

CHIASMATA AND CROSSING OVER IN A DICENTRIC CHROMOSOME IN WHEAT¹

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ONE important concomitant of the now generally accepted partial chiasmata theory of crossing over is that every chiasma constitutes a crossover. Unfortunately, as DARLINGTON (1937) points out, there has been very little opportunity to test this concept. Meiotic chromosomes of *Drosophila* are too small for an accurate determination of chiasma frequency, and maize is about the only other organism in which enough linkages have been established to insure that nearly the full genetic length of each chromosome is represented in the map. In maize DARLINGTON (1937) reported satisfactory agreement between the published map lengths of the various chromosomes and the values predicted on the basis of his own counts of chiasma frequency, and BEADLE (1932) found a good correspondence between map length and the frequency of chiasmata in a particular segment of chromosome 9 of maize. In both cases, however, the genetic and cytological data were derived from different experiments, and crossover values in maize are known to be variable. The experiments of OEHLKERS (1940) and ERNST (1938) showing similar effects of environmental changes on chiasma frequency and crossover frequency in *Oenothera* and *Antirrhinum*, respectively, argue strongly for a one-to-one correspondence between chiasmata and crossovers. COOPER (1944), on the other hand, has shown that some apparent chiasmata have no relation to crossing over but are merely "conjunctive segments."

The transmissible dicentric chromosome of wheat described by SEARS and CÂMARA (1950, 1952) appears to provide an additional opportunity for testing the concept of chiasmata-crossover correspondence. This chromosome consists of a long arm, a main or strong centromere, an intercentromeric region, a second weak or possibly defective centromere, and a minute arm (fig. 1). The arm lengths have the approximate ratio 30:10:1. The secondary centromere is evidently inactive in bivalents during the first division of meiosis; in other divisions it seems to behave like any other centromere.

When two dicentrics pair, a chiasma in the intercentromeric region, if it is a crossover, should lead to bridge formation at AI, as shown in figure 1. Bridges of the type expected do occur, and their frequency is readily determi-

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nable. The frequency of chiasmata in the intercentromeric region at MI can also be determined with reasonable accuracy. Chiasmata in this region cannot be distinguished from those that may occur in the minute end piece, but this minute arm is so short that the number of chiasmata occurring there may be assumed to be negligible. Also, if two or more chiasmata occurred in the intercentromeric region, it is doubtful that they could be distinguished from a single chiasma; but this region is short enough that two chiasmata there must be very infrequent. Furthermore, with two chiasmata, only the two- and four-strand doubles would fail to lead to bridges.

SEARS and CÂMARA (1952) found that the frequency of chiasmata in the intercentromeric region did not differ greatly from the number of bridges observed in anaphase I (13 percent). However, they used estimates rather than

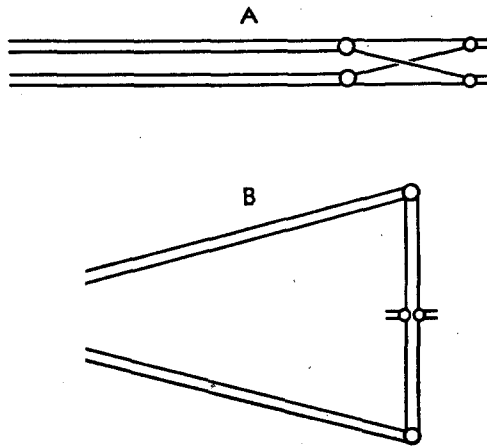


FIGURE 1.—Diagram of a pair of dicentric chromosomes with a chiasma in the intercentromeric region, at (A) pachytene and (B) anaphase I.

precise counts of chiasmata, and their preparations, which were made from spikes fixed entire, did not allow the analysis of a high proportion of cells.

In the spring of 1951 one plant with two dicentrics was grown. The anthers were fixed individually, and meiosis was studied in seven preparations, each of a single anther. In the fall of 1951 five more plants with two dicentrics were obtained, and a total of 43 single-anther preparations were studied. The results are presented in table 1. The data showed good homogeneity when tested for differences between plants, and the results may therefore be summed up as follows.

METAPHASE I

At metaphase I the dicentric chromosomes were unpaired in 21.1 percent of the cells that were analyzed. In these they lay off the plate as in figures 2 and 3 of SEARS and CÂMARA (1952). In 15 cells or 1.3 percent they were paired but lay off the plate and acted like the above univalents, both centromeres being active (*ibid.*, fig. 15). In the remaining 880 cells (or 77.5 percent)

TABLE 1
Meiotic behavior of a pair of dicentric chromosomes.

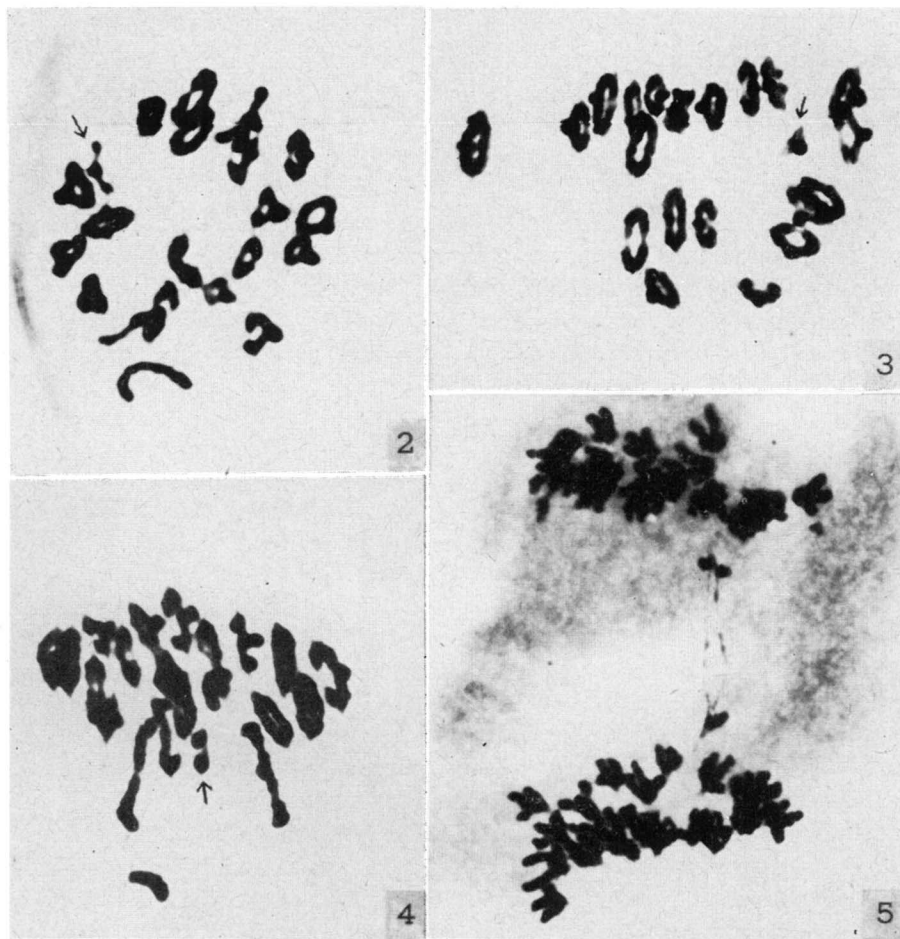
Plant No.	No. anthers	Metaphase I					Anaphase I			
		Unpaired	Paired, but acting as univalents	Xma in each arm	Xma in short arm only	Xma in long arm only	Unidentified	Bridge	No bridge	Unclassified
1	7	8	0	19	1	34	5	29
2	6	19	2	22	0	30	181	13	107	9
3	7	30	2	36	2	87	52	9	51	12
4	15	100	7	93	7	197	259	20	183	39
5	6	35	1	47	0	98	80	6	82	7
6	9	48	3	83	8	116	306	7	100	13
Totals	50	240	15	300	18	562	878	60	552	80

the dicentrics were paired, lying on the plate with the appearance of a normal bivalent in as much as only the strong centromere appeared active. The dicentric bivalent most frequently occurred as a rod bivalent with one chiasma in the long arm, and was easily recognized by its typical shape (fig. 2). Less frequently it was seen as a small ring bivalent with one chiasma in each arm (fig. 3). Very rarely, in only 18 cells or 1.6 percent of the total, it had a single chiasma in the short arm (fig. 4). Thus in 862 cells or 75.8 percent the dicentrics had a chiasma in the long arm, and in 28.0 percent of the total cells there was a chiasma in the short arm. As will be shown, this is a much higher frequency of short-arm chiasmata than would be expected from anaphase data. The question therefore arises as to whether the 878 unanalyzed metaphases, amounting to 45 percent of the total, may have included an unduly high proportion of the cells without short-arm chiasmata. This is very unlikely; in fact, the reverse is almost certainly true. Identification of dicentric ring bivalents was only possible in well-spread cells, where all 21 pairs could be studied and none discerned to be a dicentric rod bivalent. On the other hand, poorly spread cells were frequently classifiable if they contained a dicentric rod pair, because of the characteristic, easily recognizable appearance of this bivalent. Thus the unanalyzed metaphases consisted of the unfavorable cells less a considerable number in which the dicentric rod was identified. Since there is every reason to believe that the unfavorable cells were a random sample with respect to pairing behavior, it is very likely that the unanalyzed group had a higher proportion of bivalents with short-arm chiasmata than did the population as a whole.

ANAPHASE I

Anaphase I was studied and a count of dicentric bridges obtained (table 1). The characteristics of the dicentric bridge permit easy identification; it is double, and frequently the small knob or bump consisting of the minute end can be seen (fig. 5). It can be readily distinguished from the univalent dicentric bridge that would be expected from about 1 percent of unpaired dicentrics (SEARS and CÂMARA 1952). Only about 0.4 percent of the anaphases in this material would be expected to have such univalent bridges. Bridges con-

cerned with other chromosomes than the dicentrics occasionally occurred in this material, and some fragments also occurred. A few anaphases, about 12 percent of the total, too early for bridge determination, had to be excluded from the analysis.



FIGURES 2-4.—First meiotic metaphase showing paired dicentrics. $\times 960$. FIGURE 2.—Bivalent with an interstitial chiasma in the long arm. FIGURE 3.—Ring bivalent with one chiasma in each arm. FIGURE 4.—Bivalent with a chiasma in the short arm.

FIGURE 5.—First meiotic anaphase showing the double dicentric bridge. The knobs near the center presumably represent the minute arm beyond the secondary centromere $\times 960$.

Only 60 cells, or 9.8 percent of the total 612 anaphases studied, had dicentric bridges. This represents a great paucity of bridges as compared to the 28.0 percent chiasmata in the intercentromeric region that should result in such bridges. This finding will be discussed in detail below.

ANAPHASE II

Both anaphase I and anaphase II were studied to check the possibility that a chiasma in the intercentromeric region might result in early breakage at the secondary centromere without bridge formation in anaphase I. Such breakage would give rise to derivative chromosomes lacking the secondary centromere and to fragments consisting mainly of the minute arm and the secondary centromere. No such derivative chromosomes or fragments were observed. Fragments were found in six cells, but in several of these the dicentric was complete and unchanged. Apparently the occasional fragments found in this material were entirely unrelated to the dicentric chromosome.

Following its inclusion in a telophase I nucleus, each dicentric would be expected to behave as described by SEARS and CÂMARA (1950, 1952) for undivided univalent dicentrics, namely with both centromeres active but with the two centromeres of each chromatid usually directed toward the same pole. This appeared to be the case, since in only 5 out of 62 second anaphases was the secondary centromere seen to pull (ineffectively) toward the opposite pole from the primary centromere.

DISCUSSION

Although prophase analysis of bivalent dicentrics was not possible, metaphase figures indicated that the two chromosomes had in almost all cases been paired throughout their length. Thus the secondary centromeres had presumably been paired with each other; yet these centromeres ordinarily showed no poleward activity during the first division. Fifteen bivalents were found in which the secondary centromeres were active, but in each of these the secondaries had clearly not been paired with each other but with the corresponding primary centromere (cf. SEARS and CÂMARA 1952). Hence there was no exception to the rule that the secondary centromeres showed no poleward activity during first division when they had been paired with each other.

Since there are secondary centromeres in rye (KATTERMANN 1939; PRAK-KEN and MÜNTZING 1942) and maize (RHOADES and VILKOMERSON 1942) which are only active at a particular stage of meiosis, the question might be raised whether the secondaries in the wheat material are actually capable of first-division activity. The wheat secondary centromere is clearly active at this stage in univalent dicentrics, however, and in the 15 exceptional bivalents just mentioned; so there seems little doubt that it is capable of activity in the ordinary bivalents also.

So far as is known, the secondary centromeres behave normally at all other divisions, although they are weaker than the primary when opposed to it. It appears, therefore, that the secondary centromere lacks something necessary to insure the separation of the two halves of a bivalent but not required at mitotic divisions. When a secondary pairs with a primary centromere, as it evidently does in a univalent dicentric, the primary is able to initiate not only its own poleward movement but also movement of the secondary centromere. This evidence that something more is required of a centromere at meiosis than

at mitosis may be of significance in connection with problems of chromosome mechanics and the structure of the centromere. It is possible that a study of the behavior of certain derivative chromosomes will provide further information of value in this regard.

It seems clear that the bridges observed are formed in the manner indicated in figure 1—that is, by failure of chiasmata in the intercentromeric region to terminalize beyond the secondary centromeres. The bridges are double, as expected, and frequently a lump can be detected at the center of the bridge which finds ready interpretation as being the minute arm. Bridges formed in another way could occur here, in as much as SEARS and CÂMARA (1950, 1952) suggest that the intercentromeric region may contain a reversed duplication. Crossing over in this region reversely paired would result in bridges, but these would usually be single and would be accompanied by fragments.

The data thus show a real discrepancy in the frequency of chiasmata in the intercentromeric region as compared with the frequency of anaphase bridges formed. As has already been pointed out, this discrepancy cannot be due to early breakage. The disjunction without bridge formation must come about in some other way.

The possibility that most of the chiasmata counted as intercentromeric are in reality in the minute end beyond the secondary centromere, and therefore resolvable without bridge formation, has not been excluded. However, because of the small size of the minute end, this is believed to be unlikely. The relatively high frequency of such chiasmata necessary to account for the paucity of anaphase bridges would indicate a special preferential localization of chiasmata in this end. It seems unwarranted to postulate such localization, particularly since the relative frequencies of chiasmata in the long and short arm of this chromosome agree well with expectation based on their lengths.

If sister secondary centromeres could separate at AI, then terminalization of intercentromeric chiasmata could proceed, and there would be no bridges. In normal bivalents homologous centromeres pass to opposite poles at first division, while sister centromeres do not separate until second division. In univalents, on the other hand, sister centromeres separate at first division, although considerably later than do the bivalent halves. The secondary centromeres under investigation resemble centromeres of univalents in that the undivided centromere shows no poleward activity. This suggests the possibility of further resemblance, namely, in the separation of sister centromeres at the first division. The occurrence of bridges in 9.8 percent of first anaphases would then be attributed to the secondary centromeres having been active in about one-third of the bivalents with intercentromeric chiasmata, with consequent failure of terminalization across these active centromeres. No such activity of secondary centromeres was ever seen, but it could have occurred without being detected in the bivalents with intercentromeric chiasmata, since these were usually tightly paired. However, since activity of secondary centromeres was not seen in bivalents with only long-arm chiasmata, where it could scarcely have gone unobserved, it probably did not occur in the bivalents with inter-

centromeric chiasmata either. The only obvious explanation for limitation of secondary centromere activity to the latter type of bivalent would be that activity of the secondaries might be a consequence of their pairing with each other. Although this pairing would favor chiasma formation in the neighboring intercentromeric region, there seems no reason to suppose that it would invariably result in chiasma formation in this short segment.

Thus there seems to be no satisfactory way of reconciling the present data with the theory that every chiasma represents a crossover. However, of the three possibilities suggested—namely, early breakage, localization of chiasmata in the minute arm, and premature separation of sister secondary centromeres—only early breakage has been entirely excluded. The data do not, therefore, constitute critical evidence against the partial chiasmata theory. They do suggest, however, the desirability of further work with this and similar material.

SUMMARY

A transmissible dicentric chromosome in wheat has centromeres of unequal strength. In plants with two dicentrics, these usually pair, and no activity of the weaker centromere can then be detected. In 28.0 percent of the cells at MI of microsporogenesis there apparently was a chiasma in the intercentromeric region. This should have led to an equivalent frequency of bridges at AI, according to the generally accepted theory that every chiasma represents a crossover, but only 9.8 percent of AI cells had bridges. This discrepancy is not considered as critical evidence against the theory, however, since other explanations are not entirely excluded.

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