

INTERLOCKING OF BIVALENT CHROMOSOMES IN TRADESCANTIA

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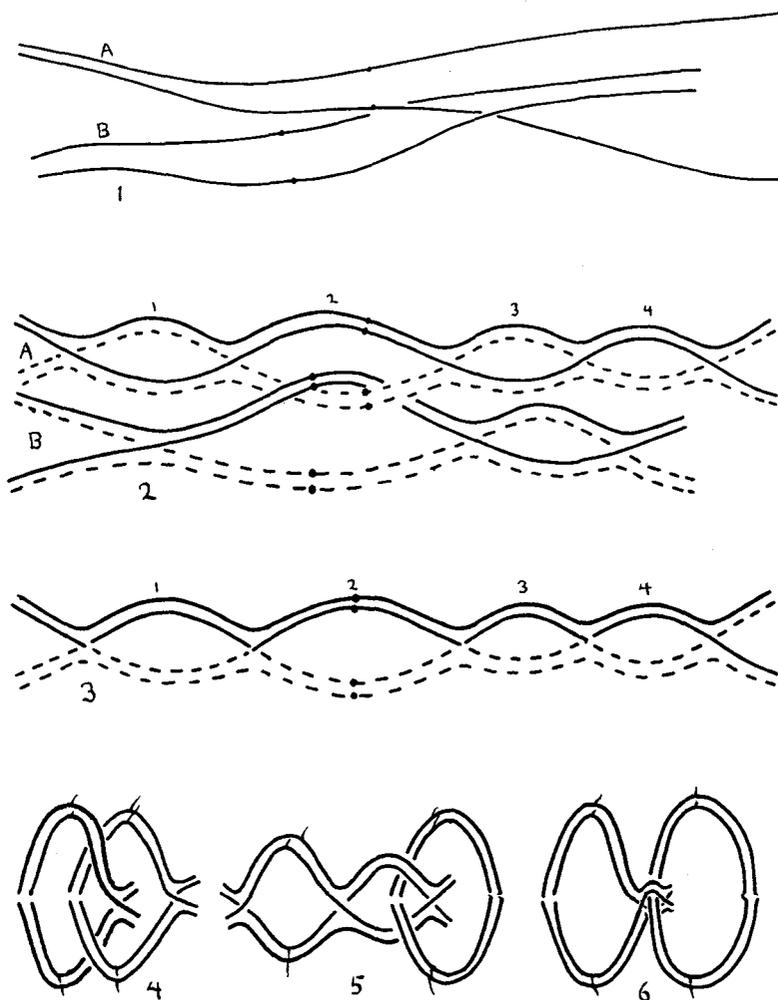
Received May 6, 1933

Interlocking of bivalent chromosomes at meiosis has been described in several genera of plants and animals. In favorable cases it may be seen at pachytene (*Dendrocoelum*, GELEI 1921) and is clearly shown at diakinesis and metaphase in *Stenobothrus* (BELAR 1928) and *Salamandra* (SCHREINER, see BELAR 1928). Interlocking occurs frequently in certain species of *Oenothera* (CLELAND 1922, CATCHESIDE 1931, et al.), *Campanula* (GAIRDNER and DARLINGTON 1931), and *Tradescantia* (SAX and ANDERSON 1933). In most genera interlocking of non-homologous bivalents is rare, if it occurs at all.

The types of chromosome interlocking and the relative frequency of these types provide evidence of value in interpreting the mechanism of chiasma formation and the subsequent behavior of chiasmata at meiosis. Because of the theoretical problems involved, a thorough analysis has been made of the more critical types of chromosome interlocking in *Tradescantia*.

Before describing chromosome interlocking in *Tradescantia* and other genera, the various types of interlocking will be described in relation to current theories of chiasma formation and behavior. At the time of pairing of homologous chromosomes, one homologue may be included between the two homologues of another bivalent, as shown in diagram 1. Such interlocking might occur if the chromosomes begin pairing at the ends, as indicated in the diagram, or if they pair first at the spindle fiber attachment points, which are indicated by dots in the figure. At pachytene the homologous chromosomes would be paired throughout their length with the exception of a short region where interlocking has occurred. Presumably such interlocking between non-homologous chromosomes could occur at any locus.

As the diplotene nodes and internodes are formed, three types of interlocking may be obtained. The two bivalents may be interlocked at the proximal or spindle fiber internodes, or the proximal loop of one bivalent may be locked with a distal loop of the other bivalent, or two distal loops may be interlocked. Proximal interlocking is shown in diagrams 2 and 4, while proximal-distal interlocking is shown in diagrams 5 and 6. The interlocking of two distal loops has not been observed in *Tradescantia*. Inter-



DESCRIPTION OF DIAGRAMS

DIAGRAM 1.—Interlocking of two non-homologous bivalents, A and B, at the time of pairing. The dots represent the spindle fiber attachment points.

DIAGRAM 2.—Interlocking at pachytene, showing the relation of the chromatids at the internodes on the classical theory of chiasma formation. Proximal interlocking, involving the internodes containing the fiber attachment points is shown. Distal interlocking could occur only at internode 4 of bivalent A and could not occur in bivalent B.

DIAGRAM 3.—Chiasma formation at diplotene on the partial chiasmotypy hypothesis. Interlocking could occur at any internode, and if at random, interlocking of distal internodes should be as frequent as proximal interlocking.

DIAGRAM 4.—Proximal interlocking at metaphase after reduction and terminalization of chiasmata.

DIAGRAM 5.—Distal interlocking.

DIAGRAM 6.—Distal interlocking of the type described in *Oenothera* and *Campanula*, but not found in *Tradescantia*.

locking involving a distal internode of one bivalent with the proximal loop of another will be referred to as distal interlocking.

As CATCHESIDE (1931) has pointed out, the types of interlocking will depend on the method of chiasma formation. If chiasmata are caused by the alternate opening-out of sister and non-sister chromatids, the "classical theory," interlocking can occur only at the reductional internodes (2 and 4, diagram 2). Only proximal interlocking is possible unless three chiasmata are formed on the same side of the spindle fiber attachment point. Distal interlocking could occur in only one of the four internodes of chromosome A and could not occur at all in chromosome B. GAIRDNER and DARLINGTON (1931) state that interlocking should occur between chromatids if the classical theory of chiasma formation is correct, but this suggestion is so obviously untenable that it need not be considered here (see SAX and ANDERSON 1933).

If chiasmata are the result of previous crossovers and only sister chromatids are associated at early diplotene, then interlocking could occur at any internode. According to the partial chiasmotypy theory, interlocking could occur at any one of the four internodes shown in diagram 3, and if interlocking is at random, distal interlocking should be at least as frequent as proximal interlocking.

The types of interlocking at diakinesis and metaphase will depend not only on the mechanism of chiasma formation, but also on the behavior of the chiasmata between early diplotene and these later stages. In the plant genera known to possess interlocked chromosomes, the chiasma frequency is from three to five per bivalent at diplotene and is reduced to about two at metaphase. On the classical theory of chiasma formation, chiasmata may be eliminated by cancellation or by breaking (SAX 1932), and in some cases chiasmata may pass off the ends of the chromosomes before metaphase. According to DARLINGTON's interpretation of the partial chiasmotypy theory, chiasmata do not break, but their frequency may be reduced by their accumulation and cancellation at the ends of the chromosomes following terminalization. As a rule, terminal affinity prevents the chiasmata from passing off the ends of the bivalents during the prophase stages (DARLINGTON 1932).

On the classical theory, proximal interlocking should be much more frequent than distal interlocking. The reduction and terminalization of chiasmata shown in the interlocked bivalents of diagram 2 would result in the configuration shown in diagram 4. Distal interlocking, as shown in diagram 5, could occur only if chromosome B were locked with internode 4 of diagram 2 followed by a break in one of the chiasmata between internodes 2 and 4. If no breaks occurred in these chiasmata, they would presumably be cancelled by the opening-out of the proximal loop and by the

limitation in terminalization of the chiasma at the left of internode 4, caused by interlocking, so that at metaphase the interlocking would involve only proximal internodes. But if one of the two chiasmata breaks, then no cancellation would occur; and the distal interlocking would persist until metaphase (diagram 5) unless the terminal chiasma passed off the bivalent, releasing the locked bivalent B. The fact that terminal chiasmata are by far the most frequent in *Tradescantia*, *Campanula* and *Oenothera* would seem to indicate that terminal affinity does prevent chiasmata from passing off the ends of bivalents in these genera.

If the partial chiasmotypy theory of chiasma formation is correct, and if interlocking is at random, distal interlocking should be at least as frequent as proximal interlocking. And if the chiasmata are simply accumulated at the ends of the bivalents, as DARLINGTON (1932) suggests, there should be frequent distal interlocking of the type shown in diagram 6. Cancellation of some chiasmata would be possible so that distal interlocking at diplotene would appear as proximal interlocking at metaphase, but a single chiasma between the proximal loop and the distal loop involved in interlocking could not be cancelled, and many of the double chiasmata between the spindle fiber and a locking internode could not be cancelled, if crossing over is at random. We should, therefore, expect distal interlocking of the type shown in diagrams 5 and 6 to be more frequent than proximal interlocking, if the partial chiasmotypy theory is correct and if interlocking occurs at random.

TYPES OF CHROMOSOME INTERLOCKING IN OENOTHERA AND CAMPANULA

Interlocking of bivalent chromosomes has been described in *Oenothera* by CLELAND (1922) and others, and has been studied critically by CATCHESIDE (1931). *Oenothera Lamarckiana* mut. *pallescens* has a ring of 6 chromosomes and 4 bivalents as the typical meiotic configuration. In 292 nuclei CATCHESIDE found 465 bivalents locked with the ring of 6 and 111 bivalents locked *inter se*. Considering the ring of 6 as 3 potential bivalents, the total amount of interlocking is about 28 percent. Almost all interlocking in this species is proximal. Distal interlocking of the type shown in diagram 6 is described, but these cases are rare and "have been seen only three times in 331 nuclei at diakinesis." If these observations are representative, proximal interlocking is more than 200 times as frequent as distal interlocking in *Oenothera*. According to CATCHESIDE, the most frequent number of chiasmata seen in the bivalents at diplotene is three, though two or four and as many as five are occasionally found. The chiasma frequency per bivalent is 2.0 at metaphase. If the partial chiasmotypy theory of chiasma formation is correct, the percentage of distal

interlocking should be much higher than that observed if interlocking is at random and if chiasmata do not pass off the ends of the bivalents.

Interlocking has been observed in *Campanula* by GAIRDNER and DARLINGTON (1931). Proximal interlocking was found in about 20 percent of the nuclei in homozygous forms. The frequency of distal interlocking is not given, but it "seems to be much rarer." From two to six chiasmata were found in each bivalent at diplotene, and the most frequent number was three, while the chiasma frequency found at metaphase was less than two per bivalent. In both *Oenothera* and *Campanula* the only types of distal interlocking described are those where terminalization has reduced the terminal internode so that it cannot be seen as such, and the two bivalents seem to be in intimate contact. These figures are interpreted as the type of interlocking shown in diagram 6. In both *Oenothera* and *Campanula*, distal interlocking is very rare.

INTERLOCKING OF CHROMOSOMES IN TRADESCANTIA

Interlocking of non-homologous chromosomes has been found at meiosis in all diploid species of *Tradescantia* investigated. In a segmental interchange plant of *T. edwardsiana* the bivalents were locked with the chain or ring of four chromosomes in about 10 percent of the cells, while interlocked bivalents were found in only five percent of the cells (SAX and ANDERSON 1933). In regular diploids the percentage of interlocked bivalents varies greatly in the same species, and there is considerable variation in the same plant. The percentage of interlocking found, ranges from 1 percent to 27 percent. The percentage of interlocking for different plants of various species and the average chiasma frequency per bivalent is shown in table 1. The data are from counts made by EDWARD KING and in most cases were based on 100 pollen mother cells from each plant. The species used include *T. gigantea* Rose, *T. edwardsiana* Tharp, *T. bracteata* Small, *T. hirsutiflora* Bush, a variety of *T. reflexa* Raf., and natural hybrids between *T. reflexa* and *T. humilis* Rose.

In all the above plants described there were usually six bivalent chromosomes at meiosis. Occasional univalents were observed at metaphase, and in rare cases two bivalents were apparently connected at, or near, the fiber constriction points. No segmental interchange rings or chains were found, although they occur frequently in a few plants of some of the same species used (SAX and ANDERSON 1933).

The correlation between chiasma frequency and the percentage of interlocking is positive ($r = .4 \pm .1$). Interlocking might be expected to decrease the chiasma frequency by preventing the normal rate of pairing, and if the chiasma frequency is greatly reduced, interlocking would necessarily be reduced. But even in plants with the lower average chiasma frequencies,

most of the meiotic chromosomes are ring bivalents, since triple chiasmata are rare. And since almost all interlocking is proximal, the lower chiasma frequencies are not low enough to account for the lower percentage of interlocking. Interlocking does not seem to decrease chiasma frequency in *Tradescantia*, and the positive correlation between chiasma frequency and percentage of interlocking may be significant.

TABLE 1
Average chiasma frequency per bivalent and percentage of interlocked bivalents in 28 *Tradescantia* plants.

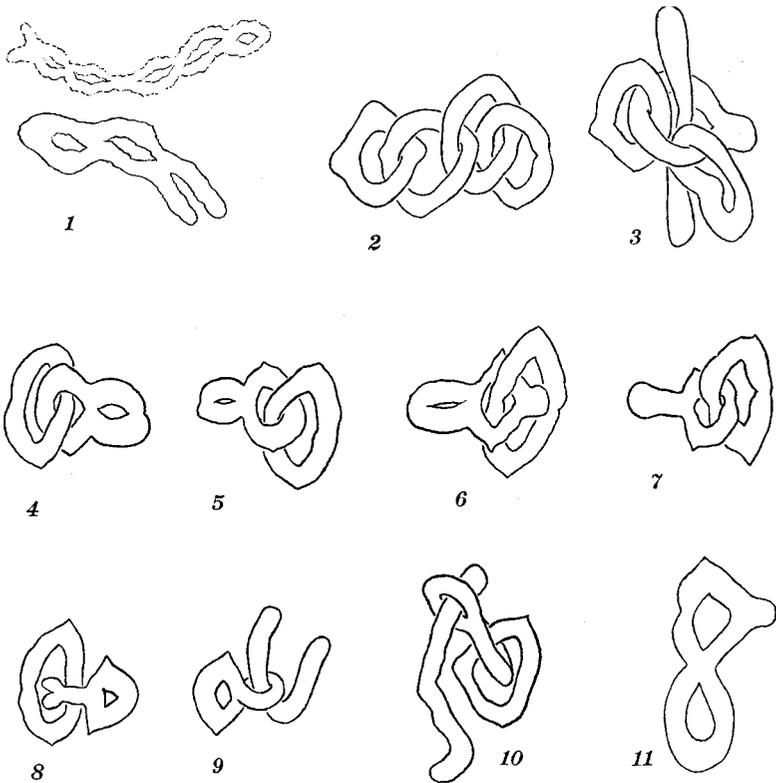
		Chiasma frequency per bivalent													
		1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	n
Percentage	0														
	5		2	2	2	2		1							9
Interlocked	5														
	10				2	2	1	1			2		1		9
Bivalents	10														
	15	1	1		1		1	1	1						6
	15														
	20								1		1		1		3
	20														
	25														
	25														
	30										1				1
		1	3	2	5	4	2	3	2		4		2	28	

Tradescantia edwardsiana seemed best suited for a detailed study of the types of interlocking because the chromosomes were less contracted at metaphase, and the spindle fiber attachment points could usually be observed. Smears of pollen mother cells were made in a modified aceto-carmin solution containing about 3 parts of aceto-carmin, 1 part of glycerine, and enough iron alum solution to intensify the stain. The smears were heated and the cover glass pressed gently with absorbant paper to remove the excess fixing fluid and to flatten the cells. Such preparations, when sealed, remain in good condition for several months or longer.

About 9 percent of the bivalents were found interlocked in *T. edwardsiana*, plant number 15. In most cases the interlocking involved two ring bivalents. Photographs of these simple types of interlocking have been shown in an earlier paper (SAX and ANDERSON 1933). More complicated cases of interlocking are found frequently. Interlocking of four ring bivalents is shown in figure 2. Occasionally a ring bivalent is interlocked with two or more rings or rods (figure 3).

Bivalents with two internodes constituted about 4 percent of the meiotic

chromosomes. Of these double looped chromosomes about 10 percent were interlocked with a ring or rod bivalent. In these cases interlocking may be either proximal or distal. Types of proximal interlocking, where the ring or rod bivalent is locked in the internode containing the spindle fiber attachment point, are shown in figures 4, 5, 6, and 7. Types of distal interlocking with the ring or rod locked in the distal loop, are shown in figures 8 and 9. A total of 20 cases of proximal interlocking was found in these double internode chromosomes, while only 3 cases of distal interlocking



Drawings from aceto-carmine preparations of chromosomes of *Tradescantia edwardsiana*. $\times 1300$.

FIGURE 1.—Two chromosomes from the same pollen mother cell. Three or four chiasmata per bivalent are often found at diplotene, and as many as five or six may occur.

FIGURE 2.—Interlocking of four ring bivalents at metaphase.

FIGURE 3.—Two ring and one rod bivalent locked in a ring bivalent.

FIGURES 4-7.—Types of proximal interlocking involving one bivalent with two internodes. Most of the interlocking found was between simple ring bivalents.

FIGURES 8-9.—Types of distal interlocking. Only 0.6 percent of all interlocking is distal.

FIGURE 10.—Proximal and distal interlocking in the same bivalent.

FIGURE 11.—An association of two bivalents attached near the fiber constriction points. These rare types of association may be the immediate result of segmental interchange following interlocking.

were found. In addition, one case of proximal-distal interlocking was found with a bivalent locked in each internode of the double loop chromosome (figure 10). In these chromosomes with two internodes the proportion of proximal to distal interlocking was 21:4, or 16 percent of the interlocking was distal. But for all interlocking, including the interlocked ring and rod bivalents, distal interlocking was found in only 0.6 percent of the cases, about the same percentage as CATCHESIDE observed in *Oenothera*.

In *Campanula* and *Oenothera* the apparent intimate contact of chromosomes at the terminal chiasmata is interpreted by GAIRDNER and DARLINGTON and by CATCHESIDE as distal interlocking of the type shown in diagram 6. No such types of interlocking have been observed in *Tradescantia*, although the chromosomes are undoubtedly more favorable for a study of interlocking than are those of *Oenothera*. Occasionally ring bivalents were found which seemed to be attached at or near the spindle fiber attachment points as shown in figure 11. In rare cases these chromosomes seemed to be in the form of a figure 8. They are not like the segmental interchange rings of four chromosomes, but they may be the immediate result of segmental interchange (SAX and ANDERSON 1933).

Interlocking in bivalents with three chiasmata occurs in the same proportion as interlocking in bivalents with two chiasmata. Nine percent of the rings were interlocked, while 10 percent of double internode chromosomes showed either proximal or distal interlocking. These proportions seem to indicate that the chiasmata do not pass off the ends of the chromosomes, thereby releasing distally interlocked bivalents.

The chiasma frequency at early diplotene could not be determined accurately. The prophase stages do not fix well, and chromosome development between pachytene and diakinesis seems to be very rapid. As many as five or six chiasmata per bivalent have been observed at diplotene, and bivalents with three or four chiasmata are often found. Two bivalent chromosomes from one pollen mother cell are represented in figure 1. One bivalent appears to have six chiasmata. The other bivalent has only three chiasmata, but it is undoubtedly further developed, and may not be typical for the chiasma frequency at early diplotene. The chiasma frequency at metaphase in *T. edwardsiana* No. 15 is 1.7 per bivalent. Apparently there is considerable reduction in chiasma frequency between diplotene and metaphase.

The chiasma frequency at diplotene in *Tradescantia* seems to be at least as high as it is in *Campanula* and *Oenothera*, where three per bivalent seems to be the usual number, and as many as five are found. With such a high chiasma frequency, distal interlocking should be at least as frequent as proximal interlocking if all internodes at diplotene are reductional,

as postulated by JANSSEN'S hypothesis, and if interlocking occurs at random at any locus. But if the classical theory of chiasma formation is correct, distal interlocking can occur only in bivalents with three or more chiasmata on the same side of the spindle fiber attachment point. Since three of the chromosomes of *Tradescantia edwardsiana* are approximately isobrachial and the other three have submedian fiber attachment points, no distal interlocking would be expected unless four or more chiasmata were formed in a bivalent at diplotene. And if distal interlocking did occur in such chromosomes, cancellation of chiasmata would change some of the distal interlocking at diplotene to proximal interlocking at metaphase, the frequency depending on the amount of crossing over. If the classical theory of chiasma formation is correct, we should expect distal interlocking to be comparatively rare. In *Tradescantia* 99.4 percent of all interlocking is proximal, and only 0.6 percent is distal. Essentially the same proportion of proximal and distal interlocking is reported in *Oenothera* by CATCHESIDE. The relative frequency of the two types of interlocking in *Tradescantia*, *Oenothera* and *Campanula* seems to support the classical theory of chiasma formation.

The results cannot be taken as conclusive evidence in favor of the classical theory because the nature of the interlocking at pachytene is not known, and it is improbable that it can be accurately determined. It is possible that the interlocking usually occurs near the spindle fiber attachment point at pachytene. If the chromosomes begin pairing at the ends, any interlocked chromosome might be forced away from the ends and finally become locked near the middle of the chromosome. It is also probable that interlocking is associated with segmental interchange, since interlocking has been found only in genera where segmental interchange is commonly found. Even though no segmental interchange rings and chains are found in any of the *Tradescantia* plants described in this paper, it is possible that segmental interchange has occurred, involving chromosome segments too short to permit the formation of interchange rings, but perhaps long enough to cause some entangling of chromosomes at the time of pairing. If these short interchange segments are most frequent near the fiber constriction point, they might have some influence in causing an excess of proximal interlocking by the attraction of homologous segments in different bivalents, or by limiting chiasma formation to the distal regions of the bivalents.

SUMMARY

Interlocking of non-homologous chromosomes at meiosis has been found in all the diploid species of *Tradescantia* so far examined. In 28 plants studied critically, 8.7 percent of all bivalents were interlocked at meta-

phase. As has been reported for *Oenothera* and *Campanula*, distal interlocking was much less frequent than proximal interlocking.

If interlocking is at random at all loci of the pachytene chromosomes, distal interlocking should be at least as frequent as proximal interlocking if the partial chiasmotypy hypothesis of chiasma formation is correct, while distal interlocking should be very rare if the classical theory is correct. The rare occurrence of distal interlocking would seem to favor the classical theory. It is possible, however, that interlocking is not at random at pachytene, and that most interlocking occurs near the spindle fiber attachment point. It is also possible that distal interlocking is often released by the passage of a chiasma off the end of the bivalent, although the frequency of terminal chiasmata and the equal proportions of interlocking in single ring and double ring bivalents would not seem to support this suggestion.

Chromosome interlocking does not decrease the chiasma frequency per bivalent, and there may be some positive correlation between chiasma frequency and interlocking. The occurrence of interlocked bivalents is probably correlated with segmental interchange between non-homologous chromosomes, even though no multivalent associations are found at metaphase.

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