

## Mating System Analysis in a Central and Northern European Population of *Picea abies*

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Mating system and genetic variability were studied in a southern and a northern population of *Picea abies*, in Czechoslovakia and Finland. Both populations had considerable genetic variability, with expected heterozygosities of 0.17 and 0.23, respectively, at nine loci. The two distant populations were more differentiated ( $G_{ST} = 0.12$ ) than has been found in studies on Scandinavian *Picea abies*. Both populations had a considerable degree of partial selfing, with estimates of outcrossing of 0.83 and 0.74 in the populations from Czechoslovakia and Finland, respectively. *Key words:* *Picea abies*, mating system, allozyme.

### INTRODUCTION

Tree breeding and domestication need as a basis a thorough understanding of the genetic variability and mating system in natural populations (Ledig, 1986; Muona, 1989). Conifers have been subjected to extensive population genetic studies by means of isozyme analysis in recent years. The studies have described the distribution of genetic variability and have characterized mating systems. In general, differentiation between populations is poor. Much work has been concentrated on species of the genus *Pinus* and on *Pseudotsuga menziesii*. Most conifers have been found to have a considerable degree of outcrossing (see Muona, 1989 for review). However, outcrossing rates vary between populations (e.g. Neale & Adams, 1985) and between individual trees (Shea, 1987; Koski & Muona, 1986).

Earlier studies on *Picea abies* have concentrated on allelic variability at isozyme loci (Tigerstedt, 1973; Bergmann, 1978; Lundkvist & Rudin, 1977; Lundkvist, 1979; Altukhov et al., 1986). Estimates of outcrossing rates, based on rare alleles, have also been obtained (Müller, 1977; Lundkvist, 1979). Tigerstedt (1973) and Brunel & Rodolphe (1985) made detailed studies on genotypic distributions within populations.

In this paper we compare two *Picea abies* populations with regard to their allelic frequencies at isozyme loci and outcrossing rates. The population from Czechoslovakia is from the southern part of the range, while the other is close to the northern tree limit. We considered two questions. First, do we find greater genetic differentiation between these distant populations than within the smaller area of Sweden studied earlier? Second, do the two populations in different environments differ in their outcrossing rates?

### MATERIAL AND METHODS

We sampled a stand of Norway spruce in the Tatra National Park, Czechoslovakia (49°12' N, 20°15' E, alt. 900 m). Cones were collected from 25 individual trees in 1981. In Finland, we sampled cones from 22 trees from the Riisitunturi National Park (66°13' N, 28°43' E, alt. 300

m) in the spring of 1984. We also sampled trees on the top of Riisitunturi mountain, but these seeds failed to germinate in sufficient numbers for analysis.

Ten seeds were studied from each tree, with the megagametophyte and embryo analysed separately. The genotype of the haploid megagametophyte equals that of the egg cell. This information, along with the genotype of the diploid embryo, allows determination of the genotype of the fertilizing pollen grain. The references for the methods of electrophoresis and staining are given by Muona & Szmidt (1985). The enzymes stained for and the loci

Table 1. Allelic frequencies at enzyme loci of *Picea abies* in Tatra National Park, Czechoslovakia, and Riisitunturi National Park, Finland, and G-test for allelic frequency differences

Locus	Allele	Tatra	Riisitunturi	G-test
<i>Aco</i>	1	0.304		
	2	0.696	1.000	120.5***
<i>Dia-2</i>	1	0.991		
	2	0.009		
<i>Dia-3</i>	1	0.478		
	2	0.522		
<i>F-Est</i>	1	0.004	0.003	
	2	0.982	0.994	
	3	0.010	0.003	
	4	0.004	0.000	2.1 NS
<i>Gdh</i>	1	0.988	0.546	
	2	0.012	0.454	280.7***
<i>Got-1</i>	1	0.014	0.007	
	2	0.986	0.990	
	3	0.000	0.003	NT
<i>Got-2</i>	1	0.412	0.376	
	2	0.564	0.624	
	3	0.008	0.000	
	4	0.016	0.000	13.0***
<i>Lap-1</i>	1	0.018	0.007	
	2	0.896	0.579	
	3	0.012	0.407	
	4	0.074	0.007	211.0***
<i>Lap-2</i>	1	0.126	0.140	
	2	0.848	0.628	
	3	0.000	0.226	
	4	0.026	0.006	63.9***
<i>Mdh-1</i>	1	1.000	0.993	
	2	0.000	0.007	NT
<i>Mdh-2</i>	1	0.014	0.004	
	2	0.004	0.000	
	3	0.966	0.996	
	4	0.016	0.000	9.7***
<i>Pgi-2</i>	1	0.042		
	2	0.305		
	3	0.010		
	4	0.589		
	5	0.054		
<i>Pgm-1</i>	1	0.959		
	2	0.041		
<i>Pgm-2</i>	1	0.022		
	2	0.961		
	3	0.017		

NT = no test, NS = not significant, \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

studied are listed in Tables 1 and 2. Muona et al. (1987) give results and references for inheritance and linkage in Norway spruce. As is evident from Tables 1 and 2, we used a slightly different set of loci for the two populations, 14 loci for Tatra, 9 for Riisitunturi. For comparisons of genetic variability, we used only shared loci.

We compared the allelic frequencies at polymorphic loci with  $G$ -tests. The proportion of genic diversity due to population differentiation was estimated using  $G_{ST}$ -analysis (Nei, 1973). In addition to allelic frequencies, we estimated fixation indices and their standard deviations for the individual populations,  $F = 1 - H_{obs}/H_{exp}$  (Brown, 1970). A positive fixation index results from excess homozygosity, possibly due to inbreeding.

The proportion of outcrossing was estimated with the multilocus method of Ritland & Jain (1981).

## RESULTS AND DISCUSSION

Allelic frequencies for the two populations are listed in Table 1, with results from  $G$ -tests for differences between populations. At most loci the two populations differed significantly. Table 2 gives the gene diversity analysis, according to which the average  $G_{ST}$  over loci was 0.12. The differentiation was most pronounced at the loci *Aco*, *Gdh* and *Lap-1*. Earlier results are variable. Tigerstedt (1973) found no differences in allelic frequencies between a southern and a northern Finnish population. Lundkvist & Rudin (1977) found little differentiation between eleven Swedish populations. Based on their allelic frequencies,  $G_{ST}$  was 0.036. Bergman (1978) found a cline in Scandinavia at one locus. Clinical variation in allelic frequencies seems to be rare at allozyme loci, as in many other conifers.

The average expected heterozygosities were 0.17 and 0.23 for Tatra and Riisitunturi, respectively. Earlier studies have found no cline in the level of variability. Tigerstedt (1973) found that the marginal and central populations were equally variable. Rudin & Lundkvist (1977), Lundkvist (1979) and Bergmann & Gregorius (1979) found some differences between populations, but the northern populations were no less variable than Central European populations. Marginal northern populations of Norway spruce could lose their variability

Table 2. Expected heterozygosities and results of gene diversity analysis for *Picea abies* in Tatra National Park, Czechoslovakia, and Riisitunturi National Park, Finland

Locus	$H_{exp}$		$G_{st}$
	Tatra	Riisitunturi	
<i>Aco</i>	0.423	0.000	0.179
<i>Dia-2</i>	0.017	-	-
<i>Dia-3</i>	0.499	-	-
<i>Got-1</i>	0.028	0.007	0.001
<i>Got-2</i>	0.512	0.469	0.003
<i>F-Est</i>	0.036	0.013	0.003
<i>Gdh</i>	0.024	0.496	0.273
<i>Lap-1</i>	0.192	0.499	0.159
<i>Lap-2</i>	0.264	0.535	0.059
<i>Mdh-1</i>	0.000	0.013	0.004
<i>Mdh-2</i>	0.067	0.007	0.009
<i>Pgm-1</i>	0.078	-	-
<i>Pgm-2</i>	0.075	-	-
<i>Pgi-2</i>	0.556	-	-
Mean	0.17	0.23	0.12

due to drift in populations of small effective size. However, the marginal northern populations are of fairly recent origin. They have colonized Northern Finland since the last glaciation, about 4000 years ago (see e.g. Schmidt-Vogt, 1974). This represents a fairly small number of generations for long-lived organisms. Northern populations of *Pinus sylvestris* are also quite variable (Muona & Szmidt, 1985).

Table 3 gives results from the mating system analysis using the Ritland & Jain (1981) method. In both populations, the single-locus outcrossing estimates were highly variable, ranging from 0.55 to 0.99. Individual locus estimates also had high standard errors. The multilocus estimates were 0.83 and 0.74 for Tatra and Riisitunturi, respectively. These are quite low estimates for conifers, and average estimates below 0.8 have only been obtained for *Larix laricina* (Knowles et al. 1987) and *Pinus radiata* (Moran, Bell and Muona, unpublished, see Muona, 1989). The low value for Riisitunturi can be partly explained, as we collected cones from low, peripheral branches. It has been shown earlier that in *Pseudotsuga menziesii* the outcrossing rate is somewhat lower in the lower parts of the crown (Shaw & Allard, 1982). Variation in outcrossing rates between parts of the crown has also been observed in *Pinus sylvestris* (Rudin & Ekberg, 1982). Another possible explanation is that flowering in the north is so irregular that the rate of outcrossing is reduced because of reduced local effective population size. Preliminary observations suggest that this may be true in *Pinus sylvestris* (Kärkkäinen & Muona, 1989). The outcrossing estimate for the central Tatra population was also fairly low. The cones were collected from the upper part of the crown and flowering and cone production were abundant in 1981.

As there was considerable partial selfing, this should be reflected also in positive fixation indices. If the mating system were the only factor causing deviations from Hardy-Weinberg proportions, the expected  $F$ -value would be  $(1-t)/(1+t)$ . This would correspond to 0.09 and 0.15 for Tatra and Riisitunturi, respectively. Table 3 shows that there was more homozygosity ( $F_{\text{obs}} = 0.23$ ) in the Tatra population than expected on the basis of the multilocus  $t$ -estimate, whereas in the Riisitunturi population there was less homozygosity than would be expected ( $F_{\text{obs}}$  was 0.07 on average). As we do not have more detailed information, the cause of the differences remains obscure.

Heterogeneity between the pollen clouds fertilizing different maternal trees can also cause lower outcrossing estimates (e.g. Ennos & Clegg, 1982). There were sufficient data in the Tatra population at four loci (*Got-2*, *Pgi-2*, *Dia-3*, and *Aco*) to examine this. The pollen clouds were significantly heterogeneous in all cases (data not shown). This may account for

Table 3. Estimates of outcrossing rates ( $t_i$ ), their standard deviations (SD), and fixation indices ( $F_{\text{obs}}$ ) for polymorphic loci in *Picea abies* in the Tatra National Park, Czechoslovakia and Riisitunturi National Park, Finland

Locus	Tatra			Riisitunturi		
	$t_i$	SD	$F_{\text{obs}}$	$t_i$	SD	$F_{\text{obs}}$
<i>Aco</i>	0.83	0.09	0.23	—	—	—
<i>Dia-3</i>	0.99	0.09	0.01	—	—	—
<i>Gdh</i>	—	—	—	0.99	0.11	0.07
<i>Got-2</i>	0.82	0.09	0.17	0.67	0.10	0.08
<i>Lap-1</i>	0.57	0.36	0.46	0.57	0.13	0.09
<i>Lap-2</i>	0.81	0.12	0.27	0.55	0.12	0.19
<i>Pgi-2</i>	0.61	0.07	0.23	—	—	—
Multilocus (mean $F$ )	0.82	0.03	0.23	0.74	0.06	0.07

some of the partial selfing. In Riisitunturi the sample size was smaller and we did not test for heterogeneity. King et al. (1984) found that there was sufficient heterogeneity between pollen pools fertilizing different trees to account for all the deviation from panmixia they observed.

Müller (1977) estimated, using rare alleles, that the proportion of selfing in a natural stand of Norway spruce varied between 7% and 18% for individual trees (mean 12%). Lundkvist (1979) used the same method for estimating selfing in some trees. His estimates ranged between 0% and 26% (mean 11%). Other species of the genus *Picea* have also been studied, with roughly similar results (Shea, 1987; King et al., 1984). However, Cheliak et al. (1985) found no selfing in *Picea glauca*.

In most conifer populations, adult genotypic frequencies have been found to conform to Hardy-Weinberg frequencies (see Muona, 1989 for review), even though there was some selfing in the population. In most cases, inbred progeny die during early growth due to severe inbreeding depression, and adult fixation indices are close to 0. Our adult samples were so small in Riisitunturi that we did not obtain any estimates. For the adult trees of Tatra, the average fixation index over loci was 0.12, but none of the individual locus values deviated statistically significantly from 0. The Tigerstedt's data (1973) can be used to estimate fixation indices for two populations. In the northern marginal population the average for adult trees over two loci was 0.19; for the central population, 0.02. This agrees with our finding of some inbreeding in the northern population.

Further studies are needed to confirm the lower outcrossing rates in northern marginal populations, and to ascertain whether they are due to genetic differences in self-fertility, or to environmental causes.

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