THE STABILITY OF BROKEN ENDS OF CHROMOSOMES IN ZEA MAYS

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I. INTRODUCTION

IF CHROMOSOMES are broken by various means, the broken ends appear to be adhesive and tend to fuse with one another 2-by-2. This has been abundantly illustrated in the studies of chromosomal aberrations induced by X-ray treatment. It also occurs after mechanical rupture of ring-shaped chromosomes during somatic mitoses in maize and is assumed to occur during the normal process of crossing-over. In a previous publication (McCLINTOCK 1938b) it was shown that following breakage of a single chromatid in a meiotic anaphase of maize, fusion occurs at the position of breakage between the two sister halves of this broken chromatid. Because of this fusion, the two sister halves cannot separate freely from one another in the following mitotic anaphase. As the two centromeres of the terminally united chromsomes pass to opposite poles in this mitotic anaphase, a chromatin bridge is produced. The tension on the bridge configuration, following the poleward migration of the centromeres, results in rupture. Once again, a chromatid with a broken end enters each sister telophase nucleus. The questions then arise: Will fusions occur at the position of breakage between the two sister halves of each of these broken chromosomes giving rise to an anaphase bridge configuration in the following mitosis? If so, will this breakage-fusion-bridge cycle continue in each successive nuclear division, or will the broken end, produced by the rupture of an anaphase bridge configuration, eventually "heal," thus discontinuing the breakage-fusion-bridge cycle? Answers to these questions were presented briefly in a preliminary publication (McCLINTOCK 1939). The results presented in this latter publication and those presented in this paper have led to the following conclusions. (1) If a chromosome, broken at the previous meiotic anaphase, is delivered to the primary endosperm nucleus through either the male or the female gametophyte, the breakage-fusion-bridge cycle will continue in the successive nuclear divisions during the development of the endosperm tissues. (2) A similarly broken chromosome delivered to the zygote nucleus by either the sperm or the egg does not give rise to bridge configurations in successive nuclear divisions in the sporophytic tissues. The broken end heals. There is a complete cessation of the breakage-fusion-bridge cycle. (3) The breakage-fusion-bridge cycle is confined to the gametophytic and endosperm tissues of the generation immediately following the initial break in the chromosome. (4) Healing of the

broken end in the embryonic sporophyte is permanent. When a chromosome with a healed broken end is introduced into gametophytic or endosperm tissues in succeeding generations, no fusions of broken ends result either between sister halves of the broken chromosome or between two such broken chromosomes when both are introduced into a single nucleus. It is the purpose of this paper to present the evidence for these conclusions.

II. THE TYPES OF GENETIC VARIEGATION PRODUCED BY THE BREAKAGE-FUSION-BRIDGE CYCLE

If a broken chromosome continued the breakage-fusion-bridge cycle in successive nuclear divisions, its presence should be made evident by genetic variegation in endosperm and plant tissues. This would follow when the

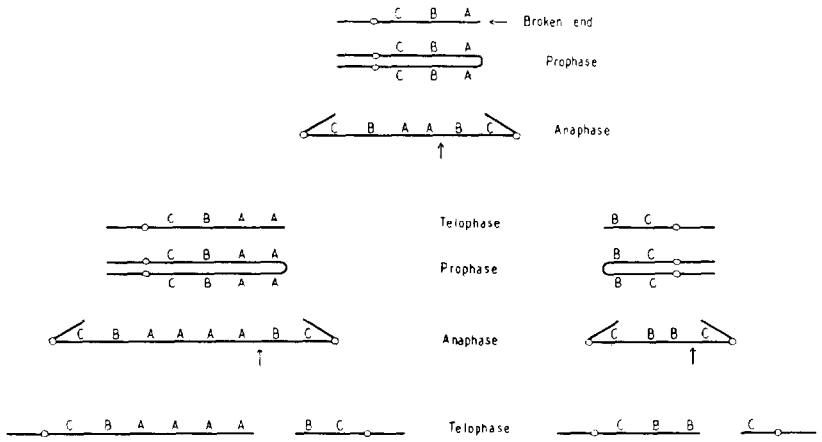

FIGURE 1.—A representative illustration of the method by which variegation may be produced in tissues carrying a chromosome with a broken end. The clear circle represents the centromere. The dominant genes *A B* and *C* are carried by the arm with the broken end, *A* being near the broken end and *C* near the centromere. The homologue of this chromosome (not diagrammed) is considered to be normal and to carry the genes *a b* and *c*. Division of this broken chromosome results in fusion at the position of breakage between the two split halves (prophase, second diagram from top). This is followed by a bridge configuration in the following anaphase (anaphase, third diagram from top). The arrow points to the position of breakage, the two broken chromosomes entering the sister telophase nuclei (telophase, right and left). This process is repeated in successive divisions. One such division is diagrammed below each of these two telophase chromatids. The diagrams illustrate how dominant genes may be deleted or reduplicated followed the breakage-fusion-bridge cycle.

broken chromosome carried dominant genes and its normal homologue carried the recessive alleles. Figure 1 illustrates the method by which variegation is produced in consequence of the breakage-fusion-bridge cycle. The line at the top of the figure represents a chromosome with a broken end. The dominant genes *A B* and *C* are carried by the arm with a broken end, *A* being close to the broken end and *C* nearest to the centromere. The

diagram immediately below represents this chromosome at the following prophase, fusion having occurred between the two sister chromatids at the position of previous breakage. Separation of the two sister centromeres at anaphase results in a bridge configuration (third diagram, fig. 1). If a break occurred at the position of the arrow, a chromosome carrying the genes *A A B C* and possessing a broken end would enter one telophase nucleus (left in the diagram) and one chromosome carrying the genes *B* and *C* would enter the sister telophase nucleus (right in diagram). All the cells arising from this latter cell would lack the dominant gene *A*. Thus, the recessive gene *a* would appear in all cells arising from this cell, and the process which results in variegation would have commenced. Each of these broken chromosomes could, in turn, repeat the process just outlined. In the telophase chromosome to the right, the broken ends of the two sister chromatids would again be fused at the succeeding prophase (see diagram), and a bridge configuration would result at the following anaphase (see diagram below). If a break occurred at the arrow, a broken chromosome carrying *B B C* would enter one telophase nucleus and a broken chromosome carrying only *C* would enter the sister nucleus. All the cells arising from this latter cell would have lost the dominant genes *A* and *B*, and the tissues would show the character of the recessive alleles *a* and *b*. In the first telophase to the left, a similar process has been diagrammed whereby the dominant gene *A* is lost to one daughter nucleus and repeated duplications of *A* genes are introduced into the sister nucleus.

The diagram (fig. 1) is merely an example. The break in the first anaphase might have occurred between the two *A* genes. Several nuclear cycles might take place before a break occurred to one side of these genes resulting in the loss of the *A* gene to one of the daughter nuclei. On the other hand, the first or successive breaks might have occurred close to one of the centromeres resulting in the loss of all three dominant genes to one nucleus and their duplication in the sister nucleus. Variegation patterns of dominant and recessive tissue resulting from this type of behavior should be very distinctive. Some tissues could be totally recessive—that is, *a b c*. In this case, all three genes would be lost from a nucleus in a single anaphase break. Other tissues could be recessive for *a* but variegated for *b* and *c*. In this case *A* would be lost from the cell following one anaphase break, while *B* and *C* would be subsequently lost. In these tissues, patches should be found which are (1) *a b c* where *B* and *C* have subsequently and simultaneously been lost; (2) *a b C* from which *B* and not *C* has been lost. In this latter patch, which is wholly *a b* in genetic character, still smaller patches should be found which are *a b c*. No tissues should be found which show the genetic constitution *a B c*. In other words, when variegation within variegation is present, the developmental pattern should show the

loss of the terminal genes before loss of genes close to the centromere. Since variegation of endosperm tissues was the means by which individuals possessing broken chromosomes were detected, a description of the types of variegation observed will be given after the method has been described by which broken chromosomes may be obtained.

In order to secure cytological evidence of the presence of a broken chromosome in a plant and to study the genetic consequences of its behavior, two methods were employed. Both methods involve the breakage of chromosome 9 at a meiotic anaphase and its deliverance to the endosperm and the zygote after successful passage through the developmental periods of the male or the female gametophytes (the pollen grain and embryo sac, respectively). This necessitates the production of a broken chromosome

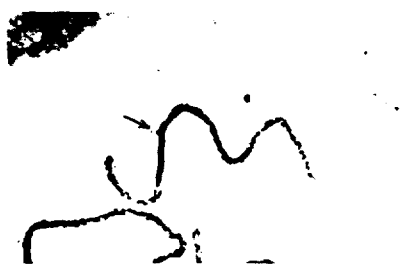

FIGURE 2.—Photomicrograph of chromosome 9 at pachytene in a microsporocyte. The centromere appears as a grey bulge (arrow). The short arm of one of the homologues terminates in a small knob. Note the deep-staining region adjacent to the centromere in the short arm and the smaller, more widely spaced chromomeres in the distal part of the arm. The proximal deep-staining segment appears relatively longer in this photograph than in the diagrams given in this paper. This is due to an overlapping of the two homologous chromosomes immediately distal to the junction of the deep-staining and lighter-staining regions.

which possesses at least a full set of genes if transmissions are to occur through the male gametophyte, or broken chromatids with only relatively short terminal deficiencies if transmissions are to occur through the female gametophyte. No deficiencies within the short arm of chromosome 9 which are transmitted through the pollen are known, but deficiencies of terminal segments up to and including one third of the short arm may be transmitted through the female gamete (McCLINTOCK unpublished).

Both methods involve the use of abnormalities in the structural arrangement of chromosome 9 of maize. A photograph of a normal chromosome 9 at the mid-prophase of meiosis is given in figure 2. The genes Yg^2, *C*, *Sh*, and *Wx* are located in the short arm of chromosome 9. *Yg* normal green plant, *yg* yellow-green plant; *C* colored aleurone, *c* colorless aleurone; *Sh* normal development of the endosperm, *sh* shrunken endosperm; *Wx* normal starch staining blue with iodine, *wx* waxy starch staining red with iodine). The linear order of these genes is *Yg-C-Sh-Wx*. *Yg* is located very

near the end of the short arm. *C* is located approximately a quarter of the distance in from the end of the short arm. *Sh* is located very close to it. *Wx* is located at approximately the middle of the short arm, although its exact position has not been determined. It should be noted that the gene *Vg* is a plant character, whereas the genes *C*, *Sh*, and *Wx* are endosperm characters.

III. THE PRODUCTION OF A BROKEN CHROMOSOME BY MEANS OF A REARRANGEMENT IN CHROMOSOME 9

(a) *Description of the rearrangement*

The first method will be described in considerable detail, since it affords particularly favorable material for a cytological and genetical study of the behavior of broken chromosomes. It involves the use of a chromosome 9

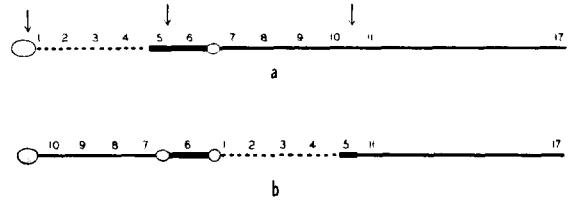

FIGURE 3.—A normal chromosome 9 terminating in a large knob (stippled). The clear oval region represents the centromere. The individual parts of the chromosome have been numbered from 1 to 17. The broken line (segments 1 to 4) represents the widely spaced small chromomeres of the distal two-thirds of the short arm. The wide line (segments 5 and 6) represents the proximal deep-staining region of the short arm adjacent to the centromere. The long arm is represented by a narrow line. The arrows point to the positions of breaks which resulted in the rearranged chromosome shown in b.

with a moderately complex rearrangement of parts which arose following X-ray treatment. A normal chromosome 9 is diagrammed in a, figure 3. In the strain of maize irradiated, the short arm terminated in a large knob (stippled). (In certain strains of maize no knob is present at the end of the short arm. In other strains small or intermediate size knobs are found. The knob substance lengthens the chromosome but does not carry essential genic material.) The proximal one-third of the short arm (fig. 2 and regions 5 and 6 fig. 3) adjacent to the centromere (clear oval region in diagram) is characterized by large, closely associated deeply-staining chromomeres, suggesting heterochromatin in appearance. It will be referred to as the pycnotic region. The distal two-thirds of the short arm (dash lines fig. 3) is composed of small, widely spaced chromomeres. The arrows in a (fig 3) indicate the positions of breaks which resulted in the rearrangement of segments shown in b (fig. 3). The large terminal knob has been broken into two unequal parts, the small part together with three quarters of the short arm being inserted into the long arm in the normal order. The section which

includes 6 to 10 became attached to the larger segment of the knob at region 10, resulting in an inverted order for the genes in this segment.

(b) *Types of chromosomes produced as the result of crossing-over*

Plants which contain one rearranged chromosome 9 and a normal homologue give rise to several types of anaphase I and II configurations following crossing over in the rearranged segments. A diagram of the meiotic prophase synaptic configuration is given in figure 4. The regions where crossing over results in aberrant configurations are labelled A, B, and C. Region A includes the segment from 1 to 5. Region B includes segment 6. Region C includes the segment 7 to 10.

Several types of altered chromosomes result from crossing over in these regions. In figure 5, the types of dicentric chromatids produced by crossing

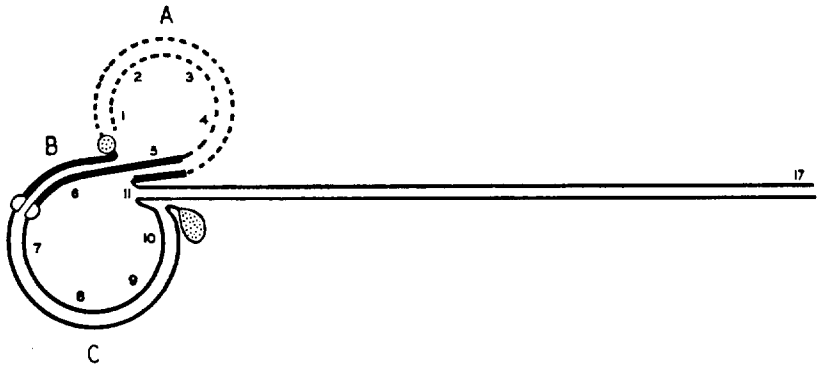

FIGURE 4.—Synaptic configuration produced following homologous associations of a normal chromosome 9 (without a knob) and the rearranged chromosome diagrammed in b of figure 3. Region A includes segments 1 to 5. Region B includes segment 6 (between the small knob and the centromere). Region C includes segments 7 to 10.

over are diagrammed. The type of crossing over which produces each type of dicentric chromatid is given in the description beneath the figure. In most cases, the dicentric chromatid produces a bridge configuration in anaphase I. The two types of crossovers which lead to bridge configurations at anaphase II are indicated in the legend. In addition to the dicentric chromatids, two new monocentric chromatids can be produced as the result of crossing over. These are diagrammed in figure 6. The various types of acentric chromatids, which are the complements of the dicentric chromatids, are mainly lost to the successive nuclei following crossing over. Thus, they have not been diagrammed.

As indicated in figure 5, most of the dicentric chromatids result in a bridge configuration in anaphase I. Only the three-strand double crossovers involving regions A and B result in bridge configurations in anaphase II.

Observations of the number of bridge configurations in the two meiotic divisions indicate the frequency of the types of crossovers. Among 238

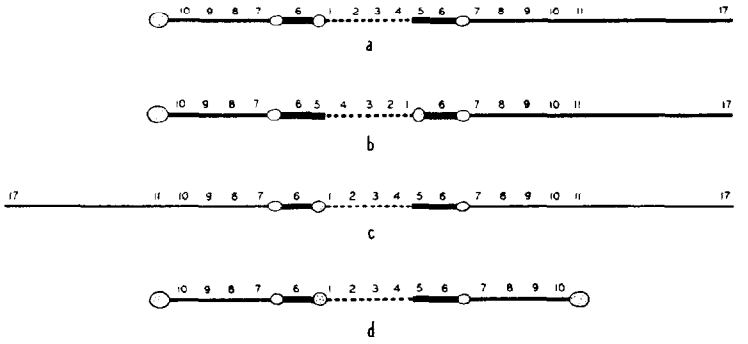

FIGURE 5.—Types of dicentric chromatids produced as the consequence of crossovers in regions A, B, and C of figure 4. a. Dicentric chromatid produced following a single crossover in region A. A bridge configuration occurs at anaphase I. b. A dicentric chromatid resulting from a two-strand double crossover involving regions A and B or A and C. A bridge configuration occurs at anaphase I. c. Dicentric chromatid resulting from a three-strand double crossover involving one chromatid of the rearranged chromosome and the two chromatids of the normal chromosome. One crossover in region A, the other in region C, will result in a bridge configuration in anaphase I. If the second crossover is in region B, a bridge configuration will occur in anaphase II. d. Dicentric chromatid resulting from a three-strand double crossover, as in c, but the chromatids involved are the reverse—that is, one from the normal chromosome and two from the rearranged chromosome.

microsporocytes in anaphase I, 77 or 32 percent showed a chromatid bridge. In two of these microsporocytes a double bridge with two acentric fragments was observed—the result of a four-strand double crossover.

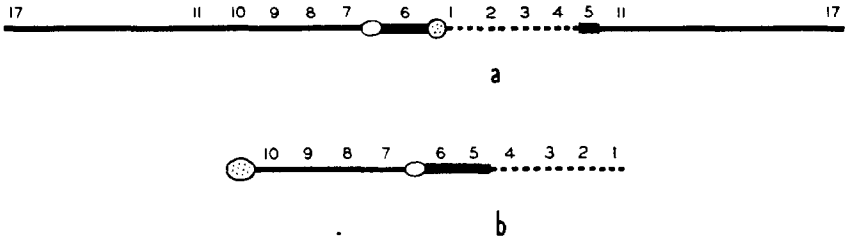

FIGURE 6.—Types of derived chromosomes with a single centromere resulting from a crossover in regions B or C of figure 4. The chromatid in a could be recovered, since it contains a complete set of genes plus a duplication of segments 11 to 17. The chromatid in b could not be recovered, since it is deficient for the segments 11 to 17.

In anaphase II, only those dyads were counted in which both sister cells could be observed at mid-anaphase. Among 271 such dyads, only five or 1.8 percent showed a chromatin bridge configuration in one of the sister cells.

The dicentric chromatid most frequently formed (a, fig. 5) is of particular significance for this study. The part of the dicentric chromatid extend-

ing from the small internal knob to the right end of the chromosome is the equivalent of a complete chromosome 9. Left of the small internal knob is the short pycnotic region 6 followed by the second centromere and the segment 7 to 10 which terminates in the large knob. At anaphase I, the two centromeres moving in opposite directions cause the chromatid to break. Analyses of anaphase I configurations and of the composition of this chromosome in microspores indicate that the break may occur at any position between the two centromeres. Breaks which occur immediately to the right of the small internal knob or to the left of this position, will result in one broken chromatid with at least a full set of genes of chromosome 9. This chromatid could result in a viable spore since no essential genic material has been lost from the chromosome at this initial break. Breaks which occur to the right of the small inner knob within regions 1 to 6 would result in a chromatid deficient for various numbers of genes of the short arm, depending in each case upon where the break had occurred. Regardless of where the break occurs, the resulting broken chromatid to the left would be deficient for a large number of genes of chromosome 9. It would not be expected to produce a functional spore. Interest centers, therefore, on the transmissions of the broken chromatid to the right.

The two dicentric chromatids shown in b and c (fig. 5) could give rise to broken chromatids with a full complement of genes. Those in d would not be expected to survive, because both of the broken chromatids would be highly deficient. In b (fig. 5) the surviving chromatid following breakage would have most of the genes in the short arm in the inverted order. In c (fig. 5) the surviving broken chromatid could have its genes in the normal order if the break occurred between the knob and the left centromere and would be indistinguishable from the broken chromatid derived from a. If the break occurred in segment 6 adjacent to the right centromere, the surviving broken chromatid would have the order of the genes reversed, as in b. Since none of the transmitted broken chromosomes so far obtained have shown an inverted order of genes, attention will be concentrated on the surviving broken chromatid arising from the dicentric chromatid of a.

(c) *Types of kernels resulting from the cross*

$$\frac{C\ Sh\ Wx \text{ rearranged chromosome } 9}{c\ sh\ wx \text{ normal chromosome } 9} \times c\ sh\ wx \text{ normal chromosome } 9.$$

The dicentric chromatid (a, fig. 5) arises from a crossover in region A of figure 4. This region carries the loci of the genes *Yg C Sh* and *Wx*. Since the chromosomes with a broken end arise from a dicentric chromatid which suffered a break at anaphase I or II, all surviving chromosomes with a broken end should be crossovers within this region A. If the breakage-fusion-bridge cycle continues after the initial meiotic anaphase break, the

endosperm tissue should be variegated when it has received from one parent a broken chromosome 9 carrying any or all of the dominant endosperm genes and from the other parent a normal chromosome 9 with the recessive alleles, *c sh* and *wx*.

The following tests were made in order to show that the variegated kernels arise following a crossover in region A of figure 4. Plants were obtained which possessed a rearranged chromosome 9 carrying the genes *Yg C Sh Wx* and a normal chromosome 9 with the genes *yg c sh wx*. These plants were crossed to those carrying two normal chromosomes 9 each with the genes *yg c sh wx*. The types of single crossovers within this region and the resulting dicentric chromatids with their genic constitutions are shown in figure 7. The rearranged chromosome 9 with the genes *Yg C Sh Wx* and the normal chromosome 9 with the genes *yg c sh wx* are diagrammed at the

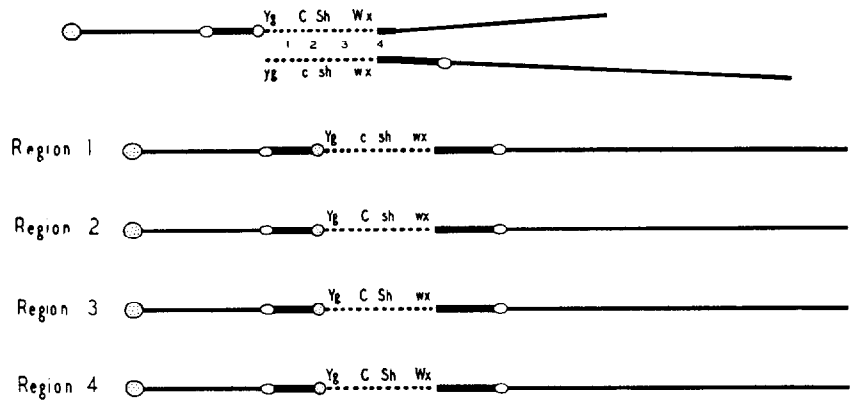

FIGURE 7.—The genic constitution of dicentric chromatids resulting from crossing over in segments 1 to 5. At the top of the diagram, homologous associations of the rearranged chromosome 9 and a normal chromosome 9 are represented only between segments 1 to 5. The genic constitution of each chromosome is indicated. Between the two chromosomes the regions where crossovers may be detected are numbered 1, 2, 3, and 4. The dicentric chromatids with their genic constitutions resulting from crossovers in each of these regions are given below.

top of figure 7. To simplify visualization of the crossovers, homologous associations of these two chromosomes are diagrammed only in the region carrying these genes (region A of fig. 4). There are four marked regions of crossing over, designated 1, 2, 3, and 4 in the diagram. The amount of crossing over which occurs between two normal chromosomes 9 in regions 1, 2, and 3 are 19 percent, 3 percent, and 21 percent, respectively (EMERSON, FRASER, and BEADLE 1935). The normal amount of crossing over in region 4 (from *Wx* to the break) cannot be stated, since there were no previous tests for this region. A crossover in region 1 (from *Yg* to *C*) would give rise to a dicentric chromatid carrying the genes *Yg c sh wx*. Following

the meiotic anaphase break, a surviving broken chromatid with the endosperm genes *c sh* and *wx* could be delivered to the primary endosperm nucleus. Since no variegation would result, this broken chromatid could not be detected by the endosperm characters.

A crossover in region 2 would give rise to a dicentric chromatid carrying *Yg C sh wx*. If the first break in the dicentric chromatid occurred to the left of *C*, a broken chromosome carrying the genes *C sh* and *wx* could be delivered to an endosperm, provided the successive breaks in the gametophyte divisions did not eliminate *C*. The resulting endosperm should be homozygous for *sh* and *wx* but variegated for *C* and *c*.

A crossover in region 3 would give rise to a decentric chromatid carrying *Yg C Sh wx*. Thus a broken chromosome with the genes *C Sh* and *wx* could be delivered to the primary endosperm nucleus. The endosperm tissue which develops should be homozygous for *wx* but variegated for *C-c* and *Sh-sh*.

A crossover in region 4 would give rise to a dicentric chromatid carrying the genes *Yg C Sh* and *Wx*. If the original break and the successive breaks in the gametophyte divisions occurred to the left of *C*, a broken chromosome with the genes *C Sh* and *Wx* would be delivered to the endosperm. The endosperm then should be variegated for all three genes, the detectible order of the loss of the dominant genes being *C*, followed by *Sh*, followed by *Wx*. In this last case, a break occurring between *C* and *Sh*, either in the first or in subsequent gametophyte divisions, could deliver a broken chromosome with the genes *Sh* and *Wx* to the endosperm tissues. The endosperm would then be *c* but variegated for *Sh-sh* and *Wx-wx*. If the original or subsequent break in the gametophyte occurred between *Sh* and *Wx*, the endosperm tissues would be *c* and *sh* but variegated for *Wx-wx*. Thus, single crossovers in regions 2, 3, and 4 should be genetically detectible by the type of endosperm variegation. Since *C* and *Sh* are close together and the genes *Sh* and *Wx* relatively distant, there should be many more *C-c Sh-sh wx* kernels than *C-c sh wx* kernels. If the normal relative rates of crossing over for these two regions is maintained in the plants heterozygous for the altered chromosome 9, the two types of variegated kernels should occur in the ratio of 1 to 7. That this ratio is maintained is seen from an examination of table 1, which gives the types of kernels resulting from the cross *C Sh Wx* rearranged chromosome 9/*c sh wx* normal chromosome 9×*c sh wx* normal chromosome 9. This table considers only the endosperm characters. In the crosses contributing to table 1, the plant character *yg* need not be considered. However, in some crosses, both parents were homozygous *Yg*. In other crosses, the heterozygous parent was either *Yg Yg*, or *Yg yg* as in figure 7, while the recessive parent was homozygous for *yg*.

In this table it may be seen that 99 percent of the kernels have the genet-

ic constitution of the two parental chromosomes of the heterozygous individual—that is *C Sh Wx* non-variegated or *c sh wx*. Only 0.8 percent of the kernels in the first column and 0.51 percent of the kernels in the second column are variegated, but the relative proportions, respectively, of the variegated kernels representing crossovers in regions 2, 3, and 4 are similar

TABLE I

Types of kernels arising from the cross.

$$\frac{C\ Sh\ Wx \text{ rearranged chromosome } 9}{c\ sh\ wx \text{ normal chromosome } 9} \times c\ sh\ wx \text{ normal chromosome } 9$$

	♀ PARENT HETEROZYGOUS		♂ PARENT HETEROZYGOUS	
Non-variegated, non-crossover kernels				
C Sh Wx	11,716		6,789	
c sh wx	11,679		5,812	
Subtotal		23,395		12,601
		98.9%		99.1%
Non-variegated, crossover kernels				
C sh wx	2		3	
c Sh Wx	3		1	
C Sh wx	12		21	
c sh Wx	8		14	
C sh Wx	0		0	
c Sh wx	1		0	
Subtotal		26		39
		0.19%		0.30%
Variegated kernels				
C-c sh wx	7		7	
C-c Sh-sh wx	78		48	
C-c Sh-sh Wx-wx	21		11	
c Sh-sh Wx-wx	2		0	
c sh Wx-wx	1		0	
Subtotal		109		66
		0.80%		0.51%
Totals	23,530		12,706	

in both crosses. Among the variegated kernels 14 were homozygous for *sh* and *wx* but variegated for *C-c*. These represent crossovers in region 2. One hundred twenty-six were homozygous for *wx* but variegated for both *C-c* and *Sh-sh*. These represent crossovers in region 3. Thirty-two were variegated for all three genes. These represent crossovers in region 4. The relative proportions of crossovers in regions 2 and 3 conform to expectancy on the basis of both the cytological and genetic evidence for the distance between the genes *C Sh* and *Wx*. The relative proportion of crossing over

from *Wx* to the break in the rearranged chromosome 9 could not be anticipated, since no previous genetic evidence was available for this region.

In both crosses there were a few kernels (0.19 percent in column 1 and 0.30 percent in column 2) carrying crossover chromatids which did not show variegation. According to expectancy, these should represent double crossover chromatids with normal, non-broken ends. The kernels should possess either a normal chromosome 9 or a rearranged chromosome 9 but not a broken chromosome 9. Whether, in any class, a normal or a rearranged chromosome is present would depend upon the positions of the two crossovers with respect to both the genes and the chromatids involved (see table 6).

Cytological evidence for the conclusion that the variegated kernels carry a chromosome 9 with a broken end and that the non-variegated kernels possess a non-broken chromosome could be obtained readily by examination of the chromosome complement of the plants arising from each of these types of kernels. However, before these results are described, it is desirable to emphasize the genetic evidence which allows one to conclude that the breakage-fusion-bridge cycle is responsible for the variegation in the endosperm. These may be enumerated as follows. (1) The frequency of the different types of kernel variegation indicates their relationship to crossing over in region A, figure 4, which, in turn, results in a dicentric chromatid and finally a broken chromosome. (2) The very low percentage of non-variegated kernels carrying crossover chromatids. This is expected on the assumption of their origin from a double crossover. These should be relatively infrequent. (3) The developmental order of loss of the genes in the variegated kernels containing *C Sh* and *Wx* is the type expected on the basis of the breakage-fusion-bridge cycle as outlined in figure 1. The genes nearer the broken end are lost before those nearest the centromere. (4) A striking confirmation of the breakage-fusion-bridge cycle appears in the various colored regions in the variegated kernels. In normal endosperm tissues the *C* allele may be present in a single dose (*C c c*), a double dose (*C C c*), or a triple dose (*C C C*). Usually the depth of color of the aleurone layer is related to the number of *C* alleles present, *C c c* being lighter than *C C c* which in turn is lighter than *C C C* (JONES 1937). When a broken chromosome 9 is introduced in the cross outlined, the depth of color in the *C* regions in the variegated kernels varies from very light to exceptionally dark. The regions are distributed in well defined patches for each intensity of color. This type of variegation is exactly what should be produced following the breakage-fusion-bridge cycle. As illustrated in figure 1, genes may not only be lost but may be reduplicated as a consequence of this cycle. Thus, various doses of the *C* allele in different regions of the aleurone are to be expected.

On the basis of the genetic evidence obtained from the variegated kernels, it was expected that the plant tissues would likewise show variegation for the *Yg* gene when the homozygous parent contained the gene *yg*, as well as the genes *c sh wx*, in its two normal chromosomes 9 while the heterozygous parent contained *Yg* in each of its two chromosomes 9. Some of the crosses contributing to table 1 were of this type. The plants arising from the variegated kernels would be expected to show variegation (*Yg-yg*) or to be wholly *yg* if the *Yg* locus had been lost either in the original or in an early subsequent break. The results were unexpected. The plants were either *Yg* or *yg*. There were no variegated individuals. Forty-eight plants were grown from the variegated kernels. Forty were *Yg* and eight were *yg*. Cytological examination showed that all the plants arising from these variegated kernels actually possessed a broken chromosome 9. The *Yg* plants had at least a complete chromosome 9. All the *yg* plants had lost a segment at the end of the short arm of chromosome 9 which included the *Yg* locus. In all cases, however, the broken end of the chromosome 9 had healed in the somatic diploid tissues, discontinuing the breakage-fusion-bridge cycle and thus the possibility of producing variegation in the *Yg* individuals. If the non-variegated crossover kernels possessed a non-broken end resulting from a double crossover, none of the plants arising from these kernels should be *yg*. All should possess either the normal chromosome 9 or the rearranged chromosome 9. Twenty-six plants were grown from the crossover non-variegated kernels. All twenty-six were *Yg;* none was *yg*. Cytological examination showed that all possessed an unbroken chromosome 9.

The cross outlined above allows one to distinguish between the crossover types which give a broken chromosome 9 and those which give a non-broken chromosome 9. As stated earlier, the single crossovers in region 1 of figure 7 cannot be detected by variegation, because the broken chromosome has the constitution *c sh wx*. Since this region between the knob and *C* in normal material shows as much crossing over as that between *C* and *Wx*, a number of broken chromosomes must have remained undetected in the above crosses. In order to detect the transmissible broken chromosomes following a crossover in region 1, as well as in the other three regions, individuals of the constitution *C Sh Wx* rearranged chromosome 9/*C sh wx* normal chromosome 9 were crossed with plants carrying two normal chromosomes 9 each with *c sh wx*. In this cross, the single crossovers in region 1 (fig. 7) would result in a dicentric chromatid with the constitution *C sh wx*. Following breakage of the dicentric chromatid, the surviving broken chromosome should have the constitution *C sh wx* and would result in a kernel variegated for *C-c* but homozygous for *sh* and *wx*. The single crossovers in regions 2, 3, and 4 would produce the same types of variegated kernels as

described in figure 7. Since single crossovers in regions 1 and 2 result in surviving broken chromosomes with similar constitutions, the class *C-c sh wx* among the variegated kernels should be relatively increased in this cross. This is shown in table 2, where the results of this cross are recorded. The first column gives the results obtained when the female parent was heterozygous. The second column gives the results obtained when the male

TABLE 2

Types of kernels arising from the cross.

$$\frac{C\ Sh\ Wx \text{ rearranged chromosome } 9}{C\ sh\ wx \text{ normal chromosome } 9} \times c\ sh\ wx \text{ normal chromosome } 9$$

	♀ PARENT HETEROZYGOUS	♂ PARENT HETEROZYGOUS
Non-variegated kernels		
C	4,532	4,116
c	1	8
Variegated kernels		
C-c sh wx	16	38
C-c Sh-sh wx	17	22
C-c Sh-sh Wx-wx	4	6
Totals	4,570	4,190
Percentage variegated kernels	0.8	1.6

parent was heterozygous. This cross was designed to test the maximum number of surviving broken chromosomes. Because some of the double crossover chromatids in the *C* non-variegated class could not be detected, classification of kernel types in the non-variegated classes have not been included in the table. Kernels with the constitution *c* should arise only following mutation of *C* to *c*, which is very rare, or following deletion of the *C* gene from the broken chromosome before its deliverance to the endosperm as a consequence of the breakage-fusion-bridge cycle. The one *c sh wx* kernel in the first column was diseased. It is possible that color did not develop because of this disease. In the second column, six of the eight *c sh wx* kernels appeared on two of the 11 ears examined. This strongly suggests that they arose following contamination. Plants have not been grown from these kernels to check the chromosome constitution.

The results of a similar cross which included *yg* are given in table 3. This cross was designed to test for the presence of *yg* in the plants derived from the various types of kernels. If the variegated kernels are the only ones which have a broken chromosome, no *yg* should appear in plants arising from the non-variegated kernels, either in the non-crossover classes or the crossover classes. However, *yg* could appear in the plants derived from the

variegated kernels. No *yg* plants appeared in more than 400 plants derived from each of the two non-variegated, non-crossover classes. The three plants derived from the non-variegated crossover kernels were likewise *Yg*. Their chromosome 9 constitution showed that a double crossover chromatid with a normal end had entered the endosperm and zygote nuclei. Nine

TABLE 3

Types of kernels arising from the cross.

$\frac{Yg\ C\ Sh\ Wx \text{ rearranged chromosome } 9}{Yg\ C\ sh\ wx \text{ normal chromosome } 9}$ ♀ × *yg c sh wx* normal chromosome 9♂

Non-variegated, non-crossover kernels	Number of kernels
C Sh Wx	2,061
C sh wx	2,016
Non-variegated, crossover kernels	
C Sh wx	2
C sh Wx	1
Variegated kernels	
C-c sh wx	3
C-c Sh-sh wx	8
C-c Sh-sh Wx-wx	1

plants were obtained from the variegated kernels. Five of these were *Yg* and four were *yg*. All nine plants carried a broken chromosome 9. The five *Yg* plants possessed at least a complete chromosome 9. Each of the four *yg* plants possessed a chromosome 9 deficient for a terminal segment of the short arm which included the *Yg* locus.

(d) *The chromosome 9 constitution of plants arising from the variegated kernels*

In the previous section, genetic evidence has been given which indicates that variegation in the endosperm tissue is due to the presence in these tissues of a chromosome 9 with a broken end. The variegation appears as the consequence of the breakage-fusion-bridge cycle in successive nuclear divisions. In contrast, the plants arising from the variegated kernels, although possessing a chromosome with a broken end, do not show variegation. The broken end of the chromosome heals in the embryo nuclei and remains permanently healed regardless of the tissue in which it may later be present.

In this section it is desired to describe the types of broken chromosomes which are present in the plants arising from the variegated kernels. In a previous publication (McCLINTOCK 1938b) it was shown that a chromo-

some broken at a meiotic anaphase gave rise to a bridge configuration in the following microgametophyte division as the result of the fusion at the position of breakage of the two sister halves of the broken chromatid. If this occurred in both the male and the female gametophytes, the broken chromosomes delivered to the zygotes should include various types of deficiencies and duplications (see fig. 1), depending upon where the breaks occurred in the anaphase bridge configurations in the successive gametophyte divisions. If the broken end, produced as the result of a bridge configuration in the last gametophyte division, healed in the zygote nucleus, all the cells of the plant would show the same type of modified chromosome 9. The meiotic prophases in these plants would then reveal the constitution of the broken chromosome 9 which each plant had received.

One hundred twenty-six plants arising from variegated kernels were examined at meiotic prophase for the constitution of the chromosome 9 delivered by the heterozygous parent. In 120 of these plants, this chromosome 9 terminated in a broken end. In three plants the chromosome 9 delivered by the heterozygous parent had undergone a secondary modification; two involved a translocation between chromosome 9 and a second chromosome, and in the third plant the chromosome 9 was in the shape of a ring. In the three remaining plants, heterofertilization had occurred. In each of these three cases, sperms from two pollen grains had contributed to the development of the kernel, a sperm from one pollen grain fusing with the polar nuclei and a sperm from a second pollen grain fusing with the egg nucleus. A normal, unbroken chromosome 9 carrying genes not corresponding with those in the endosperm tissues was present in these plants. In maize, hetero-fertilization may be expected in a low percentage of the cases (SPRAGUE 1932). For comparison, 49 plants arising from the *C Sh Wx* non-variegated kernels of table 1 were examined. All 49 plants had received the rearranged chromosome 9 with an unbroken end (b, fig. 3) from the heterozygous parent.

In 109 of the 120 plants possessing a chromosome 9 with a broken end no duplications were present of the type which could arise as a consequence of the breakage-fusion-bridge cycle in the preceding gametophytic divisions. As illustrated in figure 1, a chromosome with a broken end but possessing a duplication or repeat duplications of terminal segments could be present in these plants if the breaks in anaphase in the sucessive gametophytic divisions (two in the male gametophyte, three in the female gametophyte) did not occur at position of previous fusions—that is were non-median. To recover such a duplication, it is likewise necessary that following such a non-median break the longer segment be included in the telophase nucleus which will give rise to a gamete (first telophase to left in fig. 1 instead of first telophase to right). For descriptive purposes, therefore, the type of

recovered broken chromosome may be referred directly to the dicentric chromatid (a, fig. 5) from which it originally arose. This chromatid is reproduced in figure 8. In all cases except one (dash-line arrow, fig. 8) the recovered broken chromosome had the constitution *to the right* of the position

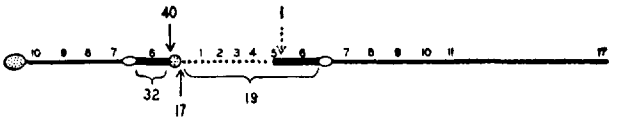

FIGURE 8.—Types of broken chromosomes which were recovered in plants arising from variegated kernels. In each plant, the type of recovered chromosome may be referred to the dicentric chromatid illustrated here. The arrows and brackets give the positions of the broken ends. The recovered broken chromosome had the constitution to the right of the arrow (except for the dash-line arrow) or to the right of any particular position within the bracket. In the case of the dash-line arrow, the recovered broken chromosome had the constitution to the left of the arrow. The numbers associated with the arrows or brackets indicate the number of plants which possessed this particular broken chromosome constitution.

of breakage as indicated by the arrows or to the right of any particular position within the brackets. The numbers placed above or below the arrows and below the brackets give the number of individuals with the constitution indicated. In the one exceptional case, the recovered broken chromosome had the constitution to the left of the dash-arrow in figure 8. This case is of particular interest and will be considered separately elsewhere in this paper.

Thirty-two of these plants showed a broken chromosome 9 with a section of the pycnotic region 6 extending beyond the small knob. In 40 plants, the broken chromosome ended in a small knob, the last break having occurred immediately to the left of the knob in most cases but through the knob in a few cases. In 17 plants, the chromosome was complete to the knob, but the knob itself was missing. In 19 plants, the recovered broken chromosome was deficient. The deficiencies ranged from loss of a single chromomere to loss of the entire short arm. In the latter case, the broken end terminated in the centromere.

Among the remaining 11 plants of the 120, the broken chromosome 9 possessed various types of duplicated segments arising in each case through secondary fusions and breakages. The broken chromosome 9 in no two plants was exactly alike, but for descriptive purposes the 11 plants may be divided into five classes according to the composition of the broken chromosome. In four of the five classes, the recovered broken chromosome showed evidence of only a single fusion and breakage following the original break at a meiotic anaphase. In the fifth case, at least two fusions and breaks followed the original meiotic break (type V, fig. 9). As will be shown later there appears to be a tendency for successive breaks to occur at positions of previous fusions, indicating a weak fusion of sister chroma-

tids following a break. A strong union is produced, however, following many of the breaks. Therefore, although the breakage-fusion-bridge cycle may have continued in all gametophyte divisions following the initial break at meiotic anaphase, the recovery of many complicated duplications

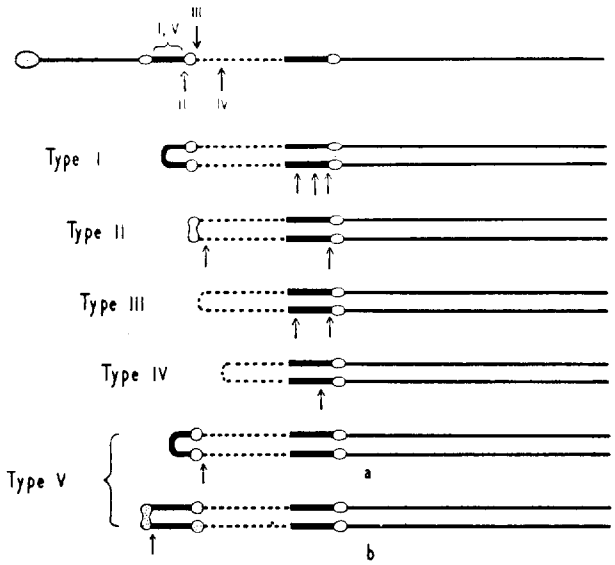

FIGURE 9.—The types of recovered broken chromosomes in plants arising from variegated kernels which demonstrate the breakage-fusion-bridge cycle. The dicentric chromatid from which each recovered broken chromosome originated is given above. Although the position of the initial break in this dicentric chromatid is not definitely known for each recovered broken chromosome, for purposes of illustrating the chromatin constitution of the broken chromosome, the break which preceded the final break in types I to IV and in a of type V may be referred to the dicentric chromatid (section of dicentric chromatid to right of arrows and bracket). This broken chromosome, with its two halves fused at the position of previous breakage, is reproduced below for each type. For types I to IV, the position of the final break is indicated by the arrows, one for each plant within a type, the broken chromatid with the upper centromere being recovered in the zygote. In type V, the recovered broken chromatid indicated that three successive breaks had occurred. The position of the first break is indicated in the dicentric chromatid. The position of the second break is indicated by the arrow in a, the chromatid with the upper centromere being in the line of descent. The position of the third break is indicated by the arrow in b, the recovered chromosome having the constitution of the broken chromatid with the upper centromere.

resulting from this process need not be anticipated. For illustrative purposes, the origin of the various classes of broken chromosomes with duplicated segments may be referred to the original dicentric chromatid with only one fusion and consequent break following the original meiotic break in types I to IV, and two successive fusions and breaks in type V. In figure 9, the dicentric meiotic chromatid in which the first break occurred is diagrammed above, the region of the first break being indicated by the arrows and the bracket for each of the five types. In all five types, the chromatid in the line of descent is that to the right of the break. This chromatid with its

two split halves fused at the position of breakage is diagrammed below for each type. The position of the second break is indicated by arrows, one for each plant in a type class except for types I and II, where the second break occurred close to the centromere in two cases in each of these two classes. The chromatid with the upper centromere was recovered in each case and represents the observed broken chromosome. Four plants were included in type I, three in type II, and two in type III. There was only one plant in

TABLE 4

The broken chromosome 9 constitution of plants derived from variegated kernels. The symbol (♀) or (♂) placed beside the kernel type indicates which parent was heterozygous for the rearranged chromosome 9.

KERNEL CHARACTER	CHROMOSOME ENDS IN KNOB	PYCNOTIC EXTENSION BEYOND KNOB	DUPLICATION OF SHORT ARM	NO KNOB, NO DEFICIENCY	TERMINAL DEFICIENCY	OTHERS
C-c Sh-sh Wx-wx (♀)	7	6	0	1	0	1
C-c Sh-sh Wx-wx (♂)	3	2	0	1	0	1
C-c Sh-sh wx (♀)	15	17	5	7	12	1
C-c Sh-sh wx (♂)	11	5	4	6	5	1
C-c sh wx (♀)	2	1	0	1	0	0
C-c sh wx (♂)	2	2	1	1	0	0
c Sh-sh Wx-wx (♀)	0	0	0	0	2	0
Totals	40	33	10	17	19	4*

* For description of these types, see text.

type IV. The recovered broken chromosome in this plant possessed a deficiency of approximately one-fifth of the distal segment of the short arm but possessed a duplication of nearly all of the remaining proximal four-fifths of the short arm. As stated above, in type V the breakage-fusion-bridge process is detected through one more cycle. The upper chromatid was in the line of descent following each of the two successive breaks.

Although the breaks in the dicentric chromatids may occur at any position between the two centromeres the evidence from all 120 cases suggests that there is a tendency for the breaks to occur at either side of the small inner knob. In approximately half of the cases, the composition of the recovered broken chromosome indicated that a break had occurred to either side of this knob (fig. 8). Similarly, in six of the 11 plants with duplicated segments (fig. 9) one or more of the breaks must have occurred to one side of the knob. In the chromosome represented in type V (fig. 9) both the second and the third breaks must have occurred adjacent to the knob. The results of these studies on the chromosome 9 constitution of plants arising from variegated kernels are given in table 4 and are summarized in table 5.

The tendency for successive breaks to occur at the positions of previous fusions probably accounts for the relatively few cases of complicated duplications which otherwise would be expected to be present following repeated fusions and breaks in the gametophyte divisions. Likewise, this tendency should work toward increasing the correspondence in the genic constitution of the broken chromosome in the endosperm and the plant tissues. The two sperms (one for the endosperm tissues and one for the plant tissues) need

TABLE 5

A summary of the various types of broken chromosomes 9 delivered to the zygote through the female and male gametophytes.

TYPE OF BROKEN CHROMOSOME	THROUGH FEMALE GAMETOPHYTE		THROUGH MALE GAMETOPHYTE	
	NUMBER OF PLANTS	%	NUMBER OF PLANTS	%
Chromosome ends in knob	24	30.7	16	35.5
Pycnotic extension beyond knob	24	30.7	9	20.0
Duplication of short arm	5	6.4	5	11.1
No knob, no deficiency	9	11.5	8	17.7
Terminal deficiency	14	17.8	5	11.1
Others	2	2.5	2	4.4
Totals	78		45	

not possess the same genic constitution with respect to the genes *C Sh* and *Wx*. A decidedly non-median break in a bridge configuration at the division of the generative nucleus could introduce a duplication of one or more genes into one sperm nucleus and a deficiency of these genes in the sister sperm nucleus. Following such a break, the genic constitution of the endosperm and embryo tissues would not be the same. Similarly, this reasoning may be applied to the embryo and endosperm tissues when the broken chromosome is introduced through the female gametophyte.

Among the examined plants the genic constitution of the broken chromosome 9 in the plant tissues was determined in all cases and compared with the genic constitution of the broken chromosome 9 delivered to the endosperm of the kernel from which the plant arose. Excluding the three cases of hetero-fertilization, as previously mentioned, correspondence was obtained in all but two cases. In these two cases, the male was the heterozygous parent; the endosperm and embryo nuclei differed in genic constitution. In both cases, the chromosome 9 delivered to the endosperm possessed the genes *C* and *Sh* as shown by *C-c* and *Sh-sh* variegation. The chromosome 9 in the plant tissues lacked both these genes because the

broken chromosome 9 was deficient for all of the short arm, in one case, and nearly all of it in the second case.

It will be noted in the summary table (table 5) that the proportion of the various types of recovered broken chromosomes are similar whether delivered through the sperm or the egg nucleus. It is known that deficiencies of the extent observed in these studies are not transmitted through the pollen when the tube nucleus possesses such a deficient chromosome. A deficient broken chromosome was observed in five plants when the broken chromosome was delivered through the male parent. This deficiency must have arisen through a non-median break in an anaphase bridge configuration subsequent to the division which produced the tube nucleus.

A deficient broken chromosome was observed in 14 plants when the broken chromosome was delivered through the female gametophyte. When all the nuclei of a female gametophyte possess a short terminal deficiency of chromosome 9, a functional embryo sac can develop. It is not certain, therefore, whether the deficiency in the broken chromosome arose at meiosis or in a subsequent division. In two cases, the evidence suggests that the deficiency may have arisen at the meiotic anaphase, for both the endosperm and the plant nuclei were deficient for terminal segments of the short arm of chromosome 9 which had deleted the same genes.

The long duplications observed in plants which have received their broken chromosomes from the male parent have some theoretical interest. All such duplications must arise in divisions subsequent to that which gave rise to the initial break—that is, subsequent to the meiotic break. This subsequent break must have occurred at a non-median position in an anaphase bridge. If the long duplications originated in the first microspore division, the generative nucleus would receive the duplication and the tube nucleus would receive a highly deficient chromosome. Such a pollen grain would not be expected to function unless the tube nucleus had received an acentric fragment with a genic complement covering the deficiency. If the long duplications originate in the division of the generative nucleus, the two sperm nuclei would differ in constitution with regard to the genes *C Sh* and *Wx*. In the four cases of recovered long duplications, the detectible genic constitution of the embryo and the endosperm were similar. This would suggest that the duplication observed in these plants could have arisen during the first division of the zygote as a consequence of an unequal break in a bridge configuration. Since it is known that acentric fragments occasionally are carried through several nuclear cycles before being lost to the telephase nuclei, it is not possible to determine directly by this analysis whether a bridge configuration actually occurred at the first zygotic anaphase. If a bridge configuration does occur at this division, healing of the broken end must occur very shortly thereafter.

Mention should be made of the four plants arising from variegated kernels whose chromosome 9 constitution was unexpected (see table 4). One of these cases is of particular interest. As stated above, all but one of the recovered broken chromosomes arising from the original dicentric chromatid, possessed the broken segment to the right of the arrow or bracket in figure 9. However, one broken chromosome possessed the segment of the dicentric chromatid to the left of the dash-line arrow in figure 8. This broken chromosome, introduced by the male parent, is deficient for a large section of chromosome 9. It is known that a pollen grain, all of whose nuclei carry such a deficient chromosome, does not develop normally and does not produce a functional grain. It is highly probable that the acentric fragment, produced during the formation of the dicentric chromatid, was included in the microspore nucleus and again included in the tube nucleus following the first microspore division (McCLINTOCK 1938b). This acentric fragment could possess the genic material which is absent in the broken chromosome. A pollen grain with such a tube nucleus, possessing at least a complete complement of genes of chromosome 9, could develop normally and function in the delivery of two deficient sperms to the endosperm and zygote nuclei, respectively.

The genes *C Sh* and *Wx* were carried by this broken chromosome 9 (fig. 10). The normal chromosome 9 in this plant carried the recessive alleles, *c sh* and *wx*. (The observed order of loss of the dominant genes in the vari-

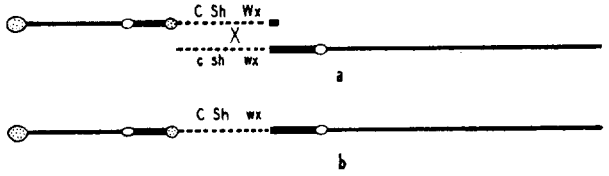

FIGURE 10.—a. Homologous associations of segments 1 to 4 of a broken chromosome (left of dash-line arrow, figure 8) and a normal chromosome. The genic constitution of each chromosome in indicated. A crossover, as indicated, would give rise to the dicentric chromatid shown in b.

egated endosperm of the kernel from which this plant arose was *Wx*, followed by *Sh*, followed by *C*. This is expected on the assumption of the breakage-fusion-bridge cycle, since the gene nearest the broken end (*Wx*) would be lost before the gene nearest the centromere (*C*).) Functional gametes carrying the dominant genes can be produced by this plant only following crossing over. All single crossovers in regions indicated by homologous associations in a (fig. 10) will give rise to dicentric chromatids (b, fig. 10) similar in constitution to that of a in figure 5. This dicentric chromatid is broken at anaphase I. Consequently, following such a crossover, all chromosomes with dominant genes must possess a broken end. In the cross of this plant with one homozygous for *c sh* and *wx*, these broken chromosomes

should give rise to kernels with variegated endosperms. Unfortunately, at the time this plant was ready for pollination, no plants homozygous for *c sh* and *wx* were available. Consequently, it was selfed. Pollen from this plant was also placed on silks of plants of the constitution *C c*. Among the 54 kernels obtained from the self, 12 showed the presence of the dominant gene *C*. The endosperms of all 12 kernels were variegated for *C* and *c*. (2 *C-c sh wx*:8 *C-c Sh-sh wx*:2 *C-c Sh-sh Wx-wx*). The remaining 44 kernels were completely recessive for all three endosperm genes.

The cross onto the heterozygous plant (*C c*) gave 1070 *C* non-variegated to 859 *c* to 133 *C-c* variegated kernels. The contribution from the female parent would lead to a 1:1 ratio of *C* non-variegated to *c*. Any distortion of this ratio would depend upon the *C* contribution of the male parent. Since variegation for *C-c* produced by the presence of a broken chromosome 9 carrying *C* and introduced by the male parent, can express itself only following union with *c* carrying nuclei contributed by the female parent, this class of 133 kernels may be added to the 859 *c* kernels to obtain the minimum number of *c* carrying gametophytes produced by the female parent. The resulting ratio of 1070 to 992 is close to the 1 *C*:1 *c* ratio which the female parent alone should contribute. The distortion in favor of the *C*-non-variegated class could be accounted for by the functioning of *C*-carrying gametes whose chromosome 9 possessed a normal end following a double crossover. In the material available, this class could not be detected. Nevertheless, the results of these two crosses are of significance in confirming the relationship between broken chromosomes and variegated endosperms.

The second plant of the four arising from the variegated kernels whose chromosome 9 constitution was unpredicted possessed a segment of chromosome 9 in the shape of a ring. This ring carried the genes *C* and *Sh* but not *Wx*. The variegation for *C-c* and *Sh-sh* in the kernel from which this plant arose was probably related to the aberrant mitotic behavior of the ring-shaped chromosome (McCLINTOCK 1938a). The origin of this ring chromosome was not determined.

The third plant possessed a translocation between the short arm of chromosome 9 and chromosome 8. The meiotic prophase figures were too poor to allow an analysis of this translocation.

The fourth plant had a complete chromosome 9 with the addition of a segment of chromatin of undetermined origin extending beyond the end of the short arm. The extra segment did not represent a duplication of part of the short arm of chromosome 9.

With the exception of the three cases of heterofertilization and the three cases just mentioned, all the other plants examined (120) showed the presence of a broken chromosome 9 in the plants derived from kernels with variegated endosperms. In 38 cases, the composition of the broken chromo-

some clearly indicated that it had arisen from the dicentric chromatid diagrammed in a, of figure 5 (arrows to the left of the small knob, fig. 8; dashline arrow, fig. 8; types I and V, fig. 9). All other cases could readily be derived from this dicentric chromatid as illustrated in figures 8 and 9.

That the variegation is the result of the breakage-fusion-bridge cycle is strongly supported by the types of recovered chromosomes illustrated in figure 9. It is not understood why this process is limited to the gametophyte and endosperm and does not continue in the embyro tissues. In all cases, the broken end of chromosome 9 healed in the embryo tissues. This is a permanent healing, for when this chromosome is again introduced into endosperm tissues in successive generations, it causes no variegation. When two such chromosomes are brought together in a single plant after each has passed through a sporophytic generation, no fusions occur between the broken ends. If the broken chromosome has a full set of genes or in addition a pycnotic extension composed of region 6, its behavior and transmissions through successive generations are comparable in every way to a normal, unbroken chromosome 9 (see f of this section).

(e) *The chromosome 9 constitution of plants arising from the non-variegated crossover kernels*

Earlier in this paper the assumption was made that the non-variegated kernels in a crossover class (tables 1 and 3) had received a non-broken chromosome 9 from the heterozygous parent as the consequence of a double crossover. Because no broken end was present, no variegation should be expected. In the classes *C sh Wx*, *c Sh Wx*, *C Sh wx*, and *c sh Wx*, the broken chromosome 9 derived from a double crossover chromatid could be either the normal chromosome 9 or the rearranged chromosome 9, depending up on where the two crossovers had occurred. The chromosome 9 constitution of 50 plants arising from the non-variegated crossover kernels are summarized in table 6. In 44 individuals, the chromosome 9 constitution was as expected—that is, the plant possessed either a normal chromosome 9 or a rearranged chromosome 9. Among the six individuals with exceptional constitutions, four arose following heterofertilization and consequently must be eliminated from consideration. In one of the two remaining exceptional individuals, a segment of the end of the long arm of chromosome 4 had been translocated to the end of the short arm of chromosome 9. The origin of this modified chromosome is not understood. The other exceptional individual showed a chromosome 9 with the same constitution as the dicentric chromatid in a, of figure 5, except that the left centromere was deleted. It is possible that this centromere was torn from the dicentric chromatid during anaphase I, the broken ends fusing on each side of the tear to give rise to the observed chromosome.

Genetic tests were conducted with some of these plants to verify the cytological determination of the presence of a double crossover chromosome. Plants of the constitution *Yg C Sh Wx* rearranged chromosome 9/*yg c sh wx* normal chromosome 9 were crossed by plants of the constitution *Yg c sh wx* normal chromosome 9. Since the locus of *yg* is almost immediately adjacent to the terminal knob in the normal chromosome 9

TABLE 6

Type of chromosome 9, delivered by the heterozygous parent, in plants arising from the non-variegated crossover kernels. The symbol (♀) or (♂) placed beside the kernel type indicates the heterozygous parent.

KERNEL CHARACTER	NORMAL CHROMOSOME 9	REARRANGED CHROMOSOME 9	OTHERS
C Sh wx (♀)	5	6	2†
C Sh wx (♂)	8	4	2*
c sh Wx (♀)	6	2	0
c sh Wx (♂)	3	5	1*
C sh wx (♀)	1	0	0
C sh wx (♂)	1	0	1*
c Sh Wx (♀)	0	2	0
c Sh Wx (♂)	1	0	0
Totals	25	19	6

* Endosperm and embryo chromosome constitution not alike due to hetero-fertilization.
† See text for description of chromosome 9 in these two plants.

(CREIGHTON 1934, McCLINTOCK unpublished), crossovers between *yg* and the knob are infrequent. If the kernels in the non-variegated crossover classes possessed a chromosome derived from a double crossover chromatid, all the plants arising from these kernels which have a normal chromosome 9 should be heterozygous for yellow-green (*Yg yg*). Those possessing a rearranged chromosome 9 should be homozygous dominant (*Yg Yg*). These tests were conducted with nine plants carrying a normal chromosome 9 and four plants carrying a rearranged chromosome 9. The results conformed with expectancy.

(f) *The genetical and cytological behavior of the broken chromosomes in successive generations. Further proof that variegation follows the introduction of a recently broken chromosome into the endosperm tissues*

To test whether the broken chromosomes, summarized in table 5, would induce variegation in the endosperm tissues of the next succeeding generation, plants in all the categories of table 5 were either selfed or crossed by those containing two normal chromosomes 9 carrying *c*. In all of these tests

the broken chromosome carried *C*, its normal homologue, *c*. In the first category of plants in table 5,—that is plants whose broken chromosome ended in a knob,—the selfing of 28 plants gave rise to 6,142 *C* non-variegated kernels, 2,048 *c* kernels, and 3 that were variegated for *C* and *c*. Two plants were crossed by plants homozygous for *c*. There resulted 242 *C* non-variegated kernels, 249 *c* kernels, and no variegated kernels. The variegation in the three kernels appearing in the selfed progenies need not be related to the behavior of the original broken end of chromosome 9. A small percentage of such kernels have been noted in many genetic crosses in maize produced through causes the exact nature of which cannot be easily ascertained. Thus, the behavior in successive generations of this broken chromosome in column 1 of table 5 is comparable in every way to a normal chromosome 9 with a normal end.

Eighteen plants in the second category of table 5 were selfed. These plants possessed a duplication of the pycnotic region 6 of chromosome 9 extending beyond the small knob. The selfing of these eighteen plants gave rise to 4,209 *C* non-variegated kernels, 1,566 *c* kernels, and no variegated kernels. In no individual case was there a marked deviation from a 3:1 ratio, although in the total counts there is a slight deviation in favor of the *c* class. This is probably due to a slight selection against the pollen grains carrying the chromosome 9 with the pycnotic extension. The backcross ratios likewise suggest this. Pollen carrying *c* was placed on the silks of seven of these plants. There resulted 1,532 *C* non-variegated kernels, 1,565 *c* kernels, and one *C-c* variegated kernel. When pollen of two of these plants was placed on the silks of seven plants homozygous for *c*, there resulted 1,087 *C* non-variegated kernels, 1,200 *c* kernels, and no variegated kernels. A lack of variegated kernels in the progeny of this second category of plants likewise shows the stability of the broken end following its healing in the embryo of the parent plant. Similar tests were carried out with plants in the fourth category, those whose broken chromosome had no knob but likewise no observable deficiency. The selfing of eight plants produced 1,818 *C* non-variegated kernels, 605 *c* kernels, and no variegated kernels. Only one plant was crossed by *c*. This resulted in 112 *C* non-variegated kernels, 139 *c* kernels, and no variegated kernels. Pollen of two plants in this category was placed on silks of *c*-carrying plants. There resulted 904 *C* non-variegated kernels, 883 *c* kernels, and no variegated kernels. Complete healing of the broken end likewise occurred in these plants. These chromosomes, although deficient for the knob, behaved strictly as a normal chromosome 9.

The broken end in plants of the fifth category likewise showed a permanent healing. Since the transmissions of the deficient chromosomes is dependent upon the extent of the deficiency, no ratios will be given here.

The plants in the third category, those whose broken chromosome possessed a duplication of the short arm of chromosome 9, gave entirely different results. Many variegated kernels resulted from the selfing and backcrossing of these plants. It will be shown that this is not due to the instability of the original broken end but to the production at meiosis in these plants of chromosomes 9 with new broken ends. These new broken ends are the cause of the variegation in the endosperms of the progeny. These new broken chromosomes become stable in the embryos of these variegated kernels and remain stable from then on. Although the number of variegated kernels arising from a self or a backcross of a plant carrying a duplication depends upon the particular duplication that is present, a summary of the results from crosses involving all the duplications is illuminating. The progeny from selfing eight plants carrying duplications gave 1,052 *C* non-variegated kernels, 841 *c* kernels, and 152 *C-c* variegated kernels. When the female parent carried the duplication, the backcross gave 397 *C* non-variegated kernels, 450 *c* kernels, and 29 *C-c* variegated kernels. When these plants were used as pollen parents, the backcross gave 179 *C* non-variegated kernels, 641 *c* kernels, and 49 *C-c* variegated kernels. Since pollen grains carrying the duplication do not function readily in competition with those carrying a normal chromosome 9, a deficiency of the *C* class is to be expected in the selfed and male backcross progenies. Among a total of 3,789 kernels resulting from these crosses, 230 were variegated for *C-c*. The variegation was of the type which would be produced by a broken chromosome. This presents an extreme contrast to the results recorded from the previous classes where four *C-c* variegated kernels appeared in a total of 24,305 kernels.

A chromosome 9 with a duplication of all or of only a part of the short arm, such as that illustrated in figure 9, can give rise to new broken chromosomes following crossing over. The method by which crossing over can give rise to broken chromosomes is illustrated in figure 11. In this illustration the plant is considered to be heterozygous for a duplication of the short arm of chromosome 9. In the diagram, the end of the short arm of the normal chromosome 9 terminated in a large knob. No knob has been diagrammed in the duplication chromosome. This constitution has been chosen, since it will apply to all the duplications which will be discussed in sections 4 and 5 of this paper. At meiosis, association of the three homologous segments of the two chromosomes 9 is 2-by-2. Two of these associations are diagrammed (a and b, fig. 11). In a, the duplicated segment is associated with the short arm of the normal chromosome 9. A crossover as indicated would give rise to a dicentric chromatid (c, fig. 11). This dicentric chromatid is the equivalent of two chromosomes 9 fused at the ends of their short arms. In b (fig. 11), the two homologous segments in the duplicated

chromosome 9 are associated. A crossover as indicated would result in the same dicentric chromatid (c, fig. 11). Disjunction of homologous centromeres in anaphase I would result in a first division bridge configuration following the crossover in a. Separation of sister centromeres in anaphase II would give rise to a second division bridge configuration following the crossover in b. In the late anaphase or early telophase of these cells, the dicentric chromatid is broken at some position between the two centromeres. If the break occurred at the position of the arrow (c, fig. 11), each of the two broken chromatids would contain a complete set of genes of chromosome 9. If the break occurred at any other position, a deficient chromatid would enter one nucleus and a chromatid with a duplication would enter the sister nucleus. In this way, chromosomes 9 could be produced with various lengths of duplication and deficiencies of segments of the short arm.

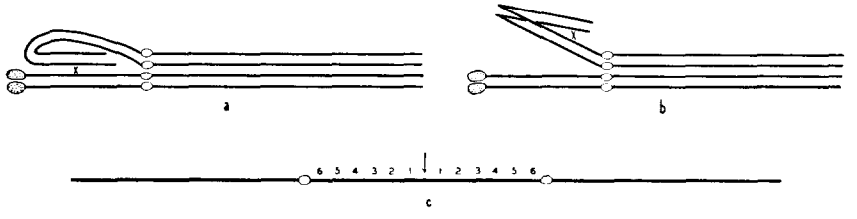

FIGURE 11.—a. Diagram of a meiotic prophase association of a normal chromosome 9 with a large terminal knob and a chromosome 9 with a duplication of the short arm but with no knob. The duplicated segment is homologously associated with the short arm of the normal chromosome 9. A crossover as indicated produces a chromatid composed of two attached chromosomes 9, as shown in c. This will produce a bridge configuration at anaphase I. b. Association of homologous regions of the duplication chromosome 9. A crossover as indicated will give rise to the dicentric chromatid shown in c. A bridge configuration will result in anaphase II.

However, each would possess a newly broken end. Each, therefore, should be capable of inducing variegation in the endosperm tissues to which it is delivered, provided the appropriate genic markers are present to allow detection of variegation. Plants arising from these variegated kernels should show a new series of various types of broken chromosomes 9. All plants with duplications of the type illustrated in figure 9 were heterozygous—that is, contained a duplication chromosome 9 with dominant genes in the duplicated segments and a normal chromosome 9 carrying the recessive alleles. In deriving the constitution of the dicentric chromosome produced following crossing over, it is necessary to insert the knobs and pycnotic regions where they occur in each of the duplicated chromosomes.

Meiotic anaphases were observed in all plants heterozygous for duplications. All showed bridge configurations in some of the anaphase I and II cells. The total percentages and proportions of bridges in the two divisions were not the same and should not be the same for all duplications. A summary of meiotic behavior and the types of progeny will be confined to a

single case, that of duplication 1276-1 (arrow to right in type I, fig. 9). In this plant, 189 microsporocytes in anaphase I were recorded. Nineteen, or ten percent of these showed a bridge configuration. In the second division, recordings were made only when the two sister cells were in mid-anaphase. Among 144 such dyads, 38 or 26 percent showed a bridge configuration in one of the cells of the dyad. This plant was selfed and produced 126 *C* non-variegated kernels, 97 *c* kernels, and 13 *C-c* variegated kernels. At the time

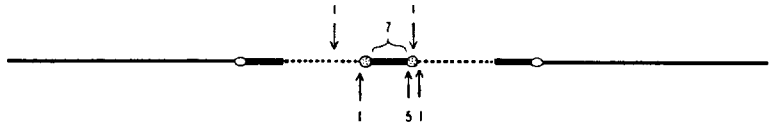

FIGURE 12.—Chromatin constitution of the dicentric chromatid produced by crossing over in plant 1276-1. The constitution of the recovered broken chromosome in plants arising from the variegated kernels may be referred to this dicentric chromatid. The recovered broken chromosomes have the constitutions to the right of the arrows and to the right of positions within the bracket. The numbers given above or below indicate the number of plants in which the broken chromosome had this particular constitution.

of pollination there were no homozygous *c* plants to which it could be crossed. Consequently, it was crossed to a normal plant of the constitution *C c*. This cross produced 187 *C* non-variegated kernels, 144 *c* kernels, and 18 *C-c* variegated kernels.

The chromosome 9 constitution was determined in 14 plants arising from the *C non-variegated* kernels of the self of 1276-1. Thirteen of these plants possessed a normal chromosome 9 and a chromosome 9 with the duplication—that is, had the same constitution as the parent. One plant possessed two normal chromosomes 9, the result of a crossover between the short arm of the normal chromosome 9 and the proximal homologous segment of the duplicated chromosome 9. Cytological determination of the chromosome 9 constitution of eight plants arising from variegated kernels of the self and of nine plants arising from the variegated kernels of the outcross were illuminating. All 17 plants possessed a newly broken chromosome 9. In eight of these plants, derivation from the dicentric chromatid produced by crossing over of the type illustrated in figure 11 was unmistakable. In the parent plant, 1276-1, a short segment of pycnotic region 6 was inserted between the two knobs and thus between the proximal and distal duplicated segments of the short arm (see arrow to right, type I, fig. 9). A crossover as diagrammed in a or b of figure 11 would give rise to a dicentric chromatid with the constitution shown in figure 12. All the newly produced broken chromosomes 9 showing a pycnotic segment extending beyond the knob may be traced to this dicentric chromatid. For illustrative purposes, the chromosome constitution of 16 of the 17 plants arising from variegated kernels may be referred to this dicentric chromatid (fig. 12). The arrows indi-

cate the positions of breakage; the numbers above or below the arrows indicate the number of plants whose broken chromosome 9 constitution corresponded to that part of the dicentric chromatid to the right of the arrow. A new duplication, derived from secondary fusions and breaks, was present in one plant. Its constitution is shown in c, figure 13. The method of deriving the origin from the dicentric chromatid (a and b, fig. 13) is the same as that used in figure 9.

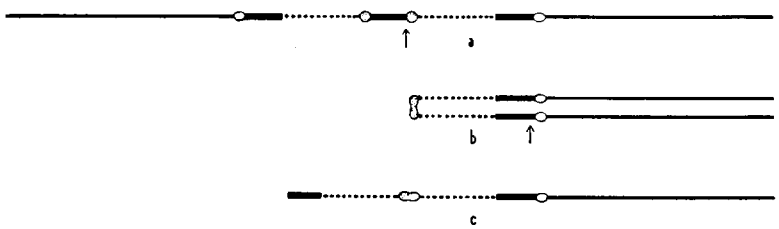

FIGURE 13.—Illustration of the origin of a new duplication chromosome 9 from duplication 1276-1 as a consequence of the breakage-fusion-bridge cycle. a. Dicentric chromatid produced following a crossover in plant 1276-1 (heterozygous for a duplication shown in type I, figure 9). The arrow indicates the position of the first break, the broken chromatid to the right being in the line of descent. b. The two split halves of the broken chromatid fused at the position of previous breakage. The arrow indicates the position of the second recognizable break which gave rise to the recovered broken chromosome with a new duplication shown in c.

The stability of these newly broken chromosomes following their passage through a sporophytic generation is indicated by kernel characters obtained from selfing seven of the plants whose broken chromosome ended either in a knob or in a short pycnotic segment extending beyond the knob. There were 1,385 *C* non-variegated kernels, 456 *c* kernels, and no variegated kernels. The ratio obtained from selfing the plant with the newly derived long duplication, illustrated in figure 13, gave 104 *C* non-variegated kernels, 80 *c* kernels, and 26 *C-c* variegated kernels. These variegated kernels were expected, for the process of forming new broken chromosomes at meiosis is the same in this plant as it was in the parent plant with the original duplication. The selfed ear from the plant with the short duplication (left-most arrow in fig. 12) was badly diseased. However, pollen of this plant placed upon silks of a *Cc* plant with two normal chromosomes 9 gave 98 *C* non-variegated kernels, 68 *c* kernels, and three *C-c* variegated kernels. From this plant, likewise, variegated kernels should appear and do appear in the progeny.

Although the original duplicated chromosome in plant 1276-1 carried *C* in each of the homologous segments, it is not certain that this genic constitution will be preserved when this chromosome is passed on to the next generation. Following crossing over at meiosis in plant 1276-1, the *c* gene carried by the normal homologue, could have been inserted into one of the

duplicated segments. As stated above, 13 of the 14 examined plants derived from the *C* non-variegated kernels of the self of 1276-1 possessed the original duplicated chromosome 9 and a normal chromosome 9. Regardless of crossing over, a *C* gene must be present in at least one of the homologous segments of the duplication. Consequently, variegated kernels should appear in the progeny of these plants following appropriate crosses because newly broken chromosomes should be produced during meiosis in these plants in exactly the same manner as occurred in the parent plant. The results of crosses involving these plants are summarized in table 7. It is obvious from this table that variegated kernels do appear. The plants arising from these variegated kernels should show, in turn, newly derived broken chromosomes.

TABLE 7

Character of kernels in the progeny of crosses involving a duplication of the short arm of chromosome 9 derived from plant 1276-1.

CROSS	*C* NON-VARIEGATED KERNELS	*c* KERNELS	*C-c* VARIEGATED KERNELS
Duplication chromosome 9 carrying *C* / Normal chromosome 9 carrying *c* ♀ × *c*♂	192	242	16
Reciprocal	179	641	49
Duplication chromosome 9 carrying *C* / Normal chromosome 9 carrying *c* selfed	131	90	16

From the numerous examples so far presented in this paper, there can be little doubt that variegation in the endosperm tissues is correlated with the presence of a chromosome with a broken end. Furthermore, if the constitution of chromosome 9 in a plant is known, it is possible to predict whether or not variegated kernels will appear in the progeny. All predictions have been completely verified.

IV. THE CORRELATION OF VARIEGATION AND BROKEN CHROMOSOMES IN THE PROGENY OF A NATURALLY ARISING DUPLICATION IN CHROMOSOME 9

Before the investigations described in section III of this paper had been undertaken, studies were underway on a chromosome 9 possessing a duplication of the short arm. Cultures containing this chromosome gave relatively high percentages of variegated kernels in their progeny. The origin of this duplication was unknown. It was discovered in a genetic strain of maize belonging to DR. L. J. STADLER. The author is indebted to DR. STADLER for use of this duplication in the study now reported. The consti-

tution of the duplicated chromosome 9 is essentially similar to the duplicated chromosome 9 illustrated in type III of figure 9. The duplication included practically all of the short arm of chromosome 9. No knob was present. This duplicated chromosome carried the genes *I* and *Wx* in the two homologous segments. *I*, an inhibitor of aleurone color development, is placed at the same locus as the gene *C* (HUTCHISON 1922). The color pattern of variegation induced by broken chromosomes derived from this duplicated chromosome is the reverse of that described in section III, for loss of the *I* gene from cells of the aleurone allows color (*i*) to appear in these cells.

Since the origin of the broken chromosome at meiosis in plants heterozygous for this duplication is exactly the same as that described in the previous section, the evidence obtained from this duplicated chromosome will be but briefly reviewed.

TABLE 8

$$\frac{I\ Wx \text{ duplication chromosome } 9}{i\ wx \text{ normal chromosome } 9} \times i\ wx \text{ normal chromosome } 9$$

CROSS	NON-VARIEGATED KERNELS				VARIEGATED KERNELS	
	I Wx	*I wx*	*i Wx*	*i wx*	*I-i Wx-wx*	*I-i wx*
♀ Parent heterozygous	438	0	23	445	17	0
♂ Parent heterozygous	62	0	29	765	27	3

In plants heterozygous for the duplicated chromosome 9,—that is, possessing one duplicated chromosome 9 and a normal chromosome 9,—bridge configurations are seen in both the first and second meiotic mitoses. Of the 193 anaphase I cells recorded, 26 or 13.4 percent showed a bridge configuration. Among 74 dyads in anaphase II, 10 or 13.5 percent showed a bridge configuration in one of the cells of the dyad.

In these plants, the duplication chromosome 9 carried *I* and *Wx* in each of the duplicated segments. No knobs were present in the duplication chromosome 9. The normal homologue carried *i* and *wx*. Its short arm terminated in a large knob. The chromosome 9 constitution of these plants was similar to that shown in figure 11. Following crossing over as diagrammed in figure 11, the order of the genes between the two centromeres of the dicentric chromatid could be *Wx I i wx*, *Wx I I wx*, or *Wx I I Wx*. Following breakage of the dicentric chromosome at a meiotic anaphase, broken chromosomes with various genic compositions should arise. Those possessing *I Wx* or *I wx*, either singly or in duplication, should produce variegation for color if delivered to an endosperm following the cross of this plant to one possessing two normal chromosomes 9 carrying *i wx*.

The results of such a cross are given in table 8. In the first line of the table, the female was the heterozygous parent. In the second line, the male was the heterozygous parent. The low frequency of the *I Wx* non-variegated class in this latter cross is due to the low transmission through the pollen of the chromosome carrying the duplication. Six plants derived from the *I Wx non-variegated* kernels in this latter cross were examined cytologically. Five plants showed the presence of the duplication chromosome 9; one plant possessed two normal chromosomes 9, the short arms of each terminating in a large knob. Since the normal chromosome 9 in all the plant of this study possessed a large terminal knob, the chromosome with *I Wx* in this latter plant obviously arose through a crossover between the normal chromosome 9 and the proximal segment of the duplication chromosome 9. The chromosome 9 constitutions were determined in 26 plants derived from the *I Wx non-variegated* class of the first line of table 8. All 26 possessed the duplicated chromosome 9 and a normal chromosome 9 terminating in a large knob.

The chromosome 9 constitution has been determined for 28 plants derived from the variegated kernels of table 8. A broken chromosome 9 was present in each plant. For illustrative purposes, the type of recovered

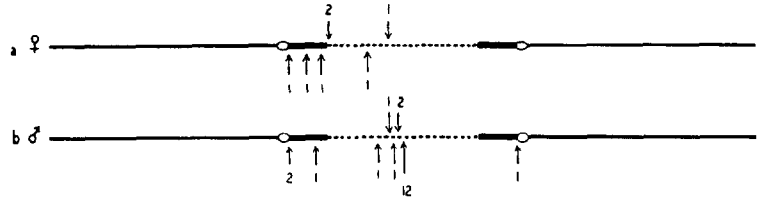

FIGURE 14.—Types of recovered broken chromosomes in plants arising from variegated kernels. The parental chromosome constitution of the heterozygous parent was exactly as diagrammed in figure 11. The dicentric chromatid produced following crossing over is shown in a and b. The constitution of the recovered broken chromosome is that to the right of the arrow in each case. The numbers above or below the arrows indicate the number of plants with this particular broken chromosome. In a, the female parent contributed the broken chromosome. In b, the male parent contributed the broken chromosome.

broken chromosome may be referred to the original dicentric chromatid from which it arose (fig. 14). The broken chromosome in seven of these plants was introduced by the female parent (variegated kernels in line 1 table 8). The broken chromosomes in the remaining 21 plants were introduced by the male parent (variegated kernels in line 2, table 8). Since the proportion of types of recovered broken chromosomes are not comparable in the two crosses, the types received from the female parent are shown in a (fig. 14), those received from the male parent in b (fig. 14). The composition of the broken chromosome is that to the right of the arrow in each case. The numbers above or below each arrow indicate the number of plants

with this particular broken chromosome. Totalling both crosses, 14 of the plants possessed a duplicated segment. These varied in length from a single chromomere in one plant to the full short arm in three plants. There was only one plant with a deficiency (b, fig. 14), but this deficiency included all of the short arm. The remaining 13 plants had a complete chromosome 9 with neither a duplication nor a deficiency. Twelve of these were introduced by the male parent. In these plants, the broken chromosome 9 could readily be distinguished from the normal chromosome 9, since the former possessed no knob whereas the short arm of the latter terminated in a large knob.

The duplications derived from the rearranged chromosome 9, described in f of section III, could be explained only as the result of successive fusions and breaks, as shown in figure 9. However, it could not be determined

TABLE 9

A. $\frac{\text{Normal chromosome 9 carrying } I}{\text{Normal chromosome 9 carrying } i} \times$ normal chromosome 9 carrying i

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	3,074	3,153	1
♂ parent heterozygous	2,213	2,129	1

B. $\frac{\text{Broken chromosome carrying } I\text{, no duplication or deficiency}}{\text{Normal chromosome 9 carrying } i} \times$ normal chromosome 9 carrying i

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	1,467	1,419	1
♂ parent heterozygous	3,008	3,011	1

C. $\frac{\text{New duplication chromosomes 9 carrying } I}{\text{Normal chromosome 9 carrying } i} \times$ normal chromosome 9 carrying i

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	1,822	2,107	49
♂ parent heterozygous	22	164	4

in some cases and was not determined in others whether the duplications illustrated in figure 14 arose directly from the dicentric chromatid or following subsequent fusions and breaks. Distinctive morphological markers such as knobs and pycnotic extensions, illustrated in figure 9, could not be present in these duplications. The presence of small extra segments inserted between the two main duplicated segments could be identified only with considerable difficulty and was not attempted. However, successive

fusions and breaks must have preceded the formation of the deficient chromosome in b of figure 14 (for discussion, see section III d).

At this point, attention should be called to the contrast in the types of broken chromosomes delivered by the sperm and the egg. Most of those delivered by the female gamete have duplications. The majority of those coming from the male parent are without duplication or possess only a very small duplication. A discussion of the possible significance of this will be postponed until more cases have been considered.

Healing of the broken end in the embryo should result in stability of the broken chromosome in successive generations. The broken chromosomes with no duplication or deficiency should behave as a normal chromosome 9. On the other hand, the broken chromosomes with newly derived duplications should give rise to variegated kernels following crossovers similar to those diagrammed in figure 11. The kernel characters obtained from crossing these various plants by those containing normal chromosomes 9 with *i* in each homologue are given in table 9. A, table 9, represents a control cross the heterozygous parent possessing two normal, unbroken chromosomes 9. In B, table 9, the *I* carrying chromosome possessed a broken end, but no duplication or deficiency was present. In C, table 9, the chromosomes carrying *I* possessed the newly derived duplications of figure 14. The results are as expected and require no further explanation.

V. THE CORRELATION OF VARIEGATION AND BROKEN CHROMOSOMES IN THE PROGENY OF A SHORT DUPLICATION DERIVED FROM A LONG DUPLICATION

All the newly derived duplications illustrated in figure 14 gave rise to variegated kernels in their progeny. Plants arising from these variegated kernels should have a newly broken chromosome. This has proved to be true. However, extensive tests were conducted with only one of these duplications, the short duplication composed of a fifth of the short arm (third arrow from left, b, fig. 14). The plant possessing this short duplication carried *I* in the duplicated chromosome and *i* in the normal homologue. When pollen carrying a normal chromosome 9 with *i* was placed upon silks of this plant, there resulted 149 *I* non-variegated kernels, 188 *i* kernels, and two *I-i* variegated kernels. When pollen of this plant was placed on the silks of a normal plant carrying *i*, there resulted seven *I* non-variegated kernels, 92 *i* kernels, and three *I-i* variegated kernels. Twenty-one plants derived from the *I non-variegated* kernels in the former cross were examined for the constitution of the chromosome 9 delivered by the female parent. The short duplication was present in 20 of these plants. In one plant, two normal chromosomes 9, each terminating in a large knob, were present—obviously the result of a crossover which inserted *I* into the normal chromosome 9 of

the female parent. Plants derived from the seven *I non-variegated* kernels in the latter cross were likewise examined. Five of these had received the short duplication chromosome 9, and two had received a normal chromosome 9—the consequence of a crossover in the parent plant.

The formation of dicentric chromatids at meiosis has been assumed to be the means of producing the broken chromosomes which give rise to variegation. The method of origin of the dicentric chromatids is the same as that diagrammed in figure 11. To verify this, meiotic anaphases were examined in plants heterozygous for the short duplication. Among 231 anaphase I configurations recorded, 20 or 8.6 percent showed a bridge configuration. At anaphase II, 150 dyads were recorded. Eleven or 7.3 percent showed a bridge configuration in one of the cells of the dyad. Plants derived from the *I Wx* non-variegated kernels in the crosses given above which were heterozygous for the duplication (carrying *I* in the duplicated chromosome and *i* in the normal chromosome) were crossed with plants possessing two normal chromosomes 9 each carrying *i*. The results are shown in table 10. These crosses produced a total of 151 variegated kernels.

TABLE 10

Types of kernels resulting from the cross.

$$\frac{\text{Short duplication chromosome 9 carrying } I}{\text{Normal chromosome 9 carrying } i} \times \text{normal chromosome 9 carrying } i$$

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	6,684	6,774	106
♂ parent heterozygous	698	3,081	45

In contrast, the three examined F_1 plants with two normal chromosomes 9, which were derived from the *I Wx* non-variegated kernels, produce no variegated kernels following similar crosses.

A cytological determination was made of the chromosome 9 constitution of 15 plants derived from the variegated kernels of table 10 to verify the supposition of the presence in these plants of a newly derived broken chromosome. This proved to be true. The constitutions of the newly derived broken chromosomes are illustrated in figure 15. The method of illustration is the same as that used in figure 14. The derived broken chromosome is referred to the dicentric chromatid from which it arose. The broken chromosomes have the constitutions to the right of the arrows. Below each arrow is given the number of plants having this particular composition of its broken chromosome; in a, the female parent contributed the broken chromosome; in b, the male parent contributed the broken chromosome. Five

of the 15 plants had a newly derived broken chromosome with no duplication or deficiency. Nine plants possessed newly derived duplications of various lengths, and one plant possessed a broken chromosome deficient for a terminal segment which included approximately one-fourth of the short arm.

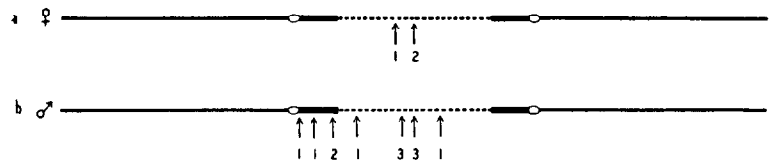

FIGURE 15.—The description for this figure is similar to that given with figure 14 except that the duplication in the heterozygous parent was short, composed of a terminal segment only one-fifth the length of the short arm. Nevertheless, following crossing over, the dicentric chromatid has the same constitution as that in c of figure 11 and in a and b of figure 14.

As in previous cases, these newly broken chromosomes were tested for their stability in the succeeding generation. The results are given in table 11. They are as expected. The five plants whose broken chromosome had neither a duplication nor a deficiency produced no variegated kernels (A, table 11). In contrast, tests of seven of the newly derived duplications (B, table 11) gave a total of 20 variegated kernels. Each ear possessed one or more variegated kernels. There can be little doubt that the plants arising from these variegated kernels would again show newly broken chromosomes. In view of the extensive analysis of the chromosome composition of plants derived from variegated kernels in the progenies of (1) the rearranged chromoxome 9 (section III, d), (2) the duplication chromosomes 9 derived from the rearranged chromosome 9 (section III, f), (3) the original long duplication which arose in a genetic culture of maize (section IV), and

TABLE 11

Tests of the stability of broken chromosomes derived from the chromosome 9 with the short duplication.

A. $\frac{\text{Broken chromosome 9 carrying } I \text{ (no duplication or deficiency)}}{\text{Normal chromosome 9 carrying } i} \times \text{normal chromosome 9 carrying } i$

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	662	661	0
♂ parent heterozygous	1,524	1,501	0

B. $\frac{\text{Newly derived duplication chromosomes 9 carrying } I}{\text{Normal chromosome 9 carrying } i}$ ♀ × normal chromosome 9 carrying *i*♂

NON-VARIEGATED KERNELS		VARIEGATED KERNELS
I	*i*	*I-i*
956	1,119	20

and (4) the short duplication derived from this latter duplication (section V), it was not considered necessary to make these tests. The evidence is conclusive from all of these cases that variegation in the endosperm tissues is related to the presence of a chromosome with a broken end, the original break having occurred in the previous meiotic divisions. The type of variegation which appeared in these kernels supports the assumption of the breakage-fusion-bridge cycle as the causal agent for this variegation, that is, the deletions of genes from some nuclei and their duplication in other nuclei.

VI. DISCUSSION

(a) *The positions of the breaks in anaphase bridge configurations in successive nuclear divisions*

In the preceding sections of this paper the determination of the chromosome constitution of 186 plants, derived from variegated kernels, has been given. In 180 of these plants, a chromosome with a broken end was found. In each case, the chromosome with the broken end carried the genes associated with the variegation, these genes being located in the arm of the chromosome which possessed the broken end. Of the six exceptional cases, three resulted from hetero-fertilization; consequently, the chromosome constitution of the plant tissues could not be related to that in the endosperm tissues. The remaining three exceptional cases showed an altered chromosome, but the relation of the alteration to the variegation appearing in the endosperm could be determined in only one case (see section III d). There can be little doubt that the variegation in the endosperm tissues, described in this paper, is related to the presence in this tissue of a newly derived broken chromosome. It is necessary that the broken end be newly derived for a broken end heals when introduced into a zygote and is no longer capable of producing variegation either in the resulting sporophytic tissues or in the gametophytic and endosperm tissues of succeeding generations. This has been clearly established.

In all cases mentioned in this paper, the broken chromosome had its origin in a meiotic mitosis. The variegation was confined to the endosperm tissues in the generation immediately following. It made no difference whether the broken chromosome was introduced through the pollen grain or through the embryo sac. In either case variegation resulted in the endosperm tissues. The character of this variegation was clearly of the type which should arise following the breakage-fusion-bridge cycle where successive breaks do not always occur at positions of previous fusions (see fig. 1). Evidence for the initiation of this cycle has been given in a previous publication (McClintock 1938b). The observations were confined to the first division of the microspore or male gametophyte. This is the first divi-

sion following the production of the broken end. The evidence for continuation of this cycle in the following gametophyte divisions is obtained inferentially in the study reported here. In several cases the broken chromosome in the endosperm and in the embryo were not alike in chromosome constitution. The difference could readily be accounted for if fusion had occurred between the broken ends of sister chromatids in the generative nucleus followed by an anaphase bridge configuration during the division of this nucleus. If the break in this bridge configuration occurred closer to one centromere than to the other, each sperm would receive a broken chromosome, but the constitution of the broken chromosome in each sperm would differ.

Evidence for the breakage-fusion-bridge cycle in the female gametophyte is likewise inferential. The duplications 1276-1 and 1503-2 (type I, fig. 9) and duplication 1269 (type V, fig. 9) were delivered to the zygote through the female gametophyte. These duplication chromosomes have obviously been derived from a dicentric chromatid in the preceding meiotic mitosis. At least one fusion and break following the original break must have occurred to give rise to duplications 1276-1 and 1503-2. At least two such fusions and breaks must have occurred to have produced the duplication of plant 1269.

On the assumption of the continuation of the breakage-fusion-bridge cycle in the gametophytic tissues, the agreement in so many cases between the genic constitution of the broken chromosome delivered to the endosperm and that delivered to the zygote might appear surprising. Two explanations are possible. First, the break in an anaphase bridge configuration may tend to occur at a median position between the two centromeres. Secondly, following an original meiotic break, the break in the successive anaphase figures may tend to occur at positions of previous fusions. Either or both possibilities must be considered only as trends; otherwise, no duplicated chromosomes would have been produced following breakage of the dicentric chromatid of figure 8 nor would extensive variegation be produced in kernels receiving broken chromosomes.

Evidence obtained from both meiotic anaphase configurations and from examination of broken chromosomes in the prophase of the first microspore division has indicated that the original break in a bridge configuration at a meiotic anaphase may occur at any position between the two centromeres. However, no data have been obtained which could be treated statistically. It is certain, though, that breaks need not occur at median positions. This is confirmed by the number of cases where the recovered broken chromosomes possessed long duplications or deficiencies.The evidence does suggest, however, that following an original break, the breaks in the succeeding anaphases may tend to occur at positions of previous fusions. This is

based on three sets of evidence. The broken chromosomes arising from the dicentric chromatid of figure 8 (see table 4) may be considered first. A functional pollen grain could arise only when the original meiotic break occurred either immediately to the right of the inner small knob or at a position between this point and the left centromere. Thus, a broken chromosome can be transmitted through the pollen only after a decidedly nonmedian break in the original dicentric chromatid. All other breaks would result in broken chromatids with deficiencies. A pollen grain whose tube nucleus does not have a complete set of genes of chromosome 9 is not functional. In the summary table 5 the percentage is given of the various types of broken chromosomes received by the embryos. Eleven and one-tenth percent possessed a deficient chromosome 9, and 11.1 percent possessed a duplication of chromosome 9. Each of these types of chromosomes could be contributed by the pollen only following a secondary break which did not occur at the position of previous fusion. Thus, it is established that in approximately one-fourth of these cases, a secondary break did not occur at the position of previous fusion. The broken chromosomes transmitted by the female gamete cannot be so analyzed, since deficiencies of approximately one-third of the distal part of the short arm can produce functional embryo sacs (McCLINTOCK unpublished). The chromosomes with duplications are the only ones which must have originated following a successive break which was not at the position of previous fusion. The high percentage of cases where the broken chromosome ended either in the knob or at positions immediately adjacent to it strongly suggests that at anaphase this substance is more readily broken than are other parts of the chromosome. This introduced an obstacle in the analysis of the randomness of the breaks. However, most of the recorded broken chromosomes gave no evidence of having undergone the breakage-fusion-bridge cycle, which suggests that breakage in successive anaphases may tend to occur at positions of previous fusions.

The evidence is likewise suggestive when the recovered broken chromosomes illustrated in figures 14 and 15 are analyzed. A surprisingly high percentage (47 percent) of the broken chromosomes delivered to the zygote by the pollen parent had neither a duplication nor a deficiency. Although the number of chromosomes examined is small, the figure for this one class is very high. The relative percentages of types of recovered broken chromosomes delivered by the male and female gametes, respectively, are as follows:

	Male gametes	Female gametes
No duplication or deficiency	47 percent	20 percent
Deficiency	6.2 percent	0.0 percent
Duplication	47 percent	80 percent

Pollen grains whose tube nucleus possesses a deficiency do not function. Therefore, chromosomes with deficiencies introduced by the male parent must have resulted from a break which was not at the position of previous fusion. Again, pollen grains whose tube nucleus contains a duplication do not function as successfully as those containing a normal genic complement. Since some of these grains are known to function, it cannot be determined whether the duplications delivered through the pollen arose at the time of meiosis or at a later stage following a break which did not occur at a position of previous fusion. More probably, both factors were involved to give the 47 percent of such cases. When the tube nucleus contains neither a duplication nor a deficiency, the pollen grain functions normally. Pollen grains whose tube nucleus contains a broken chromosome with this constitution would not meet competition. If breaks tend to occur at positions of previous fusions, the constitution of the sperm nuclei and that of the tube nucleus would tend to be similar in many grains carrying broken chromosomes. Among all the pollen grains containing broken chromosomes produced by these plants, those whose tube nucleus contains neither a duplication nor a deficiency would be selectively favored in effecting fertilization. If there were a tendency for breaks to occur at positions of previous fusions, a high percentage of the plants receiving broken chromosomes through the pollen would have neither a duplication nor a deficiency in the broken chromosome. Although the numbers are small, the results so far obtained are very suggestive of this interpretation. Since selection against megaspores and embryo sacs with duplications and in some cases small deficiencies does not occur, no such proportionality of recovered broken chromosomes should appear. The numbers of examined cases are too few to be decisive, but the percentages of types of broken chromosomes recovered from the female gametophyte does not appear to be the same as the percentages recovered from the male gametophyte.

The third line of evidence relates to the simplicity of the recovered broken chromosomes with duplications. Two divisions in the male gametophyte and possibly the first division in the zygote should follow the breakage-fusion-bridge cycle. If the microspore nucleus possessed a broken chromosome with at least a full set of genes, a decidedly non-median break in the bridge configuration at the first microspore division in most cases would result in a tube nucleus with a deficiency or a duplication. Selection against the functioning of such grains could result. However, no such effect should follow the breaks in the next divisions in the male gametophyte (or in the first division of the zygote if a bridge configuration occurs here). Since some functional pollen grains should have had duplications in their generative nuclei, repeat duplications could be produced following a non-median break in the anaphase of the division of the generative nucleus.

However, such repeat duplications have not been observed. In the female gametophyte, three divisions separate the megaspore from the egg nucleus. Since there is no selection against megaspores or embryo sacs carrying duplicated segments in chromosome 9, repeat duplications could be present in the egg nucleus following non-median breaks in the bridge configurations of the division leading to the formation of the egg nucleus. Nevertheless, repeat duplications are rare; only one such case was recognized (type V, fig. 9). Considering the facts given above, it seems reasonable to conclude that there is a tendency for the breaks to occur in the anaphase bridges at the positions of previous fusions, although it has been definitely proven that this does not occur in all such bridge configurations. Since the mechanism which results in fusions of broken ends of sister chromatids is not known, it is not possible to ascribe this breakage at the position of previous fusion to a weak or imperfect union caused by a partial healing of the broken end. The broken end must remain capable of fusion; otherwise variegation would not be produced in the endosperm tissues. This variegation can result only when the breaks do not occur at positions of previous fusions. Furthermore, once a complete fusion has occurred, it must be as strong as that between any other regions in the chromosome; otherwise the broken chromosomes derived from the dicentric chromatid of figures 12 and 13 would tend to be alike. They are not alike, however.

(b) *The type of variegation in endosperms receiving one broken chromosome contrasted with those receiving two broken chromosomes*

The presence of a single broken chromosome in the endosperm tissues was considered in the interpretation of the cause of variegation, as illustrated in figure 1. This chromosome carried the dominant genes. The endosperm tissues are 3n; two sets of chromosomes are contributed by the female gametophyte, and one set is contributed by the male gametophyte. Variegation in the endosperm results when the broken chromosomes are contributed by either the female or the male gametophytes. In the latter case, only one broken chromosome is present. In the former case, two broken chromosomes are present. The interpretation of variegation on the basis of fusion of broken ends of sister chromatids following breakage of an anaphase bridge configuration in the previous division is the only logical assumption when a single broken chromosome is present (contributed by the male parent). However, when two broken chromosomes are present (contributed by the female parent), two possibilities may occur. Either fusion of broken ends could occur between the sister halves of each broken chromosome, following breakage of the anaphase bridge configurations, or the two broken ends of each chromosome could fuse with one another. In the first assumption, each broken chromosome would produce a bridge

configuration in successive mitoses. In the latter assumption, bridge configurations would result when the two centromeres of the dicentric chromatids passed to opposite poles. Interlocking of chromatids resulting in breakage could likewise follow the passage to the same pole of the two centromeres of a chromatid. However, either of the above interpretations would lead to variegation in the endosperm tissues.

If fusions of sister chromatids occurred, the amount of recessive tissue appearing in the variegated kernels should be considerably less when two broken chromosomes are present than when one broken chromosome is present. The dominant genes from two chromosomes would have to be lost to a nucleus before the recessive character would show in the former case, whereas loss of the dominant gene from only one chromosome is necessary in the latter case. Comparative examinations of variegated kernels have not shown this difference. Although the amount of recessive tissue exhibited by individual kernels on the same ear is variable in either case, taken as a whole, the endosperms which receive two broken chromosomes do not show considerably less recessive tissue than those receiving but one broken chromosome. There is a decided difference, however, in the range of color of the various *C* spots in the two cases. The endosperms which receive two broken chromosomes carrying *C* have many more spots of extreme deep color than those which receive but one broken chromosome with *C*. This would occur if many *C* genes were included in some of the nuclei following breakage and fusion of two broken chromosomes. The more *C* genes present, the deeper the color produced. The type of variegation and the more extreme range in color of the *C* spots may be explained as the consequence of fusions between the broken ends of the two chromosomes followed by breaks in each of the dicentric chromatids at similar positions in any one anaphase spindle. That fusions may occur in sporophytic tissues between two broken ends of chromosomes rather than between the two sister halves at the position of previous breakage has been demonstrated in maize where ring-shaped chromosomes were present (McCLINTOCK 1938a). It may likewise occur in the endosperm tissue. Thus, the fusion of broken ends of sister chromatids occur in successive divisions when a single broken chromosome has been introduced in the endosperm, whereas the fusion of broken ends of two chromosomes may occur when two such broken chromosomes are present in the nuclei of the endosperm cells.

(c) *The healing of broken ends of chromosomes*

It has been repeatedly emphasized that the broken end of a chromosome becomes healed in the sporophytic tissues. The breakage-fusion-bridge cycle, which characterizes its behavior in the gametophytic and endosperm

tissues, ceases. The healing is permanent. When this chromosome is reintroduced into endosperm tissues in the following generations, no fusions occur at the broken end between the two sister halves. Furthermore, when two such broken chromosomes are brought together after each has passed through a sporophytic generation, no fusions occur between the two broken ends. No obvious explanation is available for the healing of the broken ends in the sporophytic tissues as contrasted with the lack of healing in the gametophytic and endosperm tissues. The question might arise as to whether the broken end ever heals in the gametophyte or endosperm tissues. Although this may occur in some cases, the evidence suggests that it must be rare. It will be recalled that the majority of variegated kernels arising from the rearranged chromosome 9 (see table 1) had the constitution *C Sh wx*. There were some non-variegated kernels with this constitution. It was assumed that the variegated kernels had broken chromosomes and the non-variegated kernels, non-broken chromosomes. If healing of broken ends occurs, the plants arising from some of the non-variegated *C Sh wx* kernels should have shown a broken chromosome. However, as table 6 shows, none of the plants arising from the *C Sh wx*, non-variegated kernels possessed a broken chromosome. Under certain physiological conditions it is possible that the broken end might heal in the endosperm tissues, but at present these conditions are not known.

Realization of the healing of the broken end in the sporophytic tissues came as a surprise. Evidence from the behavior of ring-shaped chromosomes had indicated that fusions of broken ends of *chromosomes* occurred in the sporophytic tissues when the break originated in this tissue. Although extensively looked for, no evidence for healing of these broken ends was obtained in the ring-chromosome material. Since it has been proven that broken ends can heal, it could be expected that healing of the broken ends arising from the breakage of a ring chromosome might occur under certain physiological conditions.

The broken chromosomes described in this paper and the breaks in the ring-shaped chromosomes all originated as the consequence of mechanical rupture in an anaphase or telophase spindle figure. Breakage induced by other means may not lead to similar consequences—that is, the fusions of broken ends. MULLER (1940) recently reviewed the evidence of the effect of X-rays on the production and behavior of broken ends of chromosomes and concluded that X-ray-induced breakage is followed by 2-by-2 fusions of broken ends, either between parts of the same chromosome, between two chromosomes, or between sister halves of a single chromosome at the position of previous breakage. However, SAX (1940)

and SWANSON (1940) in recent studies on the types of chromosomal abnormalities induced by X-radiation have reported observations which suggest that not all the induced breaks are followed by fusions of broken ends. Their observations were of the division immediately following the treatment of the microspore nucleus and of the generative nucleus of the pollen grain in Tradescantia. It was not shown whether or not these unfused broken ends healed permanently, since the behavior in successive divisions was not followed. With regard to fusions of broken ends, the breaks induced by X-rays and by mechanical rupture apparently are similar. However, the breaks induced in chromosomes by ultraviolet irradiation do not appear to be similar to those produced by X-rays or mechanical rupture. SWANSON (1940) has observed the direct effect of ultraviolet radiation on the breakage of chromosomes. His observations suggest that the breaks induced by the radiation are not followed by fusions of broken ends of chromosomes. Previous to this work, the genetic and cytological evidence of the effect of ultraviolet radiation of maize pollen (SINGLETON 1939, SINGLETON and CLARK 1940, STADLER and SPRAGUE 1936, STADLER 1939) had indicated that the breakage induced by ultraviolet radiation differed from that induced by X-radiation. Most of the recovered chromosomal abnormalities appeared to be terminal deficiencies. The broken end was completely healed. When two such broken chromosomes were present in a single nucleus, no fusion had occurred between them.

Further evidence for the non-fusibility of broken ends produced as the consequence of ultraviolet radiation is suggested by the character of the endosperm tissues which receive a nucleus derived from irradiated pollen. Studies of the sporophytic tissues mentioned above would suggest that many of the endosperm nuclei were receiving chromosomes with broken ends following ultraviolet treatment of pollen. If fusions occur between sister halves of this chromosome at the position of breakage, variegation kernels of the type described in this paper would be expected. These were not present in numbers greater than might be expected from normal, untreated material. If the endosperm possesses a broken chromosome, fusions do not occur at the position of breakage. It becomes apparent that the capacity of a broken end to fuse depends upon the method by which the chromosome becomes broken and the conditions of the cell following the breakage.

Since it has been demonstrated that broken ends of chromosomes under certain conditions retain their capacity to fuse with one another but lose this capacity permanently under other conditions, it will be necessary in the future to determine the nature of these conditions by experimental methods before an understanding can be attained of the factors responsible for fusions or for healing of broken ends.

(d) *The behavior of chromosomes initially broken in the endosperm and in the sporophytic tissues*

In this paper, the variegation in the endosperm tissues has been brought about by the introduction into this tissue of a chromosome which was initially broken in the previous meiotic divisions. The recent evidence reported by CLARK and COPELAND (1940) suggests that the breakage-fusion-bridge cycle will follow if a chromosome receives its initial break after its introduction into the endosperm. The authors examined cytologically the mitotic divisions in the endosperm of strains of maize which had consistently given high percentages of kernels with variegated sectors in the endosperm tissues. The nature of the variegation has been intensively studied by JONES (1937), who came to the conclusion that spontaneous translocations were occurring at a relatively high rate in this material. Such translocations should produce dicentric chromosomes in some nuclei. Breakage of a dicentric chromosome in a succeeding mitotic anaphase would introduce a chromosome with a broken end into the telophase nucleus. If fusions of sister chromatids at the positions of breakage resulted, the breakage-fusion-bridge cycle would be initiated. Extensive cytological examination of mitotic anaphases in this material should reveal bridge configurations many of which should not show an accompanying fragment. The authors have found this to be true and have concluded that the breakage-fusion-bridge cycle will follow a break initiated in the endosperm tissue itself. In view of the healing which results when a broken chromosome is introduced by a gamete into sporophytic tissue, a similar type of investigation needs to be conducted with sporophytic tissues to establish whether or not such healing of a single broken end will occur in this tissue if the break in the chromosome originates in the sporophytic tissue itself. SAX (1940) states that he has obtained evidence for the breakage-fusion-bridge cycle following a breakage of a chromosome in the sporophytic tissues, but a detailed description of the evidence has not been reported as yet.

(e) *Selective orientation of broken chromatids in the second meiotic mitosis*

The high percentage of bridge configurations (32 percent) observed at the first meiotic anaphase in microsporocytes of plants heterozygous for the rearranged chromosome would lead one to expect either a high percentage of variegated kernels on the ears of such plants in the crosses outlined or, if most of the broken chromosomes were highly deficient, a recognizable amount of ovule sterility due to lack of development of ovules whose functional megaspores carried such a deficient chromosome. Neither of these conditions was observed. The percentage of variegated kernels was

low, and there was no marked increase in the sterility of the ovules. Observations of normal maize plants has shown that it is the lower magaspore which develops into the embryo sac (WEATHERWAX 1919). Three explanations may be suggested for this lack of ovule sterility. First, there may be very much less crossing over in the megasporocytes. RHOADES (1940) has reported a difference in crossing over in mega- and microsporocytes in maize for genes of chromosome 5. Nevertheless, a decrease in crossing over would not fully account for the lack of ovule sterility. One could suggest as a second alternative a tendency for selection of megaspores carrying a normal chromosome 9. A third possibility seems more likely. As shown previously, most of the bridge configurations occur at anaphase I. The anaphase bridge delays the migration of the chromatids toward the two poles. Following breakage, each of the two broken chromatids may be held closer to the cell plate than the two normal chromatids. If this orientation is maintained through interkinesis, a broken chromatid would enter each of the two middle megaspores, and a normal chromatid would enter the two end megaspores. The innermost megaspore of the row of four, which normally develops the embryo sac, would then contain a normal chromosome 9. Evidence given in a previous publication (McCLINTOCK 1938b) would lead one to expect this orientation of broken chromosomes in some but not all of the second division spindle figures. This interpretation is similar to that presented by STURTEVANT and BEADLE (1936) as an explanation of low egg sterility in Drosophila in individuals heterozygous for an inversion in the X chromosome. This third alternative is favored as the probable explanation of lack of ovule sterility and is supported by similar evidence obtained from plants heterozygous for an inversion (McCLINTOCK unpublished).

SUMMARY

By use of (1) a rearranged chromosome 9, (2) a duplication arising from this rearrangement, (3) a deficiency derived from this rearrangement, (4) a duplication occurring in a genetic strain of maize, and (5) new duplications derived from this latter duplication it was possible to obtain functional gametes carrying a chromosome 9 whose short arm terminated in a broken end.

In all cases, the broken end arose following crossing over at meiosis which produced a dicentric chromatid. Rupture of the dicentric chromatid at a meiotic anaphase produced the broken end.

During the following gametophytic division, fusions occurred at the position of breakage between the two sister halves of the broken chromatid, resulting in an anaphase bridge configuration. Rupture of this bridge at late anaphase or early telophase again introduced a broken chromosome

into the sister telophase nuclei. This breakage-fusion-bridge cycle continued in the successive gametophytic divisions.

When such a chromosome, initially broken in the previous meiotic mitosis, is introduced into the endosperm tissues of the following generation through either the male or the female gametophyte, the evidence indicates that the breakage-fusion-bridge cycle continues in each successive division. When this chromosome carries dominant genes in the arm with the broken end and when the normal homologue carries the recessive alleles, variegation for these genes appears in the endosperm tissues. This is caused by non-median breaks in the bridge configurations in many anaphase figures which deletes dominant genes from one telophase nucleus and duplicates them in the sister telophase nucleus.

When such a broken chromosome is introduced into the zygote, the broken end heals, discontinuing the breakage-fusion-bridge cycle. This healing is permanent. The broken end behaves in every respect like a normal end. When a broken chromosome has passed through a sporophytic generation, it no longer is capable of producing variegation in the endosperm of the following generation. When two such chromosomes are brought together after each has passed through a sporophytic generation, no fusions occur between their broken ends.

Thus, the breakage-fusion-bridge cycle occurs only in the nuclear divisions of the gametophytic and endosperm tissues when the broken end is newly derived and has not passed through a sporophytic generation.

Evidence is presented which suggests that following an initial meiotic anaphase break, the breaks in the successive anaphase bridge configurations in the following gametophytic divisions tend to occur at the position of previous fusion, but many breaks occur at other positions.

Once a complete fusion has occurred at the position of breakage between the sister halves of a broken chromosome, the fusion results in a union which is as permanent and strong as that between other parts of the chromosome.

The factors responsible for fusions of broken ends or for the healing of a broken end are not understood but are probably related to the method by which the chromosome becomes broken and to the physiological conditions surrounding the broken end.

LITERATURE CITED

CLARK, F. J., and F. C. COPELAND, 1940 Chromosome aberrations in the endosperm of maize. Amer. J. Bot. **27**: 247–251.

CREIGHTON, H. B., 1934 Three cases of deficiency in chromosome 9 of *Zea mays*. Proc. Nat. Acad. Sci. **20**: 111–115.

EMERSON, R. A., A. C. FRASER, and G. W. BEADLE, 1935 A summary of linkage studies in maize. Cornell Agric. Exp. Sta. Memoir **180**: 1–83.

HUTCHISON, C. B., 1922 The linkage of certain aleurone and endosperm factors in maize, and their relation to other linkage groups. Cornell Agric. Exp. Sta. Memoir **60**: 1419–1473.

JONES, D. F., 1937 Somatic segregation and its relation to atypical growth. Genetics **22**: 484–522.

McCLINTOCK, B., 1938a The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315–376.

1938b The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. Missouri Agric. Exp. Sta. Res. Bull. **290**: 1–48.

1939 The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc. Nat. Acad. Sci. **25**: 405–416.

MULLER, H. J., 1940 An analysis of the process of structural changes in chromosomes of Drosophila. J. Genet. **40**: 1–66.

RHOADES, M. M., 1940 Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics **25**: 483–520.

SAX, K., 1940 An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics **25**: 41–68.

SINGLETON, W. R., 1939 Cytological observations on deficiencies produced by treating maize pollen with ultraviolent light. Genetics **24**: 109.

SINGLETON, W. R., and F. J. CLARK, 1940 Cytological effects of treating maize pollen with ultraviolet light. Genetics **25**: 136.

SPRAGUE, G. F., 1932 The nature and extent of hetero-fertilization in maize. Genetics **17**: 358–368.

STADLER, L. J., 1939 Genetic studies with ultra-violet radiation. Proc. Seventh Int. Congr. Genet. (in press).

STADLER, L. J., and G. F. SPRAGUE, 1936 Genetic effects of ultra-violet radiation in maize. III. Effects of nearly monochromatic λ 2537, and comparison of effects of X-ray and ultra-violet treatment. Proc. Nat. Acad. Sci. **22**: 584–591.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of Drosophila melanogaster to crossing over and disjunction. Genetics **21**: 554–604.

SWANSON, C. P., 1940 A comparison of chromosomal aberrations induced by X-rays and ultraviolet radiation. Proc. Nat. Acad. Sci. **26**: 366–373.

WEATHERWAX, P., 1919 Gametogenesis and fecundation in *Zea mays* as the basis of xenia and heredity in the endosperm. Bull. Torrey Bot. Club. **46**: 73–90.