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Genetic Variability of Gametophyte Growth Rate in Maize

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Summary. In order to measure differences in the pollen growth rate of numerous lines of maize and to investigate the main features of their genetic control, gametophyte growth was studied *in vitro* and *in vivo*.

In vitro pollen tube growth of twenty inbred lines and seven hybrids was measured; a remarkable variability was observed in the growth rate of the inbred lines examined: most lines were distinct, showing different levels of growth.

Analysis of frequency distribution of pollen tube lengths for pairs of inbred lines and their F_1's revealed greater variance among lengths of F_1 pollen tubes, presumably indicating the segregation of genetic factors expressed in the gametophyte.

Similar frequency distributions of tube lengths in pollen produced by two pairs of reciprocal hybrids virtually excluded the presence of a cytoplasmic component.

In vivo competitive ability of pollen tubes was measured as the increase in relative fertilization frequency from apex to base of the ear. Mixtures were made using two types of genetically distinguishable pollen, and were applied to a female common parent. Nine pairs of inbred lines furnished the pollen for the mixtures. In all cases where the B14 line was involved, this pollen type fertilized nearly all the ovules, perhaps indicating the presence of a gametophytic factor. When other lines were compared, the ears contained mixtures of the two possible seed types, the relative proportions of which indicated the differential competitive abilities of the two pollen tube types.

A comparison between *in vitro* and *in vivo* behavior was made for some genotypes. *In vivo* results generally agreed with *in vitro* results. The degree of the differences between lines however was changed, presumably because pollen-style or pollen-pollen interactions are absent *in vitro*.

Differing growth patterns between lines were also revealed *in vivo* by direct observation of fluorescent pollen tubes within the silks, a finding which may be useful in further studies.

Introduction

Abundant evidence provided by several authors demonstrates that the fertilization ability of pollen grains can not be assumed to be independent of its genetic constitution. This means that prezygotic selection frequently occurs as a result of gametophytic competition. The phenomenon has been described in several species of plants (Plonka 1968, Harding and Tucker 1969) and particularly investigated in maize (Jones 1920, Pfahler 1965, 1967, Mulcahy 1971, 1974).

In some cases fertilization ability is greatly influenced by the presence of a single-gene, Ga, "gametophytic factor" (Jones 1928, Schwartz 1950, Nelson 1952, Bianchi 1957).

In other cases differential fertilization ability is not ascribable to a single-gene effect, but rather to the genetic background. Here, the character is inherited in a quantitative manner. In both cases it can be affected by the environment and the pistillate tissues (Jones 1928, Pfahler 1965 and 1967, Plonka 1968, Mulcahy 1971 and 1974). Clearly, the competitive ability of pollen could have great evolutionary significance, particularly if a part of the genetic system is expressed in both the gametophytic and sporophytic phases of the life cycle (Ter-Avanesjan 1949, Mulcahy 1971 and 1974). However great the potential, the actual importance of differences in pollen growth rate depends on their diffusion in nature. The present study was undertaken to measure the value of this character in many different lines of maize and to ascertain the main features of the genetic control of these differences. Gametophyte growth was studied in *in vitro* and *in vivo:* in the first case, the growth rate of the pollen tube was measured; in the second, the competitive ability of pollen of different genotypes within the same silk was tested.

Material and Methods

a) *In vitro* pollen tube growth. Twenty inbred lines (W23, OH41, B14, 33-16, H2386, H2396, H2187, NI-74, Silv-91, H3025, W22, 38-11, W64A, C123, WF9, M14, H991, B37, Pop.A16, W374R) and seven hybrids (H3025 × NI-74, H2386 × H2187, H2386 × Silv-91, H2386 × B14, H2187 × Silv-91, H2187 × B14, Silv-91 × B14) were used as the pollen source.

Pollen grains were placed on a synthetic germination medium (Pfahler 1967) of the following composition: 0.6% bactoagar, 15% sucrose, 0.01% H_3BO_3 and 0.03% $Ca(NO_3)_2 \cdot 4H_2O$; one hour after inoculation (at 27 °C), all activity was stopped by bringing the plates to a temperature of 4 °C. The lengths of 15 pollen tubes chosen at random from each plate were measured on drawings obtained by a camera lucida. The total number of observations was about 60 per inbred line and 180 per hybrid.

b) *In vivo* competition ability. The competitive ability of the pollen tube was measured as the increase in

relative fertilization frequency from apex to base of the ear. Mixtures were made using approximately equal quantities of two different types of pollen. Each was marked for the presence of the normal or mutant allele of *opaque-2* (o_2), a gene that affects the structure of the endosperm and thus can be identified on the kernels. This mixture was then applied to the silks of a genetically known plant. The general design of this experiment was the following: two lines (A and B) in two versions (o_2o_2 and ++) were used for mixed pollination in every possible combination, $A+Ao_2$, $B+Bo_2$, $A+Bo_2$, Ao_2B+. The purpose of the first two combinations was to measure the effect of the marker gene. Commercial single cross opaque (o_2o_2) hybrids were used as female plants. The same F_1 was employed in all the crosses of each comparison.

The pairs of lines which furnished the pollen for the mixtures were: B14, C123; B14, B37; B14, NI-74; B14, Silv-91; WF9, B37; C123, B37; C123, RNY; RVa36, Silv-91; RVa36, H2167. A complete plan was not obtained in every case because the two versions (opaque and normal) of the lines were not always available. This was the case for the last three mixtures reported above, where only the combination $A+Bo_2$ was made. The ears obtained were divided transversely into five nearly equal segments and the relative frequencies of normal and opaque kernels in each were computed. Since the styles reaching the basal ovules of the ear are the longest, any increase in frequency of fertilization from apex (segment 1) to base (segment 5) by one of two pollen types may be taken as an index of the greater relative speed of that type. The ability of both pollen types to reach the base of ear was demonstrated by the $A+Ao_2$ and $B+Bo_2$ combinations of the experimental design.

Pollen tubes from two inbred lines (RNY and C123) were also observed using the staining technique proposed by Martin (1959) for flowering plants. Styles, fixed in formalinacetic-80% alcohol and cleared in sodium hydroxide solution, are stained in a 0.1% solution of aniline blue dye, dissolved in $0.1N\,K_3PO_4$. Under these conditions, the callose in pollen tubes exhibits a bright-yellow fluorescence and can be observed in the style with a UV dark-field microscope.

Results

a) Measurement of Pollen Tube Growth of twenty Inbred Lines

A remarkable variability was observed in the growth rate of the different genotypes. In fact, one hour after inoculation, differences between the means and no overlapping of confidence ranges were observed in many of the lines (Fig.1).

Generally, genotypes may be classified by this character. Analogous behaviour in some of these lines has been described by Gabay (1974), who found, for example, poor growth in the M14, WF9 and W64A lines, excellent growth in the B14.

The role of a gametophytic component in the genetic control of the pollen tube growth rate may be revealed by ascertaining the existence of segregation of this character in pollen produced by the F_1. In fact, in this case the gametophytic generation is the result of segregation in the heterozygous sporophyte. The increase of the variability distribution of the character is an indication of segregation; thus a comparison between F_1 variance and parental line variance makes it possible to ascertain

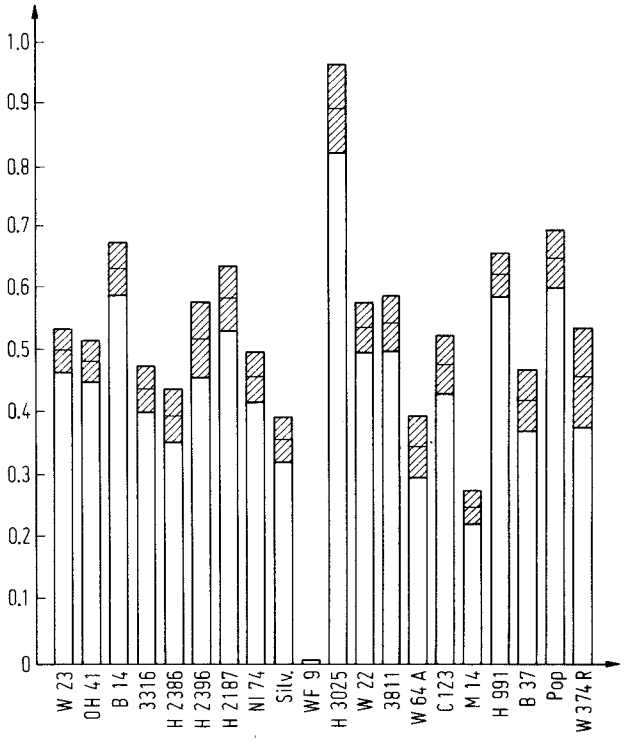

Fig.1. Variability of pollen tube length (mm.) in 20 inbred lines of maize. Shaded areas indicate confidence ranges of mean

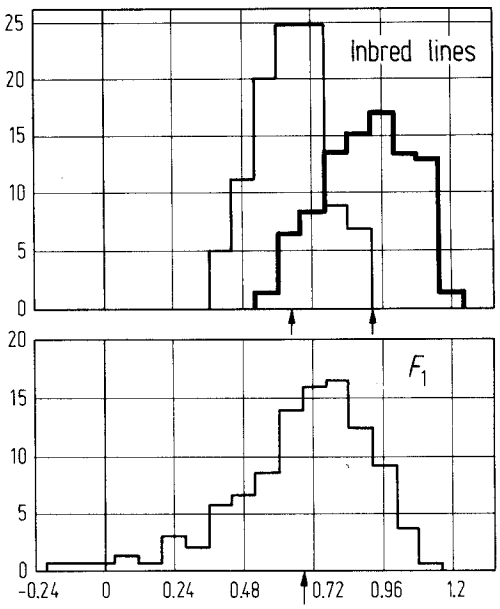

Fig.2. Frequency distributions of pollen tube length (in logarithms) of an inbred line pair and relative F_1. The arrows indicate the means

the effects of genes expressed in the gametophyte. Frequency distributions of the pollen tube length in seven pairs of inbred lines and their F_1s revealed two main kinds of genetic situation.

The first, concerning NI-74 × H3025, B14 × H2187, H2187 × H2386, is illustrated in Fig.2 (the data were transformed into logarithms to eliminate the effect of correlation between mean and variance). The means of parental lines differ and the F_1 distribution is clearly wider than either parental distribution; this is confirmed by the comparison of the relative variances $\left(\sigma^2_{F_1} = 0.0480,\ \sigma^2_{P_1} = 0.0142,\ \sigma^2_{P_2} = 0.0225\right)$. Hence it may be concluded that the parents differ with regard to the factors controlling pollen tube growth, and that segregation of these factors is responsible for the greater variance observed in F_1. These results parallel those of Johnson et al. (Univ. Massachusetts submitted for publication). In other cases (B14 × Silv-91, H2187 × Silv-91, H2386 × B14 and H2386 × Silv-91) F_1 variance was not significantly greater than that of both the parents (Table 1). Even though the differences between the parental values made this result foreseeable, it may be attributed, at least in the case of most of the combinations, to a residual heterozygosis in the parental lines; it was usually found in the presence of the Silv-91 line, whose variance value was one of the highest we obtained.

To test whether pollen growth was affected by maternal components, the frequency distributions of tube length in pollen produced by two pairs of reciprocal hybrids were also analyzed. These data can not be directly compared with the previous data, since they were obtained later, in the spring, using material produced in the greenhouse. However, the trend of frequency distributions and statistical tests indicate similarity between the two reciprocal hybrids: in the comparison NI-74×H3025 and reciprocal, with 75 observations for each, the variance ratio to test homogeneity of within-hybrid variance was 1.027; in H3025 × H2386 and reciprocal, on 75 observations for each, the value was 1.072. In both cases the difference between means turned out to be not significant. Thus, in this material, maternal effects may be excluded.

Six of the F_1 analyzed represent every possible combination between four parental lines, B14, Silv-91, H2187, H2386; these combinations may be analyzed to-

Table 1.

a) Means and variances (between pollen tubes) of four inbred lines and their F_1's

		B14	Silv	H2187	H2386	Means
B14	$\bar{X}$	0.7840	0.5869	0.5648	0.6123	0.6386
	σ^2	0.0201	0.0363	0.0519	0.0430	
Silv	$\bar{X}$		0.05242	0.6046	0.5723	0.5720
	σ^2		0.0320	0.0366	0.0463	
H2187	$\bar{X}$			0.7421	0.6134	0.6312
	σ^2			0.0258	0.0536	
H2386	$\bar{X}$				0.5719	0.5925
	σ^2				0.0311	

b) ANOVA of mean differences between genotypes

Items	d.f.	variances
Between lines	3	0.01043**
Interaction	6	0.00466*
Error (Between plates, within genotypes)	73	0.00159

Level significance is indicated in conventional manner.

gether with the parents for variability between the means, using the same criterion as for a diallel analysis. Interpreting the results is not very easy, because the effects of genes expressed in the sporophytic and gametophytic phases may overlap, but some information can be obtained which may throw light on important aspects of the competition.

Table 1 shows the parental and F_1 means and variances and the means per array; in the analysis of variance (ANOVA) the sources of variation are the "between lines" (additive effects) and the "interaction" items, both of which were significant. If the sporophytic component is supposed to be not very relevant or, in any case, not the only source of variability, the significance of interaction may be interpreted as an epistatic effect, which becomes evident, in the haploid state, because of the new genic combinations.

b) Results obtained from mixed pollinations

Frequencies (%) of opaque kernels are reported for each ear sector, together with the genotypes of the pollen mixture employed, in Figs. 3 and 4; Table 2 shows the total number of kernels per sector, and the significance of χ^2 between sectors (heterogeneity between ears was not observed).

The proportions of the two types of kernels were constant in all the segments for mixtures made using the same line (C123+ C123o_2, B37+ B37o_2 and so on); this shows that pollen tube growth was independent of the *opaque-2* marker.

Generally, when pollen mixtures of different genetic backgrounds were used, two principal types of behaviour were observed. In no cases where the B14 line was involved (Fig. 3a and Table 2) was a significant difference

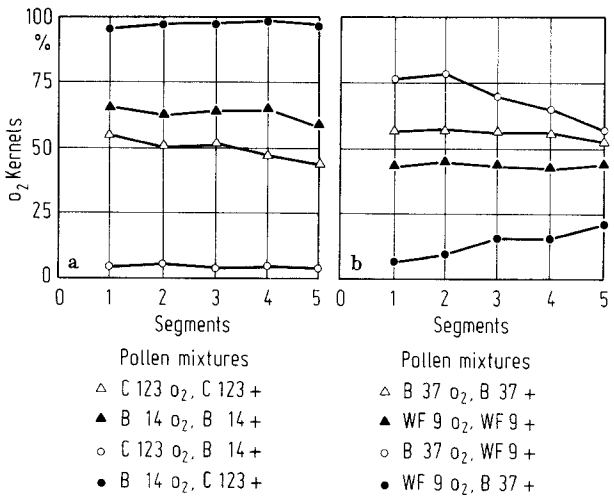

Fig. 3. Relative fertilization frequencies in 5 segments of ears resulting from mixed pollinations of a single cross *opaque-2* hybrid (o_2o_2)

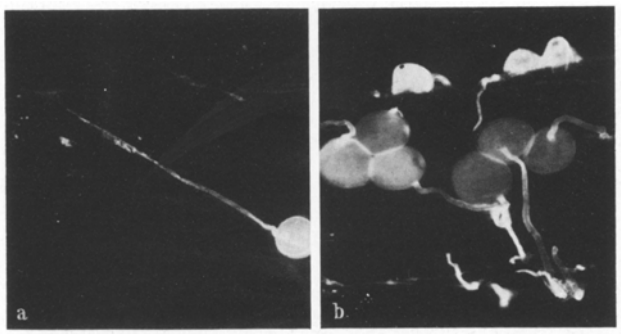

Fig. 4. Pollen tube growth pattern in the silk observed by UV dark-field photomicroscope (Leitz Ortholux, 100 x). RNY (A) and C123 (B) lines

Table 2. Relative fertilization frequencies in 5 segments of ears resulting from mixed pollinations

Pollen mixtures	No. of kernels for segment 1	2	3	4	5	between segments χ^2 significance
A. C123o_2, C123+	169	144	172	181	165	n.s.
B14o_2, B14+	388	392	435	435	395	n.s.
C123o_2, B14+	199	200	212	264	267	n.s.
B14o_2, C123+	630	548	575	559	584	*
B. B37o_2, B37+	593	548	518	514	474	n.s.
WF9o_2, WF9+	597	573	544	462	471	n.s.
B37o_2, WF9+	704	679	720	700	623	**
WF9o_2, B37+	420	388	416	391	379	**

Level significance is indicated in conventional manner.

between sectors observed, but there was a sharp difference between the numbers of normal kernels and opaque kernels. One of the two types of pollen (that of the B14 line) grows much faster than the other, or it has a notably shorter germination time, so that only a minority of the ovules - in all the sectors - can be fertilized by the slower pollen. In Fig.3a the comparison between B14 and C123 is shown; analogous results were obtained for B14 and B37 or Ni-74.

This type of result is to be expected where gametophytic factors are present; it may be that the B14 line carries one of them.

In the other genotypes, we observed competition between the two lines compared. The relative frequency of normal and opaque kernels varied according to a gradient from the apex to the base of the ear in an opposite manner in the reciprocal situations. Fig.3b shows the comparison between the WF9 and B37 lines; results of the same type were obtained with the other genotypes tested, even though the design was not complete in every case. More precisely, pollen of the C123 line had a growth speed greater than that of B37, but lower than that of RNY; RVa36 had a growth rate lower than that of Silv-91 and similar to that of H2167.

Between-line differences observed as fertilization ability and those measured as *in vitro* pollen growth are comparable.

The genotypes analyzed by both techniques can be ranged in order of merit:

in vitro: B14 > C123 > NI-74 > B37 > Silv-91 > WF9

in vivo: B14 > C123 > WF9 > B37, NI-74, Silv-91

Generally, *in vivo* behaviour reflected that observed *in vitro*. However, two points emerged: i) the WF9 line was not able to germinate *in vitro*, but showed a good competitive ability *in vivo;* ii) the B14 line proved to be the best genotype in both experimental conditions, but its superiority to the other genotypes - to C123 in particular - was much more marked *in vivo* than *in vitro*. Thus, growth on a synthetic medium does not reveal differences so great as those manifested *in vivo*, which are likely to be due to such factors as interaction with the style.

Direct observation of fluorescent pollen tubes in the styles revealed that growth patterns of different lines varied. Fig.4 shows the behaviour of RNY (a) and C123 (b) lines. In the RNY line, only scattered tubes were found and these were characterized by a linear, quite regular, growth. In the C123 line many crowded tubes were found and these had an irregular and distorted pattern of growth, not necessarily the result of crowding.

Discussion

The results of this study confirm that the fertilization ability of maize pollen is associated with its genetic constitution and that, apart from the well known Ga factors, multifactorial complexes are involved in the control of this character.

In vitro pollen growth revealed a wide genetic variability for pollen tube growth rate in the sample of lines we examined. It may, therefore, be supposed that this character is subject to selective pressures under natural conditions. Gametic selection may be an important evolutionary factor, especially for plants, where the gametophytic generation constitutes a more conspicuous part of the life cycle. The selective value of the male gametophyte may depend also on the fitness of the gametophyte itself and not only on the characteristics, such as the quantity of pollen produced, of the sporophyte from which it originates.

Evolutionary consequences of growth rate differences are to be expected where there is association between genetic effects controlling this trait and those involved in determining individual fitness. This association has been observed by Mulcahy in maize (1971, 1974), considering seed or seedling weight as sporophyte traits.

The gametophytic component appears to be important in specifying between-genotype differences in this character, while no maternal effect was revealed. Even though a sporophytic influence cannot be excluded, the evidence indicates that the phenotypical characteristics of the pollen tube depend on genes that are expressed in the gametophytic phase. In particular, these genes have an additive action, but it is probable that there is also interaction between single alleles.

The *in vivo* study of pollen competitive ability revealed two mechanisms responsible for differential fertilization ability in maize: 1.) clear-cut differences such as those due to Ga factors; 2.) differences due to quantitative inheritance. In the latter case there are only small differences in speed of pollen tube growth. These can be detected by the change in the relative fertilization frequency in the different segments of the ear.

Opaque-2 was the genetic marker employed and the results indicate that o_2 is independent of the genes controlling pollen tube growth; competition took place be-

tween pollen of different genetic backgrounds as the effect of genes not closely linked to o_2. Fertilization rate differences may be due to a different germination time, or to a differential speed of tube growth. It is possible to discriminate between these two factors: the proportion of kernels produced by the faster genotype is a function of the sector of the ear, whereas differences of germination time would produce the same result in all sectors (Jones 1928). The evidence indicates that both these factors affect the fertilization rate of maize; particularly marked are the effects of differences in growth speed.

Differences in growth pattern between two lines were directly observed in the styles by means of fluorescence microscopy. If such clearly discernable differences between pollen tube types were frequent, they could perhaps be employed in future studies of *in vivo* pollen tube competition.

The information obtained by the two techniques - *in vitro* or *in vivo* - differs. In some cases, as in alfalfa (Barnes and Cleveland 1963), a clear relationship has been observed between *in vitro* and *in situ* growth, so that the former is useful for making estimates concerning the latter. In maize, Pfahler and Linskens (1972) found that fertilization ability was not directly related to ability to germinate on an artificial medium. In the genotypes we analyzed, under both experimental conditions the agreement between *in vivo* and *in vitro* results was generally good. However, one line (WF9) was unable to grow on a synthetic medium, but revealed good competitive ability *in vivo*. Moreover, the difference between the B14 line, which proved to be the best under both conditions, and the others was much more evident *in vivo* than *in vitro*. If Ga factors are present in this line, they do not reveal gametophytic segregation and their effects are not expressed when it is grown on artificial medium.

In general it is reasonable to suppose that *in vitro* growth reflects pollen efficiency *per se*, but not the effects of interaction with stylar tissues, an important factor in the competitive ability of the pollen tube.

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