

The phenotypes of the Sh<sub>2</sub> class in this cross, line 4, and in those in lines 5 and 6, have been omitted from the table because there are a number of different types and these would be difficult to arrange in this table. None of these, however, is A<sub>1</sub> in phenotype. States 7977B and 7995, lines 5 and 6, also produced some A<sub>1</sub> sh<sub>2</sub> kernels on the testcross ears. An active Spm was not present in the heterozygous parents. In some crosses, it was introduced into many kernels by the a<sub>1</sub> sh<sub>2</sub> pollen parent that also was homozygous for wx. Some of the plants in lines 5 and 6 had wx<sup>m-8</sup> in one chromosome 9. Three of the 10 A<sub>1</sub> sh<sub>2</sub> kernels in lines 5 and 6 received wx<sup>m-8</sup> from the ear parent and Spm from the pollen parent. The A<sub>1</sub> expression in these three kernels was completely stable but that of the wx gene was not. wx<sup>m-8</sup> responded to the introduced active Spm by producing a number of endosperm sectors exhibiting various levels of Wx gene action.

Lines 7 and 8 of Table 1 are included to illustrate that no A<sub>1</sub> sh<sub>2</sub> kernels appeared in testcrosses conducted with a state of a<sub>1</sub><sup>m-5</sup> having an Spm<sup>W</sup> associated with it. This Spm<sup>W</sup> undergoes frequent mutation to a state that allows early occurring transposition and thus early occurring mutations to high alleles of A<sub>1</sub>.

Whether or not a controlling element may be removed from a locus by crossing over may well depend on the "state of the locus", as suggested by the data in Table 1, and also upon the organization of components in the comparable region of the homologue.

Two other studies aimed at removing a controlling element from the vicinity of the genes it can affect are reported below.

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## 2. Attempts to separate Ds from neighboring gene loci.

Early in the study of transposition of Ds to various locations within the short arm of chromosome 9, two instances of its insertion just distal to Sh<sub>1</sub> were found, the first instance in 1948 and the second instance in 1949. In both instances, Ds remained in this location thereafter. Although it did not transpose away from this location, it responded to Ac by producing dicentric chromatids and also a series of changes affecting the genes located to either side of it. The types of change were described in the Carnegie Institution of

Washington Year Books Nos. 51 to 55 covering the years 1952 to 1956. In the presence of Ac, one of these changes induced a modification affecting the adjacent proximal chromosome segment carrying the genes Sh<sub>1</sub> and Bz<sub>1</sub>. Gene expression of Sh and Bz was nullified. In the presence of Ac, however, return to high levels of Bz gene action occurred but no changes to Sh expression were ever noted. Tests indicated that the segment Ds sh bz<sup>m</sup> was inherited as a unit, as illustrated by the data given in Table 2. It may be mentioned that the presence of this unit has a strong influence on crossing over in the chromosome segment proximal to it. Studies of this were made, initially, with 10 sister plants. Five of these were Ds sh bz<sup>m</sup> Wx/Sh bz wx; no Ac in constitution, and five had normal chromosomes 9 with the markers sh bz Wx and Sh bz wx. The ears of these plants received pollen from plants that were homozygous for C, sh, bz, and wx. Crossing over between Sh and Wx amounted to 24.6% (3,332 kernels) in the former plants and 12.6% (3,423 kernels) in the latter plants. Crossing over between the Ds sh bz<sup>m</sup> unit and C was near normal, amounting to 4.1% in a total of 5,470 kernels on testcross ears.

Altogether 16 plants were examined, each derived from a kernel that had received a germinal Bz mutant. These Bz kernels appeared on ears produced by crosses similar to those shown in Table 2. Tests were conducted with these 16 plants and extended tests were conducted with the progeny of four of them. These tests indicated that in each case, the mutation to Bz was not accompanied by removal of Ds nor did the responsible event alter the unit of inheritance which now was Ds sh Bz. In the presence of Ac, dicentric chromatid formations occurred just distal to the locus of Bz. Return to bz<sup>m</sup> expression also occurred in all well examined cases although the frequency of this varied with the different mutants. In the absence of Ac, however, Bz gene expression was completely stable. The Bz mutants differed from one another and from the original Bz in strength of Bz gene expression. Crossing over between the Ds sh Bz unit and Wx again was very high, amounting to approximately 25% in backcross tests using the heterozygote as an ear parent, and approaching 30% when the heterozygote was used as a pollen parent.

In order to determine whether or not Ds could be removed from the vicinity of the mutant Bz locus by crossing over, tests were conducted with plants that were C Ds sh Bz Wx/C Sh bz wx and had no Ac. These were used as ear parents in crosses with plants that were homozygous for C, sh, bz, and wx and had no Ac. Among a total of 16,578 kernels this cross produced, the

Table 2  
 Phenotypes of Kernels on Ears of Plants That Were  
C Sh bz/C Ds sh bz<sup>m-4</sup> in Constitution and Had 1 Ac,  
 Not Linked with These Markers, Produced by the  
 Cross with Plants That Were Homozygous for  
C, sh, bz, and wx, and Had No Ac

	Pigment in Aleurone Layer		
	Totally <u>Bz</u>	Spots of <u>Bz</u> in a <u>bz</u> Background	Totally <u>bz</u>
<u>Sh</u> <sub>1</sub>	0	0	9,177
<u>sh</u> <sub>1</sub>	9	4,291	4,656

Table 3  
 Phenotypes of Kernels on Ears Produced by  
 Reciprocal Crosses Between Plants That Were  
C Sh Bz Wx/C sh bz wx in Constitution and  
 Had No Ac With Plants That Were Homozygous For  
C, sh, bz, and wx and Had No Ac or Were  
 Homozygous for c, sh, bz, and wx and Had One or More Ac

Parentage of hetero- zygote	Phenotypes of Kernels								Total
	<u>Sh Bz</u> <u>Wx wx</u>	<u>Sh bz</u> <u>Wx wx</u>	<u>sh Bz</u> <u>Wx wx</u>	<u>sh bz</u> <u>Wx wx</u>					
case I									
Ear	1,201	318	1	13	17	0	332	1,185	3,067
Pollen	1,546	527	2	15	23	2	606	1,401	4,122
case II									
Ear	91	24	0	2	2	0	23	101	243
Pollen	397	157	0	7	8	1	149	356	1,075

following phenotypes appeared: 8,337 sh Bz : 1 sh bz : 2 Sh Bz : 8,238 Sh bz. The one sh bz kernel was wx and the two Sh Bz kernels were Wx. Plants were grown from each of the latter two kernels. Both plants were C Sh Bz Wx/C sh bz wx in constitution and had no Ac. Thus, contamination was excluded as the reason for the phenotype of the kernel producing each of these plants. Both plants, and also the progeny of one of them (case I, Table 3), were crossed reciprocally with plants that were homozygous for C, sh, bz, and wx, and had no Ac and with other plants that were homozygous for c, sh, bz, and wx, and had one or more Ac. No evidence was given in the latter cross of the presence of Ds in the C Sh Bz Wx chromosomes. The phenotypes of the kernels that appeared on the ears produced by these crosses is given in Table 3. Transmission of the C Sh Bz Wx chromosomes is normal through pollen and egg and crossing over between the marked intervals conforms with that expected to occur between two normal chromosomes 9. It is evident from this series of tests that removal of Ds, or its effects, has restored normal crossover potentials between the loci of Sh and Bz.

That crossing over may have removed Ds in the above described cases is supported by a much more extensive series of studies that were conducted with selected progeny of plants carrying I Ds Sh Bz in this order in both chromosomes 9 and also Ac. Seven independent instances of Ds-induced nullification of gene action in the chromosome segment immediately distal to Ds, and including the I locus, were isolated and each examined extensively. (Descriptions of these cases are given in the previously mentioned Carnegie Institution Year Books.) The events responsible for these nullifications did not remove Ds. It remained just distal to Sh. The null segment in each case behaved as if it were a deficiency although no evidence of deficiency was given by the meiotic prophase chromosomes. It was decided to use these 7 cases in order to determine if crossing over could occur between the nullified region and Ds or between Ds and Sh. These tests were conducted in 1955 and 1956 but were not reported earlier because a part of the study was never completed.

Plants with no Ac that had a chromosome 9 with the null region and also the markers Ds Sh Bz Wx, and a normal homologue with the markers C sh bz wx, were used as ear parents in crosses with plants that were homozygous for c, sh, bz, and wx, and had no Ac. The resulting ears were examined for kernels in the Sh class that were Bz pigmented and for kernels in the sh class that were colorless. These were the kernels of importance to this study. The results of these tests are given in

summary form in Table 4. The percent crossing over between Sh and Bz (region 2) and Bz and Wx (region 3) observed in these tests is also given for reference. In the three cases where the same testcross was conducted both in 1955 and 1956 (cases 1, 4, and 7 in the table) a striking degree of consistency was noted with each case in the amount of crossing over that occurred in both years within each of the three tested regions.

Plants were grown in the summer of 1956 from the types of kernels indicated in the last three columns of Table 4. Tests conducted with the plants derived from the C Sh Bz Wx kernels, and continued with their progeny, were aimed at determining the following: presence or absence of Ds in the C carrying chromosome, percent crossing over between C and Sh, degree of transmission of the C Sh Bz Wx chromosome through the pollen, and the phenotype of the seedlings that are homozygous for this chromosome. Because the number of C Sh Bz kernels was significantly larger than the number of colorless, sh kernels in cases 3, 4, and 7, either contamination or some other cause was suspected to be the reason for this. Apparently, this is true. Three of the plants derived from the 20 selected C Sh Bz Wx kernels (1 from case 4 and 2 from case 7) had the same constitution as the ear parent plant. Another kernel produced a plant that had the null segment and Ds Sh Bz Wx in one chromosome 9 and c sh bz wx in the homologue. The Bz phenotype in the kernel producing this plant probably resulted from the action of blotched on the c gene as blotched segregated in one of the tested ears of this plant. A strong expression of blotched appears occasionally and unexpectedly in the cultures. The phenotype of the remaining 16 kernels did not result from contamination or misclassification. Each had received a chromosome 9 from the heterozygous parent with the markers C Sh Bz Wx. No evidence was given of the presence of Ds in any one of these 16 chromosomes. All appeared to be quite normal. Crossing over occurred with expected frequencies between the marked intervals, and the homozygotes were normal in appearance. It was concluded that each of these 16 chromosomes was produced by a crossover that had occurred between Ds and Sh.

All of the plants derived from the 14 colorless sh kernels were bz in phenotype. One plant was very small and produced no pollen or ear. Testcross ears were obtained from the remaining 13 plants, and from their progeny. All 13 plants had received a chromosome 9 with the null segment and also sh and bz. The presence of Ds in this chromosome was detected in the progeny of 4 of these initial 13 plants. Its exact location was not

Table 4  
See Text for Explanation of Contents of Table

Case No.	Year of Cross	Total No. of Kernels	Phenotypes of "crossovers" in region 1		Percent crossing over <sup>†</sup> Regions			Phenotypes of selected kernels						
			<u>C</u>	<u>Sh Bz</u>	<u>c</u>	<u>sh</u>	1	2	3	<u>C</u>	<u>Sh</u>	<u>Bz</u>	<u>Wx</u>	<u>c</u>
													<u>Wx</u>	<u>wx</u>
1	1955,1956	14,989		5	8	0.06	2.7	24.4		3				3
2	1956	7,261		6	10	0.16	2.9	27.4						
3	1956	6,066		17	8	0.54	2.4	31.2						
4	1955,1956	12,910		15	8	0.23	2.0	24.2		8				2
5	1956	7,391		14	14	0.35	2.0	31.5						
6	1956	7,100		11	8	0.31	2.3	29.4						
7	1955,1956	15,078		39	32	0.51	3.6	16.6		9		1		8
Totals		70,795		107*	88 <sup>†</sup>					20		1		13

† Calculated from the C carrying classes of kernels.

\* Includes 5 double crossovers, regions 1 and 3

+ Includes 5 double crossovers, regions 1 and 3

determined although it could be placed distal to the Wx locus. Tests of the presence of Ds in this chromosome in the progeny of the remaining 9 plants were not completed.

The project was discontinued at this juncture even though crosses had been made to obtain plants with proper constitution to determine the location of Ds in the chromosomes carrying the null segment. The main questions -- whether crossing over occurs and where this may occur -- appeared to be answered by the results already obtained. It was occurring, and between Ds and Sh and not between the null segment and Ds. At the time, it was considered that the rewards that could be expected by pursuing this project would be too meager to justify the considerable amount of effort involved in the pursuit. It should be emphasized, however, that this Ds, in the presence of Ac, causes modification in expression of Sh, located proximal to it, and this has occurred to Sh in those chromosomes that have the null segment located just distal to Ds.

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1. An analysis of chromosomal behavior during meiosis in asynaptic maize: Distribution of bivalents.

The expression of the asynaptic gene is highly variable, bivalents per cell ranging between 0 and 10. Swaminathan and Murty (Genetics 44: 1271-1280, 1959) made the interesting observation that although variation in bivalent frequency follows a binomial or Poisson distribution when the mean value per cell is low, marked deviation from a binomial distribution can be noted when this value is high and approaches half of the potential number of bivalents. This was explained on the assumption that certain pairs of homologous chromosomes entered into bivalent association more frequently than others. These authors based their conclusions on an analysis of