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1. <u>Elimination of knobbed chromosome arms in maize induced by supernumerary B chromosomes.</u>

In the 1966 Maize News Letter we reported that a genetic stock homozygous for the closely linked A_1 and Sh_2 alleles in chromosome 3 and for the Dt gene in 9 gave high frequencies of a_1 sh_2 kernels, where none should occur, when used as the pollen parent in crosses with a_1 sh_2 dt testers. This strain was designated as the high loss strain. Although self or sib contamination resulting in kernels with the recessive maternal a_1 sh_2 phenotype could occur, the exceptional a_1 sh_2 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_1 testers, marked differences were found in the percentages of exceptional kernels. A good example is given in the 1966 News Letter where plant 27342-19 produced 7.8% of wholly colorless kernels while a full sib plant 27342-27 gave 0.1% of colorless kernels. There was no difference in the percentage of fractional (mosaic) kernels in the progeny from the two sib plants. It was concluded that 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 was known from previous studies that family 27342 carried B chromosomes, known to undergo nondisjunction at the second microspore division. The loss of chromosome 3 markers in sperm cells appeared to be somewhat like the behavior of the B chromosomes or the B^A translocation chromosomes of Roman 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 chromosomes. 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. Individual plants of these two families, which were homozygous for the A allele, were sporocyted and tested as the pollen parent for 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 pollen grains are given below:

28032			28033		
No. of B's	% Loss of A in endosperm	Population	No. of 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			

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. Tests of loss rate for the A locus in chromosome 3 and for the C and Wx loci in 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. The exceptional c wx kernels have not been checked for contamination. Silks on the c wx testers were difficult to bag and some of the c wx kernels doubtless came from self or sib contamination, so the percentage of c wx kernels due to marker loss in the sperm may well be lower than the observed percentage of c wx kernels. A possible clue to the higher rate of loss for chromosome 3 markers and the lower rate for chromosome 9 markers came from the cytological observations at pachynema. In all examined plants, chromosome 3 was homozygous for a large knob in the long arm at position 0.6 while chromosome 9 had a small terminal knob on the short arms. No other conspicuous knobs were present on the remaining A chromosomes of family 28032 or 28033 except for one medium sized knob found in a heterozygous condition on either chromosome 2 or 5. The interaction of knobs and loss will be developed later.

Plant 27240-27, of A Sh/A Sh, Dt Dt, Pr Pr constitution, was used as the pollen parent in crosses with two a sh dt pr individuals. The resulting kernels on both ears consisted of the expected A Sh class and of those which were wholly colorless and shrunken. The presence of dots on the colorless kernels proved that they had not come from self contamination. One ear had 170 A Sh and 36 a sh Dt kernels. All kernels were planted in the field in the summer of 1966 and the ensuing plants testcrossed. In the family coming from the 170 A Sh 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 $\underline{A}$ and $\underline{Sh}$. In addition to the 115 normal F_1 plants 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 female parents. The progeny of 12 consisted entirely of $\underline{a}$ $\underline{sh}$ kernels borne on semi-sterile ears. They were monosomic for all or part of chromosome 3. One plant with semi-sterile pollen and ovules segregated 1:1 for $\underline{A}$ $\underline{Sh}$ and $\underline{a}$ $\underline{sh}$. This individual was presumed to be deficient for a chromosome other than 3. Two plants of normal stature arising from colored seed had pollen with a great range in size. These were triploids of $\underline{A}$ $\underline{Sh}/\underline{A}$ $\underline{Sh}/\underline{a}$ $\underline{sh}$ 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 un-expected triploids.

Fifteen mature plants came from the 36 $\underline{a}$ $\underline{sh}$ $\underline{Dt}$ kernels. Thirteen were vigorous plants with normal pollen but two were shorter in height and had ca. 50% pollen abortion. Ten testcrossed ears on plants with no aborted pollen had no ovule abortion and gave 1:1 segregations for $\underline{A}$ and $\underline{Sh}$--i.e., they were disomic for chromosome 3. The embryos of these ten plants coming from the exceptional $\underline{a}$ $\underline{sh}$ kernels arose by fertiliza-tion of an egg with a sperm cell having one chromosome 3, while the sperm cell fertilizing the polar nuclei to form the exceptional $\underline{a}$ $\underline{sh}$ endosperms lacked the $\underline{A}$ and $\underline{Sh}$ markers on chromosome 3. One testcrossed plant with no pollen abortion gave an ear with 233 $\underline{A}$ $\underline{Sh}$: 1 $\underline{a}$ $\underline{Sh}$: 63 $\underline{a}$ $\underline{sh}$ kernels. These are the proportions expected from a trisomic plant with two of the three chromosomes carrying the dominant alleles. Evidently the sperm cell fertilizing the egg possessed two chromosomes 3, each with the $\underline{A}$ and $\underline{Sh}$ alleles, to produce a trisomic for the $\underline{A}$ $\underline{Sh}$ 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 $\underline{a}$ $\underline{sh}$ $\underline{Dt}$ kernels which had semi-sterile pollen segregated 1:1 for $\underline{A}$ $\underline{Sh}$ and $\underline{a}$ $\underline{sh}$. On one ear all of the $\underline{A}$ kernels were homozygous for the recessive $\underline{pr}$ allele on chromosome 5 although all other F_1 plants were heterozygous for $\underline{Pr}$ and $\underline{pr}$. The $\underline{a}$ $\underline{sh}$ stock used in the testcross was $\underline{pr}$ $\underline{pr}$. It appears that the plant giving only $\underline{pr}$ kernels in the colored class was hemizygous for the long arm of chromosome 5. This is one of the chromosomes that might carry a medium sized knob. Inasmuch as sporocytes were not taken from the two semi-sterile plants it is not possible to identify the deficient chromosome in the second plant mentioned above. However, it was neither chromosome 3 nor 5.

Thirty-six or 17.5% of the 206 kernels on the ear from plant 27240-27 had endosperms of $\underline{a}$ $\underline{sh}$ $\underline{Dt}$ phenotype produced by union with a sperm deficient for the dominant $\underline{A}$ and $\underline{Sh}$ alleles. This percentage represents the frequency of loss of the $\underline{A}$ and $\underline{Sh}$ loci from the sperm cell which united with the polars to form the endosperm. The frequency with which the

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%, a value similar to the frequency of deficient endosperms. Judging by this small sample, selective fertilization of the egg by the non-deficient sperm does not occur as it does for the B and B^A chromosomes.

If we disregard for the moment the possibility of heterofertilization, the constitution of the second sperm cell in those pollen grains with one deficient sperm can be determined from the genotype of the embryo derived from the exceptional a sh kernels. There is in our material no way of ascertaining the genotype of the endosperm of those kernels with a deficient embryo; it can only be inferred. Non-identical sperm are produced by some mishap at the second microspore division as Roman showed for the translocated B^A chromosomes. Non-correspondence of the male contributions to embryo and to endosperm has been found in every exceptional kernel in the high loss studies that has been analyzed. The dissimilarity between the two sperm clearly arises from some event at the second microspore mitosis. To ascribe this dissimilarity to heterofertilization would require that it occurs in kernels which had lost the A allele 100% of the time and tests to date give no indication of an unusually high frequency of heterofertilization in related A/a male parents. In any event, heterofertilization does not appear to be of major importance and its occasional occurrence does not vitiate the conclusion that there is some unusual behavior at the second spore division and that unlike sperm are the consequence. If nondisjunction is responsible for dissimilar sperm, those kernels with colorless endosperms coming from the cross of a a x 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 ♀ x 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. At this stage in the analysis it could be concluded that in about 20% of the 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 no more than that the distal portion of the long arm is missing. Crosses were therefore made in which Gl_6 Lg_2 A_1 pollen from high loss plants was placed on silks of gl lg a plants. Since the Gl_6 locus is close to the centromere these three mutant loci afford excellent markers for nearly all of the long arm of 3. Loss of all three markers proved to be coincident in hypoploid plants which came from colored kernels. The colorless kernels gave only Gl Lg A seedlings. Cytological studies of somatic prophases have been made of a number of the exceptional gl lg a 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. Indeed, meiotic studies at diakinesis made from one greenhouse-grown exceptional gl lg a plant with 19 A and one fragment chromosomes disclosed a heteromorphic pair consisting of one normal chromosome and a telocentric short arm. Pachytene figures were poor so positive identification of chromosome 3 as the heteromorphic pair could not be made. However, the arm ratio and length of the heteromorphic pair strongly suggest the involvement of chromosome 3. Five of the exceptional gl lg a plants had 19 A chromosomes and no A fragment. In these instances only one chromosome 3 was present. Five of the gl lg a seedlings arising from kernels with colored aleurone apparently had 20 A chromosomes. The origin and chromosomal constitution of this unanticipated class remain to be elucidated. Meiotic studies should be revealing. An unequivocal distinction between A and B chromosomes can be made in somatic prophases when the number of B's is low but it proved more difficult when the B's were increased in number.

The cytological studies are admittedly incomplete and will be extended but the evidence at hand suggests that sperm may be deficient for all of chromosome 3 but more often is deficient for only the long arm. It is uncertain whether or not those plants giving trisomic ratios have three entire chromosomes 3 or if they possess only two and the third is a telocentric consisting of the long arm. The expected genetic ratio from a primary trisomic with two dominant and one recessive alleles might not differ significantly from that expected where the extra chromosome is a telocentric.

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 F_1 sporophytes with pollen and ovule semi-sterility 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% 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. As stated earlier, cytological examination at pachynema of high loss plants reveals that only chromosome 3 is homozygous for a large knob, 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. All other chromosomes were knobless and stable. Marker genes on chromosome 9 with its small knob are lost much less frequently than are markers on chromosome 3 with a much larger knob. Apparently knob size plays a significant role in determining rate of loss in plants with supernumerary B chromosomes.

Data from the following experiment are readily interpretable on the above hypothesis. A high loss plant of K A Sh consititution (K = knob; k = knobless) was used as the male parent in a cross with a k a Sh individual. The F$_1$ plants of K A Sh/k a Sh genotype were used as the pollen parents in testcrosses with a sh testers. The resulting progeny consisted of 416 A Sh: 448 a Sh: 35 a sh kernels. Sperm deficient for either the K A Sh or k a Sh segment would account for the exceptional a sh kernels. Elimination of all or part of the K A Sh chromosome from one pole at the second spore division results in one deficient sperm and one with the K A Sh chromosome. Fertilization of the two polar nuclei by the deficient sperm gives an endosperm that is colorless and shrunken while fusion of the non-deficient sperm with the egg produces an embryo with the K A Sh chromosome. The scutella of these embryos are colored since the necessary complementary factors for scutellum color were present. On the other hand, elimination of the k a Sh loci would yield kernels with a sh endosperms and embryos with the k a Sh chromosome. Such embryos would have colorless scutella. Twenty-seven of the 35 kernels with a sh endosperms had colored and eight, or 23%, had colorless scutella. If there was no recombination between K and the A locus, the data could be interpreted to indicate that the K A Sh segment is lost three times as frequently as is the k a Sh segment. However, a more likely explanation of the origin of the eight a sh kernels with colorless scutella is loss of all or a portion of a K a Sh chromosome derived by crossing over between K and the A locus. The 25% recombination between K and A expected to give K a Sh strands is very close to the 23% of a sh kernels with colorless scutella. That it is the knobbed chromosome 3 which undergoes loss and not the knobless one can and will be determined by cytological examination of the chromosomes 3 at pachynema in plants coming from kernels with a sh endosperms and colorless scutella. If they possess a knob on chromosome 3, then it follows that only the knobbed homologue is subject to elimination.

The above account is believed to shed light on a number of established facts which hitherto have had no rational explanation. Longley 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 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 with consequent sterility takes place in plants where the number of B chromosomes is above a critical level, it follows that there would be strong selection against any strain of maize having an appreciable number of both knobbed A chromosomes and supernumerary B chromosomes.

Obviously many tests remain to be done. A low loss strain can be converted to a high loss by selecting for increased numbers of B's, providing that some A chromosomes are knobbed. Conversely, in the selfed progeny of a high loss plant individuals with lowered numbers of B's should occur; these should be in the low loss category. Crosses which combine a high knobbed strain with a knobless one carrying many B's should produce a high loss strain, etc. The cytological mechanism leading to the partial loss and elimination of knobbed A chromosomes remains

to be determined. Some kind of interaction between the heterochromatin of knobs and that of B chromosomes which leads to loss of all or part of the knob-bearing A chromosome would appear to be likely. Unfortunately, the second spore division occurs at a stage when the cytoplasm is full of starch grains and it is not a favorable stage to observe. It may also be difficult to distinguish between B chromosomes which undergo nondisjunction at this time and aberrantly behaving A chromosomes.

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2. An interaction of B chromosomes and abnormal 10.

Y. C. Ting has proposed that abnormal chromosome 10 was derived from a normal 10 and a B chromosome. (Chromosoma: 9:286). Since then, attempts have been made to determine whether homology exists between B chromosomes and the extra chromatin of abnormal 10. Rhoades and Dempsey looked for pairing in pachytene between a single B and abnormal 10, as an indication of homology. Little if any pairing was found. (MNL:33:58). Ting found some association between B's and abnormal 10, which, however, also occurred between abnormal 10 and other heterochromatic knobs. (MNL:33:37). Even if we assume that pairing between abnormal 10 and B's is a rare event, some homology between the two cannot be ruled out. It is possible that some rearrangement in abnormal 10 has occurred since its hypothetical origin from a B chromosome. So a different approach to the problem was used.

It was first determined by Roman, and since confirmed by other workers, that the distal heterochromatin of the B chromosome is responsible for the nondisjunction of the B centromere region at the 2nd microspore division. Roman used the B-4a translocation.