JULY, 1938 RESEARCH BULLETIN 290

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

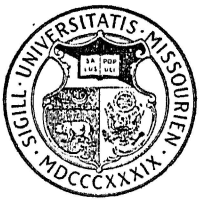
AGRICULTURAL EXPERIMENT STATION

F. B. MUMFORD, *Director*

THE FUSION OF BROKEN ENDS OF SISTER HALF-CHROMATIDS FOLLOWING CHROMATID BREAKAGE AT MEIOTIC ANAPHASES.

BARBARA MCCLINTOCK

(Publication Authorized July 12, 1938)



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The Fusion of Broken Ends of Sister Half-Chromatids Following Chromatid Breakage at Meiotic Anaphases

BARBARA MCCLINTOCK

I. Introduction.

X-ray induced chromosomal alterations have strongly suggested that breaks in chromosomes are followed by fusions of broken ends. The evidence from ring-shaped chromosomes in maize (McClintock, 1938) likewise suggests that broken ends of chromosomes will unite, for rings are continually changing in size by a mechanism which appears to break them at somatic anaphases but does not give rise to rod fragments.

To determine whether such fusions occur, breakage of chromosomes should be experimentally obtained at one division and the results of such breakage observed in the following divisions. An individual heterozygous for an inversion which does not include the spindle fiber attachment region will give rise, after a crossover within the inversion, to a chromatid with two spindle fiber attachment regions and a fragment chromatid with no spindle fiber attachment region (*a*, Figure 3). The size of the fragment thus formed is constant, provided the crossing over always occurs at homologous regions in the chromatids. Following such a crossover, a chromatid bridge is formed at anaphase I. Breakage of such a bridge is known to occur and a broken chromatid enters each telophase nucleus. If the inversion is sufficiently long, a four-strand double crossover (chromatids 1 with 3 and 2 with 4) within the inversion should occur. This produces two chromatids, each with two spindle fiber attachment regions, and two free fragments each lacking a spindle fiber attachment region (*b*, Figure 3). At anaphase I, a double bridge involving the two chromatids with two spindle fiber attachment regions, and two free fragments of similar size would be produced. Breakage of the double bridge at anaphase I would result in the entrance into each telophase I nucleus of two chromatids, each with a broken end. If broken ends of chromosomes tend to unite, the two cells resulting from division I should each show at anaphase II a chromosome involved in a bridge configuration. In maize, a cell plate is formed at the end of the first meiotic division. During the second division the two cells remain together and their division figures may be simultaneously observed. If fusions always occur, the cells with two free

fragments should always show a bridge configuration in each sister cell.

In order to secure such evidence on reattachment following chromosome breakage, it is necessary to obtain an inversion which is sufficiently long to produce a significant number of four-strand double crossovers within the inversion. For this purpose, pollen was x-rayed and placed upon silks of normal, untreated plants. The F_1 individuals were examined cytologically until an individual was found with an inversion fitting the requirements. The inversion found included a section of the long arm of chromosome-4 (Figure 1).

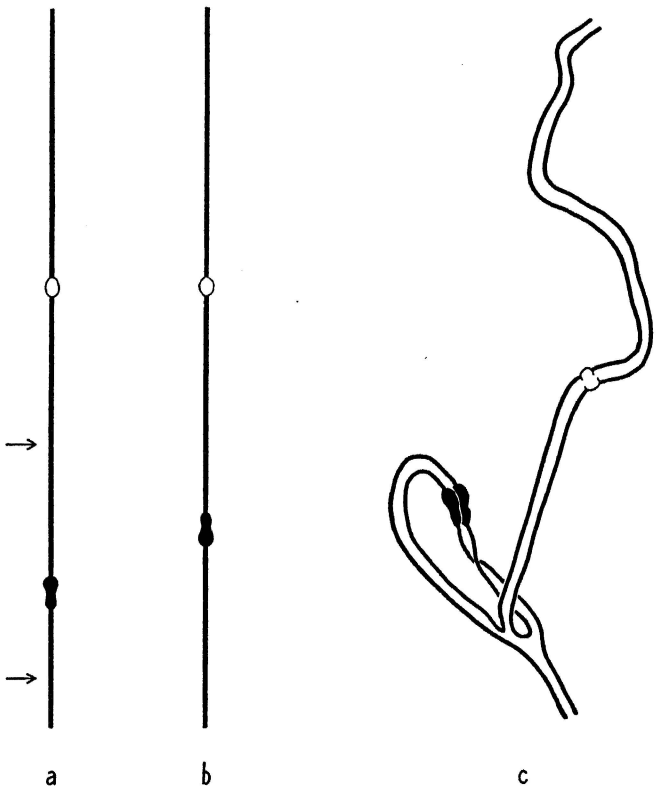

Figure 1.—*a*. Diagram of a normal chromosome-4. The clear bulging region represents the spindle fiber attachment region. The enlarged body toward the end of the long arm represents the knob. The arrows point to the positions of the breaks in the chromosome which gave rise to the inversion with the changed position of the knob shown in *b*. *c*. Outline drawing of a pachytene configuration showing the homologous synaptic association of a normal chromosome-4 and a chromosome-4 with the inversion.

Although many anaphase II figures were observed in which it was known that two broken ends had entered the telophase I nucleus, the evidence for fusions of these broken ends was mainly negative. In most cases, there was no evidence of a bridge in either cell and often, in these cells, the chromatids which had broken in anaphase I could be easily recognized in the group. In a few cases there appeared to be a very weak adherence of the two broken ends in one of the two cells. It was very clear, however, from the majority of figures that the two broken ends of the two chromatids which enter the telophase I nucleus do not usually fuse with one another.

While investigating the first nuclear division in the microspore of an individual known to produce a chromatid bridge at anaphase I or II involving chromosome-6, a number of anaphase configurations were found in which a single chromosome produced a bridge configuration. The chromosome involved in the bridge could readily be recognized as chromosome-6. Such a bridge configuration in the spores could be produced if the *chromatid* which has broken at anaphase I or II were considered as being double, *i. e.*, as having a split in preparation for the first division in the microspore and if fusions of the two split halves of the chromatid occurred at the position of breakage. Sax (1937) has diagrammed such a process to account for bridge configurations in the microspores of a Tradescantia individual known to have had bridge configurations at anaphase I or II. A similar diagram to illustrate this possibility is given in Figure 2.

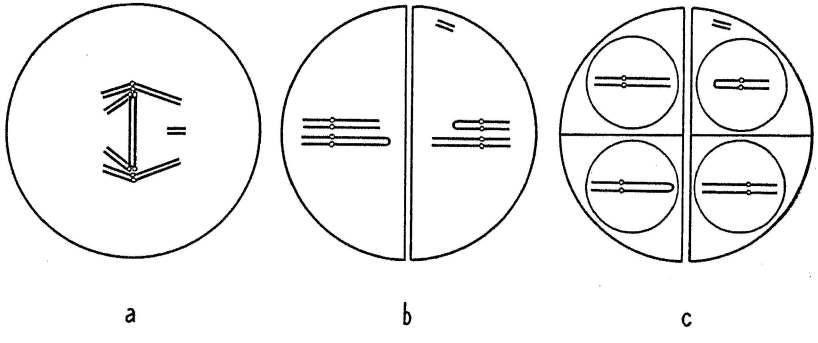

Figure 2.—Diagram to illustrate the method by which bridge configurations in the anaphase of the spore mitosis could be produced following breakage of a chromatid during meiosis. *a.* An anaphase I configuration following a crossover within the inversion. The chromatid is represented as being split in preparation for the spore mitosis. Breakage of the bridge results in the entry into each telophase I nucleus of a broken chromatid. If fusions occurred between the two halves of the split chromatid at the position of breakage a situation like that shown in *b* would exist at metaphase II. A quartet of spores would result as shown in *c*. The two split halves of the broken chromatids would be fused and would result in an anaphase bridge in the first mitosis in two of these spores.

The method of formation of anaphase I and II bridges involving chromosome-6 is distinctly different from the "inversion-crossover" process illustrated in Figure 3 and will be reported in a separate publication. However, the bridge configurations in the spores of this individual did suggest that the spores of plants which are heterozygous for the inversion in chromosome-4 should be examined to determine if such bridge configurations were likewise present. Consequently, a number of anthers with spores undergoing nuclear division were examined. Anaphase and telophase configurations were observed in 2286 spores. In 260 of these spores, a bridge configuration involving a single chromosome was observed. The question then arises: Do the spores which show a bridge configuration possess a broken chromosome-4, *i. e.,* can the bridge configuration be interpreted as the result of a fusion at the point of breakage between the two split halves of a chromatid? The answer is in the affirmative. It is the purpose of this paper to show the method by which an affirmative answer has been reached.

The method is briefly as follows: As a result of a crossover within the inversion, a chromatid bridge is formed at anaphase I (*a*, Figure 3). Coincident with the bridge formation is the production of a fragment chromatid with no spindle fiber attachment region. Breakage of the chromatid bridge occurs at anaphase or telophase I and a broken chromatid enters each telophase I nucleus. As a result of the second meiotic mitosis four spores are produced, two of which contain a broken chromosome-4 and two of which contain a normal chromosome-4. The fragment chromatid with no spindle fiber attachment region and thus no means of directed movement in the spindle figure, remains in the cytoplasm of one of these spores. Its distribution to one of the four spores of the quartet is at random. Since the majority of the spores with a broken chromosome-4 arise from a sporocyte which had a single bridge and single fragment during meiosis, one-fourth of all these spores in an anther should contain a fragment chromatid. Likewise, one-fourth of all the normal chromosome containing spores which have arisen from such sporocytes should contain a fragment. The fragment chromatid remains in the cytoplasm of the spore as a visible body throughout the development of the spore and can be seen either before, during or after the first nuclear division in the spore (Figure 21).

If 50 per cent of the microsporocytes in an anther have a bridge configuration at anaphase I or II resulting from a crossover within the inversion, 25 per cent of the spores in an anther should contain a broken chromosome-4. Twenty-five per cent of these spores with

a broken chromosome-4, in turn, should also possess a fragment chromatid. Seventy-five per cent of the spores within an anther should contain a normal chromosome-4. Only 8.3 per cent of these spores should contain a fragment chromatid. This arises from the following considerations. Two-thirds of these normal chromosome containing spores arise from 50 per cent of microsporocytes which had no crossing over or fragment formation. The remaining one-third of these spores arise from the sporocytes which had a crossover and produced a fragment. Through random distribution of the fragment to one of the four spores of a quartet, only one-fourth of the normal chromosome containing spores so produced will also have a fragment. Thus, one-fourth of one-third or one-twelfth (8.3%) of the normal chromosome containing spores will have a fragment.

If fusions of broken ends occur as shown in Figure 2, a bridge configuration at the anaphase of the first nuclear division would be present in each spore which possesses a broken chromosome-4. A fragment should be present in 25 per cent of these spores. Among the spores without a bridge configuration, *i. e.,* those with a normal chromosome-4, a fragment should be present in only 8.3 per cent of the spores. The percentage of spores with a fragment among those with a broken chromosome-4 will remain approximately constant, *i. e.,* 25, regardless of expected variations in crossing-over. The percentage of spores with a fragment among those with a normal chromosome-4 should fluctuate from anther to anther with the fluctuations in the amount of crossing-over within the inversion: the greater the number of sporocytes in an anther which had a crossover, the higher the percentage and vice versa. Barring a possible situation in which every sporocyte in the anther had a crossover within the inversion (which has never been observed and is not to be expected from knowledge of the synaptic configurations) the percentage of spores with a fragment among the normal chromosome-4 containing class will always be lower than that in the class with a broken chromosome-4. The observations to be reported in this paper indicate that it is always considerably lower.

On the basis of the fragment ratios in the two types of spores, those without a bridge configuration and those with a bridge configuration, it should be possible to determine whether the spores which show a bridge configuration likewise possess a chromosome-4 which has been broken during the meiotic mitoses. The evidence so obtained should place one in the position to conclude whether or not fusions have occurred at the position of breakage between the two split halves of the broken chromatid-4. As stated above, the evidence is clearly in

the affirmative. In the discussion to follow, supplementary evidence in support of this conclusion will be presented.

From these studies, supplemented by those involving chromosome-6, the conclusion is reached that a chromatid, broken in either meiotic mitosis, will produce a bridge configuration in the first division of the microspore through fusions of the two split halves of the chromatid at the position of breakage.

A detailed examination of meiotic mitoses and spore mitoses in plants heterozygous for the inversion in chromosome-4 has revealed several types of chromosomal behavior not previously emphasized, *i. e.,* the directed and undirected methods of bringing about the inclusion within a telophase nucleus of a chromosome without a spindle fiber attachment region and the influence and subsequent behavior of this chromosome. These features will be dealt with in their appropriate places further on.

The following sections will be devoted to (1) a description of the meiotic mitoses which furnish information regarding the expected percentage of spores in an anther which contain a broken chromosome-4, (2) the methods used in determining the random distribution of the fragment to one of the four spores of a quartet following crossing over within the inversion, (3) the method used to determine when the spores with a broken chromosome-4 undergo their mitoses and, finally, (4) a discussion of the spore mitoses with the numerical relationships shown by their bridge and fragment ratios.

II. The Processes by Which Broken Chromatids Are Produced in the Meiotic Mitoses and Their Relative Frequencies.

The inversion used in this study involved a section of the long arm of chromosome-4 which included the characteristically shaped knob, Figure 1. The production of a chromosome bridge at anaphase I or II always involves at least one crossover within the inverted section. The types of configurations seen in anaphase I and II depend upon the types of crossovers which have occurred within the inversion and between the inversion and the spindle fiber attachment region. Among all the sporocytes there are five classifiable types of anaphase I and their corresponding types of anaphase II configurations. One of these types is represented by a normal anaphase I and II resulting either from a lack of crossing over within the inversion or from a 2-strand double crossover (a compensating double crossover) within the inversion. The other four classifiable configurations result from specific types of crossovers. Several types of crossovers may result in a single type of anaphase I and II configurations. For

the purpose of this paper, however, it is only necessary to know the frequency of these types of anaphase I and II configurations. Consequently, in the diagram given to illustrate these types, Figure 3, only one method of origin of each type has been given. In the upper part of Figure 3 the inversion is diagrammed and crossover positions are indicated by the numerals 1, 2 and 3. The four types of anaphase I configurations with their corresponding anaphase II configurations are diagrammed below under a, b, c and d. In each of these cases, the configuration in the anaphase I cell is given above with the corresponding anaphase II configuration directly below. The four types of configurations are as follows:

For anaphase I:

1. A single bridge and single fragment (from a crossover at 1 or 2, giving rise to the upper cell, *a*, Figure 3).

2. A double bridge and two fragments (from a double crossover, 1 and 2, giving rise to the upper cell, *b*, Figure 3).

3. A free fragment and no bridge (from a double crossover, 2 and 3, giving rise to the upper cell, *c*, Figure 3).

4. An anaphase with two free fragments and no bridge (from a triple crossover, 1, 2 and 3, giving rise to the upper cell, *d*, Figure 3).

The corresponding anaphase II configurations are as follows:

1. A normal appearing anaphase in both cells, with a free fragment in one of these cells (from 1 above; *a*, Figure 3).

2. A normal appearing anaphase in both cells, with two free fragments variously distributed (from 2 above; *b*, Figure 3).

3. A bridge configuration in one cell and no bridge in the sister cell, with a fragment in one of these cells (from 3 above; *c*, Figure 3).

4. A bridge configuration in each sister cell, with two fragments (from 4 above; *d*, Figure 3).

To determine the percentages of the different types of configurations, counts were made at anaphase I and II. These have been recorded in Tables 1 and 2 for the anaphase I configurations and in Table 3 for the anaphase II configurations.

In column 2 of Table 1, the numbers of sporocytes with no bridge or fragment formation are recorded. The numbers of sporocytes with a single bridge and fragment (*a*, Figure 3) are recorded in columns 3 and 4. Two columns are necessary because of the following consideration. It might be expected that the fragment with no spindle fiber attachment region, resulting from a configuration diagrammed in *a*, Figure 3, would be released at anaphase I and lie free in the spindle figure. Such configurations were found in many of the sporocytes (Figure 4). In many cases, however, the fragment was as-

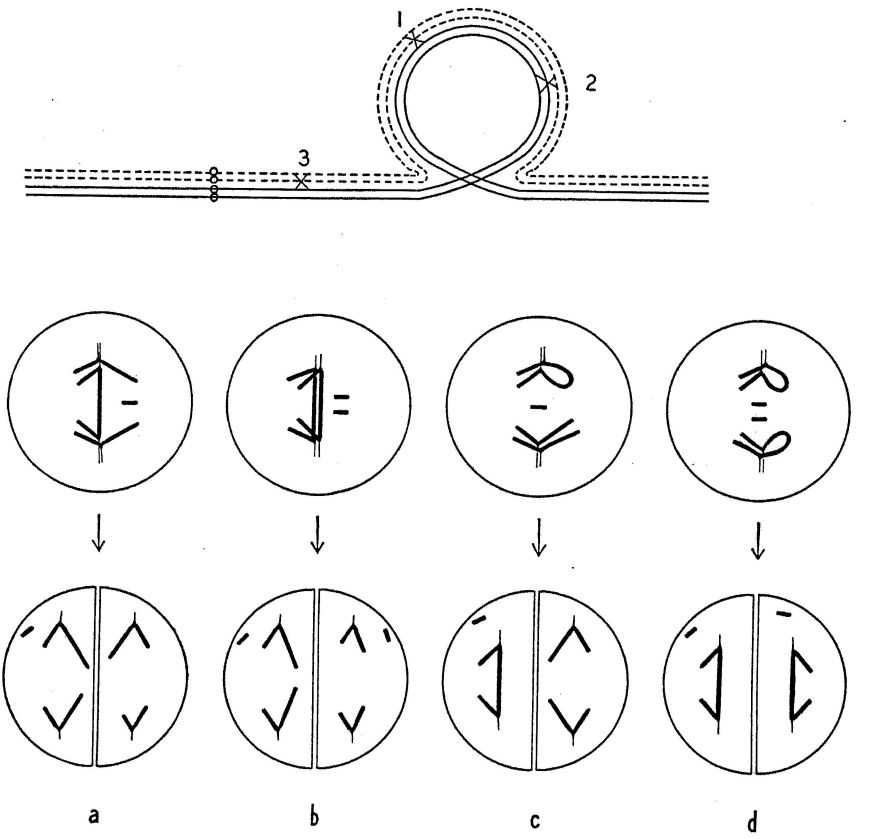

Figure 3.—Diagram to illustrate the methods of origin of recognizable types of anaphase 1 and II configurations. (For additional diagrams illustrating other methods of origin of these anaphase I and II configurations, see Darlington, 1937, pages 266-267). The synaptic association of a normal and an inverted chromosome is diagrammed above. The spindle fiber attachment regions are represented as circles. The first row of figures below this diagram represents the recognizable anaphase I configurations; the second row, the corresponding anaphase II configurations in each case. At anaphase I, disjunction of homologous spindle fiber regions occurs. *a*. Anaphase I and II configurations following a single crossover, 1 or 2 above, within the inverted segment. *b*. Anaphase I and II configurations following a four-strand double crossover, 1 and 2 above, within the inverted segment. *c*. Anaphase I and II configurations following crossovers at 2 and 3 above. *d*. Anaphase I and II configurations following crossovers at 1, 2 and 3 above.

TABLE 1.—FIRST MEIOTIC ANAPHASE CONFIGURATIONS.

Plant	No bridge; no fragment	Single bridge: Fragment free	Single bridge: Fragment attached	Double bridge; two fragments	No bridge; one free fragment	% single bridge; one fragment	% double bridge; two fragments	% no bridge; one fragment
1013-5	44	13	7	1	2	30.0	1.5	3.0
1013-11	40	29	18	5	4	48.9	5.2	4.1
"	58	20	19	4	1	38.0	3.9	1.0
"	11	6	1	0	1	36.8	0.0	0.5
"	18	10	8	2	1	46.1	5.1	2.5
1013-10	52	22	13	2	2	38.4	2.2	2.2
"	27	10	9	1	2	38.8	2.0	4.1
"	27	16	15	2	1	50.8	3.2	1.6
"	6	5	1	0	0	50.0	0.0	0.0
Totals	283	131	91	17	14	41.4	3.1	2.6

TABLE 2.—FIRST MEIOTIC ANAPHASE CONFIGURATIONS.

Plant	No bridge; no fragment	Single bridge; one fragment	Double bridge; two fragments	No bridge; one fragment	% single bridge; one fragment	% double bridge; two fragments	% no bridge; one fragment
1013-4	34	30	1	3	44.1	1.4	4.4
"	74	54	4	4	39.7	2.9	2.9
1013-9	10	6	1	1	33.3	5.5	5.5
"	18	19	0	0	51.3	0.0	0.0
"	43	40	4	4	43.9	4.4	4.4
"	16	16	1	3	44.4	2.7	8.3
Totals	195	165	11	15	42.7	2.8	3.8

sociated at one of its ends with the end of one of the normal chromatids of the bivalent (Figure 5) sometimes by a clearly discernible chromatin thread. The numbers of these two types of anaphase I configurations are given in columns 3 and 4, respectively of Table 1. It should be emphasized that when the fragment is associated with an end of a normal chromatid it is carried along during the poleward movement of this chromatid and becomes included in the telophase nucleus. The bridge produced by the chromatid with two spindle fiber attachment regions breaks in some sporocytes during late anaphase, the position of the break being variably placed between the two spindle fiber attachment regions. The broken ends enter the telophase nuclei, leaving no evidence of the previous bridge. In other sporocytes the chromatin bridge becomes very attenuated at late anaphase, and telophase sets in before breakage of the bridge is accomplished. The forming cell-plate in these sporocytes cuts through the chromatin bridge, which results in a break in the bridge at this position. Only very rarely does an intact bridge between the two telophase nuclei persist after the cell plate formation. When the breakage of the chromatin bridge is delayed until telophase, two types of

sporocytes with bridge configurations are distinguishable: (1) those in which a fragment is present in the cytoplasm (Figure 8) and (2) those in which no fragment is present in the cytoplasm (Figure 9). The former arises from the anaphase configurations entered in column 3, Table 1, the latter from the anaphases entered in column 4, Table 1. Although breakage of the chromatin strand eventually occurs at the cell plate region in these sporocytes, the broken strands are not drawn into the telophase nuclei but remain as attenuated threads extending from the nucleus toward the cell plate. This position of the broken strand is retained through the interkinesis into the following prophases (Figures 14 and 15).

The four-strand double crossovers within the inversion (*b*, Figure 3) are easily recognizable at anaphase I by the double bridge and the two free fragments, Figure 6. The numbers of sporocytes with such a configuration are entered in column 5, Table 1. The double crossover diagrammed in *c*, Figure 3, is likewise clearly recognizable. In such anaphase I configurations no bridge is present, but the free fragment is seen in the spindle figure (Figure 7). The numbers of these configurations are entered in column 6, Table 1. The triple crossover, diagrammed in *d*, Figure 3, is so rarely observed that it has not been entered in the table. In the last three columns of Table 1 the percentages of the single crossovers and the two types of double crossover are entered.

Table 2 is similar to Table 1 with the exception that the two classes of single crossover configurations, those with a free fragment and those in which the fragment is associated with the end of a normal chromatid, have been combined (column 3).

The anaphase II configurations are dependent upon the preceding anaphase I configurations, as shown in Figure 3. They have served to verify the anaphase I configurations and frequencies and have also contributed several other points of some interest. In

TABLE 3.—SECOND MEIOTIC ANAPHASE CONFIGURATIONS. THE TWO SISTER CELLS ARE CONSIDERED AS A UNIT.

Plant	No bridge; no fragment	No bridge; fragment in cytoplasm of one cell	No bridge; fragment in spindle of one cell	No bridge; two fragments in cytoplasm	Bridge in one cell; fragment in one cell	% no bridge; one fragment	% no bridge; two fragments	% one bridge; one fragment
1013-11	36	25	14	1	1	50.6	1.3	1.3
"	51	25	20	0	3	45.4	0.0	3.0
"	25	20	7	0	2	50.0	0.0	3.7
1013-13	93	32	23	4	6	34.8	2.5	3.8
Totals	205	102	64	5	12	42.7	1.3	3.0

maize, the two cells resulting from the first meiotic division remain associated. In the smear preparations the two spindle figures are oriented in similar positions in the two cells and thus can be observed together. The various types of anaphase II configuration are recorded in Table 3. In making the recordings, the two sister anaphase II cells derived from a single sporocyte are considered as a unit. The individual columns in Table 3 are related directly to the individual columns in Table 1.

In column 2 are recorded the anaphase II cells with no bridge and no fragment, *i. e.,* those in which no crossover had occurred within the inversion (or in which a reciprocal double crossover had occurred within the inversion). In the second division sporocytes entered in column 3, no bridge was present in either cell, but a fragment was found *lying in the cytoplasm* of one cell (Figure 10). Such a condition is expected to arise from the sporocytes entered in column 3, Table 1. In the second division sporocytes entered in column 4, no bridge was present but in contrast to column 3, a fragment was present *within the spindle* of one cell (Figures 11 and 17). Such a condition should arise from sporocytes included in column 4 of Table 1 and illustrated in Figure 5. In these cases, the fragment had been included in one of the telophase I nuclei and therefore should be released in the spindle figure of one of the second division cells. In nearly all cases there is no apparent connection of the fragment with any chromatid after the metaphase II period. It appears to be quite free in the spindle figure. The relative proportions of these two types of anaphase II configuration, *i. e.,* those in which the fragment is in the cytoplasm of one sister cell and those in which the fragment is in the spindle figure of one sister cell, are in agreement with the assumptions of their origin. The relative expectancy of the latter configuration from the anaphase I counts is 41 per cent. The observed proportion in the recorded anaphase II counts is 38.5 per cent.

Column 5 records the anaphase II configurations resulting from a four-strand crossover within the inversion. It is the counterpart of column 5, Table 1. These cells are recognizable by the presence of the two free fragments in the cytoplasm of these cells. The two fragments, when in the same cell, may be considerably separated from one another or may be immediately adjacent to one another.

Column 6, Table 3, is the counterpart of column 6, Table 1. A chromosome involved in a bridge is present in one of these cells, in addition there is a fragment which can be present in either cell, usually in the cytoplasm (Figure 12) but not infrequently (approximately one-sixth of these cases) in the spindle figure. When in the spindle figure it is usually in that cell which has no bridge; only rarely is it found in the spindle of the cell which contains the bridge. The probable explanation of this latter condition will be considered later.

As in Table 1, the percentages of the different types of configuration are given in the last three columns. The agreement with the percentages found in the anaphase I (Tables 1 and 2) is obvious and needs no further explanation.

The anaphase II configurations which can be interpreted as the result of a triple crossover (*d*, Figure 3) are very rare, but when present they are readily detected by the bridge in each anaphase II sister cell, together with two fragments in the cytoplasm (Figure 13)

When a bridge is present in an anaphase II cell the chromatin strand forming the bridge may break at late anaphase or not until telophase. In the latter case, the forming cell plate cuts the strand in two. When breakage is so delayed, the strand of chromatin can be seen extending from the nucleus toward the cell plate during the very early spore stage. As the spore matures, the strand becomes drawn into the nucleus.

The counts recorded in Tables 1, 2 and 3 allow an estimate to be made of the numbers of spores in an anther which could be expected to contain a normal, unbroken chromosome-4 and a deficient, broken chromosome-4. Totalling the counts of these three tables, there are 683 sporocytes with no bridge or fragment (column 2 in all three tables). Each of these should give rise to a quartet of spores with a normal chromosome-4 in each spore, or 2732 spores with a normal, unbroken chromosome-4. The 594 sporocytes with a single bridge and fragment in either anaphase I or II (columns 3, 4 and 6 in Tables 1 and 3, and columns 3 and 5 in Table 2) should each give rise to a quartet of spores, two of which contain a normal chromosome-4 and two of which contain a broken chromosome-4. Thus, these should produce 1188 spores with a normal chromosome-4 and 1188 spores with a broken chromosome-4. Each of the 33 sporocytes which had a four-strand double crossover within the inversion (column 5, Tables

1 and 3 and column 4, Table 2) would give rise to a quartet of spores each of which had a broken chromosome-4, making a total of 132 spores with a broken chromosome-4. Totalling the spore types resulting from all configurations, 3920, or 74.8 per cent, should contain a normal, unbroken chromosome-4, while 1320, or 25.2 per cent, should possess a broken chromosome-4. From these counts, one could anticipate that, on the average, approximately 25 per cent of the spores of an anther would contain a broken chromosome-4 and thus could produce a bridge configuration in the anaphase of the first microspore division if fusions occurred between the two split halves of this chromatid as diagrammed in Figure 2.

Figure 4.—Anaphase I following a single crossover within the inverted segment. Note the single bridge and free fragment. See *a*, Figure 3.

Figure 5.—Similar to Figure 4, but the fragment is attached to the end of a normal chromatid of the bivalent.

Figure 6.—Anaphase I following a four-strand double crossover within the inversion. See *b*, Figure 3.

Figure 7.—Anaphase I following the crossovers diagrammed in *c*, Figure 3.

Figure 8.—Early telophase I showing a single bridge and an U-shaped fragment chromatid.

Figure 9.—Telophase I. Note the attenuated strand connecting the two nuclei. No fragment was visible in this sporocyte.

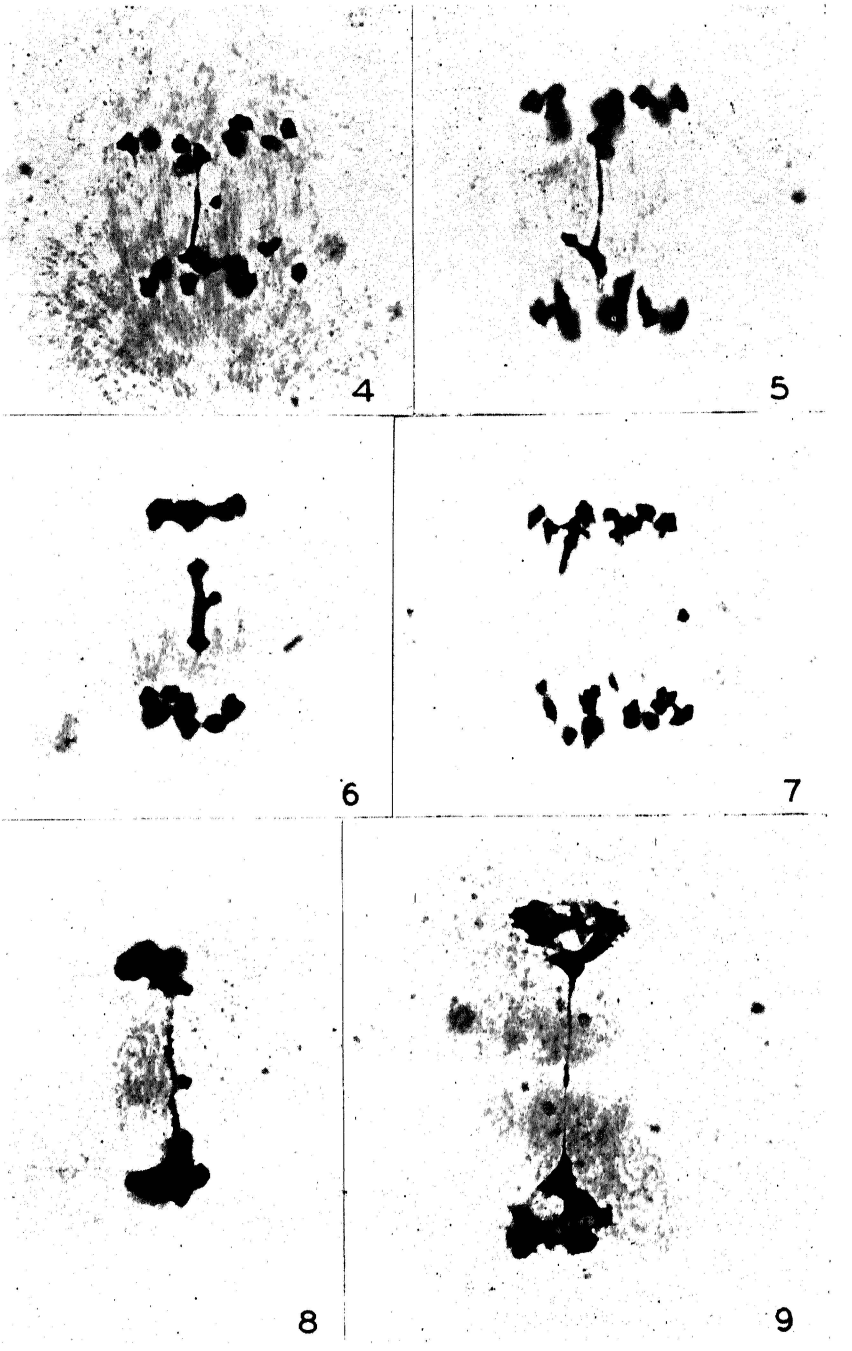
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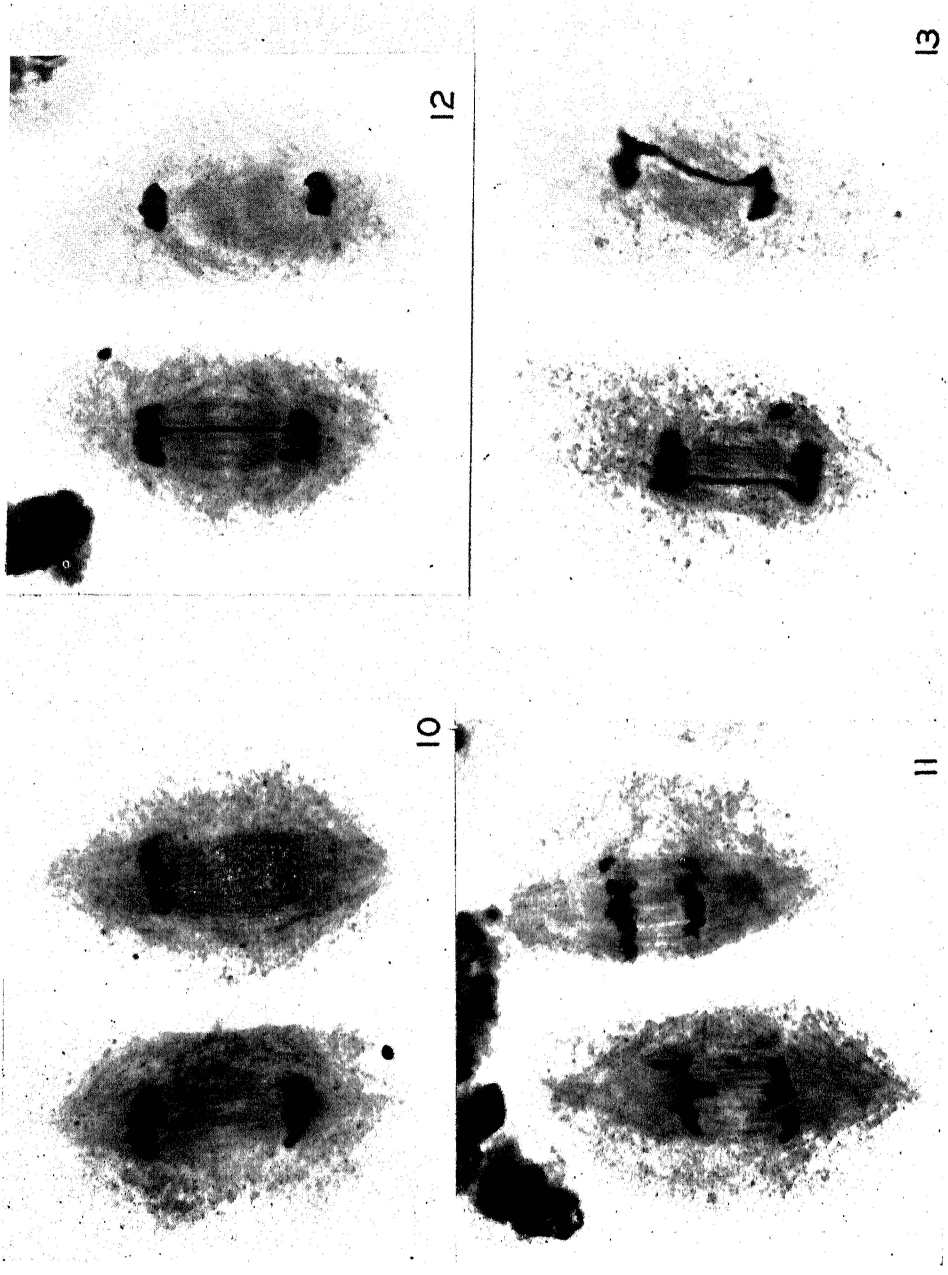
Figure 10.—Two sister cells in late anaphase II following a configuration shown in Figure 4. The fragment chromatid is in the cytoplasm at the lower part of the cell to the left.

Figure 11.—Two sister cells in mid-anaphase II following a configuration shown in Figure 5. The fragment chromatid is in the spindle figure in the cell to the left.

Figure 12.—Two sister cells in late anaphase II following a configuration shown in Figure 7. Note the bridge and the fragment chromatid in the cell to the left.

Figure 13.—Two sister cells at late anaphase II following a configuration diagrammed in *d*, Figure 3.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III. The Random Distribution of the Fragment, Produced by a Crossover Within the Inversion, to One of the Four Spores of a Quartet.

As mentioned in the introduction, the bridge configuration found in the anaphases of the spore mitosis has been related to those spores which possess a broken chromosome-4. This is made possible by the presence of the fragment with no spindle fiber attachment regions which is produced when a crossover occurs within the inversion. This fragment persists in the cytoplasm of the spore as a recognizable body from the telophase I or II period, when it is released into the cytoplasm, through the period of the spore mitosis (Figure 21). As has been shown in Tables 1, 2 and 3, the majority of crossover types give rise to a single bridge at anaphase I or II and a single fragment. The resulting spore quartet contains two spores with a normal chromosome-4 and two spores with a broken chromosome-4. If the fragment is distributed at random to one of the four spores of a quartet, the fragment expectancy in the types of spores derived from these quartets can be calculated. One-fourth of the spores in each class should possess a fragment chromatid. Since there are many sporocytes in which no crossover occurs within the inversion, there are many quartets produced which have only normal chromosome containing spores, none of which have a fragment.

The period during which the spores are in quartets is rather short: they soon become detached from one another. If counts of the spores with and without a fragment are made in these older anthers it is necessary to know the expected fragment ratios in the two types of spores produced from the non-crossover and crossover sporocytes. Since the spores with a broken chromosome-4 come from sporocytes in which a crossover occurred, the percentage of these spores with a fragment will remain constant regardless of expected variations in crossing over among the different anthers, *i. e.*, approximately 25 per cent of these spores will possess a fragment. The spores with a normal chromosome-4 are derived from both types of sporocytes, those in which a crossover occurred within the inversion and those in which it did not. Although 25 per cent of the normal spores derived from the crossover sporocytes will contain a fragment, none of the spores derived from the non-crossover sporocytes will contain one. Therefore, the fragment percentage among the total number of normal chromosome containing spores will be considerably below 25 per cent. In any one anther the percentage of normal chromosome-containing spores which likewise have a fragment will depend upon the number of sporocytes which had a crossover. Among the different anthers, varia-

tions in crossing over are to be expected. Hence, the percentage of the normal chromosome containing spores which have a fragment will vary directly with the frequency of crossing over. However, the counts given in Tables 1, 2 and 3 indicate that this percentage is always considerably lower than 25. The expected percentages among the two types of spores based on the counts of Tables 1, 2 and 3, are given in Table 6 (see adjacent text for the derivation of this table). It is realized that this table is reliable only if the fragment is distributed at random to one of the four spores of a quartet. There is, fortunately, a rather simple method of determining the distribution of the fragment to a particular spore of the quartet.

It has been stated previously that a bridge at anaphase I is either broken at late anaphase I or persists until the telophase period, when it is intercepted and broken by the cell plate. The chromatin strand thus broken at telophase remains in the cytoplasm as an extension from the nucleus and is directed toward the cell plate. This position is retained through the interkinesis period and the following second division prophase (Figures 14 and 15). At metaphase II, the attenuated strand, which is the broken chromatid, extends from the spindle into the cytoplasm and is directed toward the cell plate region of division I, where it had previously been broken (Figure 16). This strand of chromatin, extending into the cytoplasm, retards the free movement of the dyad chromosome in the spindle figure, frequently causing it to be displaced and forcing it to occupy a position close to the wall which separates the two cells. As the anaphase II progresses, this position is maintained and the extended chromatin strand, which represents the chromatid which had broken at anaphase I, lags behind in the cytoplasm, its broken end still pointing towards its previous position of breakage at the cell plate region. In these configurations it is often easy to observe the unlike positions of the breaks which had occurred in the chromatin bridges (Figure 17).

As the anaphase II progresses and telophase II sets in, the displaced chromatid becomes an extension pointing from the telophase nucleus toward the position in the cell plate of division I where it had previously been broken. Such a condition results in three types of quartets. If a long chromatin strand, running from the nucleus to the cell plate, were present in each telophase I cell (see Figure 15), the second division telophases can be of two types depending on the positions the two dyad chromosomes have taken in the spindles of the sister cells at metaphase II. The broken chromatids of chromosome-4 can pass to the same pole, relatively, in both sister cells, resulting in a configuration shown in Figure 18, or they may pass to opposite poles, relatively, resulting in the configuration shown in

Figure 14.—Two sister cells in late prophase II following delayed breakage of a bridge strand at the first meiotic mitosis. Note the unequal lengths of the extending strands and the fragment chromatid near the cell plate region in the cell to the right.

Figure 15.—Two sister cells just prior to metaphase II. Note the extended strands (broken chromatid from division I) in each cell running toward the cell plate region and the fragment chromatid in the cytoplasm at the lower part of the cell to the right.

Figure 16.—Two sister cells at metaphase II. Note the elongate chromatin strand in the cell to the left and the fragment chromatid immediately below this strand.

Figure 17.—Two sister cells in anaphase II following a single crossover within the inversion. The two chromosomes nearest the cell plate region of division I in the lower group in each cell have come from a bridge configuration which has broken at telophase I. Note their unequal size, their displacement in the spindle figure and the extension of their broken ends into the cytoplasm. The fragment chromatid is in the spindle figure of the cell to the left.

Figure 18.—Early telophase II configuration following an anaphase shown in Figure 17. The forming nucleus in the lower part of each cell contains a broken chromosome-4. The forming nucleus in the upper part of each cell contains a normal chromosome-4. The fragment chromatid does not show in this photograph.

Figure 19.—Early telophase II configuration following a bridge configuration at anaphase I with delayed breakage of the bridge. The upper telophase nucleus in the cell to the right and the lower telophase nucleus in the cell to the left each have a broken chromosome-4. The remaining two nuclei each contain a normal chromosome-4. The fragment chromatid lies immediately above the upper telophase nucleus in the cell to the left.

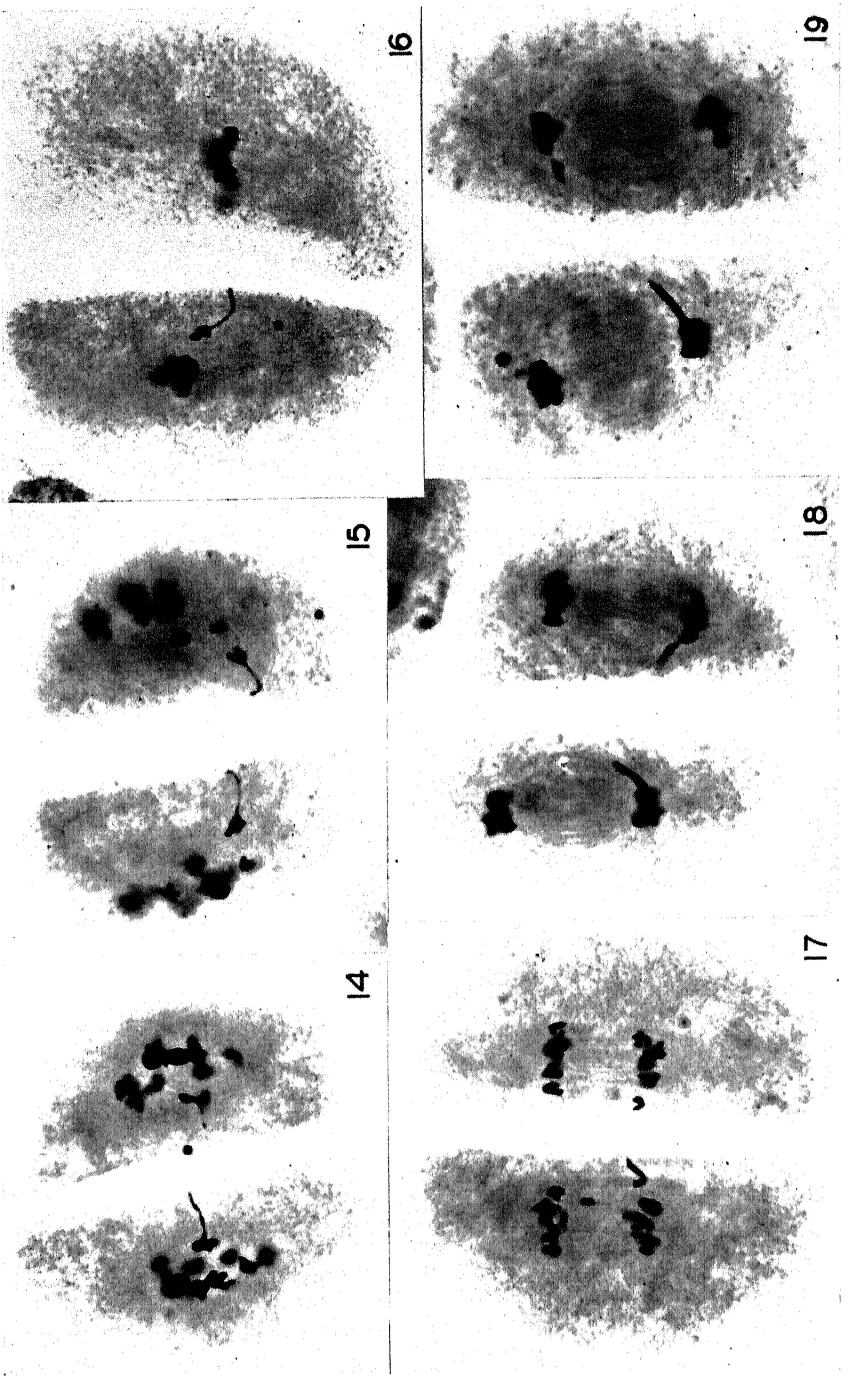
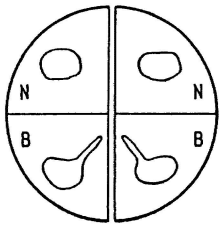
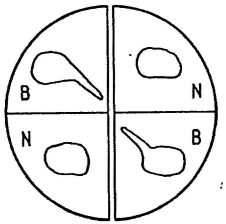
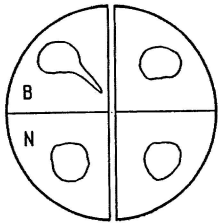
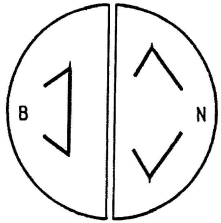

16
19
15
18
14
17

TABLE 4.—DISTRIBUTION OF FRAGMENT TO SPORES WITH A NORMAL CHROMOSOME-4 (N) AND TO THOSE WITH A BROKEN CHROMOSOME-4 (B).









Plant	Fragment in N	Fragment in B	Fragment in N	Fragment in B	Fragment in N	Fragment in B	Fragment in N	Fragment in B
1013-4	2	6	4	5	2	8	6	2
1013-5	4	1	4	4	0	3	0	3
1013-11	10	11	4	5	3	4	9	12
1013-13	26	23	25	21	26	19	71	43
1013-14	7	9	7	8	12	11	19	17
1013-17	4	1	0	1	5	3	1	4
Totals	53	51	44	44	48	48	106	81

Figures 19 and 20. It is therefore clear that in such quartets the two spores with long nuclear protuberances extending toward a common position in the telophase I cell plate region are those that contain a broken chromosome-4, and conversely, the two spores whose

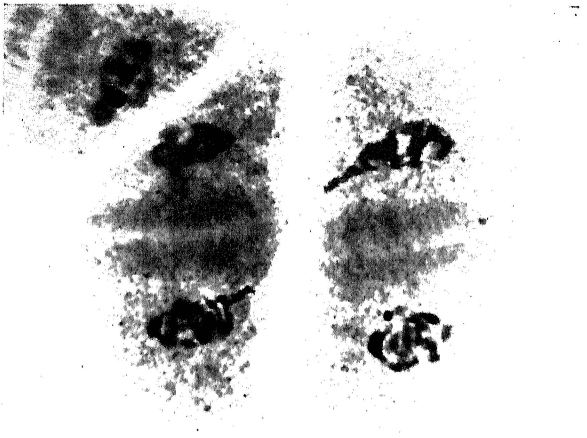


Figure 20.—Late telophase II following a configuration described in Figure 19. Note the nuclear protuberances (upper right and lower left) extending toward a common point in the cell plate region of division I. These two nuclei each contain a broken chromosome-4. The two remaining nuclei each contain a normal chromosome-4. A cell plate is forming in each cell to produce a quartet of spores. Note the fragment chromatid, the deeply stained body, immediately above and slightly to the left of the middle of the nucleus at the lower right.

nuclei have normal outlines, possess a normal chromosome-4. It is only necessary to observe in which spore of such quartets the fragment lies in order to determine whether the fragment has been distributed to a normal chromosome containing spore or to one containing a broken chromosome-4. These results of these counts are given in columns 2 to 5 of Table 4. It is clear from this table that the distribution of the fragment to one of the four spores of a quartet is at random.

A third type of quartet, one in which only one spore of a quartet has a long nuclear protuberance, has been classified in columns 6 and 7, Table 4. These quartets arise in the following manner. It frequently happens that the break in a chromatin bridge at late anaphase or early telophase occurs very unequally (see following prophase, Figure 14). In one telophase I cell a long protuberance juts

TABLE 5.—DISTRIBUTION OF THE TWO FRAGMENTS TO THE SPORES OF A QUARTET FOLLOWING A FOUR-STRAND DOUBLE CROSSOVER WITHIN THE INVERSION.

Two fragments in same spore		Two fragments in separate spores
Two fragments close together	Two fragments separated	
7	13	32

out from the telophase nucleus while in the sister cell there may be very little evidence of an extending chromatin strand. Consequently, in metaphase II (Figure 16) and anaphase II a long chromatin protuberance is present in only one sister cell, and at telophase II only one spore of the quartet has a nucleus with a long protuberance extending toward the middle of the division I cell plate region. In these quartets the presence of the fragment, when in one of these two sister anaphase II cells, can be recorded. If it lies in the spore which has the nuclear protuberance, it is in a spore with a broken chromosome-4. If it lies in the sister spore, it is in a spore with a normal chromosome-4. As column 6 and 7 of Table 5 show, the fragment is distributed at random to these two cells. When the fragment was present in either of the other two sister spores of the quartet no record could be made, since there was no means of distinguishing these two spores.

It should be emphasized that there is no difficulty in distinguishing the three types of quartets. They are very distinct. The counts recorded in columns 2 to 7 of Table 4 were made from quartets with well defined division II cell plates (Figure 20) and not from the early telophase II configurations like those in Figures 18 and 19.

It is necessary to determine the fragment distribution among the spores of a quartet which have resulted from the double crossover types, although their frequency among the spores of an anther is relatively low. The distribution of the fragment to the spores resulting from a double crossover illustrated in *c*, Figure 3, is readily observed. If, at anaphase II or telophase II, it lies in the cell that has no bridge, it will be distributed to a spore with a normal chromosome-4. If it lies in the cell which has a bridge (Figure 12) it will be distributed to a spore with a broken chromosome-4. The counts of such configurations are given in columns 8 and 9, Table 4. The distribution of the fragment to the spores with a normal chromosome-4 is more frequent than its distribution to a spore with a broken chromosome-4. There is a basis for an expected deviation from an equal distribution. It is possible that the fragment may be drawn into the telophase I nucleus by a method similar to that described for

those cells which show a single bridge at anaphase I, *i. e.*, by being associated with the end of a normal chromatid of the bivalent so that it is drawn into the nucleus containing the normal chromatids of chromosome-4. This assumption finds support in the observation that the fragment, when it lies within the spindle figure at anaphase II. is usually in the cell with the normal chromatids of chromosome-4 and not in the cell with the bridge configuration. As stated previously, in only approximately one-sixth of these cases is the fragment found within the spindle figure.

The four-strand double crossovers within the inversion give rise to very characteristic quartets. At anaphase I, the double bridge considerably delays the poleward movement of the spindle fiber attachment regions of the complex (Figure 6). Consequently, a double strand extending from the nucleus toward the cell plate is present in nearly all telophase I sporocytes after such crossing over. At prophase II and metaphase II this configuration is retained; hence at telophase II a quartet of spores is formed in which each nucleus has a protuberance extending toward a common point in the direction of the previous (telophase I) cell plate region. The two fragments may be close together in one spore of the quartet, some distance apart in the same spore of a quartet, or in two different spores of the quartet. The observations of the different distributions are recorded in Table 5. All four spores in these quartets have a broken chromosome-4. The distributions of the fragments represented in columns 1 and 2 result in a 3:1 ratio of spores without and with fragments. The distributions given in column 3 will result in a 1:1 ratio of spores without and with a fragment.

It can be concluded from these observations that there is a random distribution of the fragment to one of the four spores of a quartet. At this point it will be mentioned that no fragment could be found in some quartets of the types described above. This condition has a particular significance which will be considered toward the end of the following section.

IV. The Method of Correlating the Bridge Configurations in the Miscrospore Anaphases With the Presence in These Spores of a Broken Chromosome-4.

As shown in section II, an anther is expected to contain a number of spores with a broken chromosome-4. The exact number in any particular anther is dependent upon the number of sporocytes which had a crossover within the inversion. The number of such crossovers is not the same in all anthers but varies within limits (see Tables 1, 2 and 3). On the average, however, one may expect approximately

twenty-five percent of the spores to contain a broken chromosome-4. It is necessary to determine whether the spore containing a broken chromosome-4, and thus a deficiency in the chromosome complement, will undergo a nuclear division. Anthers in late stages of development were examined to determine the percentage of spores which had undergone a nuclear division. These examinations revealed the fact that the first division occurs in practically all of the microspores, which must include most of the spores with a broken chromosome-4. If, in these latter spores, the two split halves of the broken chromosome-4 have united at the position of breakage, as shown in Figure 2, the anaphase figures in these spores should show one chromosome involved in a bridge configuration. Since it had been determined that these spores so regularly undergo the first nuclear division, it is clear that sufficient examinations of anthers whose spores were undergoing the microspore mitosis should include many which have a broken chromosome-4.

From previous (unpublished) studies, it was known that the relative time at which a particular spore would undergo its division is dependent upon several factors: (1) the position of the spore within the anther, (2) the genic constitution of the nucleus and (3) the presence or absence of a deficiency or duplication within the nucleus. Spores at the mid-region of the anther are, on the whole, the first to commence divisions. However, the genetic constitution of the spores in heterozygous plants controls to some extent the time at which the nucleus of a spore will divide regardless of its position within the anther. The presence of a deficiency or of a duplication within the nucleus retards the relative time of division. In general, the nuclear division in these spores commences after the nuclear division in many of the normal chromosome containing spores has been completed. Thus, in a particular spore, the combination of these three factors controls the time at which the nucleus in this spore will undergo its division. These previously known facts would lead one to anticipate a delayed nuclear division within the deficient chromosome containing spore, *i. e.*, those which possess a broken chromosome-4. The normal chromosome containing spores should undergo their nuclear division in advance of the spores which have a broken chromosome-4. The observations have shown that this actually occurs. However, there is no definite interval separating the divisions of the normal spores and the divisions of the deficient spores. Instead, there are two distinct waves of division which overlap one another. The first wave of division includes the majority of the normal chromosome containing spores, the second wave, the majority of the deficient chromosome containing spores.

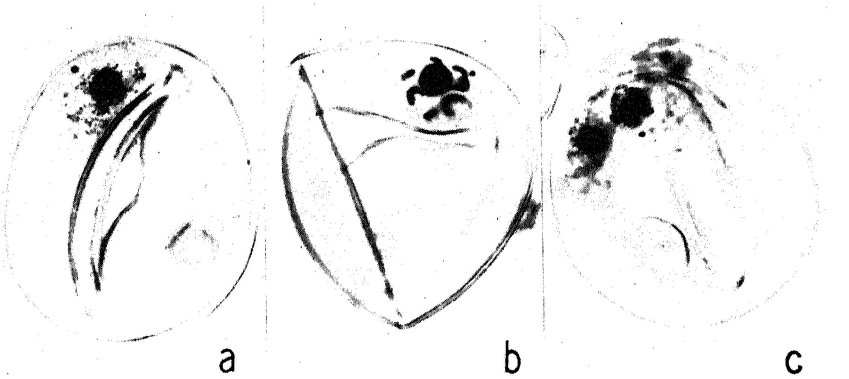


Figure 21.—Photomicrographs of microspores to illustrate the appearance of the fragment chromatid, produced by a crossover within the inverted segment, when it is left in the cytoplasm following the meiotic mitoses. *a*. Uninucleated microspore. The pycnotic fragment lies above and slightly to the left of the nucleus. *b*. Microspore at late prophase of the first nuclear division. The pycnotic fragment lies to the right of the prophase group immediately adjacent to the chromosome farthest to the right. *c*. Binucleated microspore. The fragment lies immediately below the nucleus to the right. The magnification of these photographs is approximately 560x. The magnification of all other photographs in this paper is approximately 1000x.

The method of determining the two waves of division is based upon two sets of observations: (1) the fragment ratios in the one and two nucleated spores of anthers at various stages of development and (2) the percentage of spores with a bridge configuration at anaphase (*i. e.*, those with a broken chromosome-4) in these anthers at various stages of development. This leads to a more detailed consideration of the fragment.

It has been shown in the previous sections that a bridge at anaphase I or II produced by a crossover within the inversion is accompanied by fragment formation. It has also been shown that this fragment is distributed at random to one of the four spores of a quartet. The fragment has been followed from the stage immediately after the second meiotic division to the binucleated stage in the spore. Depending upon the temperature, this period extends over a 5 to 8 day interval. At telophase II, the fragment forms a small micronucleus. As the spore develops, there is a noticeable shrinkage of this micronucleus. At the stage prior to the microspore division it is a deep staining inclusion in the cytoplasm. It remains clearly visible in the spore cytoplasm as a pycnotic body either before (*a*, Figure 21), during (*b*, Figure 21) or after the nuclear division (*c*, Figure 21).

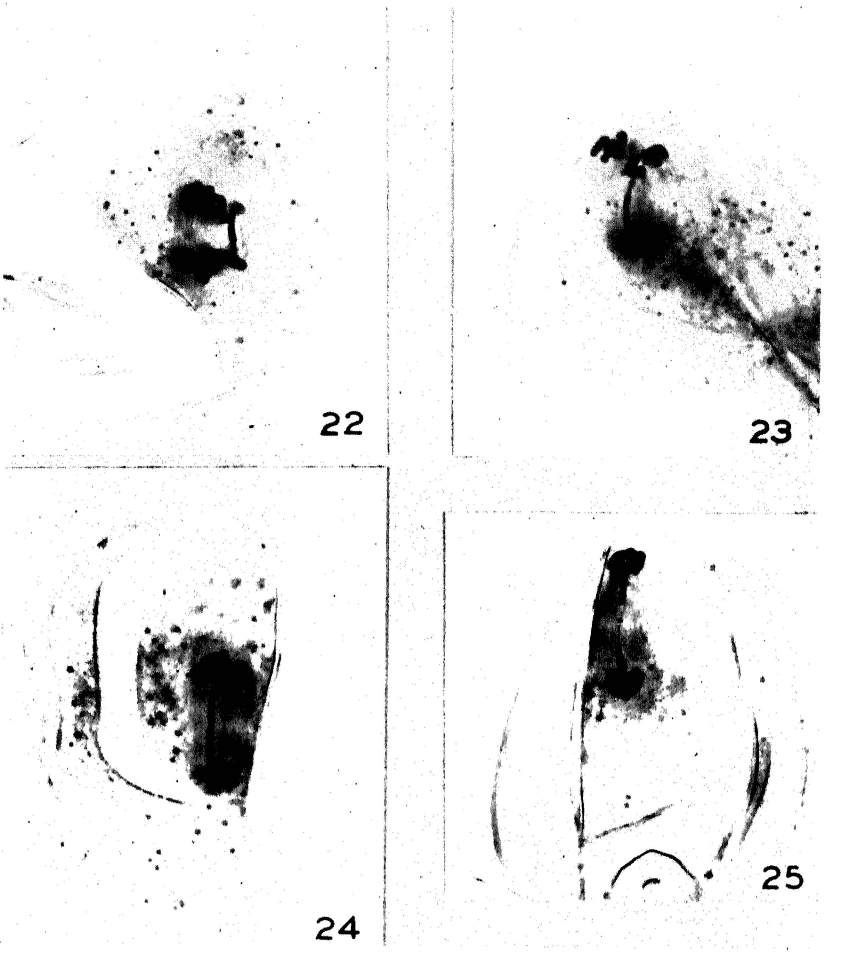
The data in Tables 1, 2 and 3, can be used to determine the expectancy of the presence or absence of the fragment in the cytoplasm in the two types of spores, those with a normal chromosome complement and those with a deficient (broken chromosome-4) complement. In the tables there are 683 sporocytes without a bridge or fragment, 594 with a single bridge and single fragment and 33 with a double bridge and two fragments. The 683 normal sporocytes would give rise to 2732 spores with a normal chromosome complement and no fragment. The 594 sporocytes with a single bridge at anaphase I or II would give rise to 1188 spores with a normal chromosome-4 and 1188 spores with a broken chromosome-4. One-fourth of the spores in each of these two latter classes, or 297, should possess a fragment, and three-quarters, or 891, should have no fragment. All of the 132 spores produced by the 33 sporocytes with a double bridge and two fragments should contain a broken chromosome-4. Since, as table 5 shows, the two fragments are retained in the same spore of a quartet in approximately one-half of the cases and distributed to two separate spores in the other half of the cases, 83 spores should contain no fragment and 49 should have one or two fragments. Totalling the spore types from all the sporocytes gives the following expectancies:

TABLE 6.—

Normal chromosome-4			Broken (deficient) chromosome-4		
No fragment	Fragment	% fragment	No fragment	Fragment	% fragment
3624	297	7.5	974	346	26.2

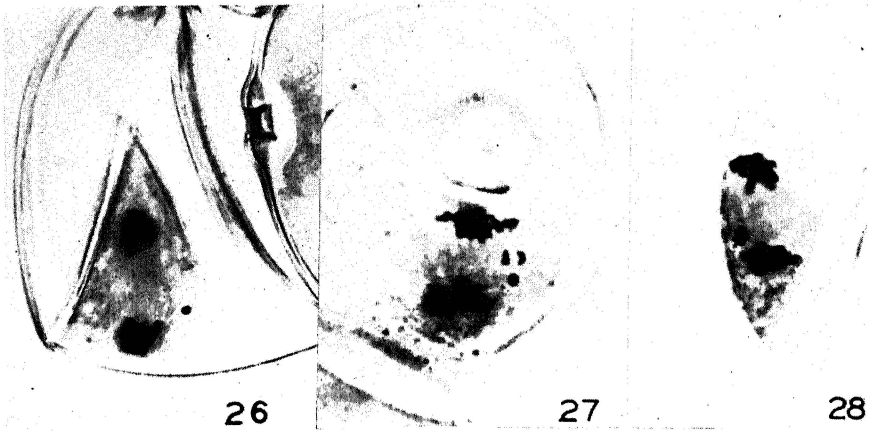
Counts of the numbers of spores with and without a fragment in the cytoplasm have been made from anthers at various stages of development, from those whose spores are all one-nucleated to those in which 95 per cent of the spores are in the two-nucleated condition. Representative counts are given in Table 7.

It can be seen from Table 7 that the percentage of spores with a fragment increases in the uninucleated class as the binucleated class increases. Such an increase is expected if the spores with a normal chromosome-4 undergo their divisions earlier than those with a broken chromosome-4. As Table 6 shows, the fragment percentages in the two types of spores, those with the normal chromosome-4 and those with the deficient (broken) chromosome-4, are very unequal. In the normal class the percentage is low; in the deficient (broken chromosome-4) class it is high. The percentage in the latter class would always remain high regardless of expected variations in crossing over within the inversion, which, in turn, produces the fragment. If nuclear divisions in the normal class occur earlier than those in the deficient



Figures 22 to 25.—Photomicrographs to illustrate the appearance of a bridge configuration in the first mitosis in the microspore. Figures 22 and 23 show a bridge configuration at anaphase. Figures 24 and 25 show the appearance of a bridge at early telophase.

class, the fragment percentage in the undivided spores (uninucleated class) should gradually rise as the anther matures, until it approaches 25 per cent where it should remain constant. If all of the normal chromosome containing spores divide before any of the deficient chromosome containing spores commence their divisions, this condition should be reached when the actual numbers of fragment containing spores are equal in the uninucleated and binucleated classes. The table clearly shows that there is a trend in this direction and indi-



Figures 26 to 28.—Early telophase configurations of the first microspore mitosis. Figure 26. Normal telophase, no bridge is present. Note the round, pycnotic fragment to the right of the spindle figure. This fragment has been present in the cytoplasm of the spore since the meiotic mitoses. Figure 27. The two sister halves of a fragment chromatid which had been included within the spore nucleus following the second meiotic mitosis. The two, small, rod-shaped chromosomes lie immediately above the round remnant of the nucleolus at the right edge of the spindle figure. Figure 28. Similar to Figure 27. The two sister halves of the fragment chromatid lie to the lower left of the spindle figure. Note that they are rod-shaped. See drawing of same, Figure 29.

cates that the spores with a deficient, broken chromosome-4, are delayed in division in comparison with the normal chromosome containing spores. The reason why the ratio in the uninucleated class does not rise to 25 per cent will be evident from the later discussion. Likewise, the reason why the fragment percentage in the uninucleate class does not reach a maximum when the numbers of spores with a fragment are equal in the uninucleated and binucleated classes will be evident after it has been shown that some of the spores with a deficient, broken chromosome-4 commence their division before all of the normal chromosome containing spores have completed their division.

Before commencing a discussion of the anaphase configurations in the spores an explanation will be given of the last column in Table 7. It represents an attempt to estimate, from the spores with and

TABLE 7.—FRAGMENT DISTRIBUTIONS AMONG THE UNI- AND BINUCLEATED SPORES OF ANTHERS IN VARIOUS STAGES OF DEVELOPMENT.

Plant	% binucleated spores	Uninucleated spores			Binucleated spores			Total % of spores with a fragment	Estimated % of sporocytes with a bridge and fragment at anaphase I or II
		No fragment	Fragment	% fragment	No fragment	Fragment	% fragment		
1013-5	0	1043	130	11.1	0	0	0	11.1	44.3
"	0	1016	91	8.2	0	0	0	8.2	32.8
1013-9	0	820	129	13.6	0	0	0	13.6	54.4
"	0	670	117	14.7	0	0	0	14.7	59.3
1013-13	0	738	93	11.1	0	0	0	11.1	44.7
"	0	898	105	10.4	0	0	0	10.4	41.8
1013-9	11.1	981	131	11.7	130	9	6.4	11.2	44.7
"	19.4	670	113	14.4	175	14	7.4	13.0	52.2
"	29.3	645	87	11.8	282	22	7.2	10.5	42.0
1013-5	34.7	461	49	9.6	261	10	3.6	7.5	30.2
1013-20	55.5	500	77	13.3	675	46	6.3	9.4	37.8
1013-9	57.3	282	54	16.1	416	36	7.9	11.4	40.6
"	57.9	331	69	17.2	508	42	7.6	11.6	45.8
"	68.3	314	82	20.7	773	81	9.5	13.2	52.2
1013-1	73.1	167	43	20.4	529	44	7.6	11.1	44.4
1013-5	82.7	147	43	22.6	836	74	8.1	10.6	42.5
1013-1	88.3	52	14	21.2	473	26	5.2	7.0	28.4
1013-5	91.0	89	27	23.2	1097	73	6.2	7.7	31.1

TABLE 8.—ANAPHASE AND TELOPHASE CONFIGURATIONS IN MICROSPORES OF ANTHERS AT VARIOUS STAGES OF DEVELOPMENT SHOWING THE PROPORTION OF SPORES WITH A FRAGMENT IN THE TWO MAIN CLASSES: (1) THOSE WITH NO BRIDGE CONFIGURATION AND (2) THOSE WITH A SINGLE CHROMOSOME INVOLVED IN A BRIDGE CONFIGURATION.

% binucleated spores in anther	No Bridge								Bridge							
	Anaphase				Telophase				Anaphase				Telophase			
	No fragment	Fragment in cytoplasm	Fragment in spindle	% fragment	No fragment	Fragment in cytoplasm	Fragment in spindle	% fragment	No fragment	Fragment in cytoplasm	Fragment in spindle	% fragment	No fragment	Fragment in cytoplasm	Fragment in spindle	% fragment
0 to 49.9	290	10	0	3.3	437	18	7	5.4	5	2	7	64.3	4	1	0	20.0
50 to 79.9	214	10	7	7.3	602	45	16	9.2	24	8	5	35.1	32	9	4	28.8
80 to 95	88	3	0	3.3	239	33	7	14.3	63	14	3	21.2	60	19	0	24.0
Totals	592	23	7	4.8	1278	96	30	8.9	92	24	15	29.8	96	29	4	25.5

without a fragment, the approximate numbers of sporocytes in the anther which had a bridge and fragment in I or II. The majority of the spores with a fragment have been derived from separate sporocytes. The total number of spores in an individual count of an anther can be divided by four to obtain the sporocyte number they represent. If each spore with a fragment is considered to have been derived from a single sporocyte with a bridge at anaphase I or II, the number of sporocytes which had such a bridge can be calculated. It is obvious that the resulting figure is only an approximation, since the double crossover types with two bridges and two fragments have not been considered in the estimate. Their effect on the estimate would be relatively insignificant. There is, however, another source of error in this calculation. The estimates in this last column are based entirely on the assumption that the fragment is always left in the cytoplasm at the conclusion of the anaphase II mitosis. Actually, the fragment is included *within* one of the four nuclei of the quartet in an appreciable number of cases. The percentage of inclusion is not great enough, however, to materially alter the estimates. The column gives an estimate which is useful for comparisons with the anaphase I and II counts of Tables 1, 2 and 3 and between individual anthers in this table.

At this point the anaphase and telophase conditions in the spores of anthers at various stages of development will be considered. The spores with a normal chromosome complement should give rise to normal anaphase configurations. Those with a broken chromosome-4 could give rise to an anaphase configuration in which a single chromosome is involved in a bridge if fusions occurred as shown in Figure 2. If the spores with normal anaphases and those with a bridge configuration are encountered, the fragment percentage in the two classes should differ markedly. If a broken chromosome-4 always produces a bridge at the spore anaphase, the expected frequency in the no-bridge class would be very low, while that in the bridge class should be close to 25 per cent. To see this relationship easily, one needs only to substitute "Spore anaphases with no bridge" for "Normal chromosome-4" and "Spore anaphases with a bridge configuration" for "Broken chromosome-4" in Table 6.

Observations of anaphases and telophases in the spores of anthers of all stages of development were undertaken. In the young anthers, the majority of the spores in anaphase or telophase had no bridge configuration. The fragment percentage among these spores was always low. In the somewhat older anthers, a number of spores each showing one chromosome involved in a bridge configuration were en-

countered, Figures 22 to 25. The fragment percentage among these spores was always high. In these anthers there were always a number of spores which were normal, having no bridge configuration at anaphase. The fragment percentage among these latter spores was always low. In the oldest anthers (85 to 95 per cent of the spores in the binucleated stage) the majority of the spores in anaphase showed a bridge and the fragment ratio in these spores approached three without a fragment to one with a fragment.

Since the division figures in the spores of a large number of anthers (more than 100) have been counted at all stages of anther development and records made of all the division figures, it has been thought advisable to condense the data into three sections: (1) counts from anthers in which the binucleated condition ranged from the lowest percentage to 49.9, (2) counts from anthers in which the percentage of binucleated spores ranged from 50 to 79.9 and (3) counts from anthers in which the percentage of binucleated spores ranged from 80 to 95. The counts of anaphase and telophase configurations with and without a bridge and with and without a fragment are summarized in table 8. Since it was observed that the chromatin bridge in a spore could break at late anaphase, all late anaphase figures in which the chromosomes had commenced to approach one another in a polar group were included in a telophase class.

The data clearly show the expected relationship between the presence and absence of the fragment in the two classes—the class with no bridge, assumed to possess a normal, unbroken chromosome-4 and the class with a bridge, assumed to contain a broken chromosome-4. The relative proportions of those two classes in anthers at the different stages of development likewise coincide with expectancy on the basis of the fragment ratios in the uninucleated and binucleated classes in the different anthers recorded in Table 7. In the young anthers the spores that showed a division figure were mainly normal, whereas in the older anthers many had a bridge configuration. From the fulfillment of these two expected correlations, *i. e.*, time of division and fragment ratios in these two classes, one is led to conclude that the two split halves of a chromosome-4 which has been broken during meiosis become united at the position of previous breakage, and that this union is responsible for the bridges seen at the spore anaphases. Since the fragment percentage in the no-bridge class does not rise in the older anthers, where many of the spores with a broken chromosome-4 are undergoing their divisions, it is concluded that this union probably always occurs. It is necessary, however, to describe in some detail the significance of the data given in the various columns of Table 8.

The first column in each section of Table 8 refers to spores which had no fragment. The second column refers to those spores which had a pycnotic fragment in their cytoplasm, this fragment having been present in the cytoplasm since the completion of the meiotic mitoses. The third column refers to those spores which had a fragment that had been included *within* the nucleus at the end of the second meiotic mitosis. Its presence in a spore is revealed only during or after the nuclear division in this spore. It should be recalled that this fragment possesses no spindle fiber attachment region and thus is incapable of self-directed movement in the spindle figure. In describing the behavior of this fragment during anaphase I it was stated that in approximately 40 per cent of the cases the fragment was drawn into the telophase I nucleus through a terminal association of the fragment with one of the normal chromatids of the bivalent, (Figure 5). In the second meiotic mitosis the fragment occasionally showed an attachment to the normal chromatid at the late prophase and early metaphase stages. This attachment, however, does not persist at anaphase. Instead, the fragment, which appears as a single, unsplit chromatid, lies free in the anaphase II spindle figure (Figures 11 and 17). It is necessary, therefore, to explain how it could become included in a telophase II nucleus when no apparent means of directed movement is present. The method of inclusion appears to be as follows. The inclusion in the telophase I nucleus is obviously predetermined by the persistent terminal association of the fragment with a normal chromatid. As stated above, this occurs in proximately 40 per cent of all the anaphase I figures showing a bridge and fragment formation (Table 1). Thus in approximately 40 per cent of the anaphase II figures where a fragment but no bridge is present, the fragment is found in the spindle figure (Table 3). However, its position in the spindle is variable. It may be present in any region. This fortuitous position is important for the following reason. The formation of a telophase nucleus in maize takes place as follows. At the mid- or late anaphase, a matrix substance appears about each chromosome. This very lightly staining substance first appears as a rim about the deeply staining chromatin of the chromosome. As the anaphase progresses, this rim of matrix substance gradually swells until it becomes a conspicuous sac in which the chromatin lies. The matrix material of two adjacent chromosomes becomes confluent as the telophase period commences. This confluence of matrix material appears to be the means of uniting the chromosomes into one telophase nucleus. Should a normal chromosome be placed in the spindle figure to one side of the main group

of chromosomes, there is, frequently, too great a separation between this chromosome and the main group of chromosomes for its matrix substance to make contact with their matrices. It is then excluded from the main nucleus and forms, instead, a micronucleus. This behavior of a normal chromosome has been seen repeatedly among the thousands of anaphase and telophase figures that the author has examined over a period of years, although its relative frequency is low. The fragment chromatid undergoes the same anaphase and telophase transformations as the normal chromosome. If it should happen to lie in the spindle figure in the region where the telophase nucleus will be formed, its swelling matrix may make contact and thus it may be included in the nucleus. If it is not in such a position, it will form a separate micronucleus as previously described. On this basis, its inclusion in a telophase nucleus will be fortuitous, depending upon its previous position in the spindle figure. This method of inclusion of a fragment in a telophase nucleus has been followed in the case of several other fragment chromosomes where inclusion could be clearly seen in some of the early telophase nuclei. The method appears to be of general occurrence.

After inclusion in the telophase II nucleus, the fragment undergoes all the transformations that the normal chromosomes undergo and apparently exerts its influence (see below) in the nucleus as a whole. At late prophase in the spore it is indistinguishable in appearance, except for its obvious size difference, from the normal chromosomes. It is normally contracted and clearly double. At metaphase it does not line up on an equatorial plate along with the chromosomes which have spindle fiber attachment regions, but lies to one side, usually close to the edge of the spindle figure. As the two halves of the normal chromosomes separate in anaphase, the two halves of the fragment chromosome usually remain quite close together (Figure 28 and drawing of the same, Figure 29). There is frequently a slight irregular separation of the two halves of the fragment chromosome, Figure 27, probably because of external and unequal pressures on the two halves exerted by the moving substance within the spindle. At telophase, the two halves of the excluded fragment produce either one micronucleus, including both halves of the fragment, or two small micronuclei lying close together. They are clearly visible in either the cytoplasm of the tube cell or in that of the generative cell.

It is believed that the fragment may occasionally be included in a telophase nucleus of the spore, since a broken chromosome-4, which is deficient for a relatively long region, has been transmitted through

the pollen. Transmission of such a deficiency through the pollen is contrary to other experience with deficiencies in maize. However, if the fragment chromosome (one or both halves) has been included in a tube nucleus which contains a broken chromosome-4, and if the

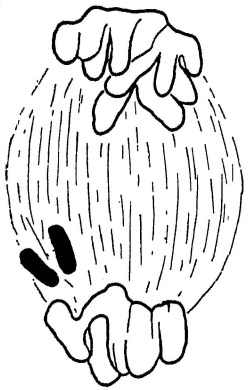


Figure 29.—Drawing to accompany the photograph of Figure 28.

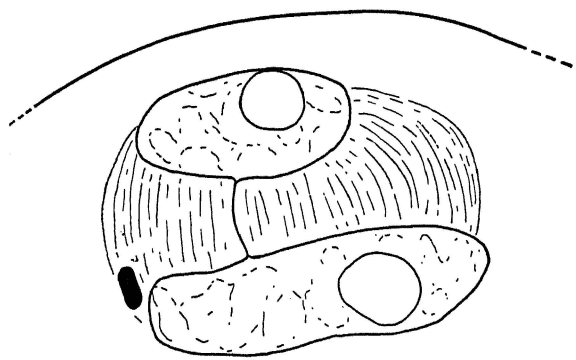


Figure 30.—Late telophase of the first mitosis in the microspore. The line above the two nuclei represents the spore wall. The two nuclei are connected by a fine chromatin bridge. Only one of the two halves of the fragment chromatid remains in the spindle figure. The other half has probably been included in the lower nucleus (tube nucleus).

fragment covers the deficiency (it would be expected to do so in at least one-half of the cases) the tube nucleus would contain at least a complete genomic complement and could be expected to function in the growth of a pollen tube. It could then deliver the two sperms with a deficient chromosome-4 into an embryo sac. The resulting individual would then be heterozygous for a broken chromosome-4 with a terminal deficiency of the long arm. Such an individual has been recovered.

Direct evidence for the inclusion of the fragment in the tube nucleus has been obtained from telophase figures in the spores. The two split halves of the fragment can ordinarily be seen in the spindle figure, as shown in Figures 27 and 28. Occasionally, however, only one of the split halves of the fragment is visible at telophase, lying in the spindle figure close to one of the telophase nuclei (Figure 30). In this case, it is probable that the other half has been included in one of the telophase nuclei, the lower or tube nucleus in the illustration given. In this figure, a fine bridge connecting the two nuclei was present, indicating that the microspore nucleus likewise possessed the broken chromosome-4.

If, following meiosis, a spore nucleus contained a broken chromosome-4 and also a fragment chromatid which completely covered the deficiency in the broken chromosome-4, the nucleus would no longer be deficient. It would be hyperploid, since the fragment itself possesses a duplicated segment of the long arm of chromosome-4. If the genes in the fragment chromosome function normally, the spore could be expected to undergo a mitosis earlier than those spores which contain a broken chromosome-4 without a covering fragment included in their nuclei. As Table 8 shows, half of the spores with a bridge at anaphase were of this type in the youngest anthers, whereas the percentage of such figures in the very late anthers was materially lower. The numbers are too small to be decisive but are highly suggestive.

Counts of the telophases in the spores of the various anthers have been included to supplement the anaphase counts although they are less useful. This is necessarily so because a bridge may break in late anaphase or early telophase, leaving no evidence of its previous presence. In some of the telophases, fixation occurred shortly after the bridge had broken and there were clear indications of its previous existence. However, in the "Bridge, Telophase" section of Table 8 only those telophases possessing an intact bridge, running from one nucleus to the other, were included. At this point it may be stated that breakage of the bridge can occur at various positions between the two spindle fiber attachment regions and is not confined to the position of fusion between the two sister halves of the broken chromatid-4. In some cases, however, the bridge in the anaphase figures appeared to show a weak association at the mid-region, the region of fusion, as if this fusion had not produced a strong union between the broken ends (Figure 25). In other figures, the fusions appeared to be sufficiently strong to withstand the pull at anaphase, resulting in breakage of the bridge at other points than the place of union. In many cases, the break did not take place until late telophase. In all such figures, the chromatin bridge had been pulled into a fine thread. In these cases, likewise, the fusion must be considered as having produced a strong union of the broken ends.

The telophases with a bridge configuration are theoretically as useful as anaphases for determining the fragment ratios. However, the presence of the one or two micronuclei, which had resulted from the exclusion of the two halves of a fragment which had previously been included in the spore nucleus, might occasionally be missed if situated immediately above or below the main nuclei. This does not apply to the pycnotic fragment which was left in the cytoplasm at the conclusion of the second meiotic mitosis. It is always conspicuous (Figure 26).

The fragment percentages in the "No-bridge, Telophase" class is always higher than in the "No-bridge, Anaphase" class, since some of these telophases in the younger anthers and many of them in the older anthers represent cases in which a bridge had been present previously but had broken. It should likewise be noted that the proportion of telophases with no bridge but with a fragment increases in the older anthers. This is to be expected because the proportion of spores with a broken chromosome-4 undergoing divisions steadily increases with the developmental progress of the anther and the fragment percentage in this class is constantly high.

Since there is an undeterminable number of spores in an anther which have a fragment included in the nucleus, it is not possible by totalling the figures of the three lines of Table 8 to derive an accurate estimate of the presence and absence of fragments in the two main classes of spores in order to compare them with the expected percentages given in Table 6. In the younger anthers, column 3 in the bridge class (fragment in spindle) would show relatively high values if the fragment in the nucleus hastened the division of the spore. On the other hand, in the older anthers, the fragment percentage in column 2 (fragment in cytoplasm) would be correspondingly depressed and thus the relative proportion of the fragment class would be depressed. It is obvious, however, from the third line of this table (80-95 per cent binucleated spores in an anther) that the inclusion of the fragment in the telophase II nuclei in which a broken chromosome-4 is likewise present, does not always insure an early division of this spore. The chromosomal constitutions of such spores would be various combinations of duplications and deficiencies and their division rates are not clearly predictable. That the inclusion of the fragment in the telophase II nuclei does not materially alter the observed as compared with the calculated ratios could be concluded by comparing the calculated fragment frequencies (derived from the spores with a fragment in their cytoplasm in those anthers which contain only uninucleated spores: last column, Table 7) with the observed frequencies of such fragment formation in the first and second meiotic mitoses (Tables 1, 2 and 3). Thus, the totalled figures in Table 8 should give a close agreement with the calculated expectancies of Table 6. This correlation is obvious upon inspection of the two tables.

In conclusion, the evidence for the contention that a chromatid which is broken in anaphase I or II will produce a bridge configuration at the first spore mitosis through fusions at the point of breakage between the two split halves of this chromatid can be summarized as follows:

1. A crossover within the inversion gives rise to a chromatid with two spindle fiber attachment regions and a fragment chromatid with no spindle fiber attachment region.

2. The chromatid with two spindle fiber attachment regions forms a bridge configuration at anaphase I or II. Breakage of the bridge at various positions in the different cells occurs either at anaphase or telophase.

3. The fragment chromatid is left in the cytoplasm at the end of telophase II in the majority of the cases. It is distributed to one of the four spores of a quartet at random. It persists in the cytoplasm as a visible body throughout the period of the first spore mitosis.

4. From counts of sporocytes with bridge configurations at the meiotic mitoses, it was possible to obtain an estimate of the number of spores in an anther which contain a normal chromosome and the number containing a broken chromosome. The values obtained were approximately 75 per cent and 25 per cent respectively. The percentage of spores with a fragment in the normal chromosome containing class should be low, approximately 7.5 per cent, while that in the broken chromosome class should be approximately 26 per cent.

5. Observations of older anthers indicated that practically all of the spores with a broken chromosome undergo the first nuclear division.

6. The spores with a broken chromosome are deficient. That nuclear division is delayed in these spores was directly shown by the gradual increase in the fragment percentage in the uninucleated class of spores as the binucleated class increased.

7. The spores undergoing nuclear division in the younger anthers show, mainly, normal anaphase configurations. In the older anthers, many or most of the spores at anaphase have a single chromosome in a bridge configuration. Regardless of the stage of development of the anther, the spores which have no bridge configuration always have a low fragment percentage; among the spores with a bridge configuration the fragment percentage is always high, 25 per cent or higher.

8. The correspondence between the time at which the normal and the broken chromosome containing spores undergo their divisions and the absence and presence of bridge configurations, respectively, coupled with the fragment ratios in these two types of spores, indicates that the spores with a bridge configuration are those which contain a chromosome which had been broken during the meiotic mitoses.

9. The fragment is included in a spore nucleus at telophase II in an appreciable percentage of the cases. The inclusion of the frag-

ment chromosome in a nucleus with a broken chromosome should produce, in at least one-half the cases, a spore with at least one full genomic complement. Such spores could be expected to undergo their mitoses ahead of those which do not contain such a covering fragment. The evidence suggests that this is what occurs.

The combined evidence points to the conclusion that when a break occurs in a chromatid at anaphase I or II, the two split halves of this chromatid become fused resulting in a bridge configuration in the anaphase of the first spore mitosis. As stated in the introduction, this conclusion is supported by evidence from another source where it has been found that the chromosome showing a bridge at the first spore mitosis is the chromosome known to be involved in chromatid breakage at anaphase I or II. It is definitely evident in this latter case that the position of fusion between the two split halves of the broken chromatid is at the place of breakage in the meiotic chromatid. In the case of the broken chromatids described in this paper, such proof is not as readily obtained. The morphology of the chromosome involved in the bridge configuration in the spore, however, definitely supports this interpretation.

V. Conclusions.

The evidence presented in the previous sections indicates that a chromatid, broken at either meiotic mitosis, will show a bridge configuration when the two split halves of this chromatid attempt to separate in the following mitosis, *i. e.,* in the first nuclear division in the microspore. The evidence indicates that the bridge is produced through a union of the two split halves of the chromatid at the position of previous breakage. The question arises: Has the chromatid at the first meiotic anaphase a split in preparation for the first nuclear division in the spore? If it were so split, one could assume that fusions occurred between the two halves at the position of breakage immediately after such breakage. The evidence from the literature of the double nature of a meiotic chromatid is contradictory. It is based on two types of observations: (1) direct cytological observations of the chromatid itself and (2) the types of induced alterations when the chromatid is x-rayed at various stages from early meiosis to the first nuclear division in the spore.

At diakinesis in maize there is occasionally a very clear indication of the double nature of the chromatid. It is only occasionally, however, that such figures are encountered. In most cases a doubleness of the chromatid is not obvious. A similar uncertainty exists as to the observable presence of a split in the spore mitoses. In the late prophase of most spores the chromosomes are clearly double. In a few spores, however, the quadruple nature of the chromosomes is so obvious as to be undeniable. This is especially clear when a knob is present in the chromosome. Its four-parted nature can not be misinterpreted. Although of uncommon occurrence, the presence of these figures in diakinesis and in spore prophases suggests that the chromosome may actually be split in advance for a division which is to follow the one in progress. A similar interpretation has been presented by Nebel (1932). For the present discussion, the evidence is quite unsatisfactory. It merely suggests that such a possibility can not be denied.

The evidence from x-ray induced chromosomal aberrations likewise does not remove the uncertainty. Huskins and Hunter (1935), Riley (1936), Husted (1937) and Mather (1937) have attempted to solve this problem by x-raying spores at all stages of development from the period immediately following meiosis to the period immediately previous to the first nuclear division in the spore. That the chromosome behaves as a double structure in the late stages in the spore is obvious from all of these investigations. That it reacts as a single

structure in the early spore stages in many of the spores is likewise obvious. However, it may react as a double structure in some of the very early spores. None of these investigations proves that the chromatid following meiosis is single. They merely indicate that it usually behaves as a single structure with regard to x-ray induced alterations in the early spore stage. With no conclusive experimental evidence available, it is not possible to affirm or deny the possibility of the split nature of the meiotic chromatid. The cytological observations on a few selected sporocytes lend support to the possibility.

If the chromatid were split at meiosis, the diagram given in Figure 2 would serve to illustrate the method by which bridge configurations are produced at the spore mitosis. If the chromatid is not split other hypotheses must be applied. Fusion might take place after the chromatid had become split or the broken end might not reproduce itself in the normal manner at the region of breakage during the splitting process, this resulting in a failure of separation of the two split halves at this position. At present it is not possible to choose between these hypotheses. The behavior of the broken chromosome in future nuclear cycles may lead to a solution. At present the evidence for the behavior in the following cell generations is somewhat contradictory. This evidence has not been completely analysed; consequently, the author wishes to make no definite statement at this time.

As stated in the introduction, there is considerable evidence which indicates that breaks in chromosomes are followed by unions of broken ends. There is no evidence to indicate whether the union follows breakage immediately or whether a significant delay can occur between breakage and the union of broken ends. If the chromatid at meiosis is not split, the four-strand double crossovers diagrammed in *b*, Figure 3, are of interest. On the basis of immediate fusions of broken ends, one would expect a bridge configuration to be present in each of the sister anaphase II cells. Bridge configurations in the anaphase II cells following such a breakage in anaphase I were only rarely encountered, and in these cells the bridge was usually produced by a weak adherence of the two broken ends. These configurations did not suggest a typical fusion. In most cases, there was no indication of a bridge configuration. If the chromatid were split and if fusions occurred 2-by-2 between the two split halves, bridge configurations in anaphase II would not be expected.

Although this investigation has served to elucidate the subsequent behavior of the broken meiotic chromatid and has indicated that fusions of broken ends do occur, it has not solved the problem of how the fusions occur or whether such behavior would apply to somatic

chromosomes. It is believed at present that the method is not strictly applicable to somatic chromosomes in maize, but a definite conclusion awaits further investigation.

Breakage of a bridge configuration at anaphase or telophase has been emphasized throughout this paper. It is desired to emphasize, also, that the position of breakage is variable. This applies to both the meiotic bridges and the bridges in the spores. It may occur close to the mid-point in the bridge configuration, close to one of the spindle fiber attachment regions or at any position between these two points. Evidence for the position of breakage in a large number of bridge configurations has been obtained from another source (unpublished). In this case, the exact position of breakage can be determined because the chromosome involved is visibly differentiated along its length. Here, likewise, breakage has been proven to occur anywhere between the two spindle fiber attachment regions.

The observations on the behavior through mitotic cycles of a fragment chromosome with no spindle fiber attachment region, produced by a crossover within the inversion, are of some theoretical interest. In the first meiotic mitosis the association of the fragment by what frequently appears to be a terminal chiasma with one of the normal chromatids of the bivalent results in its inclusion in one of the telophase I nuclei. If a fragment were very small, it might be undetected in such configurations, which may account for the bridge configurations without detectable fragments reported by Sax (1937). The association of the fragment with a normal chromatid in the inversion studied was present in such a constant proportion of the cases as to suggest some definite cause. It is possibly brought about by a chiasma beween the inversion and the end of the chromosome. Before the question can be settled, suitable inversions must be studied which will allow a discriminating answer.

As stated in the previous sections, the fragment is released from this terminal association in the second meiotic anaphase. Nevertheless, it becomes included in one of the telophase II nuclei in an appreciable number of cases. A suggestion as to the method of inclusion has been given (page 36). It has been stated, likewise, that when included, it acts as a normal chromosome both in its cytological behavior during the metabolic stage and in its genetic influence. This is in contrast to its behavior when left in the cytoplasm at the end of division II. In the latter case, degeneration processes set in during the period of spore development.

Evidence for the continued inclusion of the fragment in subsequent nuclear cycles has been presented. Such a condition might

continue for a number of cell generations. The probabilities for inclusion would be increased if, at the end of the first mitosis in the spore, both halves entered one nucleus. In the following spindle figure four fragments would be expected because of a splitting of each fragment. The more fragments in the spindle figure, the greater the chances of one of them being included in a telophase nucleus. It is possible that the "recovery" spots in the endosperm of maize, described by Stadler (1930) might find their solution in some such mechanism.

VI. Summary.

1. The object of the investigation described in this paper was to determine whether fusions of broken ends of chromosomes will occur when two such ends are included in the same nucleus at telophase.

2. The method of producing breaks in chromosomes included the use of an inversion in the long arm of chromosome-4 in maize. When a normal chromosome is synapsed with one having the inversion, a single crossover within the inversion results in a chromatid with two spindle fiber attachment regions and hence in a chromatid bridge at the first meiotic anaphase. A four-strand double crossover within the inversion (involving chromatids 1-3 and 2-4) results in a double bridge at the first meiotic anaphase. In both cases breakage of the bridge occurs at anaphase or telophase I. As the result of the four-strand double crossover, two broken chromatids enter each telophase I nucleus. If fusions of broken ends of chromosomes occur, the results of such fusions should be seen in the following anaphase II. However, fusion of these two broken ends usually does not occur. *Instead, each broken chromatid behaves as if it were split, with fusions occurring between its two longitudinal halves at the position of breakage.*

3. Union of the two halves of a split chromatid, broken at either meiotic anaphase, results in a bridge configuration at the anaphase of the first mitosis in the microspore. The evidence suggests that such a union always results when a chromatid is broken during meiosis. The method for relating the spores with a single chromosome involved in a bridge configuration to those which contain a chromosome broken at the meiotic mitoses is described.

4. Two processes which result in the inclusion within a telophase nucleus of a chromosome which has no spindle fiber attachment region are described. In one process, the chromosome is directed in its movement in the spindle figure by being associated with a normal chromatid and is necessarily drawn into one of the telophase nuclei. In the other process, the chromosome is undirected; it lies free in the spindle figure. Its inclusion within a telophase nucleus is then dependent upon its fortuitous position within the spindle figure. In this way a fragment chromosome with no spindle fiber attachment region can pass through successive nuclear cycles. Its structural changes and genetic influence within a nucleus are comparable to those of a chromosome fragment which possess a spindle fiber attachment region.

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