

EVIDENCE FOR SISTER-STRAND CROSSING OVER IN MAIZE¹

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CROSSING OVER, the exchange of chromatin between homologous chromosomes, is of utmost importance in genetic studies. Although little is known about the mechanism which is responsible for this exchange, a number of facts on this subject have been fairly well established: (1) Crossing over is produced by breakage and reunion of broken ends (even on BELLING's hypothesis, breakage and reunion have to be postulated to explain 3-strand and 4-strand doubles). (2) The position of the exchange is exactly between homologous regions of the chromosomes. (3) Crossing over occurs at prophase of the first meiotic division. (4) Only 2 of the 4 chromatids are involved in any one crossover. (5) The occurrence of one crossover decreases the probability of another occurring in its vicinity—the phenomenon of interference. It has generally been assumed that there is little or no sister-strand crossing over. This

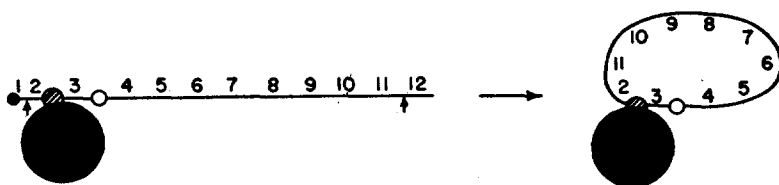


FIGURE 1.—Diagrammatic representation of the formation of the ring chromosome following breakage in the distal regions of chromosome 6. The nucleolus is represented by the large black circle and the centromere by the clear oval. The shaded area represents the nucleolar organizer. The arrows indicate the probable sites of X-ray-induced breakage. (Reprinted through courtesy of the American Naturalist.)

conclusion has been drawn from studies in *Drosophila* with attached-X chromosomes (BEADLE and EMERSON 1935) and with the Bar locus (STURTEVANT 1925, 1928; MULLER and WEINSTEIN 1933). However this is still an open question. The pioneering work of McCLINTOCK (1938, 1941b) on dicentric double-sized ring formation and the evidence to be presented in this paper suggest that crossing over does occur between sister chromatids. Such an event can be detected only with ring chromosomes with the possible exception of "unequal" crossing over in sister rod chromatids giving rise to duplicated segments.

In a previous paper (SCHWARTZ 1953) the behavior of a large ring in maize involving almost the whole of chromosome 6 was discussed. Gametophytes possessing nine chromosomes plus the ring are viable even though deficient for the terminal regions of chromosome 6 (fig. 1). Crossing over in the hetero-

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zygote between the ring and its homologous rod may result in bridges in both anaphase I and anaphase II. Only crossovers in the long arm of the chromosome are being considered since those in the short arm between the centromere and the nucleolar organizer are very infrequent (McCLINTOCK 1941b). A

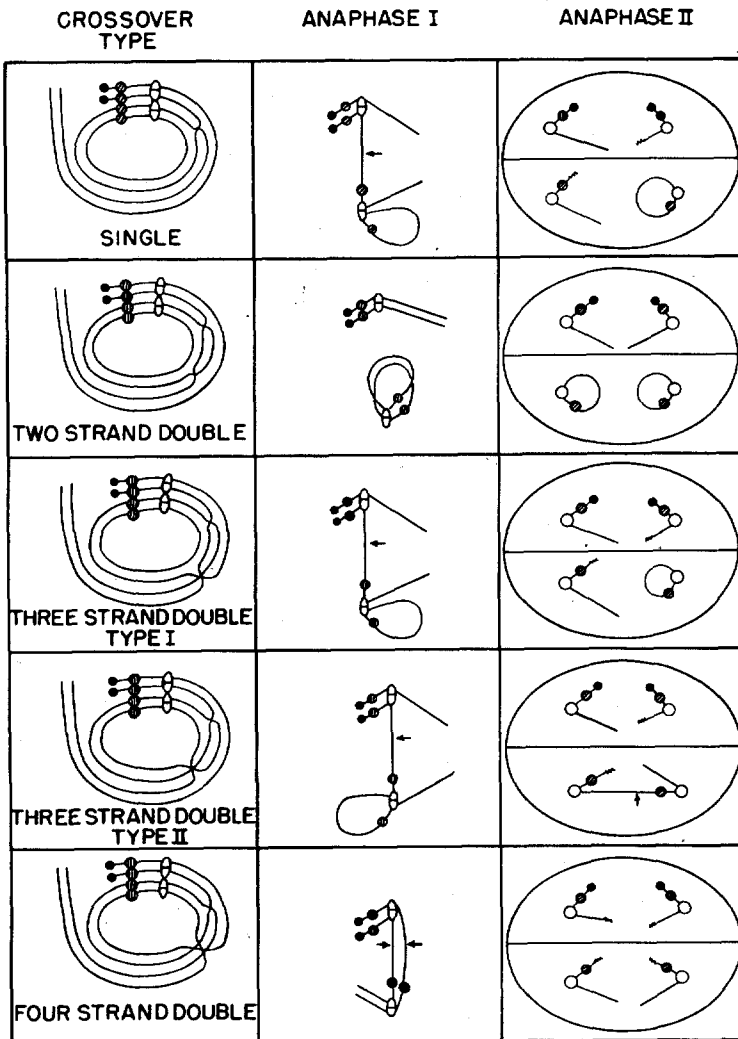


FIGURE 2.—Anaphase configurations resulting from crossing over between the ring and its homologous rod chromosome. (Reprinted through the courtesy of the American Naturalist.)

— = broken chromosome end —→ = position of breakage

single or a 3-strand double crossover (type I), in which the same ring chromatid is involved in both exchanges, gives a single bridge in AI only (fig. 2). Both 2-strand double crossovers and non-crossovers result in normal disjunction without bridge formation. A 3-strand double of the second type (II), in which the same rod chromatid is involved in both exchanges, gives a single

bridge in both AI and AII. A 4-strand double gives a double bridge in AI. These bridges are not associated with fragments.

Two of the double crossover classes can easily be distinguished—type II 3-strand doubles and the 4-strand doubles. Equal frequencies of double bridges in AI and single bridges in AII would therefore indicate the absence of chromatid interference.

RESULTS AND INTERPRETATION

Four plants heterozygous for the ring and a rod chromosome were used in this study. The frequencies of the various anaphase configurations observed are listed in table 1. The anaphase II data are given in terms of daughter cell pairs which show a single or double bridge in one of the two cells. This frequency was calculated in the following manner. All cells in AII were counted regardless of whether they were found singly or in pairs. Of these cells 166 showed single bridges, 47 double bridges (from dicentric rings), and 737 no bridges. Type II 3-strand doubles and sister-strand crossovers in the ring give rise to bridges in only one of the two daughter cells, the other being normal. There-

TABLE 1
Meiotic anaphase configurations observed in plants heterozygous for a ring and a rod.

	Anaphase I				Anaphase II (daughter cell pairs)			
	Single bridge	Double bridge	No bridge	Total	Single bridge	Double bridge	No bridge	Total
Number	368	81	171	620	166	47	262	475
Percent	59	13	28	100	35	10	55	100

fore, 213 cells (166 + 47) must be subtracted from the 737 and the remaining 524 divided by 2 (i.e., 262 as given in table 1) to determine the number of daughter cell pairs which lacked bridges.

There are two important facts which should be pointed out in the data. First, the frequent occurrence of double bridges (dicentric rings) in AII. These would not be expected from single and double crossovers and only from 1/16th of the triple crossovers. Second, the frequency of single AII bridges.

It is apparent from table 1 that single bridges in AII are much more frequent than double bridges in AI, approximately a threefold difference. Can this difference be interpreted as being due to negative chromatid interference; i.e., the participation of one strand in a crossover enhances the probability that it will be involved in the second? This might appear to be the case, since anaphase configurations resulting from 3-strand doubles where one of the strands is involved in both crossovers, are more frequent than those arising from 4-strand doubles where each strand is involved in only one crossover. However, on this basis the frequency of 2-strand doubles should be even greater than 3-strand doubles. As the data indicate, this is not true. Only 28 percent of the AI cells showed no bridges and some of these are presumably due to non-crossovers.

If the 35 percent single bridges in AII are due entirely to type II 3-strand double exchanges, an equal frequency of type I might be expected. Thus, 70 percent of the AI cells would show 3-strand double crossing over and if one then added the frequency of double bridges, no bridges, and single bridges due to single crossovers, the total would come to much more than 100 percent. It seems clear therefore that negative interference cannot explain the data and that the frequency of single bridges in AII is not a true measure of the frequency of type II 3-strand doubles. The frequent occurrence of triple crossovers between the ring and the rod is unlikely and it would not account for the ratio of observed bridge configurations.

The high frequency of single bridges in AII can be accounted for by sister-strand crossing over (fig. 3). The term "sister-strand crossing over" as used

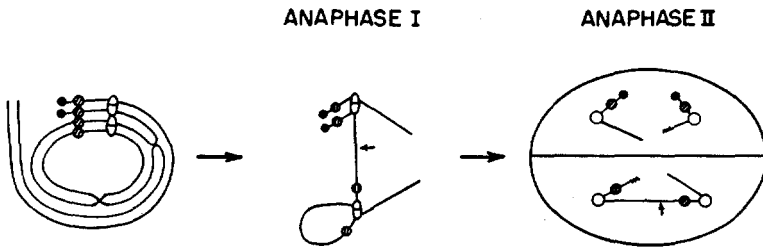


FIGURE 3.—Anaphase configurations resulting from a single non-sister crossover associated with a sister-strand crossover between the ring chromatids.

here refers simply to an exchange of chromatin between sister chromatids and does not imply any relationship to non-sister-strand crossing over in the time and manner of its occurrence, interference, etc.

There are four classes of anaphase configurations which result from double non-sister crossovers between the rod and ring (fig. 2). These are (1) single bridge in AI only, (2) single bridges in AI and AII, (3) double bridge in AI, and (4) no bridge in AI. Considering only non-sister crossing over, these classes would arise from 3-strand doubles of type I, 3-strand doubles of type

TABLE 2

*Theoretical ratio of anaphase configurations resulting from double non-sister-strand crossovers associated with sister-strand crossing over.**

No. sister-strand crossovers	Single bridge AI only	Single bridge AI and AII	Double bridge AI	No bridge AI		
				No bridge AII	Double ring AII	Total
0	1	1	1	1	0	1
1	1	1	1	0.5	0.5	1
2	1	1	1	0.5	0.5	1
3	1	1	1	0.5	0.5	1
Many	1	1	1	0.5	0.5	1

* This table is based on the assumption of no chromatid interference and was calculated by following the consequences of the four non-sister double crossover types in all possible combinations with varying numbers of sister-strand crossovers.

II, 4-strand doubles, and 2-strand doubles, respectively. The proportions of these four classes are not changed by the occurrence of sister-strand crossing over (table 2). Regardless of the frequency of the latter, whether it be one or 10 per bivalent, the proportions of these classes would remain the same as those resulting from double non-sister crossovers alone. If the number of sister-strand crossovers per bivalent is high, an odd number occurring in any one region will appear as a crossover and an even number as a non-crossover. Thus, the frequency of single bridges in AII resulting from double non-sister crossovers associated with sister-strand crossing over should still be equal to the frequency of AI double bridges, i.e., 13 percent.

Another way by which single bridges in AII can arise is by a single non-sister crossover associated with sister-strand crossing over. An odd number of sister crossovers will give rise to a single bridge in AI and AII. An even number or no sister crossovers will result in a single bridge in AI only. It is not possible to estimate from the data the number of sister-strand crossovers which occur per bivalent except that it must be one or more to account for the high frequency of single AII bridges. One sister crossover together with a single non-sister crossover will give 50 percent bridges in AI only and 50 percent bridges in AI and AII if there is an equal probability that it will occur in the ring or in the rod, since only sister crossovers in the ring result in AII bridges. Likewise, a large number of sister-strand crossovers per bivalent will give 50 percent of each type if there is equal probability of an odd or an even number occurring in the rod and ring.

Single bridges were observed in 59 percent of the anaphase I configurations. These result from the single and half of the double non-sister crossovers. If there is no chromatid interference, 26 percent of the anaphase I cells would possess single bridges due to 3-strand double crossovers, since the frequency of 4-strand doubles was 13 percent. As stated previously, the frequency of single bridges in AI only and the frequency of single bridges in AI and AII resulting from double non-sister crossovers should each be equal to the frequency of AI double bridges. This leaves 33 percent AI single bridges due to single non-sister crossovers. As a result of sister-strand crossing over, half of these or 16.5 percent will also form single bridges in AII. Thus we can calculate that 29.5 percent single AII bridges would be expected on the basis of one or many sister-strand crossovers per bivalent, i.e., 16.5 percent from the single non-sister crossovers and 13 percent from the double non-sister crossovers. The observed value was 35 percent. This difference is not statistically significant.

In somatic mitoses the frequency of sister-strand crossing over in a ring chromosome can be determined by measuring the frequency of double-sized rings. The probability of two such crossovers occurring per ring and thus effectively cancelling each other is slight in view of the low frequency of double rings. This calculation cannot be made for meiosis where the ring and rod synapse, since only a portion of the sister-strand crossovers give rise to double rings. Dicentric rings arise from sister-strand crossing over in bivalents

that had either no non-sister or double non-sister crossovers. The anaphase I cells showing no bridges, which result from double non-sister crossovers, should be equal in frequency to the cells showing AI double bridges—13 percent (table 2). Since the observed frequency of no bridge configurations in AI was 28 percent, this leaves 15 percent which did not have a non-sister crossover.

On the hypothesis of a large number of sister crossovers per bivalent, the frequency of double rings resulting from double non-sister crossovers would be one half the frequency of double bridges, or 6.5 percent (table 2). Also, half the bivalents not having a non-sister crossover, 7.5 percent, would give double rings. Thus, the expected frequency of double-sized rings would be 14 percent; the observed frequency was 10 percent.

The frequency of double rings in mitotic anaphases is quite low. McCLINTOCK (1938) counted only 8 percent double rings arising from a large ring which involved most of chromosome 2. The ring used in this study is somewhat smaller and gave 4.5 percent double rings, 57 out of 1275, as measured in meristematic root tissue. This striking difference between the meiotic and mitotic rates may offer a clue to the mechanism of sister-strand crossing over.

DISCUSSION

MATSUURA (1940, 1948) has presented evidence that in Trillium the two chromatids in each chromosome are coiled in a relational spiral system at early meiotic metaphase I and in a parallel spiral system at late metaphase I. He has shown that the chromosome can undergo this change in coiling only by breakage and subsequent reunion of the chromatids at each half coil. He has used this mechanism to explain legitimate crossing over by postulating that the paired chromatids of each chromosome are non-sisters in two-thirds of the cases. There are many objections to such a hypothesis. The frequency of crossing over would be much higher than normally found. Also, crossing over should be reduced in the distal ends of a chromosome since the twisted chromatids will be able to separate without breakage in that region, as has been pointed out by Matsuura. However, in maize one of the most striking cases of non-correlation between cytological and crossover distances occurs in the very distal end of chromosome 9 where *yg₂* and *dt*, which are cytologically very close, show 7 percent crossing over (RHOADES 1945).

Furthermore, this theory cannot explain the formation of anaphase bridges with fragments from heterozygous paracentric inversions. MATSUURA has recognized this difficulty and in a later paper (1950) made the unlikely suggestion that there is no correlation between the occurrence of paracentric inversion loops at pachytene and bridges with fragments at anaphase.

MATSUURA'S mechanism for the parallelization of the spiral system could, however, account for sister-strand crossing over if we assume that the paired chromatids in each bivalent are sister strands. The number of such crossovers per bivalent would be high, roughly equal to the number of half coils. As has been pointed out, this is consistent with the data. This hypothesis would also

explain the low mitotic rate of sister-strand crossing over since, in "somatic" mitosis, each of the two chromatids of a chromosome usually takes an independent spiral system from the beginning of its development (MATSUURA 1940).

Misdivision of the centromere is an alternative hypothesis to sister-strand crossing over which should be considered. Such a mechanism can be ruled out in a number of ways. The correlation of the frequency of dicentric ring formation with the size of the ring (McCLINTOCK 1938) is strong evidence against the production of double-sized rings by misdivision of the centromere. Double-sized rings arising from centromere misdivision would differ in the arrangement of the genes on the ring from those arising by sister-strand crossing over, and hence result in a different pattern of mosaicism (fig. 4). Endosperm mosaics resulting from the instability of a ring chromosome involving the short arm of chromosome 9 and carrying C (colored aleurone) and Wx (starchy endosperm) were analyzed (SCHWARTZ, unpublished). If the double-sized rings arise by centromere misdivision, twin sectors of colorless starchy and

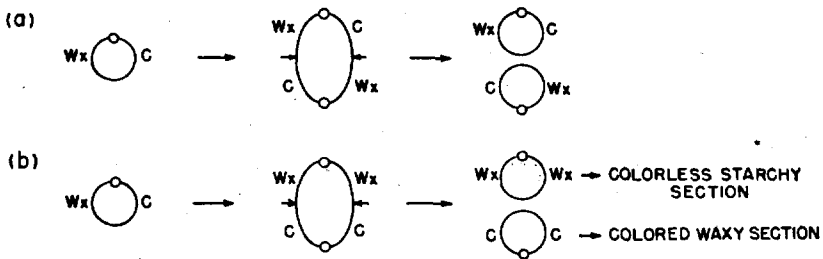


FIGURE 4.—Diagrammatic representation of endosperm mosaic pattern resulting from (a) dicentric ring arising by sister-strand crossing over, and (b) dicentric ring arising by misdivision of the centromere.

colored waxy tissue should be frequent. These would result from breakage of both bridge strands between C and Wx. No such twin sectors were found.

In order to explain the high frequency of single bridges in anaphase II by this mechanism, one would have to postulate that the undivided centromere of a dyad in which two of the arms are connected misdivides frequently while the centromere of a dyad with four free arms divides normally (fig. 5). It is difficult to visualize how such a modification in the morphology of the dyad would affect the plane of division of the centromere. Moreover, in the course of the breakage-fusion-bridge cycle described by McCLINTOCK (1941a), chromosome configurations are formed by the fusion of broken ends of sister chromatids which are similar to the dyads described above in that the centromere is undivided, and two of the arms are free and two are joined. Normal division of the centromere results in such a dicentric chromatid. Misdivision of the centromere would result in the formation of a centric ring and a centric rod. The breakage-fusion-bridge cycle, which results in a mosaic phenotype when in the proper genetic background, persists only in the gametophytic and endosperm tissue. The mosaicism is not carried over into the sporophyte since

the broken ends heal in the embryo and the cycle is halted (McCLINTOCK 1941a). If rings are formed by centromere misdivision, the mosaic pattern should be evident in the sporophyte because of the unstable behavior of ring chromosomes.

Fusion of the broken ends of an AI double bridge might be thought of as an alternative hypothesis to explain the high frequency of single bridges in AII. However, McClintock has shown that following the breakage of an AI double bridge fusion did not occur in the telophase I nucleus. In our study no cases were found where each daughter cell of a pair contained an AII bridge.

The evidence which is most frequently cited against the occurrence of sister-strand crossing over is the frequency of homozygosity in attached-X females of *Drosophila*. If the distribution of chiasmata among the four strands at meiosis is at random, there are six possible combinations of these four strands and the frequency of homozygosity cannot exceed 16.7 percent. If only non-sister strand

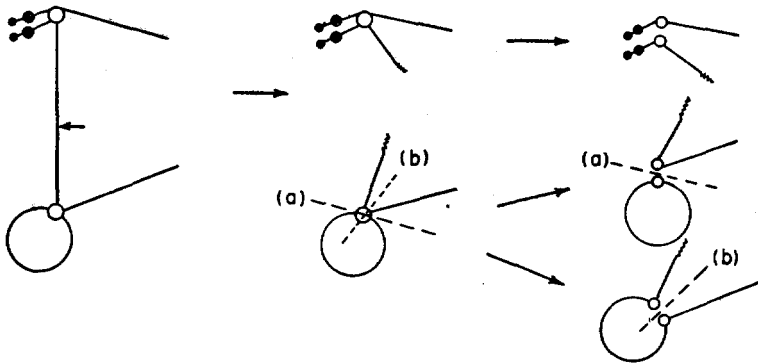


FIGURE 5.—Schematic diagram of alternative hypothesis involving misdivision of the centromere. (a) Represents normal plane of division of the centromere; (b) represents misdivision of the centromere.

crossing over occurs, the expected homozygosity value is 25 percent when one exchange occurs, 12.5 percent for two exchanges, 18.75 percent for three exchanges, etc. (SAX 1932). The finding of homozygosity frequencies significantly higher than 16.7 percent was interpreted as proving that little or no sister-strand crossing over occurs (BEADLE and EMERSON 1935). However, as these authors have pointed out, this evidence rules out only sister-strand crossing over which is equivalent to non-sister crossing over and shows chiasma interference. If the two crossover types are independent, as has been proposed in this paper, arising by different mechanisms and occurring at different times in the meiotic division, the maximum frequency of homozygosity expected from a combination of both sister- and non-sister-strand crossing over would remain at 25 percent.

From the study of the stable X^{c1} ring chromosome in *Drosophila*, MORGAN (1933) concluded that no sister-strand crossing over occurs. Her argument was as follows: Sister-strand crossing over in the ring would result in dicentric double-sized rings which would be eliminated in oogenesis. In females hetero-

zygous for the ring and a rod X chromosome this elimination would result in a decrease in the frequency of gametes carrying a non-crossover ring chromosome. The fact that equal ratios of non-crossover ring and non-crossover rod gametes were produced is certainly strong evidence against the occurrence of sister-strand crossing over in the X^{c1} ring. However this does not warrant a generalization regarding such crossing over in other organisms. If dicentric ring formation is used as the criterion, the evidence is strongly in favor of sister-strand crossing over in maize. It is an established fact that dicentric double-sized rings do arise from ring chromosomes in maize (McCLINTOCK 1938, 1941b).

The behavior of ring chromosomes in *Drosophila* is quite different from that in maize and presents an extremely complicated and little understood picture. The X^{c2} , $In(1)w^{ve}$ ring is highly unstable and gives rise to frequent gynandromorphs (BRAVER and BLOUNT 1950). The X^{c1} and X^{c2} rings are comparatively stable but the stability of the X^{c2} ring has been shown to be greatly influenced by environmental conditions (BROWN and HANNAH 1952). No stable ring chromosomes have been reported in maize.

In discussing the occurrence of those mutations at the Bar locus in *Drosophila* which were not associated with crossing over between forked and fused, STURTEVANT (1925, 1928) placed little emphasis on the possibility of explaining these mutations by unequal sister-strand crossing over. He was of the opinion that these exceptional cases arose as a result of contamination. In a similar study involving the compound A^b locus in maize, LAUGHNAN (1952) reported 8 percent exceptional cases where the separation of the α and β components of the locus was not associated with crossing over. It was possible to rule out contamination in these experiments. LAUGHNAN lists three possible explanations for these exceptional cases: (1) unequal sister-strand crossing over, (2) mutation of the β component to a null form, and (3) deficiency of the β component. The last two hypotheses are not very satisfactory. As LAUGHNAN pointed out, the exceptional cases are identical with those isolated from A^b by crossing over and are limited in the time of their occurrence to meiosis. If the non-crossover exceptions involve deficiencies of β , the deleted segments are very small including neither α nor sh_2 which are very closely linked with and situated on either side of β . While the A^b exceptional cases are not in themselves strong evidence for sister-strand exchange, they are at least suggestive and can be used as supporting evidence for such a form of crossing over.

SUMMARY

A large discrepancy in the expected ratio of anaphase configurations resulting from crossing over between a large ring involving chromosome 6 and its homologous rod chromosome is interpreted as due to sister-strand crossing over. The data indicate that at least one sister-strand crossover occurs per bivalent. An hypothesis is presented for the mechanism responsible for such crossing over based on Matsuura's observation on the parallelization of the spiral system in meiotic metaphase.

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