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Pollen competition and seed-siring success in *Picea abies*

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Abstract The aim of the present work was to study pollen-tube competition in *Picea abies*. Controlled crossings were performed with pollen mixtures including pairs of pollen lots with fast and slowly elongating pollen-tubes. Paternity analysis using isozyme markers was performed on the progenies in order to study whether the in vitro pollen-germination vigour corresponds to the proportion of seeds sired by the pollen donor. Paternal success was found to be unequal, 15 out of 23 crossings producing progeny that differed significantly from the hypothetical ratio of 1:1. The paternal contribution in the majority of the crossings was as expected: the pollen parent with more-vigorous in vitro germination sired more seeds than the less-vigorous pollen. In the case of two pollen mixtures, however, the seed-siring success summed over the maternal trees was the opposite to the expected value. Despite these aberrations, the results support the hypothesis that pollen-tube competition is one of the factors contributing to male fitness in *P. abies.* However, when all the other factors affecting pollination and seed set under natural conditions are taken into account, it is clear that the seed-siring success of a particular paternal genotype cannot be predicted reliably by measuring only the in vitro pollen vigour.

Keywords Norway spruce · Pollination · Germination of pollen · Pollen-tube growth · Paternal success

Introduction

In many forest-tree species, including Norway spruce (*Picea abies*), the genetic gain achieved by breeding is transferred to production populations through sexual reproduction in clonal seed orchards consisting of genetically superior individuals. As a result of random mating within a seed orchard, the genetic composition of the seed crop should be close to that of the original clones. However, deviations from random mating within a seed orchard are an established fact. There are large differences in flowering abundance among clones and between years (Sweet 1975; Lindgren et al. 1977; Skrøppa and Tutturen 1985; Nikkanen and Ruotsalainen 2000), as well as variation in reproductive phenology (Blush et al. 1993; Nikkanen 2001). In addition, owing to the effective pollen dispersal of this species, pollen contamination from non-orchard sources has also proved to be a serious problem for the proper functioning of seed orchards (Savolainen 1991; Paule et al. 1993; Pakkanen et al. 2000).

Male fitness depends not only on the flowering traits mentioned above, but also on the traits of a pollen grain, such as germination vigour, germination time, pollen-tube growth rate and selective fertilisation (Pfahler 1975). In Norway spruce there is no evidence that maternal plants could control the pollen-tube growth rate, as suggested for other species by Hormaza and Herrero (1996).

Competition among male gametophytes has been extensively studied and discussed in angiosperms (Mulcahy 1983 and references therein; Charlesworth 1988; Quesada et al. 1993). In a deciduous tree species, *Betula pendula* (Pasonen et al. 1999), and in a perennial marsh plant, *Hibiscus moscheutos* (Snow and Spira 1991, 1996), a faster pollen-tube growth rate of the pollen donor was associated with the siring of a larger number of seeds. The rankings of the pollen donors were consistent across the different maternal plants. Furthermore, in several gymnosperm species, e.g. *Pseudotsuga menziesii*, *Pinus radiata*, *Pinus taeda* and *P. abies*, the application of pollen mixtures has resulted in unequal paternal success of the pollen donors. Pollen competition, including different rates of germination and pollen-tube growth, has been suggested as one of the reasons for this phenomenon (Schoen and Cheliak 1987;

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Table 1 The pollen mixtures and isoenzyme loci used for paternity analysis. For isoenzyme genotypes, F=fast and S=slow allele

Pollen mixture	Sire clones	Isoenzyme locus studied	Isoenzyme genotype
Mixture 1	P491 P1203	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF SS
Mixture 2	P498 P1203	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF SS
Mixture 3	P491 P1203	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF SS
Mixture 4	P498 P1203	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF SS
Mixture 5	P675 P695	GDH, glutamate dehydrogenase, E.C. 1.4.1.2	FF SS

Nakamura and Wheeler 1992; Skrøppa and Lindgren 1994). In previous reports, we have shown differences in the average in vitro pollen-tube growth rate among pollen donors of *Pinus sylvestris* (Venäläinen et al. 1999) and *P. abies* (Nikkanen et al. 2000). However, it is not known whether the variation in this trait also affects the genetic composition of the seed produced in seed orchards.

The aim of the present work was to study pollen-tube competition in *P. abies* in more detail. Controlled crossings were performed with pollen mixtures including pairs of pollen lots with fast and slowly elongating pollen-tubes. Paternity analysis was performed on the progenies obtained in order to study whether the in vitro pollen germination vigour corresponds to the proportion of seeds sired by the pollen donor.

Materials and methods

Pollen from 66 clones was collected from *P. abies* seed orchard no. 170 (Heinämäki), located at Korpilahti, southern Finland (62°13′N, 25°24′E). Pollen grains were germinated in vitro under routinely used conditions, i.e. for 27 h at 28°C in the dark, and germination vigour was measured as described by Nikkanen et al. (2000). Pollen lots from each tail of the distribution, i.e. those showing either fast or slow tube-elongation, were considered as candidates for controlled pollinations. Based on distinguishable isoenzyme genotypes, lots P491, P498, P675, P695 and P1203 were selected for the pollen mixtures.

Five pollen mixtures were used to pollinate five seed parents (P495, P683, P689, P1206, P2579, three grafts/clone) in 1998. Each of the mixtures consisted of an equal mass of pollen from two clones. On the basis of the in vitro germination results of frozen pollen from 1996 (Nikkanen et al. 2000), one clone was supposed to have poor and the other one good in vitro pollen germination vigour. Mixtures 1 and 2 consisted of pollen collected in 1996 and stored at –20°C for 2 years, while mixtures 3, 4 and 5 comprised pollen collected just before the controlled pollinations were performed. The pollen donors in mixtures 3 and 4 were the same as those in mixtures 1 and 2 (Table 1).

The pairs of sires for controlled pollination were composed on the basis of identifiable isoenzyme genotypes. Seed paternity was determined by genotyping germinated embryos at two enzyme loci, one for each combination (Table 1). The method described for example by Muona et al. (1987, 1990) was followed. For homozygotic seed parents we needed to analyse only embryos, but for heterozygous seed parents the megagametophytes also had to be studied in order to infer the paternity (Table 2).

Table 2 Isoenzyme genotypes of the seed parents at the loci studied. For isoenzyme genotypes, F=fast and S=slow allele

Seed parent	Isoenzyme locus	
	DIA	GDH
P495	SS	FS
P683	FF	FF
P689	FS	FS
P1206	SS	FF
P2579	FS	FF

The null hypothesis of the equal contribution of the sires to the germinating seeds was examined by using a chi-square test of the goodness of fit (Sokal and Rohlf 1995).

Results

Paternity analysis showed a discrepancy in the equal paternal contribution in the germinating seeds from all the pollen mixtures (Tables 3 and 4). Table 3 shows the paternal contribution in the germinated seeds expressed as a ratio, in which the count of offspring from the more-vigorous pollen parent was divided by the count of offspring from the less-vigorous pollen parent. Since the measure for the more-vigorous sire in vitro was the numerator, all the values of the ratio would be greater than one if the outcome was in the same direction as that in the in vitro test. Of the 23 ratio values for the five pollen mixtures and five seed parents (two seed lots were missing), 15 were greater than one and eight were less than one (Table 3). The chi-square test for the goodness of fit for the 1:1 ratio of the paternal contribution showed that 15 out of the 23 ratio values differed significantly from the hypothetical 1:1 ratio (Table 4). Four of the statistically significant ratio values were in an opposite direction to that expected, and hence the less-vigorous sire in vitro had more offspring than the more-vigorous one.

Crossings with two pollen mixtures were performed using both fresh (mixtures 3 and 4) and old pollen stored at –20°C (mixtures 1 and 2). As shown in Table 3, the ratios of pollen-tube lengths in the mixtures of fresh

Table 3 Paternal success in controlled pollinations of *P. abies*. All the ratio values are expressed in the order in which the pollen parent with faster growing pollen-tubes are in the numerator, and the more slowly growing in the denominator. The ratio values which differed significantly from the 1:1 ratio are marked in bold

Pollen mixture, pollen donors	Ratio of pollen-tube length	Ratio of paternal success Seed parent					
		P495	P683	P689	P1206	P2579	Together
Mix 1 P491/P1203	6.93	0.94	**2.88**	0.58	**0.48**	**0.20**	**0.71**
Mix 2 P498/P1203	8.47	0.87	**2.09**	**2.00**	1.36	2.11	**1.61**
Mix 3 P1203/P491	1.32	**7.25**	**3.57**	**3.71**	**8.50**	**16.00**	**5.86**
Mix 4 P1203/P498	1.51	**2.00**	**2.10**	1.06	–	**2.20**	**1.75**
Mix 5 P675/P695	2.74	**0.39**	**0.39**	1.24	–	0.52	**0.60**

Table 4 Chi-square test for goodness of fit for the 1:1 ratio of the paternal contribution estimated from the germinated seeds. Statistically significant deviations from the 1:1 ratio are marked in bold

Seed parent		Pollen mixture				
		Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5
P495	*n*	31	28	33	36	32
	χ^2	0.03	0.14	**18.94**	**4.00**	**6.12**
	p	0.9>*p*>0.5	0.9>*p*>0.5	*p*<0.001	0.05>*p*>0.025	0.025>*p*>0.01
P683	*n*	31	34	32	31	32
	χ^2	**7.26**	**4.23**	**10.12**	**3.90**	**6.12**
	p	0.01>*p*>0.005	0.05>*p*>0.025	0.005>*p*>0.001	0.05>*p*>0.025	0.025>*p*>0.01
P689	*n*	30	36	33	33	38
	χ^2	2.13	**4.00**	**10.94**	0.03	0.42
	p	0.5>*p*>0.1	0.05>*p*>0.025	*p*<0.001	0.9>*p*>0.5	0.9>*p*>0.5
P1206	*n*	43	33	19	–	–
	χ^2	**4.23**	0.76	**11.84**	–	–
	p	0.05>*p*>0.025	0.5>*p*>0.1	*p*<0.001	–	–
P2579	*n*	30	28	34	32	32
	χ^2	**13.33**	3.57	**26.47**	**4.50**	3.12
	p	*p*<0.001	0.1>*p*>0.05	*p*<0.001	0.05>*p*>0.025	0.1>*p*>0.05
Sum over mothers	*n*	156	159	151	132	134
	χ^2	**4.33**	**8.61**	**75.82**	**9.82**	**8.63**
	p	0.05>*p*>0.025	0.005>*p*>0.001	*p*<0.001	0.005>*p*>0.001	0.005>*p*>0.001

pollen differed considerably from the ratios obtained for stored pollen. When comparing paternal success between mixtures of freshly collected and frozen pollen, in mixtures 1 and 3 pollen lot P1203 had a better paternal success than lot P491, independently of the ratio value for tube lengths: in seven out of ten crossings this lot sired significantly more progenies than P491. By contrast, in mixtures 2 and 4 the sire with longer pollen-tubes in vitro sired more seeds.

When the contribution of each pollen mixture was summed over the seed parents, three ratio values out of five were in agreement with the in vitro tests (Table 3). All the ratio values deviated significantly from 1:1.

Discussion

In the present study, paternal success in *P. abies* proved to be unequal, 15 of the 23 crossings producing progeny that differed significantly from the hypothetical 1:1 ratio. Similar results have been observed previously not only in *P. abies* (Schoen and Cheliak 1987; Skrøppa and Lindgren 1994) but also in some other gymnosperms such as *P. menziesii*, *P. radiata* and *P. taeda* (Nakamura and Wheeler 1992 and references therein).

The paternal contribution in the majority of the present crossings was as expected: the pollen parent that germinated more vigorously in vitro sired more seeds than the less-vigorous pollen. A similar connection between pollen-tube growth rate and parental success has previously been reported in a number of angiosperm species (Snow and Spira 1991, 1996; Pasonen et al. 1999). The present result is in accordance with the reproductive biology of *P. abies*, which provides an opportunity for male gametophyte competition. The pollen chambers of the species can accommodate more than ten pollen grains, five being the average number (Sarvas 1968). Pollen-tube formation takes place during two periods, interrupted by a resting period, resulting in fertilisation 3–4 weeks after pollination (Sarvas 1968; Christiansen 1972). Under in vitro conditions, elongation of the tubes is linear up to lengths comparable with the final distance

to the archegonia (Martinussen 1994; Martinussen et al. 1994), suggesting that the in vivo resting period may not change the order of competing tubes. Moreover, no prezygotic incompatibility mechanisms have been reported.

In the case of two of the pollen mixtures, 1 and 5, the seed siring success summed over the seed parents was the opposite to the expected one. There are several probable reasons for this. As observed in our previous study (Nikkanen et al. 2000), in vitro germination under a relatively high constant temperature does not give a complete picture of the whole variation in the germination potential of *P. abies* pollen lots. Generally, the ranking of the pollen lots remains relatively stable when the germination temperature is changed, or when stored pollen is used instead of freshly collected pollen. There are, however, pollen lots that behave differently under varying germination conditions. For example, lot P675 included in mixture 5 benefits from a higher temperature (Nikkanen et al. 2000). Thus its poor parental success in the present study could be at least partly explained by the fact that the flowering period in 1998 was colder than the long-term average (Nikkanen and Ruotsalainen 2000). On the other hand, pollen lot P1203, which was included in mixtures 1 and 3, was found to have varying vigour in different years. According to Havens (1994) and Delph et al. (1997), variation in pollen performance can be partly caused by environmental effects, such as temperature or the physiological condition of the donor plant during pollen development. The fact that the pollen in mixtures 1 and 2 was stored at –20°C before germination may also affect the results, because some pollen lots seem to suffer from freezing (Nikkanen et al. 2000). However, lot P1203 sired more seeds than its competitor, P491, irrespective of its in vitro germination result.

The reproductive biology of the species includes features that might change the outcome of gametophyte competition. In *P. abies* there is usually more than one archegonium per ovule, and in most cases two competing embryos are formed in order to ensure the formation of full seed. Thus the genotypes homozygous for lethal, sublethal or defective genes are eliminated either by early abortion of the zygotes or, later on, through embryo competition (Sarvas 1968). In the present study, however, there was no indication that these features would have been more active in any of the parental combinations than in the others.

According to Pasonen (2000), in an angiosperm tree, *Betula pendula*, genotype-environment interactions were found in pollen-tube growth rate and seed-siring success, but the changes in the rankings of the pollen donors did not translate into parallel changes in seed-siring success. In greenhouse conditions, the tube growth rate controlled the paternity of the birch seeds, but in the more heterogeneous outdoor environment a negative correlation was found between pollen elongation and paternal success. This result was assumed to be due to differences in the physiological condition of the maternal plants, caused by microhabitat variation. Since the maternal plant is known to provide carbohydrates for germinating pollen also in gymnosperms (Willemse and Linskens 1969; Johri 1992; Dawkins and Owens 1993), the same type of natural environmental variation could have affected the pollen competition in the present study.

Despite some aberrations, the present results support the hypothesis that pollen-tube competition is one of the factors contributing to male fitness in *P. abies*. There are genotype-environment interactions in pollen performance, as shown already by Nikkanen et al. (2000) and also reflected in the present study, that affect the paternal success. These G×E interactions promote the maintenance of genetic variation within populations, even if pollen performance is related to fitness (Delph et al. 1997); and in seed orchards they may contribute to the variable genetic composition of the seed produced in different years. On the basis of the present results, and taking into consideration all the other factors affecting pollination and seed set under natural conditions, it would appear that the seed-siring success of a particular paternal genotype cannot be predicted reliably by measuring the in vitro pollen vigour only.

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