recombination for the $\underline{A_2}$-$\underline{Bt_1}$-$\underline{Pr}$ region in the megasporocytes when B chromosomes were present. Furthermore, it appears that the B chromosomes had a dosage effect. The increase in crossing over and the dosage effect in both the $\underline{A_2}$-$\underline{Bt_1}$ and $\underline{Bt_1}$-$\underline{Pr}$ regions were more marked in the microsporocytes than in the megasporocytes and are similar to those obtained by Rhoades for the $\underline{C}$-$\underline{Wx}$ region of Tp9 plants. In the latter case, however, there was a corresponding decrease in the recombination value for the adjacent $\underline{Yg_2}$-$\underline{C}$ region of chromosome 9, which would indicate a shift in the distribution of crossovers along the chromosome arm (cf. "Replication and Recombination of Genetic Material", pp. 229-241. Eds. W. J. Peacock & R. D. Brock. Austral. Acad. Sci., Canberra, 1968). Ayonoadu & Rees (Genetica 39:75) have reported indications of an altered distribution of chiasmata and have found an increase in the total number of chiasmata, due to B chromosomes in Black Mexican Sweet Corn. The increased recombination in the $\underline{A_2}$-$\underline{Bt_1}$-$\underline{Pr}$ region of chromosome 5 could thus be the result of a shift in the distribution of crossovers, an increase in the total amount of crossing over, or a combination of both.

Paul Nel

5. Further studies on chromosome elimination induced by supernumerary B chromosomes.

In the 1967 Maize News Letter and in a paper appearing the same year in the Proc. Nat. Acad. Sci., data were presented showing a correlation between the number of B chromosomes and the rate of loss of the $\underline{A_1}$ allele in chromosome 3 at the second division of the microspore. In plants with low numbers of B's there was little or no loss of the $\underline{A}$ marker while in individuals with higher numbers of B's this locus was eliminated in 10% or more of the sperm cells. The earlier data did not provide a good estimate of the dosage effect of B's on loss of the $\underline{A}$ locus. Not all of the classes were represented and the data were fragmentary in some cases. Rather extensive data have since been obtained from a set of closely related plants in which the numbers of B chromosomes ranged from none to eight. The frequencies of F_1 endosperms exhibiting the recessive $\underline{a}$ phenotype in crosses of $\underline{a}\ \underline{a}$ ♀ x $\underline{A}\ \underline{A}$ ♂ where the pollen parents differed in numbers of B's are given below:

No. of B's in pollen parent	% kernels with recessive phenotype	Population size
0	0.1	1568
1	0.2	2412
2	0.2	5784
3	4.9	8490
4	11.1	10680
5	12.5	5493
6	13.3	3520
7	11.4	5280
8	13.7	2393

These data suggest that there is no significant increase in loss of the A locus when the number of B's is greater than four. The data further suggest that in this material loss of the A gene frequently takes place in microspores with two or more B's and that it rarely occurs in microspores with one or no B's. If loss of the A locus is limited to spores with two or more B's then the frequency of loss in 3 B plants should be about one-half of that in 4 B plants since in 3 B plants approximately 50% of the spores would have 2 B chromosomes and 50% would possess 1 B. It is assumed that the rate of elimination of A in the 2 B spores from a 3 B plant is the same as that in 2 B spores from a 4 B individual. Disjunction at meiosis in a 3 B plant is not invariably 2 by 1 since trivalents are not always formed and the number of 2 B spores would be somewhat less than that of 1 B microspores. If the loss of A takes place in 2 B microspores and not in 1 B spores, the rate of loss in 3 B plants should be slightly less than one half of that in plants with 4 B's where 2 by 2 disjunction at meiosis is believed to occur regularly. The observed loss rates of 4.9% in 3 B plants and of 11.1% for 4 B plants are in accord with the above assumptions.

Since the A locus is near the distal end of the long arm of chromosome 3, its loss could signify that only the distal portion of the long arm is missing. In order to test for the extent of the deletions, crosses were made in 1966 of gl lg a x Gl Lg A pollen from plants known to give a high frequency of loss for the A locus. We reported (PNAS 57: 1626-1632) that 4.7% of the seedlings from kernels with colored aleurone

were gl lg. The A allele could not be scored in these exceptional F_1 seedlings but it was assumed that most, or all, were hemizygous for the a allele. Since the Gl locus lies in 3L close to the centromere and Lg is between Gl and A, the exceptional gl lg seedlings, which were assumed to be a, came from the loss of most or all of the long arm. Cytological studies of somatic prophases of the exceptional gl lg plants disclosed that 27 individuals had 19 chromosomes of the regular complement plus a short fragment. The fragment chromosomes were apparently telocentric and almost certainly consisted of the short arm of 3, the long arm with the dominant marker genes having been eliminated. However, five exceptional gl lg plants had 19 chromosomes and no fragment, indicating the complete loss of chromosome 3. In short, these limited data indicated that sperm may be deficient for all of chromosome 3, but much more frequently are deficient for only the long arm.

In addition to the gl lg exceptions described above there were five exceptional gl lg plants with 20 chromosomes (no fragment). The suggestion was made that these were compensating types in which chromosome 3 was monosomic and another chromosome of the complement was trisomic. This is almost surely not the case; not only would such an explanation demand a high frequency of newly originating trisomes but they would have to arise in the same spore division where elimination of chromosome 3 occurred. Furthermore, segregation at anaphase must be nonrandom in that the pole disomic for one chromosome is deficient for chromosome 3.

The following hypothesis provides a more reasonable explanation, one that is in accord with all available data. We have demonstrated that the Gl locus lies in the .1-.25 segment of 3L. Breaks in the long arm proximal to Gl result in deficient chromosomes 3 consisting of 3S and portions of 3L of varying lengths. In somatic prophases it would be difficult to distinguish these modified chromosomes 3, which are not telocentric, from the shorter members of the regular complement. Consequently, these gl lg exceptions would be scored as possessing 20 A chromosomes and only examination of the meiotic prophases would disclose their true constitution.

Our published conclusion that breakage of knobbed chromosomes is restricted to the centric region is at variance with the above hypothesis

to account for the gl lg plants with 20 A chromosomes and no recognizable fragment. This conclusion is undoubtedly in error. Although the centric region is a weak spot that is susceptible to rupture by tension, it is now apparent that breaks can occur throughout the length of the long arm of a knobbed chromosome 3.

This past summer information of a genetic nature was obtained about the types and frequencies of chromatin loss induced by supernumerary B chromosomes in chromosome 3. Pollen from plants of a high loss line homozygous for dominant alleles at the D_1 Lg_2 and A_1 loci was applied to silks of d_1 lg_2 a_1 testers. The D locus is in the short arm of 3 and the Lg and A loci are in the long arm. The kernels with colored aleurone and the exceptional kernels with the recessive a phenotype that arise from sperm cells deficient for the A locus were planted in the field and the ensuing plants scored for pollen sterility and the dwarf and liguleless phenotypes. A plant that is d Lg A represents loss of the D allele in 3S, a D lg a plant loss of the Lg and A alleles in 3L, and d lg a individuals arise from loss of D in 3S and of Lg and A in 3L. Data from the cross of d lg a x D Lg A pollen from high loss plants are as follows:

colored kernels

D Lg N	D Lg ♣	D lg ♣	D Lg 3N	d lg
899	30	87	9	4

Forty-four of the D lg ♣ plants were testcrossed. Forty-three gave only a progeny, indicating loss of both Lg and A. One gave a low A ratio, indicating an internal deficiency in 3L including Lg but not A. Twenty D Lg ♣ plants were testcrossed. Twelve gave 1:1 A:a ratios, indicating a normal chromosome 3 from the pollen parent. Six gave only colorless kernels, indicating loss of A but not of Lg. Two gave low A ratios, indicating deficiency not including Lg or A.

colorless kernels

D Lg N	D Lg ♣	D Lg 3N
129	14	1

Six D Lg ♣ plants were testcrossed; all gave 1:1 A:a ratios.

Of the 91 lg plants, 87 were D and only 4 were d. These data confirm the conclusion reached in our earlier studies that loss of both arms

occurs much less frequently than does chromatin elimination from only the long arm. The 43 D lg a ♀ plants do not necessarily contain telocentric 3S chromosomes. The ratio of D:d in testcross populations will provide information on the amount of proximal 3L chromatin in the modified chromosomes deficient for Lg and A. The percentage of D plants in the testcross progenies of individuals with a normal chromosome 3 and a telocentric 3S is a measure of the recombination between D and the centromere. Higher percentages of D will occur in the progeny of those individuals heterozygous for a modified chromosome 3 possessing a proximal segment of 3L.

The 6 D Lg ♀ plants deficient for A, the 2 D Lg ♀ individuals giving low A ratios in testcrosses, and the D lg ♀ plant with the A locus present in the deficient chromosome provide unequivocal evidence that breaks in chromosome 3 are not restricted to the centric region although they occur there in a disproportionately high frequency.

A reexamination of the 1966 data lends further support to this conclusion. Among the hypoploid plants of lg phenotype were 13 offtype individuals which did not appear to be gl but classification was uncertain. These were not included in the data reported in the 1967 paper. It now appears likely that these plants were actually Gl lg and had lost only the distal portion of 3L.

In 1967 we advanced the hypothesis that knobs may be incompletely replicated at the second microspore division if B chromosomes are present and that anaphase separation is prevented by the conjoined knobs, leading to bridge formation and subsequent rupture at late anaphase. However, our earlier data were interpreted to indicate that bridge breakage was adjacent to the centric region and it was not clear why breakage should be so restricted in dicentric bridges resulting from faulty knob replication while no such restriction occurs in bridges coming from inversion crossing over. This argued against the validity of our hypothesis. We now know, however, that rupture is not restricted to the centric region and the hypothesis becomes more plausible.

All of the data presented above came from the original high loss strain which was relatively homozygous since it had been maintained by inbreeding for several generations before the loss phenomenon was

detected. Although our data clearly show that the knobbed chromosome 3 is subject to elimination at the second microspore division and that no increase in rate of loss of knobbed 3 occurs when more than four B's are present, a number of problems remain to be resolved. One is the effect of the genetic background on rate of loss, a second is whether B chromosomes from other strains are as effective in inducing loss as are the B's found in the original high loss line, and a third is whether the K3L knob in the high loss line differs in its response to B chromosomes from K3 knobs in unrelated strains.

In order to provide at least a partial answer to some of these questions a cross was made using a Black Mexican plant with nine B's onto silks of an individual from the high loss line which had 1 B chromosome and which gave no loss of the A gene in appropriate tests. All of the F_1 plants were heterozygous for the K3 knob contributed by the high loss parent and carried varying numbers of B chromosomes, of which only one at most would be a B from the high loss line. Less than half of the F_1 plants would possess the single B of maternal origin. The number of B's present in the tested F_1 plants varied from none to eight. The A allele was homozygous. The F_1 plants were used as the pollen parent in crosses with a testers and the percentage of A loss determined. The data are as follows:

No. of B's	% A loss	Population
0	0	913
1	0.08	2407
2	0.1	1450
5	1.1	3341
6	2.8	1799
7	3.6	1461
8	2.1	413

If there were no effect of the genetic background contributed by the Black Mexican parent and if all B's were equally potent in inducing loss of knobbed chromosome 3, the percentage of A loss in, for example, the 5 B class should be one-half that found for 5 B plants of the high loss strain. The former are K3 k3 and the latter K3 K3; loss of A in homozygous K3 K3 plants should be twice that in K3 k3. However, only 1.1%

of the kernels from crosses of the Black Mexican-high loss F_1 were colorless while 12.5% of the kernels from 5 B plants of the original high loss line were colorless. Since the K3 knob was identical in both types of crosses, the low rate of A loss of the F_1 plants cannot be attributed to a knob difference in susceptibility to loss. The Black Mexican line may have contributed a set of genetic modifiers for low loss rate that were partially dominant to the genome of the high loss line or else the B chromosomes derived from Black Mexican are not very effective in inducing loss of A chromatin even when the number of B's is as high as seven or eight. No distinction can be made as yet between these two explanations of the low loss rate produced by the F_1 individuals. Any confusion arising from the fact that some of the F_1 plants had a single B from the high loss line will be eliminated this summer when F_1 individuals from the cross of Black Mexican with B's by a no-B plant extracted from the high loss line will be tested.

In the experiments described in the preceding paragraphs, the F_1 plants had 50% of their genes from the high loss line and hence would possess modifiers favoring high rate of loss. A further test of the possible existence of genetic modifiers came from testing a plant with 4 B's which arose from the cross of a B-containing plant of Black Mexican with an F_1 heterozygote of Kys and a Nicaraguan strain with high knob number. The tested plant with 4 B's was heterozygous for K3 knobs and might be expected to exhibit loss of the A marker. However, only one A loss was found in a population of 1883 and self contamination has not been excluded. Here again we cannot distinguish between an inhibiting effect of the genetic background and impotency of the B's from Black Mexican to induce loss of the knobbed chromosome 3. It is also true that the K3 knob from the Nicaraguan strain may differ from the K3 knob of the high loss strain in response to induced loss by B chromosomes.

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6. Preliminary studies of the effect on crossing over of the gene ameiotic.

Bianchi (MNL 1959) reported an "asynaptic" condition which, when heterozygous, apparently resulted in a slight increase in crossing over