



# LUND UNIVERSITY

## Effects of soil pH and phosphorus on in vitro pollen competitive ability and sporophytic traits in clones of *Viola tricolor*

Lankinen, Åsa

*Published in:*  
International Journal of Plant Sciences

2000

[Link to publication](#)

*Citation for published version (APA):*

Lankinen, Å. (2000). Effects of soil pH and phosphorus on in vitro pollen competitive ability and sporophytic traits in clones of *Viola tricolor*. *International Journal of Plant Sciences*, 161(6), 885-893.  
<http://www.jstor.org/stable/pdfplus/3079999.pdf>

*Total number of authors:*  
1

### General rights

Unless other specific re-use rights are stated the following general rights apply:  
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00

## EFFECTS OF SOIL pH AND PHOSPHORUS ON *IN VITRO* POLLEN COMPETITIVE ABILITY AND SPOROPHYTIC TRAITS IN CLONES OF *VIOLA TRICOLOR*

Åsa Lankinen<sup>1</sup>

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

Greenhouse-grown clones of *Viola tricolor* were used to evaluate the importance of genetic effects on *in vitro* pollen tube growth rate in relation to the influence of two environmental soil factors—pH and phosphorus content. After 1 mo in different soil treatments, individual pollen tube growth rate was affected by a genotype by phosphorus interaction, while it was unaffected by either main effects. Though individuals differed in their response to phosphorus availability, the rank order of pollen donors remained consistent between treatments; i.e., the pollen donors with the highest pollen tube growth rate were the same in all soil conditions. Sporophytic traits were also affected by a genotype by phosphorus interaction. The response of pollen growth and sporophytic traits to soil phosphorus content were correlated within individuals in the high pH treatments. These results, in combination with a high value of clonal repeatability of pollen tube growth rate, indicate that pollen competitive ability has a genetic basis in this species. One prerequisite for this trait to have evolutionary effects on male fitness is thus fulfilled. Furthermore, if the response to phosphorus among pollen donors is more pronounced under natural conditions, variation in this environmental factor within populations has the potential to maintain some genetic variation in pollen tube growth rate even if this trait is constantly selected.

**Keywords:** gametophytic selection, genotype by environment interactions, male fitness, phenotypic plasticity, pollen competition, pollen tube growth rate.

### Introduction

In flowering plants, pollen competition can occur both among pollen grains from one pollen donor and among pollen donors that differ in average pollen competitive ability (Mulcahy 1979; Willson 1990; Delph and Havens 1998). Some recent studies indicate that differences in pollen performance among individuals can have effects on siring ability in competitive situations (Marshall and Folsom 1992; Snow and Spira 1996; Marshall 1998; Pasonen et al. 1999). This variation in pollen performance must have a genetic basis rather than a strictly environmental one if it is to affect the evolution of pollen traits (Walsh and Charlesworth 1992; Willson 1994).

Experiments involving cultivated species propose the existence of a genetic component of pollen competitive ability (Ottaviano et al. 1983, 1988; Schlichting et al. 1990; Sari-Gorla et al. 1992; Quesada et al. 1996), while in wild species, the few published studies report low heritability (Snow and Mazer 1988; Havens 1994). On the other hand, pollen performance is often shown to be highly plastic. Many recent studies have found that pollen performance can be affected by such environmental factors as herbivory (e.g., Mutikainen and Delph 1995; Quesada et al. 1995), temperature (e.g., Delph et al. 1997; Johannsson and Stephenson 1998), and soil fertility (e.g., Young and Stanton 1990; Lau and Stephenson 1993, 1994; but see Snow and Spira 1996). Since both genetic and environmental causes have been shown to influence pollen

competitive ability, it is worth investigating whether environmental conditions might differentially affect an individual's pollen competitive ability.

To investigate how selection acts on a plastic trait, it is crucial to investigate how different individuals respond to environmental factors rather than how the average trait in a population is affected (Stearns 1992; Delph et al. 1997; Schlichting and Pigliucci 1998). For this reason, I have performed an experiment in multiple environments with clones of different individuals originating from one population.

Previous studies have shown that pollen tube growth rate is a major factor determining siring ability in *Viola tricolor* (Skogsmyr and Lankinen 1999). In this study, I investigate the effects of two environmental soil factors—pH and phosphorus content—on *in vitro* pollen tube growth rate in clones of *V. tricolor*. Cloned plants of the same individual (genotype) were allowed to grow under different soil conditions in order to analyze the extent to which variation in this trait is due to genetic or environmental causes. The influence of soil condition was also studied on sporophytic traits to investigate if any potential effects are correlated in both life cycles; i.e., whether the general ability to withstand certain soil conditions has effects on both gametophytic and sporophytic traits. In addition, clonal repeatability, a measure of broad-sense heritability, was examined.

### Material and Methods

#### *Plant Material*

*Viola tricolor* is an herbaceous annual present throughout much of Europe and Asia (Lagerberg 1948; Mossberg et al.

<sup>1</sup> Telephone 46-46-222-37-70; fax 46-46-222-37-66; e-mail Asa.Lankinen@teorekol.lu.se.

Manuscript received March 2000; revised manuscript received May 2000.

Table 1

**Soil Treatments to Investigate the Effect of the Soil Factors pH and Phosphorus Content (PO<sub>4</sub>) on Cloned Plants from 15 Individuals (Genotypes)**

Soil factors	A	B	C	D	E
pH .....	High	High	Intermediate	Low	Low
PO <sub>4</sub> .....	Low	High	Intermediate	Low	High

1992). The species is predominantly outcrossing (Lagerberg 1948; Skogsmyr and Lankinen 1999). The hermaphroditic flowers show traits typical for insect-pollinated plants. When fully covered with pollen, the stigma can hold around 500 grains, which is ca. eight to 16 times the number of ovules. A theoretical comparison between 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). This suggests that pollen competition may play an important role in this species.

*Viola tricolor* is found on dry hillsides, flat rocks, sand dunes, and cultivated lime-deficient soil (Lagerberg 1948; Mossberg et al. 1992). Major factors known to limit the field distributions of such rock habitat plants are hydrogen and aluminum ion toxicity in acid soils (pH-water < 5.0) and low phosphate (PO<sub>4</sub>) solubility in neutral-alkaline soils (Tyler 1996). Soil pH and phosphorus content thus seem to be of particular relevance in investigating how plant performance is affected by environmental variation within a population. The plants used in this study originated from seeds collected in a wild population in south Sweden in the summer of 1998.

#### Micropropagation of Plants

The cloning process took place at the Botanical Garden, University of Oulu, Finland, during the winter of 1998/1999. Seeds collected from the wild were softened in NaOCl for 10 min, rinsed in sterile distilled water for several hours, and then germinated in medium (app. 1; Dirr and Heuser 1987). The seeds started to germinate after ca. 1 mo. When the seedlings were ca. 1 cm long, they were transferred to a cytokinin-rich medium (app. 1; Dirr and Heuser 1987), which made them grow shoots. During a 3-mo period, these plants were clonally propagated by cutting off shoots. Root development was then promoted by growing the shoots in a rooting medium (appendix; Dirr and Heuser 1987) containing auxins for ca. 2 mo.

Before the experiment was started, the micropropagated plants (2–4 cm in length) were transferred to soil. Since the plants do not develop functional roots when grown in the root-promoting medium, they are very sensitive at this stage. For this reason, all plants were planted in the same type of soil (a mixture of peat, sand, and nutrients). During the first week, they were also kept at high humidity. When the plants were transplanted to the experimental soil treatments (after ca. 1 mo), seedling size was 13.9 cm ± 5.08 cm (average among 15 individuals) and most individuals had started to flower ( $\bar{X}$  = 2.9 ± 1.80 flowers).

#### Experimental Design

In the summer of 1999, a total of 15 individuals (genotypes) consisting of 10–29 cloned plants per genotype were grown in five soil treatments differing in pH and phosphorus content in the greenhouse (table 1). After the maximum flowering peak had been reached (after ca. 4 wk in the soil treatments), the effect of soil condition on *in vitro* pollen tube growth rate and sporophytic traits began to be recorded. Pollen tube growth was estimated during a 2-wk period. The greenhouse temperature was held at ca. 24°C during this time. Measurements of sporophytic traits were concluded when the plants stopped flowering. At this stage, plants also terminate growth of shoots.

The soil used for the experimental treatments was collected from the same population from which the plants originated. Mixing this soil (dried for a few days at indoor temperature) with peat, lime, and PO<sub>4</sub> then produced the five soil treatments. I chose the levels of pH and PO<sub>4</sub> to be within the maximum variation found in natural populations (based on unpublished field data for 12 populations of *V. tricolor*, Tyler, G., Dept. of Ecology, Lund University). PO<sub>4</sub> content was modified by adding 0.0437 g PO<sub>4</sub>/kg dried soil for the intermediate and 2 × 0.0437 g PO<sub>4</sub>/kg dried soil for the high treatment. The pH was altered by adding 3 g lime/L dried soil for the intermediate (pH-water = 5) and 2 × 3 g lime/L dried soil for the high effect (pH-water = 6). To produce the low pH (pH-water = 4), low PO<sub>4</sub> treatment, I mixed the dried soil with unfertilized peat in the proportions 1 : 1. This should have reduced the available PO<sub>4</sub> to half that in the collected soil. Throughout the experiment, plants in the different pH treatments were given water of pH 4, 5, or 6. No additional nutrition was given.

#### Pollen Tube Growth Rate Measurement

Pollen tube growth rate was estimated *in vitro*. This eliminates maternal tissue effects seen with the *in vivo* method (Hill and Lord 1986; Fenster and Sork 1988; Cruzan 1990; Hormaza and Herrero 1996a). The *in vitro* method has further been shown to be a good indicator of siring ability in this species (Skogsmyr and Lankinen 1999). The best *in vivo* method for this species involves dyeing tubes with safranin O and aniline blue (Dafni 1992), but this only allows reliable

Table 2

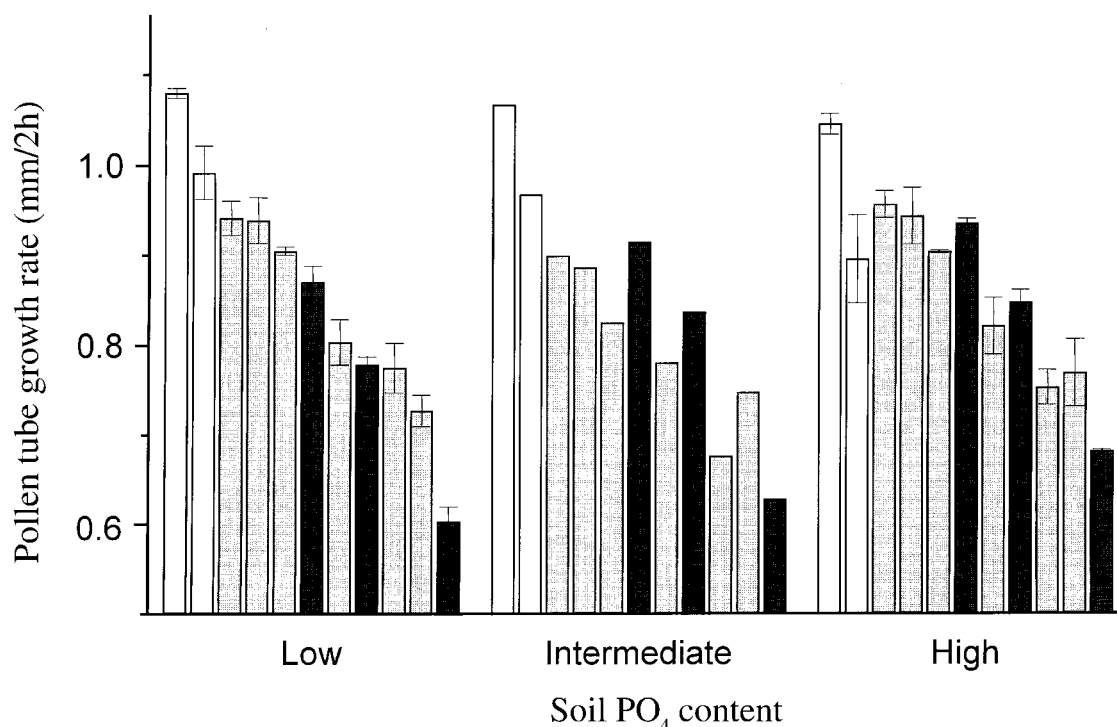
**ANOVA for Pollen Tube Growth Rate *In Vitro* (mm/2h) When Cloned Individuals (Genotypes) Have Grown in Soil with Different pH and Phosphorus Content (PO<sub>4</sub>)**

Source	df	Mean square	F ratio
Genotype .....	10	0.056	54.79***
pH .....	1	0.001	0.690
PO <sub>4</sub> .....	1	0.002	0.630
Genotype × pH .....	—	—	—
Genotype × PO <sub>4</sub> .....	10	0.003	2.61*
pH × PO <sub>4</sub> .....	—	—	—
Error .....	21	0.001	—

Note. The model was reinvestigated after the nonsignificant interactions gradually had been excluded (—).

\*  $P < 0.05$ .

\*\*\*  $P < 0.001$ .



**Fig. 1** Even though pollen tube growth rate *in vitro* was affected by a genotype by phosphorus ( $\text{PO}_4$ ) interaction, the genetic effect was too strong for any large changes in rank order of pollen donors across soil treatments. The 11 individuals (genotypes) in the figure are arranged in the same order in all treatments. Color of bars denote when the trend was decreasing (white), unaffected (grey), or increasing (black) pollen tube growth rate for increasing soil phosphorus content. For low and high phosphorus content, an average of both pH treatments are shown (error bars indicate standard error).

measures of one to four pollen tubes per sample. Since the length of pollen tubes varies within samples, the measurement error is probably large with this method.

To evaluate pollen tube growth rate, pollen from three flowers per plant was allowed to germinate in Hoekstra medium (Hoekstra and Bruinsma 1975) for 2 h in a chamber at a steady temperature of 24°C. As an indication of pollen tube growth rate, I measured pollen tube length of the first eight pollen tubes encountered in the microscope view. Pollen with tubes shorter than 0.15 mm were considered to have failed germination. The average length of these eight pollen tubes was used for statistical analysis. To determine individual pollen growth within each of the soil treatments, measurements were repeated as many times as possible, the average being 2.8 times/individual.

#### Measurement of Sporophytic Traits

In order to assess the performance of the sporophytes, I recorded lifetime production of flowers, size at the end of life (length of all shoots), and seed production. In addition, I estimated seedling size (plant height) and number of flowers before the plants were transferred to the soil treatments. Seed production was estimated as number of seed capsules multiplied by the average number of seeds per capsule of three capsules.

#### Statistics

To evaluate the effect of soil treatment on plant traits, I used a mixed-effect general linear model (SYSTAT, Wilkinson 1997). The factors included in the model were individual/genotype (random factor), pH,  $\text{PO}_4$  (fixed factors), and all two-way interactions. Nonsignificant interactions were gradually excluded from the model, and the model was reinvestigated. The average of two clones per treatment was used for all individuals.

To assess if pollen tube growth rate and sporophytic traits of individuals had a similar response to phosphorus content, I calculated  $\text{PO}_4$  quotients for these traits. The  $\text{PO}_4$  quotients were calculated both for high and low pH treatments as

$$\frac{\text{trait value}_{\text{highPO}_4}}{\text{trait value}_{\text{highPO}_4} + \text{trait value}_{\text{lowPO}_4}} \quad (1)$$

The quotient is thus an estimate of how plants respond to variation in soil phosphorus. The quotients were arcsine transformed. The slope of the functional relationship between quotients of pollen tube growth rate and sporophytic traits was calculated with the method of principal axes, since both variables are considered as random (Sokal and Rohlf 1995). If plants differ in their general ability to endure certain soil conditions, this can result in a correlation between the response of pollen tube growth rate and a sporophytic trait, since both

Table 3

ANOVA for Flower Production, Final Plant Size, and Seed Production When Cloned Individuals (Genotypes) Have Grown in Soil with Different pH and Phosphorus Content (PO<sub>4</sub>)

Source	Flower production (15 individuals)			Final plant size (cm) (15 individuals)			Seed production (11 individuals)		
	df	Mean square	F ratio	df	Mean square	F ratio	df	Mean square	F ratio
Genotype .....	14	1568	20.91***	14	16,340	11.13***	10	1,615,000	10.43***
pH .....	1	2001	10.89***	1	17,850	12.16***	1	7,446,000	48.06***
PO <sub>4</sub> .....	1	1071	4.613 <sup>a</sup>	1	9460	6.447*	1	543,000	1.042
Genotype × pH .....	14	183.8	2.452*	—	—	—	—	—	—
Genotype × PO <sub>4</sub> .....	14	232.2	3.096**	—	—	—	10	521,000	3.363*
pH × PO <sub>4</sub> .....	—	—	—	—	—	—	—	—	—
Error .....	15	74.98	—	43	1467	—	21	154,900	—

Note. The models were reinvestigated after the nonsignificant interactions gradually had been excluded (—).

<sup>a</sup>  $P = 0.05$ .

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

traits are dependent on a third factor, i.e., general fitness (cf. condition dependent traits, Andersson 1986; Iwasa et al. 1991).

To test for differences among individuals, I used a hierarchical analysis, i.e., a nested ANOVA, where different sample sizes (of individuals) are taken into account (Sokal and Rohlf 1995). I calculated clonal repeatability to get an indication of broad sense heritability, giving the extent to which phenotypic variance in a trait is explained by a genetic component,  $H^2 = V_G/V_P$  (Falconer and Mackay 1996). The genetic component in this case includes all genetic effects. Broad-sense heritability can be considered the upper limit for narrow-sense heritability. The phenotypic variance,  $V_P$ , can be divided into variance due to genetic variance and variance resulting from random environmental effects,  $V_P = V_G + V_E$ . The variance between vegetative clones (individuals) is regarded as a measure of the genetic variance,  $V_G$ , provided environmental effects are

random for all individuals.  $V_G/V_P$  is thus calculated as the proportion of the total variance that is due to variance between individuals. Since all cloned plants of an individual have a common origin, some environmental effects may be included in the measurement of  $V_G$  (Falconer and Mackay 1996). For both latter analyses, only the intermediate soil treatment (C) was used. There were no differences in variation among soil treatments.

## Results

### Effects of Soil Condition on In Vitro Pollen Tube Growth Rate and Sporophytic Traits

After 4 wk in soil treatments differing in pH and phosphorus content, *in vitro* pollen tube growth rate of the various individuals responded differently to phosphorus content, while

Table 4

Means (and Standard Errors) of Pollen Tube Growth Rate *In Vitro* and Sporophytic Traits for Cloned Plant Genotypes

Genotype	No. of flowers at 4 wk	Seedling size at 4 wk (cm)	No. of clones (seedlings)	Pollen tube growth rate (mm/2 h)	Flower production	Final plant size (cm)	No. of clones (treatment C)
3 .....	3.8 (0.44)	15.3 (0.90)	25	0.928 (0.024) <sup>a</sup>	47.8 (13.1)	131.8 (44.39)	5
5 .....	2.1 (0.24)	12.8 (0.74)	18	0.623 (0.029)	62.5 (10.5)	216.0 (27.00)	2
6 .....	3.0 (0.39)	13.6 (1.22)	16	0.856 (0.020)	...	...	2
7 .....	1.4 (0.25)	10.8 (0.87)	19	...	...	...	...
10 .....	0.73 (0.27)	5.59 (0.61)	11	1.039 (0.018)	...	...	2
11 .....	7.0 (0.40)	17.4 (0.78)	19	...	43.0 (15.0)	99.5 (25.50)	2
12 .....	2.5 (0.35)	16.0 (1.21)	28	0.907 (0.031) <sup>b</sup>	33.2 (8.20)	76.3 (18.05)	6
13 .....	2.5 (0.49)	9.77 (0.79)	24	0.952 (0.007) <sup>a</sup>	40.8 (1.83)	94.4 (4.31)	5
14 .....	2.5 (0.49)	17.4 (1.13)	29	...	35.0 (5.92)	104 (17.76)	6
16 .....	5.6 (0.37)	28.0 (7.35)	24	...	66.8 (5.29)	184.8 (13.89)	5
20 .....	3.9 (0.45)	18.4 (1.06)	19	...	...	...	...
21 .....	0.80 (0.17)	10.4 (1.04)	20	0.989 (0.038)	...	...	2
22 .....	3.0 (0.42)	23.9 (1.37)	20	...	...	...	...
23 .....	0.33 (0.21)	7.40 (1.02)	21	...	...	...	...
25 .....	4.1 (0.59)	9.68 (0.77)	25	0.924 (0.026) <sup>b</sup>	50.4 (5.20)	110.0 (18.05)	5

Note. Except for seedlings, only plants grown in the intermediate soil treatment (C) were used.

<sup>a</sup> Number of clones = 4.

<sup>b</sup> Number of clones = 3.

**Table 5**  
**Variance in Pollen Tube Growth Rate *In Vitro* and Sporophytic Traits Compared within and between Individuals and Estimates of Clonal Repeatability**

	$V_E$	$V_G$	No. of individuals	No. of plants	<i>F</i> ratio (nested ANOVA)	$V_G/V_P$
No. of flowers (4 wk) .....	3.504	3.140	15	317	19.9***	0.473
Seedling size (4 wk) (cm) .....	111.3	32.81	15	317	7.20***	0.228
Plants grown in the intermediate soil treatment (C):						
Pollen tube growth rate (mm/2 h) .....	0.001632	0.01182	8	22	20.3***	0.879
Flower production .....	297.6	108.8	8	36	2.60*	0.268
Final plant size (cm) .....	2576	1547	8	36	3.63**	0.375
Variance within individual 13 compared with variance in all individuals: <sup>a</sup>						
Seed production .....	294,600	627,200	11	...	...	...

Note.  $V_E$  refers to variance within individuals, and  $V_G$  is variance between individuals.

<sup>a</sup> Five plants; intermediate soil treatment (C).

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

there were no main effects of either phosphorus or pH (table 2). Average performance of pollen donors in the high and the low phosphorus treatments were, however, strongly correlated (Pearson correlation:  $r = 0.958$ ,  $n = 11$ ,  $P < 0.0001$ ). Thus, despite the genotype by phosphorus interaction, there was only a slight change in the rank order of individual pollen donors for different phosphorus treatments (fig. 1). During the 2 wk, pollen growth was recorded, and no effects of time could be detected.

An interaction between individual and phosphorus content was also found for flower and seed production (table 3). Contrary to pollen tube growth, all sporophytic traits were affected by pH. Low pH generally had a negative effect (flower production, 18% decrease; final plant size, 18% decrease; seed production, 33% decrease). However, flower production of the individuals was also dependent on an interaction between individual and pH (table 3).

The response to soil phosphorus content was both positive and negative among individuals. Pollen tube growth and all sporophytic traits of individuals showed a correlated response when pH was high but not when pH was low (fig. 2). There was a significant effect for flower production and plant size but not for seed production.

#### Clonal Repeatability

The individuals differed in number of flowers at 4 wk, seedling size at 4 wk, pollen tube growth rate, flower production, and final plant size (tables 4, 5). Clonal repeatability ( $V_G/V_P$ ) of sporophytic traits ranged between 0.228 and 0.473. For pollen tube growth rate, clonal repeatability was high with a genetic component explaining 88% of the variance. Seed-production variance was only measured for individual 13. The variance between clones ( $V_G$ ) was higher than that detected within clones ( $V_E$ ) (table 5). If the variance in this individual equals that in other individuals, this could indicate that seed production

All sporophytic traits were affected by seedling size, while no such effect was detected for pollen tube growth rate (table 6). Compared to pollen tube growth rate, sporophytic traits appear to be more affected by environmental effects early in life.

#### Discussion

In this study *in vitro* pollen tube growth rate of *Viola tricolor* was affected by an interaction between genotype and soil phos-

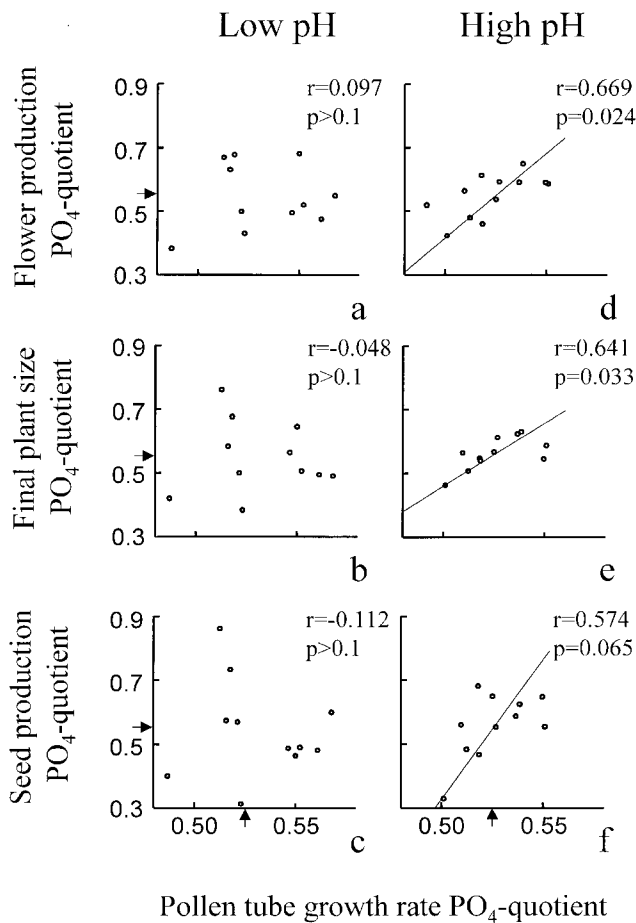
**Table 6**

**Partial Correlation Analyses between Seedling Size and Pollen Tube Growth Rate *In Vitro*, Flower Production, Final Plant Size, and Seed Production**

Trait correlated with seedling size	Partial correlation coefficient, $r_p$	No. of individuals	No. of plants
Plants grown in the intermediate soil treatment (C):			
Pollen tube growth rate (mm/2 h) .....	-0.0574	8	22
Flower production .....	0.439**	8	36
Final plant size (cm) .....	0.409*	8	36
Plants of individual 13 grown in all five soil treatments (A-E):			
Seed production .....	0.572**	24	

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .



**Fig. 2** The plastic response to phosphorus content ( $PO_4$ ) is correlated within individuals for pollen tube growth rate and sporophytic traits when pH is high (*d-f*), but not when pH is low (*a-c*). The quotients refer to the proportion of trait values that results from high phosphorus content (see methods). Since the quotients are arcsine transformed, an individual with the same response to high and low phosphorus is 0.524 (indicated with arrows). Higher values indicate a positive response to phosphorus and lower values indicate a negative response. Number of individuals (genotypes) = 11. The slope of the functional relationship between variables is calculated with the method of principal axes (*d*:  $Y_1 = -2.322 + 5.466Y_2$ ; *e*:  $Y_1 = -1.539 + 3.995Y_2$ ; *f*:  $Y_1 = -3.920 + 8.513Y_2$ ).

phorus content, while there were no main effects of either soil phosphorus or pH. The genetic influence on pollen tube growth rate was, however, the strongest. A genotype by phosphorus interaction was also detected for two sporophytic traits, and the effect of soil phosphorus within individuals was correlated in both stages of the life cycle when pH was high. Furthermore, clonal repeatability of pollen tube growth rate was found to be 88%. The combination of these results suggests that there is a genetic basis to pollen competitive ability in violets.

*Raphanus raphanistrum*, *Cucurbita pepo*, and tomato pollen produced under low soil nutrient conditions sired fewer seeds in competition with pollen produced under better soil condition (Young and Stanton 1990; Lau and Stephenson

1993, 1994; Stephenson et al. 1998). In *Hibiscus moscheutos*, however, neither salinity stress nor soil nutrients had effects on the relative siring ability of pollen donors when compared to a standard donor (Snow and Spira 1996). The genotype by phosphorus interaction affecting pollen tube growth rate in violets was not strong enough to have any large effects on the rank order of pollen donors. Even though the variation in soil factors was chosen to be within the range of that found in natural violet populations, the plants were, due to experimental design, not allowed to grow in the different soil treatments during their entire life span. Consequently, it cannot be excluded that the effect on pollen tube growth rate of phosphorus is higher under natural conditions since an environmental response is likely to be stronger after longer exposure. It seems unlikely, however, that there is an undiscovered effect of soil pH on pollen tube growth rate, as variation in soil pH was high enough to result in large effects on all sporophytic traits.

The response of pollen growth and sporophytic traits to soil phosphorus within violet individuals was correlated in the high pH treatments. An overlap in the genetic expression between the gametophytic and sporophytic phases of the plant life cycle is generally found for 60%–80% of the genes exposed in the sporophyte (Mulcahy et al. 1992; Walsh and Charlesworth 1992; Hormaza and Herrero 1994, 1996b). While the correlation could indicate that the effect of phosphorus to some degree results from genetic differences among individuals, a genetic overlap in the two stages of the life cycle need not only involve genes that determine the ability to grow. It could also incorporate a more general ability to endure certain soil conditions (cf. condition-dependent traits, Andersson 1986; Iwasa et al. 1991). Because the phosphorus response of pollen tube growth was not only associated with plant size but also with other sporophytic traits, the ability to withstand certain soil conditions may differ among individuals. The lack of connection between pollen tube growth rate and sporophytic traits when pH was low could be a consequence of ion poisoning. Tyler (1996) found that plants from rocky habitats are limited by hydrogen and aluminum ion poisoning rather than the ability to acquire phosphorus when pH is low.

Heritability of pollen performance has been found to be low in the few wild species where it has been investigated (Snow and Mazer 1988; Havens 1994). For example, in 16 clones of *Oenothera organensis* clonal repeatability measured in the greenhouse showed that ca. 9% of the variation in pollen tube growth rate could be explained by a genetic component (Havens 1994). In this study clonal repeatability of pollen tube growth rate was extremely high with a genetic component explaining more than 80% of the variance. This can be compared with only ca. 25%–45% for the sporophytic traits. Pollen tube growth rate was, however, analyzed in very constant environments. For example, temperature, which can have profound effects on pollen tube growth rate in violets (Å. Lankinen, unpublished data), was nearly constant during the entire measurement period. Although the clonal repeatability estimated for pollen tube growth rate here is very high, this result only shows that this trait has a genetic basis but does not show how large the heritability would be under natural conditions. In the wild, the higher influence of environmental factors (including temperature) is likely to hide additive genetic variance,

and measurements of heritability will be lower than under artificial conditions (Houle 1992).

The occurrence of genetic variation in pollen competitive ability is puzzling, assuming such traits are of importance for male fitness (Marshall and Folsom 1992; Snow and Spira 1996; Marshall 1998; Pasonen et al. 1999; Skogsmyr and Lankinen 1999). Variation of a trait with a high impact on fitness should theoretically decrease as selection favors the optimal genotype (Fisher 1958). Several mechanisms that could counteract the loss of variance in pollen performance have been proposed, including recombination and mutations (Schlichting et al. 1990; Charlesworth and Charlesworth 1992; Walsh and Charlesworth 1992; Mulcahy et al. 1996), negative genetic correlations between sporophytic viability and pollen competitive ability (Walsh and Charlesworth 1992; Delph et al. 1997), and genotype by environment interactions (Delph et al. 1997). Genotype by environment interactions have previously been found in studies using cultivated species (Zamir et al. 1981; Elgersma et al. 1989; Johannsson and Stephenson 1998). For example, pollen competitive ability of wild and cultivated *C. pepo* responded differently to cold or warm temperature during development (Johannsson and Stephenson 1998). This difference also had significant effects on siring ability and general viability of the resulting sporophytic generation. In a recent study on pollen performance in *Betula pendula*, Pasonen et al. (2000) found evidence for genotype by temperature interactions in a wild population. The results found in this study further indicate that such interactions might exist in natural populations.

In conclusion, this study showed that pollen tube growth rate in *V. tricolor* mainly is genetically based when individuals

grow in different soil treatments, that the detected response to soil phosphorus is correlated in both life cycles, and that there is high clonal repeatability of pollen tube growth rate. A substantial part of the variation in pollen tube growth rate found within a wild population of violets is thus likely to have a genetic basis. Since this trait previously has been found to covary with male reproductive success (Skogsmyr and Lankinen 1999), the current results suggest that in this species one of the conditions for pollen tube growth rate to be of evolutionary significance for male fitness is fulfilled. Furthermore, it was possible to detect genotype by phosphorus interactions for both pollen tube growth rate and sporophytic traits when variation in phosphorus content equals that in natural conditions; this indicates that there is variation in the plastic response to phosphorus content in violets. If the response among pollen donors is stronger under natural conditions and there exists a balance between gene flow and environmental heterogeneity of this factor within populations, this might maintain some genetic variation in pollen tube growth rate.

### Acknowledgments

I wish to thank the Botanical Garden in Oulu, Finland, for micropropagating the plants used in this experiment, G. Tyler for access to his unpublished field data, and R. Härdling, I Skogsmyr, and A. G. Stephenson for helpful comments on the text. The Nordic Academy of Advanced Study and The Swedish Research Council for Forestry and Agriculture financed the project.

## Appendix

### Media Used during Micropropagation of *Viola tricolor*

#### Germination Medium, Based on Murashige-Skoog (MS)

Media (Dirr and Heuser 1987), pH 5.8 (1 L)

MS A macroelements .....	50 mL
MS B Fe .....	100 mL
MS C microelements .....	5 mL
MS D vitamins + amino acids .....	5 mL
6-benzylaminopurine (BAP) (0.1 mg/L) .....	1 mL
Naphthaleneacetic acid (NAA) (0.05 mg/L) .....	0.5 mL
Myo-inositol .....	100 mL
Sucrose .....	30 g
Agar pH Eur. ....	6.5 g

#### Shoot Medium, Based on Murashige-Skoog (MS) Media (Dirr and Heuser 1987), pH 5.5–5.6 (1 L)

MS A macroelements .....	100 mL
MS B Fe .....	100 mL
MS C microelements .....	10 mL
MS D vitamins + amino acids .....	10 mL

BAP (0.5 mg/L) .....	5 mL
Indole-3-butyric acid (IBA) (0.25 mg/L) .....	2.5 mL
Adenine sulphate .....	20 mg
Myo-inositol .....	100 mL
Glucose .....	20 g
Agar pH Eur. ....	6.5 g

#### Root Medium, Based on Woody Plant Media (WPM) (Dirr and Heuser 1987), pH 5.5–5.6 (1 L)

WPM A macroelements .....	50 mL
WPM B macroelements .....	50 mL
WPM D microelements .....	10 mL
WPM E microelements .....	10 mL
WPM V3 vitamins + amino acids .....	10 mL
IBA (0.1 mg/L) .....	1 mL
Myo-inositol .....	100 mL
Glucose .....	15 g
Agar pH Eur. ....	6.0 g



## Literature Cited

- Andersson M 1986 Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* 40:804–826.
- Charlesworth D, B Charlesworth 1992 The effects of selection in the gametophyte stage on mutational load. *Evolution* 46:703–720.
- Cruzan MB 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. IRL, Oxford.
- Delph LF, K Havens 1998 Pollen competition in flowering plants. Pages 149–173 in TR Birkhead, A Pape Møller, eds. Sperm competition and sexual selection. Academic Press, San Diego, Calif.
- Delph LF, MH Johannsson, AG Stephenson 1997 How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology* 78:1632–1639.
- Dirr M, CW Heuser 1987 The reference manual of woody plant propagation: a practical working guide to the propagation of over 1100 species, varieties, and cultivars. Varsity, Athens, Ga.
- Elgersma A, AG Stephenson, APM Nijs 1989 Effects of genotype and temperature on pollen tube growth in perennial rye grass (*Lolium perenne* L.). *Sex Plant Reprod* 2:225–230.
- Falconer DS, TFC Mackay 1996 Introduction to quantitative genetics. 4th ed. Longman, London.
- Fenster CB, VL Sork 1988 Effect of crossing distance and male parent on *in vivo* pollen tube growth in *Chamaecrista fasciculata*. *Am J Bot* 75:1898–1903.
- Fisher RA 1958 The genetical theory of natural selection. 2d ed. Dover, New York.
- Havens K 1994 Clonal repeatability of *in vitro* pollen tube growth rates in *Oenothera organensis* (Onagraceae). *Am J Bot* 81:161–165.
- Hill JP, EM Lord 1986 Dynamics of pollen tube growth in the wild radish, *Raphanus raphanistrum* (Brassicaceae). I. Order of fertilization. *Evolution* 40:1328–1333.
- Hoekstra FA, J Bruinsma 1975 Respiration and vitality of binucleate and trinucleate pollen. *Physiol Plant* 34:221–225.
- Hormaza JI, M Herrero 1994 Gametophytic competition and selection. Pages 372–400 in EG Williams, AE Clarke, RB Knox, eds. Genetic control of self-incompatibility and reproductive development in flowering plants. Kluwer, Dordrecht.
- 1996a Dynamics of pollen tube growth under different competition regimes. *Sex Plant Reprod* 9:153–160.
- 1996b Male gametophytic selection as a plant breeding tool. *Sci Hortic* 65:321–333.
- Houle D 1992 Comparing evolvability and variability of quantitative traits. *Genetics* 130:195–204.
- Iwasa Y, A Pomiankowski, S Nee 1991 The evolution of costly mate preferences. II. The “handicap” principle. *Evolution* 45:1431–1442.
- Johannsson MH, AG Stephenson 1998 Effects of temperature during microsporogenesis on pollen performance in *Cucurbita pepo* L. (Cucurbitaceae). *Int J Plant Sci* 159:616–626.
- Lagerberg T 1948 Vilda växter i Norden. Bokförlaget Natur Och Kultur, Stockholm.
- Lau T-C, AG Stephenson 1993 Effects of soil nitrogen on pollen production, pollen grain size, and pollen performance in *Cucurbita pepo* (Cucurbitaceae). *Am J Bot* 80:763–768.
- 1994 Effects of soil phosphorus on pollen production, pollen size, pollen phosphorus content, and the ability to sire seeds in *Cucurbita pepo* (Cucurbitaceae). *Sex Plant Reprod* 7:215–220.
- Marshall DL 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 DL, MW Folsom 1992 Mechanisms of nonrandom mating in wild radish. Pages 91–118 in R Wyatt, ed. Ecology and evolution of plant reproduction. Chapman & Hall, New York.
- Mossberg B, L Stenberg, M Ericsson 1992 Den nordiska floran. Wahlström & Widstrand, Brepols.
- Mulcahy DL 1979 The rise of the angiosperms: a genecological factor. *Science* 206:20–23.
- Mulcahy DL, GB Mulcahy, KB Searcy 1992 Evolutionary genetics of pollen competition. Pages 25–36 in R Wyatt, ed. Ecology and evolution of plant reproduction. Chapman & Hall, New York.
- Mulcahy DL, M Sari-Gorla, GB Bergamini Mulcahy 1996 Pollen selection—past, present and future. *Sex Plant Reprod* 9:353–356.
- Mutikainen P, LF Delph 1995 Effects of herbivory on male reproductive success in plants. *Oikos* 75:353–358.
- Ottaviano E, M Sari-Gorla, M Villa 1988 Pollen competitive ability in maize: within population variability and response to selection. *Theor Appl Genet* 76:601–608.
- Ottaviano EM, M Sari-Gorla, I Arenari 1983 Male gametophyte competitive ability in maize: selection and implications with regard to the breeding system. Pages 367–374 in DL Mulcahy, E Ottaviano, eds. Pollen: biology and implications for plant breeding. Elsevier, New York.
- Pasonen HL, M Käpylä, P Pulkkinen 2000 Effects of temperature and pollination site on pollen performance in *Betula pendula* Roth: evidence for genotype-environment interactions. *Theor Appl Genet* 100:1108–1112.
- Pasonen HL, P Pulkkinen, M Käpylä, A Blom 1999 Pollen tube growth rate and seed siring success among *Betula pendula* clones. *New Phytol* 143:243–251.
- Quesada M, K Bollman, AG Stephenson 1995 Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* 76:437–443.
- Quesada MR, JA Winsor, AG Stephenson 1996 Effects of pollen selection on progeny vigor in a *Cucurbita pepo* × *C. texana* hybrid. *Theor Appl Genet* 92:885–890.
- Sari-Gorla M, ME Pè, DL Mulcahy, E Ottaviano 1992 Genetic dissection of pollen competitive ability in maize. *Heredity* 69:423–430.
- Schlichting CAD, M Pigliucci 1998 Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, Mass.
- Schlichting CD, AG Stephenson, LE Small, JA Winsor 1990 Pollen loads and progeny vigour in *Cucurbita pepo*: the next generation. *Evolution* 44:1358–1372.
- Skogsmyr I, Å Lankinen 1999 Selection on pollen competitive ability in relation to stochastic factors influencing pollen deposition. *Evol Ecol Res* 1:971–985.
- Snow AA, SJ Mazer 1988 Gametophytic selection in *Raphanus raphanistrum*: a test for heritable variation in pollen competitive ability. *Evolution* 42:1065–1075.
- Snow AA, TP Spira 1991 Pollen vigour and the potential for sexual selection in plants. *Nature* 352:796–797.
- 1996 Pollen-tube competition and male fitness in *Hibiscus moscheutos*. *Evolution* 50:1866–1870.
- Sokal RR, FJ Rohlf 1995 Biometry. 3d ed. W. H. Freeman, New York.
- Stephenson AG, JL Poulton, T-C Lau, RT Koide 1998 Effects of soil phosphorus level and mycorrhizal infection on the male function of plants. Pages 52–67 in JP Lynch, J Deikman, eds. Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic, and ecosystem processes. American Society of Plant Physiologists, Rockville, Md.
- Stearns SC 1992 The evolution of life histories. Oxford University Press, New York.
- Tyler G 1996 Soil chemistry and plant distributions in rock habitats of southern Sweden. *Nord J Bot* 16:609–635.

- Walsh NE, D Charlesworth 1992 Evolutionary interpretations of differences in pollen tube growth rates. *Q Rev Biol* 67:19–37.
- Wilkinson L 1997 SYSTAT. Systat, Evanston, Ill.
- Willson MF 1990 Sexual selection in plants and animals. *Trends Ecol Evol* 5:210–214.
- 1994 Sexual selection in plants: perspective and overview. *Am Nat* 144(suppl):13–39.
- Young HJ, ML Stanton 1990 Influence of environmental quality on pollen competitive ability in wild radish. *Science* 248:1631–1633.
- Zamir D, SD Tanksley, RA Jones 1981 Low temperature effect on selective fertilisation by pollen mixtures of wild and cultivated tomato species. *Theor Appl Genet* 59:235–238.