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THE EFFECT OF DELAYED POLLINATION IN PETUNIA HYBRIDA

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SUMMARY

By delaying pollination with flowers of *Petunia hybrida* for up to 5 days, the viability of aged pollen and the numbers of seeds resulting from crosses declined with aging of either staminate or pistillate parent. In the resulting generation, given enough time to overcome transient maternal effects, plants from aged pollen were significantly heavier than were other plants. This suggests that there is selective mortality among stored pollen grains. This may have both basic and applied implications.

1. INTRODUCTION

It has been postulated that pollen tube competition, because it involves very large numbers of individuals and also haploid genotypes, could be an adaptively important phenomenon, one which provides opportunities in the angiosperms for mass screening similar to those observed in populations of microbial species. It has already been demonstrated that, if different pollen types are mixed together the components of such mixtures may be separated on the basis of (1) differential pollen tube rates (CORRENS 1928; JONES 1928; PFAHLER 1967; MULCAHY 1974; OTTAVIANO *et al.* 1980), (2) differential ability of pollen tubes to grow at low temperatures (ZAMIR *et al.* 1981), and (3) differential resistance of aging (PFAHLER 1967). In all such mixtures, the nature of each pollen type is influenced by both the sporophytic pollen source and the genotypes of the individual pollen grains. The relative contributions of each cannot be assessed without further study. In other studies, pollen grains from single heterozygous plants have been subjected to a variety of selection methods and, because only a single pollen source was used, it may be assumed that the only genetic component of variance among pollen grains was in their individual haploid genotypes. Such studies, thus dealing with gametophytic genetic variation, touch a unique aspect of pollen competition. A pollen mixture may consist of two or at most, a few score, different components but a gametophytic population from a highly heterozygous pollen source (in which hundreds of loci may be undergoing genetic segregation) might contain many thousands of pollen types.

With pollen mixtures, therefore, the surviving pollen types would be the faster growing, more cold resistant, etc. of two or more component types. In contrast, with pollen from heterozygous pollen sources, the surviving types could

Dedicated to Prof. Dr. H. F. Linskens on the occasion of his 60th birthday in 1981.

ultimately be the fastest, etc., among thousands rather than among two or three. The potential significance of selection among pollen grains from single, heterozygous plants is thus very great. Earlier studies have dealt with pollen tube growth rates (TER-AVANESIAN 1949, 1978; MULCAHY & MULCAHY 1975), tolerance to sodium chloride (SACHER & MULCAHY 1981) and also with the effect, in *Zea mays,* of specific loci (*Waxy, Shrunken,* and *Sugary*) upon tolerance to storage of pollen. In the present study we measure the effect, in *Petunia hybrida,* of aging pollen from single plants. Our hypothesis has been that the various pollen genotypes produced by a heterozygous pollen source might differ in their ability to tolerate pollen storage. Aging the pollen should then change the gene frequencies in the pollen population. Because some genes which are expressed in the pollen are expressed also in the sporophyte (see MULCAHY 1979) it thus seemed possible that aging the pollen before making pollinations could have an effect upon the resulting sporophytic generation. This possibility was tested in the present study.

2. MATERIALS AND METHODS

The experimental materials consisted of two self-fertile clones of *Petunia hybrida.* The flowers on these clones stay open and receptive to pollen for five days. For five consecutive days, flowers of one clone, that chosen to serve as the staminate parent, were tagged to record the day of anthesis. With the second clone, flowers were tagged and emasculated on the day of anthesis. This latter clone served as the pistillate parent for all crosses. At the fifth day, a series of crosses was made, using flowers which had been in greenhouse conditions throughout the aging process. (Temperatures (in December) fluctuated between 18 and 24 degrees.) The crosses made are represented in *fig. 1.* In any

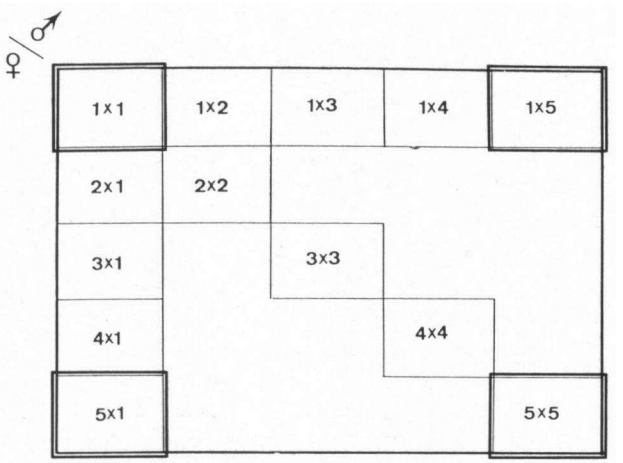

Fig. 1. Crosses made in the present study. The designation 1 × 1 indicates a cross made with both parents at the first day of their anthesis. A 1 × 2 indicates that the staminate parent was in its second day of anthesis.

cross, the age of the pistillate parent is represented on the left. Thus the notation 1 × 5 indicates that the pistillate parent was pollinated on the first day of anthesis and the staminate parent was in the fifth day of anthesis, etc. A total of 62 crosses were made. In each case, an excess of pollen, that from two anthers, was used in pollination. Pollen viability, as indicated by *in vitro* germination was also determined.

Seeds from crosses were collected, weighed, and counted. *Fig. 2* shows the linear regressions of seed number in relation to the aging of the staminate and pistillate parents.

In order to determine the effect of delayed pollination upon the resultant sporophytic generation, one capsule was chosen from each of the following crosses: 1 × 1, 1 × 5, 5 × 1, and 5 × 5. The choice of capsules was made so as to obtain capsules which differed as little as possible in seed number and weight. This was done in order to minimize the influence of maternal effects. In February, all seeds from the four capsules were individually planted in 'Jiffy Mix', a commercial germination medium consisting of finely ground vermicu-

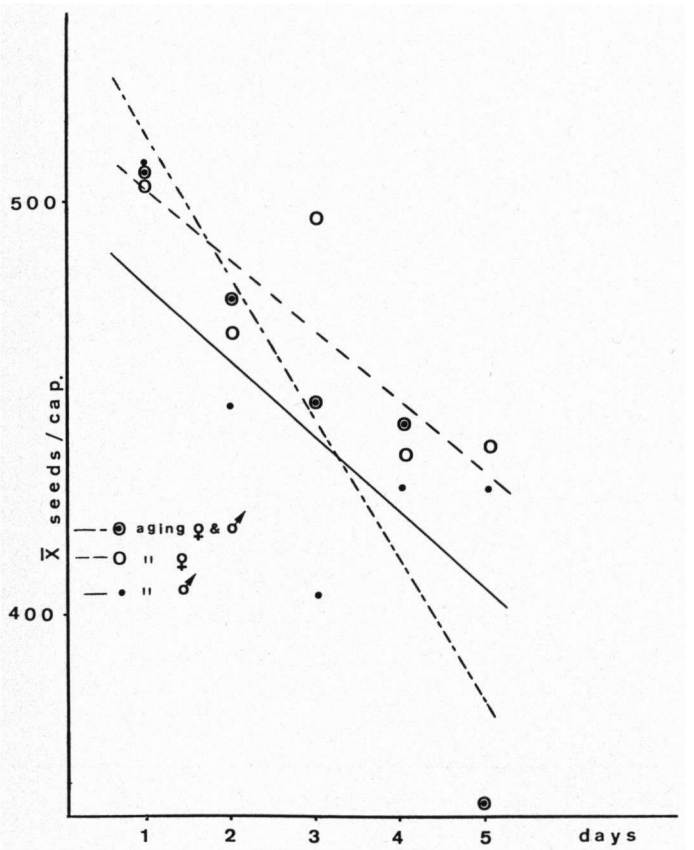

Fig. 2. The influence of delayed pollination upon the number of seeds produced by single capsules.

Table 1. Shoot weights, in grams fresh weight, of plants resulting from the various treatments.

♀ × ♂	Number of Seeds	Weight per Seed × 10^4	Percentage Germination	Shoot Weight (grams)		
				Day 35	Day 51	Day 69
1 × 1	506	1.038	92	0.63	9.04	14.97
1 × 5	455	1.011	95	0.63	9.25	20.51
5 × 1	464	1.033	88	0.61	8.69	18.17
5 × 5	360	1.085	89	0.47	8.98	17.98

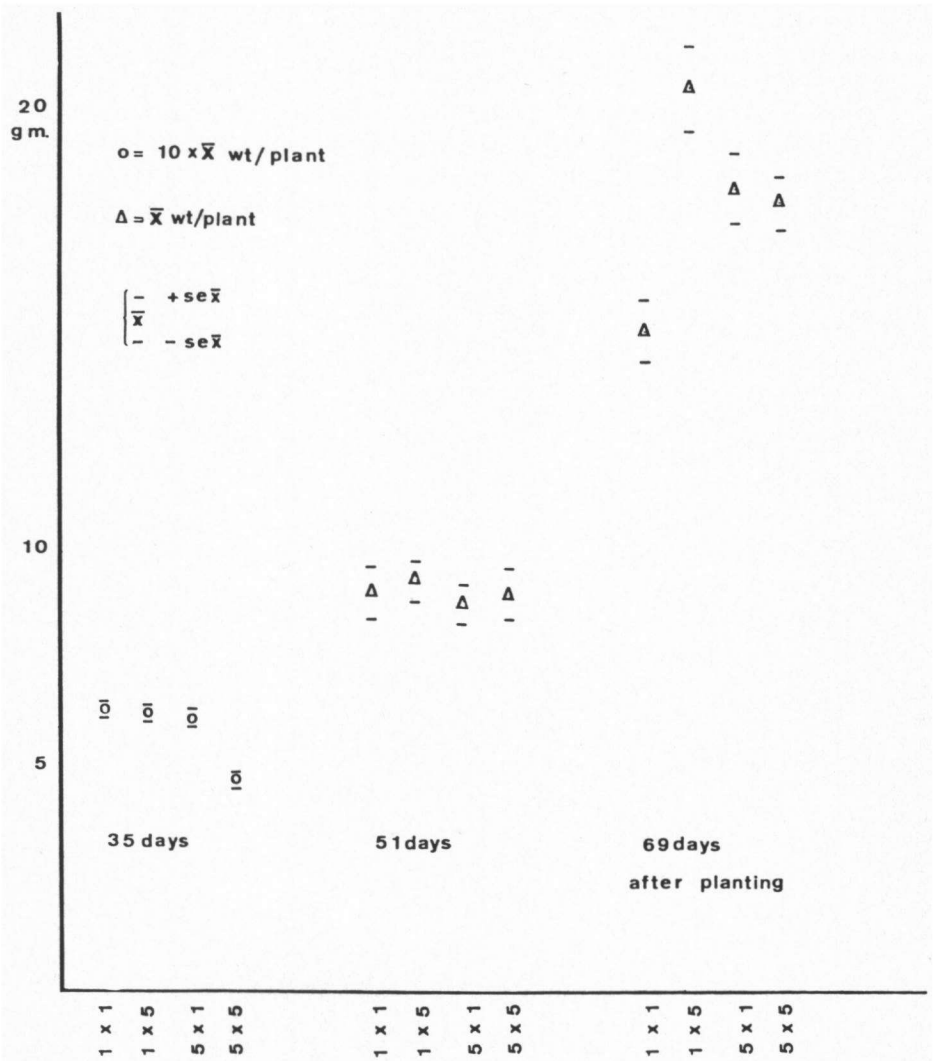

Fig. 3. The influence of delayed pollination upon the growth rate of the next generation. Data for sporophytes sampled at 35, 51, and 69 days after planting are indicated.

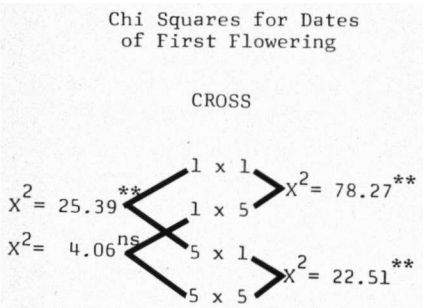

Fig. 4. Chi-square comparisons of flowering time distributions of plants produced in the four treatments.

lite and sphagnum. No supplemental light was given in order to avoid flowering. Thirty-five days after planting, 150 seedlings from each cross were transplanted to 5 cm pots of greenhouse soil. The remaining seedlings were cut at ground level and weighed. Sixteen days after this first harvest (51 days after planting) one-half of the transplanted seedlings were harvested and weighed as were the remaining plants at day 69. All of these data are presented in *table 1* and *fig. 3*.

As a further check on the effect of delayed pollination, 24 seeds were randomly selected from each of the remaining 1 × 1, 1 × 5, 5 × 1, and 5 × 5 capsules. An exception of this was for the cross 1 × 5, from which, because there was only one representative capsule, 48 seeds were selected. These seeds were planted individually, on 16 May, and transplanted at the appropriate time. Because of the season, plants were soon in flower and the data of first flowering was recorded for each plant. The data are presented in *table 2* and a chi-square analysis in *fig. 4*.

3. RESULTS AND DISCUSSION

The viability of pollen, as indicated by its ability to germinate *in vitro*, decreased by day 5 to 29.2% ± 0.66 of the value at day of anthesis. This, however, may not be a precise indication of *in vivo* germinability since VASIL (1962) has demonstrated that pollen which is incapable of *in vitro* germination may nevertheless function normally *in vivo*. Even lacking precision, however, it does suggest that the viability of pollen is decreasing with time and this allows for differential survival and thus selection.

The data presented in *fig. 2* suggest that the numbers of seeds per capsules decreases with the aging of either staminate or pistillate flowers and more rapidly when both are aged. Thirty-five days after planting, seedlings from the cross 5 × 5 are lighter than are others, but by day 51, there are no significant differences between seedlings of the four treatments. By day 69, when transient maternal influences are no longer significant, the only significant differences are between the 1 × 1 plants and those of the other three treatments. The high-

est average plant weight was observed in the treatment 1 × 5. The results suggest that aging the staminate or pistillate flowers, or both, results in progeny that are significantly heavier than are those from unaged flowers. Previous studies have shown that pollen from F_1 plants is both larger (JOHNSON *et al.* 1976) and more rapidly growing than is other pollen (MURAKAMI *et al.* 1972). These facts suggest that there may be a positive correlation between pollen diameter and pollen tube growth rate. Particularly relevant in this context is the observation by MANGELSDORF (1932) that in plants of *Zea mays* which are segregating for the gene sp_1 (small pollen), not only is a distribution of pollen sizes bimodal but the larger pollen type is distinctly larger than is pollen from homozygous dominant plants. This suggests that the resources which are not used by one pollen type (sp_1) are available to, and result in increased diameter in, the other pollen type (Sp_1). Thus it may be reasonable to assume that within a microsporangium, there are competitive interactions between pollen grains of different genotypes. These interactions could perhaps result in the variations in pollen diameter which were reported by JOHNSON et al. (1976) and possibly also in different quantities of reserve materials within pollen grains. Thus aging pollen could select for a particular subpopulation of pollen genotypes, thus resulting in some of the observations reported in the present paper. The possibility that gametophytic genetic differences could be expressed also in ovules is demonstrated quite clearly in studies by SCHWEMMLE (1968) and perhaps also by that of ANVARI & STOSSER (1978). Information on the genetic determination of ovule selection, however, it still quite limited.

Within the second part of the present study, the treatments resulted in significant differences in flowering times. *Table 2* indicates that the group with the earliest flowering date was 1 × 5, followed by 5 × 5, then 5 × 1, and finally 1 × 1. Also these results indicate that aging either gamete can result in significant differences in the subsequent generation.

In conclusion, this study confirms earlier indications by PFAHLER (1974) that selection among gametes from a single heterozygous individual may be accomplished by storage. Additionally, it suggests that this conclusion applies to both micro- and megagametophytes.

Table 2. Day of first flowering among plants produced by the various treatments.

	Day of First Flowering					
	1	2	3	4	5	
Cross	June 30 – July 3	July 4	July 5	July 6	July 7 – July 9	Average Day of First Flowering
1 × 1	6	11	14	3	30	3.63
1 × 5	16	6	5	5	5	2.38
5 × 1	11	5	11	16	22	3.51
5 × 5	20	5	8	6	12	2.71

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