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ISLAND POPULATION STRUCTURE OF NORWAY SPRUCE (*PICEA ABIES*) IN NORTHERN SWEDEN

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Picea abies, which is predominantly sexual, has been reported to propagate vegetatively through layering in a cold harsh climate, although this has not been demonstrated genetically. Using 105 amplified fragment length polymorphism markers, we analyzed 117 trees of Norway spruce from seven islands in Lake Hornavan in Lapland, northern Sweden. These islands differ in size, time since last wildfire disturbance, and vegetation successional age. A total of 96 distinct genotypes were identified among the 117 samples, and the average gene diversity was 0.37. Genetic differentiation among islands was high, $F_{st} = 0.19$. Drift, founder effect, small population size, infrequent sexual reproduction, and low seedling establishment would have contributed to the high F_{st}. Layering was found on five of the seven islands, giving an average clonal composition of 18%. A total of 11 clones, each consisting of two to five closely clustered ramets, were detected. Interestingly, layering was more common on the small islands that have not been disturbed by fire for over 1000 yr than on the larger islands with more recent fire disturbance. Our results indicate that island size and ecological conditions related to fire disturbance history on each island are important for the observed patterns of population structure.

Keywords: AFLP, clones, fire disturbance, genetic structure, island size, regeneration.

Introduction

Norway spruce, *Picea abies* (L.) Karst, is a dominant forest tree species in Sweden. It occurs widely throughout Europe, Scandinavia, and Siberia (Schmidt-Vogt 1978), and it regenerates through sexual reproduction. Climatic conditions play a crucial role in the normal functioning of the 2-yr sexual reproductive cycle of Norway spruce. Strobili bud formation is initiated at the end of growth season. Meiosis, pollen, and female strobili development take place the following early spring, followed by pollination and fertilization. The seeds continue to develop after winter and mature early next autumn. Moderate to high temperatures along with dry conditions during growth season for 2 successive years are essential for strobili initiation, optimum pollination, successful fertilization, and seeds maturation. Toward the northern distribution limit of Norway spruce, the climatic conditions suitable for successful sexual reproduction seldom occur, resulting in low seed production. In Scandinavia, good seed years can be expected only every 10–13 yr (Andersson 1965; Hagner 1965; Sarvas 1968). In alpine areas along the Scandes, where climatic conditions constrain sexual reproduction, Norway spruce has been observed to propagate naturally by layering (Skoklefald 1993; Kullman 1996*a*). Lower branches often grow drooping toward the ground and may gradually become covered with litter and humus, after which roots start to differentiate and new shoots and new standing trees develop. This phenomenon has been termed "layering," which is also observed on primary successions along the Baltic shoreline and in several other spruce species such as black spruce (*Picea mariana* [Mill.] B.S.P.; Horton and Lees 1961; Stanek 1961). However, the clustered appearance of trees cannot be taken as proof of clonal growth, and no genetic study to date has confirmed clonal development of Norway spruce.

The mode of reproduction has significant impact on plant population genetic structure and its variability. The adaptability of tree populations in a changing environment is generally determined by genetic variability in the present and future generations. Asexual reproduction such as layering reduces the population genetic variability and thus may have particularly important effects on the population fitness in changing environments. A high degree of Norway spruce layering, if confirmed, would change our perception of the syndrome of the predominant outcrossing and random mating populations of the species in the boreal forest. Thus, a more accurate quantification of layering and characterization of genetic structure of Norway spruce in relation to its population development history have important implications for its gene conservation practices in the boreal region.

Lake Hornavan in the province of Lapland is the largest lake in northern Sweden. It formed during the early Holocene period and embraces hundreds of islands of different sizes and successional ages. Island size has a direct correlation with the frequency of fire disturbances: larger islands are affected by wildfire, arising from lightning strike, more frequently than

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smaller islands (Wardle et al. 1997). Smaller islands are dominated by typical late successional species such as Norway spruce, feathermosses and ericaceous dwarf shrubs. Therefore, the island size played a role in the present-day plant community composition and structure (Wardle et al. 1997). The vegetation history on some of the islands has previously been monitored (Bradshaw and Zackrisson 1990).

For this study, we selected seven islands of different size and successional age to investigate the clonal development and island population genetic structure of Norway spruce. In addition, Norway spruce growing in Lapland represents the northwestern margin of the species distribution. Marginal populations are interesting for genetic studies since they are exposed to severe environmental stress and their genetic structure may differ from that of the central populations.

Molecular markers have been widely used to characterize population structure and the mode of reproduction in plants, and they represent the most suitable ways of identifying genotypes and clonal ramets (Parker et al. 1998; Wang and Szmidt 2001). The advantage of amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995) is the potential to offer a high number of polymorphic loci that can be used to tag genotypes. The technique is fast and reproducible (Jones et al. 1997). Most AFLP markers segregate in Mendelian fashion and can be used for population genetic studies (Mueller and Wolfenbarger 1999; Wang and Szmidt 2001). However, in diploid tissues, most of the AFLP markers behave as dominant markers. In this study we applied AFLP markers to determine the degree of Norway spruce layering on seven boreal islands; to investigate the relationship between island size, disturbance history, and layering development; and to characterize the genetic diversity and structure of Norway spruce within and between these islands.

Material and Methods

Study Site and Sampling

We selected seven islands situated in the Lake Hornavan, which is located within the northern boreal zone of Sweden. Distance between islands is 0.4–10 km. The islands differ in their sizes and successional age (table 1). The successional age refers to the years since the last fire on each island. This was determined by accelerator mass spectrometry C^{14} dating (at the Tandem Laboratory, Uppsala, Sweden) of charcoal particles sampled from the uppermost burned (charcoal) layer in organic soils at each of the investigated sites (Zackrisson et al. 1996).

All the standing spruce trees were sampled on small islands A, B, 37, 48, and 52. Distances between trees on each island ranged from 0.5 to 30 m. On island D, two groups of trees (10 and 13), one on each end of the island and separated by 200 m, were sampled. At each end of this island, a random standing tree was taken as the starting point, and trees within 45 m were sampled. These included trees growing very close together, 1 m apart, as well as trees separated by 15 m. On the biggest island, Storön, three sections were selected, at each end and the middle part of the island, separated by 550–600 m. Within each section, a random starting point was selected and 10 trees were sampled within a distance of 40–50 m. The distances between trees within each section ranged from 1 to 25 m. A total of 117 trees were sampled from the seven islands. Needles were collected from each tree and stored at −80°C prior to DNA isolation.

AFLP Procedure

Genomic DNA of each individual tree was isolated using the DNeasy Plant Mini Kit (Qiagen, Germany). About 200 ng of genomic DNA was used as starting template for the restriction digestion, with *Eco*RI and *Mse*I. Adaptors to the digested DNA were ligated using an AFLP Core Reagent Kit (Life Technologies, U.S.A.) following the manufacturer's instruction. The resulting products were diluted 10-fold and subsequently used for preamplification with primers that match the adapters' sequences but contain an additional selective base at the 3′ end (E + A and M + C). PCR amplification was performed in a PTC-100 thermal cycler (MJ Research, U.S.A.) programmed for 20 cycles of 30 s at 94°C, 60 s at 56°C, and 60 s at 72°C. The preamplification products were diluted 30-fold and used as templates in a second-round selective amplification with primers containing three (E + 3) and four (M + 4) selective nucleotides. E + 3 primers were labeled on the 5′ end with γ^{33}P-ATP (Amersham Biosciences, Sweden). Selective PCR amplification was performed as follows: 12 cycles of 94°C for 30 s; 65°C for 30 s, lowered by 0.7°C each cycle; 72°C for 60 s; followed by 23 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s. The selectively amplified products were mixed with an equal volume of loading buffer consisting of 95% deionized formamide, 20 mM EDTA (pH 8.0), 1 mg/mL bromophenol blue and 1 mg/mL xylene cyanole FF, denatured at 95°C for 3 min and rapidly cooled on ice. We loaded 2.5 μL of each sample into 6% Long Ranger (BioWhittaker Molecular Applications,

Table 1

Description of the Islands

Island	Size (ha)	Successional age (yr)[a]	Coordinates	No. trees sampled
A	0.07	2470	66°01′47″N, 17°44′60″E	All 10
37	0.14	5150	65°56′94″N, 17°50′75″E	All 17
48	0.17	3185	65°57′39″N, 17°50′77″E	All 18
B	0.22	3360	66°01′37″N, 17°45′15″E	All 9
52	1.17	1750	65°58′06″N, 17°49′16″E	All 11
D	3	1930	65°59′83″N, 17°48′28″E	22
Storön	155	195	65°58′17″N, 17°53′64″E	30

[a] Years since last fire.

U.S.A.) polyacrylamide gel (7.0 M urea, 1 × tris-borate-EDTA buffer) and separated by electrophoresis using a Sequi-Gen GT sequencing cell (Bio-Rad, U.S.A.). Gels were prerun at a constant power of 70 W for 20 min. Electrophoresis was carried out at 70 W for 2 h 45 min–3 h. Gels were vacuum dried and exposed to Kodak Biomax MR films for 3 d at −70°C. In all AFLP procedures, negative PCR control was included to verify the minimum risk of contamination.

Twenty-four *Eco*RI and *Mse*I primer combinations were screened for their potential to generate well-separated and even intensity bands across the molecular weight classes on 10 DNA samples. Finally, two out of 24 primer-pair combinations, E-ACG/M-CCTC and E-ACC/M-CCGG, were selected for the application on all the 117 samples. The repetition of the 10 samples in the final AFLP analysis gave reproducible results. An example of the AFLP profile generated by E-ACG/M-CCTC is presented in figure 1.

Data Analysis

Fragments amplified by each primer-pair combination were scored as presence (1) or absence (0) of a band. Data were scored by two persons independently and then compared. Weak and uncertain bands were discarded. Each distinct AFLP fragment was regarded as a putative locus. In the data analysis, only the polymorphic loci were used. Allele frequencies, Nei's (1978) unbiased gene diversity (h), population differentiation (F_{st}), and Nei's unbiased genetic distances among islands were computed using the Tools for Population Genetics Analysis program (TFPGA; Miller 1997). AFLPs behave as dominant markers in diploid tissue. Thus, allele frequencies cannot be estimated directly from the AFLP phenotypes. We applied two methods of calculating allele frequencies: the square root of recessive homozygotes and Lynch and Milligan's (1994) Taylor expansion method as implemented in TFPGA.

Genotypic diversity for each island and for the total samples were quantified by a normalized Shannon's diversity index (H_s) as described in Goodwin et al. (1992):

$$H_s = -\sum P_i(\ln P_i / \ln N),$$

where P_i is the frequency of the ith multilocus genotype and N is the sample size. The values of H_s range from 0 to 1, and the maximum possible value occurs when each individual tree in a group has a different genotype. Each different multilocus genotype is regarded as a genet. Trees sharing the same genotype are regarded as ramets of the same clone. The clonal fraction, that is, the proportion of trees within a group (island) derived from clonal propagation, was calculated for each island as $(N - G)/N$, where G is the number of genets and N is the sample size.

The differences among multilocus genotypes were measured by genetic distance calculated as $D = n - n_{xy}$, where n is the total number of polymorphic fragments and n_{xy} is the number of fragments shared by two genotypes. A distance matrix was

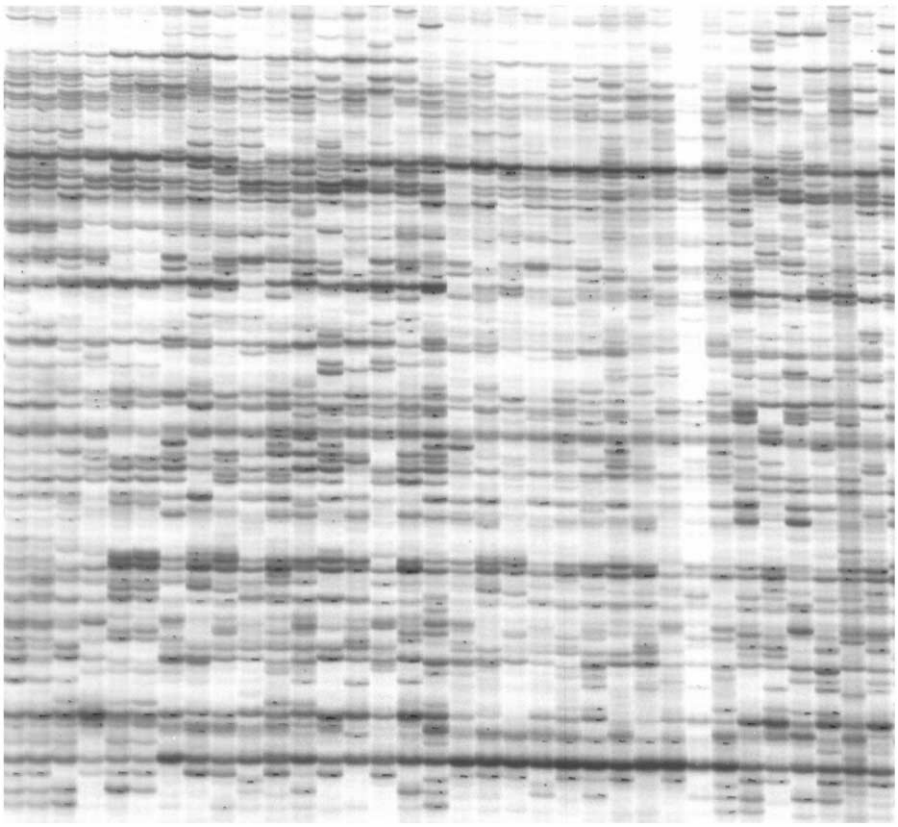

Fig. 1 AFLP profiles generated by primer combination E-ACG/M-CCTC in Norway spruce

generated for all possible pairwise comparisons among different genotypes. This distance matrix was used for Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering of the genotypes and to partition the genetic variation among and within islands using the analysis of molecular variance (AMOVA; Excoffier et al. 1992).

Results

A total of 105 distinct AFLP loci were scored for the 117 samples: 66 and 39 loci by the primer pairs E-ACG/M-CCTC and E-ACC/M-CCGG, respectively. Among the 117 samples, we detected 96 distinct 105-locus genotypes. Pairwise comparison of all multilocus genotypes revealed that majority of the genotypes were very different from each other: 98% of the genotype pairs differed by 21–49 fragments; only 0.2% differed by 1–10 fragment(s). The maximum observed difference among genotypes was 57 fragments.

Of the 96 genotypes, 85 were sampled only once and 11 were sampled more than once (table 2), of which six were sampled two times, one was sampled three times, three were sampled four times, and another one was sampled five times. Norway spruce is a predominantly outcrossing species. The probability of these 11 multilocus AFLP profiles occurring a second time by random mating is virtually nonexistent (data not shown). Thus, individuals that shared the same multilocus genotype were regarded as ramets of the same clone. On five of the seven islands (islands A, B, D, 37, and 48) genotypes shared by more than one individual trees were detected (table 2). The spatial distribution of the shared genotypes was very restricted. Ramets of the same clone were clustered in close proximity, between 1 and 8 m, on the same island. Two groups of trees were sampled from the opposite ends of island D. On this island, we detected two genets that each consisted of more than one individual tree. One of the genets had two ramets and another had four ramets. The ramets of the same clone were restricted within each section of the island; none were spread to another section. No genotypes were shared among trees from different islands. Trees from island 52 and Storön each represented a different genotype. Genotypic diversity ranged from 0.7 to 1.0 among islands, with the lowest value on the smallest island, A (table 2). In general, the small islands had higher clonal composition than the larger islands. The highest clonal fraction was found on the two smallest islands, island A and island 37, with a value of 40% and 41%, respectively (table 2). No clones were detected on the large islands Storön and 52. The overall clonal fraction of Norway spruce over all seven islands was 18% (table 2).

Two data sets were created for gene diversity estimation: data set 1 comprised all 117 samples, and data set 2 comprised one representative of each of the 96 genotypes. The two methods of calculating allele frequencies, the square root of recessive homozygotes and Lynch and Milligan's (1994) Taylor expansion method, gave very similar gene diversity and interisland genetic differentiation (F_{st}) estimates. Thus, only the results based on allele frequencies derived from Lynch and Milligan's (1994) method are presented. For data set 1, average gene diversity over the seven islands was 0.371, ranging from 0.150 to 0.375 among islands (table 2). For data set 2, slightly higher gene diversity was observed, ranging from 0.173 to 0.375. Among the seven islands, Norway spruce growing on island A (the smallest island) had the lowest gene diversity estimate; the highest was for Norway spruce growing on the biggest island, Storön (table 2). The average genetic distances from islands A and B (which had the lowest number of genotypes) to the other islands are much larger than the distances among other islands (table 3).

The partition of total gene diversity to within and among islands revealed that 19% of the total diversity was due to

Table 3

Nei's Unbiased (1978) Genetic Distance for Norway Spruce among Islands

Island	A	B	D	37	48	52	Storön
A		0.1723	0.1609	0.1619	0.1681	0.1538	0.1703
B	0.1728		0.1332	0.1505	0.1251	0.1220	0.0967
D	0.1521	0.1225		0.0925	0.0714	0.0943	0.0481
37	0.1498	0.1337	0.0809		0.0783	0.0975	0.0879
48	0.1553	0.1235	0.0685	0.0684		0.0738	0.0501
52	0.1434	0.1145	0.0943	0.0883	0.0699		0.0648
Storön	0.1605	0.0876	0.0435	0.0754	0.0483	0.0648	

Note. Above the diagonal space: data set 1 with 117 trees in seven groups; below the diagonal space: data set 2 with 96 genotypes.

Table 2

Diversity Measures for Norway Spruce from Seven Islands

		Genets					Gene diversity *h*	
Island	Polymorphic loci	Total	Sampled once	Sampled >once	Clonal fraction (%)	H_s[a]	1[b]	2[c]
A	42 (40%)	6	4	2	40	0.698	0.1500	0.1727
37	62 (59%)	10	7	3	41	0.735	0.2185	0.2297
48	79 (75%)	15	12	3	17	0.920	0.3066	0.3164
B	62 (59%)	6	5	1	33	0.719	0.2785	0.2945
52	70 (67%)	11	11	0	0	1.000	0.2560	0.2560
D	86 (82%)	18	16	2	18	0.898	0.3311	0.3336
Storön	103 (98%)	30	30	0	0	1.000	0.3752	0.3752
Total	105 (100%)	96	85	11	18	0.934	0.3710	0.3747

[a] H_s = genotypic diversity.
[b] Data set 1: all trees.
[c] Data set 2: unique genotypes.

differentiation among islands (average $F_{st} = 0.19$) using data set 1 consisting of 117 samples. When only one representative from each genotype was retained (data set 2), the F_{st} value decreased slightly to 16%. AMOVA was also employed to analyze the differentiation among islands. AMOVA results were slightly lower than the F_{st} values and revealed that 15% of the total genetic variation was due to differences among islands consisting of 117 samples. For data set 2 this value decreased to 9.6%. All these tests indicate that majority of the genetic diversity is found within each island. UPGMA clustering analysis of the 96 multilocus genotypes did not segregate individuals by islands. Rather, most of the genotypes from different islands grouped randomly (not shown).

Discussion

Layering Development

In the boreal forests of northern Sweden, fire, caused by lightning, plays an important role in shaping vegetation structure (Zackrisson 1977; Engelmark et al. 1994; Wardle et al. 1997). Island size is, in general, directly correlated with fire frequency. The bigger islands are affected by fire more often than the small islands, which as a consequence resulted in different plant communities present on different islands. Islands A, B, 37, and 48 are fairly small islands. The vegetation on these sites has developed for more than 2500 yr without disturbances. Islands D and 52 are medium-size islands and had the last fire more than 1500 yr ago. Layering was observed on five of these islands. The clonal fraction on each of islands A, B, and 37 is more than 30%. Island Storön is the biggest island in this study and is in the relatively "early" stage of vegetation development after the last fire ca. 200 yr ago. There was no clonal growth detected on this island, and regeneration through seeds is the main means of population development. Thus, layering is shown to be more frequently occurring on the smaller islands that have gone through a long time span of development without disturbances. However, this correlation should not be viewed as an oversimplified island age/size and layering relationship since it does not require 1000 yr for clones to develop. A complex of ecological factors could have jointly contributed to this observation. Since fire is more frequent on the bigger islands, lower branches and/or trees with lower branches are more prone to be eliminated, making layering impossible. In the event of intense fire disturbance, most of the Norway spruce and ground layer vegetation will be destroyed. In the boreal habitats, pioneer species such as birch (*Betula* spp.) and shrubs (e.g. *Vaccinium* spp.) are the first to invade after fire. Given enough time (close to 100 yr), spruce will establish in the secondary birch woodlands. From then on, a certain amount of time would be required for layering to develop from standing trees. Thus, the lack of clonal composition on the big island can be a result of frequent fire disturbances that prevent layering from developing. In addition, in the boreal forests, fire is important in restoring soil nutrition levels and consequently encouraging tree regeneration and growth (Zackrisson 1977; Zackrisson et al. 1996). Indeed, we observed much better growth of Norway spruce on island Storön than on other smaller islands. Faster-growing trees on the bigger islands, as a result of the generally positive effects of fire on nutrient cycling (Zackrisson and Nilsson 1992; Nilsson et al. 1993), would have few branches close to the ground, making layering difficult.

On the smaller islands with long fire intervals, the dwarf shrub *Empetrum hermaphroditum* Hagerup and pleurocarpous mosses are more dominant and form a thick carpet of ground layer vegetation that may inhibit the establishment and growth of spruce seedlings (Wardle et al. 1997; Ponge et al. 1998; Nilsson et al. 1999). Trees grow much slower on these sites because of the thick humus layer. The lower branches remain close to ground over many years. Heavy snowfall through the long winter can easily push the lower branches down to contact with ground. In addition, a thick moss layer on the old islands can provide a moist condition for fine roots emerging from the layering branches and for facilitating clonal development. Thus, the fire frequency–island size relationship has a strong impact on layering development.

The sampling scheme in the present study is aimed more toward the small and old islands for better detection of layering. Therefore, our result could be an overestimation of the average degree of Norway spruce clonal propagation in the boreal forest in northern Sweden. More site investigations, particularly on the mainland, would be needed to generate a more representative estimate. Nevertheless, our results provide for the first time genetic evidence that Norway spruce layering is an alternative way of propagation in the boreal region.

Genetic Structure of the Island Populations

The average gene diversity detected by the AFLP markers in Norway spruce from the seven islands is high ($h = 0.37$) compared with many investigations using allozyme markers in the main Norway spruce distribution range (Lagercrantz and Ryman 1990; Goncharenko et al. 1995; Müller-Starck 1995). This could be partly due to the exclusion of monomorphic loci in our AFLP analysis and the primarily noncoding DNA targeting of the AFLPs as compared with amino acid substitution in coding DNA by allozymes. Nevertheless, similar high values of gene diversity were observed for many other Scandinavian populations of Norway spruce (Tigerstedt 1973; Lundkvist and Rudin 1977). Northern marginal populations of Norway spruce are reported to have a higher selfing rate as compared to central populations (Tigerstedt 1973; Muona et al. 1990). This could reduce the genetic variability in these marginal populations. However, most of the studies have revealed little reduction in gene diversity, and marginal populations are as variable as central populations (Tigerstedt 1973; Lundkvist and Rudin 1977; Muona et al. 1990). Similarly, populations of Norway spruce from high elevations in the Alps harbor no smaller amount of genetic variation than low-elevation populations (Müller-Starck 1995). This is in general attributed to extensive gene flow among populations (especially via pollen), predominant outcrossing, high inbreeding depression, and recent origin of Norway spruce recolonization after the last glaciation (Koski 1973; Tigerstedt 1973; Schmidt-Vogt 1978; Lagercrantz and Ryman 1990).

In general, low genetic diversity would be expected for small islands with few individuals, and this was demonstrated in our study. The smallest, island A, had the lowest gene diversity and the largest genetic distance to the other islands. Genetic

differentiation among islands revealed a F_{st} of 0.19 and 0.16 for islands with and without clonal fractions, respectively. This is surprisingly high in viewing the small geographic distances separating these islands, as compared with $F_{st} = 0.05$ among 70 Norway spruce populations throughout the distribution range as revealed by 22 allozyme markers (Lagercrantz and Ryman 1990). The small differentiation among Norway spruce populations results mainly from high gene flow among populations and the recent postglacial migration history in the main distribution range. A relatively high $F_{st} = 0.118$ is found for eight populations of Norway spruce from the Italian Alps, using seven sequence-characterized amplified regions (SCAR; Scotti et al. 2000). This high value is explained by the different origin of the populations through different migration routes of recolonization in the Alps (Scotti et al. 2000). However, this factor cannot explain the high F_{st} among our islands since they share the same postglacial development history. Five of the seven islands analyzed in this study are fairly small, and so are their population sizes. Drift and founder effect usually have a bigger impact on small populations, making the interpopulation differentiation more pronounced. On the other hand, gene flow could counteract the effect of drift over generations. However, close to Norway spruce tree limit, seedlings are rare, indicating poor sexual regeneration and seedling survival. The next generation of trees is produced only after a fire (Kullman 1996*b*). The rare sexual reproduction and the difficulty for new seedling recruitment on the small, old islands could reduce the impact of gene flow as compared with populations in the central distribution where conditions are more favorable for regular reproduction and regeneration. This would preserve the impact of drift and founder effect on the initial populations for a much longer time.

The random clustering of the 96 genotypes and the large differences among genotypes indicate a lack of family structure on the islands. Similar findings of random gene distribution within stands are reported for two Finnish populations (Tigerstedt 1973) and one Italian population (Bucci and Menozzi 1995) of Norway spruce. Weak correlation between genetic relationship and topographic distance is found in a French population (Brunel and Rodolphe 1985). The lack of family structure is generally expected for predominant outcrossing species. In our samples, the random clustering of genotypes suggests the initial island population setup was done by random genotypes (seeds), and this initial structure is preserved due to the restricted sexual reproduction and regeneration. In this situation, fire disturbance can be viewed as a force of promoting gene exchanges among islands by opening the site for new seedling establishment.

In conclusion, the average genetic variation of Norway spruce over the seven islands is high, and the island populations were developed from random genotypes (seeds) after fire disturbances. Because of the small size of some of the islands, drift and founder effect had significant impact on the interisland differentiation. This differentiation is preserved by the lack of new seedling establishment. Layering is observed on islands with long fire intervals. The clonal growth within each island further increased the genetic differentiation among islands. While frequent fire promotes regeneration and growth by improving the soil conditions, it makes layering less likely to develop. As a consequence, fire disturbance would promote gene exchange among islands and increase genetic diversity within an island. Thus, environmental and ecological factors play an important role in shaping the genetic structure of the boreal Norway spruce populations.

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Literature Cited

Andersson E 1965 Cone and seed studies in Norway spruce (*Picea abies* [L.] Karst.). Stud For Suec 23:5–214.

Bradshaw R, O Zackrisson 1990 A two-thousand year history of a northern Swedish boreal forest land. J Veg Sci 1:519–528.

Brunel D, F Rodolphe 1985 Genetic neighborhood structure in a population of *Picea abies* L. Theor Appl Genet 71:101–110.

Bucci G, P Menozzi 1995 Genetic variation of RAPD markers in a *Picea abies* Karst. population. Heredity 75:188–197.

Engelmark O, L Kullman, Y Bergeron 1994 Fire and age structure of Scots pine and Norway spruce in northern Sweden during the past 700 years. New Phytol 126:163–168.

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

Goncharenko GG, IV Zadeika, JJ Birgelis 1995 Genetic structure, diversity and differentiation of Norway spruce (*Picea abies* [L.] Karst.) in natural populations of Latvia. For Ecol Manage 72:31–38.

Goodwin SB, LJ Spielman, JM Matuszak, SN Bergeron, WE Fry 1992 Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955–961.

Hagner S 1965 Cone crop fluctuations in Scots pine and Norway spruce. Stud For Suec 33:1–21.

Horton KW, JC Lees 1961 Black spruce in the foothills of Alberta. Canadian Department of Forestry, Forest Research Branch. Technical Note No. 110.

Jones CJ, KJ Edwards, S Castaglione, MO Winfield, F Sala, C van de Wiel, G Bredemeijer, et al. 1997 Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol Breed 3:381–390.

Koski V 1973 On self-pollination, genetic load and subsequent inbreeding in some conifers. Commun Inst For Fenn 78:1–42.

Kullman L 1996*a* Recent cooling and recession of Norway spruce (*Picea abies* [L.] Karst) in the forest-alpine tundra ecotone of the Swedish Scandes. J Biogeogr 23:843–854.

——— 1996*b* Rise and demise of cold-climate *Picea abies* forest in Sweden. New Phytol 134:243–256.

Lagercrantz U, N Ryman 1990 Genetic structure of Norway spruce

(*Picea abies*): concordance of morphological and allozymic variation. Evolution 44:38–53.

Lundkvist K, D Rudin 1977 Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. Hereditas 85:67–74.

Lynch M, BG Milligan 1994 Analysis of population genetic structure with RAPD markers. Mol Ecol 3:91–99.

Miller MP 1997 Tools for population genetics analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author; http://bioweb.usu.edu/mpmbio/tfpga.htm.

Mueller UG, LL Wolfenbarger 1999 AFLP genotyping and fingerprinting. Trends Ecol Evol 14:389–394.

Müller-Starck G 1995 Genetic variation in high elevated populations of Norway spruce (*Picea abies* [L.] Karst.) in Switzerland. Silvae Genet 44:356–362.

Muona O, L Paule, AE Szmidt, K Kärkkäinen 1990 Mating system analysis in a central and northern European population of *Picea abies*. Scand J For Res 5:97–102.

Nei M 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nilsson MC, P Hogberg, O Zackrisson, FY Wang 1993 Allelopathic effects by *Empetrum hermaphroditum* on development and nitrogen uptake by roots and mycorrhizae of *Pinus sylvestris*. Can J Bot 71: 620–628.

Nilsson MC, DA Wardle, A Dahlberg 1999 Effects of plant litter species composition and diversity on the boreal forest plant-soil system. Oikos 86:16–26.

Parker PG, AA Snow, MD Schug, GC Booton, PA Fuerst 1998 What molecules can tell us about populations: choosing and using a molecular marker. Ecology 79:361–382.

Ponge JF, J André, O Zackrisson, N Bernier, MC Nilsson, C Gallet 1998 The forest regeneration puzzle: biological mechanisms in humus layer and forest vegetation dynamics. BioScience 48:523–530.

Sarvas R 1968 Investigations on the flowering and seed crop of *Picea abies*. Commun Inst For Fenn 67:1–84.

Schmidt-Vogt H 1978 Monographie der *Picea abies* (L.) Karst. unter Berücksichtigung genetischer und züchterischer Aspekte. Forstwiss Centralbl 97:281–302.

Scotti I, GG Vendramin, LS Matteotti, C Scarponi, M Sari-Gorla, G Binelli 2000 Postglacial recolonization routes for *Picea abies* K. in Italy as suggested by the analysis of sequence-characterized amplified region (SCAR) markers. Mol Ecol 9:699–708.

Skoklefald S 1993 Naturlig foryngelse av gran og furu. Aktuelt fra Skogforsk 5:3–6.

Stanek W 1961 Natural layering of black spruce in northern Ontario. For Chron 37:245–258.

Tigerstedt PMA 1973 Studies on isozyme variation in marginal and central populations of *Picea abies*. Hereditas 75:47–60.

Vos P, R Hogers, M Bleeker, M Reijans, T van de Lee, M Hornes, A Frijters, et al 1995 AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414.

Wang XR, AE Szmidt 2001 Molecular markers in population genetics of forest trees. Scand J For Res 16:199–220.

Wardle DA, O Zackrisson, G Hörnberg, C Gallet 1997 The influence of island area on ecosystem properties. Science 277:1296–1299.

Zackrisson O 1977 Influence of forest fires on the northern Swedish boreal forest. Oikos 29:22–32.

Zackrisson O, MC Nilsson 1992 Allelopathic effects by *Empetrum hermaphroditum* on seed germination of two boreal tree species. Can J For Res 22:1310–1319.

Zackrisson O, MC Nilsson, DA Wardle 1996 Key ecological function of charcoal from wildfire in the boreal forest. Oikos 77:10–19.