DIFFERENCES IN POLLEN TUBE GROWTH AND MORPHOLOGY AMONG SCOTS PINE PLUS TREES

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ABSTRACT

The aim of this study was to determine whether the growth rate and morphology of the pollen tube vary among Scots pine plus trees selected from natural populations. The material for the study was chosen so that the average pollen tube growth and the height growth of the open-pollinated progenies of the plus trees could be compared, and thus estimate possibilities to apply male gametophytic selection in Scots pine breeding. The pollen samples were collected from even-aged and homogenously growing grafts of plus trees located in the same clone bank, and then germinated *in vitro*. Significant differences in pollen tube growth were found among pollen donors, thus indicating that there is genetic potential for male gametophytic competition. The importance of pollen competition, however, is restricted by other features in the reproductive biology of Scots pine. According to the results, there seems to be no possibility to apply gametophytic selection in Scots pine breeding programs for improving the growth rate of progeny. The variation in pollen tube growth rate found on population level may have an impact on the genetic composition of seed produced in seed orchards.

Key words: *Pinus sylvestris*, pollen competition, *in vitro* pollen tube growth, male gametophytic selection

INTRODUCTION

In higher plants, the gametophytic phase has traditionally been considered to be just a vector for transmission of the genome to the sporophyte, in contrast to lower plants in which selection pressure acts mainly on the haploid generation. During recent decades, however, it has been suggested that also in angiosperms the gametophyte independently expresses its own genetic information and is exposed to selection that may have an influence on the genetic constitution of the next sporophytic generation. This hypothesis is based on the overlap in genetic expression between the gametophytic and sporophytic generations and the competition among male gametophytes (MULCAHY 1979, OTTAVIANO *et al.* 1988, HORMAZA & HERRERO 1996). The fitness of male gametophytes depends both on paternal traits, which include the phenology of male flowering and amount of pollen produced, and on pollen grain traits, such as viability, germination time, pollen tube growth rate and selective fertilization (PFAHLER 1975).

In several studies, it has been shown that progeny produced by the fast-growing pollen grains outperform progeny produced by the slow-growing pollen (MULCAHY 1983 and references therein, QUESADA *et al.* 1993). According to CHARLESWORTH *et al.* (1987), it is not clear whether male-to-male competition occurs

during pollen tube growth, mainly because there is little evidence for genetic variation in pollen tube growth rates or competitive abilities in natural populations. Even if competition between pollen tubes were demonstrated, without further experiments showing that the better performance of the progeny was in fact heritable, one could not conclude that faster-growing pollen tubes yield genetically superior progeny (CHARLESWORTH 1988). So far, no population level studies on variation in the pollen tube growth of gymnosperms or on the associations between the tube growth rate and the performance of the progeny have been reported.

In breeding programs for forest tree species it would be desirable to use strategies that allow early selection of genotypes, of which gametophytic selection could be one. In this approach, different genotypes can be evaluated by screening the pollen produced by these plants. The main limitation of gametophytic selection is that it can only be used for traits that are expressed at the cellular level. The approach has been used successfully to screen for tolerance to different temperatures, osmotic pressure, heavy metals, fungal toxins, herbicides, antibiotics, or air pollution (HORMAZA & HERRERO 1996). For forest tree species, the main trait on which breeding efforts are focused, is wood production, the height growth of trees being the most frequently used selection criteria.

The breeding program for Scots pine (*Pinus sylvestris* L.) in Finland was started in 1947, and at the moment the first predictions of the breeding values of the phenotypically selected plus trees have been obtained through progeny testing (VENÄLÄINEN 1993). Information on breeding values has been used for thinning the first-generation seed orchards and for establishing new seed orchards with clones that have excellent growth and quality characters. To ensure optimal functioning of seed orchards, the amount and phenology of male and female flowering of the clones have also had to be taken into account. There is, however, no information on potential competition among male gametophytes that could also affect the genetic composition of the seed produced.

The aim of the present study was to determine whether there is variation in the pollen tube growth rate and morphology among the Scots pine plus trees selected from natural populations. The pollen tube growth was studied using *in vitro* germination method which enables easy and fast screening of numerous plus trees. The material for this study was chosen so that it was possible to compare the average pollen tube growth of the plus trees with the height growth of their open-pollinated progenies and thus estimate the possibilities to apply gametophytic selection in Scots pine breeding.

MATERIAL AND METHODS

Plus tree material

The Scots pine plus trees used as pollen donors in this study were chosen to represent the variation mainly within one breeding population. These plus trees originate in the lake district in the southern part of Finland (breeding zones 1 and 2). In order to illuminate the variation between distant geographical regions, northernmost Lapland (zone 11) was taken as a comparison area (Figure 1). The potential differences between the southern and northern plus trees are interesting, since the seed orchards for northern Finland have been established in southern Finland to enhance seed maturation. Later, it has been realised that background pollination affects the genetic composition of the seed produced in these orchards (HARJU & MUONA 1989, PAKKANEN & PULKKINEN 1991, HARJU & NIKKANEN 1996).

Of the 1354 Scots pine plus trees selected from breeding zones 1 and 2, 880 have already been ranked according to the prediction of their breeding value for height growth based on 263 separate, 10 to 15-year-old, progeny tests. In these trials, the coefficient of variation for height has been 6 %. The progeny test data have been combined using a modification of the performance
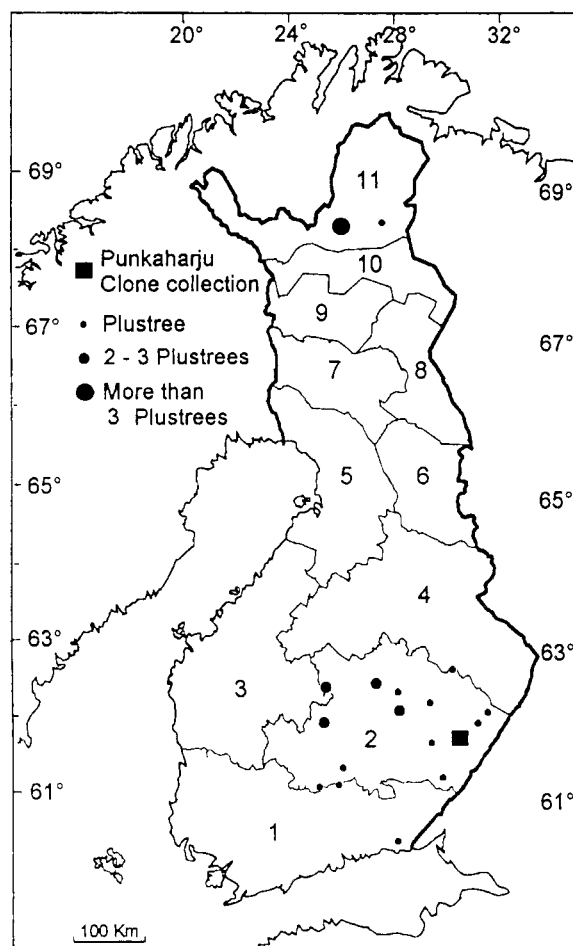
Figure 1. Location of the original Scots pine plus trees and the clone collection in which the pollen-producing grafts are situated. 1–11 refer to Finnish Scots pine breeding zones.

level method (HATCHER *et al.* 1981, VENÄLÄINEN 1993). For the present study, 20 plus trees were selected from breeding zones 1 and 2 so that half of these trees represented the top part of the height-growth ranking list and the other half the bottom part of the list. The seven plus trees originating from Lapland were taken randomly, because no proper data for their breeding value were available (Table 1).

Pollen collection

The pollen samples were collected from the grafts of the original plus trees located in an even-aged and homogeneously growing clone bank at Punkaharju (61°48′N, 29°17′ E). Pollen was collected by isolating microsporangiate strobili with paper bags on the 9th and 10th of June in 1998, about three days before the natural pollen shedding. The isolation bags were removed immediately after the pollen had shed into them, kept

Table 1. The Scots pine plus trees used as pollen donors in the germination experiments. The southern plus tree group is ranked according to the prediction of breeding value for height growth.

Plus tree	Breeding zone	Breeding value [1]
Southern group		
E2650	2	97
K818	2	96
K415	2	94
K1066	2	90
K880	2	90
E3117	2	86
E2131	2	85
E2232	2	84
K294	2	83
K297	2	82
E725	2	74
E2274	1	36
K802	2	22
E1957	2	20
K395	2	19
K806	2	19
K764	2	17
E2888	2	16
E167	2	11
K253	2	10
Northern group:		
P546	11	.
P552	11	.
P553	11	.
P555	11	.
P559	11	.
P563	11	.
P577	11	.

[1] The mean of these weighted performance level values is 50 and the standard deviation is 16.

overnight at room temperature and the pollen was harvested with a vacuum cleaner. Directly after harvesting the pollen lots were used for assays of *in vitro* germination.

In vitro germination of pollen

Pollen lots were germinated *in vitro* in 24-well plates by suspending 10 mg of dry pollen in 1 ml of modified BREWBAKER's and KWACK's (1963) medium. The suspensions were kept on an orbital shaker (Infors AG, 180 rpm) at +27 °C in the dark, as described by HÄGGMAN *et al.* (1997). Each pollen lot was germinated in two independent experiments lasting for 27 or 51 hours, both times as five replications.

Measurements of pollen tube length

Random samples of germinated suspensions of Scots pine pollen were photographed under an Olympus CK2 microscope (magnification 33 ×) by using the attached SC35 camera. The negatives of the black and white films were enlarged (10 ×) with a photograph enlarger. From these enlargements the lengths of the pollen tubes were measured using a calliper rule connected to a computer. About 30 pollen grains were measured per replication, the total number of measurements being 9 657. For non-germinated pollen grains the pollen tube length was recorded as zero. This was done because the aim was to illustrate the actual competitive ability of the pollen lot under *in vivo* conditions in which a non-germinating pollen grain also occupies space in the pollen chamber. In addition, in the 27-hour experiment very slowly germinating and non-viable pollen grains could not yet be distinguished. Moreover, the morphological features of the germinating pollen grains were observed, i.e. ramification of the pollen tube and the appearance of a second tube on a single pollen grain were recorded for each pollen grain and the tendency of pollen lots to develop pollen tubes with swollen tips was evaluated visually.

Statistical analysis

Statistical analyses of measurements of pollen tube length were carried out using the replication means as observations. Single tube lengths were not used because the tubes growing in the same well could have been dependent due to same environmental disturbances, *e.g.* growth of microbial contaminants. Hence the experimental design provides 10 observations for each lot, *i.e.* pollen collected from one plus tree graft. The analysis of variance, the comparison of means and the custom hypothesis testing were performed using the GLM, the estimation of variance components using the VARCOMP and the calculation of correlation coefficients using the CORR procedure of SAS/STAT® statistical software (SAS Institute Inc. 1989).

Differences in the morphology of single tubes were analysed in the pollen lots germinated for 51 hours by fitting logistic regression models to data with the binary response variables (second tube observed or not, tube ramified or not). The goodness of fit of the models was evaluated by the analysis of deviance and Pearson's χ^2 statistics, and the significance of the effects was tested with Wald statistics using the SAS/INSIGHT® statistical software (SAS Institute Inc. 1995). The correlation between the appearance of a second tube in the 27-hour germination and tube ramification in the 51-hour germination was calculated by using the pollen lot-wise

Table 2. Analysis of variance for average pollen tube length after 27 or 51 hours germination.

Source	d.f.	MS	F-value	p-value
in the 27-hour germination:				
pollen donor	26	1376	12.2	0.0001
contrast 'South' [vs] 'North'	1	7779	68.7	0.0001
error	107	113		
in the 51-hour germination:				
pollen donor	26	4280	13.1	0.0001
contrast 'South' [vs] 'North'	1	4857	14.8	0.0002
error	105	328		

percentages with the CORR procedure of SAS/STAT® statistical software (SAS Institute Inc. 1989).

RESULTS

Measurements of pollen tube length

Variation in the pollen tube growth rate among the Scots pine plus trees was studied using analysis of variance. The effect of pollen donor was found to be significant both in 27-hour and 51-hour experiment ($p < 0.0001$) (Table 2). As a variance component, the pollen donor explained 69 % and 71 % of the total variation, respectively. In the 27-hour germination the range of the pollen lot means for tube length was 37–96 μm, the differences among pollen donors being three-fold at maximum. The mean of pollen lot means was 70 μm and the coefficient of variation 24 %. In the 51-hour germination the range of the pollen lot means was 105–223 μm, the differences among pollen donors being only two-fold at maximum. The means, as well as the result of multiple comparison test, are presented in Table 3. The mean of pollen lot means was 153 μm and the coefficient of variation 20 %. The Pearson correlation coefficient for the pollen lot means in tube length between the 27-hour and 51-hour germination was 0.49 ($n = 27$, $p = 0.009$). The pollen lot means are plotted against each other in Figure 2.

In order to illustrate the distribution of tube lengths within single pollen lots, the 25th, 50th, 75th, and 95th percentiles, together with the distribution means and the percentages of non-germinated pollen grains germinated for 27 or 51 hours, are presented in Table 4. The fastest germinating 5 % of the pollen tubes exceeded 79–173 μm after 27 hours germination and 163–317 μm after 51 hours germination, depending on the pollen lot.

The average length of pollen tube was also calculated for the geographical groups. In the 27-hour germination the mean for the southern group was 74

Table 3. The average pollen tube length of the Scots pine pollen lots after 51 hours germination. The results of the Student-Newman-Keuls test for multiple comparison of means are presented. The means symbolised with the same letter are not significantly different ($p = 0.05$).

Pollen donor	Mean tube length	Grouping according to Student-Newman-Keuls test							
K806	223	A							
K818	209	A	B						
K294	199	A	B						
K415	188		B	C					
E2888	182		B	C	D				
K1066	180		B	C	D				
E2274	166			C	D	E			
K395	163			C	D	E	F		
E167	161			C	D	E	F		
P552	158			C	D	E	F		
K764	156			C	D	E	F		
P577	155			C	D	E	F		
K802	153			C	D	E	F	G	
K253	151			C	D	E	F	G	
P546	150			C	D	E	F	G	H
E2232	149			C	D	E	F	G	H
K880	148			C	D	E	F	G	H
P559	145				D	E	F	G	H
P553	144				D	E	F	G	H
E3117	129					E	F	G	H	I
P563	127					E	F	G	H	I
E1957	125						F	G	H	I
K297	123						F	G	H	I
P555	122						F	G	H	I
E2131	115							G	H	I
E725	112								H	I
E2650	105									I
Mean	153									
s.d.	30									

(±1.9 s.e.) μm and for the northern group 57 (±2.2) μm, and in the 51-hour germination the means were 156
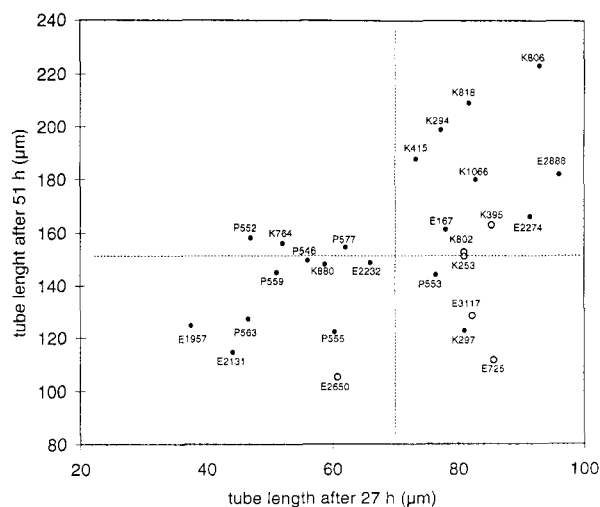
Figure 2. The average pollen tube lengths after 27 or 51 hour germination in pollen lots derived from grafts of Scots pine plus trees. The means of pollen lots are shown with dashed lines.

(±3.7) μm and 143 (±3.6) μm, respectively. In both cases the means differed significantly from each other ($p < 0.0001$ and $p < 0.0002$, respectively) (Table 2).

Correlation between the pollen tube growth rate and the progeny height growth

The value for performance level of progeny height growth was available for the 20 southern Finnish plus-trees. For these plus trees the Pearson correlation coefficient was calculated in order to ascertain whether the pollen tube growth rate and the height growth rate of the open-pollinated progeny were correlated. No correlation was found, the coefficient being –0.161 ($p = 0.497$) in the 27-hour germination and –0.165 ($p = 0.488$) in the 51-hour germination.

Morphological features of germinating pollen grains

The frequency of appearance of a second tube and tube ramification varied among the pollen lots derived from different Scots pine plus trees (Table 5). There were only a few pollen lots in which pollen grains had non-ramified single tubes (Figure 3a). The proportion of pollen grains with a second tube (Figure 3b) was between 0–38 % after 27 hours germination and no more after 51 hours germination. Ramification of pollen tubes was hardly apparent after 27 hours germination but in the samples germinated for 51 hours the proportion of pollen grains with ramified tube (Figure 3c) varied from 0 % to 56 %. For the pollen donors,

the Pearson correlation coefficient between the appearance of a second tube in the 27-hour germination and tube ramification in the 51-hour germination was 0.462 ($n = 27$, $p = 0.015$). For the single pollen grain, however, the appearance of the second tube and tube ramification were independent events, since the frequency of ramification was the same for the pollen grains with one or two tubes. The visual evaluation showed that 6 pollen lots had a clear tendency to develop pollen tubes with a swollen tip during the 51 hours germination (Figure 3d). All these pollen lots showed a remarkable reduction of the pollen tube growth rate between 27- and 51-hour germination (Figure 2).

The logistic regression models for the appearance of a second tube and for tube ramification fit the data well ($p < 0.0001$). According to the Wald statistics, in both models the effect of pollen donor was significant ($p < 0.0001$). The pollen tube length was included in the model for tube ramification and the effect of it was found to be significant ($p < 0.0001$).

DISCUSSION

Significant differences in the average pollen tube growth rate were found among the pollen lots derived from Scots pines selected from natural populations. The pollen lots used for the study were collected from the even-aged and evenly managed grafts of the plus trees growing in one clone collection area, and the variation caused by environmental factors was assumed to be minor. Thus, genetic variation should be the major component of the observed variation. This is the first report in gymnosperms revealing variation in the pollen tube growth rates at the population level and among individuals belonging to breeding material, as well as being the first study of the correlation between this trait and the rate of height growth of the progenies.

In the present study the pollen tube growth rates were measured *in vitro*. In several gymnosperm species, including Scots pine, *in vivo* and *in vitro* germination have been found to be similar, although germination is faster *in vitro* than *in vivo* (OWENS & BLAKE 1985, DAWKINS & OWENS 1993, DE WIN *et al.* 1996). In Scots pine, *in vivo* germination of pollen takes several days and then the growth of the pollen tube ceases rather soon, not continuing before the next growing season, about ten days prior to fertilization (SARVAS 1962). According to SARVAS' studies (1962), the total distance that the pollen tube has to grow to reach the archegonium can be estimated to be about 6–8 times the diameter of the pollen grain, *i.e.* 400–500 μm. In this study, the average tube length after 27 hours germination varied among pollen donors from 37 to 96 μm, the

Table 4. The means and the 25th, 50th, 75th and 95th percentiles of single pollen tube lengths for the Scots pine pollen lots germinated fro 27 or 51 hours and the percentages of non-germinated pollen grains, i.e. grains without visible pollen tubes.

Pollen donor	Tube length after 27 hours						Tube length after 51 hours					
	no tube %	Mean	Percentiles				no tube %	Mean	Percentiles			
			25th	50th	75th	95th			25th	50th	75th	95th
			µm						µm			
K806	18.8	93	65	104	132	173	1.6	226	184	230	274	311
K818	9.3	81	63	88	106	137	0.0	211	175	207	250	300
K294	29.5	77	0	94	123	153	15.2	200	169	226	268	317
K415	15.0	73	46	79	105	130	3.7	187	159	187	223	278
E2888	9.1	80	35	89	116	154	3.6	181	148	192	222	258
K1066	6.1	94	81	101	117	142	6.4	180	152	191	221	265
E2274	6.1	90	68	95	115	147	1.3	165	128	161	204	245
K395	14.6	85	68	93	114	139	7.1	162	141	171	197	247
E167	6.2	79	61	81	103	129	4.9	161	138	160	195	243
P552	28.0	47	0	51	76	110	6.4	158	136	161	193	229
K764	10.5	52	27	42	73	126	5.9	158	130	162	197	244
P577	12.5	62	34	64	88	125	5.6	153	130	163	185	224
K802	10.4	80	61	89	104	133	3.3	151	121	150	181	241
K253	9.9	78	56	83	105	138	4.9	150	104	155	195	258
P546	22.2	56	21	62	86	113	10.2	150	124	163	193	234
E2232	6.1	66	54	70	83	106	1.9	148	120	150	181	223
K880	10.6	58	30	57	84	123	8.7	147	95	157	201	259
P559	9.5	51	28	53	72	100	1.3	145	123	145	169	201
P553	5.7	76	57	78	95	127	7.4	143	107	153	192	225
E3117	7.2	81	61	81	101	135	3.7	128	106	129	155	187
P563	10.6	47	19	47	69	100	10.9	126	105	139	159	208
E1957	13.0	37	17	35	53	81	6.7	125	99	130	158	193
K297	11.3	79	52	84	109	142	9.0	122	102	132	159	192
P555	16.6	59	26	68	87	115	8.1	122	108	127	155	187
E2131	15.7	43	20	47	63	79	7.2	115	102	121	139	163
E725	7.6	84	72	88	104	133	8.1	110	91	108	138	184
E2650	6.5	60	48	61	79	105	2.7	104	85	102	127	165

fastest five percent reaching 79 and 173 µm, respectively. After 51 hours germination, the corresponding measures were 105 and 223 µm for the means, and 163 and 317 µm for the fastest five percent. Compared with the results obtained by de WIN *et al.* (1996) for Scots pine pollen, the elongation of the pollen tubes was faster in the present study. On the other hand, the present results are in accordance with the observations made by HOEKSTRA (1983) showing the rate of tube growth in Pinaceae family after 17 hours lag period being up to 10 µm/h.

The differences among the pollen lots observed *in vivo* can be expected to reflect the variation in natural populations. The plus tree-wise growth rates of pollen tubes differed significantly within one geographical region. Also the means of the northern and southern plus tree groups were different, although the ranges were overlapping. The results reveal the different genetic potential of the pollen lots to occupy *in vivo* a

position adjacent to archegonium. According to STOCK-WELL (1939), the relative positions of the pollen tubes and archegonia are important for pre-embryonal selection in pines.

In a natural population of an angiosperm, *Hibiscus moscheutos*, faster average pollen tube growth rate of the pollen donor resulted in a larger number of seeds sired (SNOW & SPIRA 1991, 1996). In several gymnosperm species, *Pseudotsuga menziesii* Mirb., *Pinus radiata* D. Don., *Pinus taeda* L., and *Picea abies* (L.) Karst., when pollen mixtures have been applied, the paternal success of the pollen donors has been unequal. Pollen competition, including different rates of germination and tube growth, has been suggested to be one of the reasons for variation in paternal success (SCHOEN & CHELIAK 1987, NAKAMURA & WHEELER 1992 and references therein, SKRÖPPA & LINDGREN 1994). The present results indicate that also in Scots pine there is a potential for pollen competition. In some species,
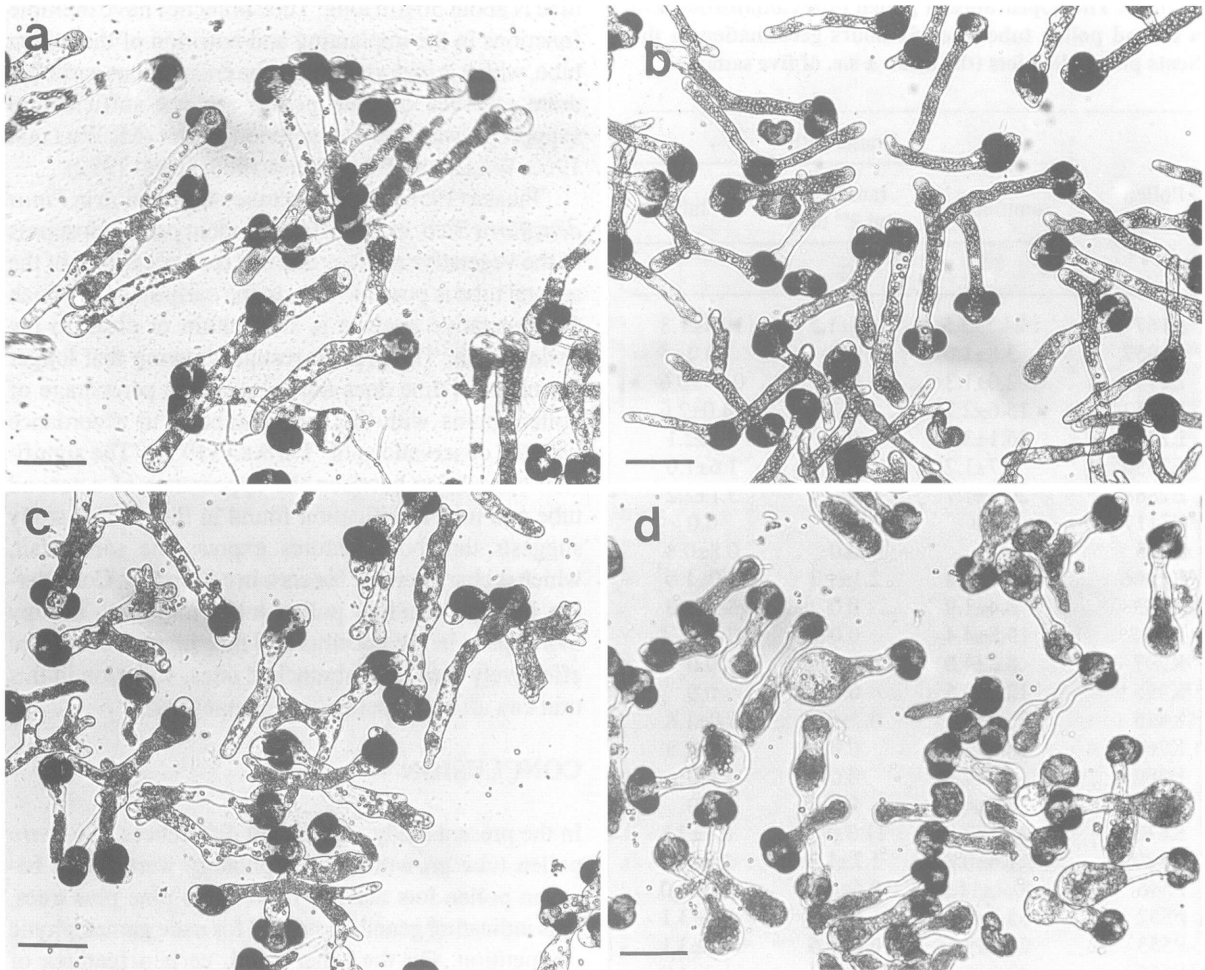
Fig. 3. Scots pine pollen lots differing in their morphological features after 51 hours germination: germinated pollen grains of (a) the plus tree K806 with non-ramified single pollen tubes, (b) the plus tree E167 with abundant second pollen tubes, (c) plus tree K1066 with ramified pollen tubes, and (d) the plus tree E725 with swollen tube tips. The scale bars represent 10 μm.

maternal plants may selectively abort fruits from low pollen loads and consequently seeds from slow-growing pollen would be less likely to survive (SNOW 1986). The fact that, also in Scots pine, insufficient pollination leads to deterioration of the female strobilus (SARVAS 1962) is in accordance with the above-mentioned hypothesis.

There are, however, several features in the reproductive biology of Scots pine that may limit the influence of pollen competition. According to SARVAS (1962), the maximum capacity of the pollen chamber is six pollen grains, but two grains is the most common number observed. The number of archegonia per ovule, which varies from one to four, often offers a possibility for more than one pollen grain to fertilize an egg nucleus. The long time span between pollination and fertilization may reduce the advantage of rapid tube growth and gives time for environmental selection pressures, such as cold, to function. Later on, the

competition among polyzygotic polyembryos finally determines the genotype of the embryo (SARVAS 1962).

In several angiosperm species, a positive correlation between the pollen tube growth rate and the performance of the progeny has been reported for traits such as seed germination rate, seedling growth rate, competitive ability, and yield (MULCAHY 1983 and references therein, QUESADA *et al.* 1993). The present results, however, show no correlation between *in vitro* growth rate of the pollen tube and height growth of the progenies in the Scots pine population studied.

In this Scots pine material, the magnitude of the variation was larger in the growth rate of the pollen tube than in the height growth of the progenies. In traits related to fitness, natural selection is expected to favour alleles linked to the ability to produce large numbers of good-quality progenies, thus reducing genetic variation (FALCONER 1981). The large variation found in the pollen tube growth rate could indicate the minor

Table 5. The appearance of pollen tube ramification and a second pollen tube after 51 hours germination in the Scots pine pollen lots (the mean ± s.e. of five samples.

Pollen donor	Grains with		
	ramified tube	ramified and 2nd tube	2nd tube
	%		
E167	24.5±3.5	4.5±1.5	19.4±1.8
E1957	3.8±1.6	0.0	0.0
E2131	2.0±1.3	0.0	0.6 ±0.6
E2232	15.6±2.2	0.0	14.0±2.6
E2274	16.1±3.3	0.0	5.1±2.1
E2650	1.7±1.7	0.0	1.6±1.0
E2888	20.7±1.7	0.6±0.6	5.1±2.2
E3117	0.0	0.0	0.0
E725	0.0	0.0	0.8±0.8
K1066	50.8±6.8	2.1±1.3	1.0±1.0
K253	6.4±1.9	0.0	5.5±2.0
K294	19.8±4.4	0.0	10.4±3.4
K297	8.8±4.0	0.0	0.0
K395	12.0±3.5	0.0	0.0
K415	35.0±6.8	0.7±0.7	3.0±1.8
K764	10.3±7.9	0.0	8.5±2.5
K802	6.2±4.4	0.0	0.0
K806	6.7±6.7	0.0	0.0
K818	33.1±7.0	11.3±9.3	8.7±2.5
K880	1.4±0.8	1.2±1.2	2.3±1.5
P546	40.4±11.5	0.6±0.6	1.6±1.0
P552	35.5±5.4	0.0	9.3±3.1
P553	23.4±9.5	0.6±0.6	34.7±3.1
P555	49.5±6.2	9.0±4.3	11.3±4.0
P559	7.5±3.0	0.0	0.6±0.6
P563	23.0±7.8	0.0	3.0±1.3
P577	36.1±6.8	0.0	5.1±2.6

importance of this trait for the fitness of Scots pine. On the other hand, besides the factors that limit the consequences of pollen competition in Scots pine, there are processes that could maintain large variation also in fitness-related traits including mutation, frequency-dependent selection, genotype-by-environment interactions, male-female interactions, and the gene flow from populations in which the selection pressure has been relaxed (SNOW 1994).

The germinated pollen lots derived from Scots pine plus trees differed in their morphological features, *i.e.* in their tendency to grow a second or ramified pollen tube. The same phenomenon has also been observed by DE WIN *et al.* (1996). The ramification of pollen tubes is characteristic for pine pollen *in vivo*, as has been shown already by FERGUSON (1904), and in the case of Scots pine by SARVAS (1962). According to DE WIN *et al.* (1996), ramification occurs *in vitro* when the pollen

tube is about 50 μm long. Tube branches have multiple functions in the implanting and nutrition of the pollen tube, which is important since the endogenous carbohydrate reserves of pine pollen are not sufficient to support growth over a long period of time (MCWILLIAM 1960, WILLEMSE & LINSKENS 1969, JOHRI 1992).

Tanaka (1956) found that tube ramification in *Pinus densiflora* Sieb. et Zucc. is dependent on the closeness of the vegetative nucleus and that the appearance of the second tube is possible only in the early stage in which the vegetative nucleus is still within or close to the pollen grain. The present results showing that longer germination time does not increase the percentage of pollen grains with the second tube is in accordance with the observations of TANAKA (1956). The significant correlation between the appearance of a second tube and tube ramification found in the present study suggests that both features express the same trait, which is characteristic for certain pollen lots. Considering that the ramified pollen tubes may both occupy more space in the nucellus and take up nutrients more effectively than the unbranched ones, variation in this trait can affect gametophytic competition.

CONCLUSION

In the present study, significant differences in *in vitro* pollen tube growth and morphology were found between pollen lots derived from Scots pine plus trees, thus indicating genetic potential for male gametophytic competition. On the other hand, certain features of Scots pine reproductive biology restrict the importance of pollen competition. The variation in pollen tube growth rates among different pollen donors may, however, have an impact on the genetic composition of seed produced in seed orchards, especially in those orchards with northern clones which have been established in southern Finland. According to the present results, there seems to be no possibility to apply gametophytic selection in breeding programs for improving the growth rate of Scots pine.

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