DIEPOXYBUTANE AS A CHEMICAL MUTAGEN IN ZEA MAYS¹

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DIEPOXYBUTANE (DEB) has been used very effectively as an inducer of gene mutations in *Neurospora* (KÖLMARK 1953; KÖLMARK and GILES 1953; KÖLMARK and WESTERGAARD 1953; WESTERGAARD 1957), chromosome deficiencies and rearrangements in *Drosophila* (BIRD and FAHMY 1953; FAHMY and BIRD 1953; FAHMY and FAHMY 1956), reversions in *Escherichia coli* (DEMEREK *et al.* 1954), and chromosome breaks in the *Vicia faba* root-tip test (LOVELESS 1951). In *Neurospora*, treatment with DEB was 20 times more effective on the induction of mutations at the adenine locus than were comparable doses of mustards, other epoxides, or ultraviolet. In *Drosophila*, DEB was found to be far less effective than X-rays in the production of major structural rearrangements in the chromosomes but considerably more efficient than X-rays in the production of small deficiencies. DEB affected the darker bands of the chromosomes preferentially although mustard gas affected the lighter ones, indicating a specificity of DEB for heterochromatin. This marked ability of DEB to induce gene mutations as well as chromosome breaks led to its being chosen for a study of chemically induced mutations in maize. The term "mutation", unless otherwise specified, is used here to include changes resulting from both chromosome breaks and so-called "point" mutations.

Maize has several characteristics which make it a very desirable object for studies of induced mutations. There are a number of endosperm characters determined by known genes of regular inheritance which are located in the short arm of chromosome 9. Maize endosperm, being triploid, is not adversely affected by considerable loss of genetic material. Chromosomes treated in mature pollen are in a prolonged resting stage and are not subject to the same stresses that exist during cell division. An F_1 generation can be observed for further test of the occurrence of point mutation or surviving chromosome abnormalities, if desired.

Although maize has been utilized extensively for studies of induced mutation by radiation, the use of chemicals for this purpose has been largely neglected. Nitrogen mustard treatments of mature pollen have been found very efficient as inducers of chromosome breaks observable in the endosperm (GIBSON, BRINK, and STAHMANN 1950; UMALI 1949). However, similar treatments with ethylene oxide were only moderately successful (FABERGÉ 1955). It is the purpose of the present paper to report on the effectiveness and specific characteristics of DEB as a chemical mutagen in maize.

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MATERIALS AND METHODS

Genetic material

The standard procedure of utilizing multiple dominant stocks as the source of pollen for treatment and multiple recessive stocks as seed parents was followed. The absence of one or more dominant marker genes in the F_1 endosperm indicated loss of the markers involved. The word *loss*, designates only the disappearance of the phenotypic effect of the dominant allele and does not imply the particular mechanism involved.

The endosperm markers used in the following experiments are all located in the short arm of chromosome 9. They include the allelic color factors *I*, (inhibitor of aleurone color, dominant to *C*), *C* (aleurone color, dominant to *c*), and *c* (no aleurone color when homozygous). The interactions of the color factor bronze (*bz*) with *I* and *C* have been described by RHOADES (1952). Bronze phenotypes are visible in the endosperm only in the absence of *I* and presence of *C*. Endosperm composition is determined by genes *Sh* (*sh*, shrunken endosperm) and *Wx* (*wx*, waxy starch). Normal starch (*Wx*) stains blue with KI plus I_2 solution while waxy starch is either colorless or stains brown with iodine solution. At pachytene the genes *I*, *Sh*, and *Bz* are located close together in chromosome 9 their general position occupying a point slightly less than the distal third of the short arm. *Wx* is located close to the middle of the short arm. (McCLINTOCK, personal communication). The crosses used in these experiments were either *c sh wx* $\times$ *C Sh Wx* or *C sh bz wx* $\times$ *I Sh Bz Wx*, permitting a study of three or four linked genes, respectively, in the short arm of chromosome 9. All F_1 kernels were scraped and stained in bulk with iodine solution, then examined microscopically for the appearance of nonstaining areas of waxy starch.

The use of chromosome 9 markers and the reading of mosaic patterns resulting from breakage-fusion-bridge (B.-F.-B.) cycles originated with the work of McCLINTOCK (1951), who described the phenotypes expected from breaks occurring at various positions along the short arm of chromosome 9. For a comprehensive discussion of this subject see FABERGÉ (1956). In these experiments any deviations from the expected order of loss were placed in the "complex" class as the first point of chromosome breakage in these kernels is often impossible to determine.

The loss of dominant marker genes occurring spontaneously or induced by DEB were classified according to the number of genes involved and to the extent of the deficient area of the kernel. Fractional or mosaic patterns occupying less than one eighth of the kernel were excluded since they are both difficult to classify accurately, and their frequency is not increased appreciably by the DEB treatment (KREIZINGER 1958).

Preparation of DL-diepoxybutane

Since diepoxybutane may exist in several isomeric forms, the method of preparing and purifying the DL-compound used in these experiments is of importance. The stereospecific chemical used was substantially pure DL-diepoxybutane,

having been made from cis-2-butene-1,4-diol by bromination followed by dehydrobromination with dry potassium hydroxide. The material was carefully fractionated through a 30-inch Podbielniak column and the fraction, b.p. 58–60° (35mm), was collected. The DL-diepoxybutane was prepared under the direction of Dr. D. D. PHILLIPS, formerly of Cornell University.

Methods of pollen treatment

Diepoxybutane was introduced into the pollen by one of two procedures. In the cut tassel method of treatment, tassels in the second or third day of pollen shedding were selected. Cut ends of the tassels were placed in flasks containing 200 ml of 0.2 percent ($2.2 \times 10^{-3}M$) DEB solution. After 18 hours of treatment pollen was taken from the tassels, and pollinations were made immediately in the field. Pollen from a single tassel was used to pollinate one multiple recessive plant. The same procedure was followed for controls except that the solutions contained no DEB.

In the second method of treatment a wick was used to introduce DEB into the plant. Tassels just commencing to shed pollen were splinted below the tassel with a piece of wood to provide support. A hole five mm in diameter was bored into the stalk of the plant four inches below the lowest tassel branch, and a piece of woven glass wicking was pushed securely into the hole with forceps. The other end of the wick was placed in a one dram vial containing 4.5 cc of 0.2 percent DEB. The vial was attached to the stalk with masking tape, and an additional piece of tape was placed over the wick area to reduce evaporation. Although the vial was usually empty within two to four hours, it was not removed until the following day. The pollen was collected 20 hours after starting the treatment and at successive 24-hour periods for four days.

Statistical analysis

The t test and analysis of variance were used throughout for comparisons between treatment means. In the wick method of treatment the frequency of mutant kernels resulting from pollination with individual treated tassels differed significantly ($P = 0.05$) from tassel to tassel. Therefore, chi-square tests, which require that sampling within treatments be from a homogeneous population, are not applicable. In each case the analysis was made on the percent mutant kernels within individual ears.

Method used to calculate maps of chromosome 9 from induced breakage frequencies

The short arm of chromosome 9 was considered to have a length of 100 percent for determination of the relationship between frequency of breakage and the distance between marker genes along the arm. The mutant kernels from each ear of corn were tabulated according to the position of the original break between marked regions along the chromosome arm. Within an individual ear the number of breaks between each two marker genes was converted to a percentage of the total number of kernels showing chromosome breaks in that ear. Means (in

percent) were calculated for each chromosome region from all ears within each of the five days of pollination in the wick method of treatment, the two methods of treatment, and the two stocks used. These means were compared by appropriate *t* tests or analysis of variance. Chi-square tests are not valid because, as will be shown later, tassels treated with DEB differ from each other in the manner that breaks are apportioned along the chromosome arm and thus do not represent a homogeneous population.

RESULTS

The effectiveness of DEB as a chemical mutagen in maize pollen

The marked ability of DEB to induce losses of endosperm marker genes in maize is evident from Tables 1 and 2. The number of mutant kernels resulting from DEB treatment is either 50 or 60 times greater than the number observed in the control depending on the method of treatment. The statistical analysis shows that the difference between the frequency of mutant kernels in the DEB treated and control ears is significant at the $P = 0.05$ level (see Table 3). This difference was unaffected by the year of treatment, the stocks used, or any of the possible interactions between them (KREIZINGER 1958). For purposes of analysis it is assumed that the action of the mutagen is mainly in breaking chromosomes since all evidence, discussed below, indicates that this is by far the most important effect. The classification of kernels by B.-F.-B. cycles, interstitial losses, and terminal losses are shown for stock *I Sh Bz Wx* in Table 1 and for stock *C Sh Wx* in Table 2.

In the wick method of treatment a single tassel was used for five successive days of pollination. Only tassels in which all five days of pollination were represented by an ear were used in the statistical analyses. However, kernels from all ears resulting from the wick method are tabulated in Table 2. The means from the five successive days of pollination did not differ significantly in either the

TABLE 1

Type and frequency of mutant endosperms induced by 0.2 percent DEB treatment of maize pollen in the cross C sh bz wx × I Sh Bz Wx using the cut tassel method of treatment

		Classification of kernels													
		B.-F.-B. cycles starting in				Interstitial losses		Terminal losses				More complex			
Treatment	Year	<i>E-I</i>	<i>I-Sh</i>	<i>Sh- Bz</i>	<i>Bz- Wx</i>	<i>ShWx</i>	<i>Wx</i>	<i>I</i>	<i>Sh I</i>	<i>Bz Sh I</i>	<i>Wx Bz Sh I</i>		Total affected	Total examined	Percent affected
0.2%	1956	117	4	4	28	3	6	15	2	8	163	7	357	3314	10.8
	1957	44	1	1	10	.	1	3	1	2	57	3	123	920	13.4
	Total	161	5	5	38	3	7	18	3	10	220	10	480	4234	11.3
Control	1956	2	2	.	.	.	.	1	.	.	9	1	15	6377	0.2
	1957	1	.	.	.	.	.	.	.	.	.	.	1	874	0.1
	Total	3	2	.	.	.	.	1	.	.	9	1	16	7251	0.2

TABLE 2

Type and frequency of mutant endosperms induced by 0.2 percent DEB treatment of maize pollen in the cross c sh wx × C Sh Wx

Treatment	Method	Year	Classification of kernels										Total affected	Total examined	Percent affected
			B.-F.-B. cycles starting in			Interstitial losses		Terminal losses			More complex				
			E-C	C-Sh	Sh-Wx	ShWx	Wx	C	CSh	CShWx					
0.2%	Cut tassel	1956	74	7	26	.	3	6	17	153	3	289	2436	11.8	
		1957	44	0	23	1	1	4	3	85	1	162	1145	14.2	
	Total	118	7	49	1	4	10	20	238	4	451	3581	12.6		
	Wick	1957	313	21	97	0	17	26	37	652	9	1168	11578	10.1	
	Grand total	431	28	146	1	21	36	57	890	13	1619	15159	10.7		
Control	Cut tassel	1956	5	.	1	.	.	1	.	2	.	9	4338	0.2	
		1957	.	.	.	.	.	2	.	.	.	2	1548	0.1	
	Wick	1957	5	1	.	.	.	5	.	2	.	13	5711	0.2	
	Total	10	1	1	.	.	8	.	4	.	24	11597	0.2		

TABLE 3

Statistical comparison of the frequency of mutant endosperms induced by DEB in c sh wx × C Sh Wx and in C sh bz wx × I Sh Bz Wx using the cut tassel method of treatment

	c sh wx × C Sh Wx				C sh bz wx × I Sh Bz Wx			
	1956		1957		1956		1957	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
No. ears	40	24	24	6	59	51	15	4
	percent				percent			
Total	670.0	5.2	342.8	0.8	780.3	13.0	193.8	0.4
Means	16.8	0.2	14.3	0.1	13.2	0.2	12.9	0.1
S _x	1.8	0.1	1.4	0.1	1.2	0.1	1.5	0.1

treated or control material. Therefore, the five days of pollination were combined to determine the mutagenic effectiveness of DEB when introduced by the wick method. The unweighted five day DEB treatment mean of 10.6 percent is significantly different from the unweighted control mean of 0.4 percent at the $P = 0.01$ level providing further evidence of the ability of DEB to induce mutation in maize. The cut tassel method of treatment is significantly ($P = 0.05$) more effective than the wick method in the induction of marker losses. However, following the wick method of treatment, ears usually bear many more kernels which results in a much greater total number of mutant kernels for the number of pollinations made (KREIZINGER 1958).

*Induced chromosome breaks related to marked regions
in the short arm of chromosome 9*

Before comparing the number of breaks between markers with the relative distances between them, it was necessary to determine what effect, if any, the two stocks, two years, and two methods of treatment had on the way breaks were distributed along the arm. This distribution is not influenced by the year of treatment or the interaction of years with stocks following the cut tassel method of treatment (KREIZINGER 1958). However, there is a surprising difference between the two stocks in the frequency of breakage induced within the region between *I* (*C*) and the end of the short arm and between *Sh* and *Wx*. The amount of breakage distal to *I* in stock *I Sh Bz Wx* is about ten percent higher than in stock *C Sh Wx* (see Table 4)—a difference which is significant at the $P = 0.05$ level.

TABLE 4

*The relative frequency of breakage within marked regions of chromosome 9 in stocks
C Sh Wx and I Sh Bz Wx*

Region between markers	<i>C Sh Wx</i> percent	Mean of stock <i>I Sh Bz Wx</i> percent	Mean difference
End- <i>C</i> (<i>I</i>)	26.0	35.7	9.7* $\pm$ 4.6
<i>C</i> - <i>Sh</i>	3.8	4.4	0.6 $\pm$ 1.7
<i>Sh</i> - <i>Wx</i>	22.2	13.9	8.3 $\pm$ 4.2
<i>Wx</i> -Centromere	48.0	46.0	2.0 $\pm$ 4.3

* Significant at the $P = 0.05$ level.

The difference in breakage frequency distal to *I* in the two stocks is almost entirely compensated for between *Sh* and *Wx* where the frequency of breaks in stock *C Sh Wx* is eight percent higher than in stock *I Sh Bz Wx*. These latter figures are highly suggestive of a real difference although the *t* value is not quite significant.

The *C Sh Wx* stock was used exclusively in the wick method of treatment. With the exception of the region between *Sh* and *Wx*, the frequency of breaks within the marked regions of the short arm of chromosome 9 did not differ significantly among any of the five days of pollination. However, in the region between *Sh* and *Wx* there was a significant difference ($P = 0.05$) among successive days of pollination as well as a highly significant difference ($P = 0.01$) among tassels in the frequency of breakage within this region (KREIZINGER 1958). Since all of the difference among days could be attributed to the fifth day of pollination, data from the fifth day were omitted from further comparisons. The analysis was based on DUNNETT's method for comparing treatment means with a control, in this case using the first day of pollination as the control (DUNNETT 1955). There was no difference between the two methods of treatment on the apportionment of breaks along the arm.

After finding that the distribution of breaks along the chromosome arm was

not influenced by the years or methods of treatment, it was possible to relate the frequency of breaks between marked loci to the actual relative distances between them in each stock. As shown in Figure 1 the distances between loci as determined cytologically and by breakage frequencies are remarkably similar. The percent of breakage between loci in the *C Sh Wx* stock compares almost exactly with the frequency expected if breakage and restitution are at random along the chromosome arm. However, in the *I Sh Bz Wx* stock there appears to be a relative excess of breaks distal to *I* and a dearth of these events between *Sh* and *Wx*. This may indicate some type of differential breakage or restitution in the *I Sh Bz Wx* stock.

*The relationship between sectoring and whole kernel effects
following DEB treatment of maize pollen*

Sectoring, in which only a portion of the kernel shows the loss of the dominant marker genes, is observed very frequently following DEB treatment of maize pollen. The loss sectors in the kernels were classified roughly according to size as one eighth, one fourth, one half, three fourths, or entire. Kernels with B.-F.-B. cycles initiated distal to *C* or *I* were not classified by sector size. Two points were revealed by the classification: (1) The frequency of sectorial to entire kernel losses of marker genes was approximately two to one, and this frequency was not influenced by the method or year of treatment or the stock used. (2) The size of the sectors formed a nearly normal frequency distribution about the mean value of one half of the endosperm (KREIZINGER 1958). The possible causes of sectoring will be considered below.

DISCUSSION

The high frequency of mutant kernels obtained following DEB treatment of the pollen places this chemical with X-rays and nitrogen mustards as powerful inducers of mutant endosperms in maize. Although no direct comparisons among these mutagens have been made, the frequency of mutation resulting from DEB treatment of maize pollen is very similar to that obtained by FABERGÉ (1956) using 1500r of X-rays on pollen from an *I Sh Bz Wx* stock. The frequency of

FIGURE 1.—Distances between marked loci in the short arm of chromosome 9 as determined cytologically at pachytene and as determined from breakage frequencies (in percent) following DEB treatment.

endosperm mutations induced by DEB is also similar to the 12 percent obtained by GIBSON *et al.* (1950) using moderately severe doses of nitrogen mustards.

Differential sensitivity of maize pollen to DEB introduced during each of the five days prior to pollen shedding was also investigated. It has been reported by SINGLETON and CASPER (1954) that the time of greatest sensitivity for mutation production in maize pollen treated with gamma irradiation is a few days before the pollen is shed. The mutation rate drops sharply for irradiations applied during the last two days before pollen shedding. In the radiation treatments of SINGLETON and CASPER maize tassels were irradiated in a timed sequence before pollen shedding. In the DEB wick method, tassels were treated at one time, and pollen was collected on successive days following treatment. While the number of days elapsing between the treatment of any individual pollen grain with DEB and its time of shedding undoubtedly varied because of environmental factors, there was no indication of increased sensitivity with earlier treatment as reported for gamma irradiation. In so far as the two groups of data can be compared directly, the results indicate some fundamental difference between the action of gamma irradiation and DEB on the production of mutations in maize pollen. Further study employing both mutagens under the same conditions are in progress.

There is no evidence that gene mutation is markedly increased by DEB treatment of maize pollen. The total number of single gene losses of *I*, *C*, and *Wx* observed was only 82 out of 22,410 kernels. This is a lower frequency of single losses of *I* (*C*) and *Wx* than the 43 observed by FABERGÉ (1956) in 6,782 kernels following X-ray treatment of the pollen although the over-all mutation rate from the two types of treatment was similar. The studies of STADLER (1954) and NUFFER (1957) have shown that all single gene losses observed following X-ray treatment of maize pollen are chromosomal deletions, not true point mutations. Therefore, since the number of single gene losses after DEB treatment was proportionately fewer than following X-ray treatment, it can be tentatively inferred that the single gene losses following DEB treatment were largely chromosomal deficiencies. A few spontaneous gene mutations would be expected in a sample of over 22,000 kernels; these would be included among the 82 single gene losses.

The paucity of gene mutations following DEB treatment is in agreement with the suggestion that maize reacts differently to mutagenic agents than does *Drosophila* or *Neurospora*. Although X-rays are known to cause true gene mutations in both these organisms, they are induced extremely rarely, if at all, in maize. Similarly, gene mutation in maize is not markedly increased by DEB treatment although in *Neurospora* this type of mutation is greatly increased by the same chemical (KÖLMARK and GILES 1953).

From the use of linked genes in the short arm of chromosome 9 it was possible to determine whether breakage and restitution were at random along this arm following DEB treatment. A study of the effect of year, stock, and method of treatment on the position of breaks revealed that the two stocks differed sig-

nificantly ($P = 0.05$) in the amount of breakage distal to *I* (*C*). The difference in the frequency of breakage between *Sh* and *Wx* in the two stocks also approached significance. There are three possibilities to account for these differences. (1) A marked region in one stock is more sensitive to breakage or less likely to reconstitute than the same region in the other stock. (2) The short arm of chromosome 9 differs in length in the two stocks either throughout the chromosome cycle or at the time of treatment. (3) The differences are due to sampling error.

The most plausible explanation for the discrepancy in the two stocks appears to be some type of differential breakage or restitution within one or both stocks. Credence for the explanation of genic control of breakage comes from three sources. (1) The studies of McCLINTOCK (1951) have established that spontaneous breakage in maize can be under genic control. However, it should be emphasized that in the present experiments there was no evidence of a Dissociator-Activator system having been induced by the treatment. (2) Crossing over appears to be under genic control in maize since stocks which differ in crossover frequency between marked loci have these loci in the same cytological position at pachytene (McCLINTOCK, personal communication). According to the recent work of TAYLOR (1957) chromosome breakage is a prerequisite to crossing over. Therefore, since crossing over is governed by the genotype, the chromosome breaks involved must be similarly controlled. (3) The work of LINNERT (1951) indicates that induced breaks are also genically controlled. Treatment of pachytene chromosomes of maize with urethane plus KCl resulted in the breaking of both homologues of a pair at the same region more often than would be expected on the basis of chance.

Differences in the length of the short arm of chromosome 9 in the two stocks at time of treatment cannot be responsible for the differences between stocks. If the region distal to *I* in stock *I Sh Bz Wx* were actually longer than the same region in stock *C Sh Wx*, all other regions in stock *I Sh Bz Wx* should show correspondingly fewer breaks rather than only the region between *Sh* and *Wx*.

The possibility that the differences between stocks are due simply to sampling error cannot be excluded. However, the excellent agreement in frequency of breakage within identical regions of the chromosome arm in the same stock under different years and methods of treatment and the wide divergence between stocks regardless of years, suggest that this is a real difference.

The relative distances between marked loci based on the number of breaks occurring between them were compared with the positions of the markers as observed at pachytene. The close agreement in the position of loci as determined by these two methods is of considerable interest since it suggests that breakage induced by DEB is approximately at random along the chromosome arm.

The relative location of breaks induced by DEB treatment within chromosome 9 were also compared with those induced by X-rays using the data of FABERGÉ (1956) in which maize pollen from an *I Sh Bz Wx* stock was treated with 1500r of X-rays and was used to pollinate a *C sh bz wx* stock. Variations in the breakage frequencies within identical regions following DEB or X-ray treatment are prob-

ably no greater than can be expected on the basis of heterogeneity in the material and differences in experimental procedure.

One factor not considered in the DEB data is the conclusion of FABERGÉ (1956) that stable, terminal deletions occur only rarely, if at all, in maize endosperm following ultraviolet or X-ray treatment of the pollen. If stable end losses do not occur, a loss not followed by fusion of the two sister chromatids must be considered to involve two breaks, one of which would not be detected if occurring distal to *I*. The fact that functionally stable chromosome ends are produced in maize following radiation by alpha particles (FABERGÉ 1959) indicates that the behavior of the chromosome is dependent on the type of treatment. There is no *a priori* reason to expect the production of stable, terminal ends to be the same following DEB treatment as following either type of radiation. Computations to determine whether stable, terminal deletions are produced following DEB treatment are not feasible since data were not from a homogeneous population and cannot be pooled.

The frequency of sectoring following DEB treatment of maize pollen was about two fractional losses to one entire loss, a ratio similar to that found following ultraviolet or nitrogen mustard treatment (STADLER and SPRAGUE 1936; GIBSON, BRINK, and STAHMANN 1950). By contrast, sectoring is relatively infrequent in maize following X-ray treatment (STADLER and SPRAGUE 1936; NUFFER 1957).

The hypotheses which have been suggested to explain the frequent occurrence of sectoring in maize and *Drosophila* following ultraviolet or chemical treatment are: (1) a multiple strand nature of the chromosome leading to the possibility that the treatment affects only a portion of the subunits rather than the entire chromosome, (2) interference with chromosome duplication resulting in subsequent chromosome or chromatid breakage, (3) induction of an unstable genic state, and (4) influence of the treatment on the chromosome matrix.

It has frequently been suggested that ultraviolet and chemicals break chromatids or smaller chromosome subunits as opposed to the action of X-rays which break whole chromosomes. Sectors smaller or larger than one half are envisioned as the result of the sorting out of subunits which were affected at the time of treatment. However, MULLER (1940) observed that if the entire chromosome is broken but held together until time of division, one of the resulting broken chromatids may reconstitute while the other heals. The loss of only one acentric fragment bearing dominant genes would result in a sector of recessive tissue.

It has also been suggested that ultraviolet and chemicals interfere with chromosome duplication rather than actually breaking chromosomes. Chemical interference with duplication is supported by the fact that epoxides react *in vitro* with both phosphate groups and amino acids (ROSS 1958; STACEY, COBB, COUSINS, and ALEXANDER 1958).

The hypothesis of genic instability resulting in point mutation as proposed by AUERBACH (1951) for mosaicism in *Drosophila* does not seem a satisfactory answer for sectoring in maize. As previously discussed, maize responds differently to X-ray and chemical treatment than does *Drosophila* in that gene mutation is

very rare in maize following these treatments. Therefore, genic instability does not seem a satisfactory explanation for sectoring in *Zea mays*.

The fourth hypothesis for sectoring involves changes in the chromosome matrix, a suggestion which has been proposed by SWANSON (1944) to explain the anomalous results obtained from combined ultraviolet and X-ray treatments in *Tradescantia*. Diepoxybutane may conceivably exert an effect on the chromosome matrix which renders it more difficult for chromatids or acentric fragments to separate. Such an effect would increase the occurrence of restitution and of sectoring when the entire chromosome is broken by the treatment.

SUMMARY

The mutagenic effects of diepoxybutane treatment of maize pollen were studied. Losses of dominant marker genes in the endosperm of the resulting kernels were used to evaluate the mutagenicity of the diepoxybutane. One stock contained three, the other four marker genes located on the short arm of chromosome 9.

Diepoxybutane was found to be a powerful inducer of mutant endosperm sectors in maize. The proportion of F_1 kernels showing single or multiple gene losses was about 12 percent. There was no evidence that the frequency of gene mutation was markedly increased by diepoxybutane treatment although some single gene losses may have been "point" mutations.

The distance between marked loci in the short arm of chromosome 9 as determined by the relative frequency of breaks occurring between these loci were very similar to the cytological distances as determined at pachytene. This was considered evidence that breakage resulting from diepoxybutane treatment was induced approximately at random along the chromosome arm. However, the two stocks used differed significantly in the number of breaks observed distal to *I*. It is suggested that this may be due either to differential sensitivity of some chromosomal regions to breakage or to sampling error.

About twice as many kernels had sectors of mutant tissue as had exclusively mutant tissue in the endosperm. The size of mutant sectors formed a nearly normal distribution about the most frequent values of one half the endosperm. The possible causes of sectoring are considered.

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