Theor. Appl. Genet. 51, 211-215 (1978)

Male Gametophyte in Maize: II. Pollen Vigor in Inbred Plants

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Summary. The competitive ability of pollen from inbred plants in mixed pollinations in this study is not merely maintained but enhanced through successive generations of selfing. The data presented suggest two conclusions: 1) the possible existence of pollen-stylar interactions during successive selfings, which select for certain pollen genotypes, those best suited for rapid growth through "self" styles; and 2) the presence of sporophytic vigor in the heterotic F_1 sporophyte, or its absence in the "depressed" F_7 sporophyte, is not necessarily demonstrated in the gametophytic generation, perhaps because it can be overwhelmed by other factors, e.g. gametophytic response to selection.

Key words: Pollen - Inbreeding - Maize - Vigor

Introduction

Although the effect of inbreeding on the vigor of the sporophytic generation is well-known, few studies supply corresponding information on the male gametophytic generation. The gametophytic generation plays a significant role in the life cycle both in gene dispersal and in adaptive processes (Mulcahy 1974). How does inbreeding affect the ability of the gametophytic generation to fulfill this role? In this study, gametophytic vigor and competitive ability are observed in a normally outbreeding species, *Zea mays*, which had been selfed for several generations.

Impetus for this study came in part from the observation that, in the cross Wf9 × OH40B, pollen size declined significantly during seven generations of inbreeding (Johnson et al. 1976). Galinat (1961) has suggested a relationship between pollen grain size and the ability to maintain tube growth through long styles. This and the probable influence of the pollen source upon pollen quality (Pfahler 1965, 1967) suggest that the observed reduction in pollen size could modify the competitive ability of the gametophyte in inbred lines. If earlier investigations of pollen tube competition are compared (see Jones 1928; Pfahler 1967), an interesting and perhaps informative difference is revealed. For example, Jones, studying mixed pollinations in inbred lines, found that "self" pollen outcompeted "other" pollen in 20 out of 23 mixes. However, Pfahler (1967), using F_1 hybrids, found no consistent pattern in the outcome of competition between "self" and "other" pollen. In a third study using mixed pollinations, Murakami et al. (1972) found that F_1 hybrid pollen in most cases had the advantage in fertilization over the pollen from the inbred parents. Murakami suggested the possibility of the existence of a kind of "heterosis" in the F_1 pollen, caused either by non-allelic interactions, or as an effect from the vigorous F_1 sporophyte.

Differential fertilization can be influenced by: 1) differential germination rate, as seems to be the case in gametophytes carrying the waxy and non-waxy genes (Sprague 1933); and 2) differential pollen tube growth rates within the stylar tissues. In this study, we investigate the relative competitive abilities of pollen from both hybrid and inbred plants, considering the relative influence of pollen germination rates and pollen tube growth rates.

Materials and Methods

The general design of the study was to compare the relative competitive abilities of the "self" pollen from each of several inbred generations against a standard "other" pollen competitor. Genetic stocks were obtained from Dr. W.G. Galinat, Suburban Experiment Station, Waltham, Massachusetts. The pistillate parent (and source of "self" pollen) was in each case a member of an inbred series, extending

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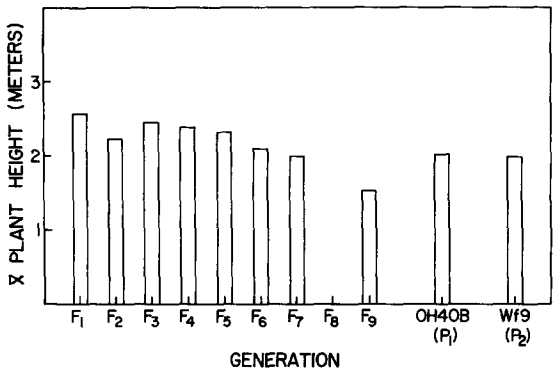

Fig. 1. Average plant height in meters of the F_1 hybrid Wf9 × OH40B, and the successively selfed generations, F_2 to F_9, expressing classical inbreeding depression in the significant reduction in height ($r = -0.913^{**}$, df = 6, $P < 0.01$)

from the F_1 (Wf9 × OH40B) to the F_9. The control line used as the source of "other" pollen was a yellow dent inbred (Wisconsin, W23). This line was homozygous for the R^{nj} ('Navajo') allele, which, in the homozygous condition, results in a purple color in the crown of the aleurone. In the heterozygote, the R^{nj} expression is limited to purple color in the tip of the embryo, or sometimes in the scutellum. The Wf9 × OH40B line did not possess the R^{nj} allele, making the presence of the purple spot a positive marker of cross (or "other") fertilization.

The cross Wf9 × OH40B produces a heterotic F_1, which exceeds its inbred parents in height and earliness of anthesis. This heterotic effect is gradually lost with successive generations of selfing; in height, the F_7 and F_9 show inbreeding depression, being significantly shorter at maturity than the F_1 (see Fig. 1).

All plants were field grown in the summer of 1973 at the University of Massachusetts, Amherst, Massachusetts. Pollen was collected by standard field techniques; actively shedding tassels were cleaned of open and empty anthers in the late afternoon, then bagged with Lawson corn pollen collecting bags. Pollen was collected the following morning and brought to the laboratory, where it was screened to remove debris.

Pollen from the control "other" line was collected each day from several actively shedding tassels and mixed thoroughly. One milliliter of this mix was then combined with an equal volume of pollen from the appropriate plant of the selfed line, Wf9 × OH40B. All sieving, mixing, and measuring of pollen was done within two hours of collection. The mixed pollen samples were then returned to the field where the appropriate pollinations were made as follows:

♀		♂
F_1	×	$(F_1 + R^{nj})$
F_2	×	$(F_2 + R^{nj})$
⋮		⋮
F_7	×	$(F_7 + R^{nj})$

It should be emphasized that, while the R^{nj} pollen from several plants was pooled, the "self" component of each pollen mixture was not a pooled sample. It was a true self pollination, not a "sib".

In addition, another sample of each pollen mix was also used to pollinate a third line, unrelated to either pollen source. This pollination was intended as a control, to evaluate the relative competitive ability of hybrid and inbred pollen against a common competitor when growing on "non-self" styles. However, extremely poor seed set of both pollen sources on the chosen third line was obtained, and the results of these pollinations were not reported.

Adjustment of the observed number of fertilizations per volume of pollen was necessary since significant differences between mean pollen diameters of F_1 and F_7 plants had been reported (Johnson et al. 1976). Accordingly, the procedure used by Pfahler (1965) was adapted to this experiment; samples of pollen from the test plant ("self") and from the Navajo (R^{nj} - "other") mix were saved and measured to establish the mean pollen diameter for each. The pollen was expanded overnight in a medium of lactic acid/aniline blue, and measurements were made using an ocular micrometer with an A.O. Spencer microscope at 430X. Thirty pollen grains were measured for each sample; these data were averaged to establish the mean for each generation and for the Navajo (R^{nj}) mix. The percentage of "self" pollen in each mix was then calculated using the formula from Pfahler (1965):

$$\%\ \text{"self" pollen in each mix} = \frac{N^3}{(S^3 + N^3)} \times 100$$

where S = the mean diameter of the pollen of the particular generation under consideration
N = the mean diameter of the Navajo (R^{nj}) pollen mixture.

For any observed number of total fertilizations, the expected deviation in representation of the two pollen types could then be calculated.

In addition, another possible source of error in estimating relative fertilization ability arose from variations in the percentages of non-fertile grains in the different pollen samples. Microclimatic differences have been observed to contribute to reduced fertility of pollen in field grown plants of *Aster* (Jones 1976). Such day to day variations in pollen fertility could be reflected in modified competitive ability in mixed pollinations, obscuring significant trends. Therefore, the pollen samples used in diameter adjustments were also scored for the percentages of non-fertile grains, using the stainability of the protoplast with aniline blue as an indication of fertility. Presence of the protoplast, whether viable or not, is indicated by the stain; aborted pollen grains lacking a protoplast remain unstained (Jones 1976).

Results and Discussion

The average heights (in meters) for the inbred parents and each succeeding generation in the cross Wf9 × OH40B are shown in Fig. 1. The F_1 exceeds its inbred parents in mean plant height, while the F_7 and F_9 generations express classical inbreeding depression in the significant reduction in height ($r = -0.913^{**}$, df = 6, $P < 0.01$).

Table 1. Mean pollen diameters of the Navajo (R^{nj}) line, the parental inbred OH40B, and the successively selfed generations used in the mixed pollinations

Pollen Source	$\bar{X}$ (μ) and Standard Deviation	
F_1	103.09	(4.20)
F_2*	102.55*	----
F_7	100.45	(2.76)
OH40B (P_1)	107.73	(3.11)
Navajo (R^{nj})	96.25	(2.91)

* F_2 pollen samples were destroyed by fungus. Therefore, the mean pollen diameter shown here was calculated for the F_2 on the basis of the difference in mean diameter between the F_1 and the F_7. The observed significant linear decrease in mean pollen diameter during inbreeding (Johnson et al. 1976) suggested this calculation

Table 2. Average percentage of non-fertility of pollen samples from plants used in the mixed pollinations.

Pollen Source	$\bar{X}$ % Non-Fertility
F_1	8.00
F_2*	7.26*
F_7	3.56
OH40B (P_1)	5.21
Navajo (R^{nj})	2.02

* As previously stated, F_2 pollen samples were destroyed by fungus; this figure is an estimate of non-fertility, based on the observed difference between the F_1 and F_7. However, the linearity of this relationship has not been determined

Table 1 shows the mean pollen diameters of the Navajo (R^{nj}) line, the parental inbred OH40B (P_1), and the successively selfed generations scored for their competitive ability in the mixed pollinations. Mixed pollinations were made on all generations, but the data presented are based on 14 well-filled ears, six from the F_1, four from the F_2, and two each from the F_7 and OH40B (P_1), a total of 3,218 fertilizations.

The average percentage of non-fertility for each generation and for the Navajo (R^{nj}) mix is shown in Table 2. In the F_1, a much higher percentage of non-fertility was observed than in the F_7, making the compensatory calculations for differences between generations absolutely necessary in this case.

Since equal volumes of "self" and "other" pollen were mixed, an approximate estimate of the relative representation in fertilizations should be 50 % "self" and 50 % "other", assuming equal competitive ability. However, differences between pollen sources in the average diameter of single pollen grains (Table 1) and also in degrees of pollen abortion, as indicated by aniline blue staining (Table 2), necessitate corrections in the 50:50 estimate. Table 3 presents the steps in correcting for differences in volume and percentage nonfertility between the various pollen sources (columns 1, 2). Also shown is the final corrected value for the percentage of fertile "self" pollen in each pollen mixture (column 3). These corrected values, which differ between mixtures, indicate the frequency of self fertilization which is expected, provided that the components of the pollen mixtures possess equal competitive ability.

Kernels on well-filled ears obtained from the mixed pollinations were scored for color to determine the number of fertilizations attributed to each pollen type. The deviation from the expected frequency of self fertilization was then calculated for the apical, middle and basal portions of each ear. The average deviation from the expected frequency

Table 3. Corrections for differences in volume and presumed viability between different pollen sources

Results of corrections for volume differences; Expected % "self" and "other" in each equal volume mix

Generation	Self	Other (R^{nj})
F_1	44.9	55.1
F_2	45.2	54.8
F_7	46.8	53.2
OH40B (P_1)	41.6	58.4

Results of corrections for both volume and presumed viability; Expected % "self" and "other" in each equal volume mix

Generation	Self	Other	Total
F_1	41.3	54.0	95.3
F_2	41.9	53.7	95.6
F_7	45.1	52.1	97.2
OH40B (P_1)	39.4	57.2	96.6

Corrected value for % "self" pollen in each mix

Generation	Self
F_1	41.3/95.3 = 43.3
F_2	41.9/95.6 = 43.8
F_7	45.1/97.2 = 46.4
OH40B (P_1)	39.4/96.6 = 40.8

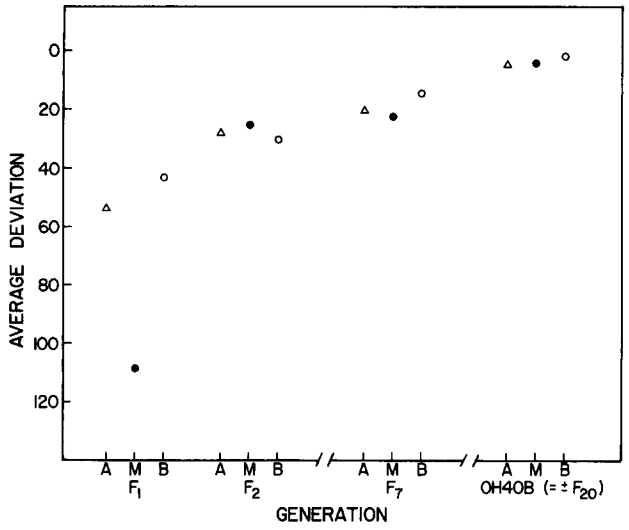

Fig.2. Average deviation from expected frequency of self fertilizations in apical (A), middle (M) and basal (B) portions of the ears of the F_1, F_2, F_7, and OH40B. Plotting the number of generations of selfing against the observed deviation from expected fertilizations revealed a significant log curve ($r = 0.78^{**}$, df = 7, $P < 0.01$). (The exact number of repeated selfings for the inbred parental line, OH40B, was not known. The regression was calculated using various estimates, from 10 to 40 generations of selfing for the OH40B. Under all circumstances, a highly significant relationship was obtained. For example, using the estimate of 10 successive selfings, r = 0.79**, using 15, r = 0.79**, using 40, r = 0.75**.)

of self fertilization for each generation is shown in Fig.2.

Since our initial question was whether or not the observed reduction in pollen size during inbreeding could be associated with modified competitive ability, the relationship between pollen size (Table 1) and the observed deviation from the expected frequency of fertilizations (Fig.2) was then analyzed by regression ($r = -0.31$, df = 10, N.S.). From this we conclude that if pollen diameter does influence the competitive ability of pollen tubes, the influence cannot be of overwhelming importance. Additional factors must be operating.

In all of the mixed pollinations, the Navajo (R^{nj}) appeared to be an extremely strong competitor; therefore, it is the trend in the outcomes of the mixed pollinations that is important in our considerations, and not the absolute values. When the relationship between the number of exposures to self pollen through repeated selfing and the observed deviation from expected fertilizations was fitted to a log curve, a highly

Table 4. Summary: Chi-square values for the average deviations from the expected frequency of self-fertilization in the apical, middle, and basal portions of ears of each generation

Generation	Portion of Ear	X^2(Exp.1:1)	Probability
F_1	Apical	29.97	$P < 0.001$
	Middle	54.40	0.001
	Basal	21.05	0.001
F_2	Apical	15.41	$P < 0.001$
	Middle	7.85	0.01
	Basal	20.38	0.001
F_7	Apical	12.05	$P < 0.001$
	Middle	6.95	0.01
	Basal	6.26	0.05
OH40B	Apical	0.89	$P < 0.5$ ns
	Middle	0.656	0.5 ns
	Basal	0.244	0.7 ns

significant positive regression was obtained ($r = 0.78^{**}$, df = 10, $P < 0.01$). In spite of the reduced pollen size and the reduction in size and vigor of the sporophyte in this line (see Fig.1 and Table 1) the competitive ability of the pollen in the repeatedly selfed generations is not only maintained, but apparently is significantly enhanced in self pollinations.

As mentioned in the Introduction, an increasing degree of self-fertilization could be due to two different factors: more rapid germination of "self" pollen; or more rapid pollen tube growth rate of the "self" gametophytes. These two effects may be separated by the following method: in pollen mixtues applied to silks in the field, not all pollen tubes start at the same level. If, in a mixed pollination, different pollen tubes penetrate the stylar tissues at different relative rates, the probability of fertilizations by faster pollen tubes will be proportional to style length (Correns 1928; Mulcahy 1971). Random effects will be more significant when styles are short. On long styles, relative growth rates will outweigh these random effects. Since basal kernels have longer styles than apical kernels, any basipetal increase in fertilization frequency by one pollen type will be indicative of a greater relative growth rate (see Mulcahy 1971). Thus it is particularly significant that in the F_7 and in the OH40B, the two most repeatedly selfed generations, the greatest competitive ability of "self" pollen (i.e. the smallest deviation from the expected) is expressed in the middle and basal portions of the ears (Fig.2 and Table 4). This suggests

that the observed increase in self pollen competitive ability is apparently due to increased relative pollen tube growth rate of "self" pollen, rather than to faster pollen germination. Moreover, the data in Fig. 2 and Table 4 appear to explain the apparent conflict between Jones (1928) and Pfahler (1967); after several generations of selfing and pollen-stylar interactions, a particular genotype of "self" pollen has been intensively selected for growth in that particular stylar environment. Selected "self" pollen then would be favored in competition with unselected "other" pollen (and thus explain Jones' (1928) results with inbred lines). But, conversely, in F_1's unexposed to self-stylar selection, the pattern of "self" fertilization versus "other" would not be predictable (and thus give rise to Pfahler's results).

An important corollary of this interpretation is that the competitive ability of pollen is determined, at least in part, by the gametophytic genotype. If pollen quality were determined solely by the sporophytic genotype, selection among pollen types from one plant would be impossible. Murakami et al.'s suggestion (1972) that sporophytic heterosis might be also reflected by the gametophyte is, of course, not excluded by this interpretation. Rather we would suggest that both sporophytic and gametophytic genotypes may influence pollen quality. However, further studies are needed to quantify the relative contributions of gametophytic and sporophytic genotypes.

Received August 2, 1977
Communicated by H.F. Linskens

Literature

Correns, C.: Bestimmung, Vererbung und Verteilung des Geschlechtes bei den hoheren Pflanzen. Handbuch der Vererbungswissenschaft Band II, 1-38 (1928)

Galinat, W.C.: Corn's evolution and its significance for breeding. Econ. Bot. 15, 320-325 (1961)

Johnson, C.M.; Mulcahy, D.L.; Galinat, W.C.: Male gametophyte in maize: Influences of the gametophytic genotype. Theor. Appl. Genet. 48, 299-303 (1976)

Jones, A.G.: Environmental effects on the percentage of stainable and presumed normal pollen in *Aster* (Compositae). Amer. J. Bot. 63 (5), 657-663 (1976)

Jones, D.F.: Selective Fertilization. Chicago: University of Chicago Press 1928

Mulcahy, D.L.: A correlation between gametophytic and sporophytic characteristics in *Zea mays* L. Science 171, 1155-1156 (1971)

Mulcahy, D.L.: Adaptive significance of gametic competition, in Fertilization in Higher Plants. Amsterdam: North-Holland Publishing Co. 1974

Murakami, K.; Yamada, M.; Takayanagi, K.: Selective fertilization in maize, *Zea mays* L. I. Advantage of pollen from F_1 plants in selective fertilization. Japan J. Breeding 22, 203-208 (1972)

Pfahler, P.L.: Fertilization ability of maize pollen grains. I. Pollen sources. Genetics 52, 513-520 (1965)

Pfahler, P.L.: Fertilization ability of maize pollen grains. II. Pollen genotype, female sporophyte, and pollen storage interactions. Genetics 57, 513-520 (1967)

Sprague, G.F.: Pollen tube establishment and the deficiency of waxy seeds in certain maize crosses. Proc. Nat. Acad. Sci. 19, 838-841 (1933)

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