56°31′N, 10°35′E	13	19.0	1.2	0.064	
19 Hjøllund (DK)	56°04′N, 09°23′E	16	42.9	1.5	0.115	
20 Foxhol (NL)	53°11′N, 06°40′E	25	47.6	1.6	0.168	
21 De Punt (NL)	53°07′N, 06°35′E	25	28.6	1.6	0.128	
22 Grolloo (NL)	52°56′N, 06°35′E	25	23.8	1.3	0.095	
23 Bornia (NL)	52°04′N, 05°18′E	18	57.1	1.8	0.170	
Mean			**36.6**	**1.5**	**0.127**	**0.166**
P. piliferum						
24 Norlund (DK)	56°02′N, 09°07′E	25	14.3	1.5	0.067	
25 Hjøllund (DK)	56°04′N, 09°23′E	23	28.6	1.4	0.098	
26 Foxhol (NL)	53°11′N, 06°40′E	25	28.6	1.5	0.118	
27 De Punt (NL)	53°07′N, 06°35′E	25	19.0	1.3	0.090	
28 Grolloo (NL)	52°56′N, 06°35′E	25	28.6	1.4	0.104	
29 Bornia (NL)	52°04′N, 05°18′E	25	19.0	1.3	0.078	
Mean			**22.9**	**1.4**	**0.094**	**0.105**

N, mean sample size; P, percentage polymorphic loci (a locus is considered polymorphic if more than one allele was detected); A, mean number of alleles per locus; H_T, total gene diversity within species (Nei, 1987); H_S, mean gene diversity within populations (Nei, 1987).

shoots. Samples were stored in plastic bags in the refrigerator (6°C) until electrophoresis.

Allozyme electrophoresis

Allozyme electrophoresis was performed for 11 enzyme systems for *P. commune*, *P. uliginosum* and *P. formosum*: aconitase (ACON, E.C. 4.2.1.3.), acid phosphatase (ACPH, E.C. 3.1.3.2.), alcohol dehydrogenase (ADH, E.C. 1.1.1.1.), glutamate-oxoacetate transaminase (GOT, E.C. 2.6.1.1.), glucophosphate isomerase (GPI, E.C. 5.3.1.9.), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42.), malate dehydrogenase (MDH, E.C. 1.1.1.37.), 6-phosphogluconate dehydrogenase (6-PGD, E.C. 1.1.1.44.), phosphoglucomutase (PGM, E.C. 5.4.2.2.), shikimate dehydrogenase (SHDH, E.C. 1.1.1.25.) and triosephosphate isomerase (TPI, E.C. 5.3.1.1.). For *P. piliferum* and *P. juniperinum*, three additional enzyme systems were investigated: hexokinase (HK, E.C. 2.7.1.1.), malic enzyme (ME, E.C. 1.1.1.40.) and superoxide dismutase (SOD, E.C. 1.15.1.1.). The electrophoretic procedures, recipes for buffers and staining solutions are identical to those described in Bijlsma *et al.* (2000) and references therein. The staining solutions for HK, ME and SOD are described by Hofman (1991). The staining of HK and SOD was performed on gels ran on a Tris-borate EDTA pH 8.6 buffer-system, also described by Hofman (1991), whereas for ME a gel ran on a LiOH-borate pH 8.3 buffer-system was used (Bijlsma *et al.* 2000).

Data analysis

After interpretation of the banding patterns, multiple loci at one enzyme system were numbered (1–3), and a letter (*a–h*) was assigned to different alleles at a single locus (see Table 2). The enzyme systems ACPH, IDH and TPI showed complex banding patterns of three or more bands, that were difficult to interpret, and when variable, the electrophoretic mobility of several bands changed in concert. Therefore, these complex banding patterns were treated as a single locus. Because of inconsistent staining of locus *Mdh-3* in *P. juniperinum* and *P. piliferum* and of locus *Gpi-3* in *P. commune*, *P. uliginosum* and *P. formosum*, these loci were only scored in those species in which they could be consistently interpreted. In the phylogenetic comparisons involving all five *Polytrichum* species, only the 15 loci that had been analysed for all species were taken into account.

Allele frequencies were determined for all loci. Mean number of alleles per locus (*A*), percentage polymorphic loci (*P*) and mean gene diversity (*H*$_S$; Nei, 1987) were calculated as measures of genetic

variability within populations using BIOSYS (Swofford & Selander, 1981). *F*-statistics, F_{ST} and F_{CT}, were calculated as measures of genetic differentiation among populations using ARLEQUIN (Schneider *et al.*, 1997). Finally, Nei's (1987) unbiased genetic identity (*I*) was calculated as a measure of genetic differentiation between species.

Results

Genetic variation within populations

Genetic variation could be scored at 16 putative loci for all populations of *P. commune*, *P. uliginosum* and *P. formosum* and at 21 putative loci for all populations of *P. juniperinum* and *P. piliferum*. Most enzyme loci showed a single band for each individual, and for polymorphic loci no heterozygotes were ever observed, confirming the haploid state of the species. Only 1–3 loci were polymorphic within each of the populations of *P. commune*, *P. uliginosum* and *P. formosum* (Table 1), and for some of these loci polymorphism was only due to rare alleles (i.e. alleles that were observed only once at these loci within one or a few of the examined populations; Table 2). In fact, only 1–2 loci showed substantial levels of variation (i.e. loci with at least two alleles with allele frequencies higher than 0.05) for each of the species (Table 2). Consequently, compared to those reported for other moss species (Wyatt *et al.*, 1989b), the different measures of the amount of genetic variability within populations of these species showed on average rather low values (Table 1): *P*: 6.3–18.8%, *A*: 1.1–1.3, and *H*$_S$: 0.008–0.059. Many more polymorphic loci were observed in populations of *P. juniperinum* and *P. piliferum* (Table 1). In addition to other loci showing only variation for rare alleles, 6–8 loci showed substantial levels of variation within each of these species (Table 2). As such, *P. piliferum* and in particular *P. juniperinum* showed considerable higher values for the different measures of genetic variability (Table 1): *P*: 14.3–57.1, *A*: 1.2–1.8, and *H*$_S$: 0.064–0.170. A one-way ANOVA revealed that these differences in mean *H*$_S$-values between the species (Table 1) were significant (*P* < 0.001). An additional multiple comparison of means (Dunn–Šidàk Method; Sokal & Rohlf, 1995) showed that there are, in fact, two homogeneous groups (*P. commune*, *P. uliginosum* and *P. formosum* vs. *P. piliferum* and *P. juniperinum*), and within these groups the means of *H*$_S$ are not significantly different from one another. Hence, *P. piliferum* and *P. juniperinum* show significantly higher levels of genetic variation than the other three *Polytrichum* species.

As there were differences in both sample size and number of loci examined between these groups, the

Table 2 Allele frequencies for polymorphic loci for all populations of *Polytrichum* studied. Population numbers correspond to the numbers in Table 1

Locus Allele	Populations of *P. juniperinum* 18	19	20	21	22	23
Pgd-1						
a	1.000	1.000	0.880	0.960	1.000	0.944
b	0.000	0.000	0.120	0.040	0.000	0.056
Pgd-2						
b	0.000	0.000	0.000	0.000	0.000	0.056
c	1.000	1.000	0.880	0.960	1.000	0.889
d	0.000	0.000	0.120	0.040	0.000	0.056
Idh						
c	0.846	0.188	0.480	0.640	0.640	0.500
d	0.000	0.500	0.520	0.080	0.280	0.278
f	0.154	0.313	0.000	0.280	0.080	0.222
Acon						
b	1.000	1.000	1.000	1.000	1.000	0.944
d	0.000	0.000	0.000	0.000	0.000	0.056
Mdh-2						
c	1.000	0.938	1.000	1.000	1.000	1.000
e	0.000	0.063	0.000	0.000	0.000	0.000
Pgm-1						
a	0.231	0.125	0.800	0.760	1.000	0.167
c	0.769	0.875	0.200	0.240	0.000	0.833
Gpi-2						
a	1.000	0.750	1.000	1.000	0.400	0.889
b	0.000	0.250	0.000	0.000	0.600	0.111
Gpi-3						
a	0.000	0.000	0.040	0.000	0.000	0.056
b	1.000	0.938	0.920	0.960	1.000	0.944
c	0.000	0.063	0.040	0.040	0.000	0.000
Hk-1						
c	0.000	0.063	0.000	0.000	0.000	0.000
d	1.000	0.938	1.000	1.000	1.000	1.000
Hk-2						
a	0.154	0.000	0.000	0.000	0.000	0.000
b	0.846	1.000	0.840	0.720	0.840	0.833
c	0.000	0.000	0.160	0.280	0.160	0.167
Me						
a	0.000	0.563	0.280	0.240	0.000	0.000
b	0.308	0.375	0.400	0.640	0.040	0.722
c	0.692	0.063	0.320	0.120	0.960	0.278
Got						
a	0.000	0.000	0.240	0.120	0.000	0.056
b	1.000	1.000	0.760	0.880	1.000	0.722
c	0.000	0.000	0.000	0.000	0.000	0.222
Acph						
a	1.000	0.938	0.280	0.200	0.400	0.278
b	0.000	0.063	0.720	0.800	0.600	0.667
g	0.000	0.000	0.000	0.000	0.000	0.056

Locus Allele	Populations of *P. juniperinum* 18	19	20	21	22	23
Tpi						
a	0.000	0.000	0.240	0.000	0.080	0.167
e	1.000	0.938	0.760	1.000	0.920	0.833
f	0.000	0.063	0.000	0.000	0.000	0.000
Adh						
a	0.000	0.000	0.000	0.040	0.000	0.000
c	1.000	1.000	1.000	0.960	1.000	1.000

Locus Allele	Populations of *P. piliferum* 24	25	26	27	28	29
Pgd-2						
b	0.000	0.000	0.000	0.000	0.040	0.000
c	1.000	1.000	1.000	1.000	0.960	1.000
Acon						
b	0.040	0.000	0.040	0.000	0.000	0.000
d	0.960	1.000	0.960	1.000	1.000	1.000
Mdh-1						
a	0.040	0.348	0.320	0.360	0.280	0.200
b	0.960	0.652	0.680	0.640	0.720	0.800
Pgm-1						
d	0.000	0.000	0.240	0.000	0.000	0.000
e	0.800	0.304	0.600	0.720	0.680	0.480
f	0.200	0.696	0.160	0.280	0.320	0.520
Hk-1						
a	0.000	0.043	0.080	0.000	0.200	0.000
b	0.960	0.783	0.920	1.000	0.800	1.000
c	0.040	0.087	0.000	0.000	0.000	0.000
d	0.000	0.087	0.000	0.000	0.000	0.000
Hk-2						
a	1.000	0.870	1.000	1.000	1.000	1.000
b	0.000	0.130	0.000	0.000	0.000	0.000
Me						
a	0.120	0.000	0.040	0.040	0.040	0.000
b	0.840	0.913	0.880	0.960	0.800	0.960
c	0.040	0.087	0.080	0.000	0.160	0.040
Got						
b	1.000	1.000	1.000	1.000	1.000	0.920
c	0.000	0.000	0.000	0.000	0.000	0.080
Acph						
d	0.960	1.000	0.720	0.600	0.680	0.400
e	0.040	0.000	0.280	0.400	0.320	0.600
Tpi						
b	0.080	0.174	0.520	0.040	0.000	0.000
c	0.760	0.826	0.320	0.720	0.920	0.960
e	0.160	0.000	0.160	0.240	0.080	0.040
Adh						
d	0.960	0.957	1.000	1.000	1.000	1.000
f	0.040	0.043	0.000	0.000	0.000	0.000

Table 2 (*Continued*)

Locus Allele	Populations of *P. formosum*					
	12	13	14	15	16	17
Pgd-2						
a	0.040	0.042	0.000	0.034	0.000	0.000
b	0.800	0.625	0.875	0.966	0.577	0.972
c	0.160	0.333	0.125	0.000	0.423	0.028
Mdh-3						
a	0.840	0.680	0.875	0.759	0.808	0.859
b	0.160	0.320	0.125	0.241	0.192	0.141

Locus Allele	Populations of *P. uliginosum*				
	1	2	3	4	5
Acon					
a	0.015	0.015	0.031	0.000	0.000
b	0.985	0.985	0.969	1.000	1.000
Mdh-2					
b	0.303	0.284	0.031	0.127	0.030
d	0.697	0.716	0.969	0.873	0.970
Tpi					
d	1.000	0.985	1.000	1.000	0.848
h	0.000	0.015	0.000	0.000	0.152
Adh					
b	1.000	1.000	1.000	1.000	0.879
e	0.000	0.000	0.000	0.000	0.121

Locus Allele	Populations of *P. commune*					
	6	7	8	9	10	11
Pgd-2						
b	0.868	0.864	0.766	0.846	0.772	0.789
d	0.132	0.136	0.234	0.154	0.228	0.211
Acon						
a	0.000	0.000	0.000	0.173	0.024	0.024
b	1.000	1.000	1.000	0.808	0.976	0.976
d	0.000	0.000	0.000	0.019	0.000	0.000
Pgm-1						
b	1.000	1.000	1.000	0.981	1.000	1.000
d	0.000	0.000	0.000	0.019	0.000	0.000
Gpi-2						
c	1.000	1.000	1.000	1.000	0.976	1.000
d	0.000	0.000	0.000	0.000	0.024	0.000

results might be biased. However, when the 'additional' loci that were analysed for *P. juniperinum* and *P. piliferum* and not for the other three *Polytrichum* species, were omitted from the analysis, the level of gene diversity (H_S) became even higher in *P. juniperinum* and *P. piliferum* (data not shown). Moreover, the highest H_S-values were observed for those species with on average the smallest sample sizes.

Genetic differentiation within species

Genetic differentiation between populations of the same species was studied by means of a hierarchical analysis (Table 3). As indicated by low F_{ST}-values, relatively low levels of genetic differentiation were observed between populations of any species, except *P. juniperinum*. At most 10% of the total variation observed could be attributed to differences between populations (Table 3), which corresponds to *Nm*-values (number of migrants per generation) of 4.5 and higher (note that $F_{ST} \approx 1/(2Nm + 1)$ for haploid organisms; Wright, 1951; Whitlock & McCauley, 1999). *P. juniperinum* was an exception, because in this species almost 25% of the total genetic variation, *Nm* < 1.6, could be ascribed to differentiation between populations.

The effect of geographical region, i.e. populations within a country (on average 51 km apart) vs. populations from different countries (on average 406 km apart), on genetic differentiation was also analysed for each species. Table 3 shows that only for *P. uliginosum* the F_{CT}-value was significantly different from zero, indicating that, for this species, genetic differentiation among regional populations was lower than between populations from different countries. However, the overall level of differentiation was still very low.

Genetic differentiation between species

The level of divergence between different *Polytrichum* species was very large (Table 4, Fig. 1). Averaged over all pairwise comparisons for all species the genetic identity was only $I = 0.222$, but it varied substantially for each of the pairwise comparisons. The identity between *P. commune* and *P. uliginosum* was significantly higher ($I = 0.611$) than for any other pair of *Polytrichum* species ($I = 0.071$–0.345). However, even for these species

Table 3 Hierarchical analysis of genetic differentiation between populations for the different *Polytrichum* species

Species	F_{ST}	F_{CT}
P. uliginosum	0.087 (0.000)	0.099 (0.009)
P. commune	0.018 (0.075)	0.006 (0.488)
P. formosum	0.099 (0.000)	0.000 (0.808)
P. juniperinum	0.241 (0.000)	0.085 (0.059)
P. piliferum	0.100 (0.000)	0.000 (0.937)

F_{ST}, the proportion of the total variation that can be attributed to the variation between populations; F_{CT}, the proportion of the total variation that can be attributed to the variation between the countries Denmark and The Netherlands. Deviations of *F*-values from 0 were tested for significance by permutation (*P*-values within parentheses).

Table 4 Average Nei's (1987) unbiased genetic identities between species (below the diagonal) and between populations within species (on the diagonal). Numbers within parentheses are standard errors

Species	P. uliginosum	P. commune	P. formosum	P. juniperinum	P. piliferum
P. uliginosum	0.997 (0.001)				
P. commune	0.611 (0.001)	0.999 (0.000)			
P. formosum	0.127 (0.001)	0.181 (0.001)	0.996 (0.001)		
P. juniperinum	0.209 (0.001)	0.345 (0.001)	0.157 (0.002)	0.942 (0.007)	
P. piliferum	0.071 (0.001)	0.080 (0.001)	0.141 (0.002)	0.301 (0.005)	0.979 (0.003)
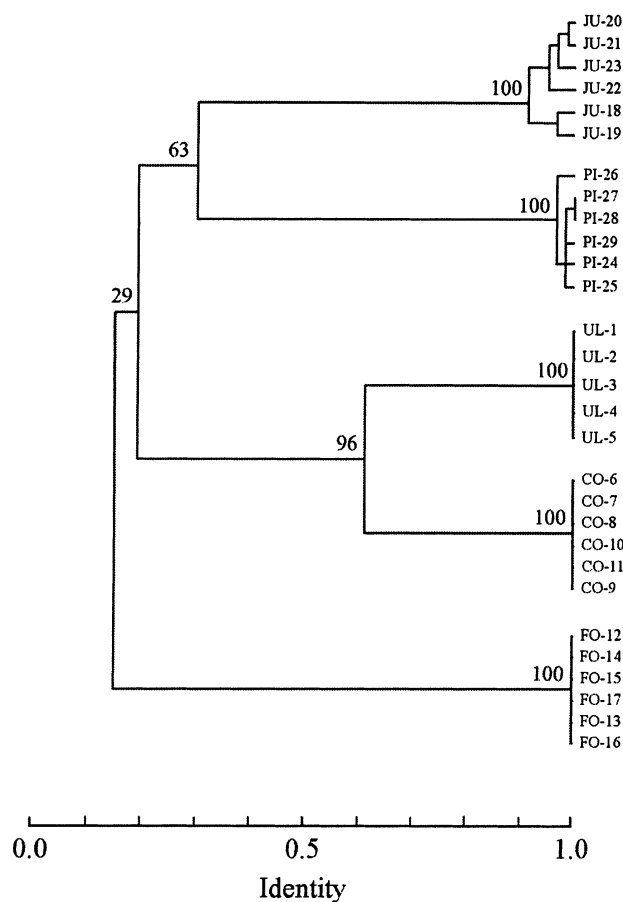
Fig. 1 Consensus UPGMA tree expressing overall levels of Nei's (1987) unbiased genetic identity between 29 populations and five species of the moss genus *Polytrichum* (CO, *P. commune*; UL, *P. uliginosum*; FO, *P. formosum*; JU, *P. juniperinum*; PI, *P. piliferum*; population numbers correspond to numbers in Table 1). This analysis is based on the 15 loci that were analysed for all species. Numbers in the tree denote bootstrap values (percentage) after 1000 resamplings.

genetic identities were still very much lower than the genetic identities observed for populations within any of the species (range $I = 0.942$–0.999; Table 4). This shows that the genetic divergence between species is of a different order of magnitude than at the population level.

As indicated by high bootstrap values, the topology of the UPGMA tree constructed for the *Polytrichum* species is quite robust (Fig. 1). The species *P. commune* and *P. uliginosum* nearly always cluster together, indicating that they are much more related to each other than to any of the other *Polytrichum* species. Although less marked, *P. juniperinum* and *P. piliferum* also seem more related to each other than to any of the other species.

Discussion

In general, we observed relatively low levels of genetic variation, mean gene diversity (H_S) ranging from 0.023 to 0.127, within populations for each of the five *Polytrichum* species (Table 1). The level of H_S was particularly low for the species *P. commune*, *P. uliginosum* and *P. formosum*. These values of gene diversity are considerably lower than those observed for vascular plants (Hamrick & Godt, 1990) and also lower than those observed for populations of most other moss genera (De Vries *et al.*, 1983, 1989; Wyatt *et al.*, 1989a; Cronberg *et al.*, 1997; Cronberg, 1998), although such exceptionally low levels have also been reported for some other moss species (Hofman, 1991; Wyatt, 1992). On the other hand, we observed significant differences in the amount of genetic variability among the *Polytrichum* species: *P. piliferum* and in particular *P. juniperinum* showed 3–5 times significantly higher values of H_S than the other three examined species. Within other moss genera also considerable ranges in H_S-values have been observed (De Vries *et al.*, 1983, 1989; Hofman, 1991; Wyatt, 1992; Cronberg, 1998), which have been accounted for by differences in ecology and/or life history of the species involved. Both Hofman (1991) and Cronberg (1998), although from a different perspective, suggested such differences in genetic variation to be the consequence of differences in breeding system: monoecy vs. dioecy. Because all *Polytrichum* species studied here are dioecious, this can not explain the differences in the level of genetic variability we observed for these species. Although it is not impossible that the observed differences in the amount of genetic variation between *Polytrichum* species might be the result of historical

events, we rather attribute them to differences in habitat and life history. *Polytrichum commune*, and in particular *P. uliginosum* and *P. formosum* are often found in more stable habitats, like peat bogs and forests (Touw & Rubers, 1989), where clonal growth might outweigh sexual reproduction and recruitment of new individuals (genets), whereas *P. piliferum* and *P. juniperinum* are more often observed in exposed and dynamic, sandy habitats (Touw & Rubers, 1989), where turnover of genets is probably much more rapid, and, as a consequence, sexual reproduction might be more important. Predominance of asexual reproduction in *P. commune*, *P. uliginosum* and *P. formosum*, and predominance of sexual reproduction in *P. piliferum* and *P. juniperinum* therefore would account for the higher level of gene diversity in the last two species. A comparable situation has been observed for the perennial grass *Agrostis stolonifera* (Kik *et al.*, 1992), where in stable meadow habitats a low number of large clones and a low level of genotypic diversity were found, whereas in dynamic habitats, such as sand dunes, there were many genets and genotypic diversity was high.

In general, differentiation between populations within species was observed to be low and, except for *P. juniperinum*, F_{ST}-values were 0.10 or less (Table 3). These F_{ST}-values corresponds to Nm-values greater than 4 (Wright, 1951; Whitlock & McCauley, 1999), indicating sufficient gene flow, probably by effective spore dispersal, to prevent substantial genetic differentiation between populations. The fact that within most of the species there appears to be hardly any differentiation between Danish and Dutch populations indicates that spores probably easily disperse over large distances, which agrees well with Van Zanten & Pócs (1981), who showed that spores can disperse over large distances. However, some caution is needed, because the number of polymorphic loci, upon which F_{ST}-values were based, is low, especially in *P. commune*, *P. uliginosum* and *P. formosum*. On the other hand, the level of genetic differentiation within *P. piliferum*, based on six substantially more polymorphic loci, is not different from that in *P. uliginosum* and *P. formosum*. Moreover, preliminary results using much more variable microsatellite loci, revealed, compared to allozymes, similar F_{ST}-values for *P. formosum*.

Compared to the other *Polytrichum* species, *P. juniperinum* shows much higher levels of genetic differentiation between populations. Under the assumption that, for bryophytes, allozyme variation is selectively neutral in most cases (Stenøien, 1999), this indicates that gene flow is reduced in *P. juniperinum* (Table 3). As spores from *P. juniperinum* have more or less the same size as those from the other *Polytrichum* species (Touw & Rubers, 1989), spore size differences are unlikely to

explain this observed difference. At the moment we do not have a satisfactory explanation for this higher level of genetic differentiation within *P. juniperinum*.

In a recent review on the population biology of the Polytrichaceae, Wyatt & Derda (1997) have summarized data on genetic variability for a number of *Polytrichum* species as observed for North American populations. They also observed relatively low levels of gene diversity for the species studied in this paper, though their results differ from ours in some respects. They observed considerably higher gene diversity values (H_S) for *P. commune* and *P. formosum*, while, on the contrary, their H_S-values for *P. piliferum* and *P. juniperinum* seem much lower than we observed for these two species. In part, the differences can possibly be explained by the fact that they sampled mainly diploid *P. formosum* populations, while for *P. commune*, they apparently did not distinguish between the different taxa recognized within *P. commune* s.l. (see Derda & Wyatt, 1990), which may have considerably inflated their estimates for these two species. For *P. juniperinum* and *P. piliferum* the differences are more puzzling, the more so because their F_{ST}-values for these species, 0.445 and 0.699, respectively (Wyatt & Derda, 1997), are considerably higher than we observed (Table 3). At the moment we do not have a satisfactory explanation for these differences, but possibly they may reflect differences between the continents in recolonization patterns after the latest glacial period.

In contrast to low levels of genetic differentiation within species, we observed substantial levels of divergence between species (Table 4; Fig. 1). Between pairs of *Polytrichum* species genetic identities ranged from $I = 0.071$ to $I = 0.611$ with an average of $I = 0.222$, a value significantly lower than an average genetic identity of $I = 0.79$ observed for 17 congeneric species of different genera of flowering plants (Crawford, 1983). This agrees well with the observation that, in general, mean genetic identities between congeneric moss species are lower than for congeneric species of vascular plants (Wyatt *et al.*, 1989b). Although *P. commune* and *P. uliginosum* show a significantly higher level of relatedness than any other pair of *Polytrichum* species ($I = 0.611$), it is still comparable to the range of genetic identities ($I = 0.51$–0.63) reported for congeneric species in other moss genera (De Vries *et al.*, 1983; Cronberg, 1998). The high level of divergence between *P. formosum* and the other examined *Polytrichum* species appears to support a division of the genus *Polytrichum* s.l. into *Polytrichum* s.s. and *Polytrichastrum*, which includes *P. formosum* and among others a number of allodiploid species (Smith, 1971; Derda & Wyatt, 2000). However, the level of divergence between the species pairs *P. commune*/*P. uliginosum* and *P. piliferum*/*P. juniperinum* is nearly of the same order of magnitude as that

observed between *P. formosum* and each of these two species pairs, suggesting that if *P. formosum* is assigned to a separate genus *Polytrichastru*m then the species pairs *P. commune*/*P. uliginosum* and *P. piliferum*/*P. juniperinum* should also be assigned to different genera.

The high level of genetic divergence between congeneric *Polytrichum* species, and in fact between most congeneric species of other bryophyte genera, suggests that the evolutionary rate per se is not constrained, but rather that morphological characters evolved at a much slower rate than allozymes in bryophytes. A comparable situation has been observed for horseshoe crabs, which show striking morphological conservatism for the last 150 million years, but show as much genetic divergence at the DNA-level as other crab genera (Avise *et al.*, 1994), indicating that the low rate of morphological change in horseshoe crabs is not due to a lack of genetic variability. Similarly, we infer, that the relative morphological stasis within the genus *Polytrichum* is not the consequence of lack of genetic variability. In addition, the considerably lower levels of genetic identity observed between most *Polytrichum* species, compared to other congeneric moss species, indicate that this genus is indeed relatively ancient, as suggested by Crum (1976) based on morphological comparisons of extant and fossil species.

In conclusion, contrasting the high levels of divergence *between* the *Polytrichum* species, we observed mostly low levels of genetic differentiation among populations *within* species, indicating a considerable degree of gene flow by wind-dispersed spores. On average low levels of gene diversity were observed within species, showing that these haploid moss species are indeed somewhat depauperate of genetic variation.

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