EFFECT OF THE WAXY GENE IN MAIZE POLLEN,—
A REPLY TO CRITICISMS

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It was independently discovered and coincidentally reported by DEMEREC (1924) and by BRINK and MACGILLIVRAY (1924) that maize pollen grains carrying the waxy gene give a unique reaction with the iodine-potassium-iodide solution commonly employed in detecting starch. LONGLEY (1924) has also reported this phenomenon. In a recent paper, KIESSELBACH and PETERSEN (1925) question the validity of our observations and conclusions.

In our original papers we showed that pollen of flint, dent, pop and sweet varieties of maize contain reserves giving the well-known blue reaction characteristic of starch when tested with iodine. On the other hand, the contents of pollen grains produced by homozygous waxy plants fail to give this blue color when similarly treated, but stain a dull yellow passing to reddish under conditions favoring gradual concentration of the test solution. It was found, moreover, that individuals heterozygous for the waxy character produce both types of pollen grains, namely, blue-staining and yellowish-staining, in approximately equal numbers. This significant fact we took to indicate an immediate effect of the segregation of the non-waxy-waxy allelomorphs.

KIESSELBACH and PETERSEN have been unable to demonstrate this difference in the nature of waxy and non-waxy pollen and hold that it does not exist. Their observations and conclusions may be briefly summarized as follows: (1) Normally-developed mature pollen from waxy as well as from non-waxy races of maize gives the starch reaction with iodine. (2) The iodine solution does not serve to differentiate the content of the pollen grains of waxy and non-waxy maize. (3) The walls of the immature pollen of all varieties assume a reddish color with iodine which

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in older material is obscured by the dark stain of the starch within the grain. (4) Starch deposition in the grains of a single anther is sometimes not completed simultaneously, resulting in a transition stage in which some of the pollen stains reddish and some blue. (5) This latter condition was mistaken by the present investigators as evidence of segregation of the non-waxy–waxy genes.

It was made clear, we thought, at the time our discovery was reported, that the difference in the characters of the male gametophytes occasioned by the non-waxy-waxy allelomorphs rests upon a distinctive color reaction with iodine, of the two types of pollen reserves. It was pointed out that such difficulties in observation as were occasioned by the pollen-grain wall may be readily overcome by resort to the simple expedient of applying enough pressure to the cover-glass of the mount to rupture the grains and force out a portion of their contents. If attention is then focussed upon the granular inclusions of the cytoplasm, a difference in these materials from non-waxy and waxy gametophytes is readily apparent. In the case of pollen from homozygous non-waxy plants numerous small, mostly rod-shaped and ovate, blue-staining starch grains are present; the corresponding bodies from waxy pollen, which are similar in size but somewhat more irregular in shape, fail to give this blue reaction with iodine. If the preparation is allowed to stand unsealed for an hour or so, a little fresh solution being added from time to time, these waxy-type granules assume a reddish color. Plants segregating for waxy produce these two sorts of pollen with equal frequency.

Since this phenomenon was first reported, one of us (Brink) has confirmed the difference in the nature of the reserve carbohydrates in non-waxy and waxy pollen by separating these substances in relatively pure form and studying their color reactions in vitro. Freed from both the spore coat and the cytoplasm in which they are found, it can be demonstrated in an unequivocal manner that the two types of reserves, when stained with iodine, provide a reliable basis for distinguishing non-waxy and waxy pollen. Although they do not stain as readily, these constituents of the pollen can be differentiated quite as clearly as the corresponding endosperm reserves. If small amounts of the granular carbohydrate reserves separated from the pollen of non-waxy and waxy plants are heated to boiling with water and a few drops of iodine added to the suspensions after cooling, the color reactions obtained are practically identical with those given by the corresponding materials from the endosperm. Following Ridgway's (1912) standards, the color of the non-waxy preparation is Helvetia Blue, while that of the waxy preparation is Rood's Violet.
We cannot give credence to the statement by KiesseIbach and Peter-

sen that mature pollen of waxy and non-waxy races alike gives the
typical starch reaction with iodine, nor can we let their observation that
no difference is shown in the color reaction of the contents of the two sorts
of crushed grains pass unchallenged. Of course, one may stain both types
of pollen so heavily that they will appear black simply through failure
to transmit light. In getting a satisfactory differentiation some discrimina-
tion as to strength of solution and time of staining must be exercised,
to be sure, but the conditions of a satisfactory test are not difficult of
fulfillment.

The argument that the difference which we have taken to indicate the
effect of segregation, is a transitory condition, not only fails to account
for the 1 : 1 distribution of the two types of pollen, but is not founded on
fact. We have samples of pollen, collected at the time of dehiscence from
non-waxy and waxy plants, which have been stored in the laboratory
over calcium chloride for some eight months, which are as readily dis-
tinguished from each other now as when they were fresh.

As proved by the breeding results we have found the iodine reaction
at pollinating time a uniformly dependable means of separating homozy-
gous non-waxy from heterozygous plants in progenies comprising these
genotypes. There is little ground for questioning the validity of a test
which satisfies this criterion.

LITERATURE CITED

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