

# Extraneous pollen advantage in *Phlox cuspidata*

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Incipient incompatibility barriers often are the incidental by-products of divergent evolution. Accordingly, one expects pollen from outside of a population to be less successful than pollen from the same population or to be similar to it. Contrary to expectation, we find that in the predominantly self-fertilising *Phlox cuspidata*, alien pollen typically is at an advantage. We crossed greenhouse grown plants with pollen from their own populations and pollen from other populations, and determined the germination percentage of pollen grains on stigmas. Alien pollen had higher germination rates in 13 of the 14 populations, and for 10 populations the differences were statistically significant. Over all populations, the mean germination was 27.1 per cent for alien pollen versus 20.8 per cent for local pollen, a statistically significant difference. There was no relationship between the germination of alien pollen and the distance between pollen source and recipient.

## INTRODUCTION

In most large plant populations, pollen grains produced from extraneous sources constitute a small fraction of the total pollen pool (Levin, 1981). The impact of extraneous pollen depends on the genetic differences between populations and on the relative fitness of this pollen. Pollen from outside a population is apt to be less cross compatible than local pollen as a consequence of genetic divergence (Levin, 1978a). On the average, plants from different populations are more divergent than plants from the same population (Gottlieb, 1977; Brown, 1979).

Contrary to expectation, there is an emerging body of data, which indicates that extraneous pollen might be more cross-compatible than local pollen. In *Styloidium spathulatum*, 52 per cent of the ovules developed into seed following interpopulation cross-pollinations compared to 42 per cent for intrapopulation cross-pollinations (James, 1979). Crosses between populations typically gave better seed set than crosses within populations of *S. elongatum* and *S. confluens* (Banyard and James, 1979). Some crosses between *S. caricifolium*, *S. affine*, and transitional forms yielded greater seed set than intraspecific crosses (Coates, 1981). In *Asclepias incarnata*, 56 per cent of interpopulation

pollinations produced mature fruits with normal seeds compared to 30 per cent for intrapopulation crosses (Kephart, 1981). In *A. verticillata* 83 per cent of interpopulation crosses yielded mature fruits versus 19 per cent for intrapopulation crosses. Interpopulation crosses in *Mimulus guttatus* produced higher seed-set per flower than intrapopulation crosses, the differential varying with the location of population (Waser and Price, 1983). We do not know whether the reproductive differentials are actually due to higher percentage germination of extraneous pollen.

Given the possibility of extraneous pollen advantage and that such a phenomenon would open the breeding structure of populations, we undertook an analysis of local and extraneous pollen–pistil compatibility in *Phlox cuspidata*. We chose this species because it is predominantly self-fertilising, with most variation distributed between populations (Levin, 1978b). We thought that extraneous pollen advantage would be more likely to occur in a species with most variation distributed between populations rather than within them.

*Phlox cuspidata* Scheele is a spring flowering annual endemic to the prairies of Central Texas, and occurs in large discrete populations composed of thousands of individuals. The species is self-compatible and self-pollinating, with seed-set from selfing and outcrossing being about the same in the greenhouse. Self pollen has the same germination rate on stigmas as outcross pollen from the

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same population (Levin, unpubl.) Cross-pollination is accomplished by several lepidopteran species. Seeds are dispersed a few meters by the explosive dehiscence of the capsule. There are no special mechanisms for long distance dispersal. Thus, it is likely that interpopulation gene exchange occurs predominantly via pollen.

#### MATERIALS AND METHODS

Plants were raised from seed collected at random in 14 natural Texas populations. Seeds from each population were bulked, and a sample was sown. The location of the *P. cuspidata* populations and the code numbers by which they are referred to in the tables are as follows: # 1—4 mi S of Giddings, Lee Co., # 2—200 mi N of population # 1, # 3—2 mi N of Giddings, # 4—1 mi N of Giddings, # 5—Carmine, Fayette Co., # 6—4 mi N of Wallis, Ft. Bend Co., # 7—Gonzales, Gonzales Co., # 8—McDade, Bastrop Co., # 9—6 mi S of Lexington, Lee Co., # 10—3 mi N of Lexington, # 11—9 mi E of Eagle Lake, Colorado Co., # 12—2 mi E of Eagle Lake, # 13—3 mi S of Columbus, Colorado Co., # 14—Hearne, Robertson Co.

Plants were raised to flowering under 12 hour daylight with 70°–80°F day and 55°F night temperatures in the University of Texas greenhouses. Hand pollinations were performed within and among populations on plants 3 to 4 months old. Plants in each population were crossed with pollen from that population, and pollen from a subset of the remaining populations. In most instances, the number of extraneous pollen sources exceeded 5.

The number of crossing combinations was limited by the number of large healthy plants. Each combination was replicated ca. 16 times, using different pollen-ovule parent pairs. Over 1500 flowers were crossed in 81 combinations.

In some previous studies of pollen-pistil compatibility in *Phlox*, the percentage of pollen grains germinating and sending tubes into the style was estimated from stained pollen tube squashes (Levin, 1970, 1976). Subsequently we found that when the stigma is removed 24 hrs or more after pollination and stained with aniline blue in lactophenol, the number of normal size and unstained pollen grains on a stigmata is the same as the number of pollen tubes in the style. The cytoplasm of the unstained grains is in their pollen tubes. The grains which fail to send pollen tubes into the pistil appear a deep blue. Aborted pollen grains in *Phlox* are smaller than fertile pollen and typically have oil droplets within them. They are easily distinguished from germinated pollen.

We chose to estimate the percentage pollen grain germination on stigmas of *P. cuspidata* using the aniline blue procedure. Stigmas were harvested three days after pollination, noting the origin of the stigma and the pollen. They were placed on glass slides, stained with aniline blue in lactophenol, and covered with cover slips. We could unambiguously score the 300 to 400 pollen grains applied to each stigma. Nearly every pollen grain was in contact with the stigmatic surface.

The effect of pollen donor population, pollen recipient population, and the interaction between the two, on the percentage pollen germination, was explored via two-way analysis of variance. All

**Table 1** Mean per cent pollen germination in *P. cuspidata* by pollen source populations and by stigma source populations. Blanks indicate that cross was not made. Row and column means are given along margins

	Pollen source populations														$\bar{X}$
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	16.4	—	27.0	32.0	22.0	—	—	17.0	—	—	24.0	—	—	—	22.0
2	21.7	26.5	33.2	—	—	35.6	39.5	—	30.3	—	—	—	—	—	32.5
3	—	12.3	13.5	—	—	11.1	15.6	—	9.4	—	—	—	—	—	12.3
4	—	—	—	30.7	—	—	43.5	—	—	27.3	—	—	—	—	31.5
5	29.6	41.0	39.8	—	18.9	26.0	—	—	30.0	31.3	—	—	—	—	28.5
6	27.3	25.8	25.9	—	—	21.7	22.7	—	28.9	—	20.3	26.8	—	—	24.2
7	—	26.0	24.1	—	—	25.4	21.9	—	—	—	—	—	—	—	25.2
8	—	34.4	14.0	—	—	—	26.7	15.3	—	—	29.0	28.4	—	—	22.3
9	—	26.6	18.1	—	—	19.6	24.4	—	18.0	—	—	—	—	—	21.2
10	—	—	32.8	—	—	—	—	47.8	47.8	19.4	37.8	30.5	—	—	31.1
11	20.0	—	36.8	38.2	—	—	33.8	26.8	—	—	22.6	32.3	—	—	28.2
12	34.9	—	—	32.5	—	—	42.5	—	44.5	—	48.3	28.9	—	—	34.8
13	30.4	31.3	29.2	—	—	28.8	—	—	30.5	—	—	—	17.8	—	26.3
14	—	—	—	—	—	—	—	32.5	—	—	39.4	—	—	14.4	27.4
$\bar{X}$	24.6	25.2	24.5	33.0	20.2	24.0	26.8	21.6	26.0	23.6	27.9	28.2	17.8	14.4	

percentages were arcsin transformed before analysis in order to improve the normality of the data.

## RESULTS

Pollen-pistil compatibilities in intra and inter-population crosses were quite heterogeneous. Pollen germination following intrapopulation crosses varied from 14 per cent to 31 per cent, whereas pollen germination following interpopulation crosses varied from 9 per cent to 48 per cent (table 1). Mean germination of pollen from different sources (averaged over all pollen sources) ranged from 12 per cent and 35 per cent (table 1). The variability down columns and across rows in table 1 illustrates the dependence of pollen germination on both pollen population and stigma population. The effect of stigma population and the interaction between stigma population and pollen population were highly significant; the effect of pollen population was not significant (table 2). There was

**Table 2** Analysis of variance of pollen germination in *Phlox cuspidata*

Source	DF	MS	F	P
Model	79	0.1516	5.00	0.0001
Stigma pop	13	0.1467	4.84	0.0001
Pollen pop	13	0.0502	1.65	0.0648
Interaction	53	0.0550	1.81	0.0004
Error	1211	0.0303		
Total	1290			

**Table 3** Mean germination rates of local and alien pollen in *P. cuspidata*

Population	Germination (%) of local pollen	Germination (%) of alien pollen	P
1	16.4	24.4	0.0133
2	26.5	33.8	0.0011
3	13.5	10.8	NS
4	30.7	34.8	NS
5	18.9	32.0	0.0054
6	21.7	24.9	NS
7	21.9	28.7	0.0218
8	15.3	29.5	0.0001
9	18.0	21.7	NS
10	19.4	37.3	0.0012
11	22.6	32.7	0.0034
12	28.9	38.8	0.0377
13	17.8	30.3	0.0001
14	14.4	33.1	0.0275
X	20.8	27.1	0.0001

no significant correlation between the quality of populations as pollen parents and stigma parents (Pearson  $r = 0.18$ ).

In general, extraneous pollen has a germination advantage over local pollen (table 3). Thirteen of 14 populations exhibited higher germination of pollen from different populations compared to pollen from the same population; 10 of the 13 differences were significant. In some populations the extraneous pollen has a two-fold advantage. Averaged over all stigma sources, extraneous pollen germination was 27 per cent compared to 21 per cent for local pollen, a highly significant difference (table 3). The significant interaction between stigma population and pollen source indicates that the extraneous pollen superiority is population dependent (table 4).

**Table 4** Analysis of variance of local and alien pollen germination in *Phlox cuspidata*

Source	DF	MS	F	P
Model	27	0.3660	11.91	0.0001
Stigma pop.	13	0.3581	11.66	0.0001
Local or alien	1	1.497	48.68	0.0001
Interaction	13	0.0626	2.04	0.0156
Error	1263	0.0307		
Total	1290			

## DISCUSSION

Incipient incompatibility barriers often are the incidental by-products of divergent evolution (Levin, 1978a). Accordingly, one expects pollen from outside of a population to be less compatible than pollen from the same population or to be similar to it. Partial pollen-pistil incompatibility is present between many populations of *P. drummondii*, a close ally of *P. cuspidata* (Levin, 1976). Contrary to expectation, we find that in *P. cuspidata*, extraneous pollen had a germination advantage over local outcross pollen; in some populations the advantage was greater than 50 per cent. Accordingly, if a stigmata had similar numbers of local and extraneous pollen grains, more than 50 per cent of the progeny would be hybrids, because there are no post-fertilisation barriers between populations (Levin, unpublished).

The basis for extraneous advantage remains to be determined. It is not the result of the inherent superiority of pollen from particular sources, because there are no significant differences between populations in pollen germinability. This is in contrast to a recent study on *Asclepias speciosa*,

in which Bookman (1984) demonstrated that pollen quality varied between populations.

Extraneous pollen advantage, whether measured in terms of cross-compatibility or seed-set, is more likely to occur in self-compatible species than in self-incompatible ones, because the pollen pools of extraneous populations typically are more divergent in selfers than in outcrossers, and local populations of the former have small effective sizes (Brown, 1979). In a self-compatible species, the seeds from extraneous pollinations are likely to be more viable than those from local pollinations, which may abort due to inbreeding depression. The species in which extraneous pollen advantage (as seed or fruit set) has been demonstrated are self-compatible (James, 1979; Banyard and James, 1979; Coates, 1981; Kephart, 1981; Waser and Price, 1983).

The superior crossability of extraneous pollen increases the evolutionary flexibility of populations. Polymorphism, gene diversity and heterozygosity will be higher than would otherwise be the case, and the response to selection and stochastic processes will be retarded. Thus, the crossability of plants from the same versus different populations warrants our serious consideration.

If novel genotypes were introduced by long distance seed dispersal, superior pollen might then arise within a population. Consequently, the impact of seed immigrants on the levels of polymorphism and heterozygosity would be similar to that of pollen immigrants. Moreover, natural selection would increase the representation of genes conferring a mating advantage until some critical frequency was reached. Minority "compatibility genotypes" would be subject to frequency-dependent selection in a manner similar to that for rare males in some *Drosophila* species (Ehrman, 1966; 1967; Ehrman and Petit, 1968), and for self-sterility alleles in populations of self-incompatible plants (Wright, 1964; Yokoyama and Nei, 1979; Yokoyama and Hetherington, 1982).

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