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Potential selection for female choice in *Viola tricolor*

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ABSTRACT

That sexual selection can be an active force in plant evolution is still under debate. When the number of pollen grains deposited onto a stigma exceeds the number of available ovules, competition among pollen grains for fertilizations will result in selection on traits that increase siring ability (e.g. pollen tube growth rate). The pistil can be regarded as an arena for pollen competition, where pistil size and shape have the potential to intensify competition and thereby increase the possibility of sorting among pollen donors. Pollen tube growth rate can function as a cue for female choice if there is a positive relationship between pollen tube growth rate and sporophytic quality of individual donors when this quality is reflected in the offspring. In hermaphroditic violets, *Viola tricolor*, we found that pollen tube growth rate *in vitro* is an indicator of donor sporophytic quality expressed as seed production. In two-donor crosses, pollen tube growth rate had a strong influence on siring ability, which, in turn, was positively related to offspring seed production. Artificial selection of offspring sired by superior pollen donors also resulted in reduced variation in seed production and pollen tube growth rate. A father–offspring regression further indicated that pollen tube growth rate is heritable to a certain degree. There is thus a potential for selection on traits that increase the probability that ovules are fertilized by fast-growing pollen.

Keywords: good genes, mate choice, maternal provisioning, pollen competition, pollen tube growth rate, sexual selection.

INTRODUCTION

One of the major issues in the theory of sexual selection is the evolution of female choice. If female choice is costly, evolution is only possible if such choice benefits the female (Iwasa *et al.*, 1991). In animals where males contribute to the rearing of offspring, this benefit is evident: choosy females get more resources and can increase their production of offspring (Johnstone, 1995). In many animals and in most sessile organisms, however, males contribute only their genes (Andersson and Iwasa, 1996). In such cases, the benefit must then lie in the quality of these genes (e.g. Zahavi, 1975; Hamilton and Zuk, 1982), a suggestion that has been met with some scepticism. The evolution of such female choice has been shown to be theoretically plausible, but firm empirical evidence is still lacking (for a review, see Andersson, 1994). The prerequisites for evolution of female choice when males only

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contribute their genes are that: (a) there exists a male trait on which females base their choice; (b) this trait is an indicator of male fitness; and (c) males with a high siring ability (i.e. preferred by females) have offspring of increased fitness (Iwasa *et al.*, 1991; Collins, 1993; Johnstone, 1995).

In plants, pollen deposited on a stigma germinates and produces pollen tubes that grow through the pistil to the ovary. When the number of pollen grains on a stigma exceeds that of the available ovules, pollen competition can occur (Mulcahy, 1979). This, in turn, can select for pollen traits that confer a high competitive ability. The pistil provides the arena where this competition takes place (Willson and Burley, 1983). The advantage of particular pollen traits is thus not independent of pistil size or shape, or the timing and amount of nutritional support in the pistil (Mulcahy, 1983; Armbruster, 1996; Dahl and Fredrikson, 1996; Herrero and Hormaza, 1996). Consequently, selection on the morphology and function of the pistil has the potential to affect pollen competition. Although it is known that the competitive ability of donors in some species is dependent on the complementarity of the recipient plant (e.g. incompatibility effects; Cruzan, 1990; Johnston, 1993; Björkman *et al.*, 1995), recent studies have shown that siring success following competition can be consistent across several recipient plants (Snow and Spira, 1996; Marshall, 1998; Pasonen *et al.*, 1999).

Although many botanists accept the idea that mating is non-random and that pollen tubes grow at different rates in the pistil, the suggestion that there are consistent differences between pollen donors in siring ability is not uniformly accepted (e.g. Richards, 1997). The possibility of selection acting on female function to increase the probability of being fertilized by donors of higher quality is viewed by many botanists as a heresy (e.g. Grant, 1995). Even so, theoretical arguments clearly show the potential for selection on mechanisms of female choice that affect pollen competition (Stephenson and Bertin, 1983; Willson, 1990, 1994). In this study, we do not examine directly the effects of potential choice mechanisms. Rather, we examine the prerequisites for a pollen trait to function as a cue for choice, which theoretically should select for female choice. If maternal plants can intensify pollen competition and thereby sort among mates, then separating the exact influence of male competition or female choice is of minor interest, since selection of female choice in that case can never be independent of selection of the male gametophyte.

We previously found that, in *Viola tricolor*, pollen tube growth rate is of major importance for siring ability following two-donor crosses over two seasons (Skogsmyr and Lankinen, 1999). By further investigating these two-donor crosses, we ask whether pollen tube growth rate reflects quality of the pollen-producing sporophyte and if this quality in some way is expressed in the offspring. More specifically:

1. We compare the inherent pollen tube growth rate (i.e. growth rate in medium) of the pollen donor to the sporophytic quality of the same individual. Since *V. tricolor* is a hermaphrodite, we measure quality as seed production; that is, ‘maternal fitness’ of pollen donor plants.
2. We compare siring ability of the pollen donor in two-donor crosses with offspring quality (seed production and pollen tube growth rate *in vitro* to evaluate both reproductive functions).
3. We test if artificial selection of pollen donors with high siring ability results in decreased variance of pollen tube growth rate *in vitro* and seed production.
4. We examine if pollen tube growth rate is heritable.

MATERIALS AND METHODS

Plant material

Viola tricolor is a predominantly outcrossing herbaceous annual (Lagerberg, 1948). The flowers show traits typical for insect-pollinated plants and have a distinctive fragrance. This particular violet species does not produce cleistogamous flowers. When fully covered, the stigma can hold about 500 pollen grains, which is more than ten times the number of ovules. A theoretical comparison between individual pollen tube growth rate differences and pistil length showed that fast-growing pollen grains would have an advantage even if deposited a considerable time after slow-growing ones (Skogsmyr and Lankinen, 1999). The plant material used in this study originated from two populations in France, Nancy and Bent, hereafter referred to as N and B (expect for one recipient plant in the first-year crosses originating from a third population in Bonne). We made each two-donor cross with one donor from each of these two populations, due to availability of genetic markers used for paternity determinations. The origin of the recipient plant did not affect the outcome of two-donor crosses (Skogsmyr and Lankinen, 1999). The populations did not differ in siring ability following the crosses or pollen tube growth rate *in vitro* (Skogsmyr and Lankinen, 1999), or in seed production (unselected populations in 1994 (generation 1) – seeds per capsule: Pop. N 30.6 ± 12.2, Pop. B 29.5 ± 21.2, *t*-test: $P > 0.1$, d.f. = 43; total seed production: Pop. N 1375 ± 654, Pop. B 2032 ± 1189, *t*-test: $P > 0.1$, d.f. = 36).

Experimental design

To assess donor siring ability, we performed controlled two-donor crosses in the greenhouse over two seasons (Skogsmyr and Lankinen, 1999; see Appendix). The first-year crosses were performed in France and the rest of the experiment was carried out in Sweden. The paternity of the offspring produced each crossing season was determined the following spring. In total, we worked with three different generations of plants over three seasons (Fig. 1). The offspring generated from the second-year crosses (generation 3) were used to investigate donor siring ability in relation to offspring quality (see Appendix). We measured offspring quality in eight randomly selected offspring – or as many as there were – from each of the 23 offspring groups with the same parents. This involved 17 pollen donors (i.e. 17 paternal offspring groups), since some individuals were used more than once. For each pollen donor combination, we only investigated the offspring produced by one recipient (see Appendix).

To facilitate paternity determinations in the second generation, we used already typed (see ‘Methods of two-donor crosses’) offspring of the first generation as parents for the second generation (Fig. 1). These (only pure N individuals) were then crossed with B individuals arising from seeds collected in the wild, since there were no pure B offspring resulting from the first-year crosses. (We generally used individuals from population N as recipient plants and as one of the donors; the other pollen donor originated from the B population; see Appendix.) The N individuals used as pollen donors in generation 2 were the offspring of pollen donors with the highest siring ability in generation 1. Because of practical problems in the greenhouse, we were only able to save paternal offspring groups with a large number of individuals (i.e. offspring sired by successful competitors). We thus artificially selected the N population for high siring ability this year (Fig. 1).

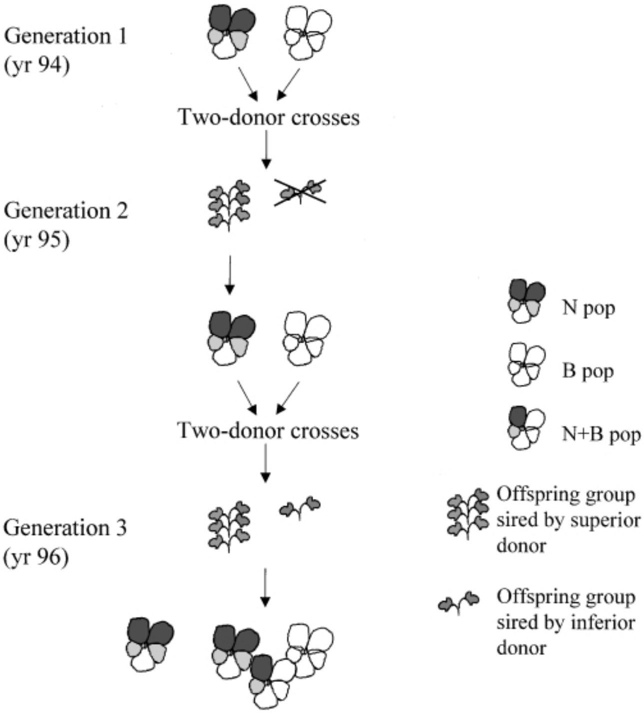

Fig. 1. To determine if pollen tube growth rate can function as a cue for female choice, we assessed pollen donor performance in two-donor crosses and the resulting offspring during three generations (1–3) in 1994–96. The N individuals in generation 2 were derived only from offspring groups sired by superior donors. The offspring generated from the second-year crosses (generation 3) were used to investigate donor siring ability in relation to offspring quality. See text for further details.

During the two crossing seasons, we made two-donor crosses with 34 pollen donor combinations involving 64 individuals, of which 38 were used as pollen donors (1994: $n = 21$, 1995: $n = 13$; see Appendix). Some individuals were used as both pollen donors and recipient plants. We generally avoided crosses with close relatives (full or half siblings). Although we found no incompatibility effects in a few two-donor crosses made between related material, such effects cannot be ruled out. In addition, post-zygotic events due to inbreeding depression could potentially affect siring ability. However, it should be noted that these effects would mask rather than be the reason for a strong correlation between siring ability and pollen tube growth rate (Skogsmyr and Lankinen, 1999).

We assessed donor siring ability by allowing two randomly chosen pollen donors to compete in pairs on randomly chosen recipient plants (see Appendix). Since our aim was to examine the consequences of particular traits on reproductive success, we included as many pollen donors as possible. Lyons *et al.* (1989) suggested that complete factorials and diallel crosses are desirable when studying plant fertilization characteristics. This design makes it possible to distinguish between maternal and paternal effects and gives a very precise estimate of the various mechanisms involved. These benefits, however, come at a cost – the number of individuals that can be included. A complete factorial cross including two types (genotypes, phenotypes or lines) necessitates 25 crosses. The number of crosses increases rapidly with the number of individuals included. This approach is thus not suitable when the aim is to acquire a general picture of relationships between specific traits and, for example, reproductive success in a population (unless the relation between trait and benefit is expected to be very high). Although our experimental design (following the Darwinian approach; for a review, see Charlesworth *et al.*, 1987) tells us little about the exact relationship between two given individuals in a population, the randomized crosses should cancel out any bias introduced by specific interactions between certain mate combinations. A separate study on maternal effects in this species further showed that such effects were not large enough to reverse the ranking of pollen donors (Skogsmyr and Lankinen, 1999).

Methods of two-donor crosses

Hand pollinations were made on emasculated flowers using the methods described in Skogsmyr and Lankinen (1999). In all two-donor crosses, we used the same amount of pollen from two different donors. In total, 400 ($2 \times 200 \pm 15$) pollen grains, counted beneath a binocular magnifying glass, were applied from both donors. This equals about 10 pollen grains per ovule. The amount of pollen applied completely covered the stigmatic surface. During the 'counting process', pollen was exposed to a slightly higher temperature (approximately 30°C) for 10 min. However, pollen germination and growth in medium is little affected by increasing the germination temperature from 22 to 32°C (Å. Lankinen, unpublished data).

We used genetic markers (a single locus with three alleles coding for PGM) identified with starch gel electrophoresis to determine paternity of seedlings (Soltis and Soltis, 1990; Skogsmyr and Lankinen, 1999). When the number of seedlings produced by a certain cross was very high, we analysed a randomly chosen subset of seeds. On average, we analysed 20 seeds per cross.

Measurement of pollen tube growth rate and fitness traits of all generations

All plants in all generations were grown in a greenhouse, so that all plants during one generation experienced similar conditions. We measured *in vitro* pollen tube growth rate of all three generations to evaluate male competitive ability as well as heritable variation. Pollen tube growth rate is more reliably measured *in vitro* than *in vivo*. Pollen tube growth rate *in vitro* strongly correlated with siring success following the two seasons of two-donor crosses (Skogsmyr and Lankinen, 1999). This suggests that pollen growth *in vitro* is a good estimator of pollen performance in the pistil. Furthermore, the only *in vivo* method we have found it possible to work with (due to problems with staining techniques) is questionable, since only between one and four pollen tubes per sample can be identified over its whole length (Å. Lankinen, unpublished data). Another advantage with *in vitro* tests is that the effect of maternal tissue is excluded.

We estimated pollen tube growth rate by measuring pollen tube length 2 h after germination in Hoekstra medium (Hoekstra and Bruinsma, 1975) in a chamber at a constant temperature of 22°C. We used pollen from three flowers per individual. We counted pollen tube length as the average of the first ten pollen tubes encountered under the microscope. This process did not require additional lights. Our estimate of pollen tube growth rate also includes the speed of germination. Siring ability of the donor is unrelated to germination rate in *Viola tricolor* (Skogsmyr and Lankinen, 1999).

To assess whether pollen tube growth rate was a reliable indicator of sporophytic plant vigour, we measured other fitness-related traits, such as seed production per capsule and total seed production, in both donors and offspring. We thus used traits traditionally regarded as giving maternal fitness. In violets, there is no apparent trade-off between the male and female reproductive functions under greenhouse conditions. Pollen production showed no relationship to seed production (I. Skogsmyr, unpublished data). We therefore judge seed production as an indicator of sporophytic vigour. We used an average of three capsules per plant to assess individual seed production per capsule. We estimated total seed production by multiplying the number of seed capsules by the mean seed production per capsule.

Statistical analyses

We determined donor siring ability as the average proportion of offspring sired by a given pollen donor in all crosses in which this donor was used (= rank of pollen donor). The values were arcsine transformed for statistical analysis. To test if higher-ranked donors produced offspring of higher quality, we examined the regression of offspring quality on donor siring ability using a hierarchical analysis; that is, a nested analysis of variance that takes different sample sizes (of paternal offspring groups) into account (Sokal and Rohlf, 1995). In the analysis, both the effects of pollen donor and offspring groups with the same parents are included. When investigating the relationship between pollen tube growth rate of pollen donors and offspring quality, we compared the difference between donors in a cross with the average difference of the two offspring groups produced in that cross. We did this to avoid effects of the specific crossing combination of pollen donors (i.e. the pollen tube growth rate of the competitor). The differences between donors can be considered as statistically independent samples. There is, however, a certain amount of dependence between crosses, since some individuals are part of more than one pair (two donors were used three times, one donor twice and 12 donors once).

To determine if selection of pollen donors with high siring ability resulted in a decrease in the variance in sporophytic and gametophytic quality (seed production and pollen tube growth rate *in vitro*) in later generations, we used a one-tailed *F*-test (Clarke, 1980). We used a father–offspring (average of group sired by a specific father) regression to measure heritability in the narrow sense (Falconer and Mackay, 1996). To analyse the significance of this father–offspring regression, we made a bootstrap analysis following Manly (1991), using SYSTAT (Wilkinson and Engelman, 1997) to generate the probability distribution. This method has been shown to be a good way of dealing with problems arising from small sample sizes (Manly, 1991; Wilkinson and Engelman, 1997).

RESULTS

Pollen tube growth rate and sporophytic quality within plants, and selection on siring ability

In the unselected plant material of both populations (the plants originating directly from the wild), pollen donors with higher pollen tube growth rate *in vitro* also had a higher total seed production (Table 1). There was thus a connection between the ability to produce fast-growing pollen and the sporophytic quality measured as total seed production within individual plants. Inadvertently, we tested the effect of artificial selection on siring ability in the experiment. After the first-year crosses, we only saved offspring from donors that sired a large percentage of the offspring in a cross (the highest ranked pollen donors in generation 1). These individuals were used as pollen donors in generation 2 (from the N population). There was no difference in siring ability between the two unselected populations (Skogsmyr and Lankinen, 1999). In the second-year crosses (generation 2), donors from the selected N population were more successful than donors from the unselected B population in seven out of the eight crosses in which we found significant differences between donors (see Appendix). Artificial selection of the N population not only resulted in an increase in siring ability between generations 1 and 2, but also a reduction in variation in both sporophytic and gametophytic quality (total seed production and pollen tube growth rate; Table 2). The relationship between pollen tube growth rate and seed production within individuals was also weakened in generation 2 in the selected N population. Among the unselected B individuals used in generation 2, however, the relationship was strong enough to give a P-value < 0.05 (Table 1).

In the last year of the experiment, we saved paternal offspring groups of donors with both high and low siring ability (generation 3). Selection of offspring sired by successful pollen donors was thus not as strong as our artificial selection in the previous year. Variation in total seed production and pollen tube growth rate was still low within this generation. The variation was not significantly different from that in generation 2 (Table 2).

Quality of offspring resulting from crosses in the previous generation

We found a positive correlation between donor siring ability in generation 2 and seed production per capsule in the offspring in generation 3 (Fig. 2, Table 3). This, however, was not large enough to have a significant effect on total seed production (= seed production per capsule × capsule production) (Table 3). Total seed production in offspring was not significantly affected by the combination of this trait in both parents (mid-parent) (hierarchical analysis: $b = 0.0222$, number of offspring groups with the same parents = 23, number of offspring = 148, $P = 0.908$). Furthermore, there was a positive relationship between the difference in pollen tube growth rate of the two competing donors in a cross and the difference in average seed production per capsule of the two paternal offspring groups (Spearman rank correlation: $r = 0.652$, $n = 10$, $P < 0.05$).

Table 1. The relationship between pollen tube growth rate *in vitro* and sporophytic quality measured as total seed production within individual pollen donors in selected and unselected material of populations N and B

	Population N					Population B				
	r	n	n_{groups}	P	Year	r	n	n_{groups}	P	Year
Unselected	0.377	26		0.058	1994	0.526[a]	19	4	< 0.05	1995
Selected	–0.086[a]	83	8	n.s.	1995					

Note: r = correlation coefficient; n = number of pollen donors; n_{groups} = number of groups. All individuals within a group had the same parents.

[a] Partial correlation compensating for relatedness of individuals.

Table 2. Variation in total seed production and pollen tube growth rate *in vitro* in the N population

Year		CV	Year	*F*	*P*
Total seed production					
1994	1375 ± 654	0.48	1994–95	$F_{28,7} = 4.10$	< 0.05
1995	1091 ± 323	0.30	1995–96	$F_{7,11} = 1.51$	n.s.
1996	2115 ± 263	0.12			
Pollen tube growth rate *in vitro*					
1994	0.828 ± 0.307	0.37	1994–95	$F_{34,7} = 4.81$	< 0.05
1995	0.707 ± 0.140	0.20	1995–96	$F_{7,11} = 1.32$	n.s.
1996	0.843 ± 0.122	0.14			

Note: A significant reduction for both traits (*F*-test, one-tailed) was seen between generations 1 and 2 following artificial selection of the donors with the highest siring ability. CV = standard deviation/mean value.

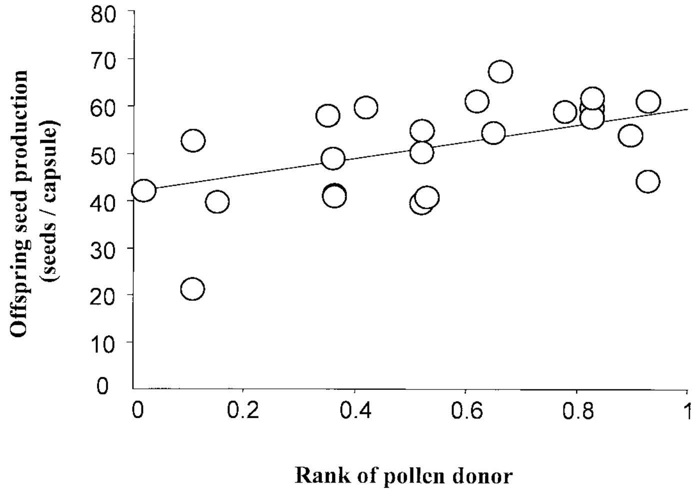

Fig. 2. A measure of offspring fitness (seed production per capsule) was positively related to competitive ability of the father (described by siring ability = rank of pollen donor). Number of paternal offspring groups = 17, number of groups with the same parents = 23.

Pollen donors that had a high siring ability produced offspring with a high pollen tube growth rate (Table 3). This strongly indicates that they also had a high siring ability, although we could not test this directly, since no crosses were made in generation 3.

Heritability of pollen tube growth rate

The heritability of pollen tube growth rate measured in a father–offspring analysis using all pollen donors in generation 2 and their offspring in generation 3 – that is, in our created greenhouse population – was comparable to, or slightly higher than, that of other studies of these kind of traits (Stearns, 1992), although it was not significant (linear regression: $b = 0.246$, standard error = 0.131, $n = 16$, $P = 0.161$; $h^2 = 2 \times b = 2 \times 0.246 = 0.492$) (Falconer

Table 3. Offspring traits in generation 3 regressed on donor siring ability in generation 2 (calculated with hierarchical analysis)

Offspring trait	$\sigma_{\text{within groups}}$	$\sigma_{\text{among groups}}$	Test for variation among groups	b	P
Seeds per capsule	9.64	7.24	4.579***	11.68	0.049
Total seed production	617.4	319.9	2.706***	178.3	0.520
Pollen tube growth rate *in vitro*	0.176	0.113	3.632***	0.194	0.045

Note: Variation among paternal offspring groups was significant for all investigated traits, ***$P < 0.001$. $\sigma_{\text{within groups}}$ = variation within paternal offspring groups; $\sigma_{\text{among groups}}$ = variation among paternal offspring groups; b = regression coefficient; number of offspring = 148; number of paternal offspring groups = 17.

and Mackay, 1996). We used a bootstrap analysis to re-evaluate the significance of this regression between generations 2 and 3. The bootstrap analysis (number of iterations = 2500, $n = 16$) showed that the probability of generating our b-value if the null hypothesis of $b = 0$ is correct was 25/2500 ($P = 0.01$). We did not test heritability between generations 1 and 2, since the number of offspring groups in generation 2 was low. However, we were able to test heritability in the N population by comparing pollen tube growth rates between grandparents in generation 1 and offspring groups in generation 3. This showed that the offspring in generation 3 had pollen tube growth rates similar to their grandparents (mid-grandparent–offspring; hierarchical analysis: $b = 0.466$, number of offspring groups with the same grandparents = 6, number of offspring = 42, $P = 0.019$). In this case, however, we cannot exclude the influence of maternal effects on the result.

DISCUSSION

Our results indicate that, in *Viola tricolor*, the prerequisites for a male pollen trait to function as a cue for female choice (Iwasa *et al.*, 1991; Collins, 1993; Johnstone, 1995) could be fulfilled. The trait of major importance for siring ability – pollen tube growth rate – reflects the sporophytic quality of the pollen donor measured as seed production. Offspring quality is further positively related to high competitive ability of the pollen donor. Since the benefit of possessing a high pollen tube growth rate is affected by the distance the pollen tube has to grow to reach the ovules, this gain cannot be independent of the morphology or function of the pistil. Our results indicate that there is an advantage for maternal plants to be fertilized by donors with fast-growing pollen tubes. By producing a long pistil or otherwise promoting pollen competition, the female increases the probability that fast-growing pollen deposited later will have a chance to fertilize the ovules. These pistil traits can then be favoured by selection.

Pollen tube growth rate in relation to sporophytic quality of pollen donors

In this study, individual pollen donors that had the highest pollen tube growth rate *in vitro* also had the highest sporophytic quality – measured as seed production – in unselected populations (*V. tricolor* is a hermaphrodite). This should not be surprising, since both traits could be affected by the ability of the plant to acquire resources. In fact, on average, 60% of the genes expressed in the sporophyte are also expressed in the haploid stage (for a review, see Hormaza and Herrero, 1994, 1996).

In the second generation, the relationship between pollen tube growth and seed production was weakened in the N population. The reason for this is probably that these individuals were artificially selected for increased siring ability, which reduced variation in both seed production and pollen tube growth rate *in vitro*. The B individuals in this generation (originating directly from the wild) had a higher variation in pollen tube growth rate and this trait was significantly related to seed production. A connection between pollen growth and sporophytic quality within individuals can result from environmental differences. In our study, all plants within each population were grown in a greenhouse with fairly constant environmental conditions. Furthermore, since the relationship between gametophytic and sporophytic quality was weakened after strong selection for high siring ability, it is probable that the differences in quality in the wild plant material is partly genetically determined.

Offspring quality following pollen competition

Offspring of individuals with a high siring ability produced more seeds per capsule. This effect was not strong enough to have a significant effect on total seed production. In the case where relationships between pollen tube growth rate and seed production within an individual were tested, both effects on seed number per capsule and on total number of capsules contributed to the overall positive relationship (even if neither effect was strong enough to be significant on its own). Between generations, on the other hand, maternal influences might distort the relationship. We did not, however, find a significant association between seed production of mid-parent and seed production of offspring. Individuals with a high siring ability produced offspring that also had high pollen tube growth rates. Since this is a trait that has a strong influence on siring ability (Skogsmyr and Lankinen, 1999), it is reasonable to assume that the offspring also have a higher siring ability. Thus, females gain an additional benefit if they increase the probability that offspring are sired by fast-growing pollen.

Higher offspring quality following pollen competition has traditionally been explained by the overlap in gene expression between the two life-stages (Mulcahy, 1979): pollen grains that are good competitors express genes that are beneficial to the sporophyte as well. Increased intensity of pollen competition (e.g. increasing the number of pollen grains deposited on a stigma) has been shown to enhance offspring quality in several species (Bertin, 1990; Quesada *et al.*, 1993, 1996a; Palmer and Zimmerman, 1994). Marshall and Whittaker (1989) found significant differences among three pollen donors with respect to offspring traits. Seed weight was positively correlated with siring ability in different inbred lines of maize (Mulcahy, 1971). Delph *et al.* (1998), however, suggested that positive correlations between pollen tube growth rate and offspring vigour resulted from maternal investment, rather than heritable genetic quality of the pollen donor. According to this view, fast-growing pollen donors give rise to higher-quality offspring simply because they sire the first seeds.

How can we separate this mechanism from the one investigated in this paper? One possible way is to determine the relationship between siring ability and offspring quality. If maternal provisioning is the only reason for variation in offspring quality, the first seeds sired should be of the highest quality and then the quality should diminish independent of who sires the seeds. It follows from this reasoning that a pollen donor that is a good competitor (e.g. sires 60% of the ovules) sires predominantly ovules of a high quality compared to a slower growing competitor. An even better competitor, on the other hand, that can sire say 90% of the ovules, will also sire the ovules that will develop into offspring of lower quality. Hence, the *average* brood quality will be lower for this pollen donor than a donor siring 60% of the ovules. We should thus not expect the relationship between pollen tube growth rate of pollen donors and offspring quality to be an increasing function. A prerequisite is that there are enough pollen grains from each of the donors to fertilize all available ovules in a cross. If, on the other hand, there were only enough pollen grains to fertilize a small proportion of the available ovules, a fast donor could constantly fertilize only the first seeds (the ones best provisioned). Then there might be a positive relationship between pollen tube growth rate and average offspring vigour as a result of the maternal provisioning outlined by Delph *et al.* (1998). In the present study, we applied enough pollen from both donors so that either one could sire all seeds. This should exclude the possibility that the maternal effect alone could explain the positive relationship between pollen tube growth rate and offspring quality.

Heritability of pollen tube growth rate

Although few studies have investigated heritability of pollen tube growth rate, some selection experiments have indicated a genetic component of pollen performance (Ottaviano *et al.*, 1983, 1988; Schlichting *et al.*, 1990; Quesada *et al.*, 1996b; but see Snow and Mazer, 1988). In 16 groups of clones in *Oenothera organensis*, about 9% of the variation in pollen tube growth rate could be explained by a genetic component (Havens, 1994). In our study of *Viola tricolor*, pollen tube growth rate was heritable in our greenhouse population (with material from two populations) when investigated with a father–offspring regression, although we could only show the regression to be *significantly* different from zero when we used a bootstrap analysis. If there is heritability of pollen tube growth rate within a wild population, then this trait can respond to selection. In the present study, selection of successful pollen donors led to reduced variation in pollen tube growth rate in further generations.

Potential selection for female choice

Pistil morphology and function may be a result of many conflicting selection pressures, such as efficient pollination, the possibility of preventing related or dysfunctional pollen from siring ovules, and promoting pollen competition between individuals (Mulcahy, 1983; Armbruster, 1996). For example, pistil form and function in *Dalechampia* species is probably an adaptive compromise between selection to improve pollination and selection to increase the intensity of pollen competition (Armbruster, 1996). For that reason, production of a structure that increases pollen competition (e.g. a long style) may be costly if the efficiency of pollination is affected. If choice is profitable for a female, it can still be selected even when it is associated with a cost (e.g. Iwasa *et al.*, 1991). This benefit can be an increase in offspring fitness, as found in the present study. However, Fisher (1958) argued that, if the heritability of fitness-enhancing traits is very high, the traits should go rapidly to fixation in a population. This could mean that the positive effect on the offspring from choosing the superior males gradually disappears: all males in the population possess the same positive genes. It must be remembered, however, that Fisher's fundamental theorem, despite its epithet 'fundamental', builds on the assumption of a constant environment. As soon as the environment varies in time or space, traits optimizing vigour will differ and so, in turn, will the type of preferred male. If this mechanism is responsible for the variability found in pollen tube growth rate, we should expect that pollen tube growth rate is a reflection of sporophyte vigour, rather than a result of specific genes. This kind of mechanism, where the cue used for mate choice reflects the bearer's general quality, has been proposed in animal studies (Andersson, 1986; Iwasa *et al.*, 1991). In plants, it has been shown that pollen tube growth rate is affected by environmental effects on the sporophyte (for a review, see Delph *et al.*, 1997). Even so, there can be constant differences between males in different treatments showing that individuals differ in their ability to withstand stress (Snow and Spira, 1996). Under natural conditions, environmental effects could keep the heritability of pollen tube growth rate at a level that allows for selection on pistil shape and size (female choice), while at the same time maintaining the variability between pollen donors. In this study, we found that variability in pollen tube growth rate diminished after selection. Our plants were kept in a greenhouse under relatively constant conditions during and between generations. If pollen tube growth rate reflects how an individual fares in a given environment, selection under constant conditions could result in a more rapid effect on variability than is the case under natural conditions. In addition, if plants sometimes are pollen-limited under natural conditions (unlike in our study), this might also promote variation in pollen tube growth rate if defective pollen grains are able to fertilize some ovules.

We conclude that, apart from increasing reproductive success of the male gametophyte, a high pollen tube growth rate could, at least in *Viola tricolor*, be an indication of a heritable superior quality of the pollen-producing sporophyte. Female traits that enhance the probability that the ovules are sired by fast-growing pollen might then be selected, since pollen tube growth rate has the potential to function as a cue for mate choice. In this study, we have looked at the female benefit indirectly, by studying the pollen donors and their offspring. The natural continuation of our work would be to investigate whether or not females that produce longer pistils, for example, also increase the proportion of offspring sired by vigorous donors and, as a consequence, produce offspring of a higher quality.

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APPENDIX

We used crosses performed in 1995 (generation 2) to investigate the relation between siring ability of donors and offspring quality (generation 3) (**bold** type in Table A1). To assess if the two donors differed in siring success in a particular cross, we compared observed to expected (1 : 1) siring ability. Since 23 of the 34 donor combinations differed in siring ability, the total significance for the occurrence of non-random mating was high (binomial test: $P_{(x \geq 20|\text{random mating})} < 0.0001$). For further discussion of paternity data, see Skogsmyr and Lankinen (1999).

Table A1. Crosses performed between a recipient plant and two pollen donors, A and B. N, O and B indicate origin. N = Nancy, O = Bonne, B = Bent. One recipient plant from Bonne was used in 1994

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

[a] Calculated with a 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 L1 N, Q10 N, Q18 N, R17 N, R30 N, **R31 N** = mother of offspring investigated for this donor combination.

[d] The number of offspring sired in six crosses.

[e] Based on the number of offspring sired in six crosses.

[f] The recipient plants are P9 N, P14 N + B, Q18 N, R15 N, R25 N, **U2 N** = mother of offspring investigated for this donor combination.

[g] The number of offspring sired in two crosses.

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

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.