ON THE MECHANISM OF CHROMATIN LOSS INDUCED BY THE B CHROMOSOME OF MAIZE

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ABSTRACT

Knobbed regions of the regular maize complement frequently are eliminated at the second microspore division in spores which have two or more B chromosomes. Evidence is presented that no or little loss occurs in spores with one B and that the rate is not increased in spores with more than two B's.——The B chromosomes from an unrelated strain proved as effective in inducing loss as did the B's of the original high loss stock.——Chromatin loss induced by B's is restricted to knobbed A chromosomes and occurs only at the second microspore division. Knobbed chromosomes 3, 5, and 9 have been tested and all interact with B's to give loss. Chromosomes with large knobs are more frequently broken than are those with smaller knobs and knobless chromosomes show negligible loss.——Although knobs and B's are essential for chromatin elimination, modifying genes can markedly affect the rate of loss.——Two knobbed heterologous chromosomes undergo simultaneous loss more frequently than expected from independent events. The data indicate that joint loss occurs in competent cells and that preferential assortment of the two deficient chromosomes to specific poles is unlikely.——B chromosomes and deficient chromosomes assort independently at the second microspore anaphase.——Genetic data from crosses with marker genes in both arms of chromosome 3 show that breakage of the postulated dicentric bridge does not occur solely at the centric region since a variety of deficient chromosomes were recovered.——Nondisjunction of B chromosomes and elimination of knobbed chromatin take place during the second microspore mitosis. The argument is advanced that the two phenomena result from faulty replication of heterochromatic segments. The position of the nonreplicating segment in the two kinds of chromosomes determines whether nondisjunction or breakage takes place.——Finally, it is suggested that all of the reported effects of the B chromosome can be accounted for if the B is a parasitic entity having no genetic function other than controlling the replication of its proximal heterochromatic knob and increasing the ability of B-containing sperm cells to compete successfully for fertilization of the egg.

AMONG the challenging problems awaiting solution by the maize geneticist is an understanding of the function and origin of the supernumerary B chromosome. The B is unique in several ways. It has no synaptic homology with members of the normal complement (the A set) and differs in its morphology, consisting in large part of heterochromatic blocks which are deeply stained by the Feulgen reaction. The B chromosome of maize replicates in the later part of the S phase of the mitotic cycle (ABRAHAM and SMITH 1966; HIMES 1967), it undergoes nondisjunction at a specific cell division (ROMAN 1947) and it was

held to have no or little genetic activity despite a heavy charge of DNA. The concept of genetic inertness was primarily based on RANDOLPH's (1941) observation that sister plants with and without B's were not perceptibly different in phenotype although a marked reduction in stature and fertility was produced when the number of B's was in excess of 12.

The first evidence of a specific genetic effect of B's in maize was the slight but significant increase in recombination rates observed by HANSON (1965, 1969). NEL (1971) found that the increased recombination induced by B's was most pronounced in the proximal segments of certain of the A chromosomes. A more striking example of the effect of B's on crossing over came from a study of a strain with a chromosome 3 segment intercalated into the short arm of chromosome 9. In plants homozygous for the transposed segment (*Tp9/Tp9*) the percentage of crossing over between two loci flanking the inserted piece was more than doubled in 1B compared to 0B plants. Furthermore, a dosage effect was observed as the number of B's was increased from one to three (RHOADES 1968). The effect of B's on recombination has also been demonstrated by a study of chiasma frequencies in maize plants with varying numbers of B's (AYONOADU and REES 1968). The cytological results are consistent with the genetic data in showing increased chiasma formation in plants with higher numbers of B's.

Unexpected and unrelated to the effects on crossing over described above was the discovery that all or parts of a knobbed chromosome 3 were frequently eliminated at the second microspore mitosis when several B chromosomes were present. Knobless chromosomes 3 were unaffected by the B's and no loss of knobbed (or knobless) chromosomes occurred in the development of the female gametophyte. In the preliminary account of this phenomenon (RHOADES, DEMPSEY and GHIDONI 1967) the following questions were raised: (1) Do the knobs on all of the A chromosomes interact with B's? (2) Does the rate of loss reach a plateau when the numbers of B's attain a certain level? (3) What is the relationship of knob size, position, and number to the rate of loss? (4) Will heterotic hybrids coming from the cross of a high knobbed with a high B strain produce progenies, when used as pollen parents, with a high proportion of hypoploid individuals having reduced vigor and high sterility?

The answers in full or in part to these and other questions are given in this communication. Consideration is also given to the ways in which the B chromosome might mediate these genetic effects.

DOSAGE EFFECT OF B CHROMOSOMES

In our earlier studies a correlation was found between the number of B chromosomes and the rate of loss of the A_1 (aleurone color) 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 *A* marker while in individuals with higher numbers of B's this locus was eliminated from one of the two sperm cells in 20% or more of the microspores. The earlier data did not provide a good estimate of the dosage effect of B's on loss of the *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 *a* phenotype in crosses of *aa* ♀♀ × *AA* ♂♂ where the pollen parents differed in numbers of B's are given below:

Number of B's in pollen parent	Percent kernels with recessive phenotype	Population size
0	0.1	1568
1	0.2	2412
2	0.2	5486
3	4.8	9529
4	11.1	11312
5	12.6	5631
6	13.3	3885
7	8.8	5245
8	13.7	2393

These data suggest that there is no marked increase in loss of the *A* locus when the number of B's in the paternal parent is greater than four. The data further suggest that 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 3B plants should be about one-half of that in 4B plants since in 3B plants approximately 50% of the spores would have 2B chromosomes and 50% would possess 1B. It is assumed that the rate of elimination of *A* in the 2B spores of a 3B plant is the same as that in 2B spores of a 4B individual. Disjunction at meiosis in a 3B plant is not invariably 2 by 1 since trivalents are not always formed and a single unpaired B may lag on the spindle and fail to be included in a telophase nucleus. Thus, the number of 2B spores would be somewhat less than that of 1B microspores and the rate of loss in 3B plants should be slightly less than one half of that in plants with 4B's if 2 by 2 disjunction at meiosis occurs regularly in 4B plants. The observed loss rates of 4.8% in 3B plants and of 11.1% in 4B plants are in accord with these assumptions. However, even 4B plants may undergo occasional 3:1 disjunction of the B's or accidental loss of single B's. Thus, a small fraction of spores in the 4B plants may receive only 1B and experience no elimination of A chromatin. The proportion of spores with less than two B's would be smaller in paternal parents with five or more B's. The slight increase in the average rate of loss in the 5–8B group (12.1%) compared to the 11.1% loss of the 4B can be ascribed to a greater frequency of spores having the requisite number of two B chromosomes necessary for induction of loss. In these data there is no evidence that rate of loss is enhanced in spores with more than two B's.

SUBSTITUTION OF B CHROMOSOMES

In the original study the B chromosomes present in the "high loss" strain were found to be the agents responsible for chromatin elimination. Unanswered was the question whether or not B chromosomes from other strains of maize

TABLE 1

Loss of A *in microspores of plants containing Black Mexican B's*

F_1 hybrids from the cross (Black Mexican sweet corn, *k*3 *k*3, with B's × high loss line, *K*3 *K*3, 0B's) were continuously backcrossed to the 0B high loss stock. Plants involved in the backcrossing program are indicated with an asterisk. Loss frequencies were determined by testing each plant as pollen parent with *a a* female parents.

Bl Mex 29717 × 0B high loss 29704-31

F_1 — 30119

Plant	#B's	Knob	Σ	%Loss A
*11	6	Kk	1079	1.3
5	5	Kk	767	3.1
1	4-5	Kk	336	2.7
4	5	Kk	479	1.3
8	5-7	Kk	1595	2.4

BC_1 — 30804

Plant	#B's	Knob	Σ	%Loss A
7	6	KK	2363	5.7
15	6	KK	1716	7.9
18	4	KK?	1351	1.3

Bl Mex 29717 × 0B high loss 29704-32

*29987-1 5B's

No Data

30123B

Plant	#B's	Knob	Σ	%Loss A
*10	6	KK	2425	9.2
12	6	KK	2143	3.5
6	4	KK	1439	5.8
2	4	KK	2099	3.3
3	2	KK	1225	0.0
*15	5	Kk	477	0.8
1	2	Kk	2418	0.4

BC_2

*10 — 30805

Plant	#B's	Knob	Σ	%Loss A
1	6-8	KK	278	7.6
6	4	KK	2514	4.6
*3	5-7	KK	421	8.6
4	4	KK	392	10.2
5	6-7	KK	215	6.1
9	6	KK	544	8.1
*13	3-4	KK	858	15.0
14	5-6	KK	379	12.7

*15 — 30803

Plant	#B's	Knob	Σ	%Loss A
1	6	Kk?	997	4.0
16	4	--	1129	8.1
8	4	--	892	8.9
*9	6	KK	751	8.0
*12	6	KK	518	21.8

BC_3

*3 — 31386

Plant	#B's	Knob	Σ	%Loss A
3	4	KK	1607	15.0
9	6	KK	4983	9.2
8	?	KK	1801	9.6
10	4	KK	1077	9.5

*13 — 31385

Plant	#B's	Knob	Σ	%Loss A
2	2	KK	408	0.2

*12 — 31387

Plant	#B's	Knob	Σ	%Loss A
8	4	KK	2644	10.7
7	2	KK	1949	1.6
20	0	KK	1127	0.0

*9 — 31388

Plant	#B's	Knob	Σ	%Loss A
8	4	KK	526	8.7

would be equally effective in inducing loss if introduced into the high loss line. Plants of the high loss line devoid of B's were tested for loss of the *A* locus. None occurred. These 0B individuals were then crossed by plants of the Black Mexican variety known to possess B chromosomes. Both chromosomes 3 of the F_1 plants had the A_1 allele, but the one derived from the high loss parent had a large knob (*K*3) in the long arm while the other chromosome 3 was knobless (*k*3). Varying numbers of B's were found in the F_1 plants, which were tested as the pollen parent for loss of the *A* gene. As can be seen in Table 1, loss of the *A* allele did occur in the microspores of the *K*3 *k*3 plants but at a rate 50% less than that found for *K*3 *k*3 plants of the high loss line with comparable numbers of B's. Although the data demonstrate that B's from Black Mexican were able to induce a low rate of loss, they do not discriminate between the hypothesis of relatively inefficient B's and that of modifiers for low loss brought in by the Black Mexican parent. The issue was resolved by the following series of crosses.

Individual plants with varying numbers of B's in the first, second, and third backcross generations of crosses to the 0B parent would have the great majority of their genes from the high loss line. The data in Table 1 show that the rate of loss in plants with four or more B's, all from Black Mexican, equalled and in some cases apparently surpassed the average loss rate of plants of the original high loss line with comparable numbers of B's. It may be concluded that the B chromosomes derived from the Black Mexican variety are as capable of producing chromatin loss as are the B's found in the high loss line when placed in a comparable genetic background. The data further indicate that the Black Mexican line carried modifiers which tended to depress the rate of loss. For example, sib plants 30123B-10 and -12 of the first backcross generation of an F_1 plant with 5B's, each having 6B chromosomes and homozygous for the *K*3 knob, gave markedly different rates of loss for the *A* allele (9.2% *vs* 3.5%). The 9.2% rate of *A* loss for plant 10 approaches that previously found for 6B plants of the original high loss line. The difference in loss rate between plants 10 and 12 cannot be attributed to sampling errors and may be ascribed to differences in the constellations of modifying genes in the two plants. The hypothesis that the B chromosomes derived from Black Mexican comprised a heterogeneous group consisting of both inefficient and efficient B's and that the low rate of plant 12 reflects the sequestering of ineffective B's is not in accord with the data in Table 1. Plant 30123B-15, a plant in the first backcross generation, had 5B chromosomes and a low rate of loss. If this were due to ineffective B's and not to modifiers, plants of the second backcross generation would also have a low loss rate. Second backcross generation plants 30803-1, -8, -9, -12 and -16 had B's derived only from 30123B-15 but contained more of the high loss background. They gave high rates of loss. When placed in a favorable genetic environment, the supposedly inefficient B's induced as high a rate of loss as the B's of the high loss stock.

Confirmatory evidence for modifiers was obtained by crossing the original high loss line with four unrelated genetic strains. F_1 plants with various numbers of B's from each of the four crosses were tested for loss of the *A* allele (Table

TABLE 2

Loss of A in F_1's of crosses involving the high loss strain and various other stocks

F_1	Pedigree	Number of B's	Chromosome 3*	Percent *A* loss	Σ
High loss / High knob	30418	4	K^L/K^M	1.0	1924
High loss / KYS/Nic.	30138	4	K^L/K^L	0.3	1011
High loss / KYS	29730	4	K^L/k	0.1	2766
High loss / In 3a	29736	1	K^L/K^L	0.4	943
High loss / In 3a	29736	3	K^L/K^L	1.6	3475
High loss / In 3a	29736	4	K^L/K^L	2.3	433
High loss / In 3a	29736	5	K^L/K^L	1.3	2278
High loss / In 3a	29736	7	K^L/K^L	3.8	1083
High loss / In 3a	29736	8	K^L/K^L	2.9	916

* K^L = large knob, K^M = medium sized knob, k = knobless.

2). In one combination the *K*3 knob was heterozygous but it was homozygous in the other three. Although the number of B's in most of the plants was sufficient to induce 11–12% of *A* loss in the parental high loss line, a marked but variable reduction in rate of loss was found in the four hybrid combinations. When one hybrid was tested as the pollen parent, 0.3% of the kernels had colorless aleurone while plants of a different F_1 hybrid had an average loss rate approximately eight times greater. Both combinations were homozygous for the large knob on 3L. The data become intelligible if it is assumed that the four lines differed in their assemblages of modifying genes affecting chromatin loss.

An attempt to synthesize a new high loss line from two unrelated stocks was made by combining the B's from one strain with the knobs from another. Hybrids were obtained between the essentially knobless Black Mexican line having several B chromosomes and a strain with many knobbed chromosomes but no B's. F_1 plants from this cross having four or more B's were tested for loss of specific knobbed chromosomes present in a heterozygous condition. The extremely low rate of loss found for all tested knobbed chromosomes (3, 4, and 5)

suggests that the presence of both B's and knobs is a necessary but not a sufficient condition to induce loss; the B's and knobs interact only when combined with the proper constellation of modifying genes.

In our 1967 paper, we proposed that the negative correlation between knob number and frequency of B chromosomes reported by LONGLEY (1938) in diverse strains of maize could be a result of selection against plants with both knobs and B chromosomes where loss of chromatin was expected to occur. The demonstration that modifying genes can inhibit the knob-B interaction makes this suggestion unlikely. Moreover, the reality of the correlation is in question since it was not found in a recent study (LONGLEY and KATO 1965).

KNOB CONSTITUTION AND LOSS

Chromatin elimination in the high loss strain involved loss of a large knob on chromosome 3. It was believed that loss occurred only when the large knob was present in a spore and not when a knobless 3 was present. Further evidence suggesting that knobless chromosomes do not undergo loss comes from a comparison of two closely related families of the high loss stock as shown below:

	K3 K3				*K3 k3*		
Pedigree	# B's	% A loss	Σ	Pedigree	# B's	% A loss	Σ
29703	3	4.8	9529	30131	3	2.0	1625
29703	4	11.1	11312	30131	5–7	4.1	4077

As mentioned earlier, loss occurs only in spores with two or more B's and the loss rate in 3B plants is one half that found in 4B sibs where nearly all spores contain 2B's. In addition, the plants heterozygous for a knob on chromosome 3 (*K3 k3*) should give half as much loss as the homozygotes (*K3 K3*) if knobless chromosomes do not undergo loss. The data support this assumption.

The interaction between B chromosomes and heterochromatic knobs resulting in chromosome loss is not restricted to the large knob on chromosome 3. A second large knob, the terminal knob on 9S, was introduced into the high loss strain and tests of loss were made with plants heterozygous for the large knob (K^L9) and a much smaller knob (K^s9), as well as with K^L9 homozygotes. In the former experiments it was possible to study the effect of knob size by comparing rates of loss of the C_1 marker (colored aleurone) from chromosomes bearing a large or small knob (genetically marked by the closely linked Yg_2 and yg_2 alleles which give green and yellow-green seedlings, respectively). In crosses of *yg c* ♀ ♀ × K^L9 *Yg C*/K^s9 *yg C* ♂ ♂, loss of chromosome 9 occurred in the endosperms of 126 kernels in a population of 1402. The embryos of these kernels gave rise to 86 *Yg* (green) and 14 *yg* (yellow-green) seedlings. Since both chromosomes are present in the same plant, the two chromosomes are subject to the same group of modifying genes for loss. The great majority of kernels in which a loss had occurred in the endosperm had the K^L marker in the embryo. In the sperm uniting with the polars the K^L chromosome was missing while the sister sperm uniting with the egg possessed the K^L chromosome. Thus, spores with the large knob undergo loss

much more frequently than those with the small knob. The disproportionate loss of the large knob may be underestimated since some of the 14 yellow-green seedlings could have arisen from heterofertilization or from self-contamination. In testcrosses of K^LK^L homozygotes, where both chromosomes carry *Yg* and *C*, the frequency of loss of *C* in the endosperm was 19.0% and loss of *Yg* in the embryo was 11.4% in a population of 790. Kernels with colorless endosperm invariably gave rise to *Yg* sporophytes and *yg* seedlings came only from *C* kernels. In every case where a deficient chromosome was produced, there was a noncorrespondence in genetic constitution of the embryo and endosperm. It is obvious that the large knob on chromosome 9 interacts with B chromosomes at the second microspore division in a manner similar to that shown by the chromosome 3 knob.

One difference was noted between the chromosome 3 and chromosome 9 data in the phenotypes of kernels exhibiting loss. In addition to the completely colorless kernels arising from sperm lacking the *A* or *C* alleles and the occasional fractional kernel with a colorless sector caused by loss of the dominant alleles during endosperm development, a class of kernels with mosaic (colored-colorless) aleurone was found in the chromosome 9 experiments. These arise when a broken chromosome 9 with the *C* allele undergoes the bridge-breakage-fusion cycle described by McCLINTOCK (1938). No kernels with a bridge-breakage-fusion pattern of *A-a* mosaicism were found in the chromosome 3 studies. This is understandable because the *A* gene lies distal to the knob in chromosome 3 while the *C* locus is proximal to the terminal knob in 9S. All of the breaks in chromosome 3 occur proximal to the *A* locus giving a deficient chromosome without the *A* allele. This chromosome may undergo a bridge-breakage-fusion cycle in the endosperm, but a mosaic pattern of colored-colorless areas could not arise since the *A* allele is missing from the deficient chromosome. However, when a knobbed chromosome 9 undergoes loss, two kinds of deficient chromosomes are produced since breaks occur either proximal or distal to the *C* locus. A break in the short arm of chromosome 9 between the centromere and the *C* locus yields a chromosome missing the *C* allele. It will produce a wholly colorless aleurone as it undergoes a cryptic bridge-breakage-fusion cycle. However, when breaks take place between *C* and the terminal knob, the deficient chromosome has the *C* allele. An aleurone mosaic for colored-colorless sectors results as the *C* allele is lost during the bridge-breakage-fusion cycles in endosperm development. In a population of 1994 kernels, 281 were whole kernel losses for the *C* locus and 19 had a bridge-breakage-fusion pattern. The 281 whole kernel losses come from breaks in 9S proximal to *C* and the 19 mosaics (6.8% of the kernels receiving a broken chromosome 9) come from breaks between the *C* gene and the terminal knob.

In the breeding program incorporating K^L9 into the high loss strain, various generations arising from self pollination or backcrossing of $K^L9\ K^S9$, $K^L3\ k3$ plants were studied. Certain of the progeny having varying numbers of B chromosomes and different knob constitutions were tested as male parents on chromosome 9 and chromosome 3 tester stocks. The results of these crosses are given in Table 3. It is evident that a large knob is necessary for loss since the *k*3 *k*3 plant

TABLE 3

Loss of A *and* C *observed in crosses of* a a *or* c c *female parents with pollen parents varying in* K3 *and* K9 *constitution and in modifying genes affecting loss*

Male parent	Number of B's	Chromosome 3	Percent *A* loss	Σ	Chromosome 9	Percent *C* loss	Σ	Modifiers
30117–25	4–5	K^L/K^L	10.5	247	K^S/K^S	0.9	2550	+
30115–4	6	K^L/K^L	0.2	1775	K^L/K^L	0.1	4264	—
30115–5	3	k/k	0.3	745	K^L/K^S	6.3	1080	+
30116–3	3	K^L/k	0.2	574	K^L/K^L	0.8	3574	—
30822–10	6–7	K^L/k	6.4	1756	K^L/K^S	7.2	607	+
30822–1	6	K^L/K^L	12.9	970	K^S/K^S	0.2	407	+
30822–5	9	K^L/k	2.6	1820	K^L/K^S	2.1	848	±
30822–28	6–8	K^L/K^L	6.3	926	K^L/K^S	1.3	552	±
30822–14	7	K^L/K^L	2.1	422	K^L/K^S	1.1	753	—
30822–23	*ca.* 8	K^L/K^L	7.9	481	K^S/K^S	0.7	535	+
30822–24	6	K^L/K^L	3.3	1067	K^S/K^S	0.5	866	±
30822A–10	4	K^L/K^L	0.4	1970	K^L/K^S	0.2	946	—

(30115–5) and the four K^S9 K^S9 compounds showed negligible loss rates for *A* and *C*, respectively. When both large knobs were heterozygous (in K^L3 $k3$, K^L9 K^S9 compounds, see 30822–5 and –10), the rate of loss is about the same for the *A* and *C* markers. However, when the chromosome 3 knob is homozygous and the K^L9 heterozygous, a higher rate occurs for *A* than for *C*.

Considerable variation was found in the frequency of loss among the different plants in Table 3, even though all contained sufficient numbers of B's to induce loss in plants of the high loss strain. A low rate of loss has been observed in the F_1 generation of outcrosses of the high loss stock, and in subsequent backcross and F_2 generations there appears to be segregation for modifiers enhancing loss. Plus modifiers are presumed to be absent in plant 30822A-10, for example, which has four B's and large knobs on 3 and 9 but shows negligible loss of the marker genes. Inspection of the various plants in Table 3 shows that when the loss rate is reduced by absence of plus modifiers, knobbed chromosomes 3 and 9 are both affected. Because of the segregation of plus and minus modifiers, it is impossible at present to determine unambiguously whether or not the presence of K^L9 detracts from the loss potential of K^L3. However, that it does not is indicated by the data from plant 30822-10, heterozygous for both knobs, which had a rate of loss for K^L3 at least as high or higher than that found in K^L3 $k3$ plants of the original high loss strain.

Other knobs which have been shown to interact with B chromosomes include medium sized knobs on chromosomes 4 and 5. Both knobs exhibit a low rate of loss, perhaps due to their smaller size. The data on chromosome 4 loss are fragmentary, but results from tests with chromosome 5 are presented in the next section.

SIMULTANEOUS LOSS OF TWO KNOBBED CHROMOSOMES

While the experiments with chromosomes 3 and 9 give information on loss of

two knobbed chromosomes in the microspores of single plants, they do not reveal whether simultaneous loss of both knobs tends to occur in certain spores and, if this is true, whether the two deficient chromosomes are distributed randomly to the two sperm cells. A test for simultaneous loss of chromosome 3 and 5 markers was made in the following way. Endosperm loss of the Sh_2 and *Pr* alleles*, in the long arms of chromosomes 3 and 5 respectively, was followed in crosses of *sh/sh*; *pr/pr* ♀♀ × *Sh/Sh*; *Pr/Pr* high loss ♂♂. The male parents had a large knob in 3L and a smaller knob in 5L. The Sh_2 locus is distal to the knob in 3L and *Pr* is proximal to the knob in 5L. Breaks in chromosome 5 between the *Pr* locus and the knob resulted in mosaic kernels as a consequence of the bridge-breakage-fusion cycle while breaks proximal to the *Pr* locus gave whole kernel deficiencies. Both types of kernels were scored as *Pr* losses. In a population of 2937, 79.3% of the kernels were *Pr* and *Sh* in phenotype. Loss of *Sh* only occurred in 14.9% of the kernels, loss of *Pr* only in 3.3%, and loss of both *Sh* and *Pr* in 2.5% of the kernels. The expected frequency of simultaneous loss if the two knobs are independently affected by B chromosomes is 5.8% (total *Pr* loss) × 17.4% (total *Sh* loss) or 1.0%. The observed frequency of double loss was 2.5%, which is 2.5 times the expected frequency. Among kernels showing loss of *Pr*, nearly half (42%) also exhibit loss of *Sh*, instead of the expected 17.4%. This nonrandom association of loss events in two chromosomes can be explained in two ways: 1) Simultaneous loss of both markers in the sperm fusing with the polar nuclei would be frequent if the two deficient chromosomes preferentially migrated to the same pole at the second microspore division. 2) Simultaneous loss would also be high if loss events were clustered in certain (competent) spores where conditions favored chromosome breakage, with losses occurring infrequently in the remaining spores of the population. Within the competent spores, loss of the two chromosome segments would occur independently and there would be no preferential assortment of deficient chromosomes to specific poles.

Both of these hypotheses would predict a frequency of simultaneous loss higher than that calculated for randomly occurring events. However, the second possibility would also lead to an unexpected association of loss events involving nonhomologous chromosomes where the two deficient chromosomes pass to opposite poles (*i.e.*, one event is detected in the endosperm and the other in the embryo of the same kernel). The experiment described above involving simultaneous loss of *Pr* and *Sh* in the endosperm does not discriminate between the two explanations. Preferential association of endosperm and embryo losses was not detected since only the endosperm phenotypes were scored. However, the data presented in Table 5 can be analyzed for unexpected association between loss of chromosome 3 in the sperm uniting with the polars and loss of another chromosome (probably 2 or 5 since both carried medium sized knobs) in the sperm fusing with the egg to form the embryo. The latter class is detected by pollen and ovule sterility in plants giving *1:1 A:a* ratios. The percentage of semisterile plants with normal chromosomes 3 among the progeny derived from *a* kernels, where loss has occurred in the endosperm, was 13.5% (52/386). The percentage of

* The *Pr* allele gives a purple colored aleurone and *pr* a red; Sh_2 yields a plump and sh_2 a shrunken endosperm.

semisteriles in the progeny coming from *A* kernels and resulting in *A* plants (*i.e.*, the class exhibiting no loss of chromosome 3) was 2.8% (72/2738). The pollen grains involved in the production of *a* kernels had experienced loss of chromosome 3 from one sperm and this group of grains apparently had a high rate of loss of a heterologous chromosome in the sister sperm cell. On the other hand, in those pollen grains with no loss of *A*, there was a low frequency of loss for a second chromosome. While the results in the chromosome 3-chromosome 5 study can be accounted for by either hypothesis 1 or 2, the data in Table 5 are best explained by hypothesis 2. It is therefore concluded that microspores vary in their capacity for inducing loss and that multiple loss events involving either or both of the two sperm cells tend to be clustered in a portion of the microspores.

ASSORTMENT OF B'S AND DEFICIENT CHROMOSOMES

Loss of knobbed A chromosomes occurs only in microspores with two or more B chromosomes. Since both nondisjunction of B's and loss of A chromatin take place at the second microspore division and since loss is controlled by B chromosomes, a relationship between the two events might be expressed as a nonrandom assortment of the deficient chromosome with respect to the nondisjoining B's. Preferential passage of the deficient chromosome to the pole with the nondisjoined B's could be construed to indicate a nexus between the knobbed A and the B at metaphase.

A test for nonrandom assortment was made by a study of chromosome numbers in the root tips of progeny from the following cross:

$$\frac{lg\ a}{lg\ a}\ 0B\,♀\,♀ \times \frac{Lg\ A}{Lg\ A}\ \text{high loss}\ ♂\,♂$$

Some of the male parents had three and some had four B's. Following meiosis in a plant with 4B's, nearly all of the microspores possess 2B's. When each B "nondisjoins" toward opposite poles the two sperm cells both receive 2B's. When the two B's nondisjoin to the same pole, one sperm receives 4B's and the other none. Preferential fertilization of the egg by the 4B sperm with a frequency of about 66% produces a 1:2 ratio for 0B:4B classes in the embryos. Preferential fertilization involving 0B *versus* B-containing sperms does not show a great variability in different stocks (ROMAN 1948; CARLSON 1968). The average value of 66% was assumed to be correct. With 100% nondisjunction, the 2B class should equal the sum of the 0B and 4B and a ratio of 1 (0B): 3 (2B): 2 (4B) is expected in the progeny of a 4B pollen parent. Male parents with 3B's will produce approximately 50% microspores with 2B's and 50% with 1B. The latter type gives 0B and 2B progeny in a 1:2 ratio because of preferential fertilization. When this distribution is combined with the output from 2B spores, the 3B males should give a ratio of 3 (0B): 7 (2B): 2 (4B) in the progeny.

Since frequencies of nondisjunction often vary widely (50–100%, ROMAN 1947, 1948; 35–100%, CARLSON 1968, 1969), it was necessary to obtain an estimate of the rate in the high loss stock. In a total of 87 progeny of 4B plants, 14

TABLE 4

Numbers of B chromosomes in the root tip cells of plants derived from crosses of $\frac{\text{lg a}}{\text{lg a}}$ *0B* ♀ ♀ *with* $\frac{\text{Lg A}}{\text{Lg A}}$ *high loss* ♂ ♂ *having three or four B's*

		0B	1B	2B	4B	Σ
3B MALE PARENT						
Group I no loss	obs.	5	0	11	3	19
A kernel, *Lg* plant	exp.	(4.3)	(1.0)	(10.9)	(2.4)	
Group II loss in embryo	obs.	2	0	11	2	15
A kernel, *lg* plant	exp.	(3.4)	(0.7)	(8.6)	(2.3)	
Group III loss in endosperm	obs.	3	2	11	4	20
a kernel, *Lg* plant	exp.	(4.5)	(1.0)	(11.5)	(3.0)	
Σ	obs.	10	2	33	9	54
	exp.	(12.2)	(2.7)	(31.1)	(8.1)	
4B MALE PARENT						
Group I no loss	obs.	8*	0	29	14	51
A kernel, *Lg* plant	exp.	(7.7)	(0)	(28.1)	(15.3)	
Group II loss in embryo	obs.	4	0	4	3	11
A kernel, *lg* plant	exp.	(1.7)	(0)	(6.1)	(3.3)	
Group III loss in endosperm	obs.	2	0	16†	7	25
a kernel, *Lg* plant	exp.	(3.8)	(0)	(13.8)	(7.5)	
Σ	obs.	14	0	49	24	87
	exp.	(13.1)	(0)	(47.9)	(26.1)	

* Includes one plant with 21 A chromosomes.
† Includes three plants with 21 A chromosomes.
Plants having three chromosomes 3 ($2n + 1$) should occur primarily in Group III. The single $2n + 1$ plant in Group I may have been trisomic for a chromosome other than 3. See also Table 5.

had 0B, 49 had 2B's and 24 had 4B's (Table 4). Nondisjunction should result in two 2B sperms as often as it gives a 0B and 4B pair. Thus, the frequency of nondisjunction is $\frac{(14 + 24) \times 2}{87}$ or 87.4%. When the ratios for the various progeny classes are recalculated on the basis of 90% nondisjunction and 66% preferential fertilization, the following expectations are obtained:

	0B	1B	2B	4B
3B male	9	2	23	6
4B male	3	0	11	6

The occurrence of 10% normal disjunction in a 3B plant gives rise to some progeny with a single B; in a 4B plant normal disjunction increases the 2B class so that it is greater than the sum of the 0B and 4B.

The root tip counts on progenies of 3B and 4B male parents are shown in Table 4. The progenies have been subdivided into Group I, showing no loss of the *A* gene, Group II with loss of *A* in the embryo, and Group III with *A* loss in the endosperm. While the total number of plants in any one group is not large, the agreement with the expected ratios is good in progeny arising after a loss event

as well as in those not undergoing loss of chromosome 3. Thus, the data, though fragmentary, suggest that assortment of B's is independent of the assortment of the deficient chromosome 3 at the second pollen mitosis.

PREFERENTIAL FERTILIZATION

Two dissimilar sperm result from chromosome elimination at the second microspore division. One sperm is usually euploid with a normal chromosome 3 and the second has a deficient 3. If the euploid and deficient sperm randomly fertilize the egg and polar nuclei, the percentages of endosperms and embryos with a deficient chromosome 3 should be equal. ROMAN (1947, 1948) demonstrated that, following nondisjunction, the hyperploid sperm with two B^A chromosomes preferentially fertilizes the egg cell while the hypoploid sister sperm with $0B^A$'s unites with the polar nuclei. The results from one test with a high loss plant indicated that the frequencies of deficient endosperms and embryos were not greatly different although the deficient endosperms were somewhat in excess (RHOADES, DEMPSEY and GHIDONI 1967). It appeared from this small sample that the deficient A chromosome, unlike the B and B^A chromosomes, did not cause selective fertilization. More extensive data suggest that this tentative conclusion is erroneous. In general, deficient endosperms are found more frequently than are deficient embryos—*i.e.*, selective fertilization does occur. However the variation found in different crosses is so great that the phenomenon requires further study.

CHARACTERIZATION OF DEFICIENT CHROMOSOMES

Information on the types and frequencies of chromatin loss induced in chromosome 3 by supernumerary B chromosomes was obtained from crosses of d_1 lg_2 a_1 testers by high loss pollen parents homozygous for the dominant alleles. The D_1 locus is in the short arm of chromosome 3 while Lg_2 and A_1 flank a large heterochromatic knob in the distal half of the long arm (see Figure 1). All of the kernels coming from this cross would have colored aleurone (A a a) unless the sperm cell fertilizing the polar nuclei to form the endosperm was deficient for the A allele, in which case the aleurone would be colorless ($-$ a a). Loss of the D and Lg alleles is manifest only in the sporophyte while loss of A may be detected both in the aleurone and in the sporophyte when the complementary factors for anthocyanin formation are present. The kernels with colored aleurone and the exceptional colorless kernels were planted in the field and the ensuing plants classified for pollen sterility, as well as for the dwarf (d), liguleless (lg) and anthocyaninless (a) phenotypes. Pollen examination affords a very sensitive test for deficient segments. Deficiencies so minute as to escape detection in pachytene chromosomes may have a deleterious effect on development of the haploid male gametophyte. It cannot be categorically denied that some minute deficiencies were overlooked but the number is believed to be negligible.

All plants from the exceptional colorless kernels were testcrossed as were those individuals arising from colored kernels which had varying degrees of pollen

TABLE 5

The numbers of plants possessing the indicated chromosome 3 contributed by the pollen parent in crosses of $\frac{d\ lg\ a}{d\ lg\ a}$ ♀ ♀ × $\frac{D\ Lg\ A}{D\ Lg\ A}$ *high loss* ♂ ♂

The deficient loci are represented by enclosing the appropriate recessive gene symbols in parentheses. The symbol *N* stands for an intact chromosome 3 and *Df* for a deficient one.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
D Lg A N	D Lg A Df	D Lg (a) Df	D (lg) (a) Df	D (lg) A Df	(d) (lg) (a) Df	D Lg A N (3n)	D Lg A N (2n + 1)	Σ
Plants arising from *A* kernels								
2425 72* 2497	7	26	166	4	5	32	1	2738
Plants arising from *a* kernels								
312 52* 364	0	0	0	0	0	5	17	386 3124

* Semisterile plants deficient for a chromosome other than 3.

abortion. In some families, every plant, regardless of its phenotype or degree of pollen abortion, was testcrossed. The manifestation of the different recessive phenotypes, either singly or in combination, in conjunction with the breeding behavior in testcrosses reveals the extent of the deficient segments.

A total of 6403 kernels was produced from the cross of *d lg a* × *D Lg A* high loss males. All kernels were planted in the field but, owing to unfavorable growing conditions and attrition due to birds and rodents, the field population consisted of 3124 mature plants. Of the 6403 kernels, 14.1% were colorless. These exceptional kernels come from loss of the *A* allele in the sperm uniting with the polar nuclei to form the endosperm while the colored kernels have the *A* allele in the aleurone. When the progenies from the colored and colorless kernels were analyzed, the data in Table 5 were obtained. Eight classes of offspring were distinguished on the basis of their chromosome 3 constitution. Class (1) includes sporophytes receiving a normal chromosome 3 from the pollen parent and giving 1:1 ratios for the heterozygous alleles when testcrossed. All of the 2497 class 1 individuals arising from colored kernels had no pollen sterility except for 72 with 50% pollen and ovule abortion presumed to be deficient for a chromosome other than 3. The semisterility was not transmitted to the progeny of these plants and therefore was not due to translocation heterozygosity. Deficient chromosomes, on the other hand, would not be transmitted because of gametophyte inviability.

The seven individuals in class 2 had 50% pollen and ovule abortion and gave

low percentages for the segregating *Lg* and *A* alleles when testcrossed. Six were deficient for an internal 3L segment flanked by the *Lg* and *A* loci. The seventh had a small terminal deficiency.

The 26 individuals in class 3 had 50% pollen and ovule abortion and gave only colorless kernels upon testcrossing; these plants possessed a paternal chromosome 3 deficient for the *A* locus but not for *D* or *Lg*. Genetic tests indicate that they are heterozygous for a terminal deficiency including the distal half of 3L. Testcrosses of class 3 individuals gave percentages of *Lg* offspring varying from 6–11. The *Lg* plants arise only after a crossover separates the *Lg* locus and the deficiency. A range in percentage of *Lg* plants would occur if the length of the segment distal to *Lg* varies in the different deficiencies, but the small size of the populations makes the significance of the differences uncertain. The same testcross populations yielding low *Lg* percentages had approximate 1:1 ratios for the *D*:*d* alleles as expected since *D* and *Lg* show about 50% recombination in testcrosses and the deficiency is distal to *Lg*. If the deficiency includes the *K*3 knob as well as the *A* allele with the point of breakage between *Lg* and *K* (See Figure 1), the *Lg–Df* segment should be shorter than the *Lg–K* segment. The 6.5% of recombination between *Lg* and *K* reported by DEMPSEY (1971) is probably an underestimate since it comes from plants with incomplete synapsis of chromosome 3 in the region of knob heterozygosity. The deficiency heterozygotes should show no such crossover reduction and recombination values of 6–11% might well occur in the segment between *Lg* and the breakpoint of a deficiency which includes the knob.

The 166 *D lg a* individuals of class 4, which had the *D* locus in 3S but were deficient for the *Lg* and *A* loci in 3L, had one of the following kinds of chromosome 3: (1) those with a break distal to Gl_2,* (2) those with a break in the proximal region between the centromere and *Gl*, and (3) those coming from a break in the centromere with loss of all of 3L. The percentage of *D* plants in the testcross progeny from individuals with a normal chromosome 3 with the *d* allele and a deficient chromosome with the *D* allele is a measure of the recombination between *D* and the deficiency. When the deficiency includes all of 3L, the percentage of *D* is equal to the *D*-centromere recombination. Individuals with a telocentric 3S should give approximately the same percentage of *D* progeny while higher percentages will occur among the offspring of plants heterozygous for a deficient chromosome possessing a proximal segment of 3L. Sampling errors are unavoidably high in small populations but most of the 43 *D lg a* tested in crosses with *d* plants appeared to constitute a homogeneous group with a mean of 24% *D* individuals in their progenies. If normal pairing occurs between the short arms of the heteromorphic bivalents, it may be concluded that there is about 24% recombination in the *D*-centromere interval. Several of the *D lg a* plants which gave higher percentages of *D* offspring (*ca.* 40%) were assumed to have an acrocentric chromosome 3 consisting of 3S and a proximal portion of 3*L*.

The relative frequencies of the three types of chromosome 3 among class 4 individuals can be approximated. We know from crosses of *gl lg a*♀ ♀ × high loss

* The glossy-2 (gl_2) locus is located in the .1—.25 segment of 3L.

Gl Lg A ♂ ♂ that 20% of 120 plants deficient for the *Lg* locus carried the *Gl* locus. It can be argued that a similar proportion of the 166 *D lg a* chromosomes (33) arose from a break distal to *Gl*. Furthermore, some *gl lg a* exceptions are not deficient for all of 3L. We reported in the 1967 paper that five of 37 *gl lg a* exceptions, whose root tips were examined, had an acrocentric chromosome instead of a telocentric 3S. It is believed that they consisted of 3S and a portion of 3L proximal to the *Gl* locus. Since the cytological location of the *Gl* locus is distal to .1 and proximal to .25 in the long arm, acrocentric chromosomes deficient for the *Gl*, *Lg*, and *A* loci could arise. Eighteen of the class 4 individuals are estimated to be of this type. The majority (115) of the *D lg a* exceptions are believed to stem from breaks in 3L adjacent to the centromere and giving rise to telocentric chromosomes, but a considerable fraction come from more distally located breaks and result in acrocentrics. All 166, however, represent loss of the region of 3L possessing the prominent knob.

Four *D lg A* semisterile individuals were found which gave low *A* ratios when testcrossed. These class 5 individuals had internal deficiencies in 3L involving the *Lg* locus but not that of *A*. Since the *K*3 knob is closely linked but distal to the *Lg* locus, it too presumably was included in the missing segment.

The five individuals in class 6 possess none of the dominant marker genes. Since *D* is in the short arm and *Lg* and *A* are in the long arm, the *d lg a* plants are evidently monosomic for chromosome 3, having only the one contributed by the female parent homozygous for recessive alleles. Of the 171 plants in Table 5 deficient for both the *Lg* and *A* loci, 166 were *D lg a* and 5 were *d lg a*. Some of the *D lg a* plants may not be hemizygous for all of 3L but the data demonstrate that loss of both arms of chromosome 3 occurs much less frequently than does elimination of all or part of the knob-bearing long arm. A similar conclusion was reached in our 1967 paper.

Somewhat surprising was the occurrence of 32 triploids of class 7, which comprised 1.2% of the total progeny. In 31* of the 32 triploids, the embryos were of *A A a* constitution indicating that the pollen parent had contributed a diploid genome to the zygote as a consequence either of dispermy or of the fertilization of the egg by a diploid sperm produced by nondisjunction. It has not been possible to decide between the two possibilities. There appears, however, to be no association between chromosome loss induced by supernumerary B chromosomes and the induction of triploidy. The data listed in Table 5 are derived from several subfamilies. In one subfamily where the percentage of *A* loss on the ears was 9.4%, there were 18 triploids in a field population of 765 (2.4% 3n) while in another subfamily with 19.5% of *A* loss on the ears there were only six triploids (0.8%) in a population of 773. Although the mode of origin of triploids in crosses involving high loss pollen parents remains uncertain, it is apparently unrelated to the mechanism responsible for the deletion of chromatin from knob-bearing members of the regular complement. This conclusion is substantiated by

* One of the 32 triploids had a 1:1 ratio of colored to colorless kernels. This individual was disomic for chromosome 3 and could have arisen if one of the two sperm cells uniting with the egg was deficient for the *A* locus or if a diploid sperm with a deficient chromosome 3 fertilized the egg.

the observation that the frequency of triploids coming from the exceptional colorless kernels is equal to that from colored kernels.

Only one trisomic individual (class 8) was found in the progeny from colored kernels. None was expected since the passing of two chromosomes 3 to one sperm cell would automatically produce a second sperm cell with no chromosome 3 which, when it fertilized the polar nuclei, would yield a kernel with colorless endosperm. Heterofertilization may account for the single trisomic plant.

The progeny arising from the exceptional *a* kernels are shown in the lower part of Table 5. The noncorrespondence in genotype of endosperm and embryo in all 386 individuals coming from colorless kernels clearly implicates the second microspore mitosis as the time when the *A* locus is deleted from one chromosome 3 but not from the other. The two resulting sperm cells are consequently dissimilar and in testcrosses give rise to kernels with a colored endosperm and a colorless embryo or the reciprocal combination. Individuals in class 1 had a normal chromosome 3 with all of the dominant marker alleles of the pollen parent and gave in testcrosses the expected 1:1 ratios for all three heterozygous loci. Although 52 or 13.5% of the 364 class 1 plants had a normal paternal chromosome 3 they were nevertheless semiserile because of the loss of all or part of another chromosome of the complement. Only 2.8% of the plants from the colored kernels had a normal chromosome 3 but were semisterile due to hemizygosity of another chromosome. The significance of this difference is discussed elsewhere.

The most striking difference in the progenies coming from colored and colorless kernels is the absence of classes 2–6 in the *a* population. Loss of the *A* locus of chromosome 3 does not occur in both endosperm and embryo of single kernels. Self contamination of the recessive female parent could give rise to individuals with apparent loss of chromosome 3 markers in both endosperm and embryo, but none was found in this experiment.

Class 7 includes five *A A a* triploids arising from colorless kernels in which the zygote received the diploid number of chromosomes from the pollen parent. As stated earlier, there is no difference in the frequency of triploids from colored and colorless kernels.

Class 8 consists of those individuals trisomic for chromosome 3 and disomic for the other members of the complement. A higher percentage of plants trisomic for chromosome 3 was found in the *a* group than in the *A* population. The trisomics contain two *A*-bearing chromosomes from the male parent. If nondisjunction at the second microspore division were responsible for the production of sperm deficient for chromosome 3, one sperm cell would have no chromosome 3 and the other would be disomic. When the former fertilized the polar nuclei and the latter the egg, the resulting kernel would have colorless aleurone and a 2n + 1 embryo. Only 18 plants trisomic for chromosome 3 were found in the total population of 3124 and 17 of these came, as expected, from kernels with colorless aleurone. However, the great majority of the exceptional colorless kernels had diploid embryos. Loss of chromosome 3 is only occasionally accompanied by nondisjunction. The 17 class 8 individuals consisted in part of primary trisomics, in

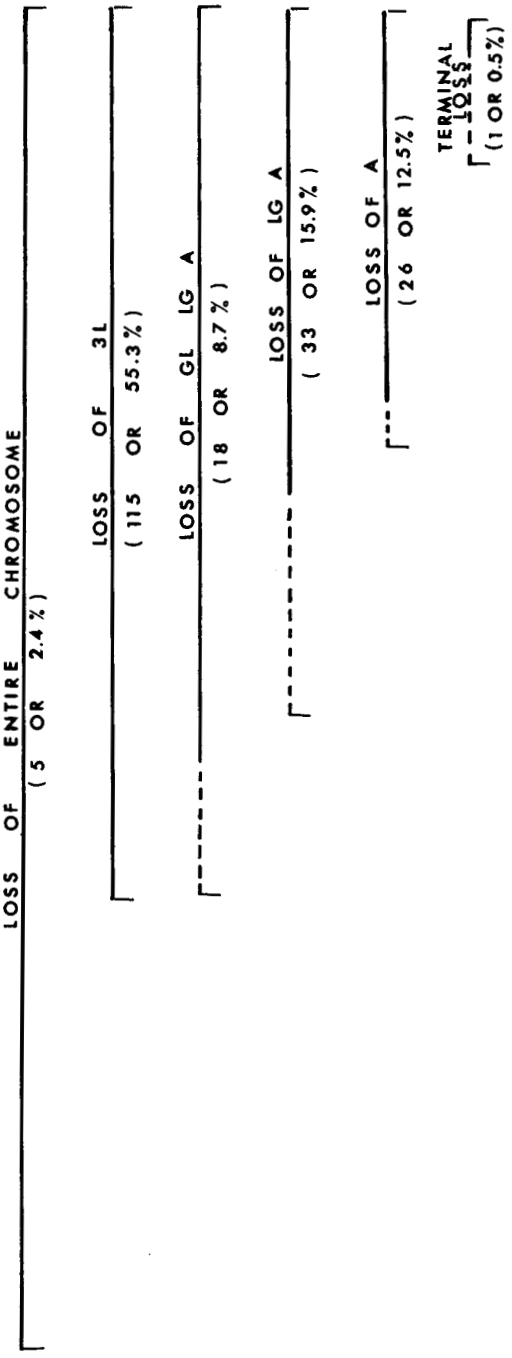

FIGURE 1.—Postulated regions of chromosome 3 lost from 208 deficient chromosomes. Extent of deficiencies determined from genetic data. Broken lines indicate segments in which breakpoints have not been precisely localized. See text for derivation of frequencies.

which the extra chromosome was an entire chromosome 3, and in part of those hyperploid for 3L. The former gave trisomic ratios for markers in both the short arm (*D*) and the long arm (*Lg* and *A*) while the latter gave disomic ratios for *D:d* and trisomic ratios for *Lg* and *A*. Both types have been found but all 18 have not yet been tested sufficiently to permit their assignment as primary trisomics or trisomics for 3L.

The reciprocal combination in which the embryo receives no paternal chromosome 3 and the endosperm two paternal chromosomes 3 presumably is represented by class 6 individuals from *A* kernels. The sporophytes are deficient for all of the dominant markers, but the presence of an addiitonal chromosome 3 in the endosperm cannot be detected.

The rare breaks distal to the *A* locus (only one in a population of 3124 chromosomes) account for the absence of F_1 kernels with a mosaic endosperm of colored and colorless patches. Such kernels would be produced when a deficient chromosome with the *A* allele underwent a bridge-breakage-fusion cycle. Endosperms receiving a chromosome 3 with a terminal deficiency coming from a break proximal to the *A* locus would undergo a bridge-breakage-fusion cycle which would not be detected on a colorless background. A significant observation is that kernels mosaic for *A-a* were found in the progeny of a high loss pollen parent with an inverted chromosome 3 (*In 3a*) in which the linear order is centromere–*Gl-A-K-Lg* (RHOADES and DEMPSEY 1953). A break between the *A* locus and the knob produces a broken chromosome with the *A* allele which will undergo a bridge-breakage-fusion cycle in the endosperm giving a mosaic kernel with colored and colorless sectors. As mentioned in an earlier section, bridge-breakage-fusion kernels were also observed following breaks in the short arm of 9 between the *C* locus and the terminal knob and when breaks occurred in the long arm of 5 between *Pr* and the knob.

Delimitation of the size and location of the deficiencies produced by chromatin elimination should be instructive in elucidating the loss mechanism. The postulated breakpoints involved in the origin of the various classes of modified chromosomes are shown in Figure 1. Although the majority of the chromosome breaks are at or close to the centromere, resulting in the loss of the long arm, they are by no means restricted to this region, occurring at different positions in 3L. Infrequently, the entire chromosome 3 is lost. In all but one of the 208 cases of a modified chromosome 3, genetic studies indicate that the deleted segment included the large heterochromatic knob. Since chromatin elimination occurs only in knobbed chromosomes and generally if not invariably results in loss of the knobbed segment, the knobs must play a vital role in the mechanism by which deletions occur.

THE MECHANISM OF CHROMATIN LOSS

Two hypotheses were advanced to explain the elimination of chromatin segments from knobbed chromosomes (RHOADES, DEMPSEY and GHIDONI 1967). The "faulty replication" hypothesis was suggested by analogy with the cytolog-

ical mechanism responsible for nondisjunction of B's in rye (MUNTZING 1954; LIMA DE FARIA 1962). In Secale, the two chromatids of a B undergo nondisjunction at the first microspore division because two regions, one in each arm equidistant from the centromere, fail to separate at anaphase. Nonseparability is controlled by a knob-bearing segment of the long arm distal to the region which fails to disjoin. This apparent stickiness at two specific sites may result from late replication. The loss of A chromosomes in maize is restricted to those possessing heterochromatic knobs and happens only when B's are present. It was postulated that the knobbed region fails to replicate normally in the second spore mitosis and that the undivided knob prevents anaphase separation. A dicentric bridge is formed as the two centromeres move to opposite poles. Breakage of the bridge will take place if the knobbed region remains effectively single during anaphase. It was formerly thought that rupture took place only at the centromere and it was difficult to see why bridge breakage should be so restricted. We now know that breaks at different positions give rise to a variety of deficient chromosomes. Breaks are not limited to the centric region. The faulty replication hypothesis becomes more plausible.

The alternative explanation, which may be called the fusion hypothesis, assumed that knobs of A chromosomes adhere to the heterochromatic segments of a B during the second microspore mitosis. Normal disjunction of the A chromatids at anaphase would be inhibited by their attachment to a nondisjoining B and the resulting stress could produce deficient chromosomes. On this hypothesis the passing of the nondisjoining B's to a specific pole should be correlated with segregation of the deficient chromosome. Data presented in Table 4 indicate that the two events are independent and do not support the fusion hypothesis. If each B chromosome provides a site for fusion with A heterochromatin, spores with 2B's should have twice as much loss as those with 1B. Instead, little or no loss occurred when a single B was present. Moreover, the effect of increased numbers of B's on loss rate is difficult to comprehend on the fusion hypothesis. The rate of loss should increase with higher numbers of B's since more opportunity would exist for physical contact between B's and knobs. No such increase was observed; the rate reached a plateau and was not enhanced in plants having more than 4B's.

On the faulty replication hypothesis, it is assumed that the B chromosomes delay the replication of the A chromosome knobs during the second microspore division. The combined activity of two B chromosomes is necessary to induce loss but no further increase in loss would be expected with additional B's if the product of 2B's reaches a threshold for inhibition of knob replication. Whether or not two B's can cause multiple losses in spores having several knobbed A chromosomes remains to be determined. Modifying genes may affect loss by controlling the time of replication of heterochromatin or by shortening the S period preceding the second microspore division. Presumably, the individual spores would vary in their response to the modifiers and the spores having the most favorable conditions for loss would be the competent ones.

DISCUSSION

The supernumerary B chromosome has been compared to a parasite (OSTER-

GREN 1945). It has no homology to the members of the normal chromosome complement and contributes nothing to the well-being of the host. In the course of its parasitic existence the B has acquired a mechanism insuring its persistence in the population even though it may adversely affect the host. The accumulation mechanism is bipartite: (1) nondisjunction of the B occurs at a specific time in the life cycle of the host and (2) the two B chromatids are preferentially included by various means in the zygote. A number of studies indicate that B chromosomes are not completely inert but have specific effects on recombination (HANSON 1965, 1969; NEL 1971) and chiasma formation (JONES and REES 1967; AYONOADU and REES 1968; JOHN and HEWITT 1965; CAMERON and REES 1967; VOSA and BARLOW 1970) during meiosis and on chromosome breakage in postmeiotic mitosis (RHOADES, DEMPSEY and GHIDONI 1967). They are also known to influence plant viability, fertility, and stature in a number of plants (See BATTAGLIA 1964). Because of their effect on vital cell processes, a passive existence as a parasite seemed unlikely. However, many of the activities of the maize B chromosome can be interpreted as a consequence of its replication, with little transcription or translation of its DNA.

The B chromosome, on this scheme, would have a negative effect on the cell in that the nucleic acid precursors and amino acids needed for its replication would be withdrawn from a limited pool to the detriment of other synthetic processes. The immediate effect on the nucleus would be a reduction in gene activity which would be reflected in an impairment of plant vigor and growth. Reduced amounts of RNA and protein per nucleus and an upward trend in the histone/DNA ratio with increasing numbers of B's in rye were found by KIRK and JONES (1970), who believe that histones produced by the B's suppress genetic activity. In maize, however, nuclei with B chromosomes show no increase in the histone/DNA ratio and the negligible change in the synthesis of chromosomal RNA is indicative that the DNA of B's is repressed (HIMES 1967). We suggest that the B's of maize have little gene activity and that they adversely affect the host either by competing for precursors or possibly by physically interfering with chromosomal movements. Inclusion of B's in a nucleus that is undergoing meiosis might require adaptation by the host to accommodate the late replicating heterochromatin of the B's. The prolonging of the mitotic cycle found in rye after addition of B's (AYONOADU and REES 1968) may extend to the first meiotic prophase and lengthen the time during which recombination takes place. If maize is like rye in this respect, the observed increases in chiasma formation and recombination become comprehensible.

B chromosome nondisjunction at the second microspore mitosis is believed to occur because the proximal heterochromatic knob fails to replicate and is single at anaphase. Control of nondisjunction is vested in a distal segment of the B which must be present if the proximal portion is to undergo nondisjunction (ROMAN 1950). We assume that the DNA of the distal segment becomes active at the second microspore mitosis and produces a gene product which delays replication of the proximal knob. Existing as an unwanted guest who feasts on the cell's resources, a parasitic B would have lost all unnecessary functions and retained only those needed for its survival. Aside from crossover enhancement,

and this may be an indirect effect, only two functions need be ascribed to the maize B chromosome. One is the control of the replication of its proximal heterochromatic knob, which results in nondisjunction, and the second is conferring upon the 2B sperm the ability to preferentially fertilize the egg cell. In the TB-A translocations, where nondisjunction produces one sperm with two B^A chromosomes and one with none, it is the hyperploid ($2B^A$) sperm which more often unites with the egg than does the hypoploid ($0B^A$) sperm. That $2B^A$ sperm are more fit is indicated by CARLSON's (1970) observation that the $2B^A$ sperm preferentially fertilizes the egg cell in those embryo sacs where the polar nuclei have been fertilized by a sperm from another pollen grain (heterofertilization). In this situation the two dissimilar sperm compete for the same egg nucleus and the successful one is usually the $2B^A$ sperm. If the generalization that the more fit sperm preferentially fertilizes the egg rather than the polars is correct, the preferential union of 2B sperm with the egg indicates that the presence of intact B chromosomes also results in a more fit sperm. Thus, control of the essential components of the accumulation mechanism, nondisjunction and preferential fertilization, resides, as might be expected of exogenous chromatin, in the B and not in one of the A chromosomes.

The gradual loss of all functions except those necessary for maintenance, postulated to occur in the case of the B chromosome, is reminiscent of a somewhat analogous situation with the Qβ RNA virus. In the *in vitro* experiments of SPIEGELMAN *et al.* (1967) the only demand made on the virus was that of replication. In their testtube universe, the virus molecules had no need to complete the normal life cycle. By reducing the incubation time between serial transfers, the most rapidly replicating molecules were selected for. As a consequence of repeated transfers, the RNA molecule diminished to 17% of its original size as it discarded dispensable regions and retained only that portion needed for its replication.

The chromatin elimination described in this paper can be explained if the DNA in the distal segment of the B also affects the replication of heterochromatic knobs on the A chromosomes. The induction of loss is fortuitous and simply reflects an overabundance, in spores with two or more B's, of the gene product inducing its nondisjunction. Why should B's undergo nondisjunction and knobbed A chromosomes be broken if late replication of heterochromatin is responsible for both phenomena? A logical answer can be provided. In the case of the B, the unreplicated segment is adjacent to the centromere. The centric region might divide normally but the two sister centromeres are prevented from disjoining to opposite poles by their juxtaposition to a block of unreplicated heterochromatin. The two conjoined chromatids undergo nondisjunction but not fragmentation because dicentric bridges would not be formed. On the other hand, the heterochromatic knobs of A chromosomes are placed at some distance from the centromere. Failure of replication in these regions leads to formation of dicentric bridges as the sister centromeres move to opposite poles. Subsequent rupturing of the bridge at various positions would lead to the different kinds of deficient chromosomes described in this report.

The finding that 1B induces relatively little chromatin elimination while 2B's

cause loss in 20% or more of the spores is not inconsistent with the suggestion of JONES and REES (1969) that B chromosomes of rye show a higher activity when they occur as a pair. However, an antithetical interpretation of the rye data appears more plausible. It can be argued that repression of B activity, rather than enhancement, occurs when they are present in even numbers. For example, rye plants with one B are more drastically affected in straw weight, tiller number and plant weight than are plants with two B's and 3B plants are more adversely affected than 4B. If B's are more active when their number is even, a greater effect on plant phenotype should be found in 2B than in 1B plants, but this was not the case. Likewise, 1B plants have a greater reduction in RNA and nuclear protein and more histone per nucleus than 2B plants. The above data appear more intelligible if the activity of B's is repressed when even numbers are present. The different behavior of odd and even numbers of B's remains an intriguing finding. In addition to the cytochemical and phenotypic effects mentioned above, the zigzag pattern with increasing numbers of B's has been reported in recombination studies in rye (JONES and REES 1967), maize (CHANG and KIKUDOME 1971) and Listera (VOSA and BARLOW 1970).

A difference in loss rates in microspores of our high loss stock with odd or even numbers of B's would be difficult to detect. The haploid microspores produced by plants with five or more B's are a heterogeneous lot with a range in numbers of B's because of an irregular meiotic assortment. In a spore population with various numbers of B's, there is no way of knowing whether the observed losses of knobbed chromatin occurred at different rates in spores with even or odd numbers of B's.

The faulty replication hypothesis can be confirmed or disproved if the time of incorporation of radioactive label into heterochromatic knobs can be determined. Cytochemical and autoradiographic techniques may also provide information bearing on the question of whether or not B chromosomes are parasitic organelles. The outcome of these studies will be awaited with interest.

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