Theor. Appl. Genet. 52, 77-79 (1978)



# The Effect of Varying the Number of Pollen Grains Used in Fertilization

D.V. Ter-Avanesian
Library of USSR Academy of Sciences, Leningrad (USSR)

**Summary**. The number of pollen grains placed upon a stigma influence both the development of pollen tubes and subsequently the progeny which result from fertilizations by gametes from these pollen tubes. The first influence is demonstrated by reduced pollen tube growth rates when pollen grains are few in number. This may indicate direct effects of pollen tubes upon the stylar tissues or perhaps more complex interactions between pollen and style. The second and potentially more important influence of limited pollination is upon the progeny. This was demonstrated with studies on three species. In each case, variation among the resultant plants was greater when pollen was limited than when normal, that is excessive, pollen was used. The mechanism of this phenomenon is not certain, but our data indicate that it is not simply an artefact of variation in seed size.

Key words: Pollen quantity – Pollen tubes – Progeny – Fertilization

## Introduction

In many species, the number of pollen grains normally found on the stigma greatly exceed the number of ovules contained in the ovary. This well-known fact takes on a new significance if we consider the possible effects of pollen competition. In our previous publication we demonstrated that in cotton, *Gossypium hirsutum*, pollination with limited quantities of pollen results in greater variability among the resultant offspring than does normal, abundant pollination (Ter-Avanesian 1949). Apparently, selection and competition among the abundant pollen gametophytes influence the quality of the resultant generation and extreme phenotypes are less frequent when excessive quantities of pollen are used. Perhaps this pollen selection represents an adaptive mechanism for increasing the proportions of medium, and presumably superior, types.

Although many factors relate to the numbers of pollen grains produced by plants, whether entomophily or anemophily, inbreeding or outbreeding, the enormous numbers of pollen grains usually produced suggest that there may be some adaptive value in pollen selection. It was to test this statement further that the following experiments were carried out.

## Methods

Pollen in quantities of 20, 100, 300 or 1000 grains were placed on the stigmas of emasculated flowers of cotton variety 'C-15' (*G. hirsutum*). In addition, there were also some natural pollinations which involved very large numbers of pollen grains. Similar crosses involving 15, 30, 50 and 100 pollen grains, as well as natural pollinations, were included in the study of *Vigna catjang*. Finally, 1, 20 and 100 pollen grains were used in a study of *Triticum aestivum*, spring wheat variety 'Grecum 433'. The flowers were emasculated the day before pollination. The results of these studies are presented in Tables 1, 2, and 3 respectively.

## Results

As seen in Table 1, the coefficients of variance for the three parameters measured are greatest when only 20 or 100 pollen grains are employed. This value was lower when 300 pollen grains were used and least in natural pollinations and in those employing 1000 pollen grains. These last two groups did not show obvious differences from each other. Tables 2 and 3 show similar patterns of heightened variation among plants resulting from limited pollinations in both *Vigna catjang* and spring wheat. For both *Vigna* and wheat, pollinations with 100 pollen grains resulted in progenies which were comparable in their range of variation to those resulting from natural or heavy pollinations.

0040-5752/78/0052/0077/$ 1.00

**Table 1.** The influence of pollen quantity upon variation in cotton (average values)

| Pollen Quality | No. of plants | Yield per plant (g) | Boll number per plant | Plant height (cm) |
|---|---|---|---|---|
| 20 grains | 5 | 175.28 (48.06) | 33.58 (44.6) | 105.0 (24.3) |
| 100 grains | 6 | 164.58 (42.5) | 33.53 (28.9) | 103.83 (19.98) |
| 300 grains | 7 | 136.35 (20.1) | 27.54 (14.6) | 106.0 (6.2) |
| 1000 grains | 8 | 135.96 (14.0) | 27.15 (11.0) | 100.0 (2.27) |
| Control, > 1000 grains | 10 | 145.31 (12.7) | 28.33 (7.8) | 100.55 (2.53) |

( ) coefficients of variance

**Table 2.** The influence of pollen quantity upon variation in *Vigna* (average values)

| Pollen Quantity | Plant Weight | Length of main stem (cm) | No. of first order branches | Length of Fruit pod | No. of seeds per pod | Wt. of 100 seeds |
|---|---|---|---|---|---|---|
| 15 grains | 381.6 (38.4) | 1.82 (24.8) | 4.64 (36.4) | 11.76 (4.6) | 10.8 (6.7) | 9,173.6 (12.9) |
| 30 grains | 335.5 (50.5) | 1.71 (36.5) | 3.88 (40.1) | 11.8 (6.4) | 11.03 (0.64) | 9,309.9 (6.3) |
| 50 grains | 488.2 (30.8) | 2.01 (30.2) | 4.6 (11.9) | 12.3 (3.6) | 11.24 (2.14) | 9,266 (3.24) |
| 100 grains | 343.0 (13.9) | 1.95 (17.7) | 5.66 (9.11) | 11.8 (3.45) | 11.16 (3.7) | 9,047.3 (3.26) |
| Control, > 100 grains | 385.4 (20.9) | 2.07 (12.7) | 5.57 (9.6) | 12.2 (3.7) | 11.24 (2.8) | 8,728.3 (4.9) |

( ) coefficients of variance

**Table 3.** The influence of pollen quantity upon variation in wheat (average values)

| Pollen Quantity | No. of plants measured | Yield per plant (g) | Straw Height (cm) |
|---|---|---|---|
| 1 grain | 7 | 3.59 (55.4) | 63.7 (13.4) |
| 20 grains | 10 | 4.49 (39.01) | 63.3 (14.4) |
| 100 grains | 10 | 4.83 (14.28) | 68.7 (2.7) |
| Control, > 100 grains | 10 | 4.72 (11.96) | 65.6 (5.3) |

( ) coefficients of variance

## Discussion

In these three studies we see a pattern similar to that reported in our previous publication (Ter-Avanesian 1949): variability among plants resulting from limited pollinations tends to be greater than when normal or large amounts of pollen are employed. This was the case for the three characteristics measured in cotton. For *Vigna*, the effect on plant weight and the number of first order branches was especially pronounced. The length of fruit and the number of seeds per fruit appear to be much more stable features and less influenced by the amount of pollen used in crosses.

In wheat, observations were limited to the agronomically important characteristics, yield per plant and straw height. These too showed the same pattern.

We thus conclude, on the basis of experiments carried out with cotton, *Vigna*, and wheat, that the quantity of pollen employed in crosses has a strong effect upon the resultant progeny. With large quantities of pollen, extreme forms are less common and the variation among plants is greatly reduced. This suggests that the excessive pollinations which naturally occur may perform the beneficial service of eliminating extreme (and presumably poorly adapted) forms. Whatever its significance, we must consider some possible explanations for the results obtained.

The first year cotton seeds resulting from pollinations made with 20, 100 and 300 pollen grains appeared to be heavier and larger in size relative to the naturally pollinated control seeds. When 1000 pollen grains were placed on the stigma of an emasculated cotton flower, there was almost no difference in average weight between the resultant seed and the control seed. Analogous results were also obtained with *Vigna*. The weight differences were recorded only in the experiments using 15-30 pollen grains; those using 50 and 100 pollen grains did not show any significant differences from those naturally pollinated. Obviously, the few seeds resulting from limited pollinations are the recipients of unusually large amounts of nutrient in the developing fruits. In addition, they are perhaps relatively free from the spacial restrictions which should exist in normally developing fruits. Both of these factors could allow genetic variation, normally present but suppressed among crowded seeds, to find expression in differences in seed size. This in turn could result in differences in the size and growth rates of the seedlings and even the mature plants. We suggest this explanation because it very possibly does play a significant part in explaining the observed results. Two other considerations, however, indicate that variation in seed weight alone is not adequate to explain these results. The first of these is that maternal effects, such as differences in seed weight, are usually limited to the first few weeks of growth. This is not to say that they are unimportant in natural populations, they very likely are. In the present study, however, where the plants were grown to maturity under cultivation, the influence of such maternal effects must be minor. A second consideration which reduces the significance of variation in seed weight is observed from the studies on wheat. This species is uniovulate, therefore seed weight must be independent of pollen quantity. Nevertheless, comparable changes in plant variation were obtained. Obviously factors other than variation in seed weight must be operative in these studies.

Further, albeit indefinite, indications come from another study we have performed, one which investigates the relationship between the speed of pollen tube growth rates and the quantity of pollen. In one case we placed 20 pollen grains on the stigmas of emasculated flowers of cotton; in a second case, emasculated flowers were pollinated with an unlimited quantity of pollen. It appeared that the pollen tubes occurring from limited pollination reached the ovule in 15 hours while those from unlimited pollination required 8 hours. The tubes developed nearly two times faster in the second case. Possibly the slow growth of tubes when their quantity is limited can be explained by the disturbance of the physiological interaction of pollen grains and stigma.

It is known from the literature that the presence of the secretion exuded by the stigma positively influences pollen germination (Martin and Brewbaker 1971). Pollen, in our own studies, did not germinate when placed on the cut surface of the style, but after placing the secretion products on such a decapitated style, we managed to obtain germination and tube growth. Golubinsky (1945) and Brewbaker and Kwack (1964) discovered that a group arrangement of pollen grains of a given species of the plant in a hanging drop stimulates the growth of the tubes.

According to this and other studies, it can be suggested that many factors influence pollen tube growth, although perhaps for normal reproduction in Angiosperms some minimum pollen quantity is required.

The present study demonstrates that limited pollination is a violation of the normal course of reproduction; one which has significant effects upon progeny. This suggests that competition among pollen tubes may serve to eliminate variant types from the progeny. This, of course, would be a mechanism of great significance to the process of adaptation. One conclusion seems relatively secure: the influence of pollen quantity is probably of substantial biological significance and should be subjected to further study (see also Mulcahy 1975).

## Acknowledgement

The author is much indebted to Professor David L. Mulcahy, University of Massachusetts, Amherst for critical reading of the manuscript and for the correction of the English text.

## Literature

Brewbaker, J.L.; Kwack, B.H.: The calcium ion and substances influencing pollen tube growth. In: Pollen Physiology and Fertilization. (ed. Linskens, H.F.), pp. 143-151. North Holland Publ. Co., Amsterdam 1964

Golubinskii, I.P.: A study of the physiology of germination of pollen. Doklady Akad. Nauk. **11** (1) (1945)

Martin, F.W.; Brewbaker, J.L.: The nature of the stigmatic exudate and its role in pollen germination. In: Pollen Development and Physiology (ed. Heslop-Harrison, J.), pp. 262-266. Butterworth, London 1971

Mulcahy, D.L.: The biological significance of gamete competition. In: Gamete competition in plants and animals, pp. 1-4. North Holland Publ. Co., Amsterdam 1975

Ter-Avanesian, D.V.: The influence of the number of pollen grains used in pollination. Bull. Appl. Genet. Plant Breeding Leningrad **28**, 119-133 (1949)

Accepted November 18, 1977
Communicated by H.F. Linskens

Prof. Dr. D.V. Ter-Avanesian
Khalturina Street 18, App. 4
192 041 Leningrad
(USSR)