

Population structure and genetic diversity of the amphiatlantic haploid peatmoss *Sphagnum affine* (Sphagnopsida)

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Nineteen populations of *Sphagnum affine* were included in a study of genetic diversity and structure in fragmented and less fragmented landscapes, and differentiation at intercontinental and three regional levels. Isozyme electrophoresis of eight enzyme systems revealed 12 variable loci, which could be used for haplotype identification. A hierarchical analysis of variance (AMOVA) revealed no significant intercontinental differentiation, and very limited differentiation among European regions. A trend of decreasing diversity with increasing latitude was apparent. Gametic phase disequilibria was high, suggesting nonrandom mating and regionally high incidences of inbreeding. The partitioning of genetic variation within and among populations in each region varied among regions, the northernmost populations having 86% of the total variation among populations, the southernmost in Scandinavia having 25% of the variation among populations, whereas the American populations displayed 89% of the variation within populations. Fifteen alleles at eight loci occurred in the U.S.A. which were not encountered in Europe, whereas only three European alleles at one locus in three populations were not encountered in U.S.A.

The differences in diversity between North America and Europe may result from loss of genetic diversity caused by founder effects during postglacial recolonization of northern Europe. In Europe, the main mountain ranges extend E–W, posing severe barriers to northwards migration of lowland species, compared to the N–S trend of mountain ranges in North America. The decline in genetic diversity and increase in population differentiation and gametic phase disequilibria towards the north in Scandinavia may be caused by a series of founder effects during postglacial migration. These may have corresponded to minor climatic oscillations that influenced the migration front/leading edge in the suboceanic lowlands of Norway. According to this model random genetic drift will be an increasingly important structuring factor with latitude.

Keywords: genetic diversity, genetic drift, glaciation, habitat fragmentation, population structure, *Sphagnum*.

Introduction

Anthropogenic fragmentation of landscapes has taken place in all parts of Europe, but some of the northernmost areas are less disturbed by human activities, leaving large areas in a natural or seminatural state. In central Norway, large mire areas remain and the landscape is generally less influenced by agriculture than landscapes in southernmost Scandinavia. The landscape of southern Sweden is generally the more fragmented, with mires scattered in an agricultural and urban landscape. The significance of habitat fragmentation on present day genetic diversity in populations of

bryophytes has attracted little attention. Theory predicts that small isolated populations will potentially experience a high degree of random genetic drift, which can be enforced by high incidences of inbreeding, and in some cases the probability of gene flow among populations may be affected (Young *et al.*, 1996). Populations in which genetic drift predominates are more prone to local extinction than populations without genetic drift because of the loss of genetic variation (Barrett & Kohn, 1991).

Sphagnum affine Ren. & Card. is a haploid, dioecious, occasionally to rarely sexually reproducing, lowland species with an ability to grow laterally by bifurcation. Formerly included in *S. imbricatum* Hornsch. ex Russ., *S. affine* received little attention

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until Flatberg (1984) resurrected it. The species is amphiatlantic with suboceanic tendencies, and rarely occurs in exposed eu-oceanic habitats (Flatberg, 1986). In Europe, *S. affine* grows in a variety of mesotrophic fens, but may also occur in more oligotrophic mires. It is considered dependent on a rather warm vegetation season (Flatberg, 1986). In North America, it occurs in forested mires, e.g. Cedar swamps and in open mire habitats similar to those in Europe. It avoids ombrotrophic mires, but may be found in poor minerotrophic fen-soaks, between ombrotrophic parts of larger mire-complexes (Table 1). In North America, *S. affine* extends northwards to Newfoundland, whereas the northernmost populations in Europe are found scattered along the west coast of Norway as far north as Meløy (66°43'N; Flatberg, 1986). Many bryophytes are distributed widely and display large disjunctions. Disjunct distribution patterns may be caused by repeated long distance dispersal events, past geological events or separation of populations during glaciations. The distribution of genetic variation in intercontinentally disjunct populations may provide important information about evolutionary rates/ages of species, as well as postglacial migration patterns. The present study is aimed at: (i) assessing the distribution of genetic variation in *S. affine* in fragmented and less fragmented landscapes along a S–N gradient, i.e. whether populations from southern Sweden are less diverse and more influenced by predicted population genetic consequences of habitat fragmentation than populations further north; and (ii) investigating whether populations on either side of the Atlantic Ocean are genetically differentiated.

Materials and methods

Sampling

Gametophytes of *Sphagnum affine* were collected from mire habitats in four geographical regions on two continents; central Norway, southern Norway, southern Sweden in Europe and New Jersey in U.S.A. (Fig. 1). A total of 640 samples from 19 populations were included (Table 1). Samples were collected every 2 m along a transect, and on each mire, 30–50 samples were collected. However, in some cases it was not possible to follow a transect owing to mire topography or vegetation structure. The 2 m separation between individual samples was never violated, nor were very small populations included where there was a possibility of sampling fewer than 15 samples. Plants were cultivated prior to electrophoresis in order to bring them into a vigorous condition.

Enzyme assays

Extractions were performed using a pestle on cooled porcelain plates. Thirty microlitres of extraction buffer (Mitton *et al.*, 1979; modification according to Cronberg, 1995) was added per capitulum sample, after rinsing in distilled water and removing excess water. Extracts were absorbed onto paper wicks (Whatman no. 3) and stored in microtitre dishes at –80 °C until electrophoresis. Electrophoresis was performed on 11% horizontal starch gels. Sodium-citrate (pH 7.0)/sodium-histidine (pH 7.0) buffer (Soltis *et al.*, 1983; no. 1) was used to screen aconitase (ACO, EC 4.2.1.3), isocitrate dehydrogenase (NADP) (IDH, EC 1.1.1.42), phosphoglucomutase (PGM, EC 5.4.2.2) and shikimate dehydrogenase (SKD, EC 1.1.1.25). Sodium borate (pH 8.0)/tris-citrate (pH 8.6) buffer (Wendel & Weeden, 1989; no. 5) was used to resolve alcohol dehydrogenase (NAD) (ADH, EC 1.1.1.1), aspartate aminotransferase (AAT, EC 2.6.1.1), formate dehydrogenase (FDH, EC 1.2.1.2) and glucose-6-phosphate isomerase (GPI, EC 5.3.1.9).

Staining methods for ADH, FDH, GPI, IDH, PGM, PGI, SKD followed Cronberg (1995), with some minor modifications of the amounts of substrates and cofactors. AAT and ACO were stained according to Wendel & Weeden (1989). Details of electrophoresis, extraction and the nomenclature of loci and alleles are described in Thingsgaard (in press).

Data analysis

Banding patterns were interpreted in terms of putative discrete loci with haploid expression. Haplotypes and allele frequencies were tabulated and used for analysis of genetic diversity and structure. Gene diversity (Nei, 1987), percentage of polymorphic loci (*P*; a locus was considered polymorphic if more than one allele was encountered), mean number of alleles per locus (*A*) and number of haplotypes were calculated for individual populations and as means over regions. The proportion of distinguishable genotypes (PD) *sensu* Ellstrand & Roose (1987) was calculated as an estimate of clonal diversity. The distribution of private alleles, i.e. alleles only found in one population was determined.

Quantification of genetic structure was carried out for a simple structure of haploid individuals within populations, as well as for hierarchical structures grouping populations according to continents or regions. Inter-continental differentiation was compared among populations in Europe and North America, respectively. Moreover, differentiation among predefined regions in Europe, namely central Norway, southern Norway and

Table 1 Localities of *Sphagnum affine* samples included in the study

Locality code	Country code*	Locality	County and general area	Altitude	Habitat
S3	SSwed	Smedseröds mossen	Bohuslän, Stenungsund. 58°04'N, 11°55'E	100 m	Mire margin dominated by <i>Myrica gale</i> L.
S6	SSwed	Maderna	Vestergötland, Partille. 57°43'N, 12°10'E	105 m	Minerotrophic rather poor fen dominated by <i>Molinia caerulea</i> (L.) Moench., <i>Sphagnum papillosum</i> Lindb. and regrowth of <i>Betula pubescens</i> Ehrh. Adjacent to ombrotrophic mire.
S7	SSwed	Övreböke	Halland, Halmstad, Övrebökesjön. 56°55'N, 12°55'E	145 m	Wooded, soligenous rich fen.
S9	SSwed	Komosse	Jönköping, Ulricehamn 57°42'N, 13°40'E	315 m	Minerotrophic fen soak with <i>Narthecium ossifragum</i> (L.) Hudson and <i>Andromeda polifolia</i> L. in ombrotrophic mire complex. Associated with <i>S. papillosum</i> , <i>S. pulchrum</i> , <i>S. rubellum</i> and <i>S. brevifolium</i> .
V01	SSwed	Mire S. Ll. Lesjön	Värmland, Arvika. 59°28'N, 12°36'E.	110 m	Mesotrophic topogenous mire dominated by <i>S. papillosum</i> .
V02	SSWed	Sülvikstjärnet	Värmland, Arvika. 59°39'N, 12°23'E	175 m	Mire margin dominated by <i>Myrica gale</i> /mesotrophic carpet.
H1	SNorw	Mire NW Solhaug	Hordaland, Stord. 59°49'N, 05°21'E	90 m	Mire margin.
H2	SNorw	Mire NW Solhaug	Hordaland, Stord. 59°49'N, 05°21'E	85 m	High mesotrophic carpet.
H3	SNorw	Storemyr	Hordaland, Lindaas. 60°50'N, 05°04'E	35 m	Poor fen/mire margin.
TE2	SNorw	E. Stavsholtmyrane	Telemark, Bø. 59°27'N, 08°57'E	530 m	Slightly soligenous poor fen with dominating <i>S. papillosum</i>
MR1	CNorw	Svanvikmyran	Møre- & Romsdal, Eide. 62°54'N, 07°26'E	20 m	Minerotrophic, poor fen-soak (soligenous) in ombrotrophic mire-complex.
T11	CNorw	Saetranmyra	Nord-Trøndelag, Naerøy. 64°49'N, 11°47'E	40 m	Poor fen-soak bordering ombrotrophic mire.
T12	CNorw	Husdalen	Sör-Trøndelag, Orkdal. W Husdalsvatnet. 63°22'N, 09°50'E	180 m	Slightly soligenous intermediate fen.
T13	CNorw	WSW Djüpdalstjärn	Sör-Trøndelag, Snillfjord. 63°22'N, 09°35'E	260–340 m	Sloping fen.

Table 1 (Continued)

Locality code	Country code*	Locality	County and general area	Altitude	Habitat
T15	CNorw	SE Hitra 1	Sör-Trøndelag, Hitra. 63°35'N, 09°03'E	50 m	Poor fen-soaks along brooklet in coastal heath.
T16	CNorw	SE Hitra 2	Sör-Trøndelag, Hitra 63°33'N, 09°02'E	70 m	Mesotrophic, soligenous fen, bordering ombrotrophic mire.
US5	NJ	Mt. Misery	Burlington, Woodland Township 39°55'N, 74°31'W	27 m	<i>Chamaecyparis thyooides</i> (L.) Britton, Sterns & Poggenb. swamp.
US7	NJ	Skit Branch	Burlington, Shamong Township. 39°46'N, 74°40'W	14 m	Abandoned cranberry farm.
US11	NJ	Firefly Bog.	Burlington, Shamong Township. 39°43'N, 74°41'W	< 50 m	Cut bog. Mesotrophic.

*SSwed, southern Sweden; SNorw, southern Norway; CNorw, central Norway; NJ, New Jersey, U.S.A.

southern Sweden, respectively, was assessed (Fig. 1). The *F*-statistics were derived from an analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992; Weir, 1996). The significance of the *F*-statistics was tested using a nonparametric method described in Excoffier *et al.* (1992).

Linkage disequilibrium between all pairs of loci was tested by the exact test of linkage disequilibrium described in Slatkin (1994) and Schneider *et al.* (1997). The proportion of linked loci (P_d) relative to the maximum number of loci (Stenøien & Sæstad, 1999) was estimated for each population. Genetic phase equilibria were calculated from haplotypic data; thus, the limited number of haplotypes encountered in most European populations renders the information obtained less straightforward to interpret than if more haplotypes were found in populations. This measure is included, despite the long overlapping generations, as it gives an indication of the relative amount of recombination taking place in the populations.

The calculations of standard genetic diversity statistics, *F*-statistics, AMOVA and test for linkage disequilibrium were performed using ARLEQUIN v.1.1 (Schneider *et al.*, 1997).

Results

Electrophoretic patterns

Twelve putative loci with haploid expression were resolved, all of which were polymorphic in at least one of the surveyed populations. All loci, except for *Aat-3*, displayed the expected haploid banding patterns. In addition, *Idh-1* consistently produced one intensely staining band and a fainter band migrating at a relative mobility identical to the other. The latter band was treated as a 'ghost band'. *Aat-3* showed the three-banded duplication-pattern found in all other species of Sphagnum section Sphagnum so far surveyed, which suggests the occurrence of an ancient duplication. However, due to variable resolution this locus was omitted from formal analysis. Detailed descriptions of banding patterns are presented elsewhere (Thinggaard, in press).

Overall genetic diversity of Sphagnum affine populations

The mean frequency of polymorphic loci (*P*) in the total sample was 43% (range 0.0–92%; Table 2), the average gene diversity (Nei, 1987) was calculated as $H_s = 0.122$ (range 0.00–0.31; Table 2) and the mean number of alleles per locus averaged 1.55 (range 1.00–2.58; Table 2). Ninety-five haplotypes were encountered

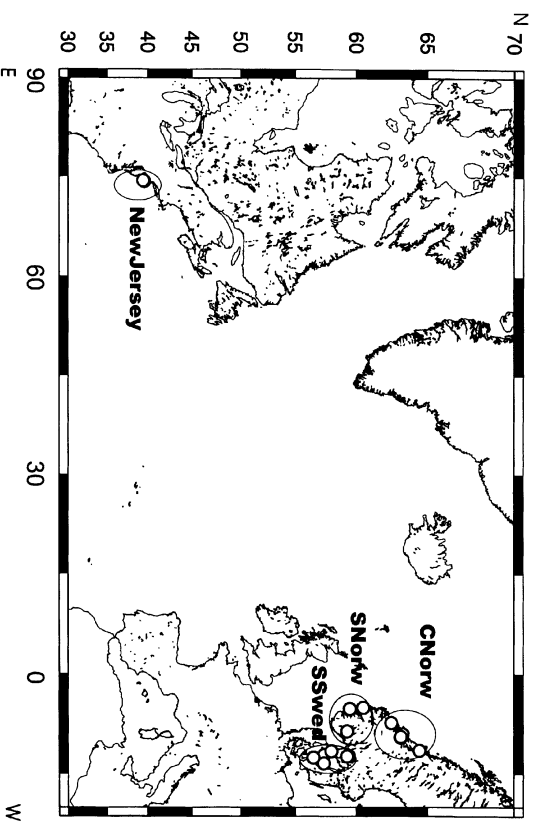


Fig. 1 Map showing regions in which populations of *Sphagnum affine* were sampled.

Table 2 Diversity indices for 19 populations of *Sphagnum affine*. Population codes according to Table 1

Population	<i>N</i>	Number of haplotypes	Frequency of polymorphic loci	PD	Average gene diversity (\pm SD)	<i>A</i>
Southern Sweden						
S3	25	9	0.500	0.360	0.1228 (\pm 0.0857)	1.583
S6	30	12	0.583	0.400	0.1983 (\pm 0.1235)	1.583
S7	37	7	0.500	0.189	0.1319 (\pm 0.0892)	1.750
S9	47	7	0.500	0.149	0.1750 (\pm 0.1104)	1.583
V01	27	7	0.500	0.259	0.1439 (\pm 0.0963)	1.417
V02	57	14	0.500	0.246	0.2026 (\pm 0.1237)	1.667
Total	223					
Mean		9.3	0.514	0.267	0.1624 (\pm 0.0344)	1.597
Southern Norway						
H1	36	9	0.583	0.250	0.1336 (\pm 0.0901)	1.833
H2	42	6	0.333	0.143	0.0526 (\pm 0.0526)	1.417
H3	17	4	0.333	0.235	0.1581 (\pm 0.1061)	1.333
TE2	33	3	0.167	0.091	0.0739 (\pm 0.0589)	1.167
Total	128					
Mean		5.5	0.354	0.180	0.1046 (\pm 0.0490)	1.438
Central Norway						
T12	36	7	0.500	0.194	0.1030 (\pm 0.0743)	1.500
T13	24	2	0.083	0.083	0.0138 (\pm 0.0216)	1.083
T11	31	2	0.083	0.065	0.0054 (\pm 0.0129)	1.083
T15	25	1	0.000	0.040	0.0000 (\pm 0.0000)	1.000
T16	39	4	0.417	0.103	0.0295 (\pm 0.0326)	1.417
MR1	48	2	0.167	0.042	0.0204 (\pm 0.0262)	1.167
Total	203					
Mean		3	0.208	0.088	0.0287 (\pm 0.0379)	1.208
New Jersey, U.S.A.						
US11	33	22	0.917	0.667	0.2522 (\pm 0.1500)	2.500
US5	17	10	0.583	0.588	0.3033 (\pm 0.1808)	1.833
US7	36	12	0.917	0.333	0.3111 (\pm 0.1787)	2.583
Total	86					
Mean		14.7	0.806	0.529	0.2889 (\pm 0.0320)	2.305

N is sample size; PD is proportion of distinguishable genotypes; Average gene diversity is over loci (Nei, 1987), *A* is mean number of alleles per locus.

Table 3 Allele frequencies at 12 polymorphic loci of populations of *Sphagnum affine*. Abbreviations of population codes correspond to Table 1

	S3	S6	S7	MR1	T12	T13	T11	S9	US11	US5	US7	V02	V01	T15	T16	H1	H2	H3	TE2
Aat-1, 90								0.500								0.027			
Aat-1, 100	0.821	0.757	0.474	0.063	0.933	1.000	1.000	0.481	1.000	1.000	0.900	0.655	1.000	1.000	1.000	0.973	1.000	1.000	1.000
Aat-1, 120	0.179	0.243	0.526	0.938	0.067			0.019			0.100	0.345							
Aat-2, 90																0.027			
Aat-2, 100	1.000	0.722	1.000	1.000	0.178	1.000	0.968	1.000	0.970	1.000	0.950	0.793	0.370	1.000	0.098	0.946	1.000	1.000	0.273
Aat-2, 120													0.630						
Aat-2, 130												0.207							
Aat-2, 140		0.278			0.822		0.032		0.030		0.050				0.902				0.727
Aco-1, 70																			
Aco-1, 90									0.030	0.588	0.210								
Aco-1, 100	1.000	1.000	0.974	1.000	1.000	1.000	1.000	1.000	0.970	0.412	0.789	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aco-1, 110																			
Aco-1, 120			0.026																
Aco-2, 100	0.036	0.156	0.105					0.510	0.273	0.059		0.068	0.074	1.000	0.049	0.108	0.024		0.636
Aco-2, 110																			
Aco-2, 120	0.964	0.844	0.895	1.000	1.000	1.000	1.000	0.490	0.485	0.353	0.684	0.932	0.926		0.951	0.892	0.220	1.000	0.364
Aco-2, 130									0.242	0.588	0.316						0.756		
Adh-1, 80			0.026						0.030		0.075								
Adh-1, 100	0.567	0.829	0.846	1.000	1.000	1.000	1.000	0.981	0.939	0.824	0.925	1.000	0.852	1.000	1.000	0.162	0.071	0.706	1.000
Adh-1, 110																			
Adh-1, 120	0.433	0.171	0.128					0.019		0.177			0.148			0.838	0.929	0.294	
Adh-1, 130									0.030										
Adh-1, 170																			
Adh-2, 70											0.075					0.027			
Adh-2, 80	0.769	0.771	0.872	1.000	0.844	1.000		0.385	0.667	0.529	0.675	0.559	0.852		0.976	0.270	0.976	0.706	
Adh-2, 90											0.025					0.460			
Adh-2, 100	0.231	0.229	0.128		0.156		1.000	0.615	0.273	0.235	0.225	0.424	0.148	1.000	0.024	0.189	0.024	0.294	1.000
Adh-2, 110												0.017				0.054			
Adh-2, 130									0.061	0.235									
Adh-2, 140																			
Adh-2, 160																			
Adh-3, 100	0.964	0.861	0.974	1.000	0.844	1.000		0.404	0.667	0.529	0.769	0.576	1.000		0.976	0.892	0.905	0.647	1.000
Adh-3, 120									0.030										
Adh-3, 140	0.036	0.139			0.156		1.000	0.596	0.242	0.294	0.231	0.407			0.024	0.108	0.095	0.353	
Adh-3, 160			0.026											1.000					
Adh-3, 170												0.017							
Adh-3, 180																			
Adh-3, 220									0.061	0.177									
Fdh-1, 70												0.025							
Fdh-1, 80												0.050							
Fdh-1, 100								0.030											

Fdh-1, 130																			
Fdh-1, 160	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.970	1.000	0.475	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Fdh-1, 170											0.450								
Idh-1, 80																			
Idh-1, 100	1.000	1.000	0.974	1.000	0.975	1.000	1.000	1.000	0.939	1.000	0.925	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-1, 120			0.026		0.025				0.061		0.075								
Pgi-1, 80									0.061										
Pgi-1, 90																			
Pgi-1, 100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.939	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi-1, 130																			
Pgm-1, 40											0.128								
Pgm-1, 60											0.051								
Pgm-1, 80	0.033	0.265	0.487	0.938	0.156	0.917		0.500	0.394	0.353	0.077	0.678	0.185		0.073	0.973	1.000	0.421	1.000
Pgm-1, 100	0.967	0.735	0.513	0.062	0.844	0.083	1.000	0.500	0.485	0.647	0.513	0.322	0.815	1.000	0.927	0.027		0.579	
Pgm-1, 110									0.091		0.051								
Pgm-1, 120									0.030		0.180								
Skd-1, 90									0.121	0.353	0.075								
Skd-1, 100	0.929	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.879	0.647	0.925	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Skd-1, 120	0.071																		

across the 19 populations surveyed. The frequency distribution of haplotypes is skewed, as most haplotypes occur in a single or few populations. The commonest six haplotypes accounted for 51% of the total variation within the entire sample. The most widespread haplotype occurred in 68% of the populations surveyed. The proportion of distinguishable genotypes ranged from 0.04 to 0.67.

Private alleles at low frequency were encountered in 13 populations. Nine of the private alleles were found in the U.S.A., two in southern Sweden and one in southern Norway. Only one population (V01) in southern Sweden had a private allele with high frequency (63%; Table 3).

Genetic variability on different continents and in different regions

Those populations originating from North America were more variable for all diversity measures compared to the European populations. Mean genetic diversity (Nei, 1987) over loci (H_s) was estimated to 0.289 compared to a European mean of $H_s=0.098$. Mean number of alleles per locus (A) was 2.3 in U.S.A. compared with 1.4 in Europe. Within Europe, pronounced differences exist among regions in terms of diversity measures. The southernmost populations (southern Sweden) were on average more variable ($H_s=0.162$) than the northernmost populations (central Norway) ($H_s=0.029$). The same trend is apparent for all diversity measures (Table 2). This might suggest clinal variation at one or more loci, but no such trend is apparent from allele frequency data (Table 3).

Clonal diversity, measured as the proportion of distinguishable genotypes (PD), was approximately three times larger in populations in southern Sweden than in central Norway. The southern Norwegian populations fall in between for all measures of diversity (Table 2). The clonal diversity (mean PD) encountered in the populations from U.S.A. was much higher than even the most diverse European region. Fifteen alleles at eight loci absent from the European sample were encountered in the three American populations (Table 3). Of these, one allele (*Fdh-1,100*) might represent a low-frequency, relict occurrence from an ancient hybridization event (Thinggaard, in press). Only three European alleles, all sampled at *Aar-2* from three of 16 populations, were absent in the three New Jersey populations. In addition, two alleles, possibly originating from very rare hybridization events between *S. affine* and *S. austrii* Sull. (Thinggaard, in press), were unique to Europe.

Population differentiation at intercontinental and regional scale

No significant substructuring among populations, regions and continents was revealed (Table 4). The fixation index ($F_{ST}=0.512$) showed that the variation was partitioned almost equally among and within populations when the total sample is considered. Among European regions, differentiation was small ($F_{RT}=0.043$), and a slightly larger fraction of the variation was found among populations compared to within European populations (Table 4).

The amount of genetic variation partitioned among populations within regions varied dramatically between regions. Most of the variation was distributed within rather than among populations in New Jersey Pine Barrens ($F_{STreg}=0.105$) and southern Sweden ($F_{STreg}=0.248$), whereas most of the variation was distributed among populations in southern Norway ($F_{STreg}=0.660$) and extremely so in the central Norwegian populations ($F_{STreg}=0.864$; Table 4; Fig. 2).

Estimate of recombination

Genetic phase disequilibrium were encountered in 94% of the population samples; linkage disequilibrium was absent in only one southern Swedish population (S3). The amount of linkage disequilibrium showed large differences among populations both within and among

regions. The percentage of linked loci relative to the maximum number (P_d) estimated as a mean of each region increased with latitude (Table 5).

Discussion

Genetic diversity

The low genetic diversity in the northernmost populations of *Sphagnum affine*, as estimated by genetic diversity and clonal diversity measures, does not support a hypothesis of lower genetic diversity in more anthropogenically fragmented areas. The pattern is better explained by random genetic drift during post-glacial recolonization. Assuming that Scandinavian populations of *S. affine* have colonized mires serially after migrating northwards from refugia in southern Europe at the onset of the Holocene or later, they may have experienced bottlenecks during minor climatic oscillations and lost genetic diversity towards the north. This genetic depletion includes the loss of the otherwise widespread allele *Adh*-1 120 in central Norwegian populations. It would have been interesting to include populations from unglaciated areas in southern Europe to assess whether they are more genetically variable than the populations included in this study. This has not been possible, mainly as a consequence of the rarity of *S. affine* south of the Scandinavian Peninsula, although reduction of genetic diversity with

Table 4 Analysis of molecular variance (AMOVA) based on a sample of 640 plants from 19 populations of *Sphagnum affine* from northern Europe and North America

Source of variation	d.f.	SS*	Variance components	Percentage of total variation	P-value
Intercontinental variation (F_{CT})	1	28.113	0.010	0.63	$P < 0.0001$
Among populations within regions (F_{ST})	17	473.324	0.810	51.15	$P = 0.0001$
Among individuals within populations	618	471.700	0.763	48.22	$P < 0.0001$
Among European regions (F_{RT})	2	85.118	0.065	4.28	$P < 0.0001$
Among populations within European regions (F_{SR})	13	373.628	0.830	55.00	$P < 0.0001$
Within populations in Europe (F_{IS})	535	328.824	0.615	40.72	$P < 0.0001$
Regional					
Among populations in U.S.A. (F_{STreg})	2	14.578	0.203	10.54	$P < 0.0001$
Within populations in U.S.A. (F_{IS})	83	142.875	1.721	89.46	$P < 0.0001$
Among populations in southern Sweden (F_{STreg})	5	65.737	0.333	24.76	$P < 0.0001$
Within populations in southern Sweden (F_{IS})	217	219.497	1.012	75.24	$P < 0.0001$
Among populations in southern Norway (F_{STreg})	3	108.062	1.147	66.03	$P < 0.0001$
Within populations in southern Norway (F_{IS})	123	72.560	0.590	33.97	$P < 0.0001$
Among populations in central Norway (F_{STreg})	5	199.829	1.202	86.44	$P < 0.0001$
Within populations in central Norway (F_{IS})	195	36.768	0.189	13.56	$P < 0.0001$

*Sum of squared deviations.

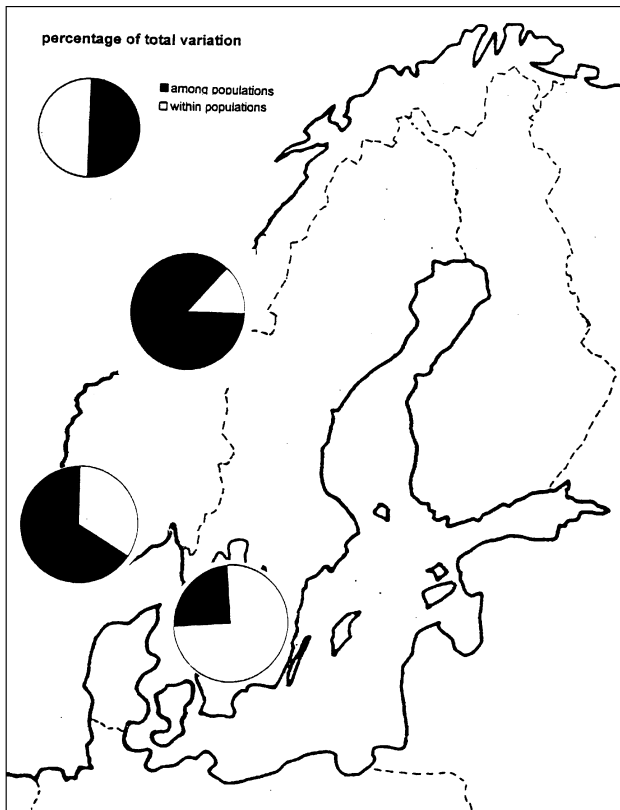


Fig. 2 Partitioning of genetic variation among and within populations of *Sphagnum affine* in three Scandinavian regions along a latitudinal gradient.

latitude suggests that southern populations may be more diverse.

A decrease of gene diversity with increasing latitude has been observed in the mosses *Meesia triquetra* (Richt.) Aongstr. in the western part of its range (Montagnes *et al.*, 1993) and *Leucodon sciuroides* (Hedw.) Schwaegr. in unglaciated vs. glaciated parts of Europe (Cronberg, 2000), as well as in angiosperms (e.g. Broyles, 1998). In comparison to the related oceanic *Sphagnum austinii*, confined to lowland atlantic bogs in Europe (Flatberg, 1986) and almost devoid of genetic diversity (Thingsgaard, in press), *S. affine* is able to grow and survive in a diverse array of mire habitats. This may be the reason why it has survived the migration with more genetic diversity preserved. For the same reason it may have had more glacial refugia in southern Europe, possibly extending further eastwards than *S. austinii*. Likewise, suboceanic *S. rubellum* is reported with reduced levels of genetic variation in Scandinavia compared to Great Britain, but little genetic differentiation among lowland populations in Scandinavia (Cronberg, 1998). *Sphagnum rubellum* extends further north and east, it occurs in a wider range of mire

habitats, and may not be as dependent on warm summer temperatures as *S. affine* (Flatberg, 1986). Cronberg (1998) speculates, that the dry climate during glaciations may have reduced the area available for survival of *S. rubellum* in southern Europe. It is likely that *S. affine* and *S. rubellum* shared refugia in southern Europe, and have faced similar constraints during postglacial migration. However, as *S. affine* is less well adapted for colder climates and has a slightly more oceanic affinity, it may have been less successful towards the north, where the effects of minor climatic oscillations were severe, than *S. rubellum*. Consequently, it has experienced more bottlenecks while colonizing Norway, and thus retained increasingly depleted populations with latitude. Similar patterns are reported for other species of animals and plants (Hewitt, 1999).

The generally higher amount of genetic diversity found in populations from New Jersey may be ascribed to the fact that the Coastal Plains, where the sample of American populations were collected, have not been glaciated during the Wisconsin glaciation (Karlín & Andrus, 1988). Comparisons of bryophytes from glaciated areas in North America and Europe show that in most cases the gene diversity in North America is higher

Table 5 Proportion of linked loci relative to the maximum number of linked loci (P_d)

S3	0.0
S6	33.33
S7	26.67
S9	66.67
V01	40.00
V02	53.33
Mean southern Sweden	27.87
H1	38.10
H2	50.00
H3	66.67
TE2	100.00
Mean southern Norway	51.59
MR1	100.00
T11	****
T12	66.67
T13	****
T15	****
T16	50.00
Mean central Norway	72.22
US5	42.82
US7	29.09
US11	10.91
Mean U.S.A.	27.62

****Gametic phase disequilibrium not calculated owing to zero or only one polymorphic locus in population.

than in Europe, i.e. glaciation does not necessarily imply that populations became genetically depleted in North America. Lower genetic diversity was reported for *Plagiomnium medium* (B.S.G.) T.Kop. in Europe compared to North America (Wyatt *et al.*, 1992), and some evidence of the same trend may be observed in *Sphagnum angustifolium* (Russ.) C.Jens. Clonal diversity of *S. angustifolium* originating from Newfoundland (calculated from data in Stenøien & Sæstad (1999)) is more than twice as high as in European populations, suggesting that the effect of glaciation on intrapopulation genetic diversity is notable in populations of *Sphagnum* in Europe compared to glaciated areas in North America. The main mountain ranges in central and southern Europe extend E–W and are a substantial barrier to north- and southward migration. In contrast, the mountain ranges in North America are orientated N–S, and thus similar barriers to N–S migrations of fauna and flora do not exist (Crum, 1972; Wyatt *et al.*, 1993). A possible explanation of the higher genetic diversity found in North American populations of *S. affine* compared to European populations may be that the species has not gone through severe bottlenecks during glaciations and subsequent recolonizations to the same extent as in Europe. Recently, Sæstad *et al.* (2000) reported lower genetic diversity in North America compared to Europe in limited samples of *Sphagnum majus* (Russ.) C.Jens from Newfoundland and New York. However, *S. majus* is a circumboreal species, only rarely extending to lowland nemoral (deciduous woodland) temperate areas. Moreover, it is rare in the western part of North America. More genetic diversity may have been preserved in this boreal species if it did not survive the glaciation in Europe in the same, probably very limited, southern refugia as *S. affine* and *S. rubellum*, but rather were able to survive in more and larger refugia located further south-east or east of the North European ice sheet than the oceanic and suboceanic species. This may also explain the rather high genetic diversity found in *S. angustifolium* and *S. capillifolium* (Ehrh.) Hedw., two species extending into continental and subalpine areas.

Differentiation at the intercontinental and regional levels

Little differentiation was found between European and North American *S. affine* populations. Similar patterns are reported in other studies of bryophytes from these areas (Wyatt *et al.*, 1992; Stenøien & Sæstad, 1999; Hanssen *et al.* 2000; Sæstad *et al.*, 2000).

Among European populations, Cronberg (1998) found low levels of differentiation in *S. capillifolium* and *S. rubellum*, indicating considerable gene flow. A low

impact of processes causing population divergence was invoked by Stenøien & Sæstad (1999) to explain the lack of intercontinental differentiation in *S. angustifolium*. However, in many clonal species the majority of genetic variation is often found among populations (Ellstrand & Roose, 1987), a pattern that is also found in *S. affine*. The genetic variation found in populations originating from southern and central Norway is strongly partitioned among populations (66% and 86%, respectively), suggesting a large impact of genetic drift (Table 4; Fig. 2). The lowest amount of interpopulation differentiation was found regionally in New Jersey (*c.* 10%) and southern Sweden (*c.* 25%). This suggests limited, but still higher, gene flow (past or present) between populations within these regions compared to the northernmost populations in central Norway.

The causal factors determining the genetic isolation among populations of *S. affine* in, for example, southern Sweden and central Norway, may be different in each region. The F_{STreg} estimates for central Norway are very high, which allows for associating the regional F_{STreg} value with a N_m value even though regional equilibrium between loss of alleles due to genetic drift and their replacement by gene flow among populations is not found (Hutchison & Templeton, 1999). The N_m value inferred via Wright's (1931) equation:

$$F_{ST} \times 1 / (4N_m + 1; \text{ for haploids } 2N_m + 1)$$

from a mean regional F_{ST} value of 0.83 is 0.102, inferring that drift is a proportionately more important structuring agent than gene flow (Kimura & Weiss, 1964) in central Norwegian populations of *S. affine*. This may support an assumption that the effect of climatic fluctuations on the populations forming the northern distributional limit or 'migration front' is reflected in the size of the effective breeding population; hence, there is a greater susceptibility to genetic bottlenecks. Severe isolation of each finite population will cause random genetic drift to become relatively more important than gene flow. It is somewhat more difficult to interpret the southern Swedish region with respect to the individual effects of interpopulation gene flow and random genetic drift on the regional population structure. Sampling of geographically close populations has not been accomplished in this region, and it is not possible to assess whether the regional structure approaches equilibrium. It is very unlikely however, that southern Swedish populations approach a migration–drift equilibrium, because populations inhabiting landscapes undergoing anthropogenic changes causing habitat fragmentation are not expected to be at equilibrium (Whitlock & McCauley, 1998). Ancient gene flow among populations and/or possibly more colonization events from less

depleted sources in the southern Sweden region, as well as a longer persistence of the conditions required for the populations to exist, compared to the Norwegian populations, may explain the comparatively lower differentiation among populations from Sweden compared to central Norway. Even if gene flow has had, or has, a more prominent role in population structuring in southern Sweden than in central Norway, interpopulation gene flow may generally be considered low among *S. affine* populations in Europe. This tendency increases with latitude, and may contribute to the partitioning of genetic variation among rather than within populations (Table 4). The existence of a private allele at high frequency at the locality near Ll. Lesjön in south-west Sweden (V01) indicates that present-day interpopulation gene flow is low. The same applies to two closely situated populations sampled in the same large mire complex in south-west Norway (H1 and H2), which are very different with respect to diversity. H1, the smaller sample, is more polymorphic, not only because of alleles occurring at low frequencies, but also because of two alleles occurring at intermediate frequency that are not represented in the nearby population. The proportion of distinguishable genotypes, i.e. the clonal diversity, is also higher at H1. Finally, the only mire site in the total sample, which housed a completely monomorphic population (T15) was situated at the island of Hitra, central Norway, only a few kilometres from another population which was variable at five loci (T16).

Recombination within populations

Some of the findings suggest that genetic recombination within populations is limited, and that it decreases with latitude. The great amount of gametic phase equilibria suggests that most populations are inbreeding, and are not panmictic. The increase in gametic phase disequilibria with latitude indicates that the northernmost populations have been established most recently through multiple colonizations. In combination with the rare production of sporophytes seen today (i.e. most reproduction may be clonal) this indicates that recombination has not been sufficient to break down the patterns of linkage disequilibria in the populations acquired during postglacial migration. When clonal size increases, the chance of successful recombination is reduced owing to limited gamete dispersal distances (McQueen, 1985; Cronberg, 1993; Daniels, 1993), unless male and female clones intermix, which they may. Even if sexual reproduction occurs, the chance that the male and female plant involved may have originated from the same sporophyte (autogamy) appears to be high, as most spores are assumed to be dispersed within a limited distance from the mother plant (McQueen, 1985).

Establishment of sporelings in existing clonal populations of *Sphagnum* is hard to assess by direct means. Little is therefore known about the germination of *Sphagnum* spores in nature (Cronberg, 1993; Daniels, 1993). It would be interesting to know, whether a spore is able to germinate, a protonema develop, and a new individual to establish in a dense carpet of other mire *Sphagnum* species, or whether new establishment is largely dependent on disturbances creating suitable niches. A recent experimental study suggests that *Sphagnum* spores may not germinate in moss carpets, but natural recruitment from spores is dependent on disturbance as well as nutrient release from litter and coverage provided by vascular plants (Sundberg and Rydin, in press).

If many European populations of *S. affine* are not panmictic, and sexual reproduction only takes place occasionally and to some extent among closely related individuals with possibly low success of establishment, the amount of haplotypic variation found must be the result of gene flow in the past. Genetic drift has been playing an important role in the structuring of genetic variation in this species. The northernmost populations confined to the suboceanic lowland of Norway have almost certainly suffered more founder effects during postglacial migration than the populations arriving on the Scandinavian Peninsula from glacial refugia in the south, and may possibly experience unfavourable breeding seasons due to climatic constraints. This will explain why populations in this region are more inbred, less diverse and more genetically isolated; a pattern expected in the more anthropogenically fragmented agricultural landscape in southern Sweden.

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