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Outcrossing Rates in Two Stands of Noble Fir (*Abies procera* REHD.) in Denmark

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Summary

The outcrossing rates in 2 stands of noble fir (*Abies procera* REHD.) in Denmark were determined from the segregation at 3 polymorphic allozyme loci in 20 progenies from 18 trees in each stand. The outcrossing rates were found to be slightly smaller than one: 0.98 and 0.90, of which only the latter value differed significantly from one. Noble fir therefore resembles other coniferous species by reproducing according to a mixed mating system, although with low level of selfing. The differentiation among the populations was relatively low, as commonly observed in wind-pollinated trees: the estimated F_{ST} value was 0.029.

Key words: Abies procera, mating systems, allozymes, gene diversity, population differentiation.

FDC: 165.4; 174.7 *Abies procera*; (489).

Introduction

Noble fir (*Abies procera* REHD.) has its natural distribution area in North America, mainly in the high elevations of the Cascade range in Oregon and Washington. It was introduced to Denmark in a small scale from the 1850s (LANGE, 1994; LARSEN, 1983). Since the 1950s, it has been planted widely in the Danish forestry, where it is used for production of Christmas greenery.

Noble fir has been reported to have relative low inbreeding depression in seed set and seedling survival (SORENSEN et al., 1976), but inbreeding depression, in vigor at a level commonly found in conifers (SORENSEN and MILES, 1982). A potential for natural inbreeding thus exists, and this concern initiated a study of selfing rates in a Danish seed orchard based on 100 clones (SIEGISMUND et al., 1996), because the mating system of this species had not been investigated previously. This study concluded that the progenies from the seed orchard clones where 100 % outcrossed.

Seed orchards are managed differently from traditional stands. One could expect the selfing rates to be higher in older and denser stands due to limited pollen movement compared to the widely spaced seed orchard with trees of lesser sizes. For this reason, the selfing rates in 2 traditional stands are estimated in the present study.

Materials and Methods

The stands

Two stands were selected for this study. One 52 year old stand (F587, [Danish seed stand approval number]) located within Ulborg State Forest District, and one 44 year old stand (F443) from Klosterheden State Forest District. Both stands are dense, but the Ulborg stand grows on a windy site in contrast to the Klosterheden stand, which grows on a more protected, gentle tract. The stands have known origin in older Danish noble fir stands from Buderupholm and Esrum State Forest Districts, respectively.

Seed collection and treatment

Cones were collected from 18 trees in each of the investigated stands in the fall of 1993, which was a year with a very dense cone crop and abundant flowering. The seeds were extracted from the cones and were stratified moist for about 6 weeks at 5 °C upon which they were germinated at room temperature. After 3 to 5 weeks the germinating seeds were partitioned into embryo and megagametophyte, which both were stored at −80 °C. From each tree 20 embryos and 10 megagametophytes were analyzed with enzyme electrophoresis. The genotypes of the megagametophytes were used to determine the genotype of the mother tree.

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In order to compare the variation at the locus *Skdh* (see below) with a sample from the seed orchard FP623, which in the previous work (SIEGISMUND et al., 1996) was reported as monomorphic, bulked seed from the seed orchard FP623 was treated as described above.

Electrophoresis

Enzymes were extracted as described by SIEGISMUND et al. (1996). In addition to the procedure used by SIEGISMUND et al. (1996) for the loci aldolase (*Ald*) UDP glucose pyrophospholyrase (*Ugpp*), 12% starch gels (Connaught, Canada) were prepared with a sodium-borate pH 8.0/tris-citrate pH 8.6 buffer (no. 5 of WENDEL and WEEDEN [1989]), which was run at 450 V for 4.5 h. This gel was stained for the enzymes glutamate oxaloacetate transaminase (EC 2.6.1.1) and shikimate dehydrogenase (EC 1.1.1.25). We used the recipes of MANCHENKO (1994), method 1, for glutamate oxaloacetate transaminase, and WENDEL and WEEDEN (1989) for shikimate dehydrogenase. The glutamate oxaloacetate transaminase stain revealed 3 zones, interpreted as coded by 3 loci *Got-1*, *Got-2*, and *Got-3*, where *Got-1* codes for the most anodically moving enzymes. Only the *Got-1* locus was polymorphic. The other 3 enzyme stains revealed variation at single zones with the loci *Ald*, *Skdh*, and *Ugpp*, respectively. In the previous *study*, *Skdh* was stained on another buffer which did not resolve the variation.

Analysis of data

The multi locus outcrossing rate was estimated with the procedure of RITLAND and JAIN (1981) as implemented in the program MLTR, version 0.9, of RITLAND (1990). We used the expectation-maximization method to find maximum likelihood estimates and used 1000 bootstraps to find confidence limits. Bootstrapping was performed by resampling among families.

The differentiation among populations was quantified with WRIGHT's *F*-statistics where we used the method of WEIR and COCKERHAM (1984). The analysis was performed on the embryo data. Standard deviations of the estimators were found by jackknifing across the populations.

Deviations from HARDY-WEINBERG proportions were tested with the permutation test of GUO and THOMPSON (1992). We used 17,000 permutations in each test.

In multiple tests we used the sequential BONFERRONI technique to test for "table-wide" significance (HOLM, 1979; RICE, 1989). The *P* values are ranked in increasing order: with n tests the smallest value (P_1) is declared significant at the "table-wide" significant level α if $P_1 < \alpha/n$. The second smallest value is declared significant if $P_2 < \alpha/(n\text{-}1)$, and so on.

Table 1. – Maternal genotype distribution and test for HARDY-WEINBERG proportion based on GUO and THOMPSON (1992) (*P*).

	Maternal genotype			
	11	12	22	*P*
Ald				
Klosterheden	3	9	6	0.630
Ulborg	0	12	6	0.040
Got-1				
Klosterheden	14	4	0	1.000
Ulborg	11	7	0	1.000
Skdh				
Klosterheden	14	2	2	0.040
Ulborg	9	7	2	1.000

Results and Discussion

The genotypic proportions at the 3 loci used for the estimation of the outcrossing rates of the 18 trees from the stands at Ulborg and Klosterheden are presented in *table 1*. None of them deviates significantly from HARDY-WEINBERG proportions on a "table-wide" level.

In the material from Klosterheden one set of embryos could not be scored at the *Skdh* locus and a single individual from another family could not be scored at any of the loci. Otherwise, the multi locus estimate of the outcrossing rate is based on 20 offspring per tree. In the material from Ulborg there is missing data at the *Skdh* locus from 10 offspring from a single tree. In Ulborg the multi locus outcrossing rate was estimated to be 0.981 with a 95% confidence interval of 0.893 to 1.000, indicating that this stand is almost completely outcrossing. In Klosterheden the multi locus outcrossing rate was 0.904 with a 95% confidence interval of 0.809 to 0.969, indicating that the outcrossing rate is significantly smaller than 1.

The level of gene diversity (expected heterozygosity, H_e) was estimated for the 3 populations in each of the 4 loci (*Table 2*). The seed orchard consists of 100 clones selected in a number of different stands (Danish Land Development Service, 1979) and the progeny can therefore be seen as outcrossed on a population level (provenance hybrid). However, on average the H_e was found to be quite similar in the 3 populations, and not larger in the seed orchard compared to the Ulborg or Klosterheden stands.

The 4 loci show a different pattern of differentiation. At the *Got-1* locus, the sample from FP623 is fixed for allele 1 whereas

Table 2. – Allele frequency distributions of the 3 populations, sample size (*N*), WRIGHT's fixation index (*F*), gene diversity (H_e), and tests for HARDY-WEINBERG proportions based on GUO and THOMPSON (1992) (*P*). The data for the variation at the *Ald* and *Ugpp* loci in the seed orchard FP623 is from SIEGISMUND et al. (1996).

		FP623	Klosterheden	Ulborg
Ald	1	0.250	0.358	0.262
	2	0.750	0.642	0.738
	N	400	359	360
	F	-0.067	-0.012	-0.112
	H_e	0.375	0.460	0.388
	P	0.227	0.911	0.043
Got-1	1	1.000	0.944	0.882
	2	0.000	0.056	0.118
	N	125	359	360
	F		0.047	-0.027
	H_e	0.000	0.105	0.209
	P		0.299	0.800
Skdh	1	0.796	0.820	0.739
	2	0.204	0.180	0.261
	N	120	339	350
	F	0.256	0.140	0.105
	H_e	0.327	0.296	0.387
	P	0.010	0.017	0.053
Ugpp	1	0.124	0.029	0.012
	2	0.876	0.971	0.988
	N	399	105	260
	F	-0.142	-0.029	-0.012
	H_e	0.218	0.056	0.023
	P	0.002	1.000	1.000
	H_e	0.230	0.229	0.251

the samples from Klosterheden and Ulborg are polymorphic (*Table 2*). A similar pattern is seen at the *Ugpp* locus, where the sample from FP623 is polymorphic but where the other 2 samples are almost fixed for allele 2. At the other 2 loci 2 alleles are found with relative high frequencies in all 3 populations. The highest differentiation measured with WRIGHT's F_{ST}-statistic according to WEIR and COCKERHAM (1984) was observed at the *Ugpp* locus with a value of 0.12. The values at the other three loci are somewhat smaller, being in the range of 0.016 to 0.027. An average across the 4 loci was 0.029. Despite the low values at 3 of the loci, the 3 populations are significantly differentiated at all loci. *G*-tests for homogeneity (SOKAL and ROHLF, 1981) on the allele numbers are significant at the 0.1% level. The populations are all differentiated from each other: a pairwise comparison of the three populations where the test statistics are summed across the loci does also reveal significant differences among them with significance levels smaller than 0.01%.

The increased level of variation found in the present study in comparison to our previous published result (SIEGISMUND et al., 1996) suggests that the import of noble fir to Denmark has not been accompanied with a severe bottleneck that has eroded the genetic variation. As noted by SIEGISMUND et al. (1996) noble fir has a level of variation at quantitative characters that is comparable to what is found in other conifer species.

The present study indicates that the outcrossing rate differs between the 2 investigated stands being lower in Klosterheden ($t_m = 0.90$) than in Ulborg ($t_m = 0.98$). However, the difference was not found to be significant on a 5% level. Both stands are uniform and fairly dense. The difference could be due to the more windy conditions in Ulborg compared to Klosterheden. SIEGISMUND et al. (1996) estimated $t_m = 1.00$ in the Viborg seed orchard, which they found puzzling considering the low level of embryonic lethal genes reported by SORENSEN et al. (1976). A similar finding has been reported for *Picea omorika* by KUITTINEN and SAVOLAINEN (1991). *Picea omorika* is self-fertile but is highly outcrossing, which is explained by the separation of male and female flowers and by a protogynous flowering.

The present study shows that outcrossing rates significantly lower than 1.00 can be found. Noble fir therefore appears to resemble other coniferous species by reproducing according to a mixed mating system, although still with a low level of selfing (see e.g. references in MUONA, 1990).

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Mode of Fertilization and its Individual Variation in *Larix gmelinii* var. *japonica*

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Summary

Fertilization mode, that is random, selective cross- or selective self-fertilization after mixed pollination (pollination with equal mixture of outcross and self pollen) was investigated for 6 clones of *Larix gmelinii* var. *japonica*. The seed fertilities expressed as proportions of filled seeds and germinatible seeds for the numbers of full size-seeds per cone after mixed pollination were examined, and these were compared with those resulting from complete outcross- and self-pollination. Five clones showed significant inbreeding depression in seed fertility. For 4 clones, fertilities of seeds from mixed pollination were similar to those resulting from