

found to be later in flowering, and slower in growth than their sibs.

For another study on the differential DNA synthesis in eu- and heterochromatin of maize, kernels with varying numbers of B-chromosomes were grown. At the early seedling stage, when length of their primary roots averaged about two inches, they were fed with  $H^3$ -thymidine in Hoagland's solution. In preparing autoradiographs a standard dipping technique was followed. Data gathered up to the present indicate that the time of DNA synthesis in eu- and heterochromatin (B-chromosomes) differs. The euchromatin of maize, or A-chromosomes, started DNA synthesis before the heterochromatin. The investigation, it is hoped, may also lead to a detailed analysis of the mitotic cycle of maize.

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### 3. Extra chromosome element.

At the first meiotic prophase of the microsporocytes of a maize plant 67-44-2, an extra chromosome element was consistently observed. It was stained as well as the regular chromosomes either with propionic carmine or with Schiff's reagent. At pachytene, it always formed a circular configuration and its length on the average measured about 20 u. Its location was not confined to a certain part of the cell.

As the division advanced to diakinesis, no evidence of shortening of this element was obtained. At metaphase I, it fragmented into two elements. No centromeres were identified. Apparently due to their lack of regular movement at anaphase I, both of these elements were always found in only one part of the spindle. However, at telophase I, they were no longer identifiable in most of the cases. Among a total of approximately 500 cells examined, these elements were definitely observed in only about two per cent of the cells.

A few years ago a similar element was seen in one of the teosinte derivatives. That element was somewhat shorter than the one reported in the present communication. But its meiotic behavior appeared to be the same. Selfings and crosses with this plant, 67-44-2, were attempted last summer in order to know more about the significance of this element.

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### 1. Genetic recombination among spontaneous and ethyl methanesulfonate-induced waxy mutants in maize.

Ethyl methanesulfonate (EMS) has been reported to produce "point mutations"<sup>4</sup> and "single locus mutations"<sup>1</sup> in maize. Since intracistron

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recombination has been demonstrated at the waxy (wx) locus in maize<sup>3</sup> an opportunity is provided to use this locus to distinguish among genetic alterations, e.g. the occurrence of intracistron recombination among mutants is evidence for "point mutations" as opposed to extensive deletions. Intracistron recombination can be studied in pollen of wx plants because of the differential staining reactions of Wx and wx pollen grains and is facilitated because large populations of pollen grains can be observed.

Results are reported for two EMS-induced wx mutants and for three spontaneous wx mutants. Treatments were made on seeds with the genotype C<sup>I</sup> Sh Bz Wx. The mutant designated wx<sup>BL-A</sup> was obtained by treating kernels with 0.025 M EMS solution for 3 days at 3° C. The mutant designated wx<sup>BL-B</sup> was obtained from 0.1 M EMS treatment for 2 hours at 25°C. These wx mutants, detected by crossing the treated material to a recessive tester C sh bz wx<sup>C</sup>, were produced by Amano and Smith<sup>1</sup> and further details on methods of EMS treatment and detection of wx mutants were reported there.

Preparatory to an analysis of the EMS-induced wx mutants it is essential to establish the induced mutant site in the homoallelic condition. In order to do this, plants arising from M<sub>1</sub> generation wx kernels (C<sup>I</sup> Sh Bz wx<sup>induced</sup>/C sh bz wx<sup>C</sup>) were self-pollinated. Portions of tassels from these M<sub>1</sub> plants were collected for a pollen analysis.<sup>2</sup> The material was segregating for the distal marker genes, and only those wx kernels with the dominant distal markers C<sup>I</sup> Sh Bz, were selected for further study. Second generation plants arising from these kernels were self-pollinated and those plants that did not recombine (give Wx pollen) at the wx locus and that were also C<sup>I</sup> Sh Bz were apparently homoallelic for the induced site as well as homozygous for the distal marker genes. Plants meeting these criteria were included in this study and were used in the final analysis. Third generation plants (C<sup>I</sup> Sh Bz wx<sup>induced</sup>) were crossed in all combinations (diallel) with the spontaneous wx mutants, wx<sup>C</sup>, wx<sup>90</sup>, and wx<sup>H21</sup>. These hybrids were planted and portions of the tassels were collected to use in the assay of pollen. A single plant was used in the first (M<sub>1</sub>) and second generations to represent each mutant. However, to make the diallel crosses several plants were used and to obtain the diallel data 3-5 plants were used.

The frequency of Wx pollen grains  $\times 10^{-5}$  that represent reversion and recombination between the wx<sup>C</sup> tester site and the EMS-induced wx site in the M<sub>1</sub> generation was  $41.39 \pm 5.17$  for wx<sup>BL-A</sup> and  $29.28 \pm 4.36$  for wx<sup>BL-B</sup>;  $155 \times 10^3$  and  $154 \times 10^3$  pollen grains respectively were used to obtain these estimates. Out of necessity the M<sub>1</sub> data and diallel data (Tables 1 and 2) were taken in separate growing seasons.

Table 1  
Average number of Wx pollen grains due to reversion from homoallelic ethyl methanesulfonate induced and spontaneous wx mutants

Alleles	Estimated no. microspores x 10 <sup>3</sup>	$\bar{X}$ no. <u>Wx</u> x 10 <sup>-5</sup> ± s $\bar{x}$
<u>wx</u> <sup>C</sup>	133	2.25 ± 1.30
<u>wx</u> <sup>90</sup>	342	0.58 ± 0.41
<u>wx</u> <sup>H21</sup>	166	0.60 ± 0.60
<u>wx</u> <sup>BL-A</sup>	266	0.0 ± 0.0
<u>wx</u> <sup>BL-B</sup>	308	1.29 ± 0.65

Table 2  
Average number of Wx pollen grains from intercrosses among homoallelic ethyl methanesulfonate induced and spontaneous wx mutants

Alleles	Estimated no. microspores x 10 <sup>3</sup>	$\bar{X}$ no. <u>Wx</u> x 10 <sup>-5</sup> ± s $\bar{x}$
<u>wx</u> <sup>C</sup> x <u>wx</u> <sup>BL-B</sup>	178	0.0 ± 0.0
<u>wx</u> <sup>C</sup> x <u>wx</u> <sup>BL-A</sup>	217	5.99 ± 1.66
<u>wx</u> <sup>C</sup> x <u>wx</u> <sup>H21</sup>	245	50.68 ± 4.56
<u>wx</u> <sup>C</sup> x <u>wx</u> <sup>90</sup>	319	86.11 ± 5.19
<u>wx</u> <sup>BL-B</sup> x <u>wx</u> <sup>BL-A</sup>	142	7.73 ± 2.33
<u>wx</u> <sup>BL-B</sup> x <u>wx</u> <sup>H21</sup>	211	3.79 ± 1.34
<u>wx</u> <sup>BL-B</sup> x <u>wx</u> <sup>90</sup>	188	0.0 ± 0.0
<u>wx</u> <sup>BL-A</sup> x <u>wx</u> <sup>H21</sup>	184	0.54 ± 0.17
<u>wx</u> <sup>BL-A</sup> x <u>wx</u> <sup>90</sup>	168	12.48 ± 2.72
<u>wx</u> <sup>H21</sup> x <u>wx</u> <sup>90</sup>	349	30.63 ± 2.96

If the attempt to obtain homoallelic induced sites fails and if instead homoallelic wx sites from the tester stock were in actuality analyzed, the so-called induced site will recombine like the wx site. The more

important evidence to indicate that the wx mutants are homoallelic for the EMS-induced site is that the EMS-induced mutant wx<sup>BL-A</sup> recombines with wx<sup>C</sup> (Table 2), but wx<sup>BL-B</sup> does not. However, wx<sup>BL-B</sup>, unlike wx<sup>C</sup>, does not recombine with wx<sup>90</sup>; also wx<sup>BL-B</sup> and wx<sup>BL-A</sup> recombine with each other.

The M<sub>1</sub> results show a higher recombination rate than the diallel results and lead to nonadditivity. In fact wx<sup>BL-B</sup> recombines with wx<sup>C</sup> in the M<sub>1</sub> generation but does not recombine in the diallel (Table 2). Nonadditivity has been previously reported at the wx locus in maize.<sup>3</sup> More important than obtaining additivity of the data is the fact that recombination occurs among both the spontaneous and induced mutants in the diallel.

The frequency of intracistron recombination of various EMS-induced wx mutants in the M<sub>1</sub> generation led to the conclusion that EMS induces independent mutations at sites within the wx locus in maize. Also the occurrence of recombination in the M<sub>1</sub> generation between mutant and tester sites indicates that "point mutations" (gene mutations) have been induced by this mutagen.<sup>2</sup> The occurrence of recombination between EMS-induced and spontaneous wx mutants crossed in all combinations confirms the earlier report<sup>2</sup> and is further indication that "point mutations," or at least minor deletions, have been induced by this mutagen.

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#### References:

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#### 2. Relative response of maize to X-rays vs. neutrons over a wide range of doses.

A problem of continuing interest in radiobiology is to determine why radiations which give different patterns of energy distribution in exposed tissues produce different degrees of response for equal amounts of total energy absorbed. A commonly used measure of this difference is the relative biological effectiveness (RBE), computed as the ratio of doses for two radiations of different quality required to produce the same effect. RBE values characteristically change with dose levels of X-rays (X) vs. neutrons (N); that is, no single ratio of X/N for equal effects holds throughout a range of absorbed doses.

Maize plants, grown from Yg<sub>2</sub>/yg<sub>2</sub> seeds that had received various absorbed doses of fission neutrons or of 250 kVp X-rays, were scored for radiation damage on the basis of 9 criteria (Table 1). The responses ranged from those caused by a sublethal genetic effect (yg<sub>2</sub> leaf sectors), to eventual gamete lethality (pollen sterility and reduced seed set), to growth retardation due to somatic cell death (reduction in plant height, survival and emergence), to complete cessation of cell division ("reversal" of emergence