The Bryologist 108(2), pp. 194–203

Genetic Structure of the Endangered Peat Moss *Sphagnum angermanicum* in Sweden: A Result of Historic or Contemporary Processes?

URBAN GUNNARSSON, KRISTIAN HASSEL, AND LARS SÖDERSTRÖM
Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway; e-mail: Urban.Gunnarsson@bio.ntnu.no

Abstract. *Genetic structure and diversity were studied in the endangered peatmoss* Sphagnum angermanicum *Melin to assess its conservation status. In total, 128 shoots from eleven populations throughout its Swedish distribution were analyzed. Among these shoots 28 haplotypes were identified by 19 ISSR loci. The most common haplotype (50% of the sampled shoots) occurred in all populations. The level of gene diversity over loci was intermediate compared to records from most other bryophytes. There was no genetic isolation between the populations and most of the genetic variation was found within populations. This implies either that the populations have originated from only a few common individuals or a high gene flow between populations. A relict population model is suggested to explain the observed pattern. Just after the last glaciation,* S. angermanicum *may have expanded its range when suitable habitat became available after the glacial ice retreated. Since then, habitats have vanished or fragmented and today only a few relict populations exist. This dioicous species has only once been reported with sporophytes in Scandinavia in modern time, but according to the genetic data, both the low level of linkage among loci and the estimated rate of recombination show evidence of sexual reproduction. However, reproduction may have been more frequent in the past. Based on the current knowledge of the species habitat requirements, life history, and the small population sizes, we conclude that the species will have an uncertain future in Sweden.*

Keywords. Genetic diversity and structure, inter simple sequence repeats (ISSR), postglacial dispersal history, rare species, recombination, *Sphagnum angermanicum*.

Comparison of genetic diversity between rare and common species has shown that rare species usually have lower genetic diversity (Cole 2003; Frankham 1996; Gitzendanner & Soltis 2000). Restricted gene flow due to habitat fragmentation or lack of reproduction is far more serious in rare as compared to common species (Cole 2003). The relative genetic differentiation among populations may, however, either be small or large (Cole 2003) depending on, for example, the species life history characteristics or historical population processes. Genetic drift is a force that becomes increasingly strong in small and isolated populations. Estimates of the proportions of the total genetic variation distributed within and among populations could provide important information for successful conservation.

Fragmentation and habitat loss have reduced the suitable area of many species. As an example about 30% of European bryophytes are currently classed rare or threatened (including insufficiently known species), partly as a consequence of fragmentation and habitat loss (ECCB 1995). Habitat fragmentation can create small, isolated populations, which due to genetic drift, are more prone to loss of genetic diversity than large populations. This could simply be an effect of a reduced population size producing a reduced genetic diversity (e.g., Frankham 1996; Prober & Brown 1994; Sampson et al. 1989; van Treuren et al. 1991), but isolation may also reduce gene flow and increase inbreeding and genetic drift (Frankham 1996; Nei 1987). Species life history characteristics, for example reproductive system, mode of dispersal, and rate of establishment at new suitable sites, may be decisive for the species genetic diversity and structure and its potential to respond to a changing environment. The reproductive system can affect the level of genetic variation within different species. For example, monoicous species usually have lower degree of genetic variation than dioicous species (Cronberg 1996; Hamrick & Godt 1989; Stenøien & Såstad 1999), as they might be affected by a high degree of inbreeding. However, many dioicous species never, or seldom, reproduce sexually, but they often have modes of asexual reproduction. Asexual propagation (reproduction) in combination with random genetic drift reduces levels of genetic diversity and quite often increases the between-population genetic differentiation (Baatout et al. 1991;

0007-2745/05/$1.15/0

Treu et al. 2001). Reliance on asexual diaspores that often have limited potential for long distance dispersal will give reduced gene flow between populations compared to species dispersed by small spores (Kimmerer 1994; Laaka-Lindberg 2001; Longton & Schuster 1983). Consequently dioicous species relying on asexual propagules will experience high inter-population differentiation (Brakefield 1989).

Historical population processes such as the occurrence of bottlenecks, founder effects, and range expansions affect the current level of genetic variation within a species. Among the most important historical events in the boreal and boreo-nemoral zones of Europe are the glaciations during the Quaternary cold periods (Ferris et al. 1999; Hewitt 1996). During these cold periods many populations went through severe bottlenecks resulting in reduced effective population sizes and loss of rare alleles and reduced heterozygosity (Luikart et al. 1998). Many of the species currently present in northern Europe (the former glaciated areas) had to expand from glacial refugia in the Mediterranean region (i.e., the Iberian Peninsula, Italy, and the Balkans, Bennet et al. 1991; Widmer & Lexer 2001) or from refugia along the European Atlantic coast (Kullman 2002) to new suitable habitats. Bottlenecks during the glacial maxima and founder effects during colonization would have allowed several opportunities for extreme range expansions for land plants during the postglacial warm Boreal and Atlantic periods after glacial retreat. Postglacial dispersal history is an important component structuring the genetic variation of populations (Newton et al. 1999; Taberlet et al. 1994). Several models to explain the postglacial colonization pattern have been suggested. The leading edge models (Ferris et al. 1999; Hewitt 1996) suggest that new suitable habitats, created after the ice retreat, were rapidly colonized by long distance dispersal from populations from the northern limit of refugial areas. This process would involve establishment of new populations by a few individuals that would expand in the new area before other dispersants arrive, resulting in large, genetically homogenous areas within the previously glaciated areas (Hewitt 1996, 2000).

Disjunct populations that occupy marginal positions should have a higher degree of genetic isolation compared to central populations, since gene flow to marginal populations may be less. In addition, since central populations may have a higher chance of regaining genetic variation during episodes of small population sizes (by inter-population gene flow) than marginal populations, habitat fragmentation will have a greater impact on the level of gene diversity in marginal populations (Prober & Brown 1994).

In this study, we investigated Swedish populations of the endangered dioicous peatmoss *Sphagnum angermanicum* Melin, a species that rarely reproduces sexually. Based on current knowledge of the species, several hypotheses can be formulated. 1) As *S. angermanicum* mainly reproduces asexually, there should be little evidence of recombination, as indicated by strong linkage among loci and low levels of genetic diversity within populations. 2) Larger populations should have more genetic diversity than smaller populations and marginal populations should have less genetic diversity than central populations. 3) The proportion of genetic variation partitioned among populations should be high in this mainly asexual species. 4) During the repeated series of glaciations, the species has undergone repeated bottlenecks that could be detected only as a recent bottleneck. In addition to the genetic variation and structure, species habitat requirements and life history are discussed in relation to the postglacial dispersal history.

Materials and Methods

The species.—*Sphagnum angermanicum* was first described by Melin (1919) on material from the boreal mire Vålandsmyren, in the Swedish province Ångermanland (our population 20, Table 1). The species is listed in the Swedish red list of endangered species as near threatened (NT, Cronberg et al. 2000). It is distributed within a limited area in central Sweden, with a few marginal populations north and east of the central area (Fig. 1). Globally it has a trans-Atlantic distribution and occurs in eastern North America from New Jersey up to southern Labrador (Maass 1967; Phillips & Miller 2001). Its European distribution is confined to Scandinavia, with most localities in Norway (Fig. 1; Flatberg & Moen 1972; Gunnarsson 2004; Hallingbäck et al. 1998) and a single report from Iceland (Jóhannsson 1992). The species is dioicous and has only once been reported with sporophytes in Scandinavia, from a locality in southwestern Norway (specimen in TRH). Sporophyte production seems to be far more common in Newfoundland (Maass 1966, 1967). Asexual reproduction can only take place by fragmentation, as specialized diaspores are not produced. In Scandinavia it occurs mainly in a specific habitat, intermediate fens (Flatberg & Moen 1972; Gunnarsson 2004).

Field sampling.—In the summer of 2001, *Sphagnum angermanicum* was sampled from 11 localities—out of the 20–25 known Swedish localities (Gunnarsson 2004). These populations were chosen to include both marginal populations (populations 12, 19, 20) and more central populations (Table 1, Fig. 1). The population sizes were estimated to correspond to a continuous cover of *S. angermanicum*, even if the populations grew in mixture with other species.

At each locality *S. angermanicum* was sampled in 10 patches with a minimum distance of 0.5 m in order to minimize the risk that the same clone was sampled more than once. In the southernmost population (population 12), only a small amount of the species was found and only five patches could be sampled. For most populations, one

TABLE 1. Swedish populations of *Sphagnum angermanicum* used for the ISSR analyses, with locality data.

Population	Locality	Province	Latitude, longitude	Elevation (m a.s.l.)	Population size (m^2)
1	Ö Vallsjön	Dalarna	13°22′ E, 60°45′ N	400	35.0
4	Ö Älgsjön	Dalarna	13°29′ E, 60°37′ N	425	5.0
9	Kulltäppssätern	Värmland	13°12′ E, 60°37′ N	400	2.0
10	Stormyren	Värmland	13°31′ E, 60°04′ N	290	25.0
11	Flämtmyren	Värmland	13°35′ E, 60°23′ N	330	1.5
12	Karvalakmossen	Västmanland	14°30′ E, 59°59′ N	250	1.0
13	Bäckemyrbäcken	Dalarna	13°10′ E, 60°48′ N	425	10.0
15	Emmådalen	Dalarna	14°43′ E, 61°19′ N	450	30.0
17	Flickran	Dalarna	14°49′ E, 61°20′ N	500	10.0
19	Kolkilamp	Gästrikland	16°20′ E, 60°50′ N	280	2.5
20	Vålandsmyren	Ångermanland	17°28′ E, 63°35′ N	300	1.5

shoot was sampled from each patch, but in population one, five shoots where sampled from six of the patches. In these patches the shoots were sampled in a quadrate (10 × 10 cm^2), one from each corner and one from the center. Shoots were placed in plastic bags and frozen within eight hours.

DNA analysis.—Before the DNA extraction, the shoots were washed in sterile water to remove soil debris and other plant material. Five inter-simple sequence repeat (ISSR) primers were used (Table 2). DNA extraction, PCR setup, and visualization follow the procedure described in Hassel and Gunnarsson (2003). Bands were scored manually and a table of presence/absence was compiled.

Data analysis.—For each population the following diversity measures were calculated: frequency of polymorphic loci, average haplotype diversity (h_S; Nei 1987), and average gene diversity over loci (H_S; Nei 1987). The pairwise genetic distances among populations were estimated by the F_{ST}-values and by the average number of pairwise differences between populations. The significance of F_{ST} and the average number of pairwise differences were tested by the non-parametric permutation approach described by Excoffier et al. (1992). Isolation by distance between populations was examined by regressions of the pairwise estimates of genetic distance $F_{ST}/(1 - F_{ST})$ and average number of pairwise differences against the logarithm of the pairwise geographical distance (Rousset 1997). The average number of pairwise differences is the genealogical relationship of the haplotypes, where a pairwise difference of one between two haplotypes implies a difference of one single step (Rohlf 1974) and is used as an absolute value of genetic distance (see Charlesworth 1998). The calculations and analyses were all done by using the computer program ARLEQUIN version 2.001 (Schneider et al. 2000). All analyses were performed at the ramet level, where each shoot is considered as its own record in the analyses.

The presence of linkage disequilibrium among different loci was inferred by using the index of association mod-

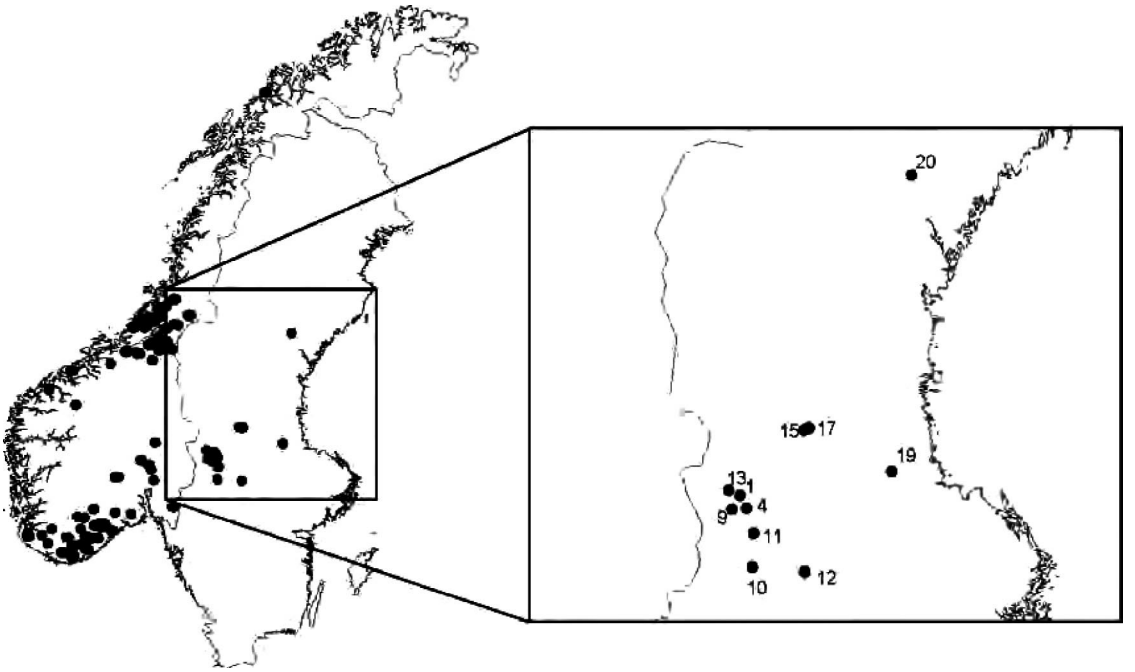

FIGURE 1. The distribution of *Sphagnum angermanicum* in Scandinavia (after Gunnarsson 2004) and the sampled Swedish populations (numbered according to Table 1).

TABLE 2. Used ISSR primer sequences in this study of *Sphagnum angermanicum*, with number of scored and polymorphic loci, scored range (base pairs), and primer specific annealing temperatures. Nucleotides within the same parenthesis indicate that either of the bases occupies the specific position.

Primer	Primer sequence (5′–3′)	Number of scored loci	Number of polymorphic loci	Bp range	Annealing temp. (°C)
808	AGA GAG AGA GAG AGA GC	6	4	350–750	50
811	GAG AGA GAG AGA GAG AC	5	5	450–800	50
818	CAC ACA CAC ACA CAC AG	4	3	500–850	50
823	TCT CTC TCT CTC TCT CC	2	2	500–700	50
841	GAG AGA GAG AGA GAG A(CT)C	2	2	250–600	52

ified to remove the dependency of sample size ($\bar{r}_d$; Agapow & Burt 2001). Calculation of statistics and tests of significance by 1,000 randomizations was performed with the program Multilocus version 1.2 (www.bio.ic.uk/evolve/software/multilocus). Another way to test the relative importance of sexual reproduction is to estimate the relative recombination rate (i.e., the influence of recombination relative to mutation on haplotypic diversity). This can be done by using the formula:

$$R_1 = (\beta_x/n_x)/\theta_x \quad (1)$$

where β_x is the mean β for a given species x, θ_x is the mean θ for all polymorphic loci, and n_x is the number of polymorphic loci examined (Stenøien & Flatberg 2000). The relative recombination rate per locus represents a crude estimate of $(u + r)/u$, where u is the mutation rate and r is the recombination rate per locus per generation. Values of R_1 close to one indicate no or little recombination (only mutations), while higher values indicate a relatively larger importance of recombination.

We looked at the occurrences of rare alleles to investigate if there have been recent bottlenecks within *S. angermanicum*. Only population one had sufficient samples to be tested with the program BOTTLENECK version 1.2.02 (Cornet & Luikart 1996). Population bottlenecks cause alleles at low frequencies to become less abundant than alleles of higher frequencies. If selectively neutral, recent bottlenecks will cause mode shift from the expected L-shape (abundance of many rare alleles) under a mutation drift equilibrium model (Luikart et al. 1998).

RESULTS

The five ISSR primers revealed 19 scorable loci among the 128 shoots sampled; 16 loci were polymorphic and 28 haplotypes were distinguished (Table 2). The most common haplotype (65 of the analyzed shoots) occurred in all populations, two other common haplotypes occurred in eight populations (14 shoots), and in six populations (10 shoots), and five other haplotypes were shared among two or three populations. One population was monotypic (the marginal population 12) the other populations had between 4–7 haplotypes and up to four unique haplotypes (Table 3). The unique haplotypes were always closely related to other haplotypes within the populations (separated by one or two mutational steps) and have probably emerged by mutations within the populations.

The average haplotype diversity (h_S), not including population 12, varied between 0.50–0.93 (Table 3) and when pooled over all populations was 0.72 ± 0.04. Average population gene diversity over loci (H_S) ranged from 0.040–0.116 (Table 3, population 12 not included), and the average for all populations was 0.072 ± 0.050. Populations 10 and 13 showed higher average gene diversity (H_S), 0.116 and 0.110, respectively. The marginal populations 19 and 20 did not have lower genetic diversity (H_S) compared to the more central populations (Table 3). The population sizes ranged from 1 to 35 m^2 (Table 1), but there was no linear relationship between the population size and genetic diversity (H_S; $r^2 = 0.07$,

TABLE 3. Standard diversity indices for the sampled Swedish populations of *Sphagnum angermanicum* and for the total dataset, using ISSR-markers. ***, $p < 0.001$; n.s., $p > 0.05$.

Pop.	n	No. of haplotypes	No. of unique haplotypes	Freq. of polymorphic loci	h_S; average haplotype diversity ± SD	H_S; average gene diversity ± SD	Average # of pairwise differences	$\bar{r}_d$; multilocus linkage disequilibrium
1	34	7	1	0.32	0.50 ± 0.10	0.048 ± 0.038	0.50	0.172***
4	10	4	2	0.15	0.64 ± 0.15	0.040 ± 0.036	0.64	−0.130$^{n.s.}$
9	10	4	0	0.16	0.73 ± 0.10	0.049 ± 0.042	0.73	−0.095$^{n.s.}$
10	10	7	4	0.32	0.93 ± 0.06	0.116 ± 0.079	0.93	0.021$^{n.s.}$
11	10	7	2	0.21	0.91 ± 0.08	0.087 ± 0.063	0.91	−0.080$^{n.s.}$
12	5	1	0	0	0.0 ± 0.0	0.0 ± 0.0	0.00	—
13	10	6	3	0.42	0.84 ± 0.10	0.110 ± 0.076	0.84	0.096$^{n.s.}$
15	10	5	1	0.32	0.76 ± 0.13	0.080 ± 0.059	0.76	0.097$^{n.s.}$
17	9	5	1	0.32	0.72 ± 0.16	0.079 ± 0.059	0.72	0.128$^{n.s.}$
19	10	5	1	0.26	0.76 ± 0.13	0.067 ± 0.052	0.76	0.081$^{n.s.}$
20	10	6	4	0.26	0.84 ± 0.10	0.077 ± 0.058	0.84	−0.012$^{n.s.}$

TABLE 4. Result of AMOVA analysis of 11 *Sphagnum angermanicum* populations sampled in Sweden and among the patches sampled within population one. ***, $p < 0.001$; n.s., $p > 0.05$.

Source of variation	d.f.	Variance component	% Variance	F_{ST}
Among populations	10	0.023	6.3	0.063***
Within populations	117	0.341	93.7	
Total	127	0.365		
Among patches	5	0.005	2.8	0.028$^{n.s.}$
Within patches	24	0.175	97.2	
Total	29	0.180		

$F = 0.70$, $p = 0.42$). The average number of pairwise differences within the populations varied from 0 in population 12 to 0.93 in the most polymorphic population (Table 3). The $\bar{r}_d$-values ranged from −0.130 to 0.172, but significant linkage occurred only in population one (Table 3). The relative recombination rate estimated over all populations (R_1) was 1.56.

The analysis of molecular variation showed that most of the variation 94% was partitioned within populations and only 6% was partitioned among populations (Table 4). Even though the proportion of variation explained by among population variation was only 6%, the overall F_{ST}-value was significantly different from zero (Table 4), which shows genetic differentiation between the populations. The pairwise F_{ST}-values varied between 0.00 and 0.33, where population 11 showed the largest F_{ST}-values and, in addition, populations 1, 4, 9, 10, 19 and 20 had three or more significant F_{ST}-values (Table 5). Almost the same pattern was revealed by looking at the average number of pair-wise differences (range 0.29–0.96; Table 5). No significant relationship was found between the geographical distance and the pairwise F_{ST}-values (Fig. 2), nor the geographical distance and the average number of pair wise differences ($r^2 = 0.1$, $F = 0.04$, $p = 0.83$).

At the local scale (the detailed study of population one) most variation (97%) was partitioned within patches, while only 3% was partitioned among patches (Table 4). The clonal structure was fragmented; one haplotype was found in all six patches, one in two patches and three in one patch. The patches had between one and three haplotypes, two monotypic patches, three with two haplotypes and one patch with tree haplotypes.

The test of a possible bottleneck in population one did not indicate that it had faced a recent bottleneck, since the distribution of allele frequencies was L-shaped.

DISCUSSION

Reproductive system, genetic diversity, and recombination rates.—The average gene diversity over loci (H_S; Table 3) in populations of *Sphagnum angermanicum* was in the same range as reported previously from isozyme studies of the species in Scandinavia ($H_S = 0.089 \pm 0.051$ S.E., Cronberg 1996) and within the lower region of the gene diversity reported in dioicous moss species, but in the mid or upper region of monoicous species (cf. Cronberg 1996, 1998; Hassel et al., pers. comm.; Stenøien & Såstad 1999; Thingsgaard 2001). The relatively low level of gene diversity within the populations of *S. angermanicum* agrees with expectations for a dioicous species that seldom sexually reproduces.

The relative rate of recombination in *S. angermanicum* ($R_1 = 1.56$) was lower than observed in

TABLE 5. Interpopulation analyses of the studied *Sphagnum angermanicum* populations, where the average number of pairwise differences between populations (above diagonal) and the pairwise F_{ST}-values (below diagonal) are shown. Figures in bold show pairwise differences significantly different from zero ($p < 0.05$).

	Pop. 1	Pop. 4	Pop. 9	Pop. 10	Pop. 11	Pop. 12	Pop. 13	Pop. 15	Pop. 17	Pop. 19	Pop. 20
Pop. 1	—	0.57	**0.71**	**0.85**	**0.91**	0.29	**0.71**	0.64	0.59	0.63	**0.71**
Pop. 4	0.02	—	0.76	0.87	**0.94**	0.40	0.75	0.69	0.66	0.68	0.75
Pop. 9	**0.08**	**0.16**	—	0.84	0.83	0.60	0.76	0.72	0.73	0.78	0.84
Pop. 10	**0.23**	**0.20**	0.01	—	0.92	0.80	0.87	0.85	0.86	0.89	0.91
Pop. 11	**0.23**	**0.33**	0.13	0.17	—	0.90	0.90	0.89	0.90	**0.95**	**0.96**
Pop. 12	0.00	0.00	0.11	0.16	**0.30**	—	0.60	0.50	0.44	0.50	0.60
Pop. 13	**0.09**	0.06	0.00	0.08	**0.11**	0.03	—	0.75	0.74	0.79	0.83
Pop. 15	0.02	0.00	0.01	0.10	**0.18**	0.00	0.00	—	0.69	0.74	0.79
Pop. 17	0.00	0.01	0.00	0.09	**0.11**	0.00	0.00	0.00	—	0.71	0.77
Pop. 19	**0.08**	0.05	0.09	**0.13**	**0.29**	0.00	0.07	0.03	0.03	—	0.79
Pop. 20	**0.09**	0.03	**0.14**	**0.16**	**0.28**	0.00	0.07	0.01	0.00	0.05	—

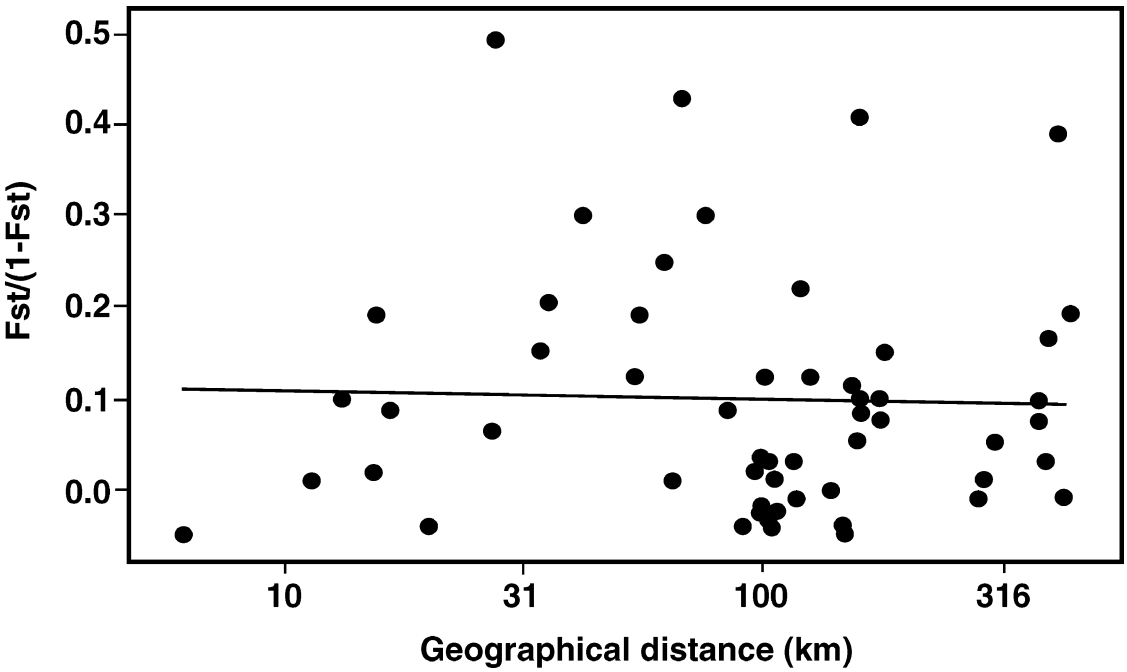

FIGURE 2. Relationship between pairwise $F_{ST}/(1 - F_{ST})$ and geographical distance ($r^2 = 0.01$, $F = 0.006$, $p = 0.93$).

the frequently sporulating dioicous species *S. angustifolium* ($R_1 = 2.25$), but higher than found in the occasionally sporulating dioicous *S. fallax* ($R_1 = 1.03$, Stenøien & Flatberg 2000). In contrast, the monoicous, selfing species *S. lindbergii* has a lower level of recombination ($R_1 = 1.03$) and the asexually reproducing Norwegian endemic, *S. troendelagicum*, has an even lower rate ($R_1 = 0.97$, Stenøien & Flatberg 2000). The $\bar{r}_d$-values showed higher linkage among loci as compared with the dioicous and frequently sporulating *Pogonatum dentatum* (Hassel et al., pers. comm.). Other studies of *Sphagnum* have found a higher linkage between loci when looking at the proportion of linkage disequilibrium (P_d) as indicators of recombination (Stenøien & Flatberg 2000; Stenøien & Såstad 1999; Thingsgaard 2001). This may indicate that recombination (contemporary or historic) has broken up the linkage between loci in *S. angermanicum* or, alternatively, that new somatic mutations have occurred. The rate of recombination observed in the species could fit a pattern of infrequent or historical sexual recombination events, with asexual reproduction dominating at the local scale without indications of recombination.

In contrast to theoretical expectation (e.g., Nei 1987) and empirical results (Frankham 1986; Nei & Graur 1984; Travis et al. 1996) there wes no relationship between the population size and the level of gene diversity within the investigated populations. This pattern may suggest that the populations are not in mutation drift equilibrium and that current population sizes may not reflect historical population size. Levels of genetic diversity may reflect the populations accumulated history of size fluctuations rather than the present population size (Linhart & Premoli 1994; Lönn & Prentice 2002). Two of the marginal populations (populations 19 and 20) of *S. angermanicum* did not show a reduction in gene diversity. The third marginal population (population 12) was the only monotypic population and was probably recently founded by one or a few colonists. An equal number of founders in the investigated populations or present or historical gene flow between populations may explain the similarities in gene diversity. In addition it is not known if the marginal populations have been marginal historically. It may be argued that the low sample sizes in most populations reduce the power to detect haplotypes in larger populations. However, in the largest and most intensely sampled population (population one) we did not detect more haplotypes than in the other less intensively sampled populations, which might indicate that the sampling design detected a large part of the actual number of haplotypes in most populations.

Genetic structure.—In contrast to the expectations for a mainly asexual species, no isolation by distance could be detected by looking at the relation between pairwise F_{ST}-values and geographical distance (Fig. 2). The wide scattering of the plotted values may indicate ongoing mutations and/or drift

within single populations that increase the population differentiation (Hutchison & Templeton 1999). The absence of isolation by distance, and the presence of several common haplotypes among different populations can be explained either by high gene flow between populations or lack of genetic variation in the founders of the populations (founders genetically uniform). The current absence of sporophytes in Scandinavian populations and the widely spread pairwise F_{ST}-distance values suggest that eventual gene flow has occurred historically. Long distance dispersal by spores from sources outside Sweden might have been important, and has been suggested to be important for other bryophyte species (e.g., van der Velde et al. 2000; van Zanten & Pócs 1981).

The clones within population one were large, but fragmented (not forming single units). Also, more than one haplotype occurred in the majority of patches. This suggests that asexual propagation is a common reproductive mode within this population and that single individuals can grow large and probably become quite old. Fragmentation of individuals, where fragments are locally dispersed, would also increase the chance of sexual reproduction, as males and females are more likely to grow within fertilization distance (Cronberg 2002), and this suggests that climatic conditions are a more probable explanation for the observed lack of sexual reproduction in these populations.

Interpretation of genetic structure on postglacial dispersal history.—The low genetic sub-structuring of the Swedish *S. angermanicum* populations can have several causes. The source populations (situated in glacial refugia) of *S. angermanicum* in Sweden may have had little genetic variation after one or repeated bottlenecks that might have occurred during repeated glaciations. These bottlenecks may not be detected by the L-shape test, since several generations have passed and new mutation drift equilibra may have established (Luikart et al. 1998). From these source populations the species has probably spread by spores over a vast area during the Boreal and Atlantic periods (9,000–5,000 years BP, Rybnícek 1973; Tolonen 1967), resulting in many weakly differentiated populations. This could be expected under the leading edge model (Ferris et al. 1999) and has been found for several other mosses (e.g., Cronberg 2000; Natcheva & Cronberg 2003). The leading edge model is, however, problematic for a dioicous organism, because both genders must occur at the same spot for sexual reproduction to occur, and sexually produced spores are more efficient for dispersal across larger distances than vegetative dispersal.

Intermediate fens can be found in the boreal parts of Scandinavia and are usually sloping. They are dominated by poor fen species (e.g., *S. papillosum*), but are always mixed with rich fen species. The water is non-calcareous and has higher pH than poor fens (between 5.5 and 6.4, see. Sjörs & Gunnarsson 2002). Intermediate fens were most probably much more abundant in Scandinavia after the last glaciation than today, before the strong expansion of poor fens and ombrotrophic bogs during the Atlantic period (Vitt & Kuhry 1992), which in this area started about 6,000 yrs. BP (Foster & Wright 1990). The intermediate fen is the mire type with the shortest lifetime (Sjörs & Gunnarsson 2002; Vitt 2000), because of a vegetation switch from dominance by rich fen bryophytes to dominance of acidifying poor fen *Sphagnum* species. This switch coincides with a minimum buffering capacity of the mire water in the pH region of intermediate fens (pH range 5.5–6.4, Sjörs & Gunnarsson 2002; Tahvanainen & Tuomaala 2003). The timescale of such switch is in the range of 50–300 years (Gunnarsson et al. 2000; Kuhry 1997; Kuhry et al. 1993; Vitt & Kuhry 1992). As the '*Sphagnum*-switch' successively changes these environments to become more acid (and less suitable for *S. angermanicum*), the area of suitable intermediate fens decreases. Today intermediate fens are not uncommon in Scandinavia, but *S. angermanicum* only occurs in some restricted areas of Sweden (Gunnarsson 2004).

Sphagnum angermanicum must, due to the relatively short habitat duration, either move to new suitable sites or to become locally extinct. Exceptions from this pattern of local extinction could occur when disturbances maintain the site in an intermediate fen or even creates new suitable sites, for instance by animal trampling or by vehicle tracks. Two alternate models can explain today's regional population dynamics of *S. angermanicum*, either the populations are of relict origin from a former large distribution area, or the species exhibits a type of metapopulation dynamics. The latter seems less probable since the formation of new. suitable sites is slow (or absent), effective dispersal between populations is lacking due to lack of spore production at present, and there is no isolation by distance that would be expected under a metapopulation dynamics model. The ongoing acidification of intermediate fens may have caused the relict population pattern and the currently observed fragmentation.

Future prospects for conservation of Sphagnum angermanicum.—The few Swedish populations of *S. angermanicum* show little genetic differentiation and are fragmented. Sexual reproduction has not been reported in the Swedish populations and only once in Scandinavia. If this is the true picture of sexual reproductive output, the chance of establishment of new populations outside its current core

area is minimal. It might even be speculated that the low level of genetic variation within a population has had effects on sexual reproduction, either as a direct effect on offspring performance (in the case of bryophytes–the formation of sporophytes) by exposing recessive deleterious alleles, inbreeding depression, or by self-incompatibility barriers (e.g., Reed & Frankham 2001). In several Scandinavian populations, both sexes have been observed in the same population, but no sporophytes have been observed (Flatberg 2002). However, asexual reproduction might give rise to new populations within the core area, since asexual diaspores generally show shorter dispersal distances (Kimmerer 1994; Laaka-Lindberg 2001; Longton & Schuster 1983) and thus assume maintenance within the core area. Deterministic processes, as the decline in area of suitable habitat (intermediate fens) will continue if no disturbances maintain the habitats in a suitable condition. The acidification rate might even have been accelerated by anthropogenic deposition during the last 50 years (Gunnarsson et al. 2000). In addition to the deterministic processes, the few populations will also face stochastic events. Taken together, the odds for survival of the species in Sweden seem low. *Sphagnum angermanicum* might, however, have the possibility to survive for longer or shorter periods in areas with regular disturbances such as cattle trampling or flooded zones around streams or lakes.

Acknowledgments

We thank Nils Cronberg, Hans K. Stenøien, and Johanne Maad for valuable comments on the manuscript and Kjell I. Flatberg for helpful discussions. This study was financially supported by NorFa and Extensus.

Literature Cited

Agapow, P. M. & A. Burt. 2001. Indices of multilocus disequilibrium. Molecular Ecology Notes 1: 101–102.

Baatout, H., D. Combes & M. Marrakchi. 1991. Reproductive system and population structure in two *Hedysarum* subspecies. I. Genetic variation within and between populations. Genome 34: 396–406.

Brakefield, P. M. 1989. The variance in genetic diversity among subpopulations is more sensitive to founder effects and bottlenecks than is the mean: a case study, pp. 145–161. *In* A. Fontdevila (ed.), Evolutionary Biology of Transient Unstable Populations. Springer Verlag, Berlin.

Bennett, K. D., P. C. Tzedakis & K. J. Willis. 1991. Quaternary refugia of north European trees. Journal of Biogeography 18: 103–115.

Charlesworth, B. 1998. Measures of divergence between populations and the effect of forces that reduce variability. Molecular Biology and Evolution 15: 538–543.

Cole, C. T. 2003. Genetic variation in rare and common plants. Annual Review of Ecology and Systematics 34: 213–237.

Cornuet, J.-M. & G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001–2014.

Cronberg, N. 1996. Isozyme evidence of relationships within *Sphagnum* sect. *Acutifolia* (Sphagnaceae, Bryophyta). Plant Systematics and Evolution 203: 41–64.

———. 1998. Population structure and interspecific differentiation of the peat moss sister species *Sphagnum rubellum* and *S. capillifolium* (Sphagnaceae) in northern Europe. Plant Systematics and Evolution 209: 139–158.

———. 2000. Genetic diversity of the epiphytic bryophyte *Leucodon sciuroides* in formerly glaciated versus non-glaciated parts of Europe. Heredity 84: 710–720.

———. 2002. Colonization dynamics of the clonal moss *Hylocomium splendens* on islands in a Baltic land uplift area: reproduction, genet distribution and genetic variation. Journal of Ecology 90: 925–935.

———, F. Eriksson, T. Hallingbäck, B. G. Jonsson, T. Pettersson & H. Weibull. 2000. Bryophytes, pp. 93–105. *In* Gärdenfors, U (ed.), The 2000 Red list of Swedish Species. Artdatabanken, SLU, Uppsala.

ECCB (European Committee for Conservation of Bryophytes). 1995. Red Data Book of European Bryophytes. ECCB, Trondheim.

Excoffier, L., P. Smouse & J. Quattro. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Ferris, C., R. A. King & G. M. Hewitt. 1999. Isolation within species and the history of glacial refugia, pp. 20–34. *In* P. M. Hollingsworth, R. M. Bateman & R. J. Gornall (eds.), Molecular Systematics and Plant Evolution. Taylor & Francis, London.

Flatberg, K. I. 2002. The Norwegian Sphagna: a field colour guide. Norwegian University of Science and Technology Vitenskapsmuseet Rapport Botanisk Series 2002 (1): 1–44.

——— & A. Moen. 1972. *Sphagnum angermanicum* og *S. molle* i Norge. Det Kongelige Norske Videnskabers Selskabs Skrifter 3: 1–15.

Foster, D. R. & H. E. Wright, Jr. 1990. Role of ecosystem development and climate change in bog formation in central Sweden. Ecology 71: 450–463.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10: 1500–1508.

Gitzendanner, M. A. & P. S. Soltis. 2000. Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87: 783–792.

Gunnarsson, U. 2004. Populations of *Sphagnum angermanicum* in Sweden: distribution, habitat requirements and threats. Lindbergia 29: 129–135.

———, H. Rydin & H. Sjörs. 2000. Diversity and pH changes after 50 years on the boreal mire Skattlösbergs Stormosse, Central Sweden. Journal of Vegetation Science 11: 277–286.

Hallingbäck, T. 1998. *Sphagnum angermanicum*, pp. 288–289. *In* T. Hallingbäck (ed), Rödlistade mossor i Sverige—Artfakta [Swedish red data book of bryophytes]. ArtDatabanken, SLU, Uppsala.

Hamrick, J. L. & M. J. W. Godt. 1989. Allozyme diversity in plant species, pp. 43–63. *In* A. D. H. Brown, M. T. Clegg, A. L. Kahler & B. S. Weir (eds.), Plant Population Genetics, Breeding and Genetic Variation in Plants. Sinauer Associates Inc., Sunderland, MA.

Hassel, K. & U. Gunnarsson. 2003. The use of inter

simple sequence repeats (ISSR) in bryophyte population studies. Lindbergia 28: 152–157.

HEWITT, G. M. 2000. The genetic legacy of the Quaternary ice age. Nature 405: 907–913.

HUTCHISON, D. W. & A. R. TEMPLETON. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influence of gene flow and drift on the distribution of genetic variability. Evolution 53: 1898–1914.

JÓHANNSSON, B. 1992. Íslenskir mosar Klukkumosaætt, dægurmosaætt og fleira. Fjölrit Náttúrufrædistofnunar 22: 27–29.

KIMMERER, R. W. 1994. Ecological consequences of sexual versus asexual reproduction in *Dicranum flagellare* and *Tetraphis pellucida*. THE BRYOLOGIST 97: 20–25.

KUHRY, P. 1997. The palaeoecology of a treed bog in western boreal Canada: A study based on microfossils, macrofossils and physico-chemical properties. Review of Palaeobotany and Palynology 96: 183–224.

———, B. J. NICHOLSON, L. D. GIGNAC, D. H. VITT & S. E. BAYLEY. 1993. Development of *Sphagnum* dominated peatlands in boreal continental Canada. Canadian Journal of Botany 71: 10–22.

KULLMAN, L. 2002. Boreal tree taxa in the central Scandes during the Late-Glacial: implications for Late-Quarternary forest history. Journal of Biogeography 29: 1117–1124.

LAKKA-LINDBERG, S. 2001. Biomass allocation to sexual and asexual reproduction in a leafy hepatic *Lophozia silvicola* Buch. Journal of Bryology 23: 3–8.

LONGTON, R. E. & R. M. SCHUSTER. 1983. Reproductive ecology, pp. 386–462. *In* R. M. Schuster (ed.), New Manual of Bryology. Hattori Botanical Laboratory, Nichinan.

LÖNN, M. & H. C. PRENTICE. 2002. Gene diversity and demographic turnover in central and peripheral populations of the perennial herb *Gypsophila fastigata*. Oikos 99: 489–498.

LINHART, Y. B. & A. C. PREMOLI. 1994. Genetic variation in central and disjunct populations of *Lilium parryi*. Canadian Journal of Botany 72: 79–85.

LUIKART, G., J.-M. ALLENDORF, J.-M. CORNUET & W. B. SHERWIN. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89: 238–247.

MAASS, W. S. G. 1966. Studies on the taxonomy and distribution of *Sphagnum* I. *Sphagnum pylaesii* and *Sphagnum angermanicum* in Quebec and some phytogeographic considerations. THE BRYOLOGIST 65: 97–100.

———. 1967. Studies on the taxonomy and distribution of Sphagnum, II *Sphagnum angermanicum* Melin in North America and its relation to allied species. Nova Hedwigia 13: 449–467.

MELIN, E. 1919. *Sphagnum angermanicum* n.sp. Svensk Botanisk Tidskrift 13: 21–25.

NATCHEVA, R. & N. CRONBERG. 2003. Genetic diversity in populations of *Sphagnum capillifolium* from the mountains of Bulgaria, and their possible refugial role. Journal of Bryology 25: 91–99.

NEI, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, NY.

——— & D. GRAUR. 1984. Extent of protein polymorphism and the neutral theory. Evolutionary Biology 17: 73–118.

NEWTON, A. C., T. R. ALLNUTT, A. C. M. GILLIES, A. J. LOWE & R. A. ENNOS. 1999. Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends in Ecology and Evolution 14: 140–145.

PHILLIPS, T. & N. G. MILLER. 2001. A new inland site in New York State for *Sphagnum angermanicum*. EVANSIA 18: 21–22.

PROBER, S. M. & A. H. D. BROWN. 1994. Conservation of the grassy white box woodlands: Population genetics and fragmentation of *Eucalyptus albens*. Conservation Biology 8: 1003–1013.

REED, D. H. & R. FRANKHAM. 2001. How closely correlated are molecular and quantitative measures of genetic diversity: a meta-analysis. Evolution 55: 1095–1103.

ROHLF, F. J. 1974. Algorithm 76. Hierarchial clustering using the minimum spanning tree. The Computer Journal 16: 93–95.

ROUSSET, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145: 1219–1228.

RYBNÍCEK, K. 1973. A comparison of the present and past mire communities of central Europe, pp. 237–261. *In* H. J. B. Birks & R. G. West (eds.), The Quarternary Plant Ecology. 14th Symposium of the British Ecological Society, University of Cambridge, Blackwell, London.

SAMPSON, J. F., S. D. HOPPER & S. H. JAMES. 1989. The mating system and population genetic structure in a bird-pollinated mallee, *Eucalyptus rhodantha*. Heredity 63: 383–393.

SCHNIEDER, S., D. ROESSLI & L. EXCOFFIER. 2000. Arlequin: A software for population genetics data analysis. Genetics and Biometry Lab, Department of Anthropology, University of Geneva, Geneva.

SJÖRS, H. & U. GUNNARSSON. 2002. Calcium estimations and pH in northern and central Swedish mire waters. Journal of Ecology 90: 650–657.

STENØIEN, H. K. & K. I. FLATBERG. 2000. Genetic variability in the rare Norwegian peat moss *Sphagnum troendelagicum*. THE BRYOLOGIST 103: 794–801.

——— & S. M. SÅSTAD. 1999. Genetic structure in three haploid peat mosses (*Sphagnum*). Heredity 82: 391–400.

TABERLET, P., L. FUMAGALLI & J. HAUSSER. 1994. Chromosomal versus mitochondrial DNA evolution: tracking the evolutionary history of southwestern European populations of the *Sorex araneus* group (Mammalia, Insectivora). Evolution 48: 623–636.

TAHVANAINEN, T. & T. TUOMAALA. 2003. The reliability of mire water pH measurements.–A standard sampling protocol and implications to ecological theory. Wetlands 23: 701–708.

THINGSGAARD, K. 2001. Population structure and genetic diversity of the amphiatlantic haploid peatmoss *Sphagnum affine* (Sphagnopsida). Heridity 87: 485–496.

TOLONEN, K. 1967. Über die Entwicklung der Moore im finnischen Nordkarelien. Annales Botanici Fennci 4: 219–416.

TRAVIS, S. E., J. MANCHINSKI & P. KEIM. 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant using AFLP markers. Molecular Ecology 5: 735–745.

TREU, R., D. S. HOLMES, B. M. SMITH, D. ASTLEY, M. A. T. JOHNSON & L. J. TREUMAN. 2001. *Allium ampeloprasum* var. *babingtonii* (Alliaceae): an isoclonal plant found across a range of habitats in SW England. Plant Ecology 155: 229–235.

VAN DER VELDE, M., H. J. VAN DER STRATE, L. VAN DE ZANDE & R. BIJLSMA. 2000. Isolation and character-

ization of microsatellites in the moss species *Polytrichum formosum*. Molecular Ecology 9: 1678–1680.

VAN TREUREN, R., R. BIJLSMA, W. VAN DELDEN & N. J. OUBORG. 1991. The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. Heredity 66: 181–189.

VAN ZANTEN, B. O. & T. PÓCS. 1981. Distribution and dispersal of bryophytes. Advances in Bryology 1: 479–562.

VITT, D. H. 2000. Peatlands: ecosystems dominated by bryophytes, pp. 312–343. *In* J. Shaw & B. Goffinet (eds.), Bryophyte Biology. 2000. Cambridge University Press, Cambridge.

——— & P. KUHRY. 1992. Changes in moss-dominated wetland ecosystems, pp. 178–210. *In* J. W. Bates & A. M. Farmer (eds.), Bryophytes and Lichens in a Changing Environment. Oxford Science Publications, Oxford.

WIDMER, A. & C. LEXER. 2001. Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. Trends in Ecology & Evolution 16: 267–269.

ms. received Nov. 29, 2004; accepted Feb. 1, 2005.