Why fast-growing pollen tubes give rise to vigorous progeny: the test of a new mechanism

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A positive correlation between the speed of pollen tube growth and the quality of the resulting progeny in several species of flowering plants has traditionally been explained as being caused by an overlap in gene expression between gametophytes and sporophytes. We experimentally manipulated the pollen tube growth rates of pollen donors, such that the genotype controlling the rate was uncoupled from the phenotype, to test the alternative hypothesis that the correlation arises because ovules fertilized early are better provisioned by the maternal plant than later-fertilized ovules. Crosses using *Silene vulgaris* individuals bearing distinctive genetic markers revealed a correlation between the order of fertilization by pollen grains and vigour of the resulting sporophytes, which was independent of the speed of growth of the pollen tubes. Seeds sired by donors with relatively fast-growing pollen germinated earlier and grew larger than those sired by slow-growing pollen when pollen from the two donors was applied simultaneously. Reversing the order of arrival in the ovary by placing slow-growing pollen on the styles earlier and closer to the ovary led to reverse results. These results suggest that differential provisioning by the maternal plant can lead to differences in progeny vigour following pollen competition.

Keywords: maternal effects; pollen competition; pollen tube; growth rate; *Silene vulgaris*

1. INTRODUCTION

Pollen competition in flowering plants, which occurs when there are more pollen grains delivered to a flower than there are ovules to fertilize, has often been shown to result in the production of more vigorous progeny than the absence of competition (Mulcahy & Mulcahy 1975; Ter Avanesian 1978; McKenna 1986; Winsor *et al.* 1987; Ramstetter & Mulcahy 1988; Bertin 1990; Richardson & Stephenson 1991; Quesada *et al.* 1993; Palmer & Zimmerman 1994; Björkman 1995; Jóhannsson & Stephenson 1997; but see Snow 1990, 1991; Mitchell 1997). One mechanism that has been widely cited as the cause of the enhanced sporophyte vigour concerns the speed or vigour of pollen tube growth, and this mechanism has been advocated as an important selective factor in angiosperm evolution (Mulcahy 1979). This hypothesis states that only the fastest growing, most vigorous pollen will achieve fertilization when pollen grains are competing for ovules. In addition, seeds sired by relatively fast-growing pollen will be more vigorous than those sired by slow-growing pollen, because of an overlap in gene expression between the gametophyte (the pollen) and the sporophyte (the progeny). In other words, when competition occurs, pollen grains with genes for relatively high vigour will sire more seeds than those with genes that make them less vigorous, and these same genes affect growth rate in the progeny, leading to a positive correlation between gametophyte and sporophyte vigour. There are two requirements for this mechanism to work. First, there must be overlapping gene expression between the two life cycle stages,

and heritable variation in pollen tube growth rate must exist (Schlichting *et al.* 1990).

Here we report results from a series of experiments designed to test an alternative mechanism to explain why a positive correlation exists between the vigour of pollen grains and the seeds they sire. We hypothesize that ovules fertilized early are better provisioned by the maternal plant than later-fertilized ovules. In other words, it is the order of fertilization, rather than the speed of pollen tube growth *per se*, that affects the vigour of the resulting progeny. This hypothesis does not require heritable variation in pollen tube growth rate, as even environmentally caused differences in growth rate will lead to variation in the timing of fertilization within a flower.

2. MATERIALS AND METHODS

(a) *Species*

Silene vulgaris is well-suited for pollen tube competition studies because the flowers have three styles that are receptive along their entire length. Each style provides access to ovules located in one of three separate locules, and this compartmentalization allows the elimination of position effects which have been shown to affect progeny vigour in other studies (Rocha & Stephenson 1990; Schlichting *et al.* 1990). Seeds of *S. vulgaris*, originally collected from a natural population near Sussex, England, by D. Charlesworth, were grown in pots and assayed for genotype at a phosphoglucosyltransferase locus (PGM-2) with three alleles using cellulose-acetate electrophoresis, so that paternity following pollinations could be determined. Cellulose-acetate gels were prepared and run according to Richardson *et al.* (1986).

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(b) Experiments

We defoliated one set of plants after the onset of flowering to produce pollen donors with relatively slow-growing pollen tubes as compared with unmanipulated control plants. This manipulation was done to decouple the genotype from the phenotype, as the treatments were assigned at random with respect to genotype. The effect of defoliation on pollen tube growth rates was determined both *in vitro* and *in vivo*. *In vitro* rates were determined by spreading pollen from the two donor types on Kwack's pollen-germination medium (Brewbaker & Kwack 1963) made semisolid with 1% agarose. After 4 h, pollen tube lengths were measured with an image digitizer. Sections of agarose were removed from the Petri dishes, placed pollen-side down on a glass slide, and projected with a Ken-a-vision projector onto a digitizer pad. The first ten tubes encountered were measured for length for each of 15 donors. In addition, we did *in vivo* pollinations that allowed the two types of donors (defoliated and control) to compete with each other for access to ovules. A total of six recipients were crossed with 20 defoliated and 20 control plants to give a direct demonstration that pollen tube growth rate affects the ability of a pollen grain to achieve fertilization. Pollen grains from same-age flowers (1–2 h after opening) of the two donors were placed in competition on styles of recipient plants by applying pollen under magnification to one side each of the tip of two styles per recipient flower. Donors and recipients assayed for genotype at the PGM-2 locus were used in crosses such that the paternity of the resulting seeds could unambiguously be determined. Following seed maturation, we randomly chose 10–20 seeds from each of the 19 fruit (one in the set of 20 failed to produce a fruit), for a total of 297 seeds, and seeds were electrophoretically scored to determine paternity. A one-way ANOVA was done to compare *in vitro* pollen tube growth rates, and a paired *t*-test was done to compare the number of seeds sired by defoliated versus control donors using the statistical package SYSTAT (SYSTAT 1989).

In addition, we performed crosses using defoliated and control pollen donors of known genotype at the PGM-2 locus to determine whether the first fertilized ovules were better provisioned for by the maternal plant and whether this influenced the vigour of the resulting progeny. We hand-pollinated two styles on flowers of 29 recipient plants with pollen from a defoliated or control donor, such that the pollen of the two donors was applied to the tip of one style each (note that this meant that the pollen tubes from the two donors were not competing for the same ovules). We hypothesized that progeny sired by the fast-growing pollen (i.e. pollen from control donors) would be more vigorous because they would have arisen from the first-fertilized, and hence best provisioned, ovules within the fruit. Alternatively, the defoliation may have affected other aspects of the pollen, besides pollen tube growth rate, which contributed to our results. To distinguish between these two hypotheses, later we did a separate set of hand-pollinations. These pollinations were similar to the first set, except that pollen from defoliated donors was given the opportunity to achieve fertilization first by placing it at the base of the style 90 min earlier than pollen from the control donor, whereas pollen from the control donor was placed at the tip of the style (pollen tubes typically take *ca.* 3 h to reach the ovules in these flowers when placed at the tip of the style).

For both sets of pollinations, one newly dehiscent anther per style was brushed across the styles of recipient flowers, thereby providing heavy pollen loads. Within each flower, one style

received pollen from a defoliated donor and one style received pollen from a control donor, giving each donor access to a separate set of ovules, as each style has connections with only one of the three locules in each flower. For the first set of pollinations, the order of pollination was randomly chosen, and within a flower both styles were pollinated within 1 min of each other (i.e. nearly simultaneously). Following seed maturation, 10–40 seeds from each cross (this number represented more than 50% of the seeds from each cross) were individually weighed, sown separately into compartments in a tray (ensuring that no root competition could occur between plants), and placed in a mist room for germination. Timing of germination was recorded, and one week after sowing, flats were moved into a greenhouse with a 16 h light–8 h dark photoperiod. After true leaves were formed, a quarter of one leaf was removed from each plant and electrophoretically scored to determine paternity. Above-ground biomass of each plant was harvested 38–39 d after sowing, dried in an 80 °C oven for one week and weighed. For the first set of pollinations, in which pollen from the two donors was nearly simultaneously applied, a total of 29 recipient plants were pollinated and 696 seeds were sown and germinated (344 from defoliated donors and 352 from control donors). For the second set of pollinations, in which pollen from defoliated plants was applied first, a total of 15 recipient plants were pollinated and 1096 seeds were sown and germinated (568 from defoliated donors and 528 from control donors). Note that the number of seeds sired did not differ significantly between the two donors in either set of crosses; this was expected given that the ovules they had access to were in different locules. One-way ANOVAs were done using SYSTAT (SYSTAT 1989) to compare seed mass, rate of germination, and above-ground biomass of the seedlings sired by defoliated versus control donors in both experiments. Above-ground biomass data were square root-transformed before analysis.

3. RESULTS

Pollen tubes from defoliated donors grew less than half as quickly *in vitro* as pollen tubes from control donors (0.59 ± 0.131 against 1.27 ± 0.156 (means $\pm$ s.e.); $F_{1,14} = 11.6$, $p < 0.001$). Furthermore, when pollen from the two types of donors was applied to the same style and therefore allowed to compete for access to ovules, defoliated donors sired significantly fewer seeds per fruit than control donors (6.6 ± 0.55 against 9.0 ± 0.58 seeds per fruit (means $\pm$ s.e.), respectively; $t = 3.3$, d.f. = 18, $p = 0.004$; 126 seeds against 171 seeds totalled over all crosses, respectively).

When pollen from defoliated donors, against control donors, was applied relatively simultaneously to the ends of separate styles, seeds sired by control donors were slightly larger ($F_{1,694} = 1.16$, $p = 0.283$), germinated significantly earlier ($F_{1,692} = 6.89$, $p = 0.009$), and grew significantly larger ($F_{1,693} = 5.84$, $p = 0.016$) than those sired by defoliated donors (see figure 1a). In contrast, when pollen from defoliated donors was applied before pollen from control donors and at the base of the style, the results were directly opposite those of the previous experiment (figure 1b): seeds sired by defoliated donors were slightly larger than those from control donors ($F_{1,1091} = 1.69$, $p = 0.193$), and seeds sired by defoliated donors significantly outperformed those from control donors in terms of timing of germination ($F_{1,1059} = 10.17$, $p = 0.001$) and above-ground biomass ($F_{1,1092} = 8.62$,

Figure 1. Performance of seeds sired by either defoliated or control donors when (a) pollen from the donors was applied to the tips of two different styles within a flower nearly simultaneously ($p =$ (i) 0.28, (ii) 0.01, (iii) 0.02), and (b) pollen from the defoliated donor was applied to the base of one style ($p =$ (i) 0.19, (ii) 0.001, (iii) 0.003), 90 minutes before application of pollen from the control donor to the tip of another style. Values are means ($\pm$ s.e.) for seed mass, number of days from planting to germination and above-ground dry mass of the progeny after 38–39 days.

$p = 0.003$). Hence, in both cases, the donors whose progeny were the most vigorous were the donors whose pollen grains were the first to fertilize the ovules within a flower (i.e. control donors in the first experiment and defoliated donors in the second experiment).

4. DISCUSSION

Our results suggest that the first ovules to be fertilized are given a resource advantage by the maternal parent, which is independent of the genes of the pollen donor that

control pollen tube growth rate. This differential provisioning could occur if carbon demand or hormones produced by the developing endosperm and zygotes determine the quantity of resources allocated by the maternal plant (Brenner 1987). This is supported by our observed differences in seed mass and germination date for seeds sired by the two types of donors: first-fertilized seeds, regardless of whether they were sired by slow- or fast-growing pollen, were consistently, though non-significantly, heavier than later-fertilized seeds and germinated significantly earlier. The overlapping gene-expression hypothesis, in which genes responsible for the growth of the pollen tube are also expressed during the growth of the sporophyte (strongly supported by selection experiments using maize (Ottaviano *et al.* 1988) and a hybrid cucurbit (Quesada *et al.* 1996)) could not have contributed to our results, as pollen tube growth rates were phenotypically modified by the experimental defoliation. Hence, our results show that the positive correlation commonly observed between the vigour of male gametophytes and the resulting sporophytes may not always be caused by an overlap in gene expression between these two life cycle stages, but may instead be caused by non-genetic maternal effects.

Experimental studies investigating how pollen competition leads to an increase in sporophyte vigour must control for the possibility that non-random seed abortion, position effects within the ovary, pollen–pistil interactions, and hormonal consequences of heavy versus light pollen loads could influence the results (Charlesworth 1988; Walsh & Charlesworth 1992). In our study, no seed abortion occurred, and position effects were unlikely given the compartmentalization of ovules into locules. Furthermore, *S. vulgaris* is fully self-compatible, and differences in pollen loads could not have contributed as these were equalized across donors in our pollinations (all high loads). Therefore, although these issues have been raised with respect to some studies on pollen competition, they are unlikely to have influenced the outcome of our experiments.

Given that reproductive success has been shown to be affected by pollen tube growth rates in our study, as well as others (Ottaviano *et al.* 1988; Snow & Spira 1991; Radha *et al.* 1993), heritabilities of growth rates might be low in natural populations, as natural selection should eliminate or greatly reduce additive genetic variation in traits that affect fitness (Fisher 1930). Whereas the overlapping gene-expression hypothesis requires heritable variation in pollen tube growth rates for there to be an affect on progeny vigour, the provisioning hypothesis does not. Few measures of heritable variation in pollen tube growth rates in wild plants have been done, and no appreciable genetic variation has been found (see, for example, Snow & Mazer 1988; Havens 1993). Instead, the speed of pollen tube growth has been found to be highly plastic (see review in Delph *et al.* 1997), responding phenotypically to many types of environmental heterogeneity, including soil temperature (Delph *et al.* 1997), soil nutrient level (Young & Stanton 1990; Lau & Stephenson 1993, 1994), temperature during pollination (Elgersma *et al.* 1989), and leaf loss as shown here, as well as more natural levels of loss that simulate herbivory (Quesada *et al.* 1995; Mutikainen & Delph 1996; Delph *et al.* 1997).

If heritable variation in the speed of pollen tube growth is found to exist in natural populations, then both the overlapping gene-expression hypothesis and the provisioning hypothesis could select for an increase in pollen tube growth rate. In addition, Haldane's hypothesis, that fast-growing pollen tubes would simply sire more seeds, would also select for an increase in growth rates (Haldane 1932). Our results are consistent with Haldane's hypothesis, as are the results of Snow & Spira (1991) with *Hibiscus moscheutos*, because greater numbers of seeds were sired by donors with fast-growing pollen tubes. Our results are also consistent with the provisioning hypothesis, because order of fertilization affected progeny vigour. Future studies of heritability in pollen tube growth rates, and the correlation with progeny vigour, are needed to determine the evolutionary significance of variation in pollen performance.

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