THE WAXY LOCUS IN MAIZE. I. INTRALOCUS RECOMBINATION FREQUENCY ESTIMATES BY POLLEN AND BY CONVENTIONAL ANALYSES¹

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THE waxy (*wx*) locus in maize conditions the type of starch which is synthesized in the triploid endosperm and in the haploid pollen grain. Further, the phenotype of a pollen grain is determined by its own genotype and not by that of the plant which produces the pollen grain (BRINK and MACGILLIVRAY 1924) and (DEMEREK 1924). This makes it possible to use the pollen grain as the unit of observation in genetical investigations on the *wx* locus. The *Wx* pollen grains stain blue with an iodine-potassium iodide stain while *wx* pollen grains stain a reddish-brown color. Since a maize plant produces millions of pollen grains, large populations can be obtained and scored easily, thus markedly enhancing genetic resolution. The investigation of low frequency events is more easily carried out with this system than with any other locus in a higher organism (NELSON 1957).

The test for intragenic recombination at the locus is to cross two *wx* mutants of supposedly independent origin, collect pollen from the F_1 plant, and score the frequency of *Wx* pollen grains. A frequency significantly above the mean of the *Wx* frequencies in the parental stocks may well be indicative of genetic recombination between two different sites of mutation which reconstitutes a functional *Wx* locus. It has been shown in a set of the ten all-combination crosses between five *wx* mutants that seven F_1 progenies show such significantly higher *Wx* frequencies (NELSON 1959). Thus, for this locus in maize, recombination within the functional unit between different mutant sites has been substantiated.

The purpose of this paper is to report that the *Wx* frequency estimated for a cross (wx^{90} times wx^{coe}) by pollen analysis is similar to the *Wx* frequency estimated for the same cross by conventional genetic techniques and to record the segregation of outside markers in the *Wx* recombinants. Further, the results from backcross progenies are in agreement with the hypothesis that heterozygosity for mutant alleles at the *wx* locus is a prerequisite for high *Wx* frequencies.

The *Wx* frequency estimated for the pollen grains produced by the cross between any two *wx* mutants should be similar to the *Wx* frequency for the same cross estimated in a backcross to a *wx/wx* female parent. In attempts to check this particular point the F_1 *Bz wx⁹⁰ V₁/bz wx^{coe} v₁* was used. The frequency of

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reversion to *Wx* in both parental stocks has been estimated as being less than 1×10^{-5} . The allele *wxc^{coe}* has the same general recombination frequencies as *wxc* (which is the standard *wx* allele) in all combinations in which it has been tested and is probably the same allele. However, it is not possible to demonstrate identity rigorously, and the allele has been given the designation *wxc^{coe}* indicating its source (Dr. E. H. COE, JR.). The alleles at the bronze (*bz*) locus control seed color in the presence of the functional alleles *A₁*, *A₂*, *C*, and *R* at the other loci affecting seed color. The *Bz* allele results in the formation of red or purple seeds owing to anthocyanin pigments. Kernels which are homozygous *bz* lack anthocyanin pigment and are tan to bronze in color. The virescent-1 (*v*) locus controls a step in chlorophyll formation in seedlings. Seedlings which are *V/V* or *V/v* are a normal green color, while *v/v* seedlings are yellow but turn green early in development. The *bz* locus is located ca. 18 units distal to the *wx* locus while the *v* locus is approximately 6 units proximal according to our data.

The preliminary test was made in 1959. Twenty-six plants of the *F₁* cross *Bz wx⁹⁰ V/bz wx^{coe} v* were grown. An estimated total population of 1.4×10^6 pollen grains from the 26 plants was scanned, and 1260 *Wx* grains detected. There were approximately equal populations of pollen grains from each test plant included in the total population. The mean frequency of *Wx* pollen grains for the 26 plants was 90×10^{-5} .

In a conventional genetic test, pollen from the *Bz wx⁹⁰ V/bz wx^{coe} v* plants was used to pollinate the tester stock *bz wx^{coe} v/bz wx^{coe} v*. Three hundred and nine testcross ears were obtained representing nearly equal numbers of pollinations from each *F₁* plant. The backcross kernels were scored visually for the *wx/wx/wx* and *Wx/wx/wx* phenotypes (the endosperm of maize being triploid with two genomes from the female parent and one from the male parent). The kernels which are *wx/wx/wx* have a dull appearance which is readily distinguished in seeds without aleurone or pericarp color from the rather translucent appearance of kernels which are *Wx/wx/wx*, *Wx/Wx/wx*, or *Wx/Wx/Wx*. Visually, the kernels with different doses of *Wx* are indistinguishable from each other.

In a total population of 61,007 kernels from the 309 ears only 12 *Wx* kernels were found. This is a frequency of 19×10^{-5} which is not quite significantly different ($t = 6.80$ and $P < .1$) from the 90×10^{-5} *Wx* frequency observed in the pollen. It has been shown that the frequency of *Wx* pollen grains per anther for any cross is in agreement with a Poisson distribution (NELSON 1959). In a Poisson population, the variance is equal to the mean, and this fact has been used in calculating the significance of the difference between the means.

Yet it is a reasonable *a priori* assumption that the estimates of *Wx* frequency by both assay methods should be similar. It was felt that the most likely source of the discrepancy would be an inability to distinguish visually between *wx* and *Wx* phenotypes in the presence of aleurone color which is present in 50 percent of the seeds in this particular cross. The genotype of the female parent (with regard to the five loci at which functional alleles must be present to allow the development of anthocyanin pigment in the aleurone) is *A₁/A₁*; *A₂/A₂*; *R/R*; *C*

bz/C bz. The genotype of the *wx⁹⁰* line is *A₁/A₁; A₂/A₂; r/r; c Bz/c Bz*. Thus in the backcross progeny there are equal numbers of *Bz/bz/bz* seeds (purple aleurone color) and *bz/bz/bz* seeds (a light tan to bronze aleurone color). The differentiation between *Wx* and *wx* phenotypes is made easily in *bz/bz/bz* seeds but with considerable difficulty in the *Bz/bz/bz* seeds. As will be shown subsequently, the majority of the *Wx* gametes are carrying the *Bz* allele.

In 1960, a second experiment of the same type was run with more pollinations being made from 13 *F₁* plants used as parents. Efforts were made to obtain similar numbers of pollinations from each *F₁* plant. After harvest and before drying, the ears were buffed lightly with an emery wheel to remove a portion of the pericarp and underlying aleurone from each kernel and thus expose the endosperm. Each kernel was then touched with a drop of dilute *I₂-KI* stain to detect the *Wx/wx/wx* kernels. Of a total of 117,819 kernels from 399 ears, 116 displayed the *Wx* phenotype (stained blue with *I₂-KI* stain). This is a frequency of 99×10^{-5} . The pollen analysis for the 13 plants gave a mean frequency of 102 *Wx* pollen grains $\times 10^{-5}$ (442 *Wx* pollen grains in a total estimated population of 435,000). Clearly there is no significant difference between the two estimates of *Wx* frequency. It appears that inability to distinguish adequately between *Wx* and *wx* phenotypes in the presence of aleurone color may well have accounted for the discrepancy between the estimates of *Wx* frequency by pollen analysis and conventional techniques in 1959.

One important point deserves consideration here. Contamination owing to pollen drifting in the air is an ever-present danger in genetic experiments with maize. Such contaminations could easily account for the observed frequency of *Wx/wx/wx* kernels. As noted, the *F₁* plants were *Bz wx⁹⁰ V/bz wx^{coe} v*, and pollen from these plants was used to pollinate the *bz wx^{coe} v/bz wx^{coe} v* tester stock. Of the 108 *Wx/wx/wx* kernels detected in the testcross progeny which were viable and hence could be scored for both markers, 93 or 86 percent were carrying one or both of the recessive alleles at the loci flanking the *wx* locus. For these gametes, contamination can virtually be excluded as a source. For the remaining 15 gametes which are *Bz V*, origin by contamination cannot be excluded. But assuming for the sake of argument that all 15 did arise by contamination and should be disregarded, there was still a gametic *Wx* frequency of 81×10^{-5} (93 plus three *bz/bz* kernels which did not germinate) in the *F₁* plants. This is not significantly different ($t = 1.55$, and $P < .4$) from the *Wx* frequency (102×10^{-5}) observed in the pollen grains considering these to be samples drawn from Poisson populations.

Since outside markers were present in the parental stocks, it is interesting to know the segregation with regard to these markers for the *Wx* gametes. Of the 108 gametes giving rise to viable kernels, 15 were *Bz V*, 63 *Bz v*, three *bz V*, and 27 *bz v*. The data suggest strongly that the order of the two alleles within the *wx* locus is (*Bz*)*wx^{coe} wx⁹⁰(V)*. At the same time, these data suggest that not all *Wx* gametes have arisen as the result of an orthodox crossing-over event between the sites of *wx⁹⁰* and *wx^{coe}*. There are fewer *Bz v* gametes and more *bz v* gametes than would be expected according to the classical precepts of crossing-over.

The proportions of high and low frequency Wx plants in backcross progenies: If, in an F_1 cross between two independently occurring wx alleles, the enhanced Wx frequency (above the parental strains) is due to recombination within the functional unit and hence a consequence of heterozygosity for the wx alleles, one would expect that in the progeny resulting from a backcross to either one of the parents, one half the plants would have pollen with a Wx frequency similar to the F_1 plants, while the other half would have a Wx frequency no higher than the parental strains. This expectation was tested in 1959 for two backcross progenies.

The backcross progeny $(wx^{90} \times wx^c) \times wx^c$ gave 34 plants with a Wx frequency of less than 2×10^{-5} and 26 plants with a mean Wx frequency of 89×10^{-5} (range 57–138). Compared with the expectation of equal numbers of each type, the χ^2 is 1.066 and $P = .30$ ($df = 1$).

In the backcross progeny $(wx^c \times wx^{H21}) \times wx^c$, the pollen from 37 plants was scored. Eighteen plants had a Wx frequency of less than 2×10^{-5} while 19 plants had a frequency roughly corresponding to the Wx frequency observed in the F_1 progeny (46×10^{-5}). This last group was not analyzed quantitatively.

The observed frequencies of low frequency Wx and high frequency Wx plants in the two backcross progenies tested do not differ significantly from the equal numbers of each plant type expected under the stated hypothesis. It should be pointed out, however, that since marker genes were not present in the crosses, heterozygosity at the wx locus as a condition for high Wx frequencies is not rigorously implicated.

In this same test of the $(wx^{90} \times wx^c) \times wx^c$ backcross progeny, a shift in Wx frequency towards a higher value than the F_1 was noticeable. The mean Wx frequency for all high Wx frequency plants in the backcross progeny was 89×10^{-5} . The mean Wx frequency of 15 F_1 plants $wx^{90} \times wx^c$ grown in an adjacent

TABLE 1

The distribution of Wx frequencies in $wx^{90} \times wx^c$ and $(wx^{90} \times wx^c) \times wx^c$ progenies, 1959*

$wx^{90} \times wx^c$	$(wx^{90} \times wx^c) \times wx^c$	
58×10^{-5}	57×10^{-5}	93×10^{-5}
59	63	98
66	63	99
67	65 (51, 78)	103
68	65	103
69	68	107
73	70	108
74	74	114
77	75	115
80	75	138 (143, 132)
80	78	140 (128, 151)
82	87	
86	87	
89	89	
89	92	

* These figures represent single preparations with populations of 40 to 50,000 pollen grains from each plant except for three estimates in the BC progeny which are means of the values given in parentheses.

† Only the high Wx frequency plants given. There were also 34 plants with a Wx frequency of less than 2×10^{-5} .

row was 74×10^{-5} . The individual plant scores for both F_1 and BC populations are given in Table 1. The range for the backcross progeny was extended considerably on the high side. A comparison of the two progenies gives a t value of 2.44 ($df = 39$). The probability is less than 0.025 that these two progenies represent different samples from the same population so that observed differences in the mean may well indicate that the populations are different with respect to Wx frequency. If so, this is most readily attributable to the effect of residual genotype on the recombinational process within the waxy locus. A corollary of this hypothesis would be the necessity for having all wx alleles in a common genetic background in order to obtain meaningful data as to recombination within the locus. This requirement is being met as rapidly as possible.

SUMMARY

For the F_1 $Bz\ wx^{90}\ V/bz\ wx^{coe}\ v$, similar estimates of the frequency of Wx gametes were obtained in 1960 by both pollen analysis (102×10^{-5}) and conventional genetic techniques (99×10^{-5}). It is necessary in the latter case to test every kernel for its reaction to a KI-I₂ stain since the phenotypic distinction between waxy and non-waxy is obscured by aleurone color conditioned by an allele (Bz) at an outside marker locus. Of the 108 putative Wx recombinants which could be scored for both outside markers, 93 or 86 percent were carrying one or both of the recessive alleles, and for these contamination can be excluded as an explanation.

Fifty-eight percent of the Wx gametes were carrying Bz distally and v proximally. Since the genotype of the F_1 was $Bz\ wx^{90}\ V/bz\ wx^{coe}\ v$, this would indicate that the order of mutant sites within the locus is $(Bz)\ wx^{coe}\ wx^{90}\ (V)$.

The proportions of plants with low Wx frequencies ($< 2 \times 10^{-5}$) and plants with high frequencies (similar to the F_1 's) in the backcross progenies ($wx^{90} \times wx^c$) $\times$ wx^c and ($wx^c \times wx^{H21}$) $\times$ wx^c are not in disagreement with those expected (1:1) if heterozygosity at the waxy locus is a prerequisite for Wx frequencies above the parental levels.

In the backcross progeny $(wx^{90} \times wx^c) \times wx^c$, the mean Wx frequency of the high Wx frequency plants is significantly higher (89.4) than the mean Wx frequency (74.4) of the F_1 ($wx^{90} \times wx^c$) grown in the same year, and the range has broadened considerably. This may indicate an effect of the residual genotype on the recombinational process leading to the formation of Wx gametes.

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LITERATURE CITED

- BRINK, R. A., and J. H. MACGILLIVRAY, 1924 Segregation for the waxy character in maize pollen and differential development of the male gametophyte. *Am. J. Botany* **11**: 465-469.
- DEMEREK, M., 1924 A case of pollen dimorphism in maize. *Am. J. Botany* **11**: 461-464.
- NELSON, OLIVER E., 1957 The feasibility of investigating "genetic fine structure" in higher plants. *Am. Naturalist* **91**: 331-332.
- 1959 Intracistron recombination in the *Wx/wx* region of maize. *Science* **130**: 794-795.