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Effective Population Sizes, Genetic Variability, and Mating System in Natural Stands and Seed Orchards of *Pinus sylvestris*

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Summary

Genetic variation at allozyme loci and outcrossing rates were estimated in three natural populations and two seed orchards of *Pinus sylvestris* L. in Finland. For the seed orchards, effective population sizes were estimated based on male strobilus and cone production, and predictions were made regarding levels of genetic variability and inbreeding in the offspring generation. The seed orchard crops had as much variation as the natural stands despite decreased effective population sizes due to variation in the number of ramets per clone and variation in gamete production between clones. Multilocus estimates of outcrossing in natural stands ($\hat{t}_m = 0.94$) and seed orchards ($\hat{t}_m = 0.98$) were higher than have been found using single locus methods. The level of inbreeding as measured by average fixation indices was low both in natural stands and seed orchards.

Key words: *Pinus sylvestris*, seed orchard, mating system, effective population size.

Zusammenfassung

Genetische Variabilität wurde in zwei Samenplantagen und drei natürlichen Beständen von *Pinus sylvestris* studiert. In den Samenplantagen wurde die effektive Populationsgröße auf Grund der Blühvariation geschätzt. Es gab keine Unterschiede in der Variabilität zwischen Samenplantagen und natürlichen Beständen mit Allozymloci gemessen, trotz kleiner effektiver Populationsgröße, jedoch wegen großer Unterschiede zwischen Klonen in der Gametenproduktion. Die Fremdbefruchtungsraten in natürlichen Beständen ($\hat{t}_m = 0,94$) und Samenplantagen ($\hat{t}_m = 0,98$) waren höher mit der Multilokusmethode als mit Einzellokusmethoden. Es gab auch weniger Inzuchteffekte,

sowohl in natürlichen Beständen als auch in Samenplantagen, als dies früher gefunden worden ist.

Introduction

Many commercially important tree species are still found primarily as natural stands and are only in the early stages of domestication. This is also true of most of Scots pine (*Pinus sylvestris* L.). For instance, in Finland more than 80% of the extant stands have originated through natural regeneration. At present, seedlings derived from seed orchards are used only for a small part of the regeneration, but the proportion is increasing. This represents a considerable change from the natural system of reproduction. The possible dangers of reduction in genetic variability or increases in the level of inbreeding have been discussed frequently (e.g. FAULKNER, 1975; ADAMS, 1981).

Scots pine is wind pollinated and in natural stands produces heavy flower crops. Pollen migration is considerable (KOSKI, 1970), and effective population sizes are expected to be large. Consequently Scots pine, like many other conifers, harbors large amounts of genetic variability at enzyme loci (MITTON, 1983; MUONA and SZMIDT, 1985; and references therein, GULLBERG *et al.*, 1985). Some seed is the result of fertilization by self-pollen (see SARVAS, 1962, for a description of the reproductive cycle), and despite embryonic mortality (see KOSKI, 1971), some selfs occur even among mature seed. Using rare isozyme markers, the percentage of viable selfed seed in the progeny of natural stands has been found to be low, varying between 5% and 10% (MÜLLER, 1977; YAZDANI *et al.*, 1985a; RUDIN *et al.*, 1986; and references therein). In the juvenile stages of develop-

ment, however, there is heavy mortality. The average annual seed crop in southern Finland may be a million seeds per hectare (KOSKI and TALLQUIST, 1978), and while a natural stand may start with 50 000 seedlings per hectare (LÖNNROTH, 1925), less than 1000 trees/ha grow in a mature stand (KOIVISTO, 1959). Because of partial selfing, there is some excess homozygosity over Hardy-Weinberg expectation at the seed stage. Most conifers are known to express severe inbreeding depression, which is expected to lead to differential mortality that selects against young inbred trees. This should lead to selective elimination of the excess homozygosity as a cohort ages. Findings on genotypic distributions supporting this idea have been made in Scots pine (YAZDANI *et al.*, 1985b) and in Douglas-fir (SHAW and ALLARD, 1982a).

Domestication and tree breeding interfere with both seed production and early juvenile stages. Because opportunity for selection to operate is much reduced in a nursery compared to natural stands, it is important that the seed have a low level of inbreeding to start with. There is also the goal of maintaining a high level of genetic variation even in cultivated stands of Scots pine. Rotation times are very long, and extensive genetic variability is considered the best insurance against crop losses. In order to produce seed that is genetically variable and non-inbred, seed orchards have to fulfill a number of requirements especially with respect to number of clones, flowering properties etc. For Finnish Scots pine seed orchards, these requirements have been listed by KOSKI (1980). The requirements that should insure variable and non-inbred seed essentially concern effective population sizes.

Genetic differences between seed crops from seed orchards and those from natural stands arise at two different stages. In the first stage, a limited number of clones are sampled to establish each seed orchard. Sampling from a wide geographical area relative to the size of a natural stand may increase variability in the orchard compared to wild populations, if the stands show geographical differentiation. On the other hand, the limited number of clones may lead to less genetic variability due to sampling effects on allelic frequencies. However, in a seed orchard setting the small population size concerns only one generation. The expected loss in variability due to drift in one generation is small when measured by change in expected heterozygosity (only $1/2N$). For example, if 50 gametes are sampled, only 2% of the heterozygosity is lost. This is in contrast to the strong depletion of genetic variability that occurs in populations where the small size is maintained for a large number of generations. Even if little variability

is lost in seed orchards according to this measure, rare alleles will be lost and allele numbers reduced. HATTEMER *et al.* (1982) have shown that with 25 clones the probability of losing alleles at frequency 0.01 in the original population is more than 0.60.

The second stage at which the genetic composition of seed orchard crops may become differentiated from that found in natural stands is during the reproductive cycle. Two factors are most likely to contribute in such finite populations. First, there is inbreeding, and second, drift may cause allelic frequency changes. The magnitude of both effects depends on the *effective population size*. The effective size of the population may be much less than the actual number of individuals, if the breeding behaviour of the population deviates from an ideal random mating population (see CROW and KIMURA, 1970). In an ideal population all individuals have the same expected progeny number (the variance is binomial). When the progeny number varies more among individuals, the effective size is smaller than the actual size.

In a small population the probability of inbreeding increases. In a random mating finite population the probability of selfing should be $1/N$. In a seed orchard there will also be some mating between and within ramets of the same clone, even with random mating. This is in addition to some selfing due to non-random union of gametes. The increase in the rate of selfing due to finite population size in an orchard can be estimated as follows. Let the probability that two uniting gametes come from the same individual be P . Each clone produces a proportion of p_i of the total pollen in the orchard. The increase in selfing of clone i due to finite population size will be exactly p_i , the probability of fertilization by its own pollen. The proportion of total seed production in the orchard of clone i is denoted by c_i . The proportion of selfed offspring due to finite population size is then $\sum p_i c_i$, measured at time of fertilization. The inbreeding effective size is defined as $N_{e(f)} = 1/P$ (CROW and KIMURA, 1970, pp. 345–361; KIMURA and OHTA, 1971, p. 36), in this $N_{e(f)} = 1/\sum p_i c_i$. If all clones are sexually symmetrical, i.e. they contribute their gametes equally through pollen and ovules, the formula reduces approximately to the standard formula

$$N_{e(f)} = (N\bar{k} - 1)/(\bar{k} - 1 + V_k/\bar{k}), \quad (1)$$

where N is the number of parents, \bar{k} is the average number of gametes contributed by parental individual, and V is the variance of gamete number (see CROW and DENNISTON, 1988). The increase in selfing will also be reflected in the

Table 1. — Latitudes and longitudes of natural stands and seed orchards of *Pinus sylvestris*, age of stand or orchard, number of clones/trees studied, number of seeds analyzed, and year of collection.

	Latitude (N)	Longitude (E)	Age	Number of trees/clones	Number of seeds	Year of collection
<u>Seed orchards</u>						
Viitaselkä	62° 15'	27° 35'	31	25	602	1984
Vilhelminmäki	62° 05'	25° 15'	27	28	965	1985
<u>Natural stands</u>						
Karvia	62° 12'	22° 50'	100	19	312	1984
Padasjoki	61° 25'	25° 00'	120	28	422	1970
Kangasniemi	61° 51'	26° 37'	80	22	176	1985

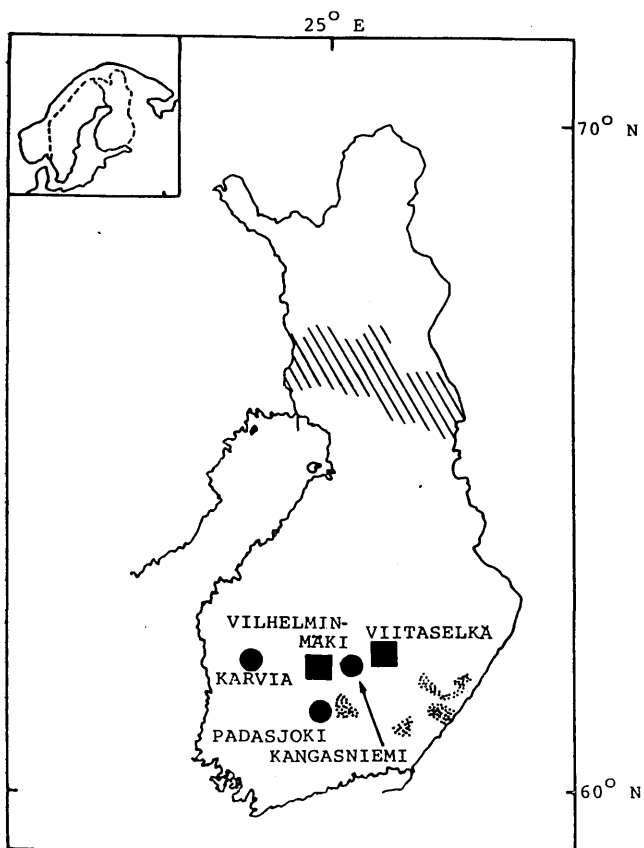


Figure 1. — Locations of natural stands (dots), seed orchards (squares), and origin of clones for southern (dotted) and northern (striped) clones of seed orchards.

offspring generation as a reduction in observed heterozygosity of $1/(2N_{e(f)})$.

Using isozyme loci, we compare the level of genetic variability and outcrossing in the seed from natural populations and from seed orchards of Scots pine. For the seed orchards, we shall also obtain estimates of their inbreeding effective population sizes given the composition and flowering properties of the orchard. This will allow us to consider whether the genetic properties of the seed crop (i.e. level of inbreeding) as reflected at isozyme loci can be predicted by the flowering properties of the orchard.

Material and Methods

Natural stands and seed orchards

The natural stands are described in Table 1 and their mapped locations are shown in Figure 1. Karvia and Padasjoki are standard pine stands of the Finnish Forest Research Institute. The third population, Kangasniemi, is a privately owned stand. For the standard stands, seed collected by single trees was provided to us by the Department of Forest Genetics, Finnish Forest Research Institute. In the Kangasniemi population, a severe storm uprooted a large number of trees on the island of Rämäinen on October 26th, 1985. On the following day, cones were collected evenly around the crown from 22 trees. The numbers of trees and seeds studied per stand are shown in Table 1.

The seed orchards are owned by the National Board of Forestry. The orchard Viitaselkä contains clones from southern Finland in a 7×7 m planting design. The number of clones is 25 and the total number of ramets is about

480. The Vilhelminmäki seed orchard contains 28 clones of northern origin, in a 5×5 m planting design. The total number of ramets in this orchard is about 980. We received seed samples from the Department of Forest Genetics, Finnish Forest Research Institute. For genetic variability statistics we used samples where seeds collected from all ramets of the orchard had been combined. For estimating outcrossing rates, we used collections from individual ramets.

Effective population sizes

To estimate effective population sizes we took into account variation in ramet number and variation in male flowering and cone production. We estimated pollen production in 1985 in the Viitaselkä seed orchard by counting numbers of male strobili in five ramets per clone. For each ramet, one branch per one meter of trunk was sampled and all strobili in that branch were counted. The total number of strobili per ramet was obtained by multiplying by the number of branches per trunk meter. The lengths of 100 male strobili were also measured for each clone to obtain an average. These data were converted into total pollen weights produced per clone by using the relationship that one cm of male strobili produces 0.028 gm of pollen (SARVAS, 1962). This is the standard method used by the Finnish Forest Research Institute (see Koski and Muona, 1986). Male flowering data for the Vilhelminmäki seed orchard for 1984 and cone production data for both orchards in 1985 were received from the Department of Forest Genetics, Finnish Forest Research Institute.

As the clones were sexually asymmetrical, we used the individual pollen and cone production data for estimating the inbreeding effective size. For each clone, p_i was the proportion of total pollen in the orchard, c_i the proportion of total cone production.

Note that for the Viitaselkä orchard our pollen production data are not for the year corresponding to the seed crop.

Electrophoresis

Standard methods for gel electrophoresis were followed (e.g. RUDIN and EKBERG, 1978). For the purposes of estimating genic diversity in the natural stands, 8 to 15 embryos (mean 13) analysed per tree. For the seed orchard crops sample sizes are given in Table 1. In addition, 12 embryos from a single randomly chosen ramet of each clone were analysed in the two orchards to obtain outcrossing rates. Before analysis, the seeds were germinated for about five days, until the radicle had emerged about 0.5 cm. We scored eight enzyme systems in the embryos, which code for 13 loci. The enzyme systems were leucine-amino-peptidase (EC 3.4.11.1), glutamate-oxalo-acetate transaminase (EC 2.6.1.1), fluorescent esterase (EC 3.1.1.1), glutamate dehydrogenase (EC 1.4.1.2), shikimate dehydrogenase (EC 1.1.1.25), malate dehydrogenase (EC 1.1.1.37), 6-phosphogluconate dehydrogenase (EC 1.1.1.44) and aconitase (EC 4.2.1.3). The first four enzyme systems were run on an ASHTON buffer system (pH 8.1) (ASHTON and BRADEN, 1961) and the remaining four on a tris-citrate buffer (pH 7.0) (SHAW and PRASAD, 1970). The locus coding the most anodal zone was numbered one, as was the fastest migrating band within each zone.

References for the staining methods and formal genetics of the banding patterns are as follows: *Lap-1* and *Lap-2* (RUDIN, 1977), *Got-1* and *Got-2* (RUDIN, 1975; RUDIN and EKBERG, 1978), *F-Est* (YAZDANI and RUDIN, 1982), *Gdh* (VALLEJOS,

1983; modified as in MUONA *et al.*, 1987), *Sdh-1* and *Sdh-2* (SZMIDT and YAZDANI, 1984), *Mdh-1* and *Mdh-2* (RUDIN and EKBERG, 1978), *6-Pgd-1* and *6-Pgd-2* (SZMIDT and YAZDANI, 1984) and *Aco* (SZMIDT and YAZDANI, 1987).

Data analysis

Average genic diversities ($1 - \sum p_i^2$) over 13 loci were estimated for all populations. Within each population, genotypic frequencies were compared to HARDY-WEINBERG expectation with G-tests (SOKAL and ROHLF, 1981). Differences between populations in allelic frequencies were tested with G-tests. The degree of differentiation between populations was measured with G_{st} -statistics (NEI, 1973). For the remaining analyses, the ten most variable loci were used (i.e. the loci above excluding *Lap-1*, *Got-1*, and *Sdh-2*). We estimated fixation indices, which measure deviations of observed heterozygosity from expected heterozygosity ($F = 1 - H_{obs}/H_{exp}$), and their variances according to BROWN (1970). The heterogeneity of fixation indices among loci within populations was tested with X^2 -tests (ELANDT-JOHNSON, 1971).

All progeny were assumed to be either the result of selfing or random outcrossing (the mixed-mating model, BROWN and ALLARD, 1970). The multilocus procedures of RITLAND and JAIN (1981) were used to estimate the proportion of progeny due to random outcrossing (t) in each population. In addition, single locus estimates were obtained with the method of BROWN and ALLARD (1970), as modi-

fied by CLGG *et al.* (1978). Mating system estimates are for the seven most variable loci (excluding a further three loci, *Lap-2*, *Mdh-1*, and *Aco*). Both average single locus (t_s) and multilocus (t_m) estimates are reported. Multilocus estimates were tested for deviation from 1.0 with z-tests.

Results and Discussion

Effective population sizes

For the natural stands we have no estimates of effective population sizes, but make the assumption that effective sizes are "large". This assumption is supported by earlier direct measurement of extensive pollen flow by Koski (1970), and by the lack of differentiation at enzyme loci between populations (GULLBERG *et al.*, 1985; MUONA and SZMIDT, 1985).

The effective size estimates for seed orchards are given in Table 2. The variation in pollen production between clones has two components: variation in the number of ramets per clone, and variation between clones in average pollen production per ramet. To give an idea of the relative importance of these factors, Table 2 gives the mean, standard deviation and the coefficient of variation of ramet number per clone, and of pollen production per ramet per clone, as well as the statistics for the joint effect, the total pollen production per clone. There was much more variation in number of ramets per clone in the Vilhelminmäki

Table 2. — Variation between clones in numbers of ramets per clone, pollen production/ramet, total pollen production, total cone production, relative gamete production per clone, and effective population sizes in two Scots pine seed orchards.

	Viitaselkä 1985	Vilhelminmäki 1984/85
Number of clones	25	28
Number of ramets		
mean	19.2	34.9
sd	6.1	25.9
C.V.	0.32	0.74
Pollen production per ramet (g)		
mean	500.9	68.2
sd	206.3	73.9
C.V.	0.41	1.08
Total pollen production per clone (g)		
mean	10 200	2 780
sd	5 770	3 810
C.V.	0.57	1.36
Total cone production per clone (l)		
mean	321	215
sd	245	222
C.V.	0.76	1.03
Total gamete production		
C.V.	0.52	0.97
Inbreeding effective population size	23.2	18.5

sd — standard deviation
C.V. — coefficient of variation

seed orchard than in Viitaselkä. This orchard was younger, the trees were smaller, and the overall pollen production was much lower. The absolute value of the variance in pollen production per clone was lower than in the Viitaselkä orchard, but the coefficient of variation, which is the important statistic, was much higher. Similar results hold for cone production in the two orchards (Table 2). Coefficients of variation for total gamete production are also given. In the Viitaselkä orchard the inbreeding effective population size was 93% of the actual size, in Vilhelminmäki only 66%.

These estimates of effective population size involve several assumptions. First, it is assumed that mating is at random. Second, these computations hold for a closed population. However, we have measured background pollination of about 30% in the Viitaselkä orchard (HARJU *et al.*, 1987). The inbreeding effects concern only the within orchard matings, all background pollinations are outcrosses.

We also made the assumption that pollen production is directly related to participation in fertilizations. This assumption is supported by the results of SCHOEN and STEWART (1986) in a white spruce seed orchard. We have ignored other components of variation in pollen production than the number of strobili, e.g. the timing of pollen production. Previous results by JONSSON *et al.* (1976) and our own observations in Viitaselkä in 1985 suggest that at least timing is of minor importance compared to strobilus number. We also assume that the total volume of cones produced is directly related to the number of seeds produced.

Previously, STERN and GREGORIUS (1972) used strobilus production data in a planted experimental stand of Scots pine to obtain estimates of inbreeding effective population sizes. Their estimates in three different years were about half the number of the actual trees in the stand. Thus, flowering variation has a large effect in such stands as well as in seed orchards.

The estimates of effective sizes in seed orchards provide background for interpreting the genetic data. We can predict expected changes in inbreeding and drift effects. In a closed population, we should expect to find a proportion of $1/N_{c(f)}$ fertilizations due to self-pollination between

ramets of the same clone in the total seed crop. This would equal 4.3% in Viitaselkä, 5.4% in Vilhelminmäki. However, these predictions concern only the matings where pollen comes from within the orchard.

Let us assume that the rate of outcrossing in large populations is t_n , and the rate of selfing due to the tree's own pollen cloud is $(1 - t_n)$. Further, assuming that the difference in rate of outcrossing between seed orchards (t_o) and large natural stands (t_n) is due to small N , then

$$t_o = m + t_n(1-m)(1-1/N),$$

where m is the rate of fertilization by pollen from background sources. When equal sample sizes are used for each clone, then N is the actual number of clones, not the effective population size. The decrease in outcrossing estimates in Viitaselkä and Vilhelminmäki should be 4% and 3.6%, respectively. Background pollination decreases the effect of small N within the orchard. A possible increase in self-fertilization can be further diluted before census stage because selfs suffer heavy embryonic mortality (KOSKI, 1971).

Similarly, background pollination will also influence the expected change in observed heterozygosity. Within the about 70% of zygotes that arise due to pollinations within the orchard, heterozygosity will decline by $1/2N_{c(f)}$. For the other 30%, there will be no decrease in observed heterozygosity due to inbreeding. In fact, if the allelic frequencies differed considerably between background pollen and the orchard, and thus between the male and female gametic pools, heterozygosity could increase in this fraction of the zygotes. As above, the influence of inbreeding on heterozygosity at the mature seed stage is lessened by embryonic mortality of selfs.

Genetic diversity

Allelic frequency data are not given, but a summary of the average genic diversities (expected heterozygosities) over all 13 loci in each population is shown in Table 3. These are not a random set of loci with respect to genic diversity, but were chosen for use in this study because they are polymorphic. However, since the same set of loci was sampled in all populations, valid comparisons of rela-

Table 3. — Average expected heterozygosity (H_{exp}), total numbers of alleles (N_{all}) over 13 loci, average fixation indices (F) over ten loci and χ^2 tests for their heterogeneity in two Scots pine seed orchards and three natural stands.

	H_{exp}	N_{all}	F	χ^2 over loci	P
<u>Seed orchards</u>					
Viitaselkä					
25 clones	0.276	29			
481 ramets	0.276	29			
seed crop	0.277	39	0.006	41.56	0.000
Vilhelminmäki					
28 clones	0.259	31			
980 ramets	0.243	31			
seed crop	0.256	40	0.000	36.98	0.000
<u>Natural stands</u>					
Karvia	0.271	33	-0.006	17.4	0.043
Padasjoki	0.293	35	0.059	11.3	0.25
Kangasniemi	0.286	36	0.060	27.3	0.002

Table 4. — Degree of genetic differentiation (G_{ST}) between seed crops from different groups of populations of *Pinus sylvestris*.

Locus	Three natural stands	Natural stands and Viitaselkä seed orchard	Viitaselkä and Vilhelminmäki seed orchards
Lap-1	0.010	0.013	0.008
Lap-2	0.002	0.007	0.001
Got-1	0.003	0.003	0.003
Got-2	0.056	0.052	0.027
F-Est	0.002	0.003	0.007
Gdh	0.028	0.031	0.029
Sdh-1	0.010	0.052	0.118
Sdh-2	0.009	0.013	0.023
Mdh-1	0.019	0.014	0.006
Mdh-2	0.010	0.026	0.045
6-Pgd-1	0.014	0.013	0.000
6-Pgd-2	0.002	0.008	0.011
Aco	0.000	0.014	0.016
Average over loci	0.017	0.024	0.029

tive amounts of allelic diversity can be made. For the Viitaselkä and Vilhelminmäki seed orchards, we show the diversities for the initial collection of clones (25 and 28), for the actual ramets in the orchard (480 and 980, respectively), and the seed crops of the orchards.

The natural populations contained much variability, with average expected heterozygosity ranging from 0.271 to 0.293. With the large sample sizes used, there were several cases of statistically significant differences in frequencies between stands. However, the genic diversity statistics showed that less than 2% of the overall variation in these population is between, and more than 98% within stands (Table 4). The populations we studied were all from a relatively small area. This result agrees with other studies on conifers that show a low level of differentiation between populations (MITTON, 1983; LOVELESS and HAMRICK, 1984; MUONA and SZMIDT, 1985; GULLBERG *et al.*, 1985).

Next, we can ask whether genic diversity in seed orchard crops is similar to that from the natural populations. We first compare the average expected heterozygosity (H_{exp}) among the parental clones to that found in the natural stands. Over the thirteen loci and three natural populations H_{exp} was 0.283. In the Viitaselkä parental clones (and ramets) H_{exp} was 0.276. Thus, no reduction took place at this stage in average expected heterozygosity. However, the total number of alleles was slightly less in the seed orchard parents than in the natural stands. For the Vilhelminmäki seed orchard, H_{exp} was 0.259 among the 28 clones and 0.243 among the 980 ramets. This may mean a slight reduction in diversity compared to southern stands (0.283), but we are not able to compare this seed orchard to the northern populations where these clones originally came from. For both orchards, losses in variability due to sampling in one generation were small, as expected, and the parental clones had about as much variability at these marker loci as there is in the natural populations. The seed crop from the Viitaselkä seed orchard had an average expected heterozygosity of 0.277, same as the parental ramets. In the Vilhelminmäki orchard H_{exp} in the seed crop (0.256) was also similar to that found in the orchard parents. No loss of variability was detected.

Background pollination has probably more than compensated for the expected loss of diversity.

The results in Table 3 show that there was hardly any difference between the orchard parents and the seed crop in genetic variability. Even though effective population sizes were reduced, the expected losses in variability in one generation are so small to be barely detectable. Background pollination makes such detection even more unlikely by introducing new alleles to the pollen pool, and influencing frequencies of others. The effect of background pollination is visible in Table 3. Both orchard crops had higher numbers of alleles than were found in the parents. The allele numbers were also higher than in the natural stands, probably due to the larger sample sizes.

Genic diversities and allele numbers in seed orchards of *Pinus radiata* from Australia and *P. sylvestris* from Sweden have been previously reviewed by ADAMS (1981). Those data also showed that there is little if any loss in variability, as measured by expected heterozygosity at enzyme loci, in the seed crops of seed orchards as compared to natural stands. Similar findings were also made in an extensive study on Scots pine by SZMIDT and MUONA (1985) in northern Sweden.

A comparison between the natural population seed samples and the seed orchard crops in Table 4 shows that despite several significant differences in allelic frequencies, the overall level of differentiation was small between the stands and the southern Viitaselkä orchard. Many of the alleles not found in the orchard parents were found in the seed crop due to background pollination. Allelic frequency differences between the northern and southern seed orchards were also small (Table 4). This last result is in accordance with earlier findings by MUONA and SZMIDT (1985), who found similar allelic frequencies in natural stands in northern Sweden as we found in Finnish populations. Also, CHUNG (1981) found that plus trees in a cone bank from northern, central and southern Finland did not differ much in their allelic frequencies. However, a share of the alleles of the Vilhelminmäki orchard crop are from the surrounding southern stands. This may also increase the similarity in allelic frequencies.

Mating system estimates and level of inbreeding

Multilocus and mean single locus estimates of proportions of outcrossed progeny are given for the two seed orchards and three natural populations in Table 5. The multilocus estimates of outcrossing ranged from 0.92 to 0.99. In two of the three natural stands (Padasjoki and Kangasniemi) the outcrossing estimates deviated significantly from 1.0

Table 5. — Multilocus (t_m) and mean single locus (t_s) estimates of proportions of outcrossed progeny in seed samples from two *Pinus sylvestris* seed orchards and three natural stands, along with estimates of standard deviations of t_m (sd_{t_m}) and z-tests for testing deviations of t_m from $t = 1.0$.

	t_s	t_m	sd_{t_m}	z	P
Seed orchards					
Viitaselkä	0.940	0.976	0.017	1.26	0.21
Vilhelminmäki	0.988	0.978	0.017	1.30	0.19
Natural stands					
Karvia	0.996	0.986	0.023	0.60	0.55
Padasjoki	0.878	0.928	0.019	3.80	0.001
Kangasniemi	0.855	0.919	0.030	2.70	0.007

($P < 0.05$), while in Karvia, t_m was nearly 1.0. Estimates of t_m were high for both orchards and not significantly different from one. The averages of single locus outcrossing rates were in most cases lower than the multilocus estimate (the average difference was 0.026). Differences between multilocus and the mean of single locus estimates have been reported in other species (see for instance FURNIER and ADAMS, 1986). When \bar{t}_s is lower than t_m , inbreeding other than that due to selfing may be indicated (SHAW and ALLARD, 1982b). This might account for the difference between t_m and \bar{t}_s in two of the natural populations.

Our multilocus estimates of outcrossing were higher than many previous estimates on Scots pine, which have mostly been based on rare alleles. Mating system estimates based on specific rare alleles are sensitive to the occurrence of the same rare allele in other trees elsewhere in the population, which may be one reason for the difference. Our average single locus estimates, which were not based on rare alleles, were only slightly lower than multilocus estimates. In general, single locus estimation methods are regarded as more susceptible to the violations of the mixed-mating model (SHAW and ALLARD, 1982b).

The trends in outcrossing rate between stands and orchards were similar for the single and multilocus estimates. There were clear differences between the natural stands, with the Karvia stand having the highest outcrossing rate. All stands were approximately even aged and there were no important differences in stand density. We do not know whether the variation is due to differences in self-fertility, or maybe to differences in flowering patterns. Variation between populations in outcrossing rates have been demonstrated for other species as well (NEALE and ADAMS, 1985; SHEA, 1987).

Our initial prediction was that due to reduced population size, we should observe higher frequencies of progeny due to self-fertilization in seed orchards than in natural stands. However, t_m was on average not lower in seed orchards than in natural stands. In fact, the predictions showed that only small decreases in estimates of outcrossing rate are to be expected, even if there is a large reduction in the inbreeding effective size. This effect is reduced after fertilization, because a large share of the zygotes due to self-fertilization are expected to die during embryonic development (KOSKI, 1971). Moreover, background pollination increases the rate of outcrossing. In all, replication of ramets is likely to have a very small influence on the rate of selfing, which is primarily determined by the probability of non-random mating within a tree. Outcrossing rates in seed orchards should resemble those in natural stands.

Previous estimates of selfing for natural or seed tree stands of *P. sylvestris* have been 6.2% for 6 trees (MÜLLER, 1977), 24% for 7 trees (RUDIN *et al.*, 1977), 11.2% for 12 trees (YAZDANI, *et al.*, 1985a) and 12% for five trees (RUDIN *et al.*, 1986). For seed orchards, the published estimates have been 2% to 5% (RUDIN and LINDGREN, 1975), 6.2% for one clone (SHEN *et al.*, 1981), 16% for 5 clones (RUDIN and EKBERG, 1982), 11.5% to 13.9% for one clone in three seasons (MÜLLER-STARCK, 1982), and 8% for five clones (RUDIN *et al.*, 1986). Estimates based on just a few trees may not give reliable population estimates, because individual trees of many species vary in outcrossing rates (SHAW and ALLARD, 1982b; RITLAND and EL-KASSABY, 1985; OMI and ADAMS, 1986; KOSKI and MUONA, 1986). Altogether, previous results and the present study do not provide evidence for consistent differences between selfing in natural stands and seed

orchards. This is also true of Douglas-fir (*Pseudotsuga menziesii*) (SHAW and ALLARD, 1982b).

Our estimates were based on approximately equal seed samples per clone or tree. The overall level of outcrossing in the total seed crop may be different, due to variation between clones in the rate of outcrossing and seed production. Individual tree or clone estimates should be weighted by their relative seed production to arrive at the proper population estimate.

The average fixation indices (Table 3) in natural stands were fairly low and in the seed orchards about zero. Most of the single locus values did not differ significantly from zero. Due to reduced inbreeding effective size, positive fixation indices of $1/N_{e(f)}$ were expected in seed orchards, but not observed. This may be due to background pollination. Small allelic frequency differences between ovules and pollen due to fertility selection may also increase the observed heterozygosity. These fixation indices were much lower than were observed by SZMIDT and MUONA (1985) for seed crops of natural stands and seed orchards of Scots pine in northern Sweden ($F = 0.085$ and higher). Those estimates were made from samples of commercial collections, where seed from different trees had been combined, and thus no mating system estimates were available. However, in equilibrium populations, the relationship between F and t is $F = (1 - t)/(1 + t)$, and thus $t = (1 - F)/(1 + F)$. Fixation indices of 0.085 and higher suggest outcrossing rates of 0.84 and lower. A possible explanation is that in the more northern conditions flowering is more irregular, and gives rise to more inbreeding. Alternatively, there may be more self-fertility in the northern trees. TIGERSTEDT *et al.* (1982) have also observed significant excess of homozygotes in a northern population of *P. sylvestris*.

In all, no large changes are expected to be found in the level of inbreeding or level of genetic variability in clonal seed orchards, even if the number of clones is fairly low. Measurements of genetic diversity and outcrossing rates showed that the values were similar between orchards and natural stands. However, this does not mean that the seed orchards are producing seed of the desired genetic quality. First, there is intense fertility selection in the orchards, as evidenced by the decreased effective population size. This does not cause large losses in genetic variability or increases in inbreeding in one generation, especially because background pollination contributes new alleles. However, those traits that have positive genetic correlations with flowering characteristics in the orchard will increase in the offspring generation (see NIKKANEN and VELLING, 1987). Such correlated responses to fertility selection may be important. Second, inbreeding, even if no more frequent than in natural stands, may still be harmful. In the wild, early mortality eliminates inbreds, but may not do so in nurseries (MUONA *et al.*, 1988). Increased mortality and poor growth in plantations may be a consequence. Third, even though background pollination may make the seed genetically variable and non-inbred, it also results in offspring of a lower genetic quality. In the case of Vilhelminmäki, background pollination makes the seed unsuitable for the target area of northern Finland, because the seedlings will not be sufficiently hardy (SARVAS, 1970).

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