Pollen Migration into a Seed Orchard of *Pinus sylvestris* L. and the Methods of its Estimation using Allozyme Markers

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Gene diversity and foreign pollen migration into a Scots pine seed orchard in northern Sweden were examined using allozyme markers. The gene diversity and fixation index varied among loci and the average values in embryos over 18 loci were 0.262 and 0.038 respectively. The observed pollen migration into the seed orchard was 30%. Two different methods were used to adjust the observed pollen migration for undetected foreign pollen. The pollen migration into the orchard after adjustment using these methods was 51% and 55% respectively. In addition, sampling procedure and other factors which can influence genetic parameters inferred from allozyme analysis are discussed. *Key words: Pinus sylvestris, seed orchards, pollen migration, gene diversity, allozymes.*

INTRODUCTION

The objective of wind-pollinated seed orchards is to produce sufficient amounts of seed of good genetic quality and to achieve high genetic efficiency. This efficiency is defined as the degree to which the parental clones transfer their genetic superiority and gene diversity to the progenies of the seed orchard. However, the aforementioned goals can be achieved only if several assumptions are met (Woessner & Franklin, 1973). One of these assumptions stipulates that the seed orchard should be isolated from external pollen which can seriously deteriorate the genetic quality of seed crops. Unfortunately, in practice it is difficult to achieve complete isolation from external pollen sources (Squillace, 1967; Squillace & Long, 1981).

Allozyme markers have been used to estimate pollen migration directly (Friedman & Adams, 1985; Smith & Adams, 1983; Nagasaka & Szmidt, 1985; El-Kassaby et al., 1989; Harju & Muona, 1989). Such estimates are important to seed orchard managers who must determine what improvements should be made in order to ensure good genetic quality of seed orchard seed.

In this paper, gene diversity and pollen migration in a 25–27 year old Scots pine (*P. sylestris* L.) seed orchard were estimated by the aid of multilocus allozyme analysis. The orchard includes the most northern clones from Sweden and is the oldest orchard of Scots pine in this country. Two different methods were employed to estimate the probability of detecting background pollen. Some possible factors which may influence such estimates were discussed.

MATERIALS AND METHODS

The seed orchard

This study was conducted in the Skaholma clonal Scots pine (*P. sylvestris* L.) seed orchard close to Umeå in northern Sweden (latitude 63°50′, longitude 20°15′). The area of this orchard is 16 ha. The initial spacing for most parts of the seed orchard was 4 × 4 m, but in some small sections 6 × 6 m spacing was used. Within some hundred meters from the seed orchard there are only a few individuals of Scots pine scarcely dispersed among stands of Norway spruce. The seed orchard was established with grafts from 34 clones, which were 25–27 years old at the time of sample collection in 1986. As losses of grafts occurred, mainly because of vole damage, an additional 8 clones have been included. These grafts were 13–18 years old in the sampling year.

Plant material

Cones from 34 older clones were collected in September 1986. Seeds from one ramet per clone have been analyzed in this study. These ramets are situated in three different blocks. Cones were dried at room temperature and the seeds were then extracted and kept at 2°C until analysis. No cones were found on the grafts of the 8 younger clones in 1986. Therefore, buds were collected from these clones to facilitate determination of their genotypes.

Electrophoretic methods

Allozyme polymorphism was assessed at 18 allozyme loci in haploid macrogametophytes, diploid embryos and buds. Before analysis seeds were germinated for approximately 7 days until a 3 mm radicle emerged from the seed coat. Protein extraction was carried out in a 0.05M Tris–0.33M glycine buffer, pH 8.3 containing 1% soluble polyvinylpyrrolidone (MW 40.000). Separation of allozymes was carried out in 12% starch gels. The buffer systems used, staining, running conditions and inheritance patterns of the enzyme markers studied have been described elsewhere (Szmidt, 1984 and references therein).

Genetic analysis

Genotypes of the parental clones were inferred from at least 10 macrogametophytes per clone. Genotypes of the 8 younger clones were inferred from buds. Genetic composition of progeny was assessed for 359 embryos. A locus was considered polymorphic if the frequency of the most common allele was less than 0.98. The expected (He) and observed (Ho) heterozygosities were computed from diploid clone and embryo data. The fixation indices were computed as described by Curie-Cohen (1982). Chi-square tests were used to test the deviation of the observed fixation indices from panmitic expectations for the embryos. The multilocus genotypes of ovule and pollen gametes were deduced from comparisons between allozyme patterns in macrogametophytes and the corresponding embryos. Pollen gametes whose multilocus genotypes could not have been produced by any of the seed orchard clones were regarded as pollen grains of external origin (Nagasaka & Szmidt, 1985).

The observed pollen migration represents a minimum estimate. The genotypes of some gametes from external sources can match those produced by seed orchard clones and will go undetected. If the genetic composition of the external pollen cloud is known, an estimate of the contribution of these indistinguishable gametes can be made (Smith & Adams, 1983). The adjusted estimate (M) can be calculated as:

$$M = C/p_d$$

where C is the observed proportion of pollen gametes with multilocus genotypes that cannot be produced by any seed orchard clone and p_d is the probability that a genotype of a foreign

pollen gamete is detected. We utilized two different methods to calculate p_d. The first method included simulation of the pollen genotypes assuming that all pollen originated from outside the orchard. This was made by replacing the experimentally determined pollen alleles with a set of randomly derived alleles based on the assumed allele frequencies in the foreign pollen population. This simulated data set was analyzed in the same way as the experimental data. The second method used to estimate p_d employed multinomial probability calculations (Smith & Adams, 1983; Harju & Muona, 1989). In this method, all gametes the seed orchard clones can produce are listed. The multinomial probability that each gamete will occur in the background pollen clound is calculated and summed. In this way the expected frequency of detectable foreign pollen gametes can be estimated. Both methods require that the allelic frequencies in the foreign pollen pool are known. We used the average of the gene frequencies in seven Swedish Scots pine seed orchards (unpublished results). In both methods, no effect of linkage was assumed. We also estimated the proportion of foreign genotypes in the ovule pool produced by the orchard. The observed contamination on the female side was subtracted from the observed pollen contamination in order to avoid overestimates of contamination.

RESULTS

Allozyme frequencies for the parental clones and their embryos are given in Table 1. Of the 18 loci studied 15 loci were polymorphic (0.98 criterion) and only Lap-1, Pgi-1 and Pgm-2 were monomorphic in both parental clones and embryos. At some loci (Got-1, Got-2, Fest, Aco, 6-pgd-1 and Shdh-1) certain allozymes observed in low frequencies in embryos could not be found in the orchard clones. These alleles have probably been contributed by pollen from the surrounding stands. The observed (Ho) and expected (He) heterozygosities in clones and embryos along with fixation indices (F) in embryos are given in Table 1. The average expected heterozygosity (=gene diversity) over 18 loci in embryos (0.262) was similar to that in orchard clones (0.264). The average fixation index in embryos was 0.038. The expected heterozygosity and F values varied among loci. Only at some loci the F values differed significantly from those expected under panmixia (Table 1). The number of different gamete genotypes at 18 loci that can be produced by the Skaholma clones was 1733. The observed number of different gamete genotypes in the ovule and pollen pools was 253 and 294 respectively. The proportion of foreign female gametes in the investigated embryos was only 0.28% which indicates that incorrect labelling of ramets or errors in genotype scoring were not important in our study. The observed pollen migration into the Skaholma seed orchard based on 18 loci was 30%. In the corresponding simulated data set the probability of detecting a foreign gamete (p_d) was 0.58. By multinomial calculation the value of p_d was estimated as 0.54. After the correction for contamination in the ovule pool and adjustment for undetected pollen the estimates of M obtained by the simulation and multinomial probability methods become 51% and 55% respectively. The observed proportion of migrant pollen grains varied considerably among individual half-sib families. Unfortunately, the size of the individual families (approximately 10 embryos) was too small to allow detailed analysis of this variation and thus has not been investigated further in this study.

DISCUSSION

Clones comprising a seed orchard are expected to contribute as much as possible to the seed crops. Hence, it is important to study the genetic diversity of seed orchard crops for it can show how much of the genetic potential of orchard clones has been transferred to the progeny.

Table 1. *Gene frequencies, observed (Ho) and expected (He) heterozygosities, fixation indices (F) in Skaholma clones and embryos*

Locus	Allele	Clones	Embryos	Ho clones embryos	He clones embryos	F embryos
Aco	1	0.036	0.024	0.214	0.196	
	2	0.893	0.931	0.138	0.131	−0.074
	3	0.071	0.043			
	4	0.000	0.002			
Adh-1	1	0.298	0.290	0.405	0.418	
	2	0.702	0.710	0.364	0.411	0.115*
Aph	2	0.012	0.010	0.333	0.317	
	3	0.810	0.788	0.331	0.342	0.036
	4	0.167	0.192			
	5	0.012	0.010			
Fest	1	0.655	0.704	0.452	0.511	
	2	0.155	0.121	0.465	0.460	0.009
	3	0.190	0.172			
	4	0.000	0.003			
Gdh	1	0.357	0.314	0.429	0.459	
	2	0.643	0.686	0.462	0.431	−0.072
Got-1	1	0.000	0.001	0.048	0.046	
	2	0.976	0.979	0.037	0.042	0.114*
	3	0.024	0.020			
Got-2	1	0.012	0.009	0.452	0.512	
	2	0.500	0.549	0.476	0.506	0.062
	3	0.488	0.439			
	4	0.000	0.003			
Lap-1	2	0.988	0.992	–[a]	–	–
	3	0.012	0.008	–	–	–
Lap-2	1	0.012	0.011	0.119	0.113	
	2	0.940	0.954	0.087	0.089	0.015
	3	0.048	0.035			
Pgi-1	2	1.000	1.000	–	–	–
Pgi-2	2	0.036	0.069	0.238	0.216	
	3	0.881	0.845	0.271	0.274	−0.033
	4	0.083	0.086			
Pgm-1	1	0.048	0.048	0.119	0.113	
	2	0.940	0.951	0.076	0.093	0.189*
	3	0.012	0.001			
Pgm-2	2	1.000	1.000	–	–	–
6-pgd-1	1	0.524	0.564	0.619	0.499	
	2	0.476	0.435	0.456	0.493	0.079
	4	0.000	0.001			
6-pgd-2	1	0.667	0.663	0.476	0.466	
	2	0.298	0.294	0.451	0.473	0.016
	3	0.012	0.014			
	4	0.024	0.029			

Table 1. *(Contd.)*

Locus	Allele	Clones	Embryos	Ho clones embryos	He clones embryos	F embryos
Mdh-1	1	0.071	0.115	0.143	0.133	
	2	0.929	0.885	0.207	0.204	−0.014
Mdh-2	1	0.655	0.663	0.548	0.481	
	2	0.298	0.320	0.427	0.458	0.084
	5	0.012	0.010			
	7	0.036	0.007			
Shdh-1	1	0.036	0.026	0.286	0.277	
	2	0.845	0.822	0.301	0.313	0.040
	3	0.048	0.081			
	4	0.071	0.065			
	5	0.000	0.004			
	6	0.000	0.001			
Clones	–	–	–	0.271	0.264	–
Embryos	–	–	–	0.253	0.262	0.038

[a] A dash indicates that Ho, He and F values were not calculated if the frequency of the most common allele was higher than 0.98.
* Significant ($p < 0.05$), see Material and methods for explanation.

The average gene diversity (He) in the embryo population from Skaholma was similar to that in the parental clones. This could indicate that most of the diversity of the parental clones has been transferred. However, in our study, an equal number of embryos was sampled from each individual family. Therefore, the maternal contribution to progenies from each clone was equal which certainly does not reflect the real situation. Differences among clones regarding female and male gamete production have frequently been found in Scots pine seed orchards (Sarvas, 1962; Jonsson et al., 1976; Müller-Starck, 1982; Muona & Harju, 1989). Thus, our sampling procedure could increase the genetic contribution of those clones which had lower seed production and decrease the contribution of clones characterized by abundant seed production. In addition, the observed high pollen migration into the orchard could cause further increase of the genetic variability in embryos. In order to determine the acutal genetic composition of seed orchard crops a sampling procedure reflecting effective contribution of individual clones should be used. Apparently, this can be achieved by random sampling of commercial seed crops.

The average fixation index for embryos was 0.038 which indicates homozygosity excess in comparison to panmictic expectations. An increased F value in embryo progenies are often observed and can probably be attributed to selfing and non-random mating (e.g. Moran et al., 1980; Shaw & Allard, 1982). It thus appears that panmictic mode of reproduction is not achieved in the investigated seed orchard.

Pollen migration from external sources can be a critical factor in reducing genetic efficiency and a serious obstacle to achieving potential genetic gains from wind-pollinated seed orchards. This problem is especially important when background pollen comes from stands poorly adapted to the intended planting site of the orchard seed crops (Eriksson et al., 1980; Sniezko, 1981). Skaholma seed orchard is the oldest seed orchard studied so far for pollen migration in Sweden. In addition, this orchard appears to be well isolated from natural

stands of Scots pine. Pollen production in Skaholma was estimated to be 30 kg/ha (unpublished results). Such pollen crop may be regarded as sufficient to guarantee a low level of contamination with background pollen (Koski, 1975). In spite of all that, the proportion of foreign pollen found in our study was unacceptably high. It thus appears that preventing excessive migration of foreign pollen appears necessary even in older seed orchards.

The most notable approaches in alleviating this problem are, e.g. supplemental-mass-pollination (Bridgwater & Trew, 1981; El-Kassaby & Ritland, 1986 *a*; Yazdani et al., 1986) and phenological time isolation (Silen & Keane, 1969; El-Kassaby & Ritland, 1986 *b*). Unfortunately, none of these methods of preventing background pollen appears to be sufficiently effective. Moreover, the latter method is hardly applicable to the Swedish seed orchards which are usually located south of the clone origin. Delaying clone flowering in these orchards would synchronize their phenology with that of the local stands. Pollen dilution zones around seed orchard have also been used as means for preventing background pollen. For most pine species, however, these zones have failed to sufficiently decrease migration of foreign pollen (Sarvas, 1970; Wright, 1953; Squillace & Long, 1981). An alternative approach to allaying the reduction in genetic gain caused by pollen migration is to locate the seed orchard in a place where the surrounding stands are not of poorer genetic quality than the base population from which the clones were selected. Otherwise, the consequences of pollen migration will be more severe than previously expected.

Nevertheless, the pollen migration into Skaholma found in this study is strikingly high when one takes into account the age and location of this orchard. It is possible that our estimates are inflated due to the occurrence of additional clones in Skaholma which have not been included in our material. In this study, the concordance of documentation with reality was assumed and only 42 ramets representing individual parental clones were analyzed. Such concordance may not exist in practice (Harju & Muona, 1989). The observed contamination in the ovule pool was very low which implies that additional female parents were practically absent. However, only one ramet per clone was analyzed in this study. Therefore, to find whether additional clones occur in Skaholma all ramets should be analyzed.

Finally, the multilocus method itself is very sensitive to various kinds of misinterpretations such as, e.g. incorrect scoring of individual allozymes. These misinterpretations may increase estimates of pollen migration based on the multilocus approach. Harju & Muona (1989) have discussed the applicability of various methods of measuring background pollination. The authors concluded that due to small differences in allelic frequencies between orchards and natural stands of Scots pine the methods based on the finite population (e.g. Smith & Adams, 1983) are more suitable than those based on differences in gene frequency (El-Kassaby & Ritland, 1986 *b*). Therefore, the method of Smith & Adams (1983) was also used in this study. In addition, we developed and used a new method which employs simulated background pollen cloud. The main reason for a simulation approach was the presence of missing data at some loci. Exact calculations are different for each multilocus constellation of missing data. With the simulation approach the data are easily simulated with the structure of missing data that is identical to that in the experimental data. The results obtained with the two methods were very similar. Therefore, we suggest that simulation method represents useful alternative in studies aimed at measurements of pollen migration.

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