CHROMOSOME ELIMINATION IN MAIZE INDUCED BY SUPERNUMERARY B CHROMOSOMES

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The regular chromosomal complement of maize consists of 20 chromosomes. These are known as the A set and are invariably present in this number in all normal diploid plants. Heteropycnotic regions known as "knobs" are found in characteristic positions on the different A chromosomes, although the number of knobs varies greatly in different strains. Knobless races are known, while others have as many as 14 knobs.[1] A specific knob is as constant in its transmission to later generations as is a mutant gene. No genetic activity has been ascribed to knobs, although judging by their strong Feulgen reaction they have an abundance of DNA.

Some races of maize possess a supernumerary or accessory type of chromosome designated as a B chromosome. Its morphology is unlike that of any A chromosome; there is no evidence of genetic homology with the A set. B chromosomes give a strong Feulgen reaction but have little genetic activity. Plants with low numbers of B's differ slightly from sibs with no B chromosomes. However, when the number of B's is 12 or greater, various modifications are produced including a marked reduction in stature and an increase in sterility.[2] The mitotic behavior of B chromosomes is essentially normal except at the second microspore division where the B's undergo a high rate of nondisjunction.[2-4] DNA synthesis in B's takes place during the last half of the S period, while it occurs throughout this time in the A chromosomes.[5] A temporal difference in DNA synthesis is not unusual for heterochromatin.[6, 7] The heterochromatic segments of B chromosomes are similar in their staining properties to the knobs of A chromosomes at pachynema, but they seldom, if ever, participate in the nonspecific fusions commonly observed between the knobs of nonhomologous chromosomes. A further difference between knobs and B's is that in plants with abnormal chromosome 10, neocentromeres are induced at knobs[8] but not at the heterochromatic regions of B's.

In his survey of the chromosomes of races of maize, Longley[1] found a negative correlation between the number of heteropycnotic knobs on the A chromosomes and the presence of B chromosomes. Strains with high knob numbers had fewer plants with B chromosomes than did those with low numbers of knobs. A similar correlation has been reported by other investigators.[9, 10] This has been interpreted to indicate that these two heterochromatic entities play the same subvital role in cellular metabolism.[11] The report that mosaicism for A chromosome genes is higher in somatic tissues of plants with translocated B's than in those without B's[12] and the finding of higher coincidence values in plants with several B chromosomes[13] are evidence of an effect of B heterochromatin on the A chromosomes. This paper presents data indicating a hitherto unsuspected interaction between the two kinds of heterochromatin, that comprising the knobs of A chromosomes and that of B chromosomes, which leads to the elimination of knobbed arms of A chromosomes at a specific cell division, the second microspore mitosis. As the

consequence of this loss, hypoploid embryos or endosperms are produced by the functioning of deficient sperm.

Results.—A genetic stock homozygous for the closely linked A_1 and Sh_2[14] alleles in chromosome 3 and for the *Dt* gene in chromosome 9 gave high frequencies of *a-sh Dt* kernels, where none should occur, when used as the pollen parent in crosses with *a-sh dt* testers. The reciprocal cross gave an extremely low frequency of exceptional kernels; loss of dominant marker genes occurred predominantly in the male gametes. This strain was designated as the "high loss" strain. Although self or sib contamination resulting in kernels with the recessive maternal *a sh* phenotype could occur, the exceptional *a sh* kernels clearly did not so arise since they were dotted as a consequence of possessing the *Dt* gene present in the pollen parent and absent in the female.

When different plants of the high loss strain were used as the pollen parent in crosses with *a* testers, marked differences were found in the percentages of exceptional kernels. For example, plant 27342-19 produced 7.8 per cent of colorless kernels, while a full sib, plant 27342-27, gave 0.1 per cent of colorless kernels.[15] There was no difference in the percentage of fractional (mosaic) kernels in the progeny from the two sib plants. The two sister plants differed in their ability to induce loss of the *A* allele in the sperm but the basis of this difference remained conjectural. It had been established from previous studies that family 27342 carried B chromosomes, known to undergo nondisjunction at the second microspore division,[3] and it seemed possible that the aberrant behavior of a member of the A set of chromosomes might be causally related to the presence of B's. Therefore, a duplicate planting of family 27342 was made in the summer of 1966 as family 28032. Plants from a sib ear comprise family 28033. Microsporocytes were taken from individual plants of these two families, which were homozygous for the *A* allele, and pollen from these plants was used in crosses with recessive testers to determine the rate of loss of chromosome 3 markers and, to a more limited extent, for chromosome 9 markers. The number of B chromosomes carried by individual plants and the rate of loss of the dominant *A* allele in the endosperm of the offspring are given in Table 1. In both families, those plants with fewest B chromosomes had the lowest rate of loss of the *A* allele. The correlation between number of B chromosomes and marker loss is consistent and can hardly be fortuitous.

Plant 27240-27, of *A-Sh/A-Sh, Dt/Dt, Pr/Pr* constitution, was used as the pollen parent in a cross with an *a-sh dt pr* individual. The resulting ear had 170 kernels of the expected *A-Sh Pr* class and 36 exceptional *a-sh Dt* kernels. The presence of dots on the colorless kernels proved that they had not come from self-contamina-

TABLE 1

28032			28033		
No. B's	Loss of *A* in endosperm (%)	Population	No. B's	Loss of *A* in endosperm (%)	Population
3	0.9	1832	1	0.7	304
7	7.7	878	2	1.1	530
7	9.0	1528	4	14.9	1037
7	13.8	1031	4	10.2	1519
7	6.5	480	6	19.9	201
8	8.4	107			
8	8.8	1503			
11	7.4	243			

tion. All kernels were planted in the field in the summer of 1966 and the ensuing plants were testcrossed. In the family coming from the 170 *A-Sh Pr* kernels, there were 115 normal-appearing plants with no pollen abortion. When used in testcrosses as the female parent, they gave 1:1 ratios for *A* and *Sh*. In addition to the 115 normal F_1 plants from the *A Sh* kernels, there were 25 individuals of reduced height and vigor. All 25 had high pollen abortion (approximately 50%). Thirteen of these 25 partially sterile plants were successfully testcrossed as the female parent. The progeny of 12 consisted entirely of *a sh* kernels borne on semisterile ears. They were deficient for all or part of the chromosome 3 contributed by the male parent, although this chromosome was present in the endosperm—i.e., there was noncorrespondence in genotype of the embryo and endosperm. The remaining plant with semisterile pollen and ovules segregated 1:1 for the *A* and *Sh* alleles. This individual possessed a normal chromosome 3 from the male parent and was presumed to be deficient for all or part of another chromosome. Two plants of normal stature arising from colored seed had pollen with a great range in size. These were triploids of *A Sh/A Sh/a sh* genotype. The occurrence of two triploids in a small population is somewhat unusual but more surprising is the fact that the pollen parent contributed the diploid number of chromosomes. Triploidy is believed, in general, to result from the union of an unreduced egg with a haploid sperm. The finding of two triploids where either a diploid sperm or two haploid sperm fertilized the egg suggests that the mechanism producing chromosome loss is also responsible for these unexpected triploids.

Fifteen mature plants came from the 36 *a-sh Dt* kernels. Thirteen were vigorous plants with normal pollen but two were shorter in height and had *ca.* 50 per cent pollen abortion. Ten of the 13 plants with no aborted pollen were testcrossed and produced ears with no ovule abortion and 1:1 segregations for *A* and *Sh*—i.e., they were disomic for chromosome 3. The noncorrespondence observed between embryo and endosperm constitution indicates that the embryos of these ten plants arose by fertilization of an egg with a sperm cell having an intact chromosome 3, while the sperm cell fertilizing the polar nuclei to form the *a sh* endosperms lacked the *A* and *Sh* markers in chromosome 3. One testcrossed plant with no pollen abortion gave an ear with 233 *A Sh*:1 *a Sh*:63 *a sh* kernels. These are the proportions expected from a trisomic plant with two of the three chromosomes carrying the dominant alleles when used as the female parent in a testcross. Evidently the sperm cell fertilizing the egg possessed two chromosomes 3, each with *A* and *Sh* alleles, to produce a trisomic for the *A Sh* segment, while the other sperm uniting with the polars was deficient for these markers. This is the consequence of nondisjunction at the second microspore mitosis.

The two plants from *a-sh Dt* kernels which had semisterile pollen segregated 1:1 for *A Sh* and *a sh*. On one ear all of the *A* kernels were homozygous for the recessive *pr* allele on chromosome 5, although all other F_1 plants were heterozygous for *Pr* and *pr*. The *a sh* stock used in the testcross was *pr pr*. It appears that the plant giving only *pr* kernels in the colored class was hemizygous for this locus on the long arm of chromosome 5. The identity of the presumed deficient chromosome in the second plant mentioned above was not determined. However, it was neither chromosome 3 nor 5.

Thirty-six or 17.5 per cent of the 206 kernels on the ear from plant 27240-27

had endosperms of *a-sh Dt* phenotype produced by union with a sperm deficient for the dominant *A* and *Sh* alleles. This percentage represents the frequency of loss of the *A* and *Sh* loci from the sperm cell which united with the polar nuclei to form the endosperm. The frequency with which a deficient sperm fertilized the egg to give a sporophyte deficient for chromosome 3 can be calculated from the above data; it comes to 14.6 per cent, a value similar to the frequency of deficient endosperms. Judging by this small sample, selective fertilization of the egg by the nondeficient sperm does not occur as it does for the B and B^A chromosomes.[16]

Noncorrespondence of the male contributions to embryo and to endosperm has been found in every exceptional kernel in the high loss studies that have been analyzed. To ascribe the observed noncorrespondence to heterofertilization would require that it occurs 100 per cent of the time when there is loss of the *A* allele from endosperm or embryo and tests to date give no indication of an unusually high frequency of heterofertilization in related *A/a* male parents. It may be concluded that some unusual event takes place at the second spore division and that sperm of unlike genetic constitution are the consequence. If nondisjunction were responsible for dissimilar sperm, those kernels with colorless endosperms coming from the cross of *a a* × *A A* should have embryos with two chromosomes 3 contributed by the male parent; they would be *A A a* and, upon testcrossing, typical trisomic ratios should result. Embryo genotypes were determined by testcrosses of the ensuing sporophytes from 64 kernels with deficient (colorless) endosperms produced in the cross of *a a* ♀ × *A A* ♂ plants of the high loss line. Twelve individuals gave a ratio of dominant to recessive phenotypes approximating that expected from duplex trisomics, while the remaining 52 gave the 1:1 ratio characteristic of the disomic condition. In 19 per cent of the pollen grain mitoses where a sperm deficient for the marker gene *A* is produced, the sister sperm acquired two chromosomes 3 by nondisjunction. Much more frequently, however, a deficiency for the *A* gene in one sperm is not accompanied by a disomic condition for *A* in the sister.

Since *A* and *Sh* are near the end of the long arm of chromosome 3, their loss from either embryo or endosperm could mean that only the distal portion of the long arm is missing. Crosses were therefore made in which *Gl-Lg-A* pollen from high loss plants was placed on silks of *gl-lg-a* plants. Since the *Gl* locus is close to the centromere and *Lg* is situated near the middle of the long arm, these two loci afford excellent markers for the proximal half of the long arm of chromosome 3. The exceptional colorless kernels arising from this cross gave only *Gl Lg* seedlings. Among the seedlings coming from colored kernels, 4.7 per cent were *gl lg*. It was not possible to score for loss of the *A* allele in the seedlings since the complementary factors necessary for anthocyanin production were lacking. Cytological studies of somatic prophases have been made of a number of the exceptional *gl lg* plants. Twenty-seven had 19 A chromosomes plus a telocentric A fragment in addition to varying numbers of B chromosomes. The size of this fragment, which was apparently the same in all 27 plants, suggested that it might consist of the short arm of chromosome 3. Meiotic studies of one greenhouse-grown exceptional *gl lg* plant with 19 A and one fragment chromosomes disclosed a heteromorphic pair consisting of one normal chromosome and a telocentric short arm. The arm ratio and length of the normal member of the heteromorphic pair strongly suggest the involvement

of chromosome 3. Five exceptional *gl lg* plants had 19 A chromosomes and no A fragment. Presumably only one chromosome 3 was present. Five of the *gl lg* seedlings arising from kernels with colored aleurone had 20 A chromosomes; they may be compensating types in which chromosome 3 is monosomic and another chromosome is trisomic. The evidence at hand suggests that a sperm may be deficient for all of chromosome 3, but more often is deficient for only the long arm.

Analysis of the progeny of plant 27240-27 as the male parent disclosed that endosperms and embryos deficient for the *A Sh* alleles occurred with approximately equal frequencies. Furthermore, the great majority of the F_1 sporophytes with pollen and ovule semisterility appear to be hypoploid for all or part of chromosome 3. If the other nine chromosomes of the haploid complement underwent the same rate of loss as did chromosome 3, there would be few if any F_1 sporophytes with the normal complement of 20 A chromosomes. However, more than 80 per cent were euploids. The argument is advanced that chromosome 3 is subject to loss at the second microspore mitosis in plants with high numbers of B chromosomes because it carries a large knob in the long arm. If, so the argument runs, it were knobless there would be little or no loss. There is a marked reduction in the frequency of loss for chromosome 3 when the number of B's is below a certain level even though it is knob-bearing. Knobless chromosomes should undergo little loss irrespective of the number of B's. The available evidence is in accord with this hypothesis. Cytological examination at pachynema of high loss plants revealed that chromosome 3 is homozygous for a large knob in the long arm, that both chromosomes 9 have a small terminal knob on the short arm, and that a medium sized knob is present in a heterozygous condition on either chromosome 2 or 5. Marker genes on chromosome 9 with its small terminal knob were lost much less frequently than were markers on chromosome 3 with a much larger interstitial knob.[17] Either knob size or position play a significant role in determining the rate of loss in plants with supernumerary B chromosomes. The other chromosomes had no conspicuous knobs and were stable in the presence of B's. Further indication of the relative stability of a knobless chromosome comes from crosses of *K A Sh/k a Sh* plants with B chromosomes as the pollen parent (*K* refers to the large knob in the long arm of chromosome 3; *k* = knobless). Although of a preliminary nature, the data strongly suggest that in these heterozygotes the knobbed homologue is eliminated much more frequently than is the knobless one.

Discussion.—The above account is believed to shed light on a number of established facts which hitherto have had no rational explanation. Longley,[1] in his survey of knob number and location in diverse strains of maize, found a negative correlation between knob number and the frequency of B chromosomes. Further, Randolph[2] reported that plants with high numbers of B's were of reduced stature and highly sterile. If, as our data indicate, frequent loss of knobbed chromosome arms takes place in plants where the number of B chromosomes is above a critical level, a modification in phenotype and a high degree of sterility should result and it follows that there would be strong selection against strains of maize having an appreciable number of both knobbed A chromosomes and supernumerary B chromosomes.

Among the many questions to be answered are the following: (1) Is a specific

segment of the B responsible for the loss of A chromosomes? (2) Do the knobs on all of the A chromosomes interact with B's? (3) Does the rate of loss reach a plateau when the number of B's attains a certain level? (4) What is the relationship of knob size, position, and number to the rate of loss? (5) Will heterotic hybrids coming from the cross of a high knobbed with a high B strain produce progenies, when used as the pollen parent, with a high proportion of hypoploid individuals having reduced vigor and high sterility?

Although the cytological mechanism responsible for elimination of knobbed arms of the A chromosomes has not been determined, a working hypothesis can be formulated based on the observed behavior of B chromosomes at the first microspore mitosis in other plants. In Secale, for example, the two chromatids of a B undergo nondisjunction at this time because two regions, one in each arm equidistant from the centromere, fail to disjoin at anaphase. Consequently, the two chromatids pass to one pole. Nonseparability leading to nondisjunction is induced by the distal knob-bearing half of the long arm of the standard B and may result from incomplete replication, producing an apparent stickiness at the two specific sites.[18, 19] Since loss of A chromosomes in maize plants with B's is restricted to those with knobbed arms, it is postulated that knobs are incompletely replicated at the time of the second microspore division. The conjoined knobs prevent anaphase separation and lead to rupture at the centromere. The B chromosomes are postulated to produce a cellular environment conducive to faulty knob replication. It is, however, not clear why bridge rupture is adjacent to the centric region in this situation while it is not so restricted in dicentric bridges coming from inversion crossing-over. Another possible mechanism is the fusion of knobs with heterochromatic segments of the B's during the second microspore division. The nondisjunction which the latter undergo at anaphase might lead to the structural alterations observed in the A chromosomes. Since B nondisjunction and loss of knobbed A chromosomes occur primarily at the second microspore division, the elimination at this mitosis and not during megagametogenesis becomes understandable on this hypothesis.

In a study of the genetic behavior of several T B-A stocks, Bianchi *et al.*[12] concluded that the increased frequency of endosperm sectors involving genes on nontranslocated A chromosomes could be attributed to nondisjunction caused by the translocated B. B chromatin was also believed to be responsible for nondisjunction of the B^A and possibly also of A chromosomes at the first microspore division. No marked difference in frequency of endosperm mosaics was observed in our plants with low and high numbers of B's[15] (1.1% and 1.6%, respectively—values only slightly higher than the 0.58% of *A Sh* sectors reported by Bianchi *et al.*[12] for plants with no B's). In our studies several cases of nondisjunction of chromosome 3 at the second microspore division were found. Among 64 tested kernels with hypoploid endosperms, 52 of the corresponding sporophytes gave normal backcross ratios but 12 displayed a typical trisomic segregation. Although no information was available on the frequency of nondisjunction in plants without B's, the incidence of trisomics is normally very low. It appears that nondisjunction of knobbed A chromosomes at the second microspore mitosis, as well as the more frequent loss of knobbed arms, is induced by B chromosomes.

Summary.—Evidence is presented that knobbed arms of members of the normal

chromosomal complement (the A set) are eliminated when supernumerary B chromosomes are present. A greater rate of loss of knobbed chromosomes occurs in plants with higher numbers of B's than in plants with lower numbers. Loss of the knobbed arms takes place at the second microspore division, thus producing dissimilar sperm cells. The behavior of knobless A chromosomes is apparently unaffected by the number of B chromosomes present in our experimental material. No or little loss of knobbed chromosomes was found in female parents with B chromosomes. The ability of B chromosomes to induce loss of knobbed A chromosomes makes understandable the negative correlation found in races of maize between numbers of knobs on the A chromosomes and the number of B chromosomes.

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[14] Explanation of gene symbols: A_1 a_1 (chromosome 3) anthocyanin vs. no anthocyanin in aleurone and sporophyte; Sh_2 sh_2 (chrom. 3) plump vs. shrunken endosperm; Dt_1 dt_1 (chrom. 9) dots of pigment vs. no dots in recessive a_1 aleurone; C_1 c_1 (chrom. 9) colored vs. colorless aleurone; *Wx wx* (chrom. 9) blue-vs. red-staining starch with I-KI; *Pr pr* (chrom. 5) purple vs. red-colored aleurone; Gl_6 gl_6 (chrom. 3) normal vs. glossy seedling; Lg_2 lg_2 (chrom. 3) liguled vs. liguleless leaves. Subscripts are omitted after first listing.

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[17] Tests of loss rate for the *A* locus in the long arm of chromosome 3 and for the *C* and *Wx* loci in the short arm of chromosome 9 were made for several plants. Plant 28032-7 with 7 B chromosomes had 7.7% loss of *A* and 0.6% loss of *C* and *Wx* (which were coincident); plant 28032-20 with 7 B's gave 13.8% *A* loss and 2.1% *C Wx* loss; plant 28032-23 with 3 B's had 0.9% *A* loss and 0.0% loss of *C* and *Wx*.

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