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IDENTIFICATION OF THE POLLEN OF MAIZE, TEOSINTE AND TRIPSACUM BY PHASE CONTRAST MICROSCOPY

BY

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IN early studies, Firbas (1937) measured the long axis of grass pollen grains from domestic and wild types in Europe and established 35μ as a division between the two, those larger being considered domestic. Subsequent studies, both in Europe and America, often showed this dividing line to be invalid.

In an effort to distinguish between the pollen of maize, teosinte and *Tripsacum*, Barghoorn, Wolfe, and Clisby (1954), investigated the pore-axis relationship in these grasses. A ratio of 5.7 or greater appeared to separate maize from the other two. Measurement of the pore included the annulus. However, this ratio has been criticized by Kurtz, Tucker, and Liverman (1960), who demonstrated that, under certain conditions, the pore-axis ratio of maize dropped well below 5.7. Their choice of a peculiar type of maize, Bikini out-crossed, probably prejudiced their findings. Further work suggesting that the pore-axis ratio is sometimes invalid as a distinguishing criterion was carried out by James Langham and Donald Whitehead of Williams College. They also investigated simple axis measurements, summarized in Text Figure

1.* A further complication of measurement results from size changes induced by various sedimentary environments and various preparatory techniques (Anderson, 1960).

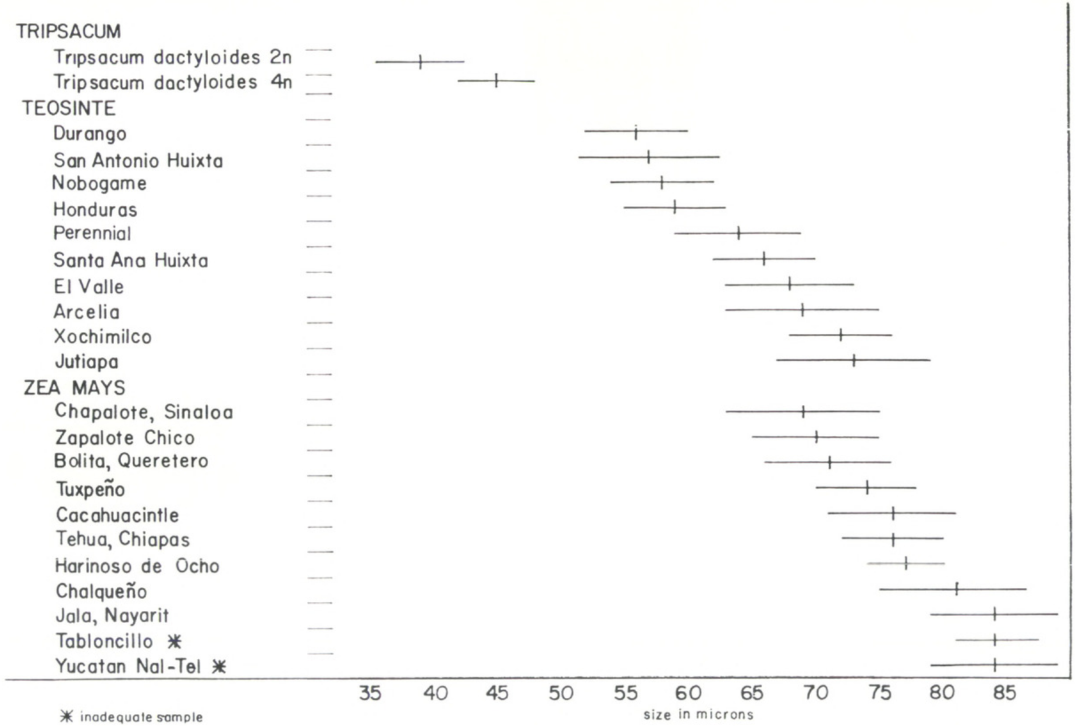
The exine itself, except for the pore, consists of an endexine, an ektexine tegillum supported by columellae, and simple spinules projecting from this tegillum and corresponding in arrangement to the columellae. With ordinary light microscopy, this exine appears smooth, while, with phase-contrast, the spinules appear as a pattern of dark dots. Using phase-contrast microscopy, Grohne (1957) investigated the pollen of "wild" and cereal type grasses in Europe and suggested that discrimination between the two was possible on the basis of certain phase changes in this exine pattern (see also Erdtman and Praglowski, 1959).

An explanation of these phase changes was offered by Rowley (1960). He found that grasses of the "wild type" have three levels of phase retardation. The lowest is that resulting from depressions between the spinules. These depressions form an incised reticulum that may have one to several spinule per lacuna. This reticulum under phase appears as a dark network. The two other regions of phase retardation are the level ektexine surface (non-incised interspine regions) and the spinules themselves. Grasses of the cultivated type have only two areas of phase retardation, the level ektexine, and that of the surmounting spinules. Rowley's investigations were aided by the use of an electron microscope.

The present paper presents results of observations of the exine, using phase-contrast light, in the New World plants maize, teosinte, and *Tripsacum*. The latter two

* The data presented in this figure were drawn from the measurements of James Langham and Professor Donald Whitehead. Used by permission of Dr. Whitehead.

TEXT FIGURE 1



DISTRIBUTION OF POLLEN SIZE OF TRIPSACUM, TEOSINTE, & MAIZE are the only New World grasses so far investigated which cannot be distinguished from maize by a simple measurement i.e. $>45\mu$ for the long axis, and $>5.7\mu$ for the pore-axis ratio.

We believe that *Tripsacum* conforms to Rowley's first type in possessing three levels of phase retardation; maize to the second type. Teosinte in some varieties appears to have a faintly incised reticulum and hence is intermediate in this optical characteristic.

In this study, pollen grains were prepared from a number of races of maize, teosinte, and two genotypes of *Tripsacum*. A list of these is found in Table I. In all cases, several anthers, selected from more than one tassel and more than one plant if specimens were available, were utilized in order to avoid the influence of an unusual genetic constitution in a single anther or single plant. All available varieties of teosinte in the Botanical Museum of Harvard University were examined. An attempt was made to choose examples from a number of groups of maize from diverse geographic sources. A maize-*Tripsacum* hybrid (WMT maize $\times$ *T. dactyloides*) and two varieties of maize bearing one or more known teosinte chromosomes were studied. In addition, several grains from the fossil record were examined.

All preparations were made by the use of standard acetolysis treatment, followed by bleaching, and the grains were mounted in glycerine jelly. Photographs were taken with a Zeiss Opton W microscope, employing phase objectives on both 35 mm. and $4'' \times 5''$ high contrast film (Microfile and Contrast Process Pan). The $2'' \times 2''$ photographs in Plates III–VII were made on $4'' \times 5''$ negatives.

As these photographs indicate, *Tripsacum* can be distinguished from maize and teosinte by use of phase optics. In *Tripsacum*, the spinules are distributed irregu-

TABLE I POLLEN TYPES STUDIED

Zea mays	*Tripsacum*	*Teosinte*	*Hybrid and Inbred Strains*
Ancient Indigenous*	T. dactyloides 2n	San Antonio Huixta	Maize-*Tripsacum* hybrid
Nal Tel	T. dactyloides 4n	Perennial	Minnesota A158 with
Chapalote, Sinaloa		Nobogame	Florida teosinte chromosome
Pre-Columbian Exotic*		El Valle	1, 3, 4, 9; and
Harinoso de Ocho		Jutiapa	3, 4, & 9
Cacahuacintle		Durango	Texas 4R3 with
Pre-historic Mestizos*		Chalco	Florida teosinte chromosome
Tehua, Chiapas		Arcelia	4, 3, & 9
Jala, Nayarit		Honduras	Nobogame teosinte chromosome
Tuxpeño		Xochimilco	3 & 4, 3
Conico		Santa Ana Huixta	New teosinte chromosome
Zapalote Chico			4 & 9
Modern Incipient*			
Chalqueño			
Bolita			
Other Races			
Huesillo			
Puno			
Confite Morocho			
Texas 4R3			
Strawberry Popcorn			
Longfellow Flint			
Minnesota A158			

*Classification according to Wellhausen *et al.*, 1952.

larly on the ektexine. Dark areas embracing several spinules can be seen, no doubt due to phase retardation of an incised reticulum, as suggested by Rowley (1960). Our sample of a maize-*Tripsacum* hybrid exhibits this pattern.

In a large number of the races of maize, the spinules are located very regularly, i.e., the spacing between each spinule being almost equal. With most varieties of teosinte, the spacing of spinules appears less regular, and in some they are rather closely aggregated, appearing as clumps.

The spinules of maize appear to produce more phase change than those of teosinte. The spinules appear stronger and darker. In teosinte, at the level of the spinules, only small areas can be brought into sharp focus. In addition, within an area so focussed, there are numerous slightly obscured or darkened regions. These may be due to one or more of the following factors: 1) teosinte may have a faintly incised reticulum; 2) the exine of most varieties of teosinte may be thinner and more easily deformed (thus thrown out of focus); 3) the spinules or columellae of maize may be slightly longer than those of teosinte.

Fossil grass grains were studied in preparation of sediment samples from the Bellas Artes core in Mexico City. On the basis of the pore-axis ratio, Barghoorn, Wolfe, and Clisby (1954) identified certain grains from the lower reaches of this core as maize. The precise age of these sediments is not known, but it is beyond reasonable doubt that they antedate man's entrance into the New World. We re-examined grass pollen grains from the Bellas Artes sediments which, on the basis of size measurements, appeared to be either maize, teosinte, or *Tripsacum*. Then, using the optical criteria described earlier in this paper, some of the grains were identified as maize, others as *Tripsacum*. None of the intermediate or teosinte type have so far been located. This lends support to the thesis that wild maize existed in the Valley of Mexico during late Pleistocene time (Mangelsdorf 1958).

In addition, our investigations have led to the following conclusions.

1. Observations on the general similarity of maize and teosinte pollen supports the thesis that teosinte is a race of maize derived from hybridization with *Tripsacum* (Mangelsdorf and Reeves 1939).

2. The more primitive races of maize (Puno, Chapalote, etc.) show the strongest, most regular pattern.

3. Inbred maize strains, Minnesota A158, and Texas 4R3, have a regular strong pattern, while the introduction of teosinte germ plasm causes them to lose the strength and regularity of the pattern, Plate VI.

4. Fossil maize from Bat Cave, New Mexico, shows a well defined, strong and regular pattern. This maize pollen dates from about 5000 years ago.

5. Several grass pollen grains, with size measurements in the *Zea* range were isolated from sediments from Lake Petenxil in Guatemala. Those were present in the lowest sample of a series of cores taken for Harvard University by Dr. George Cowgill (now of Brandeis University). This core was radiocarbon dated by the Humble Oil geochemical laboratory at 3,950 ± 130 years ago. The strength and regularity of the exine pattern suggest that these grains are maize. Present archaeological knowledge indicates that maize may have been grown in Guatemala at this date. The grains, however, could represent possibly wild maize types at that time still extant in the Lake Petenxil area.

6. In areas where teosinte does not grow wild (north of Mexico, for instance), it should prove relatively easy to establish the presence of maize in the pollen record. In these same areas, there is no present evidence of wild maize in the archaeological or palynological record. Therefore, where maize pollen is present in these areas, we can presume that the plant was cultivated. How early this occurs in the United States and where it occurs are important archaeological problems as yet unsolved.

In conclusion, in identifying gramineous pollen as maize, size measurements of large grains should first be made. Those grains which fall in the maize-*Tripsacum* range should then be examined by means of phase-contrast optics. Positive discrimination can then be made between *Zea* (maize and teosinte) and *Tripsacum*. Following certain observations outlined in this paper, maize grains can be separated from teosinte with reasonable reliability. In certain geographic areas, such as the United States, grains in the *Zea* size range with *Zea* pattern may be presumed to be maize.

Acknowledgments

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Abstract

Pollen of the Gramineae are spheroidal in shape and monoporate. The pore is surrounded by a thickened area, the annulus. Size characteristics, both of total pollen diameter and the relation of this diameter to the pore width have been used in the past to separate the pollen of maize from teosinte and *Tripsacum*. Use of measurement alone causes several difficulties, the most important being that large numbers of grains are necessary for positive identification. Morphological characteristics of the exine, studied under phase-contrast light, give more conclusive discrimination even when dealing with few grains. Maize and teosinte can easily be separated from *Tripsacum*. Primitive maize and primitive teosinte can also be separated. When dealing with hybrid races of maize of teosinte, discrimination appears to depend largely on how much germ plasm each plant has absorbed from the other. Thus, the pollen of teosinte Chalco, that grows commonly around maize fields in Mexico and frequently crosses with maize, is very difficult to tell from that of a very tripsacoid maize like Huesillo. That this pollen morphology is genetically controlled is supported by observations on pollen of derivatives of maize-teosinte hybrids. Suggestions for dealing with apparent maize pollen in the archaeological record are made.

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Explanations of Photographs in Plates III–VII

All photographs were taken through a phase-contrast microscope system.

The $1'' \times 1''$ photographs are at a magnification of 1550.

The $2'' \times 2''$ photographs are at a magnification of 4500; they are not enlargement of the smaller ones, but are of different grains, except for the fossil specimens.

EXPLANATION OF THE ILLUSTRATION

Plate III

A. *Tripsacum dactyloides* 2n (Kansas clone)

B. Maize (Nal Tel)

C. Teosinte (San Antonio Huixta)

D. Maize-Tripsacum hybrid (WMT maize × *Tripsacum dactyloides*)

Explanation

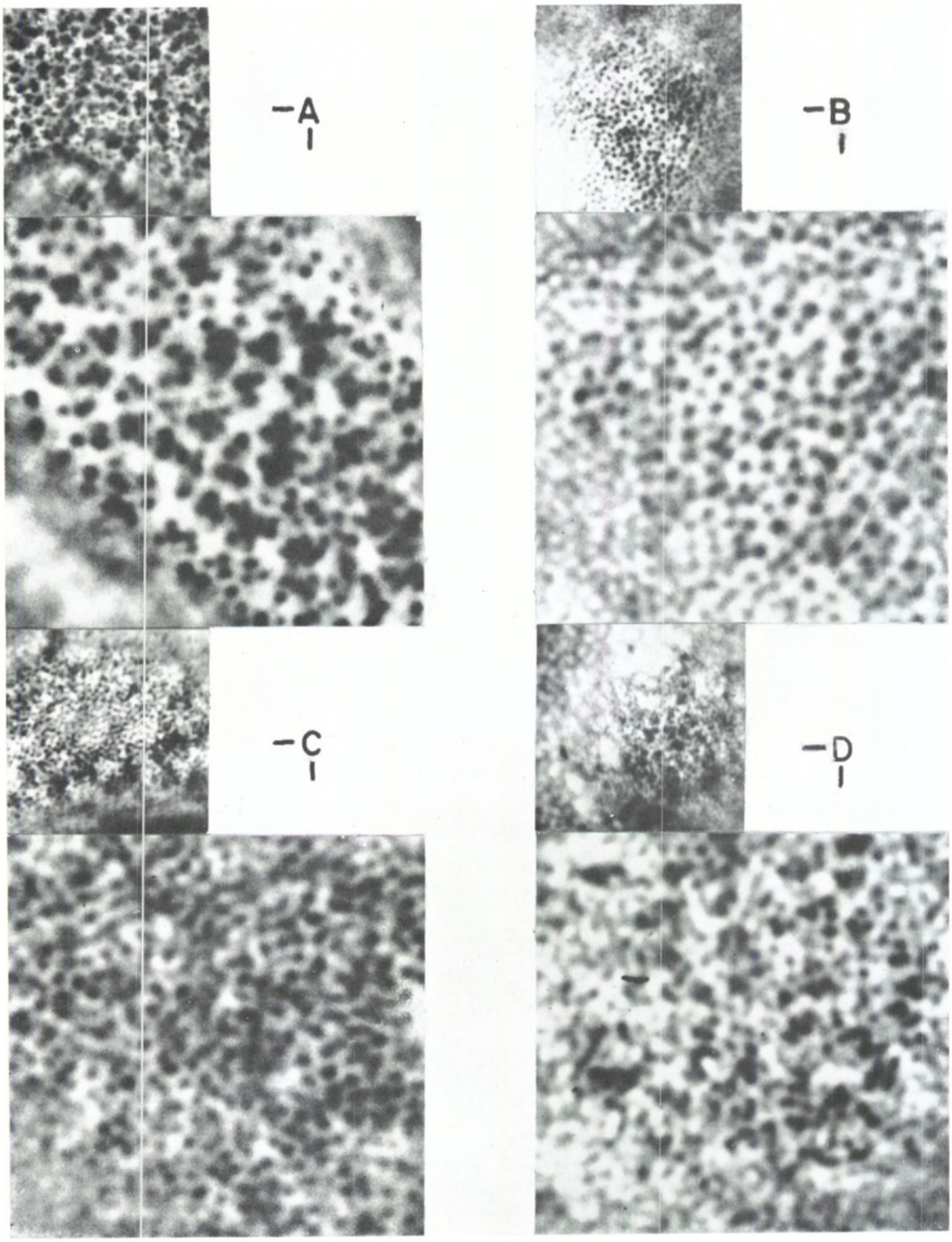
A. These two photographs show a typical "wild" exine pattern, as described by Rowley. The spinules appear clumped and are often visually merged together. In the small photograph, a faintly incised reticulum can be discerned (seen as a greyish network).

B. Typical pure maize pattern. The spinules are evenly distributed. There are only two levels of phase retardation — the spinules themselves (dark) and the ektexine surface (light).

C. Typical primitive teosinte pattern. In the small photograph, the spinules appear clumped, with patches of light and dark areas visible. The large photograph shows the spinules to be rather evenly distributed, but adjacent spinules often appear connected, so that it is difficult to isolate individuals.

D. In this hybrid, some features of both maize and *Tripsacum* are apparent. The spinules are unevenly distributed and clumped, leaving lacunae larger than those associated with either maize or teosinte, but less pronounced than those of *Tripsacum*.

PLATE III



EXPLANATION OF THE ILLUSTRATION

Plate IV

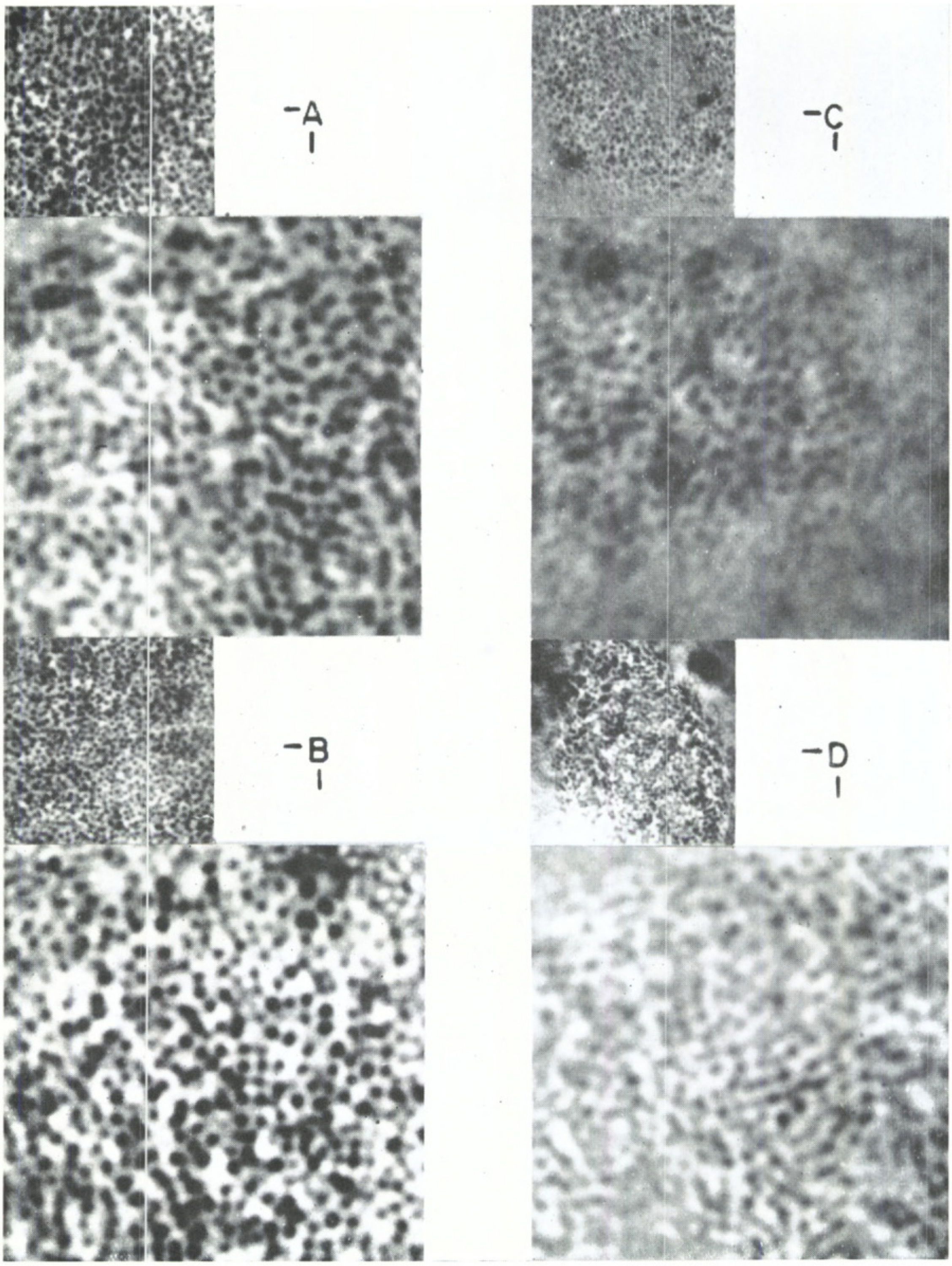
A. Maize (Chapalote, Sinaloa)
B. Maize (Confite Morocho)
C. Teosinte (Chalco)
D. Teosinte (El Valle)

Explanation

Both A and B show relatively pure maize patterns. In comparing these photographs with those of two teosinte races on the right, a second feature of maize-teosinte exine difference can be seen. The spinules of the maize grains are darker and thus more distinct. This suggests the spinules (and supporting columellae) of the maize-type exine are longer, causing more phase retardation than those of teosinte. One practical result is that larger areas of the exine of maize can be brought into sharp focus (with grains of the same size). Corresponding photographs were taken and printed under virtually identical conditions.

C and D differ in significant ways. D is a primitive teosinte (that is, with little maize contamination). C is a teosinte with a high amount of maize germ plasm. The only observable difference between the latter and typical maize is the amount of phase retardation of the spinules, discussed above. Teosinte Chalco is also one of the most maize-like of modern teosintes in other aspects of plant morphology.

Plate IV



EXPLANATION OF THE ILLUSTRATION

PLATE V

A. Teosinte (Xochimilco)
B. Maize (Puno)
C. Teosinte (Jutiapa)
D. Maize (Huesillo)

Explanation

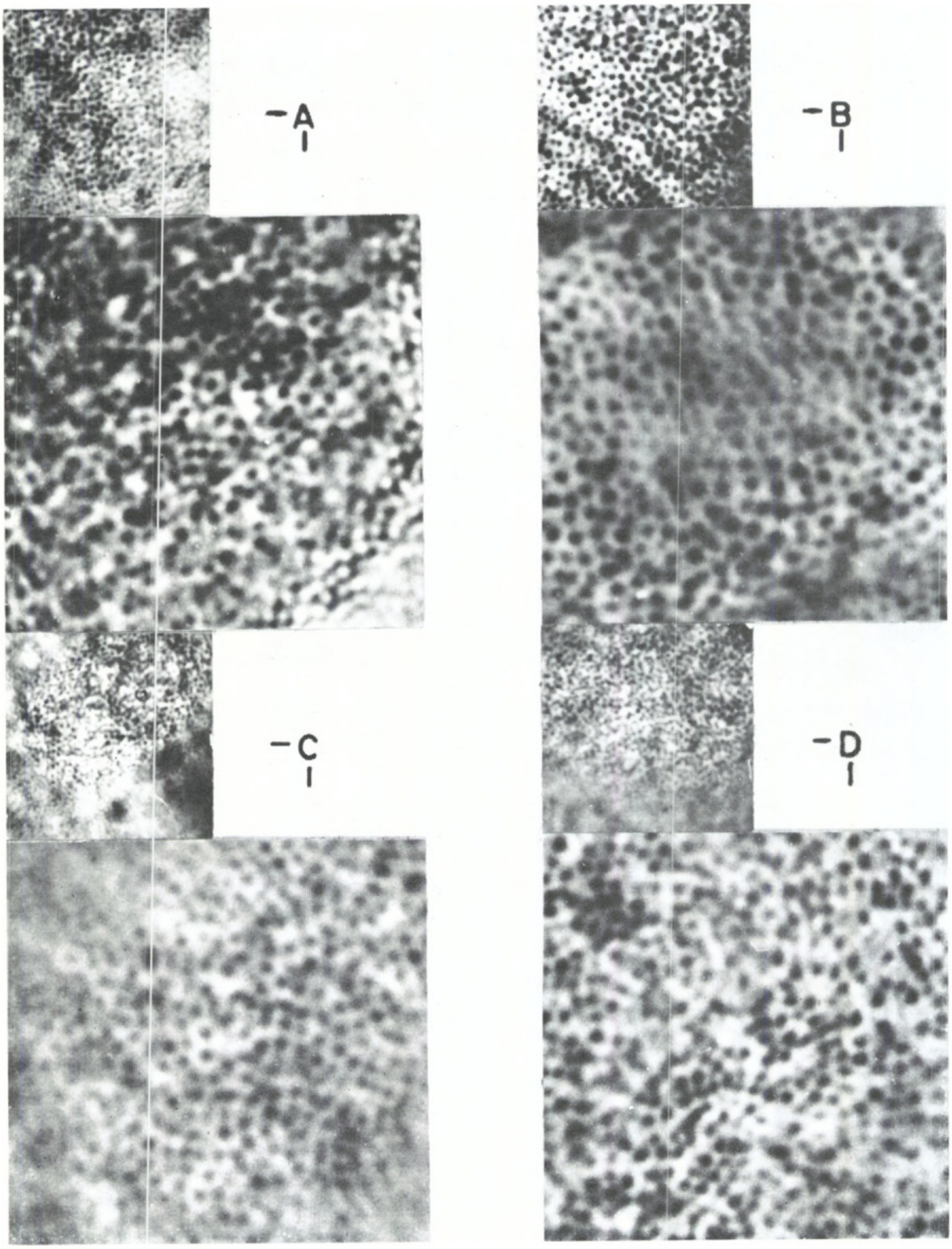
A. Another Mexican teosinte, showing considerable maize influence, according to genetic studies. The exine pattern is rather regular, the phase retardation rather strong.

B. Probably the most nearly pure maize. The spinule distribution is very regular (most clearly shown in the large photograph), and the phase retardation of the spinules pronounced (seen best in the small illustration).

C. Primitive teosinte, showing the same characteristics as El Valle teosinte shown in Plate IV (D).

D. A tripsacoid maize showing a pattern similar to maize-like teosintes (as teosinte Chalco, Plate IV, C). The spinules are pale and frequently clumped.

Plate V



EXPLANATION OF THE ILLUSTRATION

Plate VI

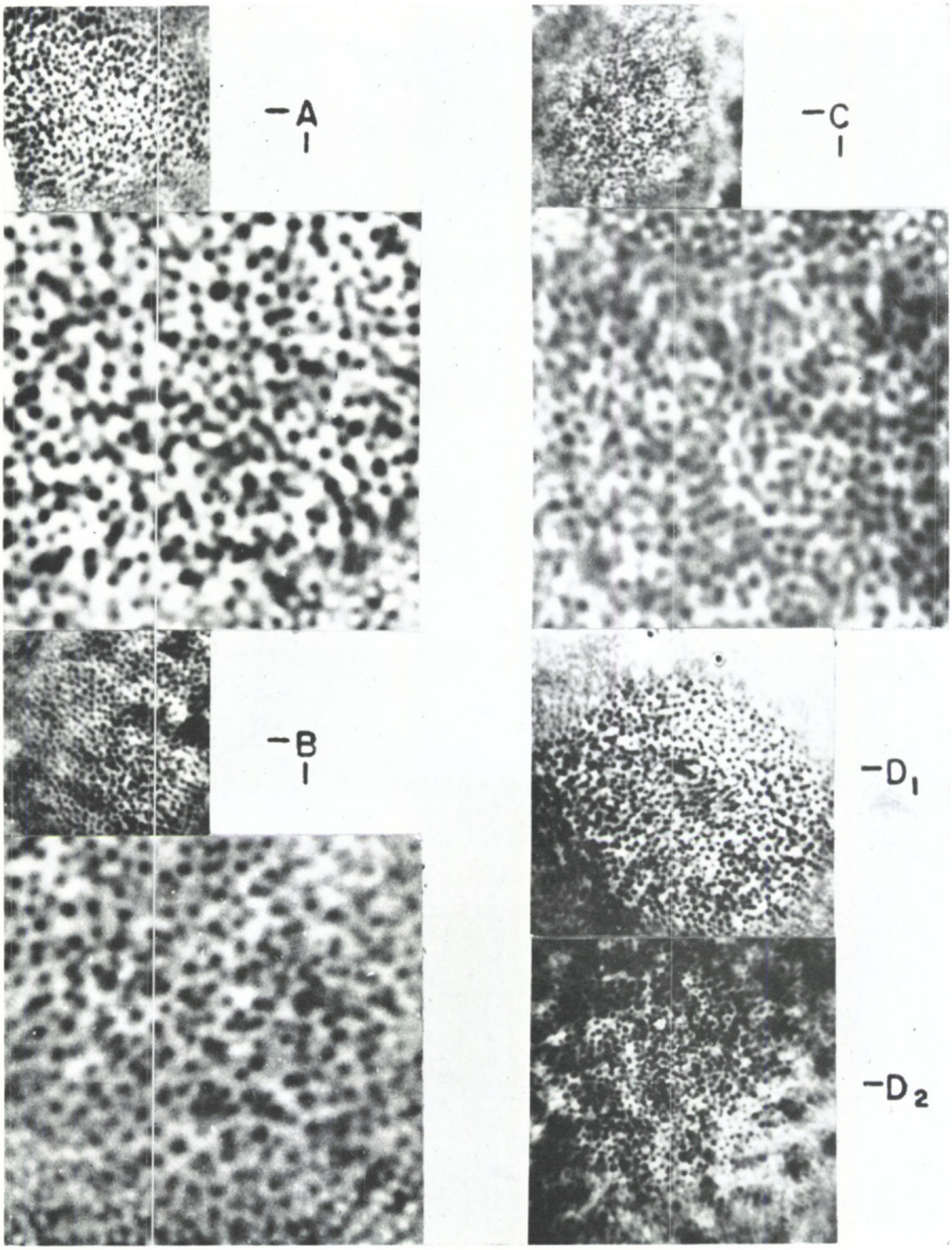
Evidences of genetic influences.

A. Maize (Minnesota A158-inbred)
B. Maize (Minnesota A158 with chromosome 1 of Florida teosinte)
C. Maize (Minnesota A158 with chromosome 3 of Florida teosinte)
D1. Maize (Texas 4R3-inbred)
D2. Maize (Texas 4R3 with chromosomes 3 and 4 of Nobogame teosinte)

Explanation

B shows no pronounced changes from A except for some increased clumping. Greater changes are shown between C and A, in the direction of teosinte: lacunae are more distinct and the contrast is poorer. D1 is an inbred maize. D2 shows a teosinte pattern in clumping of the spinules.

Plate VI



EXPLANATION OF THE ILLUSTRATION

PLATE VII

A. Fossil "*Tripsacum*" (Mexico City Belles Artes Core, depth 74 meters)

B. Fossil "maize" (Mexico City Belles Artes Core, depth 70 meters)

C. Fossil "maize" (New Mexico, Bat Cave, level VI, age ca. 5000 years ago

D. Fossil "maize" (Guatemala, Lake Petenxil, age 3950 ± years ago.

Explanation

A. Compare with *Tripsacum dactyloides*, Plate III (A).

B. Primitive maize pattern, compare with Nal Tel (a relatively pure type), Plate III (B). Both specimens A and B are in slightly eroded condition due to post-depositional processes.

C. The specimen had a maize pattern with good contrast, but with some clumping of spinules.

D. The specimen is like C, but with slightly greater clumping.

PLATE VII