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The Influence of Gametophytic Competition on Sporophytic Quality in *Dianthus chinensis*

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Summary. Pollinations were made on either the tip or the basal portions of the stigmatic surface in *Dianthus chinensis*. These two treatments provided, respectively, either good or modest opportunity for pollen tube competition. The pollen used came from a single clone. Technical and statistical methods were used to reduce greatly the influence of variation in seed weight. Seeds resulting from the two contrasting treatments were planted, and it was found that there were statistically significant differences in germination time and seedling weight between treatments. These results suggest that the quality of the F_1 generation can be significantly modified by competition between pollen tubes from a single plant.

The gametophytic phase of development is relatively inconspicuous in the angiosperms. Nonetheless, because the number of pollen grains produced so greatly exceeds the number of ovules available for fertilization, the gametophytic phase may exhibit some of the most intense competition found in the life cycle.

To what extent does this competition modify gene frequencies? That is, what is its biological significance? In answering this question, it is essential to consider that there are three modes by which gametophytic quality may be determined: gametophytic quality may be determined by loci which are transcribed only in the haploid phase of the life cycle; by loci transcribed only in the diploid phase of the life cycle; or by loci transcribed in both the haploid and the diploid phases of the life cycle. For brevity, these three modes may be referred to as haploid transcription, diploid transcription, and haplodiploid transcription, respectively. These three modes, their occurrence, and their influence upon the biological significance of gametophytic competition, are discussed briefly below.

Examples of purely haploid transcription are, by their nature, technically difficult to detect, thus their apparent rarity must be considered with caution. However, even if their occurrence is presently underestimated, Hartl (1970) has pointed out that, without a correlation between pre- and postzygotic genetic factors, gametophytic competition will have, at most, only transient, linkage mediated, effects upon the sporophyte. Some special cases, such as gametophytic incompatibility, of course, stand as notable exceptions.

Diploid transcription has been shown to determine, at least in part, the competitive ability of the gametophyte (Crosby 1949; Stephens 1956; Murakami et al. 1972). In these, and in similar cases, it is conceptually more accurate to consider that gametophytic competition is only a facet, or perhaps an extension, of sporophytic competition. This is not to detract from its biological significance but rather to emphasize that, with diploid transcription, there is no significant competition between gametophytes from a single plant.

Haplodiploid transcription, the third mode of determining gametophytic quality, has, of the three, the greatest potential for biological significance. If gametophytic quality is determined by haplodiploid transcription, then the very intense gametophytic competition could influence the dominant part of the life cycle, the sporophyte.

It should be noted that the characteristics of each of these three modes of determining gametophytic quality are such that the detection of haplodiploid transcription is relatively simple. For example, with haploid transcription, aside from linkage effects, gametophytic competition cannot modify the quality of the sporophyte. With diploid transcription, there can be no competitive interactions between gametophytes from a single sporophyte. With both haploid transcription and diploid transcription operating in the determination of gametophytic quality, there will be gene frequency changes in both phases of the life cycle, but these changes will be uncorrelated with each other. In cases of haplodiploid transcription, and only then, competition between gametophytes from a single plant will result in changes in the quality of the resultant sporophyte.

The first suggestion of haplodiploid transcription is Bond's (1926) report that, in *Pisum sativum*, aging pollen resulted in modifications of mendelian ratios. (Correns 1924 hat earlier reported modifications in the sex ratios of *Silene alba*, but sex determination, self incompatibility, and other specialized systems are not dealt with in the present paper.) The subject was not reinvestigated until Ter-Avanesian (1949) found that, in *Gossypium hirsutum* and *Vigna sinensis*, pollinations made with large amounts of pollen resulted in F_1 generations that had a smaller variance in fresh weight than did those resulting from pollinations made with less pollen. A similar report, again referring to varying quantities of pollen, was made by Matthews (See Lewis 1954). However, when pollen competition is controlled by modifying the quantity of pollen used in pollinations, the weights of the resultant seeds must be known. The few large seeds which result from low levels of pollen competition can be compared with many small seeds of heavily pollinated fruits through the analysis of covariance but only if seed weights are known. Since neither of the above two investigators presented data on seed weight, it was considered useful to reinvestigate the problem.

Materials and Methods

The species used for study was the common cultivar, *Dianthus chinensis* (Caryophyllaceae). The species is easily cloned and bears styles nearly 3 cm long, the entire adaxial surfaces of which are stigmatic. This latter quality allowed pollen competition to be varied without modifying the amount of pollen used in pollinations. Correns (1928) originally pointed out that pollination at the tip (or distal end) of such styles maximized gametophytic competition because the faster growing pollen tubes had good opportunity to surpass the slower growing ones. If pollinations are made at the basal portion of the style, separation between faster and slower growing pollen tubes will be less and fertilizations will thus represent a greater mixture of gametes from both faster and slower growing pollen tubes. That is, the degree of gametophytic selection is proportional to the distance traveled by the pollen tubes.

In the present study, two clones of *Dianthus chinensis* were employed, one served as the pistillate parent, the other as the staminate. Fifty pollinations were made at stylar tips and 50 others at stylar bases. The seed number and weight of each fruit were determined and, on the basis of these data, seeds from 15 pairs of fruits were chosen for planting. Each pair consisted of one fruit from each type of pollination, chosen so that the seed numbers of members of a pair were as nearly alike as possible. This was done to minimize differences between the seeds of the two treatments. Seeds were sown 4 cm apart, in flats, and the germination time of each was recorded. Four weeks after sowing, 50 seedlings from each fruit, fewer in some cases, were transplanted to 10×10 cm pots. The remaining seedlings were cut at ground level and weighed, fresh. Date of first flowering and the presence of asymetry in the first flower of a plant were recorded. The data are presented in table 1 and fig. 1 and 2.

As a further control on variation in seed weight, regression analyses were done on the average seed weight of each fruit versus the average germination time, average seedling weight, and average flowering time of the plants derived from each fruit. Any significant regression would indicate the need for analyses of covariance to "remove" the influence of differences in average seed weight.

Results and Discussion

Although the percentage of pollinations resulting in fruits did not differ significantly between treatments, the average number of seeds per fruit did (see table 1). This may indicate that more pollen adhered to the tip of the stigmatic surface than to the base. Within the 15 pairs of fruits selected for planting, however, no significant differences between treatments were found in seed number per fruit or in the average seed weight. This indicates that any seedling differences between treatments are probably not due to differences in seed weight.

As is shown in table 1, no significant differences between treatment were found in percentage of germination, time of flowering, or percentage of asymetric first flowers. Statistically significant differences were found, however, in germination time and seedling weight.

In neither time of germination nor seedling weight was there a significant regression against average seed weight per capsule. Analysis of covariance was therefore unnecessary. It should be recognized, however, that lacking data on individual seed weights, it was necessary to base the regression analysis on the average seed weight per fruit, thereby reducing the sensitivity of the analysis. Accordingly, the average germination time per fruit and average weight of seedlings from each fruit were also subjected to analysis of variance. Here the between treatment difference was not significant for time of germination but that for seedling weight was still statistically significant ($F = 7.1028$, df = 1,28; $P < .05$). A particularly interesting fact is that the variance in germination time for seeds from "basal" pollinations was significantly greater than it was for seeds from "tip" pollinations ($F = 2.3306$, df = 794,809; $P < .005$). A similar inverse relationship between intensity of gametic competition and sporophytic variance was reported by Ter-Avanesian (1949) and Matthews (See Lewis 1954). The primary significance of this study, however, is in its indication that, even if the possible influence of variation in seed weight is greatly reduced, competition between pollen genotypes from a single plant can indeed influence the quality of the F_1 generation. This suggests the functioning of haplodiploid transcription. Should further studies support this conclusion, it will become necessary to reconsider the biological significance of gametophytic competition.

Table 1. The products of pollinations at stylar tips (intense gametophytic competition) compared with those of pollinations at stylar bases (reduced gametophytic competition). Significant differences are indicated and irrelevant, nonsignificant ones omitted (i.e., time of flowering and floral symmetry)

Tip Pollinations	Base Pollinations	Statisticial Analysis
50 Pollinations	50 Pollinations	
48 Fruits	43 Fruits	χ^2 = .2778, df = 1;ns
↓	↓	
2503 Seeds	1714 Seeds	
($\bar{x}$ = 52.1458/Fruit)	($\bar{x}$ = 39.8604/Fruit)	F = 11.3052, df = 1,89; $P < .005$.
$\bar{x}$ weight/seed = = .001084 g	$\bar{x}$ weight/seed = = .001169 g	F = 7.5537, df = 1,89; $P < .01$
Fruits Selected for Planting		
Tip Pollinations	Base Pollinations	Statisticial Analysis
15 Fruits	15 Fruits	
↓	↓	
830 Seeds	806 Seeds	F = .0167, df = 1,28;ns
↓	↓	
$\bar{x}$ weight/seed = = .001041 g	$\bar{x}$ weight/seed = = .001099 g	F = .0434, df = 1,28;ns
↓	↓	
810 Seedlings (97.59% germination)	794 Seedlings (98.51% germination)	F = .0031, df = 1,28; ns
$\bar{x}$ germination time = = 4.4347 days	$\bar{x}$ germination time = = 4.5740 days	χ^2 = 62.7037, df = 9; $P < .005$
$\bar{x}$ seedling weight = = .3659 g	$\bar{x}$ seedling weight = = .3168 g	F = 8.9445, df = 1,183; $P < .005$

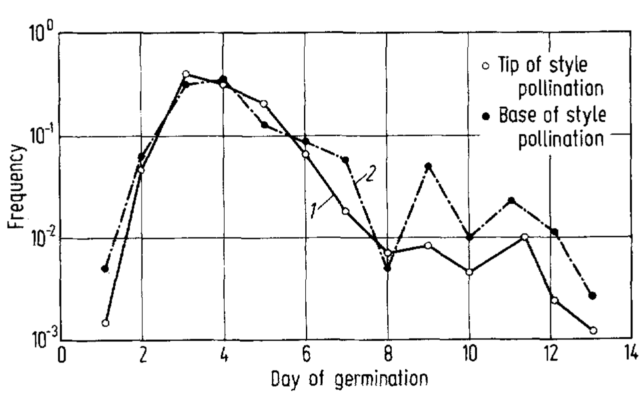

Fig. 1. Distribution of germination times for seeds produced under conditions of 1 severe pollen tube competition and 2 light pollen tube competition

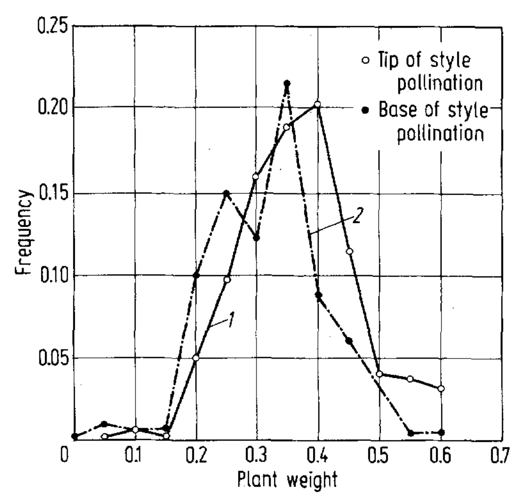

Fig. 2. Distribution of plant weights (28 days after planting). 1 refers to plants from seed produced under conditions of severe pollen tube competition. 2 refers to conditions of light pollen tube competition

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