THE CYTOLOGICAL MECHANISM FOR CROSSING OVER

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The genetic analysis of Drosophila by MORGAN and his associates has contributed more to our knowledge of chromosome behavior at meiosis than have the investigations of the cytologists. Any theory of chromosome pairing and crossing over must conform to the rigid requirements of the geneticist. As a foundation for the discussion of the mechanism of crossing over, it is essential to consider the facts which have resulted from the genetic investigations of Drosophila during the past twenty years.

It is known that the four linkage groups in *Drosophila melanogaster* correspond to the four pairs of chromosomes. The genes are arranged in a linear order in these chromosomes. Chromosome pairing must involve a gene by gene association in order to account for the great precision of crossing over. The frequency of crossing over is not the same for all regions of the chromosome and is reduced in the region of the spindle-fiber attachment. Chromosome pairing and disjunction are regular in both males and females, but no crossing over occurs in the male.

The phenomenon of interference is of special importance in any interpretation of the mechanism of crossing over. If one crossover occurs, a second one is never found in adjacent regions of the same chromosome (STURTEVANT 1913). Interference is complete for 10 to 20 units, depending on the region of the chromosome involved (MULLER 1916, WEINSTEIN 1918).

The first meiotic division in Drosophila is usually, if not always, reductional at the spindle fiber attachment point, that is, the sister chromatids are held together at the fiber constriction at this division (BRIDGES and ANDERSON 1925, ANDERSON 1925, 1929, L. V. MORGAN 1925, REDFIELD 1930, RHOADES 1931, STURTEVANT 1930).

Crossing over occurs at early prophase of meiosis, after the chromosomes have paired, and between only two of the four chromatids at any one locus (BRIDGES 1916, BRIDGES and ANDERSON 1925). It has also been shown that only two of the four chromatids are involved in a crossover in Zea (RHOADES 1932) and in Habrobracon (WHITING unpublished).

Any factor which reduces crossing over is associated with a reduction in regularity of pairing and disjunction (GOWEN 1928, ANDERSON 1929). Crossing over is decreased in individuals heterozygous for an inversion (STURTEVANT 1926).

Crossing over is not limited to two of the four chromatids throughout their length, because crossing over is found in more than 50 percent of the

emerging X chromosomes of Drosophila, and about 75 percent of the third chromosomes have one or more crossovers (ANDERSON and RHOADES 1931, REDFIELD 1930). Crossing over occurs more or less at random between homologous chromatids (ANDERSON 1925). There is no evidence that crossing over occurs between sister chromatids (STURTEVANT 1925, 1928).

It has generally been assumed that genetic crossing over is correlated with an actual physical interchange of chromosome segments. This assumption has been confirmed by the brilliant investigations of CREIGHTON and McCLINTOCK (1931) with Zea, and of STERN (1931) with Drosophila.

THE MECHANISM FOR CROSSING OVER

Janssens' interpretation of chiasma formation

JANSSENS' (1924) partial chiasmatypy hypothesis has been supported by BELLING (1931a, 1931b), DARLINGTON (1930, 1931b), MAEDA (1930a), and others. It is assumed that every chiasma represents a crossover which has occurred between two of the four chromatids at pachytene. As the homologous chromosomes open out at diplotene, only sister chromatids are paired, and the chiasmata indicate the point of interchange in crossing over.

The only plausible explanation of how crossing over could occur, on JANSSENS' theory, has been presented by BELLING (1931b). According to BELLING, the homologous chromosomes pair as single threads. Half twists occur in the paired chromosomes before the new chromatids are formed. Each chromomere, or gene, then divides. The connecting fiber between chromomeres may remain with the old chromomere or pass to the new one at random. New connecting fibers are then formed to unite the free chromomeres. At the half twist formed by the original chromatids, the connecting fibers unite the free genes by the shortest path, so that a crossover occurs at random between non-sister chromatids. It is, of course, also necessary to assume that sister-strand crossovers are very frequent. As the writer (SAX 1932a) has pointed out, such a random assortment of connecting fibers would result in numerous twists in paired chromatids in both somatic and meiotic chromosomes.

BELLING's theory can be made more plausible if it is assumed that crossovers always involve the two new chromatids, as BELLING (1931a) suggested in his second paper on crossing over. An occasional crossover between sister chromatids would result in apparent random crossing over between homologous chromatids (SAX 1932a).

An interchange of segments between two of the four chromatids should invariably produce an asymmetrical arrangement of the chromatids. This

situation was recognized by JANSSENS, but has received little or no attention from the recent supporters of his theory. The asymmetrical relations of the chromatids, if each chiasma represents a crossover, are shown in figure 1, diagram A. Chromatids which were adjacent at the four-strand stage form the cross at each chiasma. As a result of the sister-strand crossover at the second internode, the genetically detectable crossovers are not confined to two of the four chromatids. Sister chromatids are always paired at early diplotene.
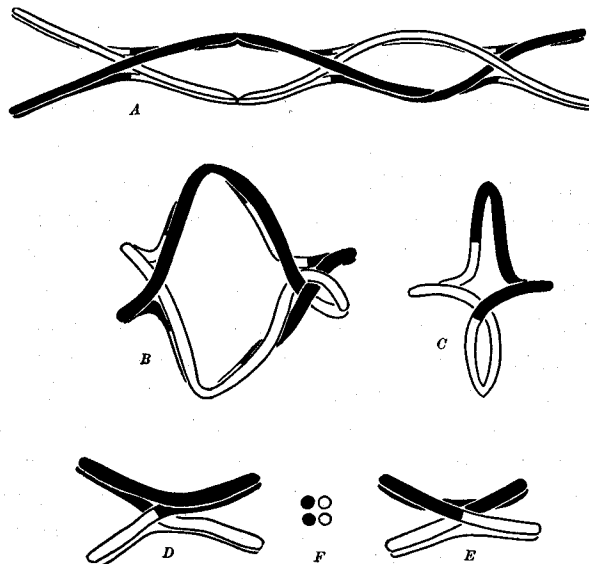

FIGURE 1

As the chromosomes open out in subsequent stages of meiosis, the chiasmata are often terminalized until, at metaphase, only terminal or sub-terminal chiasmata are found (DARLINGTON 1931, 1932, GAIRDNER and DARLINGTON 1932, and others). It is assumed by DARLINGTON that the attachment loop, in expanding, pushes all the others together at the end. As a result of such terminalization, a rather complicated association would result where two or more chiasmata are pushed together at metaphase (figure 1, diagram B). The two uppermost chromatids form the cross at the chiasma to the left of the spindle-fiber attachment. If this chiasma were opened out to form the typical cross-shaped figure, there would be a twist in one pair of chromatids, as shown in figure 1, diagram C. If only one chiasma is formed, it is possible that the torsion would rotate the paired chromatids at the

proximal end so that a symmetrical cross would result; but where a chiasma is formed on each side of the spindle fiber, few symmetrical chiasmata would be expected.

Two consecutive reciprocal chiasmata on the same side of the fiber should produce interlocking of chromatids at metaphase in half the cases if one chromatid passes above or below the other at random.

The chiasmata represented in figure 1, diagrams A to C, are all reciprocal crossovers, which is the type expected most frequently on BELLING'S hypothesis and observed most frequently by DARLINGTON (1932) in Stenobothrus. These diagrams are based on BELLING'S latest theory, with a few favorable modifications. If, however, the interchange of segments occurs in the chromatids which form the cross, the results will be the same, so far as the relation of the chromatids is concerned. Either a reciprocal (adjacent) or an equational (diagonal) crossover will result in an asymmetrical relation of the chromatids (figure 1, diagrams D and E). Viewed from the end, each chromatid will not lie in the same quadrant at all loci. In the diagram of the equational crossover, as viewed from the left end at the four-strand stage (figure 1, diagram F), the chromatid at the upper right quadrant changes to the lower left quadrant, owing to the crossover, while in the reciprocal crossover the chromatid in the upper right quadrant changes to the upper left quadrant.

BELLING'S theory of crossing over seems to be untenable for several reasons. He assumes, as does DARLINGTON, that homologous chromosomes pair as single threads at meiosis. According to KAUFMANN (1931), the chromosomes at the telophase of the last premeiotic division are two-parted in several plant species. In several Orthopteran species the chromosomes are split longitudinally before pairing at meiosis (ROBERTSON 1916, 1931a, 1931b, McCLUNG 1928). RANDOLPH (1932) finds a longitudinal split in the early leptotene threads in a haploid Zea plant. These observations can not be reconciled either with BELLING'S theory of crossing over or with DARLINGTON'S theory of meiosis. The random assortment of connecting fibers between chromomeres, postulated by BELLING, would mean that sister strand crossovers and many twists in paired chromatids would be expected. There is no evidence that sister strand crossing over occurs, and few twists are found in paired chromatids of either somatic or meiotic chromosomes. The third objection to BELLING'S theory—and this applies to any interpretation of JANSSENS' hypothesis—is the prevalence of symmetrical relations of the chromatids in many genera, even where several chiasmata are found in a bivalent chromosome. It is also difficult to account

for interference in crossing over and the absence of crossing over in the Drosophila male, on any modification of JANSSENS' theory.

DARLINGTON (1932) has recently revived the torsion hypothesis to explain how crossing over occurs. It is significant that he shows no diagrams illustrating how this mechanism might cause exact crossing over between only two of the non-sister chromatids at any one locus.

McClung's theory of chiasma formation

According to McCLUNG, chiasmata are caused by the alternate opening out of pairs of sister and non-sister chromatids. This interpretation has
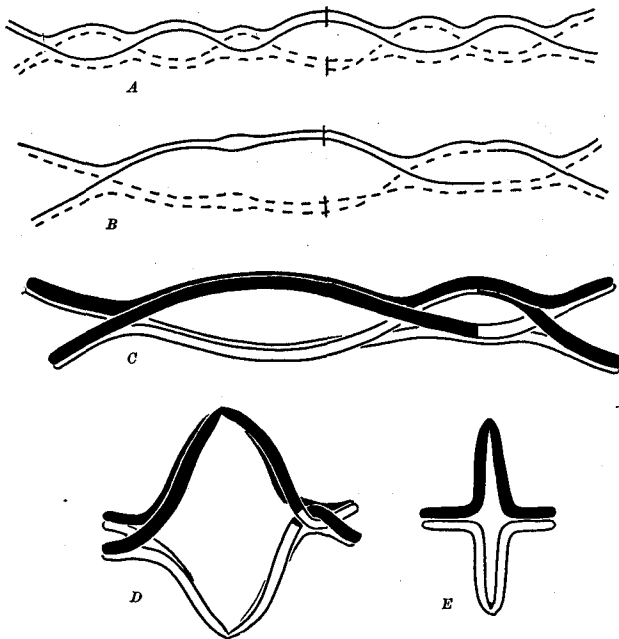
FIGURE 2

been supported by WENRICH (1916), ROBERTSON (1916), WILSON (1925), BELAR (1928), CAROTHERS (1926), and NEWTON (1926). As McCLUNG (1927) has pointed out, such an origin of the chiasmata should usually result in a symmetrical relation of the chromatids. The apparent cross at a chiasma is formed by chromatids which were diagonal at the four-strand stage, and each chromatid may lie in the same quadrant at all loci (figure 2, diagram A). The relations of the chromatids at metaphase, when no crossover has occurred, are shown in figure 2, diagram E.

The writer's theory of crossing over

The writer (SAX 1930, 1932a) has suggested that crossing over is caused by breaks in two of the four chromatids at a chiasma. At very early diplotene many chiasmata may be formed in each bivalent chromosome, as shown in figure 2, diagram A. These chiasmata are formed by alternate pairing of sister and non-sister chromatids. Occasional half-twists in paired sister chromatids permit more or less random crossing over between any two homologous (non-sister) chromatids. Between the earliest diplotene stage and diakinesis there is considerable reduction in the number of chiasmata. Unequal opening out of certain internodes will result in the elimination of certain chiasmata by cancellation, as shown in figure 2, diagram B. It is also possible that some chiasmata may be terminalized to such an extent that they will pass off the end of the chromosome. Crossovers occur only when two of the chromatids break at a chiasma, and the segments reunite in a new association, as shown in figure 2, diagrams A and B. When no crossover occurs, the chromatids at a chiasma are symmetrical, each chromatid lies in the same quadrant on both sides of the chiasma, and the apparent cross at the chiasma is formed by chromatids which were diagonal at the four-strand stage. If the distal ends of the chromosomes open out in the same plane, a symmetrical cross is formed, as shown in figure 2, diagram E.

A crossover, or a twist in sister threads, will produce an asymmetrical arrangement of the chromatids, as shown in figure 2, diagrams B and C. A crossover formed between two intact chiasmata will result in interlocking of chromatids at metaphase (figure 2, diagram D).

This theory of crossing over seems to meet the rigid genetic requirements, and is in accord with most of the cytological evidence. The lengths of the internodes, or loops, between chiasmata would account for interference. The reductional loop at the fiber attachment point is usually larger than the others, which would reduce crossing over in the spindle-fiber region. Chiasma formation and normal chromosome pairing can occur without crossing over, as is presumably the case in the Drosophila male. Crossovers between sister chromatids should be rare. Reciprocal and equational crossovers may occur with equal frequency (resulting in a genetic ratio of 2:1 in attached XX's), but under certain conditions there should be an excess of equational crossovers. A symmetrical relation of the chromatids would be expected where no crossover has occurred, but half twists in paired sister chromatids or a crossover would produce asymmetrical chiasmata.

CYTOLOGICAL EVIDENCE

The arrangement of chromatids

If McCLUNG's theory of chiasma formation is correct, the chromatids should usually show a symmetrical arrangement at each chiasma if little or no crossing over has occurred. Chromosome configurations which seem to support this interpretation of chiasma formation have been shown in Orthopteran species by SUTTON (1902), WENRICH (1916), ROBERTSON (1916), JANSSENS (1924), BELAR (1928), CAROTHERS (1926), McCLUNG (1927), DARLINGTON and DARK (1932), and others. Similar figures have been shown for other groups of animals (WILSON 1925).

In plant species the individual chromatids are not clearly differentiated until metaphase, and then only when the smear technique is used. This technique has long been used by the zoologists, but has been adopted by botanists only since TAYLOR pointed out its application to plant cells in 1924. Symmetrical chiasmata have been found in Gasteria (TAYLOR 1930), Uvularia (BELLING 1926), Tulipa (NEWTON 1926), Pæonia (SAX 1932b), Secale (SAX 1930), and in Larix (H. J. SAX 1932). According to NEWTON, the hypothesis "which explains the diakinetic figures as due to the opening out in two planes at right angles of what are originally four parallel chromatids, is adequate to explain the events of diakinesis and division in Tulipa and Fritillaria."

Asymmetrical chiasmata have been described in Orthopteran species by JANSSENS and DARLINGTON, but these figures are apparently not typical for this group. As McCLUNG has pointed out, the clearest figures shown by JANSSENS show the chromatids in the same quadrants at all loci. DARLINGTON and DARK (1932) believe that most of the chiasmata in Stenobothrus are reciprocal and involve the same two chromatids at successive chiasmata. Of the two figures referred to, one (figure 7, chiasmata A and B) shows clearly that both chiasmata are symmetrical and diagonal, as is the case in most of the figures shown, where the chromatids can be observed. The interlocking of chromatids at metaphase does indicate that some crossing over has occurred.

MÆDA (1930a) finds a large proportion of asymmetrical figures in Lathyrus chromosomes, as might be expected, since crossing over is known to occur in this genus. Crossing over is also indicated by interlocked chromatids at metaphase.

Asymmetrical chiasmata are frequently found in Pæonia chromosomes (SAX 1932b). In most cases these figures could be attributed to half twists in sister chromatids, but some figures do seem to support JANSSENS' hypothe-

sis. These exceptional figures may be due to an association of non-sister chromatids at the fiber attachment point, accompanied by a half twist in one pair of sister chromatids.

The prevalence of symmetrical chiasmata in the Orthoptera and in certain plant species seems to indicate that JANSSENS' partial chiasmatypy theory of chiasma formation is untenable. The asymmetrical chiasmata found in both plant and animal species would be expected on McCLUNG's theory of chiasma formation if twists occur in sister chromatids, or if crossovers occur in some of the chiasmata which disappear between early diplotene and metaphase stages of meiosis. If no crossovers occur, almost all the chiasmata may be symmetrical, as seems to be the case in some species.

Reduction in chiasma frequency

If crossing over is caused by breaks in the chiasmata, as the writer assumes, then there should be a reduction in chiasma frequency between earliest diplotene and metaphase stages of meiosis. Reduction in chiasma frequency can also be attributed to the meeting and cancellation of chiasmata, as shown in figure 2, diagrams A and B. There is also the possibility that some chiasmata may pass off the ends of the chromosomes.

At earliest diplotene there are numerous nodes and internodes along the bivalent chromosomes in many species of plants and animals. The frequency of these nodes, most of which are probably chiasmata, may be as high as 7 or 8 in certain chromosomes. Bivalent chromosomes with numerous nodes and internodes at early diplotene have been shown in Stenobothrus by JANSSENS (1924, figure 242), in reptilian chromosomes by NAKAMURA (1932), and in Ophyotrocha by GRÉGOIRE and DETON (1906). In plant species, chromosomes with an apparent high chiasma frequency have been shown in Pinus (FERGUSON 1904), Lilium and Allium (BERGHS 1904), Crepis (BABCOCK and CLAUSEN 1929), Tulipa (NEWTON 1926), Nothoscordum (BEAL 1932), and in Callisia (SAX 1932a). In these genera the chiasma frequency may be reduced from five or six or more at early diplotene to one or two at metaphase. A reduction in chiasma frequency between diplotene or early diakinesis and metaphase has been found in Tulipa (NEWTON 1926, DARLINGTON 1932), Lilium (BELLING 1931b), Primula (DARLINGTON 1931a), Rosa (ERLANSON 1931), Matthiola (PHILP and HUSKINS 1931), Campanula (GAIRDNER and DARLINGTON 1932), and Callisia (SAX 1930). In these genera the average number of chiasmata lost per bivalent between diplotene and metaphase is somewhat more than one. The loss of chiasmata reported in most cases would undoubtedly have been

greater if the earliest diplotene stages were favorable for examination of chiasma frequency.

In some genera there is probably little reduction in chiasma frequency between early diplotene and metaphase. In these cases the chiasmata should be symmetrical, and little crossing over would be expected.

The reduction in chiasma frequency is often associated with an increase in the proportion of terminal chiasmata, and, in many species, only terminal chiasmata are found at metaphase, even though there may be from three to five chiasmata at diplotene. DARLINGTON and DARK (1932), in discussing chiasma terminalization, assume that "the attachment loop, in expanding, pushes all the others together at the end." But if each chiasma represents a crossover, the accumulation of two or more chiasmata would often result in a complex association of chromatids, as shown in figure 1, diagram B. In numerous species, terminal or sub-terminal chiasmata show no interlocking or asymmetrical relations of the chromatids, even where three or more chiasmata are found at diplotene.

If two crossover chiasmata on the same side of the spindle fiber are terminalized without passing off the ends of the chromosomes, they will be reduced to one interlocked chiasma, or canceled, depending on the types of crossovers. If each chiasma is reciprocal and both crossovers occur in the same two chromatids, the two chiasmata will be canceled, or reduced to one, with equal frequency. If crossing over is at random between homologous chromatids, the two chiasmata would be reduced to one interlocked chiasma in 50 percent of the cases, and reduced to none in 50 percent of the cases. In Primula and Campanula the chiasma frequency is reduced from three or more at diplotene to two terminal chiasmata at metaphase, but there is no evidence of interlocked chromatids in these genera.

On the writer's hypothesis, the reduction in chiasma frequency between early diplotene and metaphase can be attributed to cancellation of chiasmata and breaks in some of the chiasmata as the early diplotene loops open out. Two chiasmata might be reduced to none by cancellation and reduced to one if the other chiasma were broken. Both the genetic and the cytological evidence support MORGAN's (1925) suggestion that crossing over is an accidental by-product of meiosis.

SANSOME (1932) has described a case of chiasma formation in a ring of six chromosomes in Pisum, which seems to support the partial chiasmatypy hypothesis. In 62 out of 78 figures one or two chiasmata were found in the "X segment" of the ring, resulting in a figure-of-eight configuration. Occasionally two chiasmata were found in this region, but the exact

proportions of double and single chiasmata are not given, presumably owing to the difficulty in studying the chromatids in this region. On the writer's theory, two chiasmata must be formed in the X segment if only two chromosomes can pair at the same locus. If one of the chiasmata breaks, the remaining one represents a crossover. The remaining chiasma would be under a special strain, but it seldom breaks.

A much more critical case of the same type has been shown in Zea by BEADLE (1932). Chiasma frequency was obtained at both diplotene and metaphase, and the crossover length of the segment was known. The ring or chain of 4 chromosomes, resulting from segmental interchange, is so constituted that crossing over in one arm is limited to about 12 crossover units between the translocation point and the region where a teosinte chromosome segment has been introduced. When a chiasma is formed in this region, a ring with two free arms is found at diakinesis and metaphase, and when no chiasma is present, the four chromosomes are arranged in a chain. At diakinesis 20 percent of the associations of four chromosomes were in the form of rings, but at metaphase only 10 percent of these chromosomes were rings.

On the writer's theory, at least two chiasmata must be formed in this w_x-translocation region. No terminalization of chiasmata can occur because of the change in homology of the distal segments. As the ring opens out, all the chiasmata in this region may disappear by cancellation (compare figure 2, diagram B). If one chiasma breaks, the remaining chiasma represents a crossover. This chiasma can not be canceled or terminalized. The frequency of such a crossover chiasma in the w_x-translocation segment at diakinesis (20 percent) corresponds with the crossover frequency found in the derived Zea-teosinte chromosomes as bivalents. But at metaphase the chiasma frequency was only 10 percent. If the frequency of ring formation at metaphase is significant, this must mean that two chiasmata were often present at diakinesis and were canceled before metaphase, or that the crossover chiasma was broken. If the crossover chiasma breaks, a double crossover might result, but the two crossovers would probably be too close together to be detected. The effect of a break in the single crossover chiasma on the crossover length of the segment would depend on types of double crossovers obtained.

If chiasma frequency is high at early diplotene, the writer's explanation of chiasma formation in the w_x-translocation segment is not improbable. Two chiasmata might be formed. Unless one chiasma breaks, the original chiasmata probably would be canceled, owing to the strain imposed by the

opening of the ring and limitations in terminalization. If a chiasma breaks, cancellation of the remaining chiasma is impossible, and since the remaining crossover chiasma can not be terminalized, it is found in the w_x-translocation region at diakinesis and metaphase.

Behavior of unequal homologues

In all recorded cases of pairing of unequal homologues, the long chromatids are paired with long and the short ones are paired with short at meiosis. With only one chiasma and a terminal spindle-fiber attachment, it is difficult to explain the occurrence of both pre- and post-reduction in the same bivalent (SAX 1932a, b) as found by WENRICH (1916). This behavior is explained by DARLINGTON (1932) on the assumption that the fiber attachment is median, but the assumption is clearly erroneous, as shown by WENRICH's figures of somatic chromosomes in Phrynotettix.

On the McCLUNG interpretation of chiasma formation, the chromatids must separate equationally at the fiber attachment, where a single chiasma is formed between the fiber and the unequal segments if no crossing over has occurred. This assumption may be valid for some species, although it is apparently not the case in Drosophila or Zea.

Interlocked bivalents

Interlocked non-homologous bivalents have been found in Oenothera, Campanula, Tradescantia, and other genera. According to GAIRDNER and DARLINGTON (1932), three types of interlocking would be expected: (1) proximal interlocking, where the loops containing the spindle fiber are involved; (2) distal interlocking, or locking of terminal loops; and (3) proximal-distal interlocking. If chiasmata are formed by alternate opening of sister and non-sister chromatids at diplotene, interlocking of bivalents could occur only in alternate internodes where sister chromatids are paired. If each chiasma represents a crossover and sister chromatids are always paired at early diplotene, interlocking can occur at any internode. In Campanula (GAIRDNER and DARLINGTON 1932) from two to six chiasmata are found in each bivalent, and the most frequent number seems to be three at diplotene. If each chiasma represents a crossover, distal or proximal-distal interlocking should be at least as frequent as proximal interlocking. But if McCLUNG's theory of chiasma formation is correct, distal or proximal-distal interlocking should be very much less frequent than proximal interlocking. These conclusions are based on the assumption that chiasmata do not pass off the ends of the chromosomes but are pushed together at

the ends of the chromosomes, as postulated by DARLINGTON and DARK (1932). GAIRDNER and DARLINGTON find, in Campanula, that "proximal interlocking occurs in about 20 percent of nuclei in homozygous groups. Distal interlocking seems to be much rarer." Similar observations have been made in Oenothera by CATCHESIDE (1931). Proximal interlocking is common in Tradescantia, but distal interlocking is rare (SAX and ANDERSON 1932). These results indicate that McCLUNG's theory of chiasma formation is correct, or that chiasmata do pass off the ends of the chromosomes before metaphase.

According to GAIRDNER and DARLINGTON, interlocking should occur between the chromatids of paired or unpaired chromosomes if the chromatids ever separate equationally at diplotene. This conclusion is obviously untenable (SAX and ANDERSON 1932).

<div align="center">GENETIC EVIDENCE</div>

<div align="center">*Non-disjunction and crossing over*</div>

In high non-disjunction lines of Drosophila, crossing over is greatly reduced in both the normal progeny and in the exceptional females (ANDERSON 1929). The genetic evidence indicates that non-disjunction is caused by a failure of chromosome pairing at the first meiotic division. The segregation of the univalent homologues will result in no-X and XX eggs which give rise to the exceptional males and females.

ANDERSON found only 7.3 percent crossing over in exceptional females, and most of these crossovers were at the distal end of the chromosome. This distribution of crossovers would indicate that the few chiasmata formed are usually near the distal end of the chromosome, and that they either break or are prematurely terminalized, so that univalent XX's result at the first meiotic metaphase. If non-disjunction is due to premature terminalization, it is difficult to account for the crossovers near the forked locus, if chiasmata represent crossovers, because such a chiasma would have to be terminalized almost the entire genetic length, and for about half of the cytological length, of the X chromosome. If such premature terminalization could occur, one might expect frequent non-disjunction in normal stocks of Drosophila, where the formation of a single chiasma would be expected in the distal half of the X in about two-thirds of the meiotic divisions. But non-disjunction occurs only once in about 2000 times in normal stocks. It would, of course, be impossible to explain crossing over in non-disjunction XX's on the partial chiasmatypy hypothesis if terminal affinity prevents the chiasmata from sliding off the ends of the chromosomes,

as suggested by DARLINGTON (1932). If only one or two chiasmata are formed, and they break, crossing over would be found occasionally in non-disjunction XX's, even in the region of the forked locus. The writer's theory of crossing over seems to provide a more plausible explanation of crossing over in non-disjunction chromosomes than does the partial chias-matypy theory, although neither theory is entirely satisfactory (SAX 1932a).

According to DARLINGTON (1931), chromosome pairing is invariably dependent on chiasma formation. If every chiasma represents a crossover, no normal chromosome can have a crossover length of less than 50 units. Little or no crossing over has been found in the fourth chromosome of *Drosophila melanogaster,* although this chromosome is as regular in pairing and disjunction as the X, which is 70 units long. DARLINGTON (1932) assumes that this fourth chromosome may have a crossover length of 50 units, but that the number of genetic factors is not sufficient to prove it. Regular chiasma formation might be expected in a short bivalent if the chromatids open out reductionally at one end of the chromosome and equationally at the other, as WENRICH (1916) assumes. It has been clearly demonstrated that chromosome pairing is not always dependent on chiasma formation (SAX 1932b), although in many species with long chromosomes pairing at meiotic metaphase does seem to be dependent on chiasmata.

Randomness of crossing over

The genetic evidence shows clearly that crossing over is not confined to two of the four chromatids (ANDERSON and RHOADES 1931, REDFIELD 1930, and others). The first crossover from the spindle fiber in the X chromosome of Drosophila is at random between the homologous (non-sister) chromatids, as shown by the proportion (2:1) of equational and reciprocal crossovers in attached XX's (ANDERSON 1925) and by the percentage of homozygous recessives at the forked locus in the attached XX's (RHOADES 1931, STURTEVANT 1930).

If crossing over is at random at all crossovers, the homozygosis in attached XX's at the distal ends of the chromosomes should be about 20 percent (SAX 1932a). The percentage of homozygosis found by STURTEVANT was 17.1, and by RHOADES 18.6. The deficiency of homozygosis may not be significant, however, owing to the lower viability of the homozygous recessive segregates.

If crossing over is at random for all crossovers at all loci, the first crossover in attached XX chromosomes should be equational or reciprocal in the proportions of 2:1. If the first crossovers are not at random, there

should be an excess of equationals on the writer's theory, and an excess of reciprocals on BELLING's theory. Three types of second crossovers should be found in the ratio of 2:8:1, as follows: (1) equationals homozygous at the distal end; (2) equationals homozygous at the proximal end; and (3) reciprocals. If the second crossover is not at random, the second class of crossovers will be decreased and the third class increased. Random crossing over can occur, on the writer's theory, only if some half twists occur in paired sister chromatids (SAX 1932a). The genetic analysis of attached XX's in Drosophila indicates that the first crossover occurs at random. The data on second crossovers and the percentage of homozygosis at different loci are not adequate for a critical test of random assortment of chromatids at the second crossover.

Absence of crossing over in the Drosophila male

No crossing over occurs in the Drosophila male, but pairing and disjunction of all chromosomes are regular (METZ 1926, GUYÉNOT and NAVILLE 1928). DARLINGTON (1931) assumes that chiasma formation and crossing over are essential for chromosome pairing. He assumes that in the male Drosophila there are always two chiasmata in each bivalent, one on each side of the spindle fiber; that the two chiasmata are very close together; that no mutations occur in the region between chiasmata; and that both crossovers are reciprocals and involve the same two chromatids. These assumptions are not only highly improbable, but also they can not be reconciled with either BELLING's or DARLINGTON's explanation of the mechanism for crossing over. The assumption that all crossovers in the male are reciprocal is difficult to reconcile with the fact that both equational and reciprocal crossovers occur in the female.

The writer assumes that some chiasmata are formed in the bivalents of the Drosophila male, but that these chiasmata are terminalized without breaking, as seems to be the case in several genera. The apparent differences in the duration of the metaphase stages at meiosis in males and females seem to support this assumption.

CHIASMA FREQUENCY AND CROSSING OVER

If each chiasma represents a crossover, it should be possible to estimate the crossover length of the chromosomes in certain species, especially where little or no terminalization of chiasmata is found. DARLINGTON (1931a) attempted to correlate chiasma frequency with crossover length of one of the chromosomes in Primula, but with rather unsatisfactory results (SAX

1932a). Zea should be satisfactory for this purpose, but little is known concerning chiasma frequency at prophase stages.

There is sufficient cytological and genetic evidence to warrant a comparison of chiasma frequency and crossover lengths of the chromosomes of *Vicia faba*. In this species MÆDA (1930b) found, at metaphase and diakinesis, an average of 8.1 chiasmata in the long bivalent and an average of 3.5 chiasmata for each of the five short bivalents.

SIRKS has studied 26 genetic factors in *Vicia faba*. Of these 26 factors, 19 were found in four linkage groups, and only 7 were independent. Of the 19 factors, 7 were found in the first linkage group, 4 in the second, 5 in the third, and 3 in the fourth. In no case did any linkage group exceed approximately 50 crossover units.

If each chiasma represents a crossover, there should be more independent chromosome units than there are factors. The long chromosome should have a crossover length of 400 units, and the average length of the short chromosomes should be 175 units. With such a high frequency of crossing over, it would be highly improbable that 19 of the 26 factors would be found in four linkage groups, none of which exceeds 50 crossover units.

SUMMARY

According to JANSSENS' partial chiasmatypy hypothesis, each chiasma represents a crossover which occurred at pachytene. BELLING has offered the only plausible explanation of how crossing over might occur on this theory, but his explanation is not in accord with certain critical cytological and genetic observations. If each chiasma represents a crossover, it is difficult to account for the prevalence of symmetrical chiasmata in many species and the reduction in chiasma frequency often found between early diplotene and metaphase stages of meiosis. If, as DARLINGTON believes, chromosome pairing at meiotic metaphase is dependent on chiasma formation, it is difficult to account for the absence of crossing over in the Drosophila male, and difficult to explain the crossover length of the fourth chromosome of *D. melanogaster*. If each chiasma represents a crossover, it is difficult to reconcile the cytological observations with the genetic results in *Vicia faba*.

The writer's theory of crossing over is based on the assumption that chiasmata are caused by an alternate opening out of pairs of sister and non-sister chromatids. Crossing over occurs only when two of the four chromatids break at a chiasma. This theory is in accord with the following genetic evidence: only two chromatids cross over at any one locus; one

crossover interferes with a second one; crossing over is reduced in the spindle-fiber region; no crossing over occurs in the male Drosophila; and crossing over is more or less at random between any two homologous chromatids. This theory is supported by the cytological evidence that there is often considerable reduction in chiasma frequency between earliest diplotene and metaphase stages of meiosis, and by the prevalence of symmetrical chiasmata in many species.

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