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# Selection on pollen competitive ability in relation to stochastic factors influencing pollen deposition

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## ABSTRACT

Pollen competitive ability will only be selected if competitive situations are frequent under natural conditions. A trait must also exist that, in various instances, increases competitive ability and has a variance large enough to allow differences to persist even when affected by stochastic factors imposed by pollinator behaviour. An indication of these prerequisites is that non-random mating is common in a species. In this study on pollen competition in *Viola tricolor*, one donor was superior at siring ovules in 23 of 34 crosses, which indicates that non-random mating is common in this species. We also found maternal effects on siring ability, although they were not large enough to reverse ranking order of donors. Of two pollen traits potentially important for pollen competitive ability (i.e. tube growth rate and germination ability), only pollen tube growth rate had significant effects on siring ability. Variability in pollen tube growth rate was high enough to allow fast tube growth to be beneficial even when the effect of stochastic factors (i.e. time and place of pollen deposition) was taken into account. Taken together, these results make it plausible that sexual selection can act on pollen tube growth rate in *Viola tricolor*.

*Keywords*: mate choice, non-random mating, plants, pollen tube growth rate, sexual selection, *Viola tricolor*.

## INTRODUCTION

The theoretical development and understanding of sexual selection has been influenced by the fact that the phenomenon has been studied predominantly in animals. This has led to a certain reluctance to view selection on reproductive characters in plants in the light of these theories (Willson, 1994). When plants exhibit traits that increase their ability to compete for matings or fertilizations, the conceptual potential for such traits to be favoured by sexual selection clearly exists (Stephenson and Bertin, 1983; Willson, 1990, 1994).

When the number of viable pollen grains deposited on a stigma exceeds the number of ovules, pollen traits that increase the ability of a pollen donor to compete for fertilizations could be selected (Mulcahy *et al.*, 1996). However, selection cannot operate effectively on a character unless the variation in siring ability among individuals is large enough to overcome the stochastic factors that affect pollination. When the variation is large, non-random

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mating will be a common occurrence. Non-random mating has been reported in several species (reviewed in Marshall and Folsom, 1991). Some of these studies (Snow and Spira, 1991a,b; Björkman *et al.*, 1995) demonstrated that non-random mating results from differences in competitive ability between pollen donors, but only a small number of donors were used. The *frequency* of non-random mating in a species, which has received little attention, is a good indicator of the potential for sexual selection. This frequency can be assessed by comparing siring ability of a larger number of randomly chosen pollen donors.

When differences in siring ability have been shown to be frequent, the next step is to study which characters are important for pollen competitive ability. Snow and Spira (1991a,b) showed that *in-vivo* pollen tube growth rate in *Hibiscus moscheutos* was positively correlated with siring ability in ten controlled crosses, eight of which included self-pollination. This result is somewhat weakened by the fact that the outcross pollen in the selfed crosses originated from only four different individuals. The present study is, to our knowledge, the first to show this correlation directly, even though many authors tacitly assume this relation to exist (e.g. Lee, 1984; Niesenbaum and Casper, 1993; Björkman *et al.*, 1995). Pollen competition is also affected by germination ability, which can have direct effects in the sense that quick germination provides a head start in the race towards the ovules (Bertin, 1988).

One problem when applying sexual selection theory to plants is the recognition that plants are sessile and therefore mating is done by proxy. In most cases, the vectors are pollinators; recently, however, sexually selected characters have been addressed in wind-pollinated plants (e.g. Dahl and Fredrikson, 1996). The movement of pollen by external agents increases the stochastic influence on mating – that is, when and where pollen will be deposited is not predictable.

A few detailed studies have investigated the potential for pollen competition to occur in natural populations (Mulcahy *et al.*, 1983; Snow, 1986; Spira *et al.*, 1992, 1996; Mitchell and Marshall, 1998). Spira *et al.* (1996) conducted hand-pollination experiments in *Hibiscus moscheutos* with intervals corresponding to pollinator behaviour. They concluded that the success of late-arriving pollen declines to very low levels after about 2 h. Another way to study the effect of timing of pollen deposition is to compare the variance in pollen tube growth rate with the length of the path the pollen tube has to grow. This length can also be variable as a result of the exact positioning of pollen on the stigma. If the variance induced by timing and positioning of the pollen is lower than that of pollen tube growth rate between individuals, the potential for pollen competition is relatively high.

In this study of *Viola tricolor* L., we assessed whether non-random mating occurs more often than expected by chance. We also analysed the influence of pollen tube growth rate and germination ability on siring ability. To study the effect of pollen deposition site and time, we compared variance in pollen tube growth rate to size of the stigma and pistil length. In this study, our focus was on selection of the pollen donor, but we also assessed maternal effects to ensure that our results of pollen performance were not an artefact of female influence.

## METHODS AND MATERIALS

### Plant material

*Viola tricolor* is a diploid, annual and herbaceous plant found on dry hillsides, flat rocks, sand dunes, and cultivated, lime-deficient soil throughout most of Europe and Asia

(Lagerberg, 1948; Mossberg *et al.*, 1992). The plant has branched stems, heart-shaped leaves and produces flowers (15–25 mm) composed of five petals and a spur. The hermaphroditic, self-compatible flowers have five anthers and one pistil with a ball-shaped stigma. Pollen adheres to most of the stigma. The species is normally outcrossing and pollinated by insects (mainly Hymenoptera), although some self-pollination can occur (Lagerberg, 1948).

We found no indication of incompatibility between individuals. Plants forced to self-pollinate in the absence of pollinators made considerably fewer seeds per capsule compared to plants pollinated naturally (paired *t*-test: $t_{35} = 10.2$, mean difference = 23.1, $s = 13.5$, $P < 0.0001$; I. Skogsmyr, unpublished data). There was no difference between the number of seeds produced by hand pollination and naturally pollinated plants (paired *t*-test: $t_{35} = 1.4$, mean difference = 4.8, $s = 20.9$, $P = 0.17$; I. Skogsmyr, unpublished data). Plants typically produce several flowers each day, which senesce after 3–4 days. The flower is slightly protandrous and the stigma becomes receptive between 12 and 24 h after flower opening. An individual produces some 80 flowers over 3 months. The pollen grains are relatively large (70 $\mu$m). Flowers develop into seed capsules containing up to 65 seeds. Only on rare occasions did the seed capsules contain aborted seeds. The plants used in this study originated from three genetically isolated populations in France (Nancy, Bonne and Bent). We used plants from different populations to give us donors with different markers.

## Two-donor crosses

To determine whether pollen donors of *V. tricolor* differed in siring ability, we performed controlled two-donor crosses. The plants were grown in the greenhouse and the crosses were made over two seasons (1994, first generation; 1995, second generation). We used genetic markers (a single locus with three alleles coding for PGM) identified with starch gel electrophoresis to determine paternity of the seedlings (Soltis and Soltis, 1990). We selected pollen donors with four specific combinations of contrasting alleles at the locus of PGM. To minimize the impact of maternal effects, we used a wide range of randomly chosen recipient plants for the crosses. Some individuals were used as both pollen donors and recipient plants.

We emasculated maternal flowers as they opened but before their stigmas became receptive. These flowers were bagged before and after hand pollinations. In the crosses, we used 200 pollen grains from each donor. To obtain the right amount, we counted the pollen grains under a binocular magnifying glass. We then placed the pollen directly on to the stigma from the slides used during counting. We applied the pollen from the two donors separately. The reason for not mixing pollen loads was the difficulty of moving pollen loads either on or between slides without losing pollen grains. Applying pollen loads separately can, of course, result in masking of direct pollen–pollen interactions. For example, Marshall *et al*. (1996) showed that placing pollen from different donors side by side on a stigma decreased interference competition between pollen donors, since the two kinds of pollen were not in direct contact. To overcome this problem, we applied both pollen loads evenly all around the stigma. We applied the two pollen types immediately after one another so that the time interval between their deposition was kept to a minimum (<2 min). The total amount of pollen applied to each stigma (400) equalled about 10 pollen grains per ovule.

We made a total of 34 crosses with different donor combinations. These included 64 individuals, of which 38 were used as pollen donors. Twenty-six of the donors were used in only one combination, seven in two combinations and five in three to nine combinations. Since we wished to assess whether there was a difference in siring ability after a given fertilization event (to test the occurrence of non-random mating), we chose to analyse a relatively high number of offspring per cross (on average 20 per cross). This provides less information on the difference in siring ability between two donors than using the same donors on many recipient plants. We argue, however, that maternal effects are kept to a minimum by using a large number of (randomly chosen) crossing combinations. The availability of genetic markers made it impossible to use only one population for our crosses. We thus chose to make the crosses between two populations. In the first year (1994), however, we assessed the potential for pollen competitive ability to be selected within one population by allowing 17 pollen donors from one of the populations (Nancy) to compete with three 'standard' pollen donors from another population (Bent). In other words, we compared the individuals within one population by studying their relative competitive ability to one of three external individuals. (There were not enough flowers to use only one.) Siring ability did not differ between the three 'standards' (one-way ANOVA: $n = 17$, $F = 2.07$, $P = 0.16$). In this way, we were able to compare the competitive ability of donors originating from one population (Nancy). The crosses were random in the sense that the recipient plant, like the competitor to the 'standard', was chosen at random. In 1995, our aim was to include as many individuals as possible (from both populations). The individuals originating from Nancy were the offspring from the 1994 crosses, while the Bent individuals were raised from seeds collected in the wild. In 1995, we performed two series of tests on maternal effects. We applied pollen from the same two donors to six randomly chosen recipient plants.

We sowed all seeds resulting from a given cross the following spring. On average, 80% of the seeds germinated. We then analysed the surviving seedlings for the presence of genetic markers. We were only able to analyse a proportion of the seedlings, so we excluded some (randomly chosen) individuals when the number of seedlings produced by a certain cross was very high.

### Pollen performance in medium and pistil size

We recorded *in-vitro* pollen performance for the pollen donors, because *in-vitro* measurements avoid any influence of maternal effects in the pistil when assessing pollen performance.

To determine pollen performance, we allowed pollen from three flowers per donor to germinate in Hoekstra medium (Hoekstra and Bruinsma, 1975) for 2 h in a chamber at a steady temperature of 22°C. As an indication of pollen tube growth rate, we measured the pollen tube length of the first 10 pollen tubes viewed under the microscope that were longer than 0.15 mm. Because of difficulties encountered during development of our method, we only have reliable estimates of pollen tube growth rate for 13 individuals in 1994. For statistical analysis, we used the average length of pollen tubes originating from a given individual. We assessed germination ability as the percent pollen that germinated of approximately 100 grains.

To determine if variation in pollen performance was high enough to override stochastic factors associated with pollen deposition, we estimated average pistil length and stigma size.

We measured the length of three pistils per individual under a binocular magnifying glass. For these pistils, we also measured the length of the stigma, which is normally about half that of total length (Fig. 1). The stigma is receptive all around.

## Statistics and ranking of donors

To determine whether the two pollen donors differed in siring ability, chi-square analysis was used to analyse frequencies of alternative alleles in the offspring. Differences giving *P*-values less than 0.05 were considered significant. When the number of resulting offspring in a cross was less than 10 (i.e. the expected frequency was less than 5), a binomial test was applied (Siegel and Castellan, 1988, p. 50). To establish whether non-random mating occurs more often than expected by chance, we used another binomial test (Siegel and Castellan, 1988, pp. 38–44). The null hypothesis in this case was that mating is completely random; that is, significant differences between the two competing donors will only be found in 5% of cases at most. The two categories were: (1) all crosses where donor performance is significantly non-random, and (2) all crosses where there are no differences between the two competing donors.

When investigating which pollen trait was important for individual performance, we ranked pollen donors according to siring ability. Siring ability of a given donor was calculated as the proportion of seedlings sired in a cross. When a donor was used in more than one cross, this proportion was counted as an average between crosses. To provide an indication of the ability of germination and pollen tube growth rate to evolve, we calculated the coefficient of variation (CV) as suggested by Houle (1992):

$$\mathrm{CV} = s^2/\bar{x}$$

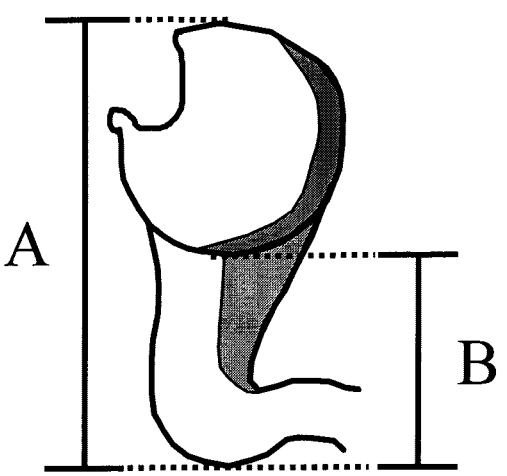


**Fig. 1.** The pistil of *Viola tricolor* consists of a spherical stigma approximately the same diameter as the length of the style. Measurements A and B = the longest and shortest distance a pollen tube can grow to the ovaries.

## RESULTS

### The occurrence of non-random mating

In the two-donor crosses, significant ($P = 0.05$) non-random mating was noted in 23 of 34 donor combinations (see Appendix 1). This gives a very high total significance for occurrence of non-random mating (binomial test, $P_{(x \geq 20|\text{random mating})} < 0.0001$). However, it should be noted that there was a certain amount of dependence, since some individuals were used in several combinations. The result should not depend on incompatibility effects (violets are fully compatible). We excluded five of the crosses in the analysis of total significance because of the rather small number of offspring tested (5–9 seedlings). However, a binomial test showed that the two donors were significantly different in three of these five crosses. In the two non-significant crosses, we cannot exclude the possibility that significant differences in male performance were concealed by a very low *n*-value. The occurrence of non-random mating might, in fact, be even higher.

The two populations used for the controlled crosses (Appendix 1) did not differ in siring ability. Individuals belonging to the Nancy population sired significantly more seeds in six crosses and significantly fewer seeds in eight crosses, when compared to the 'standard' competitors (from Bent population) in 1994. Thus the numbers of 'wins' and 'losses' did not differ ($\chi^2_1 = 0.3$, $n = 14$, $P > 0.1$), which indicates that siring ability is independent of plant source. The origin of the recipient plant did not affect which of the pollen donors were significantly better at siring offspring ($\chi^2_1 = 0.05$, $n = 19$, $P > 0.1$). The occurrence of non-random mating should thus not be an effect of inbreeding at the population or individual level (no self-pollen was used).

A presumption when using genetic markers is that they are selectively neutral so as to avoid erroneous results. We were unable to detect any effect of the markers on siring ability ($\chi^2_3 = 2.2$, $n = 15$, $P > 0.1$). There was also no effect of order of pollen application on siring ability ($\chi^2_1 = 1.8$, $n = 20$, $P > 0.1$).

We did detect some maternal effect on siring ability (see Appendix 2). In one case, one donor was significantly better in four and equally good in two crosses. In the other case, one donor was significantly better in two recipients and equally good in three. One cross was excluded because it produced only five seeds. When we compared the total number of offspring produced by each donor, there was a significant difference in siring ability in both cases (Appendix 1).

### Traits that can affect siring ability

The average pollen tube growth rate differed among individuals within a population (1994, Nancy, one-way ANOVA, d.f. = 12, $F = 5.90$, $P < 0.0001$). The large coefficients of variation for pollen performance showed that the populations were not genetically fixed in this respect (pollen tube growth rate: 1994, $\bar{x} = 0.43$ mm/2 h, $n = 13$, CV = 0.47; 1995, $\bar{x} = 0.50$ mm/2 h, $n = 17$, CV = 0.35; pollen germination ability: 1994, $\bar{x} = 0.34$, $n = 18$, CV = 0.53; 1995, $\bar{x} = 0.20$, $n = 17$, CV = 0.67). The estimates of pollen tube growth rate in 1994 include only pollen donors originating from the same population (Nancy). Pollen tube growth rates did not differ between the two original populations (compare Nancy 1994 and Bent 1995; $\bar{x} = 0.40$ mm/2 h, $n = 7$, CV = 0.32). The second Nancy generation in 1995 had a slightly higher pollen tube growth rate ($\bar{x} = 0.57$ mm/2 h, $n = 10$, CV = 0.30) than the Bent

individuals (paired $t$-test: $t_{15} = 2.218$, $P = 0.042$). This generation was dominated by offspring sired by the more competitive donors used in 1994.

In both years, siring ability increased with pollen tube growth rate *in vitro* (Fig. 2). In violets, *in-vitro* pollen tube growth rate is an indicator of growth rate in the pistil (Pearson correlation, $r = 0.343$, $n = 37$, $P < 0.05$; Å. Lankinen, unpublished data). Because of problems with the staining method, only 1–4 pollen tubes were measured in each pistil. Furthermore, it turned out that pollen tube growth rate was slower in the pistil so that differences in growth rate *in vivo* were not as pronounced as those *in vitro*. It was impossible, however, to measure pollen tubes if they were allowed to grow for a longer time. This estimate is thus somewhat questionable. Since pollen tube growth rate *in vitro* explained about half of the variation in siring ability in both years, we surmise that the estimate is too low.

Pollen germination ability showed a tendency to increase siring ability in 1995 (Pearson correlation, $r = 0.46$, $n = 17$, $P = 0.066$). When we controlled for the effect of pollen tube growth rate (partial correlation), the apparent effect of germination ability disappeared ($r = 0.136$, d.f. = 14, $P > 0.1$). In 1994, there was no relation between germination and siring ability (Pearson correlation, $r = 0.02$, $n = 17$, $P > 0.1$).

## Comparison between pistil size and variation in pollen tube growth rate

As in most theoretical work, our aim here it so provide a realistic estimate of what happens in nature. We wished to determine whether pollen competition is theoretically feasible in the given circumstances and using our experimental data. We aimed to establish whether the difference in pollen tube growth rate is large enough to override differences in deposition times. Our goal was not to identify the exact time difference that allows for

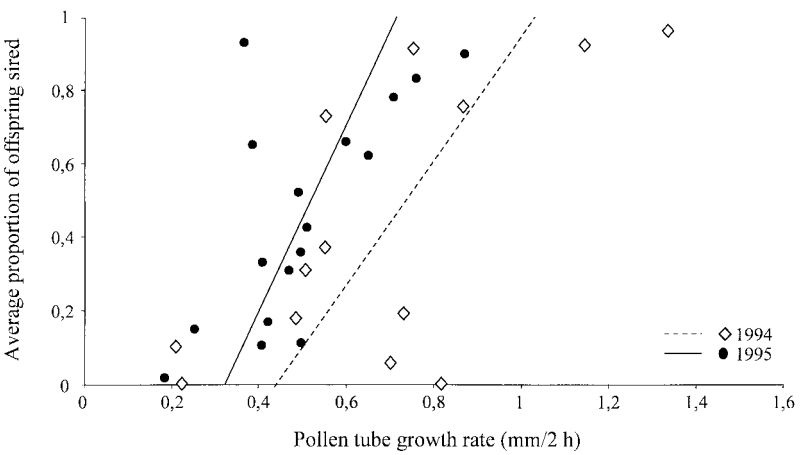


**Fig. 2.** Pollen tube growth rate is positively correlated to siring ability measured as the average proportion offspring sired by a given male (Pearson correlation, 1994: $n = 13$, $r^2 = 0.47$, $P = 0.01$; 1995: $n = 17$, $r^2 = 0.46$, $P = 0.002$). Growth rate is not comparable between years, because the plants experienced different environments (the 1994 plants were grown in France, the 1995 plants in Sweden). In 1994, pollen donors from one population (Nancy) competed with three 'standard' donors from a different population (Bent). The 'standard' donors are excluded in the 1994 analysis.

competition for this specific pair of donors on every female. It is important to note that our analysis of the effect of inherent pollen tube growth rates and the possibility of selection on this trait does not mean that we regard other traits or female influence as being unimportant.

In the following comparison, we used the difference between pollen grains from the individual (hereafter called FAST) that had the highest average pollen tube growth rate *in vitro* ($r_{(FAST)} = 0.669\ mm \cdot h^{-1}$) and that of the individual (labelled SLOW) that had the slowest rate ($r_{(SLOW)} = 0.105\ mm \cdot h^{-1}$). We used the average pollen tube growth rate of an individual, as suggested by Snow and Spira (1996), because this is the selected unit. As we were interested in the potential for pollen competition to occur under natural conditions (i.e. within one population), FAST and SLOW are the fastest and slowest pollen donors of the 13 donors in the first generation (1994) that had the same origin (Nancy). To establish at what time, $T$, later-deposited pollen grains (given that they come from the FAST individual) can still fertilize most of the ovules, we calculate

$$T = \frac{L_{(SLOW)}}{r_{(SLOW)}} - \frac{L_{(FAST)}}{r_{(FAST)}} \quad (1)$$

where $L$ is the distance from the site of pollen deposition to the ovules. Based on measurements of 35 individuals in the first generation (all originating from the Nancy population), $L$ ranged from A = 1.62 mm to B = 0.76 mm (see Fig. 1). We compared four different deposition scenarios (Figs 3a–d). In the most favourable case for the FAST individual (Fig. 3d), the pollen grains are able to fertilize ovules even if they are deposited 14.3 h after the pollen from the SLOW individual. In the most unfavourable circumstances – that is, when the SLOW pollen is deposited close to the ovules and the FAST pollen is deposited at the top of the stigma (Fig. 3a) – the fast pollen grains can still fertilize the ovules if they are deposited within 4.8 h of the earlier deposition. In favourable circumstances, violets receive enough pollen to cover the stigma within this time lag. It should be noted that we only used variation in *in-vitro* pollen tube growth rate in this comparison. Since pollen tube growth rate is faster (even if it covaries) in medium than in the pistil in violets (Å. Lankinen, unpublished data), this time lag is probably even longer in nature.

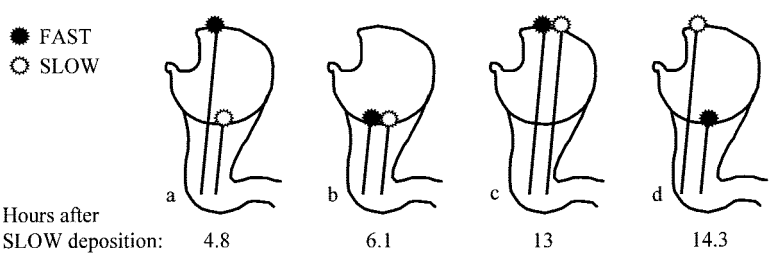


**Fig. 3.** The times at which the later-deposited pollen grains (originating from the individual with the fastest growing pollen tubes) are able to fertilize the ovules in competition with pollen grains from the individual with the slowest pollen tube growth rates, depending on deposition site on the stigma.

## DISCUSSION

Using a greenhouse experiment, we have shown that non-random mating frequently takes place in *Viola tricolor*. This makes it plausible that pollen competition is a common occurrence in this species and it is thus reasonable to assume that selection on pollen performance occurs. We also found that pollen tube growth rate strongly affects competitive ability, while pollen germination ability is unlikely to be sexually selected for in this species.

We argue that selection on pollen tube growth rate is possible because the variation in this trait is large enough to provide individuals with a high growth rate an advantage even in the unfavourable circumstances that result from stochastic influences on pollen deposition.

### Frequency of non-random mating

One of the prerequisites for selection on pollen performance is that there is variability in siring ability between donors. In most studies, the emphasis has been on the repeatability of siring ability and the detection of even small differences in siring success (Ottaviano *et al.*, 1988; Cruzan, 1990a; Marshall, 1991; Snow and Spira, 1991a,b; Stanton, 1994; Björkman *et al.*, 1995; but see Snow and Spira, 1996). As a consequence, the number of individuals involved in the crosses has usually been low. The results of these studies thus tell us little about the frequency of non-random mating under natural conditions. In violets, it is common that one donor sires a significantly large proportion of the offspring from a given cross (as in this study). There should thus be ample opportunity for traits that increase competitive ability to be selected. In nearly all crosses in this study, the two pollen donors were from different sources. This could be a limitation when investigating the possibility for pollen competitive ability to be selected within a population, since crosses between populations may not be representative of crosses within populations. We think it unlikely, however, that the common occurrence of non-random mating in our study is the result of using two populations. Pollen donors belonging to the Nancy population did not do significantly better or worse than the 'standard' donors from the Bent population. We were also unable to detect any effects on seed siring success of origin of the recipient plant. This indicates that the frequent occurrence of non-random fertilization is the result of some other factor than plant source.

We believe it unlikely that our results stem from assortative mating, since we found no covariance between female quality (measured as total lifetime seed production) and donor siring ability (I. Skogsmyr and Å. Lankinen, unpublished data).

### Traits affecting pollen competitive ability

Such traits as viability, size, germination ability and pollen tube growth rate have been suggested to be important for competitive ability of pollen (Bertin, 1988). We measured the latter two *in vitro* to identify some of the mechanisms involved in pollen competition.

When studying pollen competition, the trait that is most readily considered a candidate for sexual selection is pollen tube growth rate (Walsh and Charlesworth, 1992). Indeed, this trait has previously been shown to be positively correlated with siring ability in *Hibiscus moscheutos* (Snow and Spira, 1991a,b). In our study, pollen tube growth rate was the trait

that affected siring ability most significantly. As variation in pollen tube growth rate is also substantial when the donors come from the same population, it is probable that the pollen deposited on a stigma often differ in their competitive ability. Individuals producing fast-growing pollen will then have a competitive advantage. In the present study, we found no effect of germination ability on siring ability. These results are in line with those of Snow and Spira (1991a,b) for *Hibiscus moscheutos*. It is possible that germination ability could be important in more natural circumstances where a flower can be visited several times and there is a time lag between pollen depositions.

Snow and Spira (1991a,b) studied pollen tube growth rate in the pistil and measured length of the tube by counting the number of callose plugs formed. A more simple, but possibly less precise, way of measuring pollen tube growth rate is to allow the pollen grains to germinate in medium. However, *in-vitro* pollen tube growth rate in violets is an indicator of pollen tube growth rate *in vivo* (Å. Lankinen, unpublished data). One advantage of *in-vitro* studies is that influences from the maternal tissue can be avoided. Maternal effects on pollen performance in the pistil have been noted in several species (Hill and Lord, 1986; Fenster and Sork, 1988; Cruzan, 1990b; Hormaza and Herrero, 1996). Although we found some evidence of maternal effects in violets, these were not strong enough to reverse the ranking of the donors in our tests. We thus infer that female 'preference' does not differ to the same extent as pollen competitive ability. The maternal effects are more likely the result of differing ability to 'favour' donors with fast-growing pollen (e.g. through having differently sized pistils) than preferences for specific compatibility types. To answer this question, however, we require an experiment designed to elucidate maternal effects.

### Pollen tube growth rate in relation to stochastic factors

The process of pollination involves two different kingdoms – plants and pollinators – with widely different and sometimes conflicting objectives. Pollinator behaviour should, of course, optimize pollinator foraging success and not pollen transfer. From the plant's point of view, an element of stochasticity is introduced in terms of the timing and positioning of pollen depositions. For selection to take place under such variable circumstances, differences in expressed traits must be large enough to overcome this variability. Previous studies have been concerned in the main with such stochastic factors as how frequently pollinators visit flowers and the magnitude of pollen loads (Mulcahy *et al.*, 1983; Snow, 1986; Spira *et al.*, 1992, 1996). In many species, stigmas are large enough for positioning of pollen grains on the stigma to have an influence on male reproductive success. It is thus important to include this effect in any evaluation of the possibility for selection on pollen tube growth.

When the weather is warm, the stigmas of violets can be covered with pollen within a couple of hours (I. Skogsmyr, unpublished data). More flowers open in warm than in cold weather. In *V. tricolor*, the difference in pollen tube growth rate is large enough to allow the fastest growing pollen grains to fertilize most of the ovules even if they are deposited nearly 5 h after the pollen grains from the individual with the lowest tube growth rate. This time lag is valid for the most unfavourable circumstances – that is, when the slowest growing pollen is deposited near to the ovules and the fastest growing pollen is deposited at a distance from the ovules. When deposition site on the stigma is more important for the fast-growing pollen grains, the time lag can be more than 14 h. The difference in pollen tube growth rate used

in this study was based on measurements of pollen tube growth rate under conditions that correspond to those of sunny weather and high pollinator activity. In violets, there is consequently a strong possibility that faster growing pollen grains will indeed have a competitive advantage even when they are not deposited at the same visit.

During cold weather, when pollinator activity is lower, it may take several days for a stigma to become completely covered with pollen (I. Skogsmyr and Å. Lankinen, unpublished data). Pollen tube growth rate slows down at colder temperatures, but we are unsure if this effect is strong enough to counterbalance the longer time lag between visits in cold weather. If not, stochastic factors will have a stronger influence on siring ability in cold weather. This might then contribute to the persistence of large differences in pollen tube growth rates under natural conditions.

In this study, we compared two extremes of 13 individuals. In many instances, the differences will not be as great and the corresponding time lag required for the fastest growing pollen to win will be shorter. Since we have based our calculations on *in-vitro* rather than *in-vivo* growth, we are unsure how long the time lag is for a fast-growing pollen grain in a population. Furthermore, a more thorough evaluation of pollination under natural conditions is important for the significance of our results. Other factors, not evaluated here, that affect pollen competition are the composition of the pollen load, how fast fertilization takes place once the pollen tubes have entered the ovary, and other maternal effects. We conclude that, if the variation in pollen tube growth rate between pollen donors is as high as indicated in this study, there should be ample chance for pollen tube growth rate to be favoured by sexual selection.

## CONCLUDING REMARKS

In *Viola tricolor*, it is possible to identify a trait – pollen tube growth rate – that increases competitive ability of the male gamete. We have shown that, in a wide range of pollination circumstances, including positioning on the stigma and timing of pollen deposition, the variance in mean growth rate between individuals is large enough to allow for selection on this trait.

The differences in siring ability between individuals used in this study are large enough to detect even in relatively small samples. Pollen tube growth rate seems to be heritable in violets (I. Skogsmyr and Å. Lankinen, unpublished data). This, together with the results discussed above, indicates a possible strong selection on pollen tube growth rate that could result in fixation of genes coding for the trait (cf. Walsh and Charlesworth, 1992). In animals, sexually selected traits are often condition-dependent (Andersson, 1994). The trait is then a signal of condition, reflecting heritable viability of an individual; that is, the phenotypic expression of the trait is a result of genes *both* coding for the trait *and* coding for heritable viability (Andersson, 1986; Iwasa *et al.*, 1991). It is possible that pollen tube growth rate is condition-dependent in plants as well. For example, environmental factors affecting the sporophyte during pollen development have been shown to have a large impact on pollen performance (Delph *et al.*, 1997). Since individuals differ genetically in their ability to withstand stress, these differences will then be expressed in the pollen tube growth rate. As environmental circumstances vary, so will the (genetically determined) traits that affect viability. The viability genes influencing pollen tube growth rate will vary accordingly. This, in turn, allows for ongoing sexual selection on pollen tube growth rate.

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## APPENDIX 1

*Crosses performed between a recipient plant and two pollen donors, A and B. Pollen donor A was applied as the first pollination. We compared observed to expected (1 : 1) siring ability. N, O and E indicate origin, where N = Nancy, O = Bonne and E = Bent*

| Recipient plant | Pollen donor A | Number of offspring sired | Pollen donor B | Number of offspring sired | $\chi^2$ |
|---|---|---|---|---|---|
| **1994** | | | | | |
| 31 N | 110 E | 2 | 178 N | 24 | *** |
| 108 O | 110 E | 21 | 87 N | 1 | *** |
| 126 E | 110 E | 6 | 163 N | 0 | *[a] |
| 127 N | 110 E | 8 | 112 N | 1 | *[a] |
| 144 N | 110 E | 6 | 126 E | 11 | *[b] |
| 144 N | 126 E | 7 | 110 E | 2 | |
| 4 N | 126 E | 4 | 127 N | 12 | * |
| 4 N | 126 E | 17 | 175 N | 3 | ** |
| 64 N | 126 E | 7 | 196 N | 3 | N.S. |
| 110 E | 126 E | 2 | 74 N | 15 | ** |
| 112 N | 126 E | 8 | 104 N | 14 | N.S. |
| 127 N | 175 N | 11 | 126 E | 8 | N.S. |
| 189 N | 126 E | 3 | 198 N | 32 | *** |
| 198 N | 189 N | 22 | 126 E | 1 | *** |
| 18 N | 78 N | 22 | 143 E | 4 | *** |
| 58 N | 143 E | 29 | 46 N | 2 | *** |
| 64 N | 157 N | 2 | 143 E | 13 | ** |
| 74 N | 143 E | 8 | 58 N | 0 | ***[a] |
| 78 N | 87 N | 15 | 143 E | 10 | N.S. |
| 112 N | 56 N | 6 | 143 E | 28 | *** |
| 144 N | 104 N | 18 | 143 E | 11 | N.S. |
| 194 N | 143 E | 4 | 31 N | 1 | N.S.[a] |
| **1995** | | | | | |
| L10 N | J25 E | 16 | R24 N | 2 | *** |
| R8 N | S7 N | 13 | J25 E | 18 | N.S. |
| U4 N | C1 N | 9 | J25 E | 1 | * |
| M3 N | J15 E | 1 | M1 N | 22 | *** |
| M10 N | M1 N | 19 | F44 E | 2 | *** |
| Q9 N | J17 E | 9 | M1 N | 16 | N.S. |
| J15 E | Q10 N | 11 | J29 E | 2 | * |
| L11 N | Q10 N | 14 | J15 E | 0 | *** |
| Six various[c] | F32 E | 50[d] | Q21 N | 81[d] | **[e] |
| Q21 N | F32 E | 11 | R22 N | 21 | N.S. |
| Six various[c] | F2 E | 27[d] | B4 N | 93[d] | ***[e] |
| Q15 N | P9 N | 1 | F2 E | 5 | N.S.[a] |
| R26 N, R34 N | F22 E | 20[f] | P13 N | 11[f] | N.S.[g] |

[a] Calculated with binomial test. [b] Based on the number of offspring sired in both crosses between recipient plant 144 and the two donors 110 and 126. [c] The recipient plants are presented in Appendix 2. [d] The number of offspring sired in six crosses. [e] Based on the number of offspring sired in six crosses. [f] The number of offspring sired in two crosses. [g] Based on the number of offspring sired in two crosses. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

## APPENDIX 2

*Crosses performed to analyse maternal effects. Two pollen donors, A and B, competed on six different recipient plants. Pollen donor A was applied as the first pollination. We compared observed to expected (1 : 1) siring ability. N and E indicate origin, where N = Nancy and E = Bent*

| Recipient plant | Pollen donor A | Number of offspring sired | Pollen donor B | Number of offspring sired | $\chi^2$ |
|---|---|---|---|---|---|
| **1995** | | | | | |
| L1 N | F32 E | 2 | Q21 N | 3 | N.S.[a] |
| Q10 N | Q21 N | 26 | F32 E | 4 | *** |
| Q18 N | Q21 N | 21 | F32 E | 20 | N.S. |
| R17 N | F32 E | 11 | Q21 N | 8 | N.S. |
| R30 N | F32 E | 0 | Q21 N | 11 | *** |
| R31 N | F32 E | 13 | Q21 N | 12 | N.S. |
| P9 N | F2 E | 1 | B4 N | 9 | * |
| P14 N+E | B4 N | 16 | F2 E | 5 | * |
| Q18 N | B4 N | 19 | F2 E | 1 | *** |
| R15 E | F2 E | 9 | B4 N | 12 | N.S. |
| R25 N | F2 E | 7 | B4 N | 26 | *** |
| U2 N | F2 E | 4 | B4 N | 11 | N.S. |

[a] Calculated with binomial test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.