Pollen competitive ability: the effect of proportion in two-donor crosses

Lankinen, Åsa; Skogsmyr, Io

Published in:
Evolutionary Ecology Research

2002

Evolutionary Ecology Research, 2002, **4**: 687–700

Pollen competitive ability: the effect of proportion in two-donor crosses

Åsa Lankinen* and Io Skogsmyr

Department of Theoretical Ecology, Ecology Building, Lund University, S-223 62 Lund, Sweden

ABSTRACT

Pollen competitive ability depends on the innate capacity of a pollen donor to produce pollen that reaches the ovules fast, but could also be a consequence of the ability to interfere with pollen from other donors. In a greenhouse study on *Viola tricolor*, we examined the relative importance of both of these effects by performing crosses where we varied the pollen load composition of two donors. We found that when a pollen donor had higher *in vitro* pollen tube growth rate than a competitor, this donor sired proportionally more seeds in most cases. At very low proportions, however, there was no benefit of producing fast growing pollen. We further investigated the potential for pollen interactions by comparing *in vitro* performance in single- and mixed-donor batches of the same density. Pollen tube growth rate differed between treatments in some donor combinations, indicating that pollen from different donors interact. Only donors with the faster growing pollen tubes in the single samples showed signs of interference in the mixtures. Donors with slower pollen tube growth had an *increased* growth rate when mixed. Although our results suggest interactions between pollen grains from different donors that might affect siring ability, the intrinsic pollen tube growth rate was more important for siring ability in this species.

Keywords: pollen competition, pollen interactions, pollen tube growth rate, sexual selection in plants, *Viola tricolor*.

INTRODUCTION

To date, several studies have shown that pollen competitive ability can affect the siring success of pollen donors (e.g. Snow and Spira, 1991a,b, 1996; Marshall, 1998; Pasonen *et al.*, 1999; Skogsmyr and Lankinen, 1999). In most of these studies, hand pollination was performed to ensure that all donors contributed an equal amount of pollen. This allowed differences in siring ability to be related to individual variation and to intrinsic pollen traits. The pollen trait that is most often found to have a large impact on pollen competitive ability is pollen tube growth rate (e.g. Snow and Spira, 1991a,b, 1996; Pasonen *et al.*, 1999; Skogsmyr and Lankinen, 1999). In the wild, however, pollen loads deposited on stigmas are mixed, with an unequal contribution from different donors (Schaal, 1980; Thomson and Plowright, 1980; Price and Waser, 1982; Waser and Price, 1984; Thomson, 1986; Thomson

* Author to whom all correspondence should be addressed. e-mail: asa.lankinen@teorekol.lu.se

et al., 1986). It is possible that other traits might become important in determining siring success when the proportion of pollen differs between donors. One such trait could be the ability of pollen to inhibit germination or growth of pollen from another donor, resulting in interference competition.

A few studies have indicated that the genetic composition of the pollen load can influence the outcome of pollen competition (Landi and Frascaroli, 1988; Cruzan, 1990; Mitchell and Marshall, 1995; Havens and Delph, 1996; Marshall *et al.*, 1996; Pasonen and Käpylä, 1998). In maize, for example, donors that contributed more than half of the pollen load had a disproportionately high siring success (Landi and Frascaroli, 1988), indicating that pollen competitive ability was enhanced by the presence of related pollen.

To elucidate potential selection of high inherent competitive ability and/or a capacity to interfere with other pollen, it is important to include both aspects of pollen competition in the same study (Marshall *et al.*, 1996). One way to do this is to determine how high intrinsic pollen competitive ability affects the outcome of pollen competition when the pollen load consists of different proportions from two donors.

In a previous study on *Viola tricolor*, we applied equal proportions of pollen from two donors (Skogsmyr and Lankinen, 1999). Pollen tube growth rate was the most important trait for siring ability in these circumstances. In this study, we investigate siring ability when there is variation in the proportion of pollen contributed by two donors. In one set of crosses, the pollen tube growth rate *in vitro* differs between donors. In the other, there is no difference, which should make interference competition easier to detect. Thus, we are able to establish whether pollen tube growth rate is important when the proportion of pollen differs and compare this effect to one of interference competition. In a second experiment, we examine the possibility for interference competition by comparing the *in vitro* pollen tube growth rate of single- and mixed-donor batches.

MATERIALS AND METHODS

Plant material

Viola tricolor is a hermaphroditic annual growing on dry hillsides, flat rocks, sand dunes and cultivated lime-deficient soil in nearly all of Europe and Asia (Lagerberg, 1948; Mossberg *et al.*, 1992). The species is normally outcrossing and pollinated by insects (mainly Hymenoptera) (Lagerberg, 1948; Elfving, 1968). Some self-pollination can occur (Lagerberg, 1948). Flowers prevented from self-pollination in the greenhouse produced fewer seeds (Skogsmyr and Lankinen, 1999). A theoretical comparison between *in vitro* pollen tube growth rate differences and pistil length within a population showed that fast growing pollen grains would have an advantage even if deposited a considerable time after slow growing ones (Skogsmyr and Lankinen, 1999). This suggests that there is an opportunity for pollen competition to take place in this species.

The plants used in the crosses originated from two isolated populations in France (Nancy, $n = 26$ plants; Bent, $n = 9$ plants). Plants from Nancy were of a second greenhouse generation, while the plants from Bent originated directly from the wild. In the second experiment, we used only individuals originating from Nancy.

The distribution of genetic markers used for paternity analysis constrained the choice of donors so that we always used one donor from each population. We have found no effect of population origin on the recipient plants in earlier experiments with these populations

(Skogsmyr and Lankinen, 1999). The wild populations did not differ in siring ability or in pollen tube growth rate *in vitro* (Skogsmyr and Lankinen, 1999, 2000). Furthermore, there is no effect of relatedness between donor and recipient on siring ability in *Viola tricolor*. Abortion is very uncommon in our study material and germination ability is about 80%. Thus, although we actually test who sired the mature seeds, this is similar to determining who fertilized the ovules and we will henceforth only refer to 'siring ability'.

Experiment 1

Two-donor crosses with different pollen load compositions

We performed controlled two-donor crosses to determine whether a high *in vitro* pollen tube growth rate affected siring ability when the relative contribution to the pollen load differed between donors. We divided donor pairs into two groups depending on *in vitro* pollen tube growth rate. In group A there was a significant difference in *in vitro* pollen tube growth rate, whereas in group B there was none. For simplicity, the two competing donors are referred to as 'faster' and 'slower' in both groups. Pollen from the same pair of pollen donors was allowed to compete in up to five pollen load compositions in a replacement series design: 10 : 90, 30 : 70, 50 : 50, 70 : 30 and 90 : 10. We made the crosses in a greenhouse during the summer of 1995. For paternity analysis, we used genetic markers (a single locus with three alleles coding for PGM) identified with starch gel electrophoresis (Soltis and Soltis, 1990; Skogsmyr and Lankinen, 1999). Pollen donors were selected with four specific combinations of contrasting alleles at the PGM locus.

We made 40 two-donor crosses in 13 donor combinations (Appendix 1), of which 7 combinations belonged to group A and 6 combinations to group B. In all, we used 22 pollen donors and 13 recipient plants. Rather than aiming at a complete factorial crossing table, we used the Darwinian approach (see review by Charlesworth *et al.*, 1987). We thus included as many different combinations of individuals as possible, rather than determining exactly what happened within a specific donor–recipient combination (Snow, 1994; Skogsmyr and Lankinen, 2000). This allows us to understand the generality of how a specific trait (e.g. pollen tube growth rate) affects an evolutionarily important aspect (e.g. siring ability). By arbitrarily selecting crosses within groups (fast/slow donor and recipient plant), the effects of individual differences in compatibility, for example, should cancel out. As a result, our statistical analysis is more conservative than would have been the case if we had used full factorials. In other words, we get significant relationships in spite of, not because of, not having controlled for effects of crossing combinations.

Although we performed most crosses only once, we analysed a relatively large number of seedlings per cross (18 on average). This allowed us to identify even small differences in siring ability between donors during a specific crossing event. All crosses with a certain donor pair were performed on the same recipient plant, so that within each replacement series we kept maternal impact constant. Although maternal influence has been detected in this species, such effects were not large enough to reverse the rank order of pollen donors (Skogsmyr and Lankinen, 1999). Some individuals were used as both pollen donors and recipient plants. We generally avoided crosses with close relatives.

Hand pollinations were made on emasculated maternal flowers using the methods described in Skogsmyr and Lankinen (1999). We used a total of 200 pollen grains from both donors in all crosses. This equals about eight pollen grains per ovule. To determine the

amount of pollen, we counted pollen grains from each donor on a microscopic slide under a binocular magnifying glass. The counted pollen was applied to the stigma directly from the slides. Pollen from each donor was applied evenly all over the stigma immediately after one another (see Skogsmyr and Lankinen, 1999).

We determined paternity of the seedlings in the spring of 1996. The number of seeds produced by hand pollination did not differ from that in naturally pollinated plants (Skogsmyr and Lankinen, 1999). Since we were able to screen only a certain number of seedlings for genetic markers, we randomly excluded some individuals when the number of seedlings from a cross was very high. We cannot exclude the possibility that germination differed between offspring from different donors. Again, any effect would obscure the difference between series from group A (with a difference in pollen tube growth rate) and group B (with no difference), rather than creating a false correlation.

Measurement of in vitro pollen tube growth rate of donor pairs

We evaluated pollen tube growth rate of donor pairs in Hoekstra germination medium (Hoekstra and Bruinsma, 1975). We germinated pollen collected from three flowers per donor. Pollen was allowed to grow for 2 h in a dark chamber at a constant temperature of 22°C. As an indication of average pollen tube growth rate of donors, we measured pollen tube length of the first eight pollen tubes encountered in the microscope view. The repeatability of this *in vitro* pollen tube growth rate *within* a donor is very high (e.g. Skogsmyr and Lankinen, 1999; Lankinen, 2001). We used a *t*-test (Appendix 1) to determine whether there was a difference in pollen tube growth rate between donors (group A) or not (group B).

One reason for using the simpler *in vitro* method instead of the *in vivo* method (e.g. Snow and Spira, 1991b) was that it made it possible to estimate innate pollen performance without any maternal influence. Another reason was that, in this species, the *in vitro* method is probably the more reliable of the two. Pollen tube growth rate *in vitro* has been shown to correlate strongly with siring success (Skogsmyr and Lankinen, 1999). On the other hand, because of problems with dyeing techniques, the only *in vivo* method we have been able to use (involving a dye solution with safranin O and aniline blue; Dafni, 1992) is questionable, since the number of pollen tubes that can be measured reliably per sample is too low (Lankinen, 2001).

Statistical analysis

In both groups, we assessed whether the relation between siring ability and the proportion of pollen could be explained by a linear association. To do this, we computed a regression using an analysis of variance (ANOVA) where each value of x (pollen load proportion) had several values of y (arcsine-transformed siring ability) (Sokal and Rohlf, 1995: 477–479). This allowed us to separate the two sources of variation that cannot be explained by the linear regression; that is, deviations from regression and errors among y's for a given value of x. In other words, it separates a true representation of how y responds to treatment x (e.g. by a curvilinear function) from sources of experimental error (cf. an ANOVA with replication). It thus provides us with additional information compared with the more commonly used regression analysis without replication where the only information gained is the fit to a linear regression (cf. an ANOVA without replication). In the analyses, we excluded crosses where the number of seeds was less than 10.

To determine whether specific crosses were significantly different from what was expected from composition in the pollen load, we used chi-square analyses. When any expected value was less than or equal to 5, we used a binomial test (Siegel and Castellan, 1988: 50). Our *n*-values *within* crosses were sometimes too low to reliably test differences in siring ability between donors, particularly when the pollen percentage of the donors was very divergent (e.g. 90 : 10). Therefore, we combined treatments with similar distributions (Siegel and Castellan, 1988). This allowed us to establish whether the difference in pollen tube growth rate *in vitro* between donors was correlated with siring ability. For each donor pair, we based siring ability on all crosses available in the interval between 30 and 90% of the faster donor. The lowest frequency was excluded, since the distribution around the expected value was different from that in the other pollen compositions. We included all donor pairs that were used at least twice. The siring ability of the faster donor that is not explained by the proportion of pollen deposited, 'competitive siring ability', can be written as

$$\frac{n_{\text{faster donor}}}{n_{\text{faster donor}} + n_{\text{slower donor}}} - E(p)_{\text{faster donor}} \quad (1)$$

where n is the number of seeds produced in all pollen proportions and $E(p)$ is the expected proportion of seeds sired in all proportions given that both donors are equally good competitors.

The difference in pollen tube growth rate is relatively larger when the absolute pollen tube growth rates of the donors are smaller. We therefore standardized differences by dividing the tube growth rate of the faster pollen donor with the sum of growth rates form both donors. All proportions were arcsine-transformed.

In all analyses, the donor pairs used in the crosses can be considered statistically independent samples. It should be noted, however, that since some individuals were used in more than one pair, there was a certain amount of dependence between crosses.

Experiment 2

In vitro pollen performance in single-donor and mixed-donor batches

To determine whether pollen from different pollen donors interacted *in vitro*, we compared pollen tube growth rate in single-donor batches with that in mixed-donor batches. In the summer of 1996, pollen grains from 16 individuals were germinated both alone and in mixtures of two unrelated donors in a Hoekstra germination medium (Hoekstra and Bruinsma, 1975) (see above for methods). We used pollen grains from three flowers per individual for each test.

Both single-donor and mixed-donor batches of the same individuals were made at the same time. We used an approximately equal amount of pollen in the single-donor samples and in the mixtures, although we did not actually count the grains. Counting was not practical, since a large amount of pollen is needed to avoid effects of density on pollen tube growth (cf. pollen population effect; Brewbaker and Majumber, 1961; Schemske and Fenster, 1983; Cruzan, 1986; Thomson, 1989) and the medium will dry if exposed to strong light for a long time. In the mixtures, the pollen loads consisted of equal amounts of pollen from both donors (1 : 1). By subsequently applying pollen from the two donors in several sparse layers, we produced an even mix of pollen on the surface of the germination medium.

As an indication of pollen tube growth rate, we measured 8 and 16 pollen tubes per sample in the single and mixed batches, respectively. We were unable to differentiate between the donors in the mixed batches.

Statistical analysis

We assessed whether *in vitro* pollen performance in the mixed batch differed from the average of the two single batches. The overall significance of interactions between pollen donors was determined with a binomial test (Siegel and Castellan, 1988: 38–44). The null hypothesis was that pollen donors do not interact; that is, pollen performance in two-donor mixtures equals the average in the corresponding single-donor samples. We should then only find significant differences between these types in ≤ 5% of all cases. The two categories are: (1) all cases in which pollen performance in mixed batches is significantly different from the average of the single batches and (2) all non-significant cases.

The proportional change of pollen tube growth rate in the two-donor batches compared with the single-donor ones was calculated as

$$\frac{\text{pollen tube growth rate}_{\text{two-donor batch}} - \text{pollen tube growth rate}_{\text{single-donor batch}}}{\text{pollen tube growth rate}_{\text{single-donor batch}}} \quad (2)$$

RESULTS

Experiment 1: Pollen competitive ability in different pollen load compositions

There was a non-linear rather than a linear relationship between siring ability and the proportion of pollen from the faster donor when there was a significant difference in pollen tube growth rate between donors (group A) (Table 1, Fig. 1a). When there was no difference in pollen tube growth rate between donors (group B), siring ability increased linearly with the proportion of pollen (Table 1, Fig. 1b). In group A, the fast donor had a higher siring ability than expected in all pollen proportions between 30 and 90% of this donor (Fig. 1a). However, when the fast donor contributed 10% of the pollen load, siring ability was disproportionately low for all four crosses performed. Indeed, there was a tendency for donors with a high intrinsic pollen tube growth rate to perform worse than those with a slower tube growth rate (binomial test; $n_{\text{above exp}} = 0$, $n_{\text{below exp}} = 4$, $P = 0.062$).

The standardized difference in pollen tube growth rate *in vitro* between donor pairs was positively correlated with competitive siring success (Fig. 2). This indicates that the magnitude of difference in pollen tube growth rate between competing donors is important for siring ability when 30–90% of the pollen load consists of pollen produced by a superior competitor.

Experiment 2: Potential interactions between pollen from different donors *in vitro*

In five of the eight comparisons between single-donor and mixed-donor batches *in vitro*, there was a significant difference in pollen tube growth rate (Fig. 3). This gives a high total significance for the occurrence of interactions between pollen from different donors (binomial test; $P_{(x \geq 20 \mid \text{no interaction})} < 0.0001$).

In vitro pollen tube growth rate in the mixed samples was stimulated in three cases and inhibited in two cases (Figs 3, 4). Furthermore, the nature of the interaction – that is,

Table 1. Siring ability of the faster donor in competition with the slower donor regressed on composition of the pollen load (for pollen percentages 10, 30, 50, 70 and 90 of the faster donor)

Source of variation	d.f.	SS	MS	F_s
Group A: crosses where average pollen tube growth rate *in vitro* differs [a]				
Among proportion groups	4	4.016	1.004	8.164**
linear regression	1	2.702	2.702	4.110
deviations from regression	2	1.315	0.657	5.345*
Within groups	15	1.845	0.123	
Total	19	5.861		
Group B: crosses with no difference in average pollen tube growth rate *in vitro* [a]				
Among proportion groups	4	3.016	0.754	10.304***
linear regression	1	2.784	2.784	24.060*
deviations from regression	2	0.231	0.116	1.582
Within groups	12	0.878	0.073	
Total	16	3.895		

Note: The relationship between siring ability and pollen load composition of the faster donor could be described by a linear function in group B ($y = -0.112 + 1.35x$), but not in group A. In group A, the significant deviations from the regression instead indicated a non-linear relationship. The regressions were calculated with analyses of variance where each value of x (pollen load proportion) had more than one value of y (arcsine-transformed siring ability). In the analyses of variance, among groups SS = linear regression SS + deviations from regression SS.
[a] See Appendix 1 for details.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

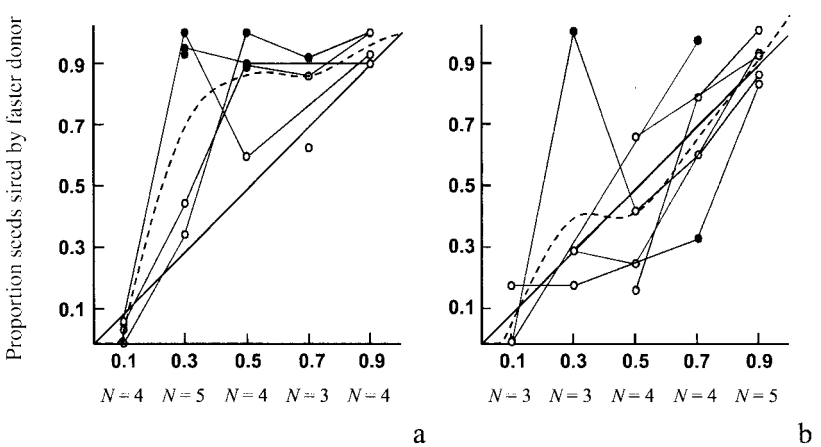

Fig. 1. Proportion of seeds sired by the faster donor after two-donor crosses where the relative pollen contribution to the pollen load was varied. (a) Average pollen tube growth rate *in vitro* when there was a significant difference in pollen tube growth rate between donors (group A). (b) No significant difference (group B). Solid circles denote that the cross was significantly different from that expected due to composition of the pollen load (indicated by a solid line). Connected circles denote crosses with the same pollen donor pair. The dashed line is fitted to the data with least squares. N = number of crosses. See Appendix 1 for further details.

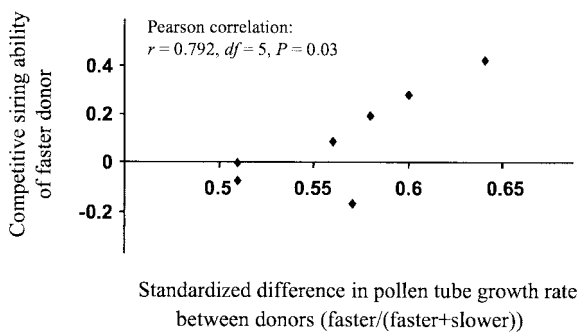

Fig. 2. Competitive siring ability of the faster pollen donor increased with the standardized difference in pollen tube growth rate *in vitro* between two competing donors. Competitive siring ability was calculated as the difference between observed and expected proportion seeds sired for the faster donor, where the expected siring ability depended only on proportion (see Methods). The measurement is based on all pollen proportions except the lowest (0.1), since the distribution around the expected value was different from that in the other pollen compositions. In the statistical analysis, all proportions were arcsine-transformed. See Appendix 1 for further details.

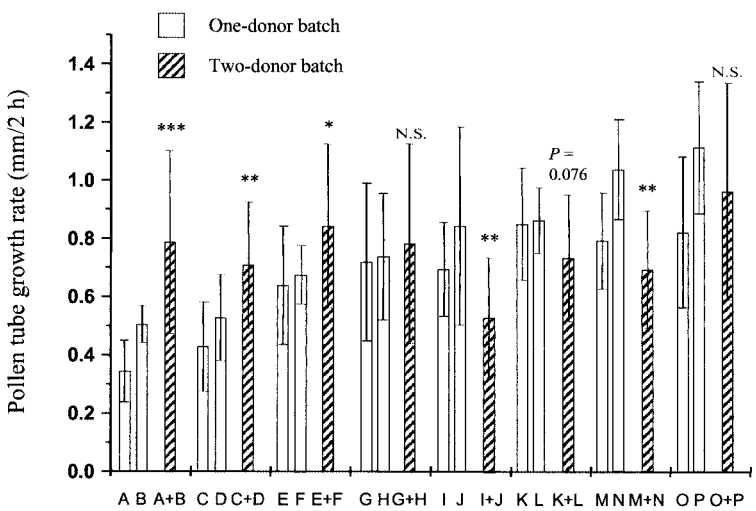

Fig. 3. In five of eight cases, there was a significant difference in pollen tube growth rate *in vitro* between single-donor and two-donor batches with the same density. Error bars indicate standard deviation. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; N.S. = $P > 0.1$.

stimulation or inhibition – depended on the absolute pollen tube growth rate of the two donors in single samples (Fig. 4). With slower absolute pollen tube growth rate of the two pollen donors, the growth rate in two-donor mixtures was higher/stimulated, while with faster absolute pollen tube growth, the growth rate in mixtures was slower/inhibited.

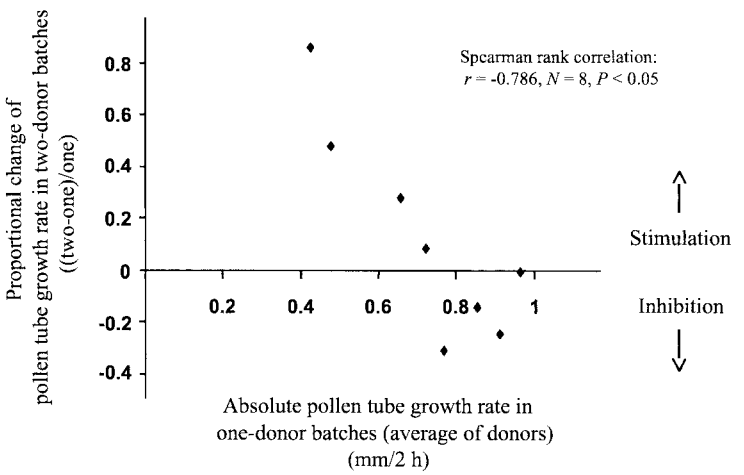

Fig. 4. The nature of pollen interactions – that is, stimulation or inhibition – depended on the absolute pollen tube growth rate *in vitro* of both pollen donors in single batches. When donors with slower growing pollen tubes were mixed, pollen tube growth rate increased compared with the average of single-donor samples. Mixing donors that produced faster growing pollen tubes resulted in a decrease in pollen tube growth rate.

DISCUSSION

In *Viola tricolor*, a donor producing faster growing pollen tubes had a siring advantage except when the proportional contribution to the pollen load was very low. The difference between donors in pollen tube growth rate (*in vitro*) was positively related to siring ability, showing that it is the relative speed of growth that is important. At very low proportions (10%), however, there was no advantage of having a fast intrinsic pollen tube growth rate. If anything, the fast pollen donors seemed to do worse than expected in these cases. Furthermore, *in vitro* pollen tube growth rate differed between mixed-donor batches and single-donor batches, indicating that pollen from different donors interact.

Although several studies have shown that pollen tube growth rate can influence siring ability (e.g. Snow and Spira, 1991a,b, 1996; Pasonen *et al.*, 1999; Skogsmyr and Lankinen, 1999), little is known about how this trait affects pollen competitive ability when the proportion of pollen is varied, as is the case in natural pollination. In wild radish, pollen donor identity was still important for seed siring ability when crosses were made with unequal amounts of pollen from two donors (Marshall and Ellstrand, 1986; Marshall *et al.*, 2000). Marshall *et al.* varied the number of flowers used to make the crosses, rather than counting the number of pollen grains. Thus, in some cases, it is possible that differences in siring ability are influenced by differences in pollen grain productivity. In our study on violets, we found that a pollen donor with a high *in vitro* pollen tube growth rate sired more seeds when the pollen from this donor varied between 30 and 90%. The difference in pollen tube growth rate between competing donors was also positively correlated with the siring success in these pollen proportions. This suggests that a fast pollen tube growth rate can be favourable when the composition of the pollen load differs. Unless

the pollen load proportion of a donor with fast growing pollen tubes is very low, this pollen trait can confer an advantage even when the proportion of pollen is lower than that of a competitor.

Interference competition between pollen of different donors from the same species has so far only been reported in a few species and little is known about the mechanisms involved (Marshall *et al.*, 1996; Mulcahy *et al.*, 1996). Some studies have indicated that there is a positive effect on germination when pollen from different donors are mixed (Mitchell and Marshall, 1995; Pasonen and Käpylä, 1998). Marshall *et al.* (1996), on the other hand, found that in mixed pollinations of wild radish pollen, germination was lower than in single or adjacent pollinations of the same density. This result is in accordance with the effect found in maize (Landi and Frascaroli, 1988). Interference between pollen from different species appears to have a chemical basis, since mixing pollen with *extracts* of pollen can produce the same results as mixtures with pollen (Kanchan and Jayachandra, 1980; Thomson *et al.*, 1981; Jiménez *et al.*, 1983; Murphy and Aarssen, 1989). In violets, we found that the nature of the response in two-donor mixtures was affected by the absolute pollen tube growth rate of the donors in the single samples. Pollen tube growth rate *increased* when donors with slow tube growth in single batches were mixed. When donors that produced faster pollen tubes were mixed, however, pollen tube growth rate *decreased*. Not only does this indicate that pollen from different donors interact, it also suggests that interference only occurs between superior competitors – that is, donors that produce faster growing pollen tubes. Unfortunately, it was not possible to determine what would happen with a combination of a fast and a slow grower. Since we do not know of a way to identify pollen identity in mixed batches, we cannot separate no effect from when one increases and the other decreases.

It is well known that density of the pollen load can have a large effect on germination ability and pollen tube growth rate (Brewbaker and Majumber, 1961; Schemske and Fenster, 1983; Cruzan, 1986; Thomson, 1989). Since we tried to use the same amount of pollen in both single and mixed batches, we believe it unlikely that the results can be explained by differences in density.

By providing the opportunity for interference competition (mixing pollen from different donors), germination as well as siring ability was affected in wild radish (Marshall *et al.*, 1996). The results of our *in vitro* studies indicate that pollen from different violets interact at least during the initial phase of pollen germination and tube growth. Even so, we did not find a general effect of interference in the donor crosses with equal pollen tube growth rate. This is not surprising, since our *in vitro* studies indicate that the response to interference varies between individuals – that is, some are stimulated while others are inhibited. Contrary to our expectations, an interference effect was seen in the crosses with a difference in pollen tube growth rate. The donor with faster pollen tube growth rate sired fewer seeds than expected in all cases where the proportion was at its lowest. This might indicate some kind of interference when slow growing pollen tubes are in excess and competing with fast growing pollen tubes. Thus, further studies are required to determine the function and importance of these pollen interactions.

Even though our results imply that a high inherent pollen tube growth rate is more important for siring ability, they also suggest that pollen–pollen interactions occur and might be of significance in some circumstances. If, after all, donors with slow growing pollen tubes really stimulate each other, whereas there is inhibition between donors of higher pollen tube growth rates, this could result in unpredictable outcomes of pollen

competition in certain pollen mixtures. In *Hibiscus moscheutos*, a pollen donor with faster growing pollen often sired more seeds in competition with slower growing pollen (Snow *et al.*, 2000). This difference disappeared in some but not all donor combinations when there was a short time delay in pollen arrival.

In conclusion, our results suggest that interactions between pollen grains from different donors can occur in *Viola tricolor*, although intrinsic pollen tube growth rate is more important for siring ability. Donors with a low innate pollen tube growth rate are stimulated by the presence of each other *in vitro*, whereas donors that produce pollen with fast tube growth rates are inhibited. Even so, when there is a significant difference in pollen tube growth rate, most offspring will be sired by the superior donor when at least a third of the pollen load originates from this donor. Only in cases of very low proportions can the interactions play a major role, so that a fast donor does worse than expected from the inherent pollen tube growth rate. Although the exact effect of pollen interactions is hard to determine from this study, it is clear that the intrinsic pollen tube growth rate is more important in most cases. Since our previous studies also indicate that this pollen trait is heritable (Lankinen, 2000; Skogsmyr and Lankinen, 2000), it should be possible for selection to act on intrinsic pollen tube growth rate under natural conditions – that is, when pollinators deposit unequally mixed pollen loads.

ACKNOWLEDGEMENTS

We wish to thank L. Delph and R. Härdling for helpful comments on the text. This work was supported by the Swedish Research Council for Forestry and Agriculture and the Royal Swedish Academy of Sciences.

REFERENCES

Brewbaker, J.L. and Majumber, S.K. 1961. Cultural studies of the pollen population effect and the self-incompatibility inhibition. *Am. J. Bot.*, **48**: 457–464.

Charlesworth, D., Schemske, D.W. and Sork, V.L. 1987. The evolution of plant reproductive characters: sexual versus natural selection. In *Sexual Selection: Testing the Alternatives* (J.W. Bradbury and M.B. Andersson, eds), pp. 317–335. New York: Wiley.

Cruzan, M.B. 1986. Pollen tube distributions in *Nicotiana glauca*: evidence for density dependent growth. *Am. J. Bot.*, **73**: 902–907.

Cruzan, M.B. 1990. Pollen–pollen and pollen–style interaction during pollen tube growth in *Erythronium grandiflorum* (Liliaceae). *Am. J. Bot.*, **77**: 116–122.

Dafni, A. 1992. *Pollination Ecology: A Practical Approach*. Oxford: IRL Press.

Elfving, R. 1968. Die Bienen Finnlands. *Fauna Fenn.*, **21**: 3–69.

Havens, K. and Delph, L.F. 1996. Differential seed maturation uncouples fertilization and siring success in *Oenothera organensis* (Onagraceae). *Heredity*, **76**: 623–632.

Hoekstra, F.A. and Bruinsma, J. 1975. Respiration and vitality of binucleate and trinucleate pollen. *Physiol. Plant.*, **34**: 221–225.

Jiménez, J.J., Schultz, K., Anaya, A.L., Hernández, J. and Espejo, O. 1983. Allelopathic potential of corn pollen. *J. Chem. Ecol.*, **9**: 1011–1025.

Kanchan, S. and Jayachandra. 1980. Pollen allelopathy – a new phenomenon. *New Phytol.*, **84**: 739–746.

Lagerberg, T. 1948. *Vilda växter i Norden*. Stockholm: Bokförlaget natur och kultur.

Landi, P. and Frascaroli, E. 1988. Pollen–style interactions in *Zea mays* L. In *Sexual Reproduction in Higher Plants* (M. Cresti, P. Gori and E. Pacini, eds), pp. 315–320. Berlin: Springer.

Lankinen, Å. 2000. Effects of soil pH and phosphorus on *in vitro* pollen competitive ability and sporophytic traits in clones of *Viola tricolor*. *Int. J. Plant Sci.*, **161**: 885–893.

Lankinen, Å. 2001. *In vitro* pollen competitive ability in *Viola tricolor*: temperature and pollen donor effects. *Oecologia*, **128**: 492–498.

Marshall, D.L. 1998. Pollen donor performance can be consistent across maternal plants in wild radish (*Raphanus sativus*, Brassicaceae): a necessary condition for the action of sexual selection. *Am. J. Bot.*, **85**: 1389–1397.

Marshall, D.L. and Ellstrand, N.C. 1986. Sexual selection in *Raphanus sativus*: experimental data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. *Am. Nat.*, **127**: 446–461.

Marshall, D.L., Folsom, M.W., Hatfield, C. and Bennett, T. 1996. Does interference competition among pollen grains occur in wild radish? *Evolution*, **50**: 1842–1848.

Marshall, D.L., Avritt, J.J., Shaner, M. and Saunders, R.L. 2000. Effects of pollen load size and composition on pollen donor performance in wild radish, *Raphanus sativus* (Brassicaceae). *Am. J. Bot.*, **87**: 1619–1627.

Mitchell, R.J. and Marshall, D.L. 1995. Effects of pollination method on paternal success in *Lesquerella fendleri* (Brassicaceae). *Am. J. Bot.*, **82**: 462–467.

Mossberg, B., Stenberg, L. and Ericsson, M. 1992. *Den nordiska floran*. Brepols: Wahlström & Widstrand.

Mulcahy, D.L., Sari-Gorla, M. and Bergamini Mulcahy, G.B. 1996. Pollen selection – past, present and future. *Sex. Plant Reprod.*, **9**: 353–356.

Murphy, S.D. and Aarssen, L.W. 1989. Pollen allelopathy among sympatric grassland species: *in vitro* evidence in *Phleum pratense* L. *New Phytol.*, **112**: 295–305.

Pasonen, H.L. and Käpylä, M. 1998. Pollen–pollen interactions in *Betula pendula in vitro*. *New Phytol.*, **138**: 243–251.

Pasonen, H.L., Pulkkinen, P., Käpylä, M. and Blom, A. 1999. Pollen tube growth rate and seed siring success among *Betula pendula* clones. *New Phytol.*, **143**: 243–251.

Price, M.V. and Waser, N.M. 1982. Experimental studies of pollen carryover: hummingbirds and *Ipomopsis aggregata*. *Oecologia*, **54**: 353–358.

Schaal, B.A. 1980. Measurement of gene flow in *Lupinus texensis*. *Nature*, **284**: 450–451.

Schemske, D.W. and Fenster, C. 1983. Pollen–grain interactions in a neotropical *Costus*: effects of clump size and competitors. In *Pollen: Biology and Implications for Plant Breeding* (D.L. Mulcahy and E. Ottaviano, eds), pp. 405–410. New York: Elsevier.

Siegel, S. and Castellan, Jr., N.J. 1988. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.

Skogsmyr, I. and Lankinen, Å. 1999. Selection on pollen competitive ability in relation to stochastic factors influencing pollen deposition. *Evol. Ecol. Res.*, **1**: 971–985.

Skogsmyr, I. and Lankinen, Å. 2000. Potential selection for female choice in *Viola tricolor*. *Evol. Ecol. Res.*, **2**: 965–979.

Snow, A.A. 1994. Postpollination selection and male fitness in plants. *Am. Nat.*, **144**(suppl.): 69–83.

Snow, A.A. and Spira, T.P. 1991a. Pollen vigour and the potential for sexual selection in plants. *Nature*, **352**: 796–797.

Snow, A.A. and Spira, T.P. 1991b. Differential pollen-tube growth rates and nonrandom fertilization in *Hibiscus moscheutos* (Malvaceae). *Am. J. Bot.*, **78**: 1419–1426.

Snow, A.A. and Spira, T.P. 1996. Pollen-tube competition and male fitness in *Hibiscus moscheutos*. *Evolution*, **50**: 1866–1870.

Snow, A.A., Spira, T.P. and Liu, P. 2000. Effects of sequential pollination on the success of 'fast' and 'slow' pollen donors in *Hibiscus moscheutos* (Malvaceae). *Am. J. Bot.*, **87**: 1656–1659.

Sokal, R.R. and Rohlf, F.J. 1995. *Biometry*, 3rd edn. New York: W.H. Freeman.
Soltis, D.E. and Soltis, P.S. 1990. *Isozymes in Plant Biology*. London: Chapman & Hall.
Thomson, J.D. 1986. Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. *J. Ecol.*, **74**: 329–341.
Thomson, J.D. 1989. Germination schedules of pollen grains: implications for pollen selection. *Evolution*, **43**: 220–223.
Thomson, J.D. and Plowright, R.C. 1980. Pollen carryover, nectar rewards and pollinator behavior with special reference to *Diervilla lonicera*. *Oecologia*, **46**: 68–74.
Thomson, J.D., Andrews, B.J. and Plowright, R.C. 1981. The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Capriofoliaceae). *New Phytol.*, **90**: 777–783.
Thomson, J.D., Price, M.V., Nicolas, D.A. and Stratton, D.A. 1986. Comparative studies of pollen and fluorescent dye transport by bumble bees visiting *Erythronium grandiflorum*. *Oecologia*, **69**: 561–566.
Waser, N.M. and Price, M.V. 1984. Experimental studies of pollen carry-over: effects of floral variability of *Ipomopsis aggregata*. *Oecologia*, **62**: 262–268.

APPENDIX 1

Crosses performed between a recipient plant and two pollen donors. Donor combinations with significantly different *in vitro* pollen tube growth rate belong to group A, while the other donor combinations belong to group B. The pollen load proportions of the two competing donors were varied in replacement series of up to five compositions. In all, 13 donor combinations involving 22 pollen donors and 13 recipient plants were used.

Recipient plant	Faster pollen donor	No. of offspring sired (expected)	Slower pollen donor	No. of offspring sired (expected)	χ^2	Difference in average *in vitro* pollen tube growth rate, faster donor – slower donor ($mm \cdot 2\ h^{-1}$) (*t*-test)
Proportion pollen of faster donor/slower donor, 10 : 90						
P9	B4	1 (1.6)	F2	15 (14.4)	N.S.[a]	0.71–0.35***[b]
C1	M1	2 (2.9)	J17	27 (26.1)	N.S.[a]	0.76–0.50***
J15	M3	1 (2.3)	F44	22 (20.7)	N.S.[a]	0.76–0.50***
R30	Q21	0 (1.8)	F32	18 (16.2)	N.S.[a]	0.65–0.47*
F32	F44	2 (1.1)	Q10	9 (9.9)	N.S.[a]	0.50–0.37
S7	J25	0 (1.6)	R8	16 (14.4)	N.S.[a]	0.49–0.38
R8	S7	0 (2.5)	J25	25 (22.5)	$P = 0.072$[a]	0.51–0.49
Proportion pollen of faster donor/slower donor, 30 : 70						
U4	C1	19 (6)	J25	1 (14)	***	0.87–0.49***
P9	B4	9 (6)	F2	11 (14)	N.S.	0.71–0.35***[b]
C1	M1	20 (6)	J17	0 (14)	***	0.76–0.50***
R18	S11	13 (4.2)	F5	1 (9.8)	***[a]	0.65–0.44**
R30	Q21	6 (5.1)	F32	11 (11.9)	N.S.	0.65–0.47*
F32	F44	3 (5.1)	Q10	14 (11.9)	N.S.	0.50–0.37
R8	S7	8 (2.4)	J25	0 (5.6)	***[a]	0.51–0.49
R26	P13	3 (3)	F22	7 (7)	N.S.[a]	0.41–0.39
Proportion pollen of faster donor/slower donor, 50 : 50						
U4	C1	9 (5)	J25	1 (5)	**[a]	0.87–0.49***
P9	B4	9 (5)	F2	1 (5)	**[a]	0.71–0.35***[b]

Appendix—*continued*

Recipient plant	Faster pollen donor	No. of offspring sired (expected)	Slower pollen donor	No. of offspring sired (expected)	χ^2	Difference in average *in vitro* pollen tube growth rate, faster donor – slower donor (mm · 2 h^{-1}) (*t*-test)
C1	M1	15 (12.5)	J17	10 (12.5)	N.S.	0.76–0.50***
R30	Q21	11 (5.5)	F32	0 (5.5)	***	0.65–0.47*
Q21	R22	21 (16)	F32	11 (16)	N.S.	0.60–0.47
Q15	P9	1 (3)	F2	5 (3)	$P = 0.094$[a]	0.42–0.35[b]
R8	S7	13 (15.5)	J25	18 (15.5)	N.S.	0.51–0.49
R26	P13	3 (6)	F22	9 (6)	N.S.	0.41–0.39
Proportion pollen of faster donor/slower donor, 70 : 30						
P9	B4	24 (19.6)	F2	4 (8.4)	$P = 0.07$	0.71–0.35***[b]
U8	L4	17 (18.9)	J29	10 (8.1)	N.S.	0.60–0.26***
R30	Q21	23 (17.5)	F32	2 (7.5)	*	0.65–0.47*
F32	F44	5 (10.5)	Q10	10 (4.5)	**[a]	0.50–0.37
S7	J25	31 (22.4)	R8	1 (9.6)	***	0.49–0.38
Q15	P9	15 (13.3)	F2	4 (5.7)	N.S.	0.42–0.35[b]
R8	S7	15 (17.5)	J25	10 (7.5)	N.S.	0.51–0.49
Proportion pollen of faster donor/slower donor, 90 : 10						
U4	C1	9 (9)	J25	1 (1)	N.S.[a]	0.87–0.49***
P9	B4	22 (19.8)	F2	0 (2.2)	$P = 0.0580$[c]	0.71–0.35***[b]
P9	B4	5 (4.5)	F2	0 (0.5)		
C1	M1	14 (13.5)	J17	1 (1.5)	N.S.[a]	0.76–0.50***
R30	Q21	18 (16.2)	F32	0 (1.8)	N.S.[a]	0.65–0.47*
Q21	R22	24 (23.4)	F32	2 (2.6)	N.S.[a]	0.60–0.47
F32	F44	19 (20.7)	Q10	4 (2.3)	N.S.[a]	0.50–0.37
Q15	P9	18 (16.2)	F2	0 (1.8)	N.S.[a]	0.42–0.35[b]
R8	S7	12 (12.6)	J25	2 (1.4)	N.S.[a]	0.51–0.49
R26	P13	13 (12.6)	F22	1 (1.4)	N.S.[a]	0.41–0.39

[a] Calculated with binomial test.

[b] Estimated as the average of four full siblings (the same mother and father). Standard deviation = 0.086, max = 0.44 mm · 2 h^{-1}, min = 0.28 mm · 2 h^{-1}. We consider this estimate exact enough to place crosses with this donor in either group A or B, since the pollen tube growth rate of full siblings is similar (Skogsmyr and Lankinen, 2000). In the correlation analysis between pollen tube growth rate and the combined siring ability, we are interested in the influence of the precise difference between two donors. In that test, therefore, we excluded all crosses involving this donor.

[c] Based on the number of offspring sired in two crosses.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; N.S. = $P > 0.1$.