FERTILIZATION ABILITY OF MAIZE POLLEN GRAINS. I. POLLEN SOURCES[1]

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THE ability of the male gamete to effect fertilization is generally assumed to be independent of its genetic constitution. The validity of this assumption has been reinforced by the fact that for the majority of the loci studied, gametes from heterozygous individuals have united at random. However, for a relatively small number of loci, aberrant ratios resulting from differential transmission of alleles by male gametes have been reported (BRINK and MACGILLIVRAY 1924; DUNN 1960; JONES 1924). The results from pollen and sperm mixtures have also indicated that male gametes from various sources differ in fertilization ability presumably because of their genetic constitution (BEATTY 1962; EDWARDS 1955; JONES 1920, 1922).

In those plant species which are naturally outbreeding and are usually considered to be mating at random, pollen from many genetic sources have the opportunity to compete in fertilization. Therefore, if differences in fertilization ability existed, a selective mechanism would be present that could alter the gene and zygotic frequencies in succeeding generations.

The purpose of this study was to measure, by the use of pollen mixtures, the fertilization ability of pollen grains from various single crosses of maize. The experiment was designed to investigate not only the existence of differences in fertilization ability of pollen grains from various genetic sources but also to examine possible influencing factors. These included the effect of the genetic relationship between the pollen source and the female parent, the genetic relationship between pollen sources unrelated to the female parent and the date of collection.

MATERIALS AND METHODS

The fertilization ability of pollen grains produced by five single crosses, Wf9×H55, Wf9×H50, H49×H55, H49×50 and Ky49×Ky27 was studied. The first four single crosses listed are homozygous dominant at the yellow endosperm locus (*YY*), have kernels with yellow endosperm and produce 100% *Y* pollen. Ky49×Ky27 is homozygous recessive at the yellow endosperm locus (*yy*), possesses kernels with white endosperm and produces 100% *y* pollen. The general procedure used was to measure the ability of each of the four single crosses producing *Y* pollen (yellow single crosses) to fertilize Ky49×Ky27 in competition with the *y* pollen from Ky49×Ky27.

Actively shedding tassels from at least 20 plants from each of the five single crosses were removed by severing the stem 25 to 50 cm below the tassel. The cut ends were then immediately submerged in water. When tassels are removed in this manner between 4 and 5 PM after the

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daily cycle of pollen shedding is completed, no more pollen shedding was found to occur until 7 AM the next day. The pollen shed from the cut tassels between 7 and 8 AM the day after cutting, probably represented the pollen that would have been shed in the next daily cycle if the tassel had remained on the plant. In terms of size, staining characteristics and percentage unfilled grains, pollen collected in this manner was found to be indistinguishable from pollen collected from tassels remaining on the plants. Large sheets of paper were placed under the cut tassels, and only pollen shed to 8 AM the day after cutting was used. The pollen obtained from all tassels within each single cross was combined and mixed thoroughly. Empty anthers were screened from the pollen to increase accuracy in measuring.

Relatively large quantities of pollen from each of the five single crosses were collected in this manner on June 2 and June 8, 1964. On each date, four combinations, each involving pollen from each yellow single cross and Ky49×Ky27, were required. For each combination, eight samples of pollen, each containing equal volumes of Y pollen from the single cross involved and y pollen from Ky49×Ky27, were made. All volume measurements were made with the same measuring device (approximately 1 ml in capacity) in the same manner. Therefore, each sample contained about 2 ml of pollen, 1 ml from the yellow single cross involved and 1 ml from Ky49×Ky27. Each of these eight samples was thoroughly mixed and subdivided into two approximately equal subsamples. Each subsample was used to pollinate one ear of Ky49×Ky27. After harvest, two samples of 100 kernels each were selected from each ear. The number, which would also be the percentage of light yellow and white kernels in each sample, was determined. Therefore, for each combination at each date, 32 samples of 100 each were classified, a total of 3200 kernels.

The light yellow kernels were assumed to be the result of fertilization by the Y pollen from the yellow single cross involved in the mixture while the white kernels were assumed to be the result of fertilization by the y pollen from Ky49×Ky27. However, since equal volumes of y pollen from Ky49×Ky27 and Y pollen from one of the yellow single crosses were mixed, differences in the pollen diameter of the single crosses would influence the percentage of Y and y pollen grains in each combination. Therefore, the number or percentage of light yellow kernels in comparison to the number or percentage of white kernels would not necessarily indicate the the fertilization ability of the pollen grains from each single cross in the mixture. To reduce this factor, on each date of collection, a sample of pollen from each of the five single crosses was placed in FAA killing and preserving solution (SASS 1951). Later, 50 pollen grains from each sample were chosen at random and measured in microns with microprojection equipment at 400× magnification. Using the mean pollen diameter of each single cross (Table 1), the expected percentage of Y pollen grains in each of the four combinations at each date could be estimated. The following formula was used:

$$\text{Percentage of } Y \text{ pollen grains in combination} = [y^3/(y^3+Y^3)] \times 100$$

where y = mean pollen diameter of y pollen grains from Ky49×Ky27 and Y = mean diameter of Y pollen grains from the yellow single cross involved in the combination. The value obtained

TABLE 1

Mean diameter (microns) of pollen grains collected from each single cross on each date of collection. The value subtracted from the number of light yellow kernels in each 100 kernel sample is bracketed

Single cross	Date of collection	
	June 2	June 8
Wf9×H55	96.7(1)	92.0(3)
Wf9×H50	93.4(4)	90.9(4)
H49×H55	95.4(2)	92.3(3)
H49×H50	92.2(5)	90.0(5)
Ky49×Ky27	98.3	96.1

from this formula not only represented the expected percentage of *Y* pollen grains in the combination but also the number of light yellow kernels expected in each 100-kernel sample if the fertilization ability of the *Y* and *y* pollen grains in the combination was equal. In order to compare the fertilization ability of the *Y* and *y* pollen grains within each combination and the *Y* pollen grains from each yellow single cross, the observed number of light yellow kernels in each 100-kernel sample in each combination was adjusted so that 50 would represent equal fertilization ability of the *Y* and *y* pollen in all combinations. The adjusted number of light yellow kernels in each 100 kernel sample in each combination was determined as follows:

Adjusted number of light yellow kernels = Observed number of light yellow kernels — (Expected number of light yellow kernels — 50)

The adjustment involved subtraction of the same number determined from the formula for each combination at each date from the number of light yellow kernels in each of the 32 samples in each combination. In all cases, the mean pollen diameter of Ky49×Ky27 was larger than those of the yellow single crosses so that the adjustment involved a reduction of from one to five from the observed number of light yellow kernels obtained in each 100-kernel sample from each combination (Table 1). The adjusted number of white kernels in each sample was obtained by subtracting the adjusted number of light yellow kernels in the sample from 100.

Chi-square tests were made using the adjusted numbers in each color class.

RESULTS

The number and percentage of light yellow and white kernels obtained from each combination on each date are presented in Table 2. Since Ky49×Ky27 was not only the female parent but also the source of *y* pollen used in each combination with each yellow single cross on each date, the percentage of white kernels would indicate if the genetic relationship between the pollen source and the female parent influenced the fertilization ability of the pollen. Only 39.2% white kernels were obtained out of the 25,600 classified. This represented a highly significant departure from 50%, the percentage which would indicate equal fertilization ability between the *Y* and *y* pollen grains in the combination. Moreover, for each yellow cross on each date, a highly significant deficiency of white kernels was obtained indicating that on both dates, pollen from Ky49×Ky27 was considerably less effective in fertilizing Ky49×Ky27 than any of the four yellow single crosses which were genetically unrelated to Ky49×Ky27.

TABLE 2

Number and percentage (bracketed) of light yellow and white kernels obtained after pollination with pollen mixtures

Yellow single cross	Date	Color of kernels		Chi-square (Expected 1 LY:1 W)	Probability
		Light yellow (LY)	White (W)		
Wf9×H55	June 2	2020(63.1)	1180(36.9)	220.50	<.005
	June 8	1834(57.3)	1366(42.7)	68.44	<.005
Wf9×H50	June 2	2138(66.8)	1062(33.2)	361.80	<.005
	June 8	1945(60.8)	1255(39.2)	148.78	<.005
H49×H55	June 2	1924(60.1)	1276(39.9)	131.22	<.005
	June 8	1882(58.8)	1318(41.2)	99.40	<.005
H49×H50	June 2	1917(59.9)	1283(40.1)	125.62	<.005
	June 8	1908(59.6)	1292(40.4)	118.58	<.005
Total		15568(60.8)	10032(39.2)	1197.16	<.005

TABLE 3

Number and percentage (bracketed) of light yellow kernels resulting from fertilization by each yellow single cross

Date	Yellow single cross				Chi-square (Expected 1:1:1:1)	Probability
	Wf9×H55	Wf9×H50	H49×H55	H49×H50		
June 2	2020(63.1)	2138(66.8)	1924(60.1)	1917(59.9)	16.06	<.005
June 8	1834(57.3)	1945(60.8)	1882(58.8)	1908(59.6)	3.44	.50–.25
Total	3854(60.2)	4083(63.8)	3806(59.5)	3825(59.8)	12.79	.01–.005

The question now arises as to whether the pollen from the four yellow single crosses which were genetically unrelated to the female parent differed in fertilization ability. The chi-square value for heterogeneity between the yellow single crosses and dates in Table 1 was 77.18 ($df = 7$, $P<.005$) suggesting that the *Y* pollen from the different sources on either date did not have equal fertilization ability. The number and percentage of light yellow kernels produced by the pollen from each single cross on each date and the total of both dates are presented in Table 3. On June 2 and the total of both dates, a highly significant chi-square value was obtained indicating that a difference between the fertilization ability of pollen from the four yellow single crosses was present. However, on June 8, apparently the fertilization ability of pollen from each yellow single cross was equal. Therefore, the environment in terms of date of collection, exerted some influence on the expression of this characteristic.

Since all combinations of two inbreds (Wf9 and H49) as female parents and two inbreds (H55 and H50) as male parents were represented in the four yellow single crosses tested, the influence of parentage on the fertilization ability of the single cross can be determined from this data. Therefore, a comparison of the contribution of the female parents, the male parents and the interaction was made. On June 2, the fertilization ability of pollen from those single crosses having Wf9 as the female parent was higher (Table 4). No significant difference

TABLE 4

Number and percentage (bracketed) of light yellow kernels resulting from fertilization by each yellow single cross on June 2

Female parent	Male parent		Total
	H55	H50	
Wf9	2020(63.1)	2138(66.8)	4158(65.0)
H49	1924(60.1)	1917(59.9)	3841(60.0)
Total	3944(61.6)	4055(63.4)	7999(62.5)

Chi-square tests

Comparison	Degrees of freedom	Chi-square value	Probability
Female parent (Wf9 *vs.* H49)	1	12.56	<.005
Male parent (H55 *vs.* H50)	1	1.44	.50–.25
Interaction	1	1.82	.50–.25

TABLE 5

Number and percentage (bracketed) of light yellow kernels resulting from fertilization by each yellow single cross summed over both dates

Female parent	Male parent H55	Male parent H50	Total
Wf9	3854(60.2)	4083(63.8)	7937(62.0)
H49	3806(59.5)	3825(59.8)	7631(59.6)
Total	7660(59.8)	7908(61.8)	15568(60.8)

Chi-square tests

Comparison	Degrees of freedom	Chi-square value	Probability
Female parent (Wf9 *vs.* H49)	1	6.02	.025–.010
Male parent (H55 *vs.* H50)	1	3.96	.050–.025
Interaction	1	2.70	.25–.10

was obtained between those having different male parents nor was any difference noted as a result of the interaction between female and male parents. Considering the total of both dates, the fertilization ability of pollen from those single crosses having Wf9 as a female parent was still significantly higher (Table 5). However, the total of both dates indicated that the male parent may exert some influence on the fertilization ability of the pollen produced by the single cross.

DISCUSSION

The results of this study indicate that differences in the fertilization ability of pollen grains from various genetic sources do exist. The factor which appeared to contribute the most to differences in fertilization ability was the genetic relationship between the pollen source and the female parent. In this study, the ability of the pollen grains from Ky49×Ky27 to fertilize Ky49×Ky27 was much lower in comparison to the four single crosses which were unrelated to the female parent. With a percentage of 50 indicating equal fertilization ability, the percentage of fertilization of Ky49×Ky27 by Ky49×Ky27 pollen ranged between 33.2 and 42.7 depending on the yellow single cross competing in the combination. This would be expressed as negative assortative mating and would result in the maintenance of heterozygosity in a heterogeneous, naturally outbreeding population. WORKMAN (1964) concluded that a system of mixed negative assortative mating and random mating can maintain heterozygosity even when there are strong selective forces favoring one of the homozygotes.

Using pollen mixtures, JONES (1920) obtained a range of 98 to 36% fertilization by pollen from the same genetic source as the female with the majority of values exceeding 50%. It should be pointed out that the percentages he obtained, were not adjusted for differences in pollen diameter between genetic sources. However, unless the pollen diameters between the genetic sources were greatly different, it is doubtful whether the results would have been appreciably altered by adjusting the percentage for this factor. As evidenced by the range, wide variation between genetic sources was obtained. From this data, JONES (1920)

concluded that pollen from the same genetic background as the female parent was more efficient in effecting fertilization than pollen from plants having a different genetic background and that self-fertilization would predominate in naturally outbreeding, heterogeneous populations. He further suggested that as the range of genetic differences between individuals in the population increased, this high frequency of self-fertilization or fertilization of likes would lead to stratification within the population and ultimately result in the erection of essentially cross-incompatibility barriers between formerly compatible groups. Since wide variation existed in the frequency of self-fertilization, the extent of stratification would be influenced by the number and diversity of the genotypes included in the population. Further studies by JONES (1922) indicated that the rate of pollen tube growth was partially responsible with the foreign pollen growing more slowly through the stylar tissue.

The influence of the genetic constitution of the stylar tissue in controlling the rate of pollen tube growth has been extensively reported in self-incompatibility studies (LEWIS 1949, 1954). There is some indication of stylar tissue influence on fertilization in those species in which self-incompatibility systems are not formally recognized. In self-pollination studies with heterozygotes at the sugary and waxy locus in maize, the pollen carrying the recessive allele was found to be less efficient in effecting fertilization (BRINK and MACGILLIVRAY 1924; JONES 1924; SPRAGUE 1933). JONES (1924) suggested that pollen containing the dominant allele was better able to accomplish fertilization than pollen carrying the recessive allelomorph only in stylar tissue which had the dominant factor in the homozygous or heterozygous condition. SPRAGUE (1933) concluded that pollen tube establishment or rate of pollen germination rather than the rate of pollen tube growth was primarily responsible for the lower fertilization efficiency of pollen grains carrying the recessive allele. Studies with the alleles at the *T* locus in the house mouse have indicated that fertilization ability of sperm was conditioned by the alleles they carried and the mechanism responsible was physiological in nature (BRADEN 1958; DUNN 1960; YANAGISAWA, DUNN, and BENNETT 1961).

Differences in fertilization ability were found in this study not only resulting from the genetic relationship between the pollen source and the female parent but also between pollen sources that were unrelated to the female parent. Since single crosses were used and as a result, the pollen produced by each source was genetically heterogeneous, only general relationships regarding genetic influence are possible. However, from these results, the fertilization ability of the pollen of the yellow single crosses appeared to be influenced more by the inbreds used as the female parent than those used for the male. This may be interpreted as indicating that the cytoplasm of the pollen source influenced the fertilization ability of the pollen produced or differences in fertilization ability were present only between the inbreds used as female parents. In the latter case, the cytoplasm of the pollen source would be independent of the fertilization ability of the pollen produced. With *in vitro* studies, BARNES and CLEVELAND (1963) obtained genetic variation for rate of pollen tube growth and theorized that this may be responsible for variation in fertilization ability.

The environment in terms of date of pollen collection considerably affected the fertilization ability of pollen grains from the various genotypes. The laboratory pollen collection method utilized in this study, would probably tend to reduce variability in comparison to field collection since at both dates, the cut ends of the tassels were placed in water approximately 12 hours before pollen shedding and were exposed to about the same temperature at the time of pollen shedding. The moisture stress and temperature before and during pollen shedding and the temperature after pollen shedding before pollination were approximately the same at both dates. Therefore, differences between dates were probably produced by the environment to which the plants were exposed in the field before the tassels were removed.

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SUMMARY

The ability of pollen grains produced by the white (yy) maize single cross Ky49×Ky27 to fertilize Ky49×Ky27 in competition with pollen produced by each of four yellow (YY) single crosses, Wf9×H55, Wf9×H50, H49×H55, and H49×H50, was measured using pollen mixtures. The resulting kernels were classified into two groups on the basis of endosperm color: (1) Light yellow (Yyy) assumed to be the result of fertilization by the Y pollen grains from the yellow single cross involved in the mixture; and (2) White (yyy) assumed to be the result of fertilization by the y pollen grains from Ky49×Ky27. Since the single crosses differed in their mean pollen diameter and the pollen from each was mixed volumetrically, the observed data were adjusted so that 50% would represent equal fertilization ability of the Y and y pollen grains in the mixture. The percentage of fertilization of Ky49×Ky27 by pollen grains from Ky49×Ky27 ranged between 33.2 and 42.7. Therefore, in this study, partial negative assortative mating was indicated since pollen grains from the same genetic source as the female parent were less effective in fertilizing than pollen grains from genetic sources unrelated to the female parent. The percentage of fertilization by pollen grains from the four yellow single crosses ranged between 57.3 and 66.8, suggesting genetic control of fertilization ability. However, the results indicated that the expression of this character may be considerably modified by the date of collection.

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