CHIASMA FORMATION IN LARIX AND TSUGA*

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From the standpoint of chiasma formation and the relation of the chromatids, two essentially different theories have been advanced. The first is JANSSENS' "partial chiasmotypy" hypothesis (1924). This theory enjoins a crossing over between two of the four chromatids, a crossover at every chiasmata, and a pairing of only sister threads at early diplotene. The Janssens' "partial chiasmotypy" theory, with various modifications, is supported by BELLING (1931), DARLINGTON (1931), and MAEDA (1930).

The other hypothesis is that of McCLUNG (1927). The work of WENRICH (1916), ROBERTSON (1916), CAROTHERS (1926, 1931), NEWTON (1927), BELAR (1928), and many others supports this theory. Chiasmata are produced by an alternate pairing of sister and non-sister strands. Chiasmata represent an exchange of partners among the chromatids. According to SAX (1930), a crossover occurs only when two chromatids break at a chiasma. On this theory chiasmata may be present without crossing over.

McCLUNG (1927) has pointed out clearly that when there is crossing over, according to JANSSENS' theory, there should be an asymmetrical arrangement of the chromatids. JANSSENS based his arguments for his theory on cases where the asymmetrical arrangements have been found in certain Orthoptera, but as McCLUNG has pointed out the clearest figures show that symmetrical figures are the usual and most prevalent types. On any theory, crossing over between two of the four chromatids should usually result in an asymmetrical relation of the chromatids. If a single crossover occurs it is possible that the chromatids might be subsequently oriented to produce a symmetrical relation of the chromatids, but in case two crossovers occur in a single bivalent a symmetrical relation of chromatids should be exceptional. Viewed from the end, each of the four chromatids should not lie in the same quadrant at all loci after a crossover has occurred at the four strand stage. As the homologues open out, the chromatids which were adjacent may form the cross at the chiasma if the chiasma is the result of a reciprocal crossover (text figure 1A). As such a chiasma is terminalized there should be a twist in one pair of chromatids. These reciprocal crossovers should occur as frequently as equationals if crossing over is at random. Equational crossovers should also result in asymmetrical relations of the chromatids although it is possible that chro-

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matid movements in subsequent stages might produce symmetrical arrangements of chromatids in some cases. If each chiasma represents a crossover as is postulated in JANSSENS' "partial chiasmotypy" hypothesis, the bivalent chromosomes with one or more chiasmata should show a prevalence of asymmetrical chromatid relationships.

TEXT FIGURE 1.—A. Chromatids at metaphase stage of meiosis according to the Janssens hypothesis. The arrangement of the chromatids is asymmetrical. The chiasmata represent crossing over. The chiasma at the left is formed by adjacent chromatids. On the right the chiasma has terminalized. There is a twist in the upper pair of chromatids. B. Chiasma formation according to the McClung theory. The cross in the chiasma at the left is formed by diagonal chromatids. At the right the chiasma has terminalized and the asymmetrical arrangement is due to a half twist at early prophase.

On McCLUNG's theory, chiasma formation is not the result of previous crossovers, so that a symmetrical arrangement of chromatids might be expected if there are no crossovers or no twists in sister chromatids. Each chromatid should lie in the same quadrant at all loci at the four strand stage, and as the paired chromatids open out at diplotene and metaphase the apparent cross at the chiasma is formed by chromatids which were diagonal at the four strand stage (text figure 1B). A half twist in sister chromatids before or after pairing of homologous chromosomes would produce an asymmetrical relation of chromatids even if no crossover has occurred (text figure 1B). A crossover resulting from a break in two chromatids at a chiasma (SAX 1932) would result in an asymmetrical relation of chromatids.

A prevalence of symmetrical chiasmata have been shown in Orthopteran chromosomes by WENRICH (1916), ROBERTSON (1916) CAROTHERS (1927) and DARLINGTON (1932). As McCLUNG (1927) has pointed out, JANSSENS' (1924) clearest figures also show a symmetrical relation of chromatids. Both symmetrical and asymmetrical chiasmata have been found in *Paonia* (SAX 1932b). The symmetrical double chiasmata show clearly

that no crossovers have occurred in these chromosomes and that chiasma formation is not dependent on a previous crossover. Asymmetrical chiasmata would be expected if a crossover has occurred at another chiasma or if a half twist was formed between sister chromatids. In some cases the cross at the chiasma was formed by the two threads which were adjacent at the four strand stage. If, as the work of WENRICH (1916) and CARTHERS (1931) suggests, sister chromatids are not always associated at the fiber attachment point, these chiasmata might be attributed to an equational separation at the first meiotic division associated with a half twist in sister chromatids.

MAEDA (1930) has observed asymmetrical chiasma formation in *Lathyrus odoratus*. This would be expected as crossing over occurs in *Lathyrus odoratus*, and on any theory of crossing over asymmetrical chiasmata are formed in crossing over.

CHIASMATA AND CHROMATIDS IN LARIX AND TSUGA

The present investigation is concerned with chiasma formation and the relation of the chromatids in Tsuga and Larix. *Tsuga canadensis*, *Larix decidua*, *Larix Kaempferi*, and the hybrid *Larix eurolepis* Henry (*L. Kaempferi* × *L. decidua*) offer very good material for the study of the chromatids.

Permanent smears and paraffin sections of the microspore mother cells of Larix and Tsuga were made. The material was fixed in Navaschin's, La Cours', and Darlington's modification of Flemming's stronger fixatives. Permanent smears with the latter fixative gave the best results. Crystal violet iodine gave the best results in staining the chromosomes. Flemming's triple stain was a better stain for the fibers.

The divisions in the microspore mother cells of *Tsuga canadensis* are especially favorable for the study of chiasmata. The term "chiasma" will be used here to designate the region where there is an exchange of partners among the chromatids. The chiasmata are very similar to those found in Larix (SAX 1932, plate 51), although the chromosomes are somewhat larger. During diakinesis the configurations of chromosomes and the number and position of chiasmata can be observed. The prevalence of interstitial chiasmata suggests that there is very little terminalization before the metaphase stage.

Figure 1, plate 1, shows all the chromosomes drawn from a side view of a late metaphase stage in the first division of a microspore mother cell of *Tsuga canadensis*. They are drawn separately to show the detail of each one. The number and position of the chiasmata can be clearly seen in some of the chromosomes, while in others some of the chiasmata have opened out because of the lateness of the stage. This point is easily inferred

LEGEND FOR PLATE 1

All drawings were made from the first division of the microspore mother cells, with the aid of a camera lucida. The magnification is $\times 3100$.

FIGURE 1.—Twelve chromosomes, a, b, c, d, e, f, g, h, i, j, k, and l are drawn from a single late metaphase stage in the first division of the microspore mother cell of *Tsuga canadensis*. The number and type of chiasmata are shown.

FIGURES 2 and 3.—Bivalents of *Tsuga canadensis*.

FIGURE 4.—Bivalent from *Larix eurolepis* at diakinesis stage with two interstitial chiasmata.

FIGURE 5.—Chromosome from *L. eurolepis* in late diakinesis. Two chiasmata, one subterminal, the other interstitial are present. The chromatids are separated in the homologues.

FIGURE 6.—Chromosome—late metaphase in *L. eurolepis*. Chromatids symmetrical.

FIGURE 7.—Chromosome at late metaphase in *L. eurolepis*. Chromosome shows stretching between chiasma and fiber attachment.

FIGURE 8.—Bivalent, late metaphase in *L. eurolepis*. One interstitial chiasma. Symmetrical arrangement of chromatids.

FIGURE 9.—Bivalent, late metaphase in *L. Kämpferi*. Two subterminal chiasmata.

FIGURES 10 and 11.—Metaphases of bivalents of *L. eurolepis* showing symmetrical arrangement of chromatids widely separated with two interstitial chiasmata.

FIGURE 12.—Metaphase of chromosome of *L. eurolepis* with chromatids separated on left side. Symmetrical arrangement of chromatids.

FIGURES 13 and 14.—Chromosomes with fiber attachment, terminal or subterminal. Chromatids separated. Chromatids symmetrical.

FIGURE 15.—Chromosome, *L. eurolepis*, early anaphase. Asymmetrical arrangement of chromatids.

FIGURE 16.—Chromosome in late metaphase, *L. eurolepis*. One chromatid from each homologue separated, the remaining chromatids still paired. Symmetrical arrangement of chromatids.

FIGURE 17.—Anaphase stage of bivalent, *L. eurolepis*. Asymmetrical arrangement.

FIGURE 18.—The same, showing symmetrical arrangement.

from the large number of stages at diakinesis and metaphase where the chromosomes have not started to divide. There are several chromosomes (figure 1a, d, h, i and k), where the homologues have opened out at one end. In figure 1l, the homologues have completely separated. There are interstitial and subterminal chiasmata which are very distinct in most of the chromosomes shown in figure 1. Some of the chromosomes show an opening where the chromatids change partners. There is considerable strain on the homologues between the chiasma and the point of fiber attachment. They are very narrow in this region. This is shown in *Tsuga* (figure 1a, d, i, and k) and in *Larix* (figure 7).

With very good fixation and lighter stain only the chromatids are seen. The relations of the chromatids at the chiasmata are somewhat more distinct. Figures 2 and 3 from *Tsuga* show a twisting of the free ends of the chromatids near the chiasmata.

The chromosomes in the divisions of the microspore mother cells of *Larix* were especially favorable for the study of the chiasmata and the relations of the chromatids in the bivalents. In the hybrid *Larix eurolepis* the pairing between the chromatids appears to be a little less close, and, in some cases, the individual chromatids are distinctly separated. Though perhaps not so distinct, the chromatids are found to have a similar arrangement in the parents, *L. Kaempferi* and *L. decidua*. In most cases, however, in both parents and hybrids the pairing was too close to follow the chromatids throughout, but the chiasmata are quite distinct and the chromosomes very similar in the hybrids and parents. However, a large number of diakinesis, metaphase, and anaphase figures were found in the hybrid in which the chromatids were distinct, and from these most of the drawings were made.

Figure 4 is drawn from a late diakinesis stage. There are two interstitial chiasmata. The homologues are widely separated between the chiasmata and at the free ends beyond the chiasmata. The chromatids are not separated in this case.

Figure 5 represents a late diakinesis, or very early metaphase stage. The chromatids in the two homologues of the bivalent are separated from each other so that they may be clearly seen. There may be a twisting in the chromatids in this case in the lower half. One chiasma is median between the fiber attachment and the end of the chromosome; the other is subterminal. The homologues are again widely separated except at the chiasmata.

In figure 7, a late metaphase, there is an interstitial chiasma with a distinct opening where the chromatids exchange partners, a slight coiling of the free ends of the chromosomes beyond the chiasma, and a stretching of the chromosome between the chiasma and the fiber attachment, indicating some strain in the separation of the paired chromatids.

In figure 6, which is a very late metaphase, the chiasma is subterminal. The paired chromatids are distinct and are separating at the chiasma. The arrangement of the chromatids is symmetrical.

The details in the separation of the paired chromatids in an interstitial chiasma are easily seen in figure 8. If one imagines the uppermost pair of chromatids rotated from the plane of the plate through the vertical position and to a position making an angle of 180° with the present position, one will recognize that the arrangement of the chromatids in this case is similar to that in figure 6; but the chiasma is interstitial. The arrangement of the chromatids in figure 8 is obviously symmetrical. The diagonal chromatids form the cross. In other words, the union of the homologues here is face to face, and not at an angle and lateral.

Two chiasmata, both subterminal, with a comparatively wide space between the chromatids, are seen in figure 9, which was drawn from *L. Kaempferi*. The arms of the bivalent between the fiber attachment and the chiasma show some coiling.

In figure 10, the chromatids are widely separated so that, except at the point of fiber attachment, they are very distinct and easy to follow. The chiasmata are interstitial. The chromatids from the upper homologue pair separately with those from the lower homologue. Two of the pairs of chromatids are touching at the ends. The other two are paired, but they are not in contact in any place. The chromatids are symmetrical on both sides. No asymmetrical arrangement is possible with such wide separation of the chromatids. If the cross had been made by adjacent chromatids, they would not be free to separate so widely in interstitial chiasmata.

Figure 11 is similar to figure 10, with somewhat thicker chromatids. The chromatids to the left have a connection near the distal end, which may be due to a twisting about each other. It is obvious, however, that the arrangement of the chromatids is symmetrical, since the chromatids of the homologues are separated beyond the region of the chiasmata. On the left side of the bivalent shown in figure 12, the chromatids are symmetrical. They are separated throughout their length.

In figures 13 and 14, the symmetrical arrangement of the chromatids is apparent. The spindle-fiber attachment is near the end of the chromosome. The paired chromatids are separated, and the ends of the homologues are separating.

In figure 15, the chromatids are all distinct, and the ends, where the homologues have paired, are separating. There is an appearance of an asymmetrical arrangement in this case.

A slightly more advanced stage is found in figure 16. The symmetrical arrangement of the chromatids is the only one possible in this case. The fiber attachment is in the middle of the homologues of a bivalent with two chiasmata. The chromatids are very distinct. One chromatid on each side

has separated completely at the region of pairing with its homologue, and has its free end in the position characteristic of the anaphase. The other two chromatids of the homologues were still paired at the ends. The arrangement of the chromatids is obviously symmetrical. An asymmetrical arrangement of the chromatids is impossible in this case.

The arrangement is asymmetrical in figure 17. In this case there is a distinct twist in the chromatids in the upper half of the figure.

Figure 18 is an anaphase. The configuration of the chromatids appears to be symmetrical. The chromatids are distinct. They are held together only at the spindle fiber attachment. In the lower half of the figure, they are loosely associated at the region of the spindle fiber attachment point.

DISCUSSION

The figures described above show a great preponderance of symmetrical figures. Though a thorough search was made among the figures of *Larix* showing separate chromatids, to find chromosomes where the arrangement of the chromatids was obviously asymmetrical, only the few possible cases mentioned above were found. There was manifest almost a total uniformity of symmetrical configurations in the chromatids of *Larix*.

The greater frequency in the occurrence of symmetrical configurations in *Larix* supports the McClung theory. They could not occur if the chromatids had paired according to the Janssens hypothesis.

Very few cases were found where an asymmetrical arrangement of chromatids is found in *Larix*. It is possible in some figures, where a symmetrical arrangement is found, that an asymmetrical arrangement existed earlier and that through a readjustment in the chromatids this asymmetry was straightened out. This may occur where the spindle fiber attachment is near the end of the chromosome. Figures 13 and 14 represent chromosomes of this type. The configurations are obviously symmetrical.

However, a large number of bivalent chromosomes with two chiasmata were found where there was no possibility of an asymmetrical arrangement of the chromatids, which would be expected on the Janssens hypothesis. This arrangement is in agreement with the McClung theory. According to SAX, some cases of asymmetry would be expected on the McClung theory if there was a half twist in sister chromatids before pairing in the early prophase, or if crossing over had taken place.

SAX (1932) found some cases in *Paeonia* where adjacent chromatids form the cross at the chiasma. This may seem to support JANSSENS' "partial chiasmotypy" theory. These cases may be explained on the McClung theory if non-sister threads are held together at the fiber attachment associated with a half twist in one pair of sister chromatids. From the behavior of heteromorphic chromosomes in Orthoptera, according to WENRICH and CAROTHERS, an equational first meiotic division seems to be possible.

The preponderance of the symmetrical arrangement of the chromatids in *Larix* suggests that there is little crossing over in *Larix*, even though the chiasma frequency is relatively high. Previous investigations have shown that the rarity of polyploidy in the conifers may be attributed in part at least to the high chiasma frequency (SAX 1932). The symmetrical chiasmata indicate that little or no crossing over occurs in this group of plants. These two factors, uniformity in chromosome numbers and absence of crossing over between chromosomes, may be of importance in explaining the relative stability of the conifers. In the genus *Larix*, distinct species which have been geographically isolated for long periods of time produce fertile hybrids, and in general the genera of conifers are not highly polymorphic.

SUMMARY

The divisions in the microspore mother cells of *Larix* and *Tsuga* are especially favorable for the study of the arrangement of chromatids because the chromatids are often seen separated in the homologues. In most cases the chromatids are symmetrical in arrangement, and the cross in the exchange of partners is formed by diagonal chromatids. These configurations seem to be incompatible with the present interpretations of the Janssens hypothesis. In a very few cases there are asymmetrical configurations. This arrangement of chromatids would be expected on the McClung theory if either a half twist of sister chromatids or a crossover had occurred in the prophase. The preponderance of symmetrical figures, together with little terminalization of the chiasmata up to metaphase, would indicate that little crossing over occurs and that chiasma formation may be independent of crossing over.

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