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Genotype-environment interactions in pollen competitive ability in an anemophilous tree, *Betula pendula* Roth

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Abstract This study describes genotype-environment interactions in pollen competitive ability expressed as pollen-tube growth rate and seed-siring success in *Betula pendula* Roth. A factorial crossing design was applied using the same maternal and paternal clones in two different environmental conditions, in a *B. pendula* seed orchard established in a greenhouse and at an outdoor clone collection. Both single donor and two-donor pollinations were employed. Female inflorescences were collected after a fixed time of germination, pollen-tube lengths were measured for each cross, and paternity of the seeds sired by two-donor pollen mixtures was analyzed using isozyme markers. The pollination site had a significant influence on pollen-tube growth rate and seed-siring success. Significant interactions between pollination site and pollen donor indicated genotype-environment interactions in pollen-tube growth rate and seed-siring success. A highly significant positive correlation between pollen-tube growth rate and seed-siring success was found in the greenhouse but not at the outdoor clone collection. These results suggest that the pollen-tube growth rate can be a predictor of seed-siring success in controlled greenhouse conditions, where differences among maternal plants are mainly of genetic origin, but not in more heterogeneous outdoor conditions. In natural birch stands, environmental maternal effects probably diminish the significance of pollen competition for sexual selection in *Betula pendula*. At seed orchards, the effects of environmental conditions on pollen competitive ability can have important consequences for the genetic composition of the seed crop.

Keywords *Betula pendula* Roth (silver birch) · Genotype-environment interaction · Pollen-tube growth rate · Seed orchard · Seed-siring success

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Introduction

Pollen competitive ability, usually expressed as pollen-tube growth rate or seed-siring success, has often been considered as a trait that can influence male reproductive success and lead to nonrandom fertilization (Stephenson and Bertin 1983; Marshall and Ellstrand 1986; Snow and Spira 1991, 1996; Marshall 1998; Mitchell and Marshall 1998; Pasonen et al. 1999). According to theoretical predictions, genetic variation in traits closely related to fitness should have largely been eliminated by natural selection in equilibrium populations (Walsh and Charlesworth 1992; Falconer and Mackay 1996). Because there are studies providing evidence for genetic variation in pollen-tube growth rate (e.g. Ottaviano et al. 1988; Sari-Gorla et al. 1992; Lankinen 2000), there has been speculation over the mechanism that might maintain variation in this trait, if it is an important component of male fitness. The mechanisms that have been suggested to explain the maintenance of genetic variation in pollen performance include mutation, recombination, gene flow, antagonistic pleiotropic effects between gametophytic and sporophytic stages of the life cycle, pollen-pistil interactions, direct pollen-pollen interactions and genotype-environment interactions (Snow and Mazer 1988; Walsh and Charlesworth 1992; Mulcahy et al. 1996).

Pollen-pistil interactions have been widely studied and observed in many species (Cruzan 1993; Johnston 1993; Herrero and Hormaza 1996; Hormaza and Herrero 1999),

and gene flow can be substantial, especially among wind-pollinated species (e.g. Starfinger and Stöcklin 1996). On the other hand, the effects of direct pollen-pollen interactions are largely unknown and there is no evidence for antagonistic pleiotropic effects. Because many species cover a range of different environmental conditions and substantial spatial variation can often be observed even within a range of one population, genotype-environment interactions can have an important role in maintaining variation in fitness-related traits (Via and Lande 1985; see also Haldane and Jayakar 1963; Hedrick et al. 1976; Hedrick 1986; Gillespie and Turelli 1989). The existence of genotype-environment interaction can mean that the best phenotype in one environment is not the best in another environment. A useful approach for studying G × E interactions is provided by a comparison of reaction norms of different genotypes. Variation in reaction norms contributes to significant G × E interactions and the evolution of adaptive plasticity (de Jong 1990).

To interpret the evolutionary significance of pollen competition, more has to be known about the effects of environmental variation on pollen-tube growth rates and the outcome of pollen competition. It is known that environmental conditions during pollen development (see e.g. Delph et al. 1997) and pollen-tube growth (Stephenson et al. 1992; Pasonen et al. 2000) have an influence on pollen performance. The results from our previous study in *Betula pendula* revealed that the rankings of the pollen donors in pollen-tube growth rate changed across different germination temperatures and pollination sites, indicating differential responses of the genotypes to environmental change (Pasonen et al. 2000). Non-parallel reaction norms constitute a basis for significant G × E interactions. However, at the moment we do not know whether pollen donors with the fastest-growing pollen tubes sire most of the seeds in different environmental conditions and whether there are any G × E interactions in seed-siring success, which is the most direct measure of pollen competitive ability.

In this study we examined the effects of environmental conditions on pollen competitive ability expressed as pollen-tube growth rate and seed-siring success with particular attention to G × E interactions. A clonal seed orchard established in a greenhouse and an outdoor clone collection of *B. pendula* provided an opportunity to study the variation in pollen competitive ability among the same genotypes across two different environments. In this paper we present the relationship between pollen-tube growth rate and seed-siring success among the same maternal and paternal genotypes in two different environments, and discuss the possible consequences of G × E interactions in pollen competitive ability for gametophytic selection. From the practical point of view, this study provides new information about the functioning of greenhouse seed orchards and the possible effects of environmental conditions during sexual reproduction on the genetic composition of the seed crop. This study addresses the following questions: (1) are the reaction norms of different pollen donors with regard to pollen-tube growth rate and seed-siring success parallel, and (2) is there a positive relationship between pollen-tube growth rate and seed-siring success in different environmental conditions?

Materials and methods

Study species and study sites

B. pendula is a common, anemophilous, monoecious and self-incompatible tree ranging throughout most of Europe. It has 200–300 male flowers in each catkin (Dahl and Fredrikson 1996) and approximately 600 female flowers in each pistillate inflorescence (personal observation by H.-L. Pasonen). Each female flower consists of a single two-locular ovary with two linear, dry stigmas. A mature ovary generally contains two ovules of which only one develops into a mature seed (Sulkinoja and Valanne 1980; Dahl and Fredrikson 1996).

The study was carried out in a plastic house seed orchard (= in a greenhouse) at Haapastensyrjä Forest Tree Breeding Centre of the Finnish Forest Research Institute in Läyliäinen (lat. 60°30′N, long. 24°E), and at an outdoor clone collection in Röykkä (lat. 60°30′N, long. 24°39′E). The seed orchard consists of 36 different *B. pendula* clones originating from southern Finland. The clones were originally selected for the seed orchard on the basis of the results from the field trials (Raulo and Koski 1977). These field trials aimed to select genotypes with superior heritable growth characters (e.g. straight stem). The plastic house provides favourable conditions for flowering and seed development, and isolates the seed-orchard clones from outside pollen sources. The paternal clones used in the present study originated from an area between latitudes 60°30′ and 62°10′, and maternal clones between latitudes 60°15′ and 63°15′.

Hand-pollination experiments

Pollen for the hand-pollination experiments was collected in 1995 and 1997 from several paternal clones growing in the greenhouse and subsequently stored at –20 °C. Germination percentages of the pollen samples varied between 31and 51%. No difference in the germination percentages with respect to the year of pollen collection was observed. We have found that if *B. pendula* pollen is properly collected (in dry conditions) and dried at room temperature for 24 h before storage at –20 °C, the germination ability of the pollen samples remains fairly unchanged for several years. Pollen from the same paternal clones (and from the same pollen samples) collected in the greenhouse was used in all hand-pollinations in the greenhouse and at the outdoor clone collection. Single-donor hand-pollinations were conducted at both pollination sites to study the effects of environmental conditions during pollen germination on pollen-tube growth rate. At both pollination sites, seven maternal plants (different *B. pendula* clones) were pollinated with pollen from seven paternal clones. The same maternal clones were used in the greenhouse and at the outdoor clone collection.

Before dehiscence of the anthers and receptivity of the female inflorescences, branches with three female inflorescences were isolated with paper bags from each maternal plant to prevent uncontrolled pollinations. Self-pollinations were prevented by removing all male inflorescences from the bagged branches. When female inflorescences became receptive, an equal volume of pollen from each pollen donor was applied to each pollination bag by using a pollination syringe. The amount of pollen applied to the pollination bags exceeded the number of ovules in the bags. The female inflorescences were collected 12 h after pollination in order to measure the pollen-tube lengths of each cross.

Two-donor hand-pollinations were conducted in the greenhouse and at the outdoor clone collection to study G × E-interactions in seed-siring success, and the relationship between pollen-tube growth rate and seed-siring success. The same maternal and paternal clones were used in the two-donor pollinations as in the single-donor pollinations. All the two-donor pollinations were per-

Table 1 Minimum, maximum and mean temperatures (°C) during 12 h of pollen-tube growth in the greenhouse and at the outdoor clone collection

Date	Greenhouse (60°30′N, 24°E)			Clone collection (60°15′N, 24°30′E)		
	Min.	Max.	Mean	Min.	Max.	Mean
28.4	19.0	36.0	27.0			
29.4	13.0	31.0	23.4			
30.4	14.0	27.0	21.2			
7.5				3.5	15.5	8.9
8.5				3.5	16.0	8.6

formed at the same time as single-donor pollinations. Pollen from seven paternal clones was mixed with pollen from a standard donor (clone E 1970) by measuring out an equal volume of two pollen clones into a small glass bottle. Clone E 1970 was chosen as a standard donor (= pollen donor 1) because it exhibited a rare isozyme genotype (13 in Pgi-2) and could thus be unambigiously distinguished from the common genotypes that all the other donors and the maternal plants exhibited (22 in Pgi-2). Prior to the onset of flowering, seven branches (one for each pollen mixture) with ten female inflorescences were isolated with paper bags from each maternal plant to prevent uncontrolled pollinations. Once receptive, each maternal plant was hand-pollinated by seven different pollen mixtures. All the hand pollinations were performed in the greenhouse on the 28th–30th of April and at the outdoor clone collection on the 7th–8th of May. Temperature during pollen-tube growth varied substantially between these two sites (Table 1).

Pollen-tube growth rate measurements

In this study, pollen-tube growth rate is expressed by pollen-tube length after 12 h of germination. Samples of female inflorescences pollinated by pollen from a single pollen donor were collected at both pollination sites 12 h after pollination. In the sampled inflorescences, the fastest pollen tubes had almost reached the base of the elongate stigma, but had not entered the funiculus. Three inflorescences per cross were detached and immediately stored in glacial acetic acid and 60% ethanol (1:9). The inflorescences were stored in a refrigerator at 4 °C until they were examined. The flowers were scraped off with a scalpel and stained with a solution of 0.1% aniline blue in aqueous K_3PO_4 (0.3 mol). As a result the pollen-tube callose became fluorescent and distinguishable in the darker stylar tissue when examined by UV fluorescence microscopy. Approximately 50 pollen tubes (from three inflorescences and from several flowers) per cross were measured.

Analysing the seed-siring success

The seed-siring success of the studied clones in two-donor pollinations was expressed as the proportion of seeds sired by pollen donor 2 when compared to the number of seeds sired by the standard pollen donor. Seeds from the two-donor pollinations were collected in July, 1998, in the greenhouse and at the outdoor clone collection, and subsequently stored at 4 °C. The seeds were germinated for the isozyme analyses under a plant lamp with a photoperiod of 15 h day and 9 h night on Petri-dishes covered with sand and moist filter paper. The samples were collected when the cotyledons had fully opened, and the whole plantlet was immediately ground in 50 µl of 0.12 M Tris–HCl extraction buffer, pH 7.5 (slightly modified from Bousquet et al. 1987) and fine granular quartz, imbibed into the wicks, and stored for 1–2 weeks at –20 °C prior to electrophoresis. The samples were assayed for phosphoglucoisomerase (Pgi-2) by standard starch-gel electrophoresis (10% Sigma Hydrolyzed Starch) using a Tris–citrate buffersystem [modified from Shaw and Prasad (1970)], for details, see Pasonen et al. (1999); and 100–120 seeds per sample were analyzed.

Data analysis

A mixed-effects ANOVA was performed to study the effect of parentage (random effects) and pollination site (fixed effect) on pollen-tube growth rate and the relative seed-siring success of the pollen donors. Pollen tube lengths were square-root transformed and the proportion of seeds sired by pollen donor 2 was arcsine square-root transformed to normalize the data. Coefficients of concordance (W) were calculated for pollen tube lengths and seed-siring success to study whether the rankings of the pollen donors changed across maternal plants (Sokal and Rohlf 1981, p 609).

To study the relationship between pollen-tube growth rate and seed-siring success, Spearman and Pearson correlation coefficients were separately calculated for each maternal parent at the two pollination sites. To summarize the Pearson correlation coefficients, a Schmidt-Hunter meta-analysis method with Fishers z-transformation was used to obtain a weighted mean correlation coefficient between pollen tube length and seed-siring success (Hunter et al. 1982; Hedges and Olkin 1985). The relationship between pollen germination percentage and seed-siring success was studied by Pearson correlation coefficients calculated separately for each maternal plant. The Pearson correlations were summarized by using the Schmidt-Hunter meta-analysis method.

Results

G × E interactions in pollen-tube growth rate

The pollination site had a significant main effect on pollen-tube growth rate in the mixed-effects ANOVA (Table 2). Significant interaction between the paternal parent and the pollination site was found (Table 2) indicating G × E interaction in pollen-tube growth rate among the studied clones between the two pollination sites (Fig. 1). Neither paternal nor maternal parent had a significant main effect on pollen-tube growth rate (Table 2). A significant concordance in pollen-tube growth rates across different maternal plants was found in the greenhouse (W = 0.55, $P < 0.001$). A coefficient of concordance could not be calculated for the outdoor clone collection because no germinated pollen tubes were found on some maternal-paternal combinations (presumably due to the unsuccessful hand-pollination procedure). There was a significant negative correlation between pollen tube lengths in the greenhouse and at the outdoor clone collection (weighted mean correlation = –0.39, $0.001 < P < 0.01$).

G × E interactions in seed-siring success

Mixed-effects ANOVA of the effect of parentage and pollination site on seed-siring success of the pollen do-

Table 2 Mixed-effects ANOVA of the effects of parentage (random effects) and pollination site (fixed effects) on pollen-tube growth rate

Source	*df*	MS	*F*	*P*
Maternal parent	6	0.049	0.65	n.s.
Paternal parent	6	0.116	0.36	n.s
Pollination site	1	9.263	33.52	0.001
Mother × father	36	0.064	0.86	n.s
Mother × site	6	0.078	1.71	n.s.
Father × site	6	0.303	6.97	<0.001
Mother × father × site	22	0.053	4.42	<0.001
Error	3,389	0.012		

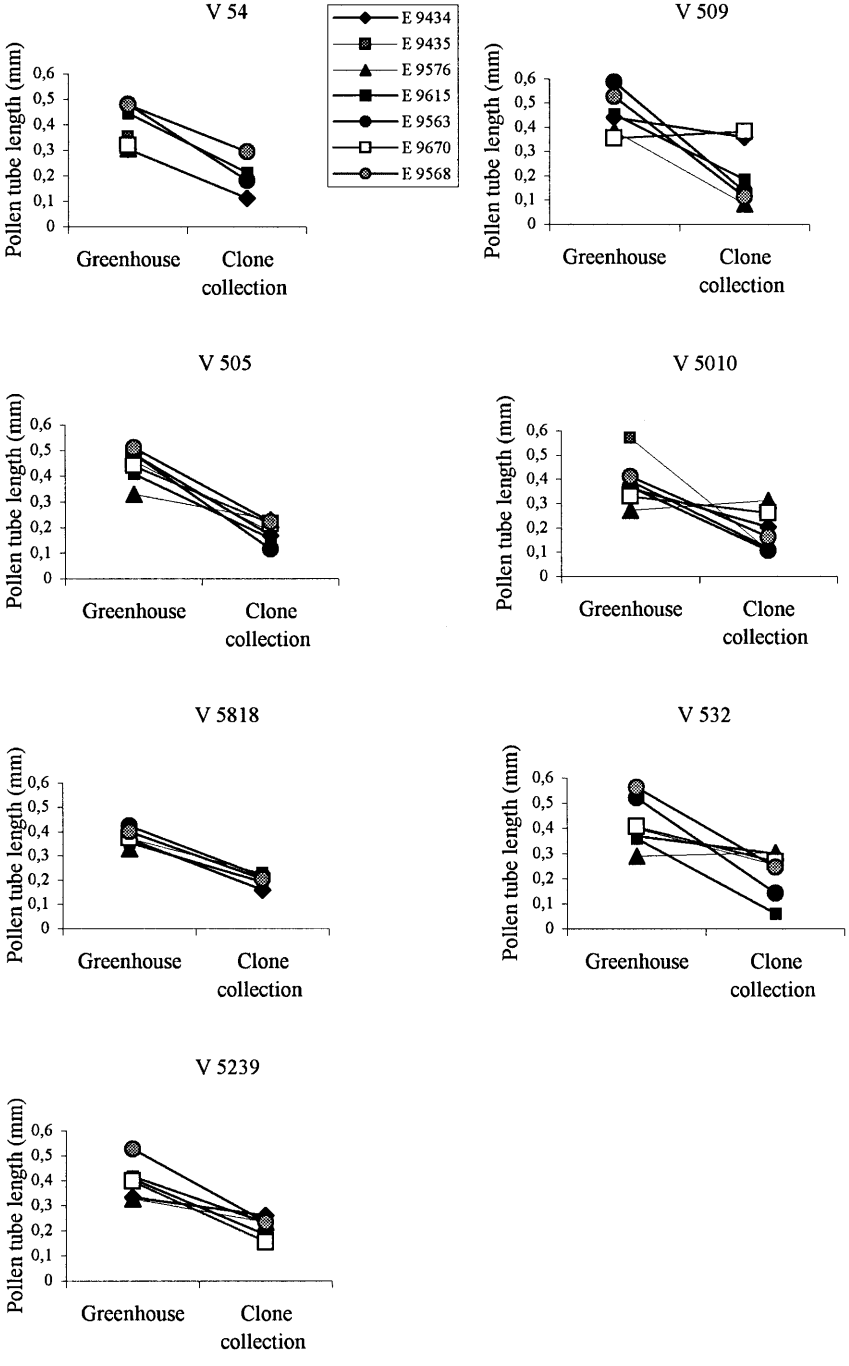

Fig. 1 Reaction norms, demonstrating genotype-environment interactions in pollen-tube growth rate on different maternal plants

Table 3 Mixed-effects ANOVA of the effect of parentage (random) and pollination site (fixed effects) on seed-siring success of the pollen donors

Source	*df*	MS	*F*	*P*
Maternal parent	6	0.025	3.68	<0.01
Paternal parent	5	0.147	21.62	<0.001
Pollination site	1	0.797	23.93	<0.001
Mother × father	26	0.007	–	–
Mother × site	6	0.024	6.49	<0.001
Father × site	5	0.013	3.51	<0.05

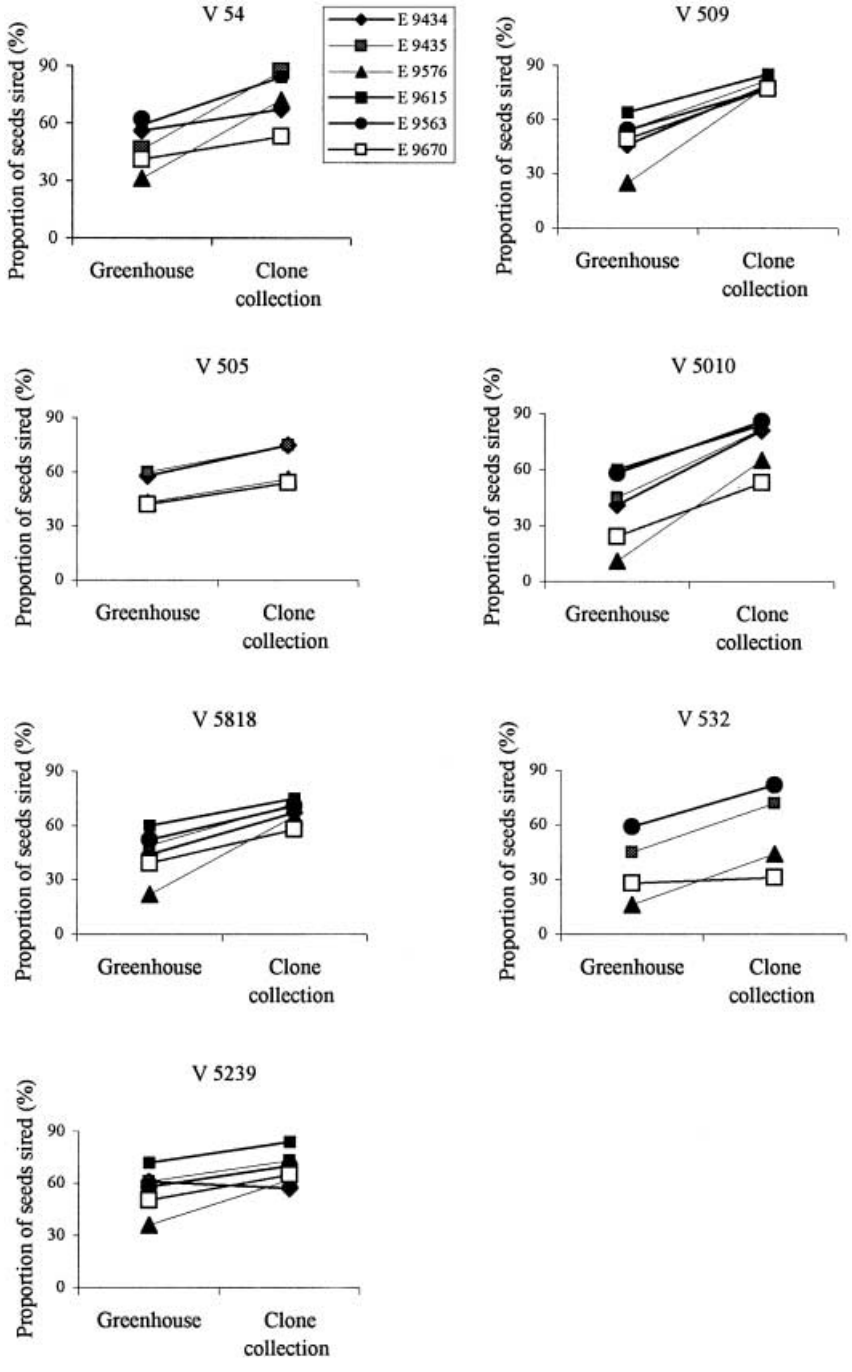

Fig. 2 Reaction norms, demonstrating genotype-environment interactions in seed-siring success on different maternal plants

Table 4 Spearman correlation coefficients between pollen tube length and seed-siring success in the greenhouse and at the outdoor clone collection. Weighted mean correlations are based on the Pearson correlations between pollen-tube length and seed-siring success calculated separately for each maternal plant

Recipient	Greenhouse			Outdoor clone collection		
	Spearman *r*	*P*	*n*	Spearman *r*	*P*	*n*
V 5239	0.75	0.084	6	–0.60	0.285	5
V 509	0.64	0.173	6	–0.21	0.734	5
V 505	0.60	0.40	4	–0.74	0.262	4
V 5010	0.83	0.042	6	–0.87	0.019	6
V 5818	0.26	0.623	6	0.40	0.600	4
V 532	0.80	0.200	4	–0.80	0.200	4
V 54	0.66	0.156	6	–	–	–
Weighted mean *r*	0.65	<0.001	7	–0.54	<0.01	6

nors revealed significant main effects and two-way interactions between both parents and pollination site (Table 3). The degrees of freedom of the paternal parents is five instead of six because the mixture E 9568 + E 1970 constantly produced no seeds at either of the pollination sites (it should also be noted that the *F*-value of the 3-way interaction and the mother × father interaction could not be counted due to the lack of replication). The significant interaction between the paternal parent and the pollination site implies significant G × E interaction in seed-siring success (Table 3). Interestingly, however, there was a significant positive correlation between the greenhouse and the outdoor clone collection in seed-siring success (weighted mean correlation coefficient = 0.80, $P < 0.001$). This indicates that the same pollen donors, on the average, are superior in siring seeds at both sites. For example, clones E 9615 and E 9563 are performing well on most maternal plants in the greenhouse and outdoors, while the proportion of seeds sired by clones E 9576 and E 9670 is fairly low on most maternal plants at both sites (Fig. 2). Despite significant G × E interactions in seed-siring success, there seems to be substantial parallelism in the rankings of the pollen donors between the greenhouse and the outdoor clone collection (Fig. 2).

A coefficient of concordance (*W*) was calculated to study whether the same pollen donors sire most of the seeds across all the maternal plants. The concordance coefficient for the pollen donors in seed-siring success was 0.68 ($0.001 < P < 0.01$) in the greenhouse and 0.56 ($0.01 < P < 0.05$) at the outdoor clone collection, indicating that the rankings of the pollen donors do not statistically change across maternal plants at either of the pollination sites.

Relationship between pollen-tube growth rate and seed-siring success

Spearman correlation coefficients between pollen-tube growth rate and seed-siring success varied between 0.26 and 0.83 in the greenhouse and between –0.87 and 0.40 at the outdoor clone collection depending on the maternal plant (Table 4). No correlation coefficient was calculated for clone V 54 at the outdoor clone collection because the number of paternal parents with data on both pollen-tube lengths and seed-siring success was only two. A weighted mean correlation coefficient between pollen-tube growth rate and seed-siring success was 0.65 ($P < 0.001$) in the greenhouse and –0.54 ($0.001 < P < 0.01$) at the outdoor clone collection (Table 4). There seems to be a positive relationship between pollen-tube growth rate and seed-siring success in the controlled greenhouse environment, and a negative relationship in more heterogeneous outdoor conditions. A positive relationship between pollen germination percentage (in vitro) and seed-siring success of pollen donor 2 was found in the greenhouse (weighted mean correlation = 0.36, $0.001 < P < 0.01$) but not at the outdoor clone collection (weighted mean correlation = 0.15, $P > 0.05$).

Discussion

In this study, significant interactions between the paternal parent and the pollination site were found in both pollen-tube growth rate and seed-siring success. These results indicate significant genotype-environment interactions in pollen competitive ability across two different pollination sites in *B. pendula* Roth. Despite evidence of G × E interactions in many floral and reproductive traits (e.g. Mazer and Schick 1991; Boose 1997; Vogler et al. 1999) extremely little is known about G × E interactions in pollen competitive ability. Vogler et al. (1999) concluded that pollen traits are well buffered against environmental variation in *Campanula rapunculoides*. In their study, the only pollen trait in which significant G × E interactions were found was pollen viability. With regard to pollen germination ability and pollen-tube growth rate, implicit information about variation in the direction of the response of different genotypes to changing thermal conditions can be obtained also from other studies (e.g. Polito et al. 1988; Elgersma et al. 1989; Travers 1999). However, there are no previous studies available on the G × E interactions in seed-siring success that is the most direct measure of pollen competitive ability and male reproductive success.

The consequences of the G × E interactions in pollen competitive ability found in *B. pendula* can be extended from the evolution of adaptive plasticity to practical tree breeding and commercial production of *B. pendula* seed.

Maintenance of genetic diversity in fitness-related traits is likely to be partly due to G × E interactions (e.g. Via and Lande 1985) although G × E interactions do not entirely guarantee the maintenance of genetic variation (see Prout and Savolainen 1996). With regard to pollen-tube growth rate, selection for faster-growing pollen tubes does not necessarily apply only to pollen-tube growth rate but also to many sporophytic traits, like seed germination and seed mass. This assumption is based on gametophytic-sporophytic genetic overlap, meaning that pollen tube growth and plant growth both depend on the basic metabolic activities controlled by the same genes (Mulcahy 1979; Ottaviano and Mulcahy 1989). From the practical point of view, G × E interactions can have implications for the functioning of seed orchards. It is possible that different pollen donors will be selected depending on the environmental conditions during pollination and pollen tube growth (Pasonen et al. 2000), which leads to random variation in the genetic composition of the seed crop. Consequently, changes in the quality of seed and seedling material can occur.

To estimate the evolutionary significance of G × E interactions in pollen competitive ability in *B. pendula*, we need to know more about the heritabilitiy of pollen performance in this species. Although some studies document heritable variation in pollen-tube growth rate in some other species (Ottaviano et al. 1988; Sari-Gorla et al. 1992; Lankinen 2000), there are no estimates on the heritabilities of pollen traits in *B. pendula* available at the moment. Furthermore, interpreting G × E interactions when only one pollen donor has been used as a standard donor may sometimes be difficult if significant environmental or maternal effects on the performance of the standard pollen donor exist. In this study, the seed-siring success of the standard donor (clone E 1970) in relation to other donors is higher in the greenhouse than outdoors. We suggest that environmental conditions have a substantial effect on the performance of the standard donor. The same pollen donor has previously been found to have the longest pollen tubes at 30 °C (the common temperature in *B. pendula* greenhouse seed orchards during flowering) when compared with five other pollen donors (Pasonen et al. 2000), and the differences in pollen-tube growth rates are known to translate into parallel differences in seed-siring success in controlled greenhouse conditions (Pasonen et al. 1999). In natural pollination conditions, pollen donors have to compete with several other pollen donors and further insight into G × E interactions would be obtained if more than one pollen donor would be used as standard donors in future studies.

In this study, the pollination site had a significant influence on pollen-tube growth rate and seed-siring success in *B. pendula*. Environmental factors during pollen development are known to affect pollen performance (see e.g. Delph et al. 1997). In natural habitats soil fertility varies from one microsite to another, which can lead to differential abilities of plants to provide developing pollen grains with resources (e.g. Lau and Stephenson 1993, 1994). Storage products provided by the pollen-producing parent are metabolized during pollen tube growth (e.g. Jackson and Linskens 1982) and are thought to play important roles during pollen germination (Mulcahy and Mulcahy 1982). Herbivory (Quesada et al. 1995; Mutikainen and Delph 1996) and temperature (Johannsson and Stephenson 1998) are also known to affect pollen-tube growth rates and the seed-siring ability of pollen. In our study, all the pollen donors were grown under similar greenhouse conditions and, thus, significant environmental effects on pollen performance are due to variation in prevailing environmental conditions during pollen germination.

One of the main interests of this study was to investigate the relationship between pollen-tube growth rate and seed-siring success under different environmental conditions. The significant positive correlation between pollen-tube growth rate and seed-siring success in the greenhouse is in concordance with our previous results from the study carried out in similar greenhouse conditions (Pasonen et al. 1999). Interestingly, the relationship between pollen-tube growth rate and seed-siring success was not parallel at the two pollination sites used in this study. At the outdoor clone collection, which represents a more heterogeneous outdoor environment and conditions in natural *B. pendula* stands, the weighted mean correlation between pollen-tube growth rate and seed-siring success was significantly negative. At the outdoor clone collection, environmental maternal effects could have caused differences, for example, in maternal provisioning for germinating pollen-tubes. Maternal plants provide pollen-tubes with nutrients during pollen germination (Labarca and Loewus 1973; Sanders and Lord 1989; Wu et al. 1995; Herrero and Hormaza 1996), and their ability to do this can vary from one microsite to another depending, for example, on the nutrient content of the soil. In the greenhouse, all the maternal plants were grown under similar temperature, water and nutrient conditions. Microclimatic differences and variation among microsites are much more substantial in natural birch stands than in the greenhouse.

In the present study, a significant positive correlation in the seed-siring success of the pollen donors between the two pollination sites was detected. This indicates consistency in the rankings of the pollen donors in their ability to sire seeds across different pollination environments despite the existence of significant G × E interaction. The fact that the correlation in pollen-tube growth rates between the two pollination sites was negative implies that the changes in pollen-tube growth rates do not lead to parallel changes in seed-siring success. It seems that pollen-tube growth rate might not be of crucial importance in determining seed-siring success in natural pollination conditions. The other mechanisms determining seed-siring success might include some form of female choice. Female choice can act during pollen-tube growth allowing only some pollen tubes to achieve fertilization (e.g. Cruzan 1993; Herrero and Hormaza 1996), or during embryo and seed development when seeds sired by certain pollen donors are aborted more frequently than seeds sired by other

donors (e.g. Marshall and Ellstrand 1988). However, further studies are needed to verify the role of female choice in the selection of pollen donors in *B. pendula*.

The result that there is a significant consistency in the rankings of the pollen donors in pollen-tube growth rate across different maternal plants in the greenhouse is also in concordance with the results from our previous study carried out in similar greenhouse conditions (Pasonen et al. 1999). Significant concordance in the rankings of the pollen donors was also found in seed-siring success across different maternal plants at both pollination sites. This indicates that the same pollen donors, on the average, are the most successful in siring seeds independent of the maternal parent. Consistent rank ordering of the pollen donors in seed-siring success after mixed-pollinations across different maternal plants has previously been reported at least in *Hibiscus moscheutos* (Snow and Spira 1996), *Lesquerella fendleri* (Mitchell and Marshall 1998) and *Raphanus sativus* (Marshall 1998).

The results of this study suggest that pollen-tube growth rate can be a predictor of seed-siring success in controlled greenhouse conditions where differences among maternal plants are mainly of genetic origin, but not in more-heterogeneous outdoor conditions. In natural birch stands, environmental maternal effects are likely to diminish the significance of pollen competition for sexual selection in *B. pendula*. From this perspective, environmental variability and genotype-environment interactions are likely to explain a large part of the variation observed in pollen-tube growth rates. To obtain a more accurate insight into the consequences of pollen competition for sexual selection in natural stands of *B. pendula*, further studies are needed.

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