

MITOTIC NONDISJUNCTION IN THE CASE OF INTERCHANGES INVOLVING THE B-TYPE CHROMOSOME IN MAIZE¹

HERSCHEL ROMAN

University of Washington, Seattle, Washington

Received April 19, 1947

INTERCHANGES between the B-type chromosome and members of the basic set (A-type chromosomes) in maize provide a means of clarifying the anomalous behavior of the B-type itself. They also make possible a method whereby plants deficient for a specific A-type segment or carrying the segment as a duplication may be regularly produced for use in various cytogenetic studies.

The characteristics of the B-type have been described in detail by RANDOLPH (1941). The chromosome occurs as a supernumerary in many strains of maize. Within a strain, the number of B-types may vary considerably from plant to plant. RANDOLPH (1928) found a range of zero to eight among 43 plants of the Black Mexican variety. The chromosome does not produce a specific genetic effect even when present in relatively large numbers; a plant devoid of B-types is not noticeably different in appearance from one carrying as many as ten (RANDOLPH 1941).

The source of the variability in B chromosome number within a strain has been investigated in crosses in which only one of the parents furnished the B-types. The results of two such crosses and their reciprocals are given in table 1. On an orthodox basis, the progeny from these crosses should not have more than one B chromosome. Each of the crosses, however, yielded plants with two or more B-types and the proportion of these exceptional plants was particularly high when the B-types were transmitted by the male parent (i.e., in the $0B \times 1B$ and $0B \times 2B$ crosses).

These results are due only in small part, if at all, to aberrant meiotic behavior. In the microsporocytes of a 2B plant, for example, the B chromosomes usually form a bivalent in the first meiotic prophase and disjunction follows. The regularity of this procedure may be verified by chromosomal counts in the microspore nuclei formed as a result of meiosis. In 130 figures counted, RANDOLPH (1941) found 116 with one B-type and 14 with none. Since male gametes with two or more B-types are obtained from a 2B plant (as shown by the results of the $0B \times 2B$ cross), these must have been produced from 1B microspores. The proportion of 0B progeny from the $0B \times 2B$ cross is also greater than would be expected from the microspore counts. This suggests that 0B gametes are also derived from 1B microspores.

The indication that both 0B and 2B gametes are obtained from 1B microspores suggests the possibility that the B chromosome is undergoing "mitotic nondisjunction" in one, perhaps rarely in both, of the divisions which produce

¹ This investigation was begun and largely completed at the UNIVERSITY OF MISSOURI, Columbia, Missouri.

the gametes. By this mechanism, a B chromosome would be distributed in such a way that one of the daughter nuclei would receive two B-types and the other would receive none. The result of the $0B \times 1B$ cross lends support to this view. Although a sizeable number of plants produced by this cross received two or more B chromosomes, the average number of B-types per plant was approximately 0.5. This may be interpreted to mean that the production of 2B gametes from 1B microspores is balanced by the production of a numerically

TABLE I
*Distribution of B chromosomes among progeny of crosses
between 0B and 1B or 2B parents.*

NUMBER OF B-TYPES IN PARENTS	B-TYPE NUMBER IN F ₁				NUMBER OF F ₁ PLANTS	AVERAGE NO. B-TYPES PER F ₁ PLANT	INVESTIGATOR
	0	1	2	3 4			
0 × 1	76	22	15		113	.49	Longley 1927 Randolph 1941
	32	13	5	2	52		
	108	35	20	2	165		
0 × 2	22		37	2	61	1.34	Randolph 1941
1 × 0	109	58	3		170	.35	Longley 1927 Randolph 1941
	46	19			65		
	155	77	3		235		
2 × 0	8	41	3		52	.90	Randolph 1941

equivalent class of 0B gametes, as would be expected from the hypothesis of mitotic nondisjunction.

A direct cytological determination of what is happening during the microspore divisions is not feasible for technical reasons. A determination by genetic methods is also precluded since the B chromosome cannot be identified by a phenotypic effect. However, through interchanges with members of the basic set (these will be referred to as A-B interchanges), it should be possible to attach A-type segments carrying known genes to B-type segments and thus to trace the behavior of the latter. This method rests on the assumption that the anomalous behavior of the B chromosome does not depend on its remaining intact. The validity of this assumption is indicated by observations of B-type fragments (RANDOLPH 1941). It should also be possible, by a comparison of the various B-segments produced by breakage at different points in the chromosome, to locate the element within it that is responsible for its aberrant performance.

MATERIALS AND METHODS

Mature pollen from plants with from four to ten B chromosomes was treated with X-rays and applied to silks of plants devoid of B-types. The

radiation was produced by a Coolidge tube operated at 138 K.V.P. and was unfiltered except for the anther wall and the damp paper towelling in which the anthers were wrapped. The doses applied ranged from 600 to 1,600 r-units.

Only a small fraction of the progeny was expected to carry the A-B interchanges. The plants most likely to carry them were screened from the F_1

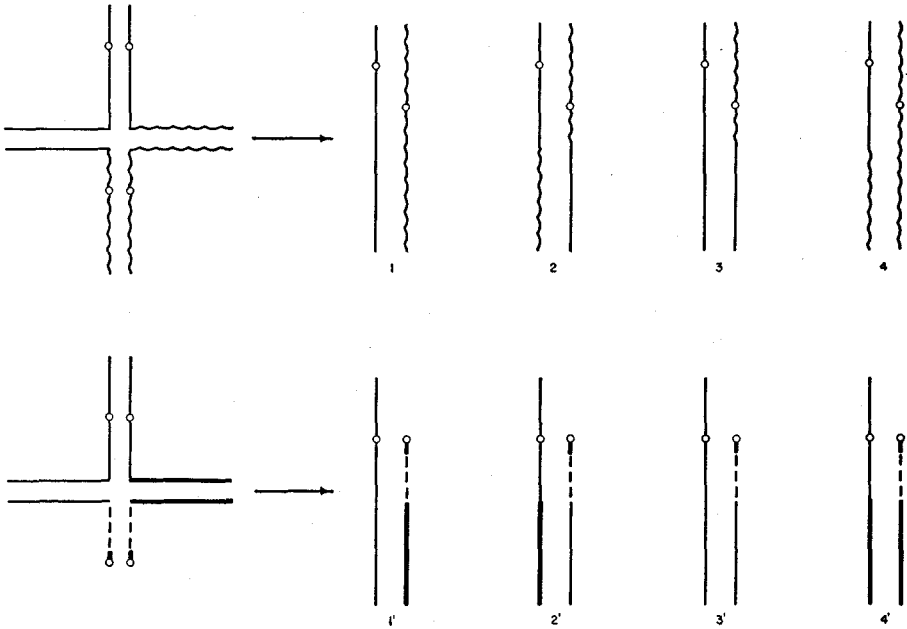


FIG. 1.—Main spore types expected from a plant heterozygous for an A-A interchange (above) compared with those expected from a plant heterozygous for an A-B interchange (below). (Only the chromosomes involved in the interchange are shown.) Note that types 3 and 4 are both deficient for A-chromatin whereas only type 4' is thus deficient. The respective pachytene configurations are given at the left. The B-type is represented as having a terminal centromere; see p. 405 for a fuller discussion of this.

population by an examination of the mature pollen of each plant. Normal maize plants in healthy condition produce pollen with nearly all grains uniform in size and well-filled with starch. There are usually a few defective grains present, seldom as high as five percent. Plants heterozygous for a deleterious deficiency produce normal and defective grains in approximately equal numbers. Plants heterozygous for an interchange between A-type chromosomes (A-A interchanges) usually yield the same pollen picture since two of the four main types of spores formed are deficient for A-chromatin (fig. 1). On the same basis, plants heterozygous for an A-B interchange would be expected to produce 25 percent of defective pollen since one of the two deficient spore types is deficient only for B-chromatin and this does not alter pollen appearance. This spore type also carries a duplication for A-chromatin but duplications ordinarily have no visible effect on pollen development.

The A-B interchanges could therefore be distinguished by pollen examination from deficiencies and most A-A interchanges. There are, however, some A-A interchanges in which one of the deficient spore types produced by the heterozygous plant develops into a visibly normal pollen grain with the result that only 25 percent of the grains are defective in appearance. Examples of this type, involving the satellite of chromosome 6, have been reported by BURNHAM (1932) and CLARKE and ANDERSON (1935). Plants heterozygous for an inversion also produce defective pollen, in proportions depending on the amount of crossing over within the inverted region. Microsporocyte samples were taken from the F_1 plants and were stored until after pollen examination. The identification of those plants heterozygous for an A-B interchange, among the 25 percent group, was accomplished by cytological examination.

The presence of an A-B interchange could be detected either in pachytene or in diakinesis. In pachytene, the interchange, in heterozygous condition, assumes the familiar cross-configuration if an intact B-type is present. If an intact B-type is not present, a T-configuration is frequently formed, as a result of the homologous pairing of the A-segments and the nonhomologous pairing of the B-segments. In either case, the interchange complex may be detected readily owing to the singular pachytene appearance of the B-type (McCLINTOCK 1933). Plants heterozygous for an A-B interchange show, in diakinesis, nine A-bivalents and the interchange complex, in contrast with plants heterozygous for an A-A interchange, in which eight bivalents are formed. The A-B interchange complex is in a chain configuration if an intact B-type is not present. If it is present, it usually associates with the complex to form a ring configuration. This offers especially convincing evidence of an A-B interchange since B chromosomes ordinarily do not pair with members of the basic set. When two or more extra B-types are present, some or all of them often associate with the complex in multivalent pairing; otherwise, they form separate univalents, bivalents, or multivalent groups.

EXPERIMENTAL RESULTS

Eight A-B interchanges were obtained from approximately 500 plants screened by the method just described. Chromosomes 1 and 7 each were involved in two interchanges and chromosomes 4, 6, and 9 each were involved once. In one of the interchanges, the A chromosome has not yet been identified.

This report deals only with the interchange involving chromosome 4, designated as TB-4a. The investigation of the other interchanges is still in a preliminary stage. It should be noted, however, that in five of the latter, the investigation has proceeded far enough to indicate that the behavior described for TB-4a applies to these as well.

In the case of TB-4a, the point of interchange in chromosome 4 is in the short arm, approximately one-eighth of the distance from the centromere to the end of the arm. In the B-type, the point of breakage is at or very near the junction of the euchromatic and the main heterochromatic segments (fig. 2). Approximately 25 percent of the pollen grains produced by a plant heterozy-

gous for TB-4a are devoid of starch. The pollen grains of the homozygote are normal in appearance.

Evidence of mitotic nondisjunction

The hypothesis of mitotic nondisjunction, set forth to account for the anomalous transmission of the B-types, has different consequences depending on whether nondisjunction occurs in the first or the second division of the microspore. The formation of the male gametes in maize proceeds as follows. The microspore nucleus derived from the meiotic divisions divides to form a generative and a vegetative, or tube, nucleus. The generative nucleus divides again to form the two gametic nuclei. Thus the mature pollen grain is trinucleate; it carries two crescent-shaped sperm and the tube cell (WEATHERWAX 1917). The latter presumably controls the growth of the pollen tube down the

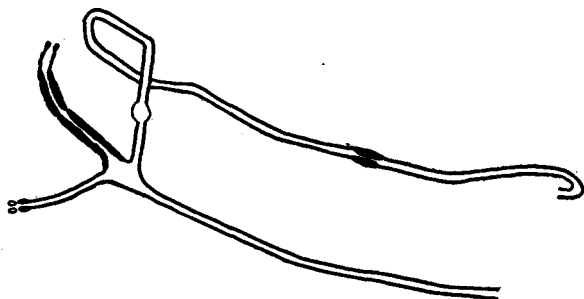


FIG. 2.—Camera lucida drawing of TB-4a in heterozygous condition in the pachytene stage of the microsporocyte.

silk and is not directly involved in the fertilization process. One of the gametic nuclei fuses with the polar nuclei in the embryo sac to form the primordial endosperm nucleus. The other gamete fertilizes the egg.

If, starting with a 1B nucleus, nondisjunction occurs only in the first division of the microspore, either the generative nucleus or the tube nucleus would receive two B-types and the other would receive none. If the generative nucleus receives the two B-types, the gametic nuclei derived from it would each have two B-types. If the generative nucleus does not receive the B-types, the gametes would also lack them. In either case, the two gametes within a single pollen grain would be identical with respect to B chromosome number. If, on the other hand, mitotic nondisjunction occurs only in the second division of the microspore, both the generative and the tube nuclei would have one B-type. The gametic nuclei of a pollen grain in this case would not be alike in B chromosome number; one would receive two B-types and the other would receive none.

In appropriate crosses, fertilization involving male gametes that are alike in B chromosome number would produce a seed with an endosperm and embryo also alike in this respect. If the gametes of a pollen grain are not alike, the endosperm and embryo would also differ. From a $\sigma B \times 2B$ cross, for example, we would expect from nondisjunction in the first division of the micro-

spore that the embryo and endosperm would both contain either two B-types or none. If nondisjunction occurs in the second division, we would expect some of the seeds to have a 2B endosperm and a oB embryo and others to have a oB endosperm and a 2B embryo, depending on the manner in which the oB and 2B gametes of the pollen grain are engaged in fertilization. The time of nondisjunction could therefore be fixed by an examination of these tissues; unfortunately, the endosperm does not lend itself readily to cytological study.

Crosses involving TB-4a were made to test the hypothesis of mitotic non-

TABLE 2
*Results obtained when Su Su plants homozygous for TB-4a were
crossed as seed parents with normal su su plants.*

CROSS	TOTAL SEEDS	SUGARY SEEDS
34.3-2×6.1-1	228	o
34.4-4×6.1-1	165	o
35.1-1×3.4-4	84	o
35.1-2×6.2-6	285	o
35.2-7×6.1-1	244	o
35.2-8×6.1-1	151	o
Totals	1,157	o

disjunction and to decide between the foregoing alternatives. Plants homozygous for the interchange were intercrossed with plants that were normal and lacked B chromosomes. (These crosses are analogous to the oB×2B and 2B×oB crosses insofar as B-chromatin is concerned except that the B-types involved in the interchange are not intact.) The normal parent was homozygous for *su* (sugary endosperm), a recessive gene located in the short arm of chromosome 4 (ANDERSON and RANDOLPH 1945). The interchange parent carried the dominant allele, *Su*, in homozygous condition.

The results of these crosses were widely different depending on whether the interchange parent served as the male or the female. When TB-4a was transmitted by the seed parent, all of the F₁ seeds were nonsugary (table 2), as would be expected from normal behavior. When the interchange parent served as the pollen source, approximately half of the F₁ seeds were sugary (table 3).

The sugary seeds obtained from the latter cross were interpreted as indicating a deficiency in the endosperm of the interchange chromosome bearing the *Su* gene. These seeds were grown for a cytological examination of the microsporocytes to determine the chromosomal content of the embryo. Twenty-four plants were examined and each was found to have an extra B⁴ chromosome, the interchange chromosome with a B-type centromere. They contained two B⁴ chromosomes in addition to a normal chromosome 4 and a 4^B chromosome (the interchange chromosome with the chromosome 4 centromere).

It remained to be determined whether the chromosome that is absent in the endosperm of the sugary seeds is the same one that is present as a duplica-

tion in the hyperploid embryo. This was accomplished by using the hyperploid plants as seed parents in crosses with normal plants that were homozygous for *su*. It was observed in diakinesis of the hyperploids that in a majority of the cells, the normal chromosome 4 paired with the 4^B chromosome and the two

TABLE 3

Results obtained when Su Su plants homozygous for TB-4a were crossed as pollen parents with normal su su plants. (34.3-7 had no intact B-types; 34.4-7 had two intact B-types.)

CROSS	TOTAL SEEDS	SUGARY SEEDS	
		%	χ^2 *
3.3-1 × 34.3-7	222	48.2	.16
6.2-3 × 34.3-7	166	44.0	2.4
6.2-6 × 34.3-7	270	55.2	2.9
78.1A-2 × 34.3-7	278	48.6	.23
78.1-2 × 34.4-7	201	53.2	.84
7.2-2 × 34.4-7	209	57.9	5.2
7.3-3 × 34.4-7	200	48.0	.32
8.4-3 × 34.4-7	245	57.6	5.6
Totals	1791	51.9	2.7

* Calculated for deviation from 50%.

TABLE 4

Results of crosses involving hyperploid plants for location of Su with respect to breakage point in chromosome 4. See text for description of cross.

TOTAL SEEDS	SUGARY SEEDS %
150	5.3
234	5.1
287	5.9
220	11.4
300	5.3
266	5.6
305	3.3
158	4.4
261	11.1
Totals 2,181	6.4

B⁴ chromosomes formed a separate bivalent. In some cells, all four chromosomes were associated in a single complex. It would be expected from these observations that two types of spores would predominate, one containing 4^B and B⁴ and the other containing chromosome 4 and B⁴ (fig. 3). If *Su* is on 4^B, we would expect, since the normal chromosome 4 carries *su*, that half of the seeds obtained from the cross would be sugary. If *Su* is located on B⁴, each of the two main types of spores would have this allele and only a small percentage of sugary seeds would be expected, these resulting from meiotic disjunction

of the relatively rare complex association. The results of the cross (table 4) show that *Su* is on the B^4 chromosome.

As already indicated, the noncorrespondence in the chromosomal composition of the endosperm and embryo in the sugary seeds is evidence of a parallel noncorrespondence in the composition of the gametes contained in the pollen grains furnished by the parent homozygous for TB-4a. Thus the gametic nucleus that fused with the polar nuclei to form the primary endosperm nucleus was deficient for the B^4 chromosome and that which fertilized the egg carried this chromosome in duplicate. This means that nondisjunction of the B^4 chromosome occurred in the division of the generative nucleus. Figure 4

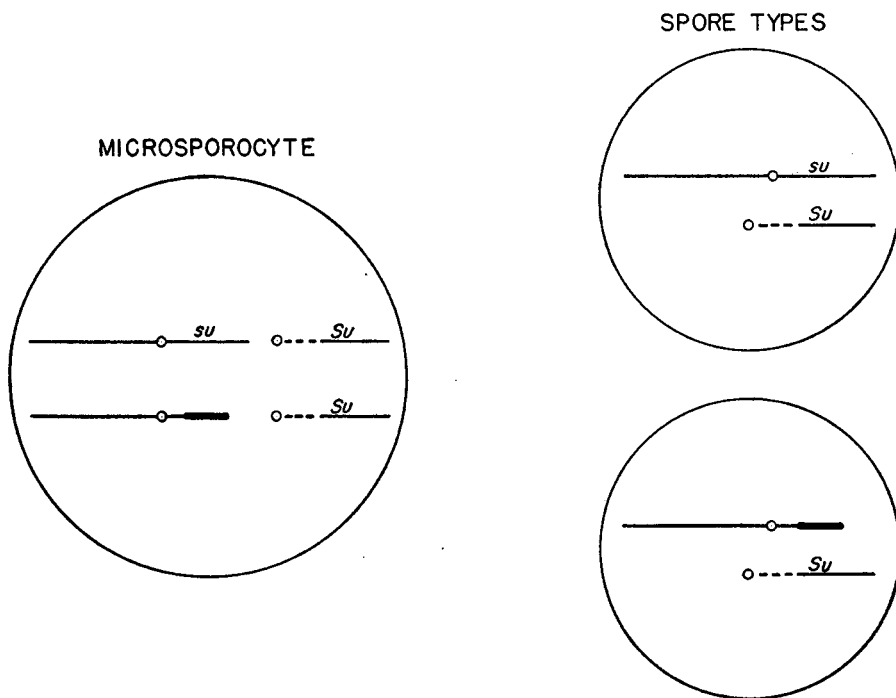


FIG. 3.—Association of chromosomes in diakinesis of microsporocyte and expected spore types.

illustrates the probable course of events in the formation of a pollen grain on the basis of nondisjunction in this division.

Another type of seed is also expected as a result of mitotic nondisjunction. If the gamete deficient for the B^4 chromosome fertilizes the egg and the gamete carrying two doses of this chromosome fuses with the polar nuclei, we should obtain seeds that are nonsugary and have a deficient embryo. Twenty-seven plants grown from nonsugary seeds included twenty-four that were deficient for the B^4 chromosome and otherwise carried a normal chromosome 4 and 4^B . The remaining three were simply heterozygous for the interchange, as would be expected from normal disjunction in the division of the generative nucleus.

In summary, when a plant homozygous for TB-4a and used as the pollen

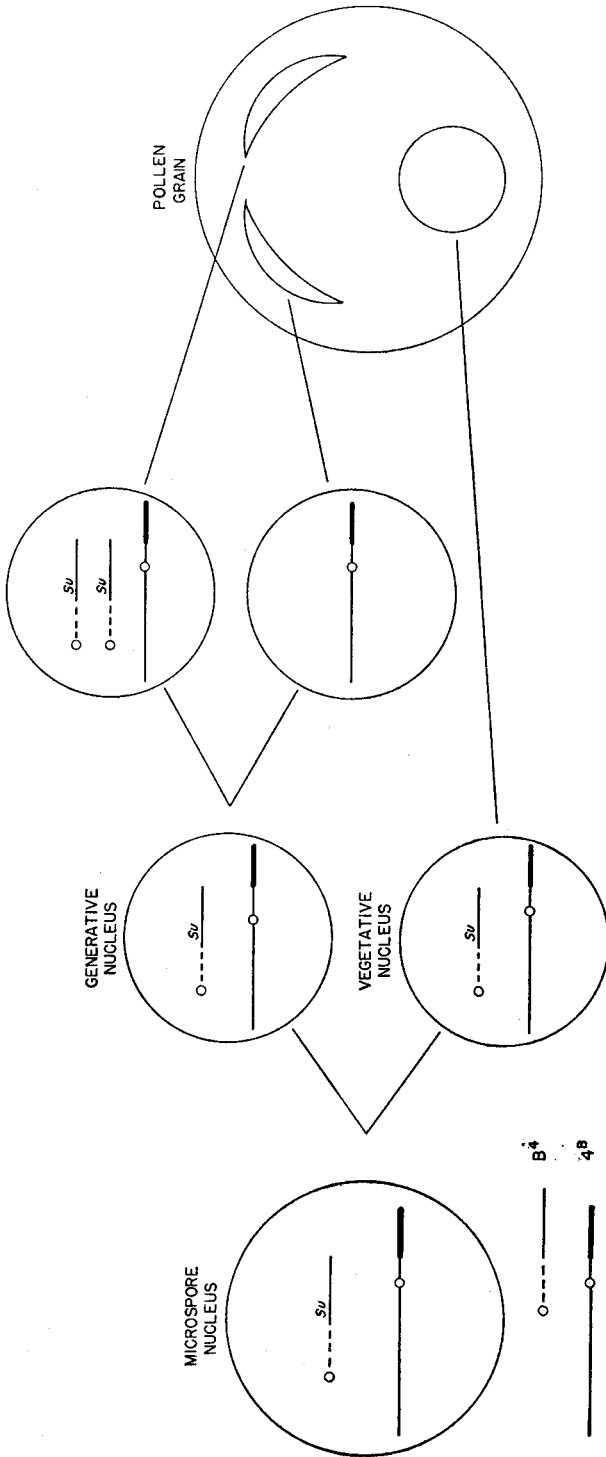


FIG. 4.—Probable course of events in the development of the pollen grain in which nondisjunction has occurred in the division of the generative nucleus.

parent is crossed with a normal plant, three types of seeds are produced. One of these has a hyperploid embryo and a deficient endosperm. Another has a deficient embryo and a hyperploid endosperm. The third type, which does not occur as frequently as the other two, has a euploid embryo, heterozygous for the interchange, and presumably also a euploid endosperm. The first two types are the result of mitotic nondisjunction of the B^4 chromosome in the second microspore division; the last, of normal disjunction.

If mitotic nondisjunction occurs in the development of the female gametophyte, its products are not transmitted to the viable eggs. When TB-4a was transmitted by the eggs in the cross of TB-4a (*Su Su*) × Normal (*su su*), none of the F_1 seeds were sugary (table 2). Twenty-one of the seeds were grown and all were found to be simply heterozygous for the interchange. It may be that the occurrence of nondisjunction, in the development of the female gametophyte, produces an inviable gamete which prevents seed formation or results in a seed lacking an embryo. However, there was no marked evidence of the elimination of inviable gametes on the ears obtained from this cross and all of the seeds had normal-appearing embryos. If mitotic nondisjunction occurs at all in the formation of the egg, it is infrequent compared with the rate with which it occurs in the second division of the microspore.

Rate of mitotic nondisjunction

The three types of seed obtained from the cross described in the preceding section are referable to three types of fertilization (fig. 5). Types I and II are reciprocals and involve male gametes that are aneuploid as a consequence of mitotic nondisjunction. In type III, the male gametes are euploid and are the product of normal disjunction.

The rate of nondisjunction in the division of the generative nucleus may be estimated from the data in table 3 if two assumptions are made. One is that the fertilization types I and II occur in equal numbers. The second assumption is that the three types of seed are equally viable. The ears obtained from the cross carried a full set of seed and the sugary and nonsugary seeds were normal in appearance. There was no gradation in seed size to suggest a possible elimination of either kind.

The consequence of each occurrence of mitotic nondisjunction would be either a sugary or a nonsugary seed and, on the first assumption, either type would be equally probable. The frequency of sugary seeds would thus be half of the rate of occurrence of mitotic nondisjunction. If the latter were 100 percent, that is, if it occurred in all of the second microspore mitoses, 50 percent of the seeds obtained from the cross should be sugary. The sugary seeds comprised 51.4 percent of the total population obtained from eight crosses involving two different interchange parents (table 3). By the chi-square test, this value does not deviate significantly from 50 percent but the deviation from 45 percent appears to be significant. It would seem, therefore, that the rate of mitotic nondisjunction is very high, between 90 and 100 percent. The evidence that it is not 100 percent has already been presented; three of the

twenty-four plants grown from nonsugary seeds were euploid and heterozygous for the interchange and were therefore the products of normal disjunction.

The calculation of the rate of nondisjunction must be made with some reservation. In two of the crosses listed in table 3, the chi-square values, 5.2 and 5.6, suggest that the deviations from 50 percent may not be due to sampling error alone. The same pollen parent was used in both crosses but it was also used in two other crosses, made under comparable conditions, which did

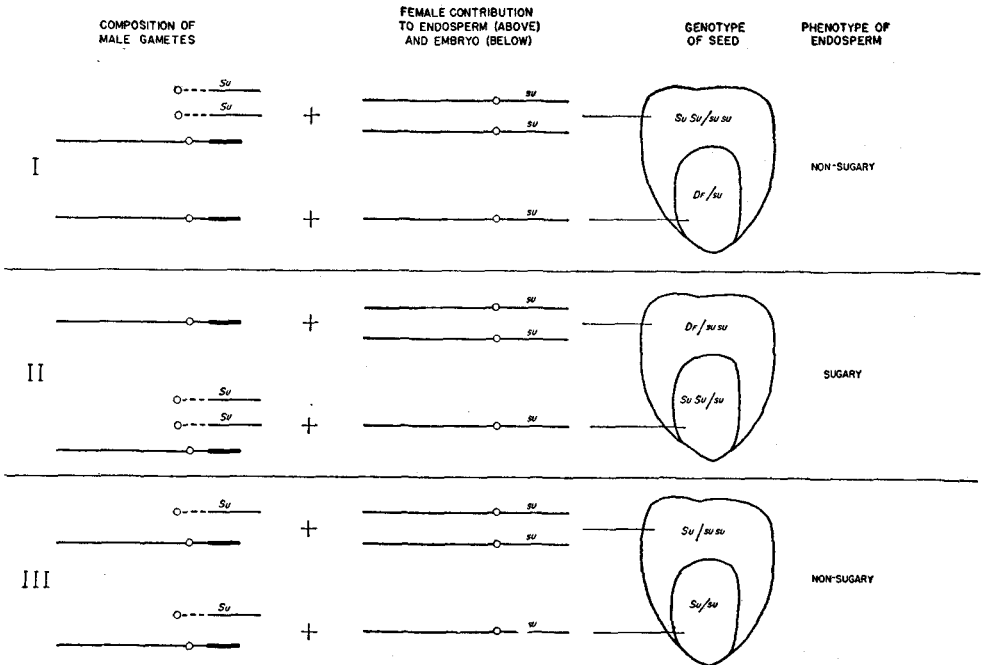


FIG. 5.—Types of double-fertilization as a result of mitotic nondisjunction (I and II) and normal disjunction (III). Types I and II are reciprocals.

not give a marked deviation. This suggests an influence of the female parent on the sugary seed frequency.

If a significant deviation from a 50 percent segregation of sugary seeds persists in additional crosses, the assumption that fertilization types I and II occur with equal frequency will require revision. Otherwise, another source of sugary seeds, not indicated by the results so far obtained, will be required. The excess of sugary seeds in some crosses suggests that fertilization type II occurs more frequently in these crosses than type I. The ratio of these two types cannot be determined directly from seed counts, since nonsugary seeds are derived from both types I and III. These could be separated if the seeds were grown and the resulting plants examined for the presence or absence of chromosome B^1 . This experiment is in progress.

Stability of the B⁴ chromosome during endosperm development

In view of the high rate of mitotic nondisjunction of the B⁴ chromosome in the division of the generative nucleus, it would be of interest to test for its occurrence in other divisions. Evidence has already been presented that mitotic nondisjunction is rare or absent in the divisions of the female gametophyte that lead to the formation of the egg. The study can be extended to the endosperm through the use of the sugary character.

The endosperms of the nonsugary seeds produced by the cross of Normal (*su su*) × TB-4a (*Su Su*) and its reciprocal are expected to be of three types, with respect to the sugary gene. When the interchange is transmitted by the seed parent, the expected composition of the endosperm is *Su Su su*. When it is transmitted by the pollen parent, two endosperm types, *su su Su Su* and *su su Su*, are obtained (fig. 5). The B⁴ chromosome carries *Su* and the normal chromosome 4 carries *su*.

The occurrence of mitotic nondisjunction of the B⁴ chromosome in the first division of a primary endosperm nucleus of composition *su su Su* would result in a sugary sector deficient for this chromosome in an otherwise nonsugary seed. Assuming uniform endosperm development, such a sector would comprise half the area of the endosperm. Smaller sectors would be obtained from mitotic nondisjunction in later divisions. In either case, the relationship between the frequency of sectors and the rate of nondisjunction would be a simple one; each occurrence of nondisjunction should yield a sugary sector.

The relationship is much more complicated in the seeds which have two B⁴ chromosomes in the endosperm. The "loss" of both of these would be a requirement for the occurrence of a sugary sector. It is conceivable that the loss could occur in either of two ways as a consequence of nondisjunction. First, one of the B⁴ chromosomes could undergo nondisjunction and the other disjoin normally to produce daughter cells with three and one B⁴ chromosomes, respectively. The recurrence of nondisjunction in a subsequent division of the cell containing one B⁴ chromosome would yield a cell line deficient for this chromosome, hence a sugary sector. Or, secondly, the B⁴ chromosomes might undergo nondisjunction simultaneously, with either of two results. If the B⁴ chromosomes move to one pole, the daughter cells would have four B⁴ chromosomes in one and none in the other. If they move in opposite directions, the original condition would be restored in each of the daughter cells. If these alternatives are equally probable, only half of the cases of simultaneous nondisjunction would lead to sugary sectors.

A simple example will illustrate the relationship between the rate of nondisjunction and the frequency of sugary sectors when two B⁴ chromosomes are present. A sugary sector comprising half the endosperm area would be expected from half the cases of simultaneous nondisjunction occurring in the division of the primary endosperm nucleus. If x is the rate of nondisjunction for each B⁴ chromosome per cell division and if each acts independently, the expected frequency of sectors of this size would be given by $x^2/2$. For a rate of nondisjunction of ten percent (one occurrence per ten divisions), the frequency of

"1/2"-sectors would be 0.5 percent, or five per thousand seeds. Smaller sectors would be expected to occur more frequently owing to a greater opportunity for both simultaneous and recurrent nondisjunction in later divisions. Sectors comprising less than one-sixteenth of the endosperm are detectable (STADLER and SPRAGUE 1936).

The seeds obtained from the crosses between normal (*su su*) plants and plants homozygous for TB-4a (*Su Su*) were examined carefully for sugary sectors. None were found among 1157 seeds produced when the interchange was transmitted by the seed parent. The reciprocal cross yielded seven sectors among 1678 seeds examined. The production of endosperms containing a single B⁴ by the latter cross (type III, fig. 5) probably accounts for the difference in the frequency of sugary sectors in the two crosses.

It should be made clear at this point that the occurrence of a sugary sector does not necessarily mean that mitotic nondisjunction of the B⁴ chromosome is involved. These sectors occur spontaneously in appropriate crosses involving normal parents, with a frequency of approximately one-tenth of one percent (STADLER and SPRAGUE 1936). They could arise as a result of mutation or the production of deficiencies including the *Su* locus due to fragmentation of the B⁴ chromosome. The B-type chromosome is subject to fragmentation (RANDOLPH 1941; DARLINGTON and UPCOTT 1941) and since the B⁴ chromosome contains the proximal third of the B-type chromosome, it is not unlikely that this mechanism furnishes at least a fraction of the sugary sectors found. With regard to nondisjunction, the results indicate that the B⁴ chromosome is remarkably regular in its behavior in the endosperm mitoses, in contrast with its aberrant behavior in the division of the generative nucleus of the microspore.

DISCUSSION

Mitotic nondisjunction, as demonstrated for the B⁴ chromosome, explains the transmission of the intact B-chromosome in most particulars. The exceptional 0B and 2B plants obtained from the 0B × 1B and 0B × 2B crosses (table 1) have their counterparts in the deficient and hyperploid plants produced by the crosses involving TB-4a as the male parent. The relatively rare 3B and 4B progeny from these crosses, however, cannot be accounted for on the basis of a single occurrence of nondisjunction. They suggest that this process may also occur occasionally during some division other than the second microspore mitosis, possibly in meiosis.

The uniformly high rate of nondisjunction, approximating 100 percent, estimated for the B⁴ chromosome does not apply in all cases to the intact B-type. A high rate is evident in the results of the 0B × 2B cross, in which no 1B progeny, indicative of normal disjunction, were obtained. In the 0B × 1B cross, however, a considerable number of 1B plants were produced and a good match with the observed data can be obtained if a rate of nondisjunction of only 50 percent is assumed (table 5). The calculations in this table are made on the basis that 0B and 1B microspores are produced in equal numbers by the 1B parent. This assumption affects the proportion of the 0B progeny in the F₁

population. It does not affect, however, the ratio of the 1B and 2B progeny to each other, and this ratio is in itself a sufficient indicator of the rate of nondisjunction.

It is unlikely that the rate of nondisjunction is influenced by whether the parent has one B-type or two, since in either case the microspore in which nondisjunction occurs has one B-type. Different rates are found for different plants of the same B chromosome number, as is shown by the results of five $0B \times 1B$ crosses, each involving a different 1B parent (LONGLEY 1927). One of these crosses yielded eight plants with two B-types and none with one (100 percent nondisjunction) and another gave none with two B-types and nine with one (no nondisjunction). The effect of environmental variation on the rate of nondisjunction is not known. There is a possibility that we are dealing

TABLE 5
Comparison of data from $0B \times 1B$ crosses with distribution expected from various rates of mitotic nondisjunction.

	B CHROMOSOME NUMBER IN F_1			
	0	1	2	3
Observed*	108	35	20	2
Expected from:				
50% nondisjunction	103	41	21	
75%	114	21	31	
100%	124	0	41	

* Combined data of LONGLEY and RANDOLPH.

with two or more types of B chromosomes (a view held for different reasons by DARLINGTON and UPCOTT 1941) which differ in their capacity for normal disjunction. It is also possible that variations in the basic genotype are responsible for these differences in the rate of nondisjunction. The work with TB-4a (and several other A-B interchanges) has not shown these differences to a marked extent but the results are not extensive enough to warrant the conclusion that they do not occur.

Localization of the chromosomal element responsible for mitotic nondisjunction

Fragment chromosomes derived from the B-type chromosome by terminal deletion give transmission results that are similar to those obtained for the intact B-type itself (RANDOLPH 1941). The smallest of these is the "F" chromosome which consists "merely of a centromere with an almost insignificant amount of attached chromatin." The evidence that this diminutive chromosome undergoes nondisjunction suggests that the B-type centromere is responsible for the aberrant process.

Similar evidence has been obtained from the investigation of the A-B interchanges. In the case of TB-4a, the interchange chromosome which undergoes mitotic nondisjunction carries the centromere and the proximal third or so of the B-type chromosome. The other interchange chromosome, carrying the

distal two-thirds of the B-type, disjoins normally. The study of five other A-B interchanges has proceeded far enough to show that the same relationship holds in these as well; the aberrant chromosome in each case is that one which possesses the B centromere.

In these interchanges, the points of breakage in the B-type chromosome range from one which occurred near the end of the chromosome to another well within the proximal euchromatic segment. The size of the A-segment in the B^A chromosome also varies widely. In one interchange, the A-segment consists of only the tip of the long arm of chromosome 7. In another, almost all of the short arm of chromosome 1 is involved, to form an interchange chromosome which is longer than the shorter members of the basic set. The length of the chromosome undergoing mitotic nondisjunction evidently has little or nothing to do with determining the occurrence of this process.

Other examples of mitotic nondisjunction are found in rye (HASEGAWA 1934; MÜNTZING 1946), in sorghum (DARLINGTON and THOMAS 1941), and in the fungus gnat *Sciara* (METZ 1938). In these, as in maize, nondisjunction occurs for the most part only in certain mitoses. The supernumerary in rye undergoes nondisjunction in the first microspore mitosis and also at some point in the development of the female gametophyte. In sorghum, nondisjunction occurs in a supernumerary division of the vegetative nucleus of the pollen grain. In *Sciara*, it occurs in the division of the secondary spermatocyte. In the four cases, a specific chromosome (or a derivative of this chromosome) undergoes mitotic nondisjunction; the other chromosomes of the set disjoin normally. Thus there are two conditions which are essential to the occurrence of mitotic nondisjunction: (1) a chromosome of a particular kind, and (2) a mitosis that imposes a restriction to normal disjunction that is specific for this chromosome.

A comparison of the aberrant chromosomes in the examples cited above reveals a striking relation with respect to the position of the centromere. The centromere in rye is subterminal and in maize it is terminal or nearly so. In *Sciara*, the position is different in different species. It is at or near the end of the chromosome in *S. coprophila* whereas it is median in *S. pauciseta* (SCHMUCK 1934). The aberrant chromosomes in sorghum, as reported in two plants, also have median centromeres and are of two distinct sizes (DARLINGTON and THOMAS 1941). The larger of these has been identified as an iso-chromosome. It has been shown in rye that large and small isochromosomes are derived from the standard supernumerary which has a subterminal centromere (MÜNTZING 1944). The aberrant chromosomes in sorghum (and perhaps also those with median centromeres in some species of *Sciara*) may have been similarly derived. Thus, in each case, the chromosome that is undergoing mitotic nondisjunction either is or may have originated from a chromosome with a subterminal or terminal centromere, in contrast with the normal chromosomes of the respective sets (again expecting *Sciara*) which have distinctly internal, usually median or submedian, centromeres.

There is some argument concerning the position of the centromere of the B-type in maize. McCLINTOCK (1933) described it as terminal on the basis of examination in the pachytene stage of the microsporocyte. RANDOLPH (1941)

reported a diminutive short arm, seen in pachytene, which was not evident in all figures. He believes that the centromere assumes a spurious terminal appearance in some figures because the short arm folds back against the pycnotic knob adjacent to the centromere in the long arm. DARLINGTON and UPCOTT (1941) reported a sizeable short arm, long enough to permit the formation of observable chiasmata. They suggest that the B-type may vary structurally to give various positions for the centromere. The B-types involved in the A-B interchanges all appear to have terminal centromeres when examined in pachytene and no marked structural differences were found. It is however possible that a very small arm, such as RANDOLPH observed, could have been missed in the examinations.

The similarity between the aberrant chromosomes of the different organisms suggests a relationship between mitotic nondisjunction and the position of the centromere. A chromosome with a subterminal or terminal centromere could be derived from a chromosome with a more centrally located centromere in one of two ways, either by rearrangement of the chromosome or by loss of a segment in one arm. Either case would require a break at or near the centromere. An aberrant chromosome might be produced if, in the process of rearrangement or loss, there is an impairment of the effectiveness of the centromere or adjacent chromatin vital to normal disjunction. If this were the case, it should be possible to produce a chromosome showing mitotic nondisjunction as a result of such structural changes in a normal chromosome.

The telocentric chromosome studied by RHOADES (1940) in maize may represent a case of this kind. This chromosome was found in a plant obtained from a cross involving a parent trisomic for chromosome 5. It consists of the entire short arm of chromosome 5 and has a centromere that is unquestionably terminal. When plants hyperloid for the telocentric chromosome are crossed with normal diploids, the progeny include hyperloid plants of two types. One of these contains a single telocentric chromosome in addition to the diploid complement; the other has two doses of the telocentric chromosome in the form of an isochromosome. These two types are produced in about equal numbers when the male parent transmits the telocentric chromosome. When it is transmitted by the female parent, the progeny containing an isochromosome comprise only 0.57 percent of the hyperloid types. The formation of isochromosomes from telocentric chromosomes is thus much more frequent in the male germ line than in the female germ line.

This dependence on sex is remarkably similar to that found for the occurrence of mitotic nondisjunction of the B-type and the B⁴ chromosomes. The tests made by RHOADES show that the formation of the isochromosome is a postmeiotic process. In the light of the behavior of the B⁴ chromosome, the results obtained for the telocentric chromosome may be interpreted as another case of nondisjunction in the division of the generative nucleus. The results differ in one respect from those obtained for the B⁴ chromosome. In the case of the latter, two chromosomes are distributed as individuals to one of the gametes. The telocentric chromosome, however, forms an isochromosome in the process. It is doubtful that nondisjunction occurs first and is followed by

a fusion of the terminal centromeres since RHOADES found that two telocentric chromosomes could exist in one nucleus without forming an isochromosome. It would seem, rather, that the centromere of the telocentric chromosome either fails to divide or divides transversely (DARLINGTON 1940) to produce the isochromosome. In this respect, the telocentric differs from both the B-type and the B⁴ chromosomes. In all three cases, however, it is probable that the centromere is responsible for the aberrant behavior.

Cytological observations by MÜNTZING (1946) in rye suggest that the centromere may not be the only chromosomal element responsible for mitotic nondisjunction. The aberrant chromosome in rye lags in the first microspore mitosis and is usually included in the generative nucleus. The lagging is due to the failure of a region near the centromere to divide in concert with the rest of the chromosome. The activity of the centromere appears to be normal.

Some other uses of A-B interchanges

The A-B interchanges provide a means of obtaining aneuploid plant types that are difficult to produce by other methods. The duplications and deficiencies that are obtained in this way are useful in the study of various cytogenetic problems. The examples that follow show some uses of the aneuploid types in problems under investigation.

It has been shown in the case of TB-4a that functional deficient and hyperploidy male gametes are produced by mitotic nondisjunction in the division of the generative nucleus. As a result, progeny are obtained which carry a specific segment of chromosome 4 in one, two, and three doses. It should be possible, by appropriate crosses, to obtain the segment in even higher numbers. For example, a plant hyperploidy for the B⁴ chromosome forms hyperploidy eggs. If these are fertilized by sperm that are also hyperploidy for the B⁴ chromosome, plants should be obtained that carry four doses of the segment of chromosome 4. The limit to continued accumulation would depend only on the degree of unbalance caused by the segment. Preliminary results have shown that a dosage series may also be established in the case of other A-B interchanges as well. The mechanism of mitotic nondisjunction thus provides a method for the study of the effect of specific chromosomal segments in various numbers. In the same way, it makes possible a determination of the effect of known recessive or dominant genes located in these segments.

The production of functional deficient gametes may also be utilized to locate recessive genes within the chromosome. A plant which contains the recessive gene is crossed as the seed parent with a plant carrying an A-B interchange and homozygous for the dominant allele. The deficient progeny will show the recessive character if the locus of the gene is distal to the point of breakage in the A chromosome. This method is illustrated by the use of TB-4a in locating *su*. The TB-4a parent was homozygous for *Su*. Crosses with *su su* plants gave kernels that were of the sugary phenotype. These kernels were deficient for the B⁴ chromosome thus indicating that *Su* is carried on this chromosome.

Intercrosses involving different A-B interchanges may provide information

as to whether or not maize is of polyploid origin. In appropriate crosses, plants can be produced that have two different A-segments in duplication. For example, a cross between a plant that has TB-4a with one that has TB-7a will yield plants in which the B⁴ and the B⁷ chromosomes are present as duplications. If the segment of chromosome 4 has a region within it that is a duplication of a region in the chromosome 7 segment, the homology might be revealed by the regular synapsis of these regions.

Another interesting aspect of these interchanges is the possibility of an interaction between the heterochromatin of the B-type and the adjacent A-chromatin in the interchange chromosomes. *Drosophila* investigations have shown that the action of a gene may be altered when, following translocation, it is brought adjacent to or relatively near a heterochromatic region. Such an effect has not been observed in maize although many interchanges between A chromosomes have been studied. No marked effect has been apparent in the case of TB-4a, but the break in the B-type in this interchange is probably not within the heterochromatic segment. In other A-B interchanges, the B-type was broken well within this segment with the result that B heterochromatin is in direct contact with A euchromatin. Interchanges of this kind provide material for a study of the heterochromatin-euchromatin relationship in maize.

SUMMARY

Eight interchanges between A-type and B-type chromosomes were obtained from B-bearing pollen treated with X-rays. The behavior of one of these, involving chromosome 4 of the basic set, is reported in this paper.

The results of crosses involving plants homozygous for this interchange (designated TB-4a) show that one of the interchange chromosomes (B⁴) undergoes nondisjunction in the second division of the microspore. As a consequence, the two gametes of a pollen grain carry different chromosomal complements. One has the aberrant chromosome in duplicate; the other is deficient for this chromosome. Both gametes are functional.

Nondisjunction of this chromosome occurs in most but not all of the second microspore mitoses. The seeds obtained from a cross between a pollen parent homozygous for TB-4a and a normal seed parent are therefore of three kinds. One has an endosperm that is deficient for the B⁴ chromosome and an embryo that carries the chromosome in duplicate. Another has a hyperploid endosperm and a deficient embryo. The third has a euploid endosperm and is simply heterozygous for TB-4a in the embryo.

The B⁴ chromosome possesses the centromere and proximal third of the B-type chromosome. The interchange chromosome bearing the centromere of chromosome 4 disjoins normally. Preliminary studies in five other A-B interchanges have shown that it is the interchange chromosome which bears the B-centromere that is aberrant in each case.

The B⁴ chromosome is relatively stable in endosperm development and undergoes nondisjunction rarely, if ever, in the development of the egg.

The anomalous male transmission of the intact B-type chromosome may

also be accounted for on the basis of nondisjunction in the second microspore division.

ACKNOWLEDGMENTS

I wish to express my appreciation to PROFESSOR L. J. STADLER for his guidance during the course of this investigation; to DOCTOR BARBARA MCCLINTOCK for her valued advice and criticism; and to PROFESSOR L. F. RANDOLPH, who generously supplied me with some of the B chromosome stocks used in the experiments.

LITERATURE CITED

- ANDERSON, E. G., and L. F. RANDOLPH, 1945 Location of the centromeres on the linkage maps of maize. *Genetics* **30**: 518-526.
- BURNHAM, C. R., 1932 An interchange in maize giving low sterility and chain configurations. *Proc. Nat. Acad. Sci.* **18**: 434-440.
- CLARKE, A. E., and E. G. ANDERSON, 1935 A chromosomal interchange in maize without ring formation. *Amer. J. Bot.* **22**: 711-716.
- DARLINGTON, C. D., 1940 The origin of isochromosomes. *J. Genet.* **39**: 351-361.
- DARLINGTON, C. D., and P. T. THOMAS, 1941 Morbid mitosis and the activity of inert chromosomes in Sorghum. *Proc. Roy. Soc. London* **130**: 127-150.
- DARLINGTON, C. D., and M. B. UPCOTT, 1941 The activity of inert chromosomes in *Zea Mays*. *J. Genet.* **41**: 275-296.
- HASEGAWA, NOBUMI, 1934 A cytological study on 8-chromosome rye. *Cytologia* **6**: 68-77.
- LONGLEY, A. E., 1927 Supernumerary chromosomes in *Zea Mays*. *J. Agric. Res.* **35**: 769-784.
- MCCLINTOCK, BARBARA, 1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea Mays*. *Z. Zellf. Mik. Anat.* **19**: 191-237.
- METZ, C. W., 1938 Chromosome behavior, inheritance and sex determination in *Sciara*. *Amer. Nat.* **72**: 485-520.
- MÜNTZING, ARNE, 1944 Cytological studies of extra fragment chromosomes in rye I. Iso-fragments produced by misdivision. *Hereditas* **30**: 231-248.
- 1946 Cytological studies of extra fragment chromosomes in rye III. The mechanism of nondisjunction at the pollen mitosis. *Hereditas* **32**: 97-119.
- RANDOLPH, L. F., 1928 Chromosome numbers in *Zea Mays* L. *Cornell Agric. Exp. Sta. Memoir* **117**: 44 pp.
- 1941 Genetic characteristics of the B chromosomes in maize. *Genetics* **26**: 608-631.
- RHOADES, M. M., 1940 Studies of a telocentric chromosome in maize with reference to the stability of its centromere. *Genetics* **25**: 483-520.
- SCHMUCK, M. LOUISE, 1934 The male somatic chromosome group in *Sciara pauciseta*. *Biol. Bull.* **66**: 224-227.
- STADLER, L. J., and G. F. SPRAGUE, 1936 Genetic effects of ultraviolet radiation in maize. I. Unfiltered radiation. *Proc. Nat. Acad. Sci.* **22**: 572-578.
- WEATHERWAX, PAUL, 1917 The development of the spikelets of *Zea Mays*. *Bull. Torrey Bot. Cl.* **44**: 483-496.