# THE RELATIONSHIP OF CROSSING OVER TO CHROMOSOME SYNAPSIS IN A SHORT PARACENTRIC INVERSION[1]

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CLASSICAL cytogenetic theory in eucaryotic organisms predicts that the frequency of crossing over in a given chromosome region will be proportional to the extent of *genetic* map which becomes involved in homologous synapsis (with an average expectancy of one crossover for each 50 map units). PRITCHARD (1960) has suggested as an alternative interpretation of map data that crossover frequency may instead be proportional to the extent of genetic map *available* for homologous pairing. This proposal in effect adds premeiotic interphase to the list of those stages during which crossing over may conceivably occur (zygotene, pachytene, diplotene).

Although the common assumption of a basic relationship of crossover frequency to extent of map synapsis may not be justified, tests of such a relationship must be complicated by the fact that in aberrant material (where pairing of chromosome parts might be studied) crossover frequency frequently appears to be affected by the existence of the aberration. Furthermore, genetic maps do not correspond precisely to cytological maps, and the only organism with a detailed genetic map available (*Drosophila melanogaster*) is an unsuitable subject for meiotic prophase study. Thus the relationship of changes of crossover frequency (induced by chromosomal aberrations or dosage effects) to actual changes in extent of genetic map which synapses is obscure.

It was recently found (MAGUIRE 1965) throughout an array of $2n + 1$ constitutions containing maize-Tripsacum interchange chromosomes that pachytene and metaphase I trivalent frequencies were so similar as to require an unorthodox interpretation: either the existence of trivalent configurations at pachytene is dependent upon prior crossing over in the appropriate region (as it presumably is at metaphase) or crossing over almost always follows trivalent formation regardless of the extent of genetic map available for homologous pairing in these plants.

It was considered a question of interest whether a similar correspondence of synapsis and crossover frequencies is displayed by other sorts of aberrant constitutions. A search of the literature revealed no previous relevant work. Material is required in which synaptic frequency and crossover frequency can be estimated simultaneously for a region known to contain substantially less than 50 crossover units. This paper is a report of such estimations from plants heterozy-

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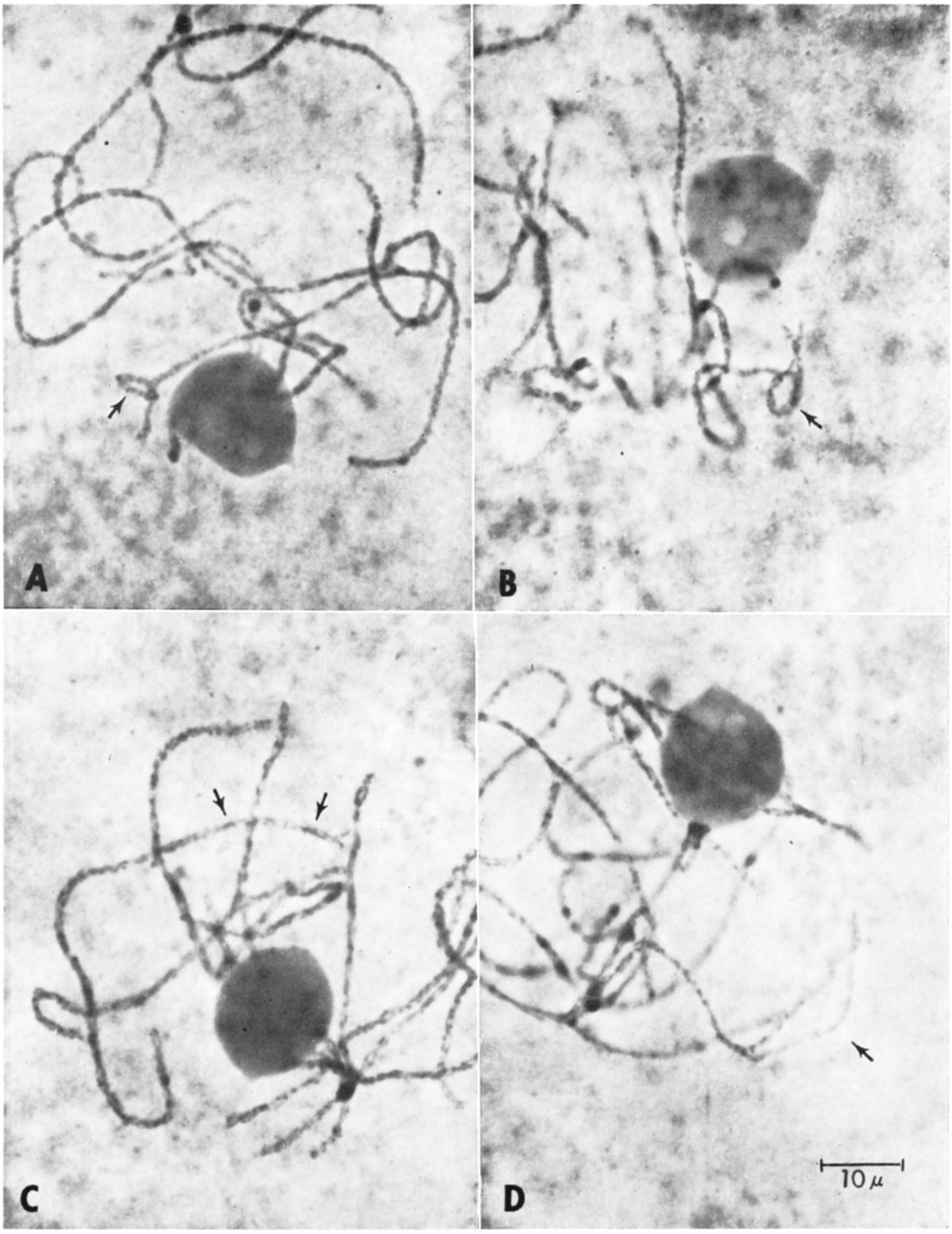
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gous for a short paracentric inversion in maize, which were deemed suitable in these respects for such a study.

## MATERIALS AND METHODS

Inversion 5083 is located in the long arm of chromosome 1 with breakpoints at .70 and .87 of the cytological distance from the centromere to the distal end (LONGLEY 1961). Since the long arm of chromosome 1 has a map length of approximately 112 units, if the inverted region were average, it would be expected to contain approximately 19 units of the genetic map. While there is no basis for an assumption that the inverted region is average in this respect, it seems unlikely that it contains as much as 50 units. In this case nearly half of the entire genetic map of the long arm of chromosome 1 would have to be contained within .17 of its physical length.

Maize plants were grown in the field which were heterozygous for inversion 5083, with a parental background which included L289 and KYS (inbred lines noted for excellent quality of pachytene spread). Microsporocyte samples were collected, fixed in alcohol-acetic 3:1 mixture and stored in a freezer until examined in acetocarmine smears.

Pachytene and anaphase I slides from each of three plants heterozygous for the inversion were systematically scanned. In pachytene slides all cells in which the chromosomes appeared under low power (100×) to be sufficiently spread for analysis were examined under high power (900×). Each pachytene cell examined under high power was listed either as containing reverse synapsis as in Figure 1A and B (of any observable length) in the region of the inversion, nonhomologous rod pairing throughout it (Figure 1C), complete pairing failure in the inverted region (Figure 1D), or as hopelessly unclassifiable with respect to pairing or lack of it in the inversion. Homologous synapsis in the region heterozygous for the inversion requires reverse pairing. Each anaphase I cell with poleward progress of 50% or greater was examined (under high power) for absence of bridge and fragment, presence of bridge and fragment, or presence of fragment only. Virtually all anaphase I cells with such an extent of poleward progress were easily classifiable in these respects, for normal bivalents, devoid of bridges, are unequivocally separated at this stage, the region proximal to the inversion is sufficiently long to form an extended bridge, and the resulting fragment, though small, is not easily missed.

## RESULTS

Results are summarized in Tables 1, 2 and 3. There appears to be no reason to suspect that position within the tassel influences the frequency of reverse synapsis at pachytene or bridge and fragment frequency at anaphase I since these data among flowers within plants are homogeneous at the 5% level in chi-square tests. Data are somewhat more variable with respect to whether the failure of reverse synapsis at pachytene is expressed as nonhomologous rod pairing or as complete pairing failure (with data for two of the plants heterogeneous at the 5% level). The unclassifiable pachytene cells introduce a source of bias if they in-

FIGURE 1.—Photomicrographs of microsporocytes at pachytene from one of the plants heterozygous for the inversion, showing various synaptic configurations in the inverted region. A and B—Homologous, reverse synapsis in inversion loops. C—Rod pairing. Since arrows (in C) indicate approximate locations of breakpoints, pairing is nonhomologous in the region between them. Short terminal pairing failure such as that seen at one end of this chromosome is not uncommon in normal maize bivalents at this stage. D—Complete pairing failure in the inverted and adjacent regions. Chromosome 1 (the chromosome carrying the inversion) has no knob in the inversion arm and a small knob near the end of the other arm in this stock.

TABLE 1

*Pachytene pairing frequencies*

| Plant | Nonhomologous rod pairing | | Pairing failure | | Reverse synapsis | | Unclassifiable | |
|---|---|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent | Number | Percent |
| 1. | 235/505 | 46.5 | 88/505 | 17.4 | 182/505 | 36.0 | 164/669 | 24.5 |
| 2. | 282/495 | 57.0 | 64/495 | 12.9 | 149/495 | 30.1 | 172/667 | 25.8 |
| 3. | 192/544 | 35.3 | 162/544 | 29.8 | 190/544 | 34.9 | 127/671 | 18.9 |

TABLE 2

*Anaphase I bridge and fragment frequencies*

| Plant | No bridge or fragment | | Bridge and fragment | | Fragment only | |
|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent |
| 1. | 837/1303 | 64.2 | 272/1303 | 20.9 | 194/1303 | 14.9 |
| 2. | 720/1023 | 70.4 | 182/1023 | 17.8 | 121/1023 | 11.8 |
| 3. | 818/1244 | 65.8 | 232/1244 | 18.6 | 194/1244 | 15.6 |

TABLE 3

*Total frequencies of homologous synapsis and bridge and fragment formation*

| Plant | Frequency of reverse synapsis at pachytene | | Combined anaphase I bridge and fragment, and fragment-only frequency | |
|---|---|---|---|---|
| | Number | Percent | Number | Percent |
| 1. | 182/505 | 36.0 | 466/1303 | 35.8 |
| 2. | 149/495 | 30.1 | 303/1023 | 29.6 |
| 3. | 190/544 | 34.9 | 426/1244 | 34.2 |

clude disproportionate frequencies of the various classes. The number of such unclassified cells was therefore tested (in rank difference tests) for correlation to frequencies of cells with reverse synapsis in each plant. Since no significant rank difference (at the 5% or even 10% level) was found, it is thought that the samples give reliable estimates of the frequency of any homologous pachytene synapsis in the region heterozygous for the inversion, although more complex sources of bias, if any, are not necessarily excluded.

Extensive linear measurements of actual length of reverse synapsis were not made, but it should be noted that in many cases this length of homologous synapsis was only a small proportion of the total inverted region, while the remainder (and often adjoining regions as well) were unpaired. This fact lends further support to the supposition that the average extent of homologous synapsis per cell in the inverted region was substantially less than 50 map units. Two measurements of tightly paired loop configurations gave breakpoint estimates close to those of LONGLEY (.70 and .71 for the proximal break and .90 and .86 for the distal).

Each case of single crossing over within the inverted region is expected to yield a bridge and a fragment at anaphase I if there is no additional crossing over proximal to the inversion. A fragment only at anaphase I (with a bridge at anaphase II) results from three-strand double crossovers in the inversion and the region proximal to the inversion. Other types of double crossovers with one located in the inversion and one external to it produce a bridge and a fragment at anaphase I. Two-strand double crossovers within the inversion (which produce no bridge or fragment) are expected to occur with negligible frequency in a short region. Four-strand doubles within the inversion, which yield double bridges and fragments at anaphase I, and are expected to occur with a frequency equal to that of two-strand doubles within the inversion in the absence of chromatid interference, were rarely observed (four in the entire experiment). Evidence for absence of chromatid interference within a maize inversion has been reported by RHOADES and DEMPSEY (1953). Three-strand doubles within the inversion (also expected to be rare) give a bridge and a fragment at anaphase I. Higher orders of crossovers are expected to occur with negligible frequency.

Although an additional very short heterozygous inversion in chromosome 8 exists in some plants of the stock used, the plants selected for use here showed no pachytene abnormalities (other than the single case of inversion heterozygosity under study) and no anaphase cell was seen with bridges from two or more bivalents.

Thus the combined frequencies of bridge and fragment and fragment only at anaphase I are thought to provide a good estimate of the frequency of crossing over within the inversion in this experiment. Approximate estimates from the three plants of 18, 15 and 17 map units for the inverted region (calculated from observed crossover frequencies of 35.8, 29.6 and 34.2% respectively) are surprisingly close to the average of 19 crossover units calculated earlier for regions of this length in the short arm of chromosome 1. Such correspondence may, however, be coincidental and should not be interpreted as sound evidence that the region actually is average in extent of map inclusion. To what extent crossing over may be suppressed within heterozygous inversion regions in maize is not known. In Drosophila the degree of actual suppression (not to be confused with frequency of recovery of recombinant progeny) has been found to vary with the size of the inversion (STURTEVANT and BEADLE 1936) and with its proximity to heterochromatin (NOVITSKI and BRAVER 1954). No obvious heterochromatic region exists in the entire chromosome arm containing the inversion in this study (see Figure 1).

When pachytene and anaphase data from all flowers studied are pooled for each plant, a striking similarity is seen in each case between frequency of cells with *any* reverse synapsis at pachytene in the region heterozygous for the inversion and frequency of cells at anaphase with bridge and fragment or fragment only (Table 3).

## DISCUSSION

The data provide an additional case comparable to those reported earlier

(MAGUIRE 1965) in that crossover frequency is estimated to match closely the *frequency* of homologous synapsis at pachytene. They do not rule out either of the following alternatives:

1. Homologous synapsis of a region at pachytene may almost invariably follow and depend upon the existence of prior crossing over within it. (The *extent* of map synapsed at pachytene may not be, or appear to be, a function of number of crossovers but may depend mostly or entirely upon other variables.) Under these conditions (barring complicating factors) the *frequency* of pachytene synapsis of a region of less than 50 units (which cannot pair homologously by simple zipping up from adjacent regions) will match closely the frequency of occurrence of crossing over within it. This is true whether homologous regions are generally aligned throughout their length prior to pachytene-type synapsis (see MAGUIRE 1965) or are brought close together only in very short regions of "effective pairing" at this stage (PRITCHARD 1960).

2. The normal distribution and/or frequency of crossovers may be changed by the existence of aberrant synaptic configurations, such as trivalents and inversion loops (or partial loops). In the trivalents studied earlier by this author and the inversion configurations reported here the change would apparently be such that any pachytene synapsis of the short regions tested (less than 50 units) almost invariably leads to crossing over within them, presumably due to the action of a regulating mechanism. (Although the literature contins widespread reports of effects of aberrations on crossover frequency, previous quantitative comparisons to pachytene synaptic frequencies seem to be lacking.)

The fundamental question is raised of the stage or stages of meiosis or premeiosis during which crossing over occurs. Recent work with Drosophila has suggested that crossing over may occur at a stage close to that of DNA replication (GRELL and CHANDLEY 1965) and that homologous pairing may not be continuous at the time of crossing over (ROBERTS 1965; JUDD 1965).

The results presented here warrant caution in interpretation of the significance of the pachytene stage and perhaps also therefore of the synaptinemal complex.

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## SUMMARY

Crossover frequency in a heterozygous short paracentric inversion in maize (estimated from frequency of bridge and fragment formation) was found to correspond closely to the frequency of (any) homologous synapsis within it at pachytene. As is the case with certain trivalent configurations (reported earlier), the interpretation seems to be required that either crossing over is a precondition for homologous pachytene synapsis or invariably follows synapsis of the tested region, even when its genetic length is substantially less than 50 units.

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