No indication is yet available as to whether $\underline{lo}_x$ is proximal or distal to wx.

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1. Further studies of x-ray-induced mutations at the Sh and Bz loci.

Preliminary data were presented in the 1965 Maize News Letter on the nature of x-ray-induced mutations at the bronze locus. One mutant in particular ($\underline{bz\text{-}x}_1$), which appeared originally as a possible intragenic change, was studied extensively. Since then, three additional alterations involving bronze which were suspected of being other than gross changes have been subjected to genetic and cytological tests. This report includes data collected on all four mutants.

Pollen from plants homozygous for the Sh, Bz and Wx alleles was x-rayed and applied to silks of sh bz wx tester stocks. (For a complete presentation of the materials and methods employed in these experiments, refer to the 1965 MNL, p. 98.) Three classes of bz mutants were identified--sh bz and Sh bz wx types, showing loss of two dominant markers, and Sh bz Wx mutants, exhibiting loss of Bz only. Table 1 lists the mutants observed in the endosperm and embryo. Of the endosperm mutants, only those of Sh bz phenotype were tested for Wx versus wx. Bronze mutants identified at the seedling stage were scored for Sh and Wx by progeny tests. Putative sh bz embryo mutants may include plants of Sh bz*[1]/sh bz constitution in which the Sh bz* gametophytes were non-functional, as well as the expected - -/sh bz class in which both dominant markers have been lost. Simultaneous mutations of Sh and Bz are nearly twice as frequent in the endosperm as in the embryo. Either the viability of the double mutants is lower in the embryo than in the endosperm or selective fertilization occurs in which the sperm nucleus containing the normal chromosome 9 preferentially fertilizes the egg nucleus. Many F_1 seeds

[1]The symbol bz* refers to any change at the Bz locus resulting in the bz phenotype.

Table 1

Mutants obtained in crosses of sh bz wx ♀♀ x Sh Bz Wx ♂♂ following irradiation of the male parent

Experiment #	Population	sh bz mutants			Sh bz Wx mutants			Sh bz wx mutants			bz embryo mutants not surviving to maturity	Bz-bz mosaics in endosperm
		Endosperm	Embryo and endosperm	Embryo	Endosperm	Embryo and endosperm	Embryo	Endosperm	Embryo and endosperm	Embryo		
1	66,337	601	7	178	2	0	1	0	0	0	160	272
2	66,620	790	42	235	7	0	1	10	0	1	212	166
Total	132,957	1391*	49*	413*	9	0	2	10	0	1	372	438

*The mutants in these classes may range from deletions of the entire chromosome 9 to deficiencies which include only the Bz locus. If functional gametes with the mutant chromosome are not produced, only the sh bz phenotype will be expressed in the testcross progeny.

germinated but did not survive to the stage at which the bronze phenotype could be observed and a large proportion of the lethal seedlings may have been double mutants.

Of the 837 bronze mutants obtained in the embryo, the two exhibiting the Sh bz Wx phenotype were saved for further analysis. These are designated bz-x$_1$ and bz-x$_2$. In addition, two sh bz mutants (sh-bz-x$_1$ and sh-bz-x$_2$) which had little aborted pollen were maintained. All of the remaining embryo mutants exhibited a high frequency of pollen abortion and, when backcrossed to sh bz wx tester plants, proved to be double mutants of sh* bz*/ sh bz or bz* wx* / bz wx constitution. It was assumed that these plants were heterozygous for a chromosomal aberration affecting the short arm of chromosome 9 and that no gene mutation had been induced. Such mutants were not analyzed further. No plants mosaic for Bz and bz sectors were observed in the population of embryo mutants. Such individuals could arise if only a single DNA strand of the bronze gene had been mutated in the sperm nucleus.

A description of the original bz-x$_1$ heterozygote was presented in the 1965 News Letter. The F_1 plant heterozygous for the induced bz-x$_2$ mutation was normal in stature. It bore an ear which, when testcrossed, had slightly reduced seed set. The progeny consisted of 78 Sh bz Wx, 17 Sh bz wx, 45 sh bz Wx and 132 sh bz wx kernels. Aborted pollen was lower in frequency than in the majority of the bronze embryo mutants. (Pollen analyses of the original bz-x and sh-bz-x plants were made with a hand microscope on unstained pollen and exact frequencies of abortion were not obtained.)

The F_1 heterozygote carrying sh-bz-x$_1$ was normal in stature and gave rise to an ear with a low amount of ovule abortion. Testcross progeny of this sh-bz-x$_1$ Wx/sh bz wx individual included 102 sh bz Wx and 120 sh bz wx kernels. A low amount of aborted pollen was present but the exact percentage was not ascertained.

The original sh-bz-x$_2$ heterozygote appeared to be completely normal. The ear produced 177 sh bz Wx and 153 sh bz wx kernels when backcrossed to a sh bz wx tester. Seed set was normal and the aborted pollen frequency appeared no greater than that of normal sibs. Except for the shrunken-bronze phenotype, neither the plant nor the ear could be distinguished from normal sibs.

In backcross progenies of all four mutant heterozygotes, the dominant contamination markers from the treated pollen parent were present: hence, the original mutant plants possessed a genome derived from an irradiated sperm and did not arise by fertilization with foreign pollen.

Pollen from individuals heterozygous for each of the mutants and a normal chromosome 9 was stained with IKI and frequencies of aborted grains and grains of sub-normal size were obtained. Control values were obtained from plants of similar background containing two normal chromosomes 9. The results are presented in columns 2 and 3 of Table 2. The frequencies observed in bz-x$_1$, bz-x$_2$ and sh-bz-x$_1$ heterozygotes indicate that the alterations in each case affected gene loci controlling pollen development in addition to affecting Bz and Sh. The 10.7% abortion of sh-bz-x$_2$ is higher than that observed in control plants; however, the wx:Wx ratio of the normal grains from sh-bz-x$_2$ Wx/sh bz wx plants was close to unity (1:0.98). If the mutant region was influencing pollen development, Wx grains would be observed less frequently than wx grains. Hence, the greater abortion frequency cannot be ascribed to the presence of sh-bz-x$_2$.

Transmission of the mutants through the male and female gametophytes was tested in crosses of bz-x/Bz and sh-bz-x/Sh Bz plants with tester stocks. Frequencies were determined by dividing the amount of bz or sh bz kernels by the number of Bz or Sh Bz progeny, respectively, in the following generation. Columns 4 and 5 of Table 2 list the results of these crosses. The data indicate that all mutants except sh-bz-x$_2$ affect male gametophyte viability drastically and embryo sac development to a lesser degree. In the case of sh-bz-x$_2$, all plants tested showed full viability of female gametophytes carrying the alteration. Transmission of the mutant chromosome through the pollen ranged from 60% to 82.5% in four individuals but in two additional plants, the values were 109.5% and 116.0%. Tests indicated that, in these two latter cases, a viability factor on the normal homolog closely linked to wx may have caused the deficiency of Sh-Bz kernels. In the progeny from self-pollinations of four Sh Bz/sh-bz-x$_2$ individuals, the percentages of sh bz kernels were 25.4, 22.8, 22.0 and 21.7. Hence, the viability of pollen carrying the mutant homolog may reach 100% in some cases; however, further tests must be made

Table 2

Effects of the bz-x and sh-bz-x mutants on pollen abortion, gametophyte viability and crossing over

Plant constitution	% aborted pollen	% sub-normal pollen	% gametophyte viability		% recombination		% reduction in crossing over	
			pollen	embryo sac	Sh-Bz	Bz-Wx	Sh-Bz	Bz-Wx
bz-x1 / normal	9.7	35.2	0	32.0	0.93	16.6	59.4	10.7
bz-x2 / normal	19.5	39.3	0.001	51.5	0.86	18.6	62.4	0
sh-bz-x1 / normal	6.2-22.8	2.13	1.05	63.3	0	14.2	100	23.7
sh-bz-x2 / normal	10.7	0	60-100	100	0	19.9	100	---
normal / normal	6.8	0	---	---	2.29	18.6	---	---

with isogenic lines in which the viability of the normal homolog is known. These tests are in progress.

The effects of bz-x and sh-bz-x mutations on crossing over in flanking regions were tested in crosses of sh Bz wx/Sh bz-x Wx and Sh Bz wx/sh-bz-x Wx heterozygotes as pollen parents with sh bz wx testers. The results of these crosses are listed in columns 6-9 of Table 2. Both $bz\text{-}x_1$ and $bz\text{-}x_2$ reduce crossing over in the Sh-Bz interval, but only the $bz\text{-}x_1$ alteration has any effect on the region between Bz and Wx. In heterozygotes containing the sh-bz-x alterations, no exchanges occurred in the Sh-Bz interval. The $sh\text{-}bz\text{-}x_1$ mutation reduces crossing over between Bz and Wx to the greatest degree of all the mutants. On the other hand, $sh\text{-}bz\text{-}x_2$ appears to increase slightly the frequency of exchange in this region over that found in normal sibs. The difference is small but statistically significant.

Self-pollinations of sh-bz-x/Sh Bz individuals were made to determine whether or not plants homozygous for these alterations are viable. Since these plants were heterozygous for the a_1 allele, the sh phenotype was used as an indication of homozygote viability. No self pollinations were made with the bz-x mutants since functional pollen carrying the mutant allele was either not produced or very rare. Intercrosses of sh-bz-x/Sh Bz and bz-x/Bz individuals were also performed to determine the viability of plants heterozygous for the various mutations. Results of these crosses are listed in Table 3. Progeny from self-pollinations of $sh\text{-}bz\text{-}x_1$/Sh Bz plants consisted of less than 1000 kernels, none of which were sh in phenotype. If homozygotes of this mutant are viable, their survival rate is too low to be detected in a population of this size. Data in Table 3 indicate that $sh\text{-}bz\text{-}x_2$ has little or no effect on the viability of homozygous sporophytes. In compounds of $sh\text{-}bz\text{-}x_2$ with the other mutations, the chromatin present in the $sh\text{-}bz\text{-}x_2$ homolog is sufficient to support growth of the heterozygotes to varying degrees.

Extensive cytological examinations of sporophytes heterozygous for each of the mutations and a normal homolog revealed no abnormal pairing in the short arm of chromosome 9; however, known deficiencies such as the a-x mutations reported by Stadler and Roman have no visible effect on synapsis in chromosome 3. Consequently, the cytological observations

Table 3

Tests of viability of sh-bz-X2 homozygotes and of sh-bz-X1/sh-bz-X2, bz-X1/sh-bz-X2 and bz-X2/sh-bz-X2 compounds

Cross	Plant #	Progeny		% sh kernels	X^2
		Sh	sh		
sh-bz-X2 Wx / Sh Bz wx ⊗	1451-1	311	106	25.4	0.04
	1457-1	357	105	22.8	1.27
	1467-3	411	116	22.0	2.51
	1467-4	245	68	21.7	1.79
sh-bz-X1 Wx / Sh Bz wx ♀♀ x sh-bz-X2 Wx / Sh Bz wx ♂♂	1486-1	239	45	15.8	
		Bz	bz	% bz kernels	
Sh bz-X1 Wx / sh Bz wx ♀♀ x sh-bz-X2 Wx / Sh Bz wx ♂♂	1443-1	242	34	12.3	
	1443-2	172	29	14.4	
	1443-3	185	17	8.4	
Sh bz-X2 Wx / sh Bz wx ♀♀ x sh-bz-X2 Wx / Sh Bz wx ♂♂	1447-1	210	27	11.3	
	1447-2	278	45	13.9	
	1448-1	265	36	11.9	

of the present study do not rule out deficiencies as the cause of the mutations.

The effects of $bz\text{-}x_1$, $bz\text{-}x_2$ and $sh\text{-}bz\text{-}x_1$ on gametophyte viability and crossing over indicate that the three mutations constitute deletions of varying size. On the other hand, since $sh\text{-}bz\text{-}x_2$ does not reduce crossing over in the Bz-Wx interval, and since mutant homozygotes are viable, the origin of this mutation remains open to speculation. McClintock (1956) reported four cases of simultaneous loss of Sh and Bz expression induced by Ds. It was demonstrated that three of these mutations represented deletions. The behavior of the fourth, however, was

strikingly similar to that of sh-bz-x_2. McClintock stated that crossing over within the affected segment of some mutant heterozygotes was reduced in varying degrees or completely absent. However, crossover reduction in the fourth mutant was not specifically mentioned.

If sh-bz-x_2 represents suppression of the two alleles by a controlling element, the mechanism by which this element inhibits crossing over may be similar to that of recombination genes.

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1. Observed and "expected" heterosis in interracial crosses of maize.

When epistasis is negligible, heterosis of an interpopulational hybrid can be expressed as a function of gene frequencies and dominance effects.

An attempt is made to compare actual heterosis values for yield in 9 interracial crosses of corn with calculated heterosis parameters. Observed heterosis ($\hat{h}_{ij}$) was measured as the excess in yield of a hybrid over the midpoint between its parents. Experiments conducted in three locations with three rep./location provided the yield averages. Based on knob frequencies obtained by Kato (1964) and Blumenschein (1968) and on hypothetical dominance values, "expected" heterosis parameters were computed as follows:

$$h^*_{ij} = \sum_k \left[(p_{ik} - p_{jk})^2 + 2 \cdot \bar{\Delta}_{ijk} \right] \cdot \delta_k$$

p_{ik} being the knob frequency [of any type of knob (large, medium or small)] at chromosome position k for population i; $\bar{\Delta}_{ijk}$ is the average Hardy-Weinberg disequilibrium parameter for populations i and j and chromosome position k, i.e., $\bar{\Delta}_{ijk} = (1/2)(\Delta_{ik} + \Delta_{jk})$, and Δ_{ik} = the observed frequency of homozygous knobbed plants minus the corresponding expected frequency under Hardy-Weinberg equilibrium, for position k.