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GENETIC VARIATION AND STRUCTURE IN THE EXPANDING MOSS *POGONATUM DENTATUM* (POLYTRICHACEAE) IN ITS AREA OF ORIGIN AND IN A RECENTLY COLONIZED AREA[1]

KRISTIAN HASSEL,[2,4] SIGURD M. SÅSTAD,[2] URBAN GUNNARSSON,[3] AND LARS SÖDERSTRÖM[3]

[2]Museum of Natural History and Archaeology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway; and
[3]Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway

Genetic variation in the expanding moss species *Pogonatum dentatum* was studied using intersimple sequence repeat (ISSR) markers. The genetic consequences of range expansion were studied by comparing source populations in a mountain area with populations from a recently colonized lowland area in Sweden. Indices of genetic variation show slightly lower number of alleles per locus in the lowlands and a similar gene diversity in both areas. Three of four lowland populations had evidence of a recently passed bottleneck. Considerably higher haplotype diversity was found in the recently colonized lowlands compared to source populations in the mountains. Patterns of allelic diversity suggest that *P. dentatum* experiences loss of genetic variation through founder effects and genetic drift when expanding its distribution range. Higher haplotypic diversity, less linkage disequilibrium, and fewer compatible loci indicate that sexual recombination is relatively more important in the lowlands compared to the mountains. A likely explanation is higher success of establishment from spores in the lowlands, while clonal propagation predominates in the mountains. Less genetic differentiation among lowland populations indicates more gene flow in the lowland area, involving more spores and/or fragments moving among populations.

Key words: bottleneck; bryophyte; clonal plant; gene flow; inter-simple sequence repeats (ISSR); invasive species; recombination; Sweden.

Genetic diversity of species that are expanding their ranges, and of invasive species in particular, has been given increased attention during the last decade due to easier access to molecular methods (e.g., Stiller and Denton, 1995; Tsutsui et al., 2000; Marston and Villalard-Bohnsack, 2002). This has increased the knowledge of genetic consequences of range expansions. Population bottlenecks through founder effects are thought to be important in reducing genetic diversity in populations of expanding species (Amsellem et al., 2000; Le Page et al., 2000), but exceptions exist. Holland (2001) found no evidence of founder effects in invasive populations of brown mussel (*Perna perna* L.), and Berg et al. (2002) found that the effects of founder events measured as heterozygosity became undetectable within 7 years in the crustacean *Bythotrephes longimanus* Leydig. The success of a species expanding its range does not seem to depend on extensive genetic variation (Marston and Villalard-Bohnsack, 2002; Xu et al., 2003), although the level of genetic variation should have consequences for evolutionary potential and adaptation by natural selection to new environments.

Life-history characteristics may affect the genetic diversity and structure in a species expanding its range. Species characteristics such as generation time, fecundity, reproductive system, and mode of dispersal will influence levels of genetic variation and, hence, the ability to respond evolutionarily to environmental change. Dispersal potential (i.e., number, size and transportability of diaspores) and frequency of sexual reproduction is likely to affect the degree of genetic variation and structure. Populations with restricted dispersal ability, producing only large asexual diaspores, should have lower levels of genetic differentiation and haplotype diversity compared to outcrossing populations with small dispersible diaspores (Baatout et al., 1991; Treu et al., 2001). Many invasive plants undergo evolutionary shifts to more selfing (cf. Amsellem et al., 2001) or asexual reproduction (Pellegrin and Hauber, 1999), which may reduce genetic variation in newly founded populations. Species expanding their range often have good dispersal ability, and a high degree of gene flow is expected (Sakai et al., 2001). This could lead to maintenance of genetic variation in the expanding populations and little differentiation among populations.

Bryophytes are unique among land plants in that the haploid gametophyte is the longer-lived, independent, and more photosynthetically active generation. *Pogonatum dentatum* (Brid.) Brid. (Bryophyta: Polytrichaceae) is a dioicous moss that commonly reproduces sexually. Originally, *P. dentatum* was mainly restricted to arctic and alpine areas in Fennoscandia (Vaarama, 1967; Hedenäs, 1983). With the industrialization of forestry and extensive building of forest roads during the 20th century, *P. dentatum* started to colonize lowland localities. Now it is regularly found on disturbed soil in the lowlands east and south of its formerly known distribution area (Hassel, 2000).

Comparison of the genetic composition of recently established populations with populations in the original range of an expanding species can provide valuable information about evolutionary processes and temporal and spatial patterns of species range expansions (Sakai et al., 2001). This study investigates genetic diversity and structure in the expanding

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[4] Author for correspondence (e-mail: kristian.hassel@vm.ntnu.no)

moss species, *P. dentatum*, in its original range and in a recently colonized area in northern Sweden using ISSR (intersimple sequence repeats) as molecular markers. Comparing the genetic composition in the two areas seeks to describe the pattern of molecular genetic variation, and to obtain knowledge about the processes shaping these patterns.

Two alternative hypotheses mainly differing in the underlying dispersal mechanisms can be formulated. Hypothesis 1: Expansion of *P. dentatum* is a result of easily dispersed and sexually produced spores. Prediction 1.1: Effective spore dispersal and establishment will result in high haplotype diversity and spore establishment is found to be common in the lowlands while rare in the mountains (Hassel and Söderström, 2003). Prediction 1.2: Little genetic differentiation will take place among populations, due to effective dispersal. Hypothesis 2: The expansion of *P. dentatum* is a result of occasional dispersal of vegetative fragments by, e.g., cars or other human activities. Prediction 2.1: Restricted dispersal will keep haplotype diversity low for a long time after initial establishment. Prediction 2.2: Genetic differentiation will take place among populations due to low gene flow (restricted dispersal). In addition, low levels of genetic variation are expected within populations due to local recruitment by locally dispersed asexual fragments.

If sexual reproduced spores are the main agent of dispersal, no linkage among loci is expected. In contrast, high linkage (linkage disequilibrium) is expected if asexual reproduction by fragments is dominating. For both scenarios of dispersal, we predict that newly established populations would experience a bottleneck with loss of rare alleles.

MATERIALS AND METHODS

Study species—*Pogonatum dentatum* has acrocarpous growth and is frequently recorded with sporophytes in both mountain and lowland areas. Spores are small (17–22 μm diameter), produced in large quantities (200 000–450 000 per capsule), and are potentially important in long-distance dispersal (Hassel and Söderström, 2003). It lacks specialized asexual diaspores, but fragmentation is potentially important for local dispersal. Asexual reproduction by fragmentation may take place from a single leaf or larger fragments like part of a stem (Hassel and Söderström, 2003). Vegetative reproduction by rhizoid wicks such as known for *Polytrichum* species (Wiggelsworth, 1947) has been searched for in *P. dentatum*, but not found (K. Hassel, unpublished). Establishment from spores and fragments is common in the lowland area, but seems to be less frequent in the mountain area (Hassel and Söderström, 1999, 2003). In the study area, *P. dentatum* typically occurs on disturbed mineral soil. In the mountains, the main disturbance factors are frost heaving and wind, whereas in the lowlands road construction and maintenance are the most common causes of soil disturbances that lead to creation of new habitats.

Study area—Populations were sampled from two areas in northern Sweden (Fig. 1) in August 2001. The mountain area is in the alpine region at Stekenjokk (65°05′N, 14°30′E; 800 m a.s.l.) on a mountain heath above the tree limit. The lowland area is in the boreal region at Junsele (63°45′N, 17°15′E, altitude 300 m a.s.l.) in spruce forest. The mountain and lowland areas are part of the same valley and river system, Ångermanälven. If range expansion takes place by stepwise dispersal, this would be a natural colonization route, because expansion of forestry activity followed the valley system toward the mountains.

Sampling and DNA analysis—Four populations of *P. dentatum* were sampled from both the mountain and lowland areas (Table 1). A population was delimited as a group of patches of *P. dentatum* shoots occurring at a restricted site. In each of the eight populations, a transect was placed from the population edge towards its centre. Along each transect, five patches (cluster of shoots that consist of one or more individuals/genets) separated by 2–2.5 m were sampled. From each patch, five shoots (gametophores/ramets) were sampled from a quadrat (10 × 10 cm), one shoot from each corner and one from the center. Shoots were put in paper bags, air dried, and stored at 4°C until analyzed.

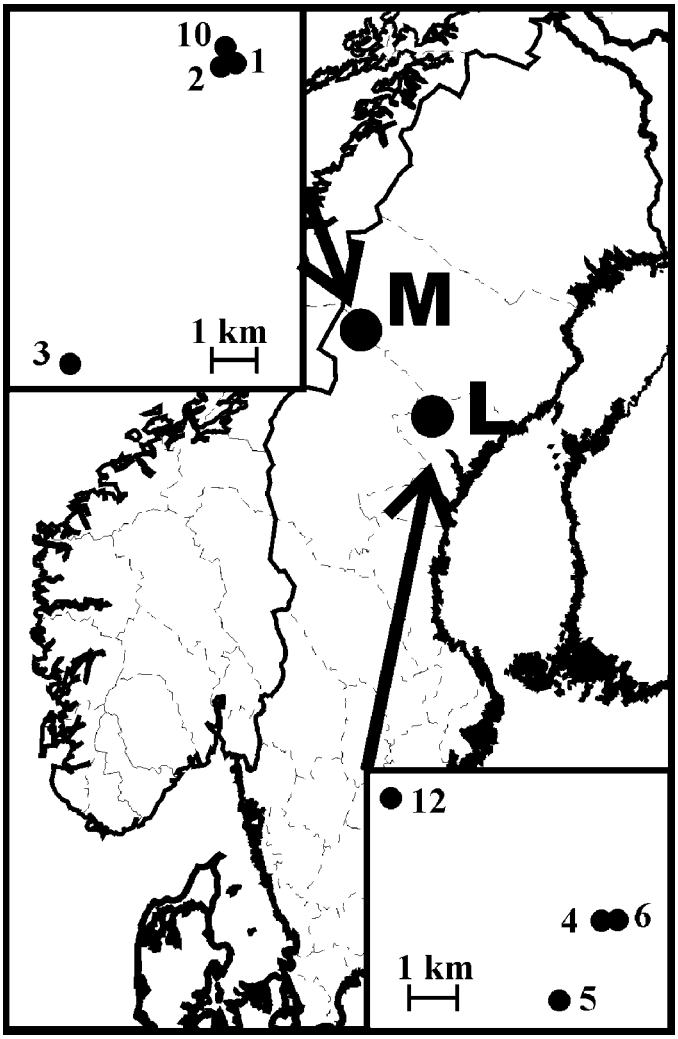

Fig. 1. Map of Scandinavia indicating the Swedish study sites in the lowland area (L) and mountain area (M). The small maps indicate the distance between the studied populations (1–6, 10, 12) within each area.

ISSR markers where chosen because they are easy and inexpensive to apply and have been successfully used in population studies of vascular plants (e.g., Wolfe et al., 1998; Yannic et al., 2004) and bryophytes (e.g., Korpelainen and Virtanen, 2003; Vanderpoorten et al., 2003; Gunnarsson et al., 2005). The shoots were washed in sterile water before DNA extraction. DNA extraction, PCR setup, and visualization of PCR products followed Hassel and Gunnarsson (2003). The intersimple sequence repeat (ISSR) primers, annealing temperature, and the number of scoreable fragments per primer are shown in Table 2. Bands were scored manually, and a table of presence/absence of ISSR fragments was compiled.

Data analysis—Assessment of genetic variation from sampling of ramets in clonal organisms is problematic. Estimating allele frequencies at the ramet level runs the risk of pseudoreplication because some haplotypes may be represented more than once in the population, whereas in genet-level calculations different genets with identical multilocus haplotypes cannot be distin-

TABLE 1. Area occupied by a population (Pop. area), the size of the largest patch in a population (Max. patch size), and the total number of patches constituting a population of *Pogonatum dentatum* in the mountains (M) and lowlands (L) of northern Sweden.

Population	Latitude	Longitude	Altitude (m)	Pop. area	Max. patch size	No. of patches
M1 Stekenjokk	65°05′55″ N	14°27′35″ E	822	18 × 12 m	40 × 40 cm	12
M2 Stekenjokk	65°05′47″ N	14°27′24″ E	814	36 × 15 m	150 × 150 cm	20+
M3 Gervenåkko	65°03′28″ N	14°21′56″ E	868	16 × 4 m	40 × 40 cm	8
M10 Stekenjokk	65°06′02″ N	14°27′33″ E	812	10 × 14 m	50 × 50 cm	20
L4 Tärnickberget	63°45′52″ N	17°14′25″ E	371	70 × 4 m	40 × 40 cm	20+
L5 Asptjärnen	63°44′46″ N	17°12′29″ E	290	47 × 5 m	continuous	1
L6 Tärnickberget	63°45′50″ N	17°14′07″ E	353	19 × 3 m	20 × 20 cm	15
L12 Båsberget	63°47′35″ N	17°06′53″ E	273	50 × 3 m	30 × 30 cm	20+

guished (McLellan et al., 1997). Whether identical haplotypes could have originated independently from random assortment of alleles was tested by calculating the probability of observing as many or more ramets with a particular haplotype than actually observed under a null hypothesis of free recombination (Tibayrenc et al., 1990). Whether the addition of more loci would increase haplotypic resolution was tested by generating plots of haplotype diversity against the number of loci in 100 random samples of one to 21 loci, and determining whether the relationship reached a plateau.

The number of polymorphic sites (*S*), mean gene diversity over loci (H_S; Nei, 1987), mean haplotype diversity (h_s; Nei, 1987), occurrence of shared haplotypes, and pairwise genetic distance among populations estimated by F_{ST} values were analyzed using the computer program ARLEQUIN 2.001 (Schneider et al., 2000). Allelic diversity (*A*) estimated as mean number of alleles per locus was calculated for populations and areas (corrected for sample size).

Bottlenecks generate heterozygosity excess because allelic diversity is generally lost faster than gene diversity during a bottleneck (Luikart and Cornuet, 1998). To reveal if populations had gone through a bottleneck the occurrence of rare alleles was investigated. Alleles were considered rare at two levels, if they occurred in ≤5 and ≤15% of the samples. Using the computer program BOTTLENECK version 1.2.02 (Luikart and Cornuet, 1999), a sign test for revealing populations that recently have passed through a bottleneck from allele frequency data at polymorphic loci under assumption of the stepwise mutation model (SMM) and the infinite allele model (IAM) was performed. To be statistically conservative, one should only use the SMM when analyzing microsatellite data, but because the true model of mutation for most loci is intermediate between IAM and SMM, using both models is recommended (Luikart and Cornuet, 1998). ISSRs and microsatellites are of similar origin (simple sequence repeats, Godwin et al., 1997) and should follow the same mutation model.

Linkage disequilibrium and character compatibility analyses were performed to assess whether marker distributions originated from sexual or asexual reproduction in the different populations. Multilocus linkage disequilibrium was inferred using the index of association modified to remove the dependency of sample size (r_d; Agapow and Burt, 2001). Calculation of statistics and tests of significance by randomization were performed with the program Multilocus v1.2 (http://www.bio.ic.uk/evolve/software/multilocus).

Compatibility methods search for the largest set of mutually compatible characters. For a pair of bi-allelic markers, this means that no more than three of the four possible genotypes should be present. Asexual lineages are assumed to accumulate unique somatic mutations arranging the genets in a tree-like structure. Therefore, none or a small fraction only (due to parallel mutations) of the loci, are expected to be incompatible. The sum of incompatible loci over all pairwise comparisons (matrix incompatibility, Wilkinson, 2001), can hence be used as a measure of recombination (Mes, 1998; van der Hulst et al., 2000). Matrix incompatibility and the observed conflict compared to the conflict in randomly permuted data (incompatibility excess ratio, Wilkinson, 2001) in all populations were estimated.

To investigate the genetic structure an analysis of molecular variance (AMOVA) of the ISSR data (analogous to RFLP data), ARLEQUIN 2.001 (Schneider et al., 2000) was used to examine genetic variation at area, population, and patch levels for both ramets and genets. The level of genetic differentiation was measured by F_{CT}, F_{SC}, and F_{ST}, that refer to distance among groups, among subgroups within groups and within subgroups, respectively (see Table 3). The data were analyzed in a hierarchical manner to estimate variance components at the different spatial scales.

RESULTS

PCR products corresponding to 39 alleles at 21 loci, amplified by the four primers were scored in 194 shoots. Eighteen loci were polymorphic and a total of 64 haplotypes could be distinguished. The number of ramets of the most common haplotype in all populations was significantly higher from what we would expect if the populations reproduced solely sexually i.e., free recombination (P_{hap}, Table 3). This suggests that predominant haplotypes are asexually produced and that some genets are sampled more than once. The addition of more loci increased the haplotypic resolution in the lowlands, while resolution seemed to be satisfactory in the mountains where the number of haplotypes reaches a maximum (Fig. 2). Thus, haplotype diversity in the lowlands should be considered as a minimum estimate. Results from analyses at the genet level are presented next. However, results from the ramet level are included in Table 3 for comparison.

Haplotype diversity was higher in the lowlands than in the mountains (mean of 11.5 and 6.0 haplotypes, respectively; N_g, Table 3). Mean percentage of polymorphic loci (P_p) was highest in the lowland populations (39 vs. 32% in the mountains).

TABLE 2. Primers used in the intersimple sequence repeat (ISSR) analysis of *Pogonatum dentatum*, the number of scored and polymorphic loci, and the primer-specific annealing temperature.

Primer name	Primer sequence (5′-3′)	No. of loci	No. of polymorphic loci	Annealing temp. (°C)
UBC-811	GAG AGA GAG AGA GAG AC	7	6	50
UBC-825	ACA CAC ACA CAC ACA CT	2	2	48
UBC-841	GAG AGA GAG AGA GAG AYC	3	2	52
UBC-888	BDB CAC ACA CAC ACA CA	9	8	52

Note: Y = C, T; B = C, G, T; D = A, G, T.

TABLE 3. Genetic variability within populations of *Pogonatum dentatum* in the mountains (M) and lowlands (L) of northern Sweden, detected by intersimple sequence repeat (ISSR) analysis.

Population	N_r/N_g	S	P_p	A_r/A_g	$h_s \pm$ SD	$H_{Sr} \pm$ SD/$H_{Sg} \pm$ SD	$\bar{r}_d$	IER	P_{hap}
M1	25/6	8	38.1	1.44/1.66	0.833 ± 0.034	0.151 ± 0.090/0.181 ± 0.122	0.040^{ns}	0.339^{ns}	8.2×10^{-6}
M2	25/5	4	19.0	1.24/1.49	0.300 ± 0.118	0.038 ± 0.032/0.095 ± 0.075	0.056^{ns}	ND	0.0176
M3	25/5	9	42.9	1.49/1.79	0.690 ± 0.056	0.158 ± 0.093/0.219 ± 0.151	0.016^{ns}	0.162^{ns}	6.2×10^{-13}
M10	25/8	6	28.6	1.34/1.47	0.757 ± 0.060	0.120 ± 0.074/0.153 ± 0.100	0.045^{ns}	0.050^{ns}	2.8×10^{-6}
L4	25/12	8	38.1	1.44/1.51	0.910 ± 0.032	0.148 ± 0.089/0.154 ± 0.096	0.023^{ns}	0.001^{ns}	1.1×10^{-9}
L5	23/12	10	47.6	1.54/1.61	0.909 ± 0.036	0.186 ± 0.108/0.187 ± 0.113	0.033^{ns}	0.174^{ns}	1.5×10^{-7}
L6	25/11	9	42.9	1.49/1.57	0.907 ± 0.032	0.157 ± 0.093/0.168 ± 0.104	0.010^{ns}	0.034^{ns}	0.0028
L12	21/11	6	28.6	1.35/1.41	0.891 ± 0.045	0.138 ± 0.084/0.145 ± 0.092	0.042^{ns}	0.058^{ns}	0.0001

Note: N_r/N_g = number of sampled ramets and genets (haplotypes), respectively; S = number of polymorphic sites; P_p = percentage of polymorphic loci; A_r/A_g = mean number of alleles per locus (corrected for sample size) at the ramet and genet level, respectively; h_s = mean haplotype diversity; H_{Sr}/H_{Sg} = mean gene diversity at the ramet and genet level, respectively; $\bar{r}_d$ = multilocus linkage disequilibrium; IER = incompatibility excess ratio and P_{hap} = the probability of observing as many ramets of the most common haplotype as actually observed in a population in a sexually reproducing population; L and M = lowland and mountain populations, respectively; ND = no data; ^{ns} = non significant.

The mean allelic richness (A_g) was higher in the mountain populations (1.53 in the lowlands vs. 1.60 in the mountains). However, there were no clear differences between the mountains and lowlands in gene diversity (H_{Sg}; Table 3). Multilocus linkage disequilibrium ($\bar{r}_d$) and the incompatibility excess ratio (IER) did not reveal significant deviations from the assumption of free recombination in the two study areas. However, there was a trend toward higher linkage and more compatible loci in the mountains relative to the lowlands (Table 3).

There were three rare alleles in both areas using the 5% criterion. The mean number of rare alleles in the populations was 0.75 and 0.50 in the mountains and lowlands, respectively. Using the 15% criterion, there were more rare alleles in the mountains (9, population mean 2.75) than in the lowlands (4, population mean 1.25). Analysis of bottlenecks showed that three of the lowland populations (4, 5 and 12) had gone through a bottleneck, while population 6 was in mutation-drift equilibrium (Table 4). In the mountain population 1 had gone through a bottleneck, while populations 2, 3, and 10 were in mutation-drift equilibrium (Table 4). These analyses where done on the ramet level; reliable analyses could not be done at the genet level due to low sample size.

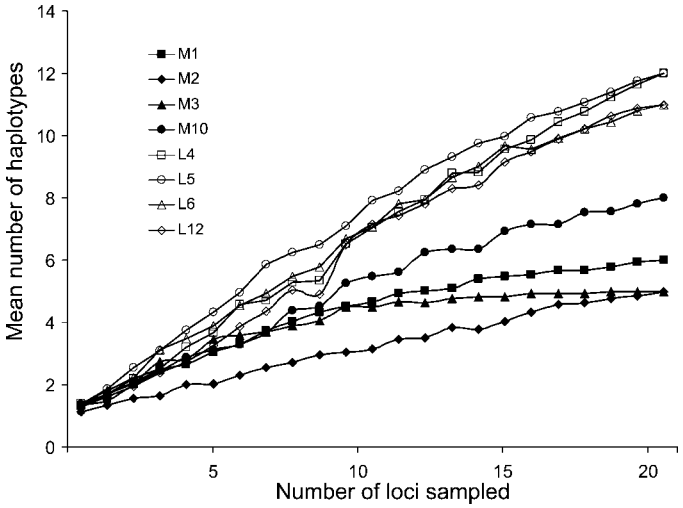

Fig. 2. Investigation of sampling scale to see if addition of more loci would increase haplotypic resolution. Plots of haplotype diversity against number of loci, in 100 random samples of 1–21 loci, were generated to see if the relationship reached a plateau. L and M refers to lowlands and mountains, respectively, numbers (1–6, 10, 12) identify populations.

Hierarchical analysis of the genetic structure showed that the proportion of total genetic variation that can be ascribed to differentiation between the mountain and lowland areas (F_{CT}) is close to zero (Table 5a). Genetic structuring appears to take place among populations within each area (F_{SC} = 0.229) and within populations (F_{ST} = 0.223). About 80% of the total genetic variation was due to variation within populations and ca. 20% was due to variation among populations within the two areas. Separate analyses showed clear differences between the mountains and lowland areas with regard to the partitioning of genetic variation among populations. In the mountains ca. 30% of the genetic variation was among populations in contrast to ca. 15% in the lowlands (Table 5b). Genetic distances were greater among populations in the mountains than among the lowland populations and among the mountain vs. lowland populations (F_{ST}, Fig. 3).

Two haplotypes occurred both in the mountain and lowland areas, but no haplotypes were shared among the mountain populations. The lowland population L4 shared three haplotypes with population L5, and population L6 shared one haplotype with L12. In the mountain populations edge patches shared one or more haplotypes with the interior patches. In contrast, no haplotypes were shared between the edge patch and interior patches in the lowland populations. In fact, the same haplotypes occurred in more than one patch only within two of the

TABLE 4. Results from bottleneck analysis of population of *Pogonatum dentatum* in the mountains (M) and lowlands (L) of northern Sweden.

Population	N	H_e/H_d	Sign test	
			SMM	IAM
M1	25	7/1	0.033	0.019
M2	25	1/3	0.075	0.393
M3	25	7/2	0.233	0.055
M10	25	4/2	0.337	0.271
L4	25	7/1	0.031	0.023
L5	23	8/2	0.055	0.023
L6	25	6/3	0.227	0.178
L12	21	6/0	0.023	0.006

Note: P values are shown for a sign test under the stepwise mutation model (SMM) and the infinite allele model (IAM). N = no. of ramets sampled per polymorphic locus, H_e/H_d = the heterozygosity excess/deficiency ratio.

TABLE 5. (A) Analysis of molecular variance (AMOVA) of *Pogonatum dentatum*, at the genet level, sampled from four populations in each area and five patches from each population. L and M denote lowland and mountain areas, respectively. (B) Separate analysis of the mountain and lowland areas.

Source of variation	df	Variance component	Variance (%)	Fixation index
A) Between areas (L & M)	1	−0.020	−0.91	$F_{CT} = -0.009^{ns}$
Among populations within areas	6	0.511	23.17	$F_{SC} = 0.229^{***}$
Within populations	62	1.714	77.74	$F_{ST} = 0.223^{***}$
Total	69	2.205		
B) Among M populations	3	0.669	30.78	$F_{CT} = 0.308^{***}$
Among M patches within M populations	16	−0.136	−6.24	$F_{SC} = -0.090^{ns}$
Within M patches	14	1.639	75.46	$F_{ST} = 0.245^{*}$
Total	33	2.172		
Among L populations	3	0.385	17.47	$F_{CT} = 0.175^{***}$
Among L patches within L populations	16	0.383	17.38	$F_{SC} = 0.210^{***}$
Within L patches	29	1.436	65.15	$F_{ST} = 0.349^{***}$
Total	48	2.204		

Note: F_{CT} = variation among groups divided by total variation, F_{SC} = variation among sub-groups within groups divided by the sum of variation among sub-groups within groups and variation within sub-groups, F_{ST} = the sum of variation among groups and variation among sub-groups within groups divided by total variation, ns = $P > 0.05$, * = $P \leq 0.05$, *** = $P \leq 0.001$.

four lowland populations (two shared haplotypes between two patches in population L4 and one shared haplotype between two patches in population L6).

DISCUSSION

Genetic diversity—The lower allelic diversity (number of polymorphic loci) in the lowlands compared to the mountains could be explained by loss of rare alleles during colonization events (Widmer and Lexer, 2001). Moreover, founder effects is supported by the bottleneck analyses. No decrease in gene diversity (expected heterozygosity), but reduced allelic diversity in the recently colonized lowland area matches the pattern found by Comps et al. (2001). They studied postglacial colonization of the tree *Fagus sylvatica* L. and found decreased allelic richness and even increased gene diversity towards the north (more recently colonized areas). Studies of postglacial colonization of bryophytes have, however, found both reduced allelic richness and gene diversity towards the northern parts of the distribution (Cronberg, 2000; Thingsgaard, 2001). A possible explanation for the different patterns of gene diversity is differences in dispersal potential. Species with effectively dispersed diaspores or gametes might be expected to show high gene diversity throughout their range, due to admixture and frequent sexual reproduction. Allelic richness will be (periodically) reduced by colonization events. In contrast, species restricted to short-distance dispersal (step-by-step) would experience series of population bottlenecks and genetic drift resulting in both decreased allelic richness and gene diversity, unless they experience secondary contact (admixture) with populations from other areas.

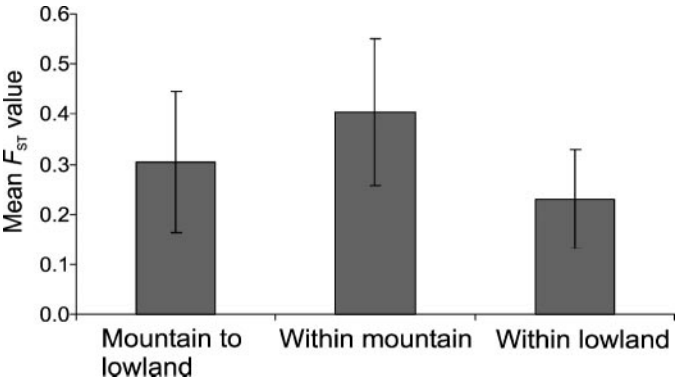

Fig. 3. Genetic distance (± 1 SD) among populations between areas, within the mountain area, and within the lowland area, based on pairwise F_{ST} values among populations. Mean geographical distance among populations: mountain to lowland, 197.8 km; within mountain, 3.3 km; and within lowland, 4.3 km.

The lower haplotype diversity and higher percentage of shared haplotypes among mountain patches suggests that asexual reproduction is more prevalent within the mountain populations. All edge patches in the mountains share one or more haplotypes with the interior patches, and recruitment by fragmentation is a likely explanation. Production of spores is high in both areas, but Hassel and Söderström (1999, 2003) showed in spore germination experiments that successful establishment of sexually produced spores is most common in the lowlands. Higher linkage disequilibrium and more compatible loci in the mountain populations further suggests relatively more frequent recombination events in the lowlands compared to the mountains. In addition to being more favorable for spore germination per se, the lowlands probably have more available substrate with a more continuous distribution (roads), increasing the chance of finding a suitable site for establishment. Although three of four populations in the lowlands and one population in the mountains showed evidence of recent bottlenecks, frequent recombination combined with gene flow may be an efficient way of removing effects of founder events (Berg et al., 2002). Because production of sporophytes is common in both areas, the limiting factor seems to be spore establishment and not gamete dispersal or gametangium and sporophyte production. The process of removing founder effects may thus take less time in the lowlands.

Genetic structure—No genetic differentiation was found between the lowland and mountain areas. This is consistent with some other studies of bryophytes that have found very little differentiation between even widely separated areas (e.g., Såstad et al., 2000; Thingsgaard, 2001; van der Velde et al., 2001; Gunnarsson et al., 2005). Stenøien and Såstad (1999) argued that lack of differentiation among populations, even between intercontinental disjuncts, may be due to large effec-

tive population sizes and little genetic drift, and hence retention of ancestral polymorphisms rather than recurrent gene flow. Derda and Wyatt (1999a, b, 2003), however, found a high degree of differentiation between regions in four *Polytrichum* species, so there seems to be no general pattern. Based on the historic expansion of *P. dentatum*, it is reasonable to assume that a high proportion of the diaspore flow is from mountains to the lowlands or within the lowlands in this species. This would potentially involve many founding events in the lowlands, and we could thus expect some degree of differentiation between the mountain and lowland areas because of genetic drift (cf. Barrett and Husband, 1999). The reason that genetic differentiation is not observed may be due to both high rates of gene flow and the short time since range expansion.

Genetic structuring among populations is most prominent in the mountain populations. This fits our interpretation, with more gene flow in the lowlands due to more frequent establishment from spores. When a ''window'' for diaspore establishment opens, diaspores of different origin must arrive. Whereas this ''window'' is small in the mountains it is probably larger in the lowlands in the form of forest roads. Recruitment in the mountains may instead take place mainly via asexually produced diaspores (e.g., Longton and Schuster, 1983; Kimmerer, 1994), which would lead to low haplotype variation within populations as seem to be the case in the mountains. Our results from the lowlands seems to agree with van der Velde et al. (2001) who found that sexual reproduction is the most important determinant of genetic structure in *Polytrichum formosum* Hedw. However, effective dispersal and successful establishment from spores seems to vary between species in the Polytrichaceae as indicated by the low variation within populations in *P. commune* Hedw., *P. juniperinum* Hedw., and *P. piliferum* Hedw. (Derda and Wyatt, 1999a, b, 2003).

The recent range expansion of *P. dentatum* therefore seems to have been facilitated by effective spore dispersal and extensive areas with suitable substrate in the lowlands. Both historical factors such as the extensive building of forest roads and the predominant establishment from spores in the lowlands vs. clonal propagation in the mountains, seem to be responsible for the observed differences in genetic variation and structure between the mountain and lowland areas.

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