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Male Gametophyte in Maize: Influences of the Gametophytic Genotype

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Summary. Size and variation in pollen samples were investigated in several successively selfed generations. A significant decrease in mean diameter of pollen grains accompanied inbreeding; also decreased variation in pollen size from individual plants was observed. Since loss of developmental homeostasis in the sporophyte could affect variation in the gametophyte, sporophytic characters were observed as a control. The main conclusion reached in this study was that pollen diameter is influenced by the gametophytic genotype.

Introduction

The effect of inbreeding on the sporophytic generation is very well documented, but corresponding information for the gametophytic generation is sparse. Here we report investigations on the influence of inbreeding upon pollen diameters in successive generations in *Zea mays*.

This study was undertaken with the knowledge that many other factors can affect pollen size; for example, environmental factors such as: water relations, temperature, and photoperiod (Kurtz, Liverman, and Tucker 1960); mineral nutrition (Bell 1959); position of the flowers on the plant; size of anthers, and time of anthesis (Banerjee and Barghoorn 1971). Also, genetic factors are known to be significant in the determination of pollen size (Sax 1935; Barber 1941). In corn, for example, pollen size is influenced by gametophytic expression of the sp allele, resulting in small pollen grains (Singleton and Mangelsdorf 1940). Furthermore, Peterson and Munson (1962) found slightly greater variability in pollen grain size in plants having one to five B-chromosomes (accessory chromosomes), although the mean sizes remained the same.

The study of pollen diameters in successively selfed generations is of particular interest because relative variability could indicate the extent to which the gametophytic genotype determines the gametophytic phenotype. For example, one effect of repeated selfing (and resulting increase in homozygosity) would be to decrease genetic heterogeneity among pollen grains from a single plant. If variance in pollen size also decreased as heterozygosity of the sporophyte decreased, this would suggest a large component of gametophytic determination of pollen size. Conversely, if pollen size is determined largely by genetic factors transcribed in the sporophyte, then pollen size variation should either remain constant, or as explained below, increase with inbreeding. (Since the gametophytic generation is hemizygous, the terms *heterozygous* and *homozygous* refer, of course, to the condition in the sporophyte.)

Recently, Sari-Gorla *et al.* (1975), using *in vitro* techniques, found greater variance for pollen tube lengths from F_1 plants than from their inbred parents; this increased variability in the F_1 was attributed to genes transcribed in the gametophytic generation. However, there are two difficulties with interpreting the possible source of gametophytic variation: first, parental characteristics are only moderately useful as predictors of the hybrid gametophytic quality (Pfahler 1970; Sari-Gorla *et al.* 1975); secondly, it is necessary to consider the relation between homozygosity and loss of developmental homeostasis in the inbred sporophyte. This loss could be expressed in the microspore mother cells, and thus also in the gametophytic generation.

The first difficulty may be solved by observing the trend in gametophytic variability with increasing homozygosity in successively selfed generations, rather than in parent-hybrid comparisons. The second difficulty may be offset by using comparative information on developmental homeostasis of the sporophyte. Such a control is necessary for two reasons; first, inbreeding is known to have an effect on the developmental homeostasis of the sporophyte (Mather 1953; Lerner

1954). In corn, this may be expressed as decreased variability in phenotypic characters in heterozygous hybrids as compared to homozygous lines (Shank and Adams 1960). In the present study, loss of developmental homeostasis in the increasingly homozygous sporophyte could be manifested in greater variability in pollen grain sizes, thus possibly masking gametophytic influence on pollen size. Secondly, although Lerner's thesis of the relationship between developmental homeostasis and heterozygosity is often supported, exceptions do exist. Levin (1970), for example, has indicated that poorly co-adapted gene complexes may result in lowered developmental homeostasis in some hybrids. Cases in which heterozygous hybrids exceeded their inbred parents in variability of some phenotypic characters have been reported also by Jones (1918, 1920) and by Dobzhansky and Wallace (1953). It must be concluded, therefore, that the F_1 may sometimes show an increase in variability when compared to its inbred parents and this, of course, could influence pollen mother cell development. Accordingly, in studies dealing with the relative variability of hybrid pollen and that of their inbred parents, it is clearly necessary to determine the relationship between heterozygosity and developmental homeostasis in the sporophyte for each case.

In the present study, size and variance in F_1 pollen grains are compared, not only in the inbred parents, but also in several subsequent generations. In addition, data on sporophytic characters are presented as a control index of developmental homeostasis in each generation.

Material and Methods

Pollen was collected from 42 plants representing successive generations of inbreeding from the cross Wf9 × OH40B, grown in Florida during the winter of the 1972 season. Measurements were made using an ocular micrometer with an A.O. Spencer microscope at 430×. Pollen samples were mounted in a medium of lactic acid and IKI. Two slides were made from each sample, and 50 observations were made from each slide. The slides were prepared 24 hours before the observations were made: thus all samples were allowed to expand in the lactic acid medium for equal periods before measurements. Measurements were made of the longest outside diameter of the pollen grains, and the data were analyzed through use of the Bio-med program BMDOIV, Analysis of Variance for one-way design, a program from the Health Sciences Computing Facility, UCLA, version of June 11, 1964.

Plants used in the study were from 5 generations, the F_1, F_2, F_3, F_6, and F_7, and the two inbred parents, Wf9 and OH40B. Six plants from each generation were sampled.

The upper portion of the flag leaf was also collected from each plant sampled for the pollen studies, for use as an index of developmental homeostasis in the sporophyte. Stomatal length was measured to give an estimate of variability of cell sizes in the sporophyte. Also the numbers of stomates in fields of constant size were counted as a second index of developmental stability in each generation. The stomate measurements were made using an ocular micrometer with an A.O. Spencer microscope at 430×. A portion of the leaf about 5 cm below the tip was mounted in the lactic acid-IKI medium used for pollen expansion. Stomates and epidermal cells were easily visible on the abaxial surface, due to the clearing effect of the medium on the leaf tissue. Twenty measurements per leaf were made in an area on either side of the mid-rib, avoiding large veins.

Stomate counts were made in a similar manner, in ten fields per leaf, at 220× (the method used by Heichel 1971).

The standard deviations, means, and coefficients of variation ($S.D./\bar{x}$) were calculated for each plant, and regression analysis was used to test for significant trends. Also the F-test (Sokal and Rohlf 1969) was used to determine whether variation in the F_1 pollen was significantly different from that of the inbred parents.

Results and Discussion

Table 1 shows the mean pollen grain diameters, in microns, for each of the two parental lines, and also for the five subsequent generations studied. Statistical analysis (see Fig. 1) indicated a highly significant, and negative, linear relationship between generations F_1, F_2, F_3, F_6, F_7, and mean pollen diameter ($r = -0.6164^{**}$, $df = 28$, $P < 0.01$).

The frequency distributions of pollen measurements of the two inbred lines, Wf9 and OH40B, and their F_1 are shown in Fig. 2. An F-test of the variances showed that F_1 variance was not significantly different from that of the female parent, Wf9 (P1) ($F_{599,599} = 1.2096$, N.S.). However, F_1 variance was significantly greater than that of OH40B, the male parent (P2) ($F_{599,599} = 1.55$, $P < 0.01$).

Fig. 3 summarizes the frequency distributions of the pollen measurements for the successively inbred generations, F_1, F_2, F_3, F_6, and F_7. These frequency distributions clearly show a trend toward reduced variability in the inbred generations. However, the aspect of particular importance to this investigation is the extent of phenotypic variation in the pollen sample of each individual sporophyte rather than each generation. Therefore, the coefficient of variation was calculated for the pollen sample from each plant. (The

Table 1. Mean diameters (in microns) of the pollen samples studied from each generation. Each generation mean represents a total of 600 observations, 100 from each of the 6 plants in each generation

Generation	Pollen Grain Diameter Means (μ)
F_1	100.85 ($\pm$ 0.618)
F_2	99.04 ($\pm$ 0.537)
F_3	98.77 ($\pm$ 0.816)
F_6	97.14 ($\pm$ 0.517)
F_7	94.03 ($\pm$ 0.497)
P1 (Wf9)	93.71 ($\pm$ 0.657)
P2 (OH40B)	100.22 ($\pm$ 0.577)

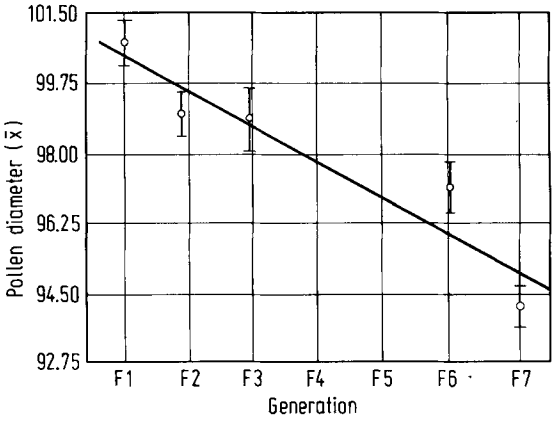

Fig.1. Relationship between pollen diameter and generation. The generation means are shown as data points; the regression equation for the line which fits these points is: $\bar{Y} = 98 + (-0.1070)\bar{x}$. The data points are in microns; the standard errors of the means are indicated by the vertical lines

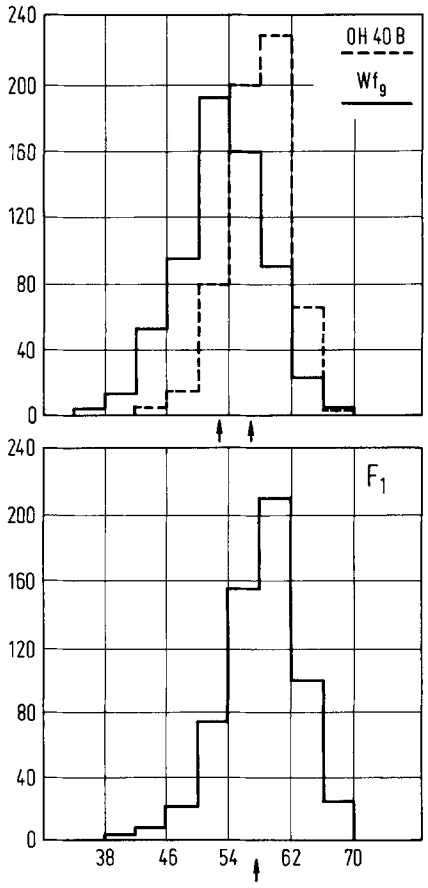

Fig.2. Frequency distributions of pollen grain diameters of an inbred line pair and their F_1. Measurements are in optical units (one unit = 1.75 microns). The mean for each distribution is indicated by an arrow

coefficient of variation (C.V. = S.D./$\bar{x}$) allows comparisons of variability between populations having different means. The higher the calculated C.V. value, the more variable the population is for that character.) These data, summarized in Fig.4, were used in a regression analysis to determine whether variation in pollen samples from individual plants changed significantly during inbreeding, F_1 through F_7. The decrease in variation in pollen diameter through increasingly inbred generations was also highly significant ($r = -0.5530^{**}$, df = 28, $P < 0.01$).

It is important to aks what influence the quality of the sporophyte has upon pollen variation; certainly the decrease in pollen means from the F_1 through F_7 indicates a significant sporophytic effect (see Fig.2). Also, the increasing similarity between members of increasingly inbred generations would result in smaller differences between pollen from separate plants (see Fig.3). However, neither of these can account for the observed decrease in variation in pollen sizes within individual plants (see Fig.4). Additional evidence on this point is provided by the control measurements on developmental homeostasis in the sporophyte. A regression analysis of the coefficients of variation based on stomatal frequency revealed no significant change from the F_1 through the F_7 ($r = -0.219$, df = 33, N.S.). Similar analysis of the stomate lengths also showed no significant change in variation from the F_1 to the F_7 ($r = 0.163$, df = 28, N.S.). On the basis of the characters observed in these two controls, it must be concluded that inbreeding did not have a detectable effect upon developmental homeostasis in the sporophyte. In this study, therefore, it seems unlikely that changes in gametophytic variance

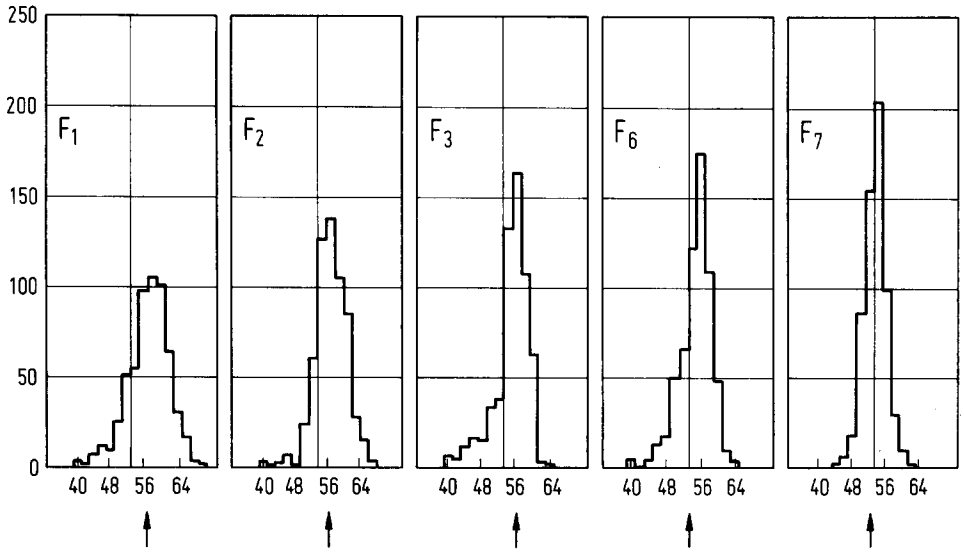

Fig. 3. Frequency distributions of pollen grain diameters of successively selfed generations. Measurements are in optical units (one unit = 1.75 microns). The arrows indicate the mean diameter for each generation. Plotting each of these five distributions on normal probability paper revealed no obvious deviations from normality, except for some slight leptokurtosis in the smaller size classes

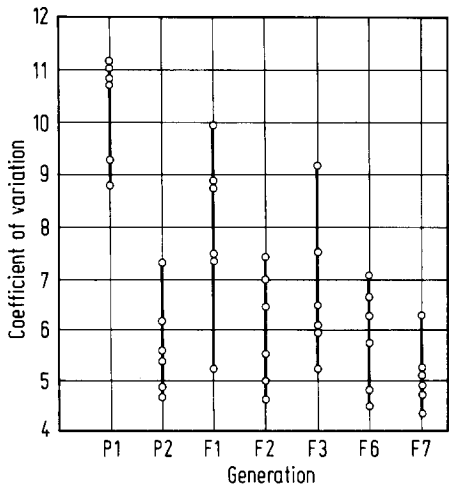

Fig. 4. Relationship between coefficient of variability of pollen diameters and generation. The data points indicate the coefficient of variability for the individual plants in each generation. The higher the calculated C.V. value, the more variable the population is for the character in question. Regression analysis using these data showed a highly significant decrease in variation in pollen diameter over the five successively selfed generation ($r = -0.5530^{**}$, $df = 28$, $P < 0.01$)

could be attributed to changes in sporophytic variance. Accordingly, a more reasonable interpretation of the observed reduction in gametophytic variance is that pollen size is significantly influenced by the gametophytic genotype.

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