

# White pollen in maize

**ABSTRACT:** White pollen in corn (in contrast to normal yellow) is determined by the double recessive condition for the anthocyanin factor *c2* with a newly discovered factor, *whp*. The pigmentation is determined by the genotype of the sporophyte bearing the pollen rather than by the genotype of the pollen grain itself. Pollinations made with white pollen have been unsuccessful. Deposition of flavonoids in the pollen grain appears to be essential to normal pollen function.

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WHITE POLLEN is an unusual variation in higher plants. It has been reported in one variant individual of bristle cone pine, *Pinus aristata* Engelm., and in an isolated grove of *P. resinosa* Ait.<sup>4</sup>; G. A. Marx has noted white pollen in peas resulting from the combined effects of two independent recessive factors, *yp* and *yp-2*, for yellow (vs. normal orange) pollen<sup>7</sup>. This report defines the inheritance and characteristics of white pollen in maize, *Zea mays* L. It is determined by the double recessive condition of *c2*, a previously known anthocyanin factor, with *whp*, a new factor. White pollen is nonfunctional, but not aborted.

White pollen was first noted as a trait segregating among plants in an F<sub>2</sub> progeny also segregating for two anthocyanin factors, *C2/c2* and *R-r/r-r*. White pollen subsequently has been observed in *c2 c2* plants of several strains, all of which have in common in their pedigree the inbred line K55. In one group of related pedigrees a puzzling past failure to obtain self-fertilized progeny is explained by the fact that the unfruitful plants had white pollen, unrecognized at the time.

## Plant Pigments: the Anthocyanins and the Flavonols

The yellow flavonoid pigments in corn pollen and the red/purple anthocyanin pigments of various plant parts have pathways in common in their respective syntheses. The control of enzymatic glucosylation at the 3-position of both flavonols and anthocyanins has been defined to the *Bz1* locus<sup>5,6</sup>, and the pathway has been proposed to include at least one other pair of

gene-controlled parallel reactions<sup>13</sup>. Understanding of the genetic control of anthocyanin pigmentation in maize is well advanced<sup>1</sup>. Pigment expression is tissue-specific. In data to be discussed, the complementary factors, other than *C2* and *R*, necessary for pigmentation in the aleurone tissue of the kernel and the anther walls, were constant in all crosses described.

The effects of the *R* locus can be represented in a generalized way (in the presence of *C2*) by a series of anthocyanin-conditioning alleles:

|            | Aleurone tissue | Anther wall |
|------------|-----------------|-------------|
| <i>R-r</i> | purple          | red         |
| <i>R-g</i> | purple          | green       |
| <i>r-r</i> | colorless       | red         |
| <i>r-g</i> | colorless       | green       |

Anthocyanin is expressed as a dominant trait.

The *C2* locus (Coe, unpublished observations) determines the following expressions (in the presence of *R-r*):

|                 | Aleurone tissue | Anther wall                 |
|-----------------|-----------------|-----------------------------|
| <i>C2 C2 C2</i> | purple          | red                         |
| <i>C2 C2 c2</i> | purple          | dilute red                  |
| <i>C2 c2 c2</i> | dilute purple   |                             |
| <i>c2 c2 c2</i> | colorless       | green (sometimes faint red) |

In both tissues the level of anthocyanin pigmentation is dependent on dosage of *C2*.

## Genetic Data and Interpretation

The failure of white pollen to function restricts simple testcross analysis; pedigree analyses have defined the inheritance of

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white pollen as a duplicate-factor (15:1 segregation) system involving *c2* and a newly designated factor, *whp*. From the original F<sub>2</sub> progeny segregating *C2/c2* and *R-r/r-r*, 38 plants grown from the colorless-aleurone class showed 17 with red and 21 with green anthers (expect 16.3 *C2-r-r* *r-r* and 21.7 *c2 c2--*), all of which produced yellow pollen except for four of the green-anthered plants, which produced white pollen reminiscent of white sand. If a recessive factor, either *r-r* or a new factor, *whp*, in combination with *c2* were responsible for white pollen, the expectation would be 5.4 white-pollen plants. Initial tests showed the *r* was not involved, and that *c2* combined with an independent, recessive factor determines the character.

In F<sub>2</sub> progenies from *C2 c2 R-r R-r Whp whp*, 36 plants grown from the colored-aleurone class showed red anthers and yellow pollen; from the colorless-aleurone class 28 plants showed green anthers and yellow pollen while 5 plants showed green anthers and white pollen (expectation with independence would be 24.75:8.25). Linkage studies are in progress.

Stocks have been derived that are homozygous for *whp* and *R-r*, segregating for *C2 c2*. For example in a self-pollinated progeny (from *C2 c2 whp whp*), 22 plants from the colored-aleurone class produced yellow pollen and 10 plants from the colorless class produced white pollen. We routinely derive and maintain stocks by crossing white-pollen plants, *c2 c2 whp whp*, with yellow-pollen male parents, *C2 c2 whp whp*. From this cross colored kernels can be chosen to be planted for yellow-pollen male parents, and colorless kernels for white-pollen ear parents, to continue the stock. In plantings from such crosses, 118 plants grown from the colored-aleurone class produced yellow pollen while 202 grown from the colorless class produced white pollen. In each of these classes, one exceptional individual occurred for which progeny tests demonstrated a genotype, respectively *c2 c2* and *C2 c2*, noncorrespondent with the endosperm classification. Such noncorrespondence is expected at frequencies typically around 1 percent due to heterofertilization<sup>11</sup>.

#### Expression of White Pollen and its Determination by the Sporophyte

Classification for pollen color is difficult unless the samples are compared under the same conditions of drying. Freshly shed normal pollen is creamy yellow and turns

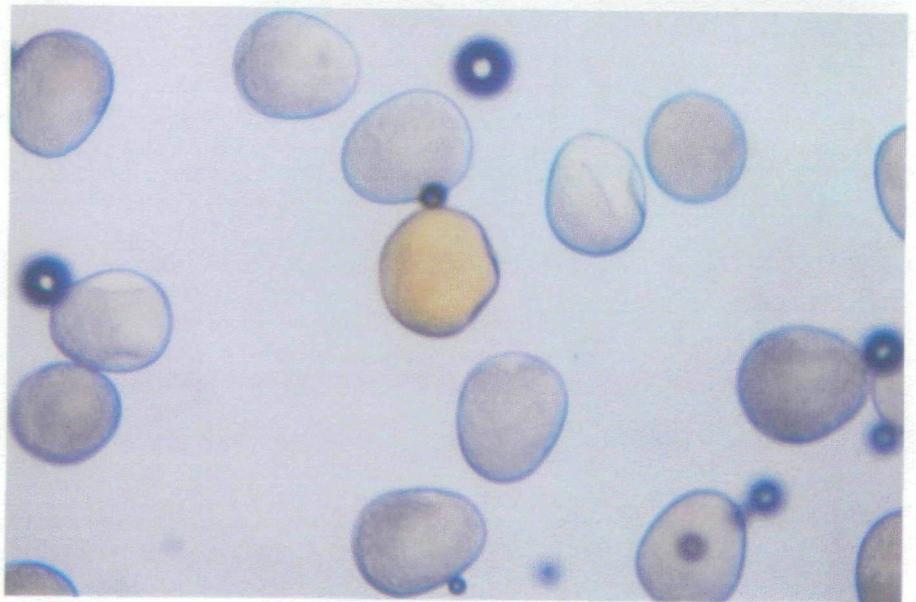


FIGURE 1 Top: white (nonfunctional) and yellow (normal) pollen of maize. Bottom: artificial mixture of dried, normal and white pollen grains, rehydrated in ammoniacal glycerol.

rapidly golden upon drying; freshly shed white pollen is like white sand and turns cream-colored upon drying. Mixed dry and fresh pollen, as typically collected overnight in a pollinating bag, has been confusing in our experience; we have found that better discrimination can be made on a sample freshly shaken from a newly shedding branch (especially the first shedding from the central spike). A brown paper pollinating bag (creased to localize the pollen in bulk) provides better background contrast than a white surface (Figure 1).

Pollen produced by plants homozygous for *C2 Whp*, *C2 whp*, or *c2 Whp*, or by the heterozygote *c2 c2 Whp whp* is normal yellow in color when viewed in bulk, Pol-

len produced by *C2 c2 whp whp* plants may be yellow to varying degrees in differing backgrounds or conditions. Collections from some plants have been distinctly lighter yellow on some occasions of collection than on others, while collections from other plants of this genotype have been consistently recorded as light yellow or, on the other hand, as normal yellow. Considering that the *C2* locus has a conspicuous dosage effect on anthocyanin pigmentation in the aleurone tissue, anthers, husks, and sheaths (Coe, unpub. observations), an influence of dosage on the flavonoids in the pollen wall would not be unexpected.

Fresh pollen from heterozygous plants,

either *C2 c2 whp whp* or *c2 c2 Whp whp*, does not appear to segregate as observed under the microscope, but the color is so light that enhancement of the intensity of the pigments is necessary. Dry pollen samples from heterozygous plants of each of these constitutions were rehydrated briefly in glycerol-water-NH<sub>4</sub>OH (10:10:1 by volume) to heighten the yellow flavonoid colors. These samples did not show any evidence of segregation for yellow vs. white pollen grains. Thus yellow pollen color is determined by the genotype of the sporophyte on which the pollen is borne. Ammoniacal glycerol applied to artificial mixtures defines the yellow grains clearly, even from dried, rehydrated samples (Figure 1).

Starch in pollen samples tested with iodine-potassium iodide solution stains similarly in both white and yellow grains, and both types expand and burst at similar rates in the test solution. Pollinations have been attempted in four seasons with white pollen, but as yet no unequivocally valid progeny have resulted. Markers for reliable detection of accidental outcrossing are currently being incorporated toward controlled experiments on transmission. We are also exploring several aspects of the functional behavior of white pollen.

Normal maize pollen contains considerable amounts of two flavonols, quercetin and isorhamnetin (3'-methoxy quercetin), and trace amounts of kaempferol (3'-deoxy quercetin)<sup>6,10,15</sup>. In peas, R. K. Crowden and I. C. Murfet<sup>4</sup> have found that the major pigments are carotenoids, and that yellow pollen, determined by the *yp* gene<sup>9</sup>, contains only approximately 5 percent of the carotenoid level of normal orange pollen. Although  $\beta$ -carotene has been reported in maize pollen<sup>14</sup>, the greater quantity of flavonols (which are specifically heightened by ammonia exposure) can be assumed to be the primary source of the yellow color. No flavones or flavonols could be detected in white pollen in preliminary tests in our laboratory and in more discriminating tests by Susan McCormick at the University of Texas (pers. comm.).

### Discussion

White pollen is determined by the double recessive condition for *c2 whp*. The first, *c2*,

is a previously known factor controlling anthocyanin pigmentation. The *whp* factor is identified and designated in this report.

Deposition of the major yellow pigments in corn pollen requires that the plant bearing the pollen have the dominant condition for one or the other of these two factors (*C2*, *Whp*). Deposition of pigment is a function of sporophytic tissue rather than of the pollen grain, according to these genetic data and studies of development morphology<sup>12</sup>. Gametophytic determination is characteristic of internal pollen characters<sup>8</sup>. In fact the 3-glucosylation of these pigments controlled by *Bz1* has been supposed in previous studies to be determined by the genotype of the pollen grain rather than of the sporophyte<sup>5</sup>. While a linear relationship between enzymatic activity and the dosage of *Bz1* is found for pollen, a linear relationship also holds for seedlings and other stages of the sporophyte; hence, the same relationship may be expected in pollen if the sporophyte is determinative.

Styles and Ceska<sup>13</sup> have proposed that *C2* (allele *C2-Idf*) acts very early in the pathway that leads in common to anthocyanins and flavonols. Dooner has reported<sup>3</sup> that this locus may control the enzyme flavanone synthase, which acts in an early step in the common part of the pathway. The two factors *C2* and *Whp* could be interpreted simply as controlling duplicate functions at this point in the pathway. Since pollen in corn does not accumulate anthocyanin, it is not possible to determine whether anthocyanins and flavonols are separately controlled in the pollen. Inasmuch as the *C2* locus determines anthocyanin pigmentation in the kernel while *Whp* does not, any suggestion of duplicate functions must be reserved without further biochemical information on the constituents and pathway involved in the deposition of pollen pigments.

Healthy appearing, white pollen does not function normally, which suggests that pigment synthesis or deposition is vital to pollen function. Ideas on the role of flavonoids in pollen function<sup>12</sup> include growth-stimulating activity (upon which both creditable and discredited studies have been reported) and screening against ultraviolet radiation damage. The germinating ability of white pollen from a variant bristle cone pine tree, although somewhat

lower than that of yellow pollen, was not further altered by direct sunlight exposure (L. C. Johnson, pers. comm.). It is difficult to attribute to ultraviolet damage the failure of white pollen in maize to function. The exposure of white pollen in these experiments has been minimal, since the collections were made from tassels covered overnight (out of yet-unopened florets covered by multiple tissue layers), onto silks promptly covered with two bag layers. If the presence of flavonoid compounds is necessary for pollen function, the basis for this requirement is not evident from current knowledge of the pathway and functions involved.

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