THE INTERLOCKING

of

NON-HOMOLOGOUS BIVALENTS

in

TRILLIUM ERECTUM L.

by

ERIC ROGER BOOTHROYD

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Table of Contents.

Table of Contents(cont.)

1. INTRODUCTION.

In the course of the cytological studies of cereals conducted in this department it was noticed, in a number of cells, that two or more bivalents were interlocked with each other at meiotic metaphase. The percentage of cells containing such interlocked bivalents is unusually high in the speltoid mutants of wheat (Triticum vulgare Host), unbalanced types having 40 or 42 chromosomes with a homozygous deficiency instead of the usual 42, and resembling Triticum Spelta more than T. vulgare.

When, during the making of Trillium erectum L. smear preparations in January, 1940, certain slides were found which contained a high percentage of interlocked bivalents, Dr.S.G.Smith suggested that a study of this most favourable cytological material, in which all five chromosomes are morphologically distinguishable, and in which all four chromatids can be traced, might throw some light on the question of interlocking, the cause of which had worried investigators for so long in their wheat studies.

Many investigators have noted the interlocking of non-homologous bivalents, but few have studied it. Of these few none has studied it in such favourable material as Trillium, several investigations, in fact, being carried out on mass stained preparations.

Considering these facts, the present investigation was undertaken in the hopes of being able to discover some of the factors concerned with the interlocking of non-homologous bivalents.

II REVIEW OF LITERATURE.

As early as 1906 bivalent interlocking had been seen in Salamandra maculosa (Schreiner and Schreiner), and since that time it has been noted by many workers in a great number of different organisms. In spite of its early discovery, however, no comprehensive study of interlocking has been undertaken.

Mechanics of Interlocking.

The interlocking of non-homologous bivalents is thought by most workers to be the result of a mechanical accident occurring during zygotene pairing - Catcheside (1931), Gairdner and Darlington (1931), Mather (1935). With few exceptions, chromosomes are fortuitously distributed in the nucleus before they pair in meiotic prophase. When two homologues come together, therefore, one or both members of another pair may be caught between them, resulting, with the formation of chiasmata, in true or false interlocking respectively. Such interlocking has been seen taking place at zygotene in Dendrocoelum (Gelei 1921) and in Viviparus (Belar 1928).

Dark (1936), working with Paeonia, and Upcott (1939), working with Tulipa, found interlocking to be far more common in tetraploid than in diploid plants. The possible explanation given by both writers was that crowding of the chromosomes in the tetraploids would increase the chances of an interlock occurring.

Sax and Anderson (1933 and 1934) suggest that bivalent interlocking may not be entirely mechanical in its origin, but

may be the result of the interchange of very short segments which would cause intertwining and subsequent interlocking of the chromosomes. These interchanges would be too small to be morphologically distinguishable. Dark agrees that true interlocking occurs at zygotene, but suggests that false interlocking, where both members of one pair of chromosomes pass between those of another pair, may be the result of chromosome movements after zygotene. In Eremurus spectabilis Upcott (1936) found false interlocking rare at metaphase compared with diplotene and diakinesis. She explains this as probably due to the bivalents slipping apart. Considering chromosome repulsions this seems more probable than that they should get pushed through each other, as Dark suggests.

In all the cases of bivalent interlocking which he noted in Paeonia, Dark found that the chromosomes were morphologically similar. This suggested that they might be homologous, and become sufficiently closely associated to interlock either (a) through secondary pairing, or (b) through pachytene pairing to form a quadrivalent which did not persist owing to failure to form the necessary chiasmata.

With regard to the position of interlocking Mather (1935) states that if pairing occurs at random along the chromosomes the position of interlocking should be random, and if there is no terminalisation of chiasmata it should remain so. If, however, pairing starts at the ends of the chromosomes interlocking should be most frequent in the central region, and consequently if the

attachment is submedian most of the interlocking would be proximal.

Catcheside (1931), working with a mutant variety of Oenothera Lamarkiana having four bivalents and a ring of six chromosomes, found frequent interlocking of the bivalents both with the ring and with each other. He found that the relative chances of a bivalent interlocking with the ring or with another bivalent (3.13:1) are similar to the relative lengths. The interlocking in this Oenothera appeared to occur at random between the bivalents.

Darlington (1931) found two kinds of interlocked rings in Oenothera, two rings locked as links of a chain, and rings locked to form an apparent multiple chiasma. A ring of four chromosomes is formed by pachytene association in the form of a cross, and terminalised to form a ring at metaphase. Interlocked rings result when one chromosome passes between two others near the attachment, chiasmata being formed on either side of the attachment and distal to the interlock and subsequently terminalising. Interlocking of the apparent multiple chiasma type presumably results from one chromosome passing through two others in the distal region, with chiasmata formed on either side in that arm and terminalised in the same direction.

Marquardt (1937) compared two varieties of Oenothera, Oe. Hookeri and Oe.Hookeri flava sulfurea (genetically Oe.Hookeri, but in suaveolens cytoplasm). In Oe. Hookeri nearly all the bivalents were rings with terminal chiasmata, while in Oe. Hookeri flava sulfurea a high percentage were rods at diakinesis. The

difference (0.82% and 5.86%) was very significant. When compared with regard to interlocking, however, they were found to be the same (21.5% and 22.4%). He explained this independence of interlocking and chiasma frequency by suggesting that interlocking occurs only in the central heterochromatic region of the chromosome, while chiasmata are always formed distal to this region. Such a supposition, however, would not explain distal interlocking as indicated in Darlington's second type of interlocked rings, which simulate multiple chiasmata.

Anderson and Sax(1936) found the percentage of interlocking varied greatly within species of Tradescantia, the variation between plants being from 0 to 27%. Finding no corresponding variation in chiasma frequency, although according to Sax and Anderson (1934) " the correlation between chiasma frequency and the percentage of interlocking is positive " they state that this variation in the percentage of interlocking is due to minor environmental factors not affecting chiasma frequency. In Eremurus spectabilis, Upcott (1936) found a variation of from 0 to 15% of the cells containing interlocked bivalents. She suggests that such local variation in the amount of interlocking might be due to variations in the speed of early prophase stages. If rapid, the chances of homologues lying together before pairing begins would be remote, and interlocking would be probable. If slow, on the other hand, the homologues would have time to approach each other before pairing actually starts, and the chances of interlocking occurring would be small. These two explanations may be the same, since minor

environmental factors, such as change in temperature, might affect the rate of prophase.

Interlocking and Chiasma Formation

There are two main theories of chiasma formation, the alternate opening out, or " classical " theory (McClung 1914 et seq.) and the partial chiasmatype theory (Janssens 1909 and 1924). According to the classical theory chiasmata are formed by the alternate opening out of the chromatids in the equational and reductional planes. The partial chiasmatype theory states that sister chromatids are paired throughout their length, and chiasmata occur as the result of breaking and rejoining of the chromatids, that is they are actual genetical cross-overs. The configurations of interlocked bivalents may afford some evidence for distinguishing between these two theories, and it is with this fact in mind that a great deal of the work on interlocking has been done.

(a) Classical Theory

Sax (1932) quoting Gairdner and Darlington (1931) states that three types of interlocking are expected at metaphase, proximal with the attachment loops involved, distal with one or more chiasmata between the interlock and the attachment, and proximal-distal, proximal in one bivalent, distal in the other. If chiasmata are formed by the alternate opening out of the chromatids in the equational and reductional planes, interlocks could occur only in alternate internodes. If, on the other hand, chiasmata represent cross-overs, and sister chromatids are always paired at early diplotene, interlocking could occur at any internode.

In Campanula Gairdner and Darlington found an average of three chiasmata per chromosome at diplotene. If each chiasma represents a cross-over Sax states that distal and proximal-distal interlocking should be as common as proximal. If, however, McClung's alternate opening out theory of chiasma formation is correct, proximal interlocking should be much more frequent than the other types. They found proximal interlocking to be far more common than the other types, which Sax considers to be evidence either for McClung's theory, or that chiasmata pass off the ends of the chromosomes before metaphase.

In Tradescantia Sax and Anderson (1934) found 99.4% of the interlocks were proximal, and a similar percentage was reported by Catcheside (1931) in Oenothera. Sax and Anderson considered these to be strong evidence in favour of the classical theory of chiasma formation. Later, however, they reconsidered the importance of this excess of proximal interlocking in Tradescantia (Anderson and Sax 1936), suggesting that it may be due to the fact that the chromosomes start pairing at the ends. At this time chiasmata are formed. By the time the central regions pair chiasma formation is inhibited. If this is the case it invalidates such evidence in favour of the classical theory of chiasma formation.

(b) Partial Chiasmatype Theory.

Gairdner and Darlington (1931) state that the character of the interlocking observed at metaphase in organisms with terminalisation will depend on two factors. (1) the way chromatids separate

in chiasma formation (equational and reductional or only reductional and (2) the way terminalisation takes place (with or without breaking of chiasmata). They reiterate the hypothesis that identical pairs of chromatids (sister chromatids) fall apart together at diplotene, so that chiasmata only arise as the result of crossing-over. Chiasmata do not break, but move along towards the ends of the chromosomes, this terminalisation taking place away from the attachment.

They state that the occurrence of distal interlocking shows that the two chiasmata on either side of the interlock are able to withstand the exceptional strain without breaking during the terminalisation. It cannot, therefore, be supposed that they ever break in the absence of any exceptional strain. Further, interlocking was not found between chromatids of paired or unpaired chromosomes, yet such interlocking should occur, both with and without terminalisation, if the chromatids ever separate equationally at diplotene. These two facts are in accord with the chiasmatype hypothesis, and exclude earlier suggestions that crossing-over is conditioned by breaking of chiasmata, and that chiasmata arise by the meeting of alternate equational and reductional loops.

Four cases of double interlocking have been reported, where a loop in one bivalent is locked with two adjacent loops of another, and around the chiasma between them. The first such case was reported by Mather (1933) in Lilium regale, and it has since been seen by Upcott (1936) in Eremurus spectabilis, by Beal (1936) in

Lilium elegans, and by Straub in Gasteria (Oehlkers 1937).

Mather states, that, in the case of his double interlock, if chiasma formation were by alternate opening out of the chromatids then in one loop chromatids of one chromosome are on opposite sides of the interlocking chromosome. Since interlocking must occur before chiasma formation, this is absurd, therefore the central chiasma must have arisen by genetical crossing-over.

The configuration could have arisen by opening out if there had been two chiasmata in the centre, one of which had subsequently broken. This is unlikely unless interlocking causes chiasma breakage, since reduction in the number of chiasmata from diplotene to metaphase is slight in Lilium. Mather's material , however, was mass stained, and therefore it would be difficult, if not impossible, to distinguish between one chiasma and two very close together. Upcott states that in Eremurus there is no reduction in chiasma frequency from diplotene to diakinesis. The central chiasma must therefore have arisen by crossing-over.

These cases of double interlocking are strong evidence in favour of the chiasmatype theory.

On the classical theory of chiasma formation interlocking should tend to reduce the chiasma frequency of the affected bivalents (Mather 1935), for if an equational loop were to open out in the interlocked region of one bivalent it would not persist since this would necessitate chromatid interlocking. The formation of an equational loop would, therefore, be prevented and the chiasma frequency reduced. If the chiasmata were due to crossing-over,

however, interlocking could occur at any loop, and would not reduce chiasma frequency since crossing-over would be unaffected by interlocking at pachytene. He found no noticeable reduction in chiasma frequency in the interlocked bivalents of Lilium.

In an analysis of ten true interlocks Mather found that five were in the loop next to the attachment loop, where interlocking would be impossible according to the classical theory of chiasma formation.

Huskins and Smith (1935), Dark (1936), and Darlington (1937) all state that interlocked bivalent configurations were found which were explicable only on the partial chiasmatype theory of chiasma formation, and not on the classical theory.

Interlocking and Terminalisation of Chiasmata

Gairdner and Darlington (1931) have stated that the occurence of distal interlocking shows that terminalisation had occurred without breaking of chiasmata. It cannot, therefore, be assumed that chiasmata ever break during the terminalisation in the absence of this exceptional strain imposed by interlocked chromosomes.

According to Upcott (1939) movement of chiasmata is slight in Tulipa. When two bivalents each with one chiasma in each arm are interlocked, however, there is complete terminalisation of the chiasmata, a condition never observed in free bivalents of this type. This throws further light on the mechanics of terminalisation. Evidently the body repulsions of the chromosomes, increased by interlocking, reinforce their " centric " repulsions, and are strong enough to bring about terminalisation of the chiasmata.

Pellew and Sansome (1931) found in Pisum savitum a configuration in which two pairs of segments of a segmental interchange ring quadrivalent were interlocked. The number of chiasmata was unusually high, ten being observed, four more than had been seen in any other configuration. They suggest that interlocking might increase the number of chiasmata by increasing the number of breaks in the chromatids at earlier stages or by hindering terminalisation.

Since this configuration contained distal interlocking, and cancellation of chiasmata is not possible, it may reasonably be assumed that distal interlocking would tend to hinder terminalisation, whereas proximal interlocking would tend to aid it.

General Appearance of Interlocked Bivalents.

Mather (1835) found in Lilium that when bivalents were interlocked the loops involved were generally enlarged. A similar condition was observed by Upcott (1936) in Eremurus. She also found that the two bivalents orientate themselves at right angles, as do successive loops of one bivalent, this obviously being the position of equilibrium. In the case of false interlocks both Mather and Upcott state that the outer bivalent is always found to be around a chiasma in the inner one. Anywhere else the repulsion of the expanding arms of the loop would tend to push it towards one or other chiasma. One exception was found by Upcott, but the inner loop was not fully expanded. She suggests that in this case the outer bivalent must have been in the central equilibrium position, where the forces tending to push it towards one chiasma equalled those tending to force it towards the other.

The appearance of these interlocked bivalents and the cases of terminalisation of chiasmata in Tulipa noted by Upcott indicate the non-specific nature of the repulsions between the chromosomes.

As a result of interlocking the proper orientation of the chromosomes on the metaphase plate is frequently upset.

Belling (1927) suggested that interlocking of chromosomes may cause segmental interchange. Sax and Anderson (1933) found an apparent correlation between interlocking and segmental interchange in several species of Tradescantia. Koller (1936) found a configuration of two bivalents which indicated that they had been interlocked, and then broken. The ends had rejoined in such a way that they would give translocations.

111. MATERIAL AND METHODS.

Corms of Trillium erectum L. were collected at Ste. Agathe, Quebec, in September and October 1937 and 1939. Table 1 is a summary of the history of the material from the time it was collected until the slides were prepared. The corm from which slide 58-5d3 was prepared was placed directly in the temperature chamber when collected. All other material was first kept in the refrigerator. When meiosis occurred the anthers were removed, smeared, desiccated for twenty to thirty seconds, and fixed in La Cour's 2 BD fixative for six to eight hours. The slides were then washed in water, bleached in a solution of equal parts of hydrogen peroxide and 70% alcohol for one or two hours, and stained with crystal-violet according to the method described by Huskins and Smith (1935)

Slide 58-5d3 was prepared by Dr. G. B. Wilson, 65-M-9a and 65-M-9c were prepared by Dr. H. B. Newcombe, and slides 66-T-81-c, 66-T-85-d, 66-T-85-f, and 66-T-89-1 were prepared by Dr. S. G. Smith.

Observations were made with a Zeiss 1.5 mm., 1.3 N. A. objective combined with 7x, 15x, and 20x oculars. Drawings were made with the camera lucida at an original magnification of 3875x.

TABLE 1.

History of Material

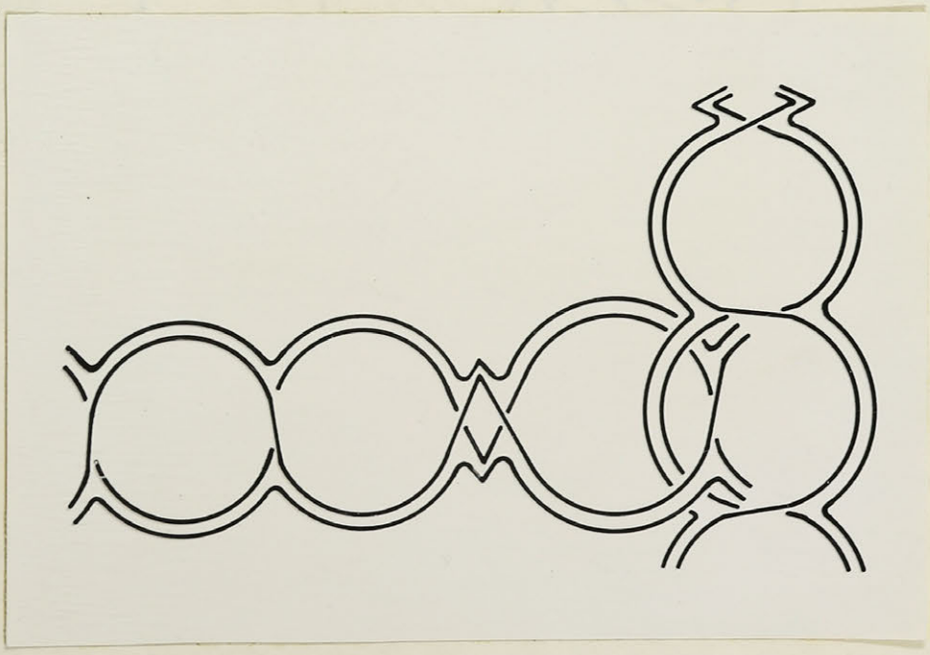
Material	Pretreatment	Where Meiosis Occurred	Temperature	Dates of Treatments.
58-5d3		Temperature chamber 5.	16° C	Sept.30-Nov. 23, 1937.
65-M-9a	refrigerator		3°- 4° C	Oct.4-Nov.11, 1937.
65-M-9c		"Mouse Room"	22°- 24° C	Nov.11-Dec.1, 1937.
66-T-81-c	refrigerator		3°- 4° C	Sept.-Oct.13 1939.
		Temperature chamber 3	8°- 10° C	Oct.13,1939- Jan.6,1940.
66-T-85-d	refrigerator		3°- 4° C	Sept.-Oct.12, 1939
66-T-85-f		Temperature chamber 2	4°- 6° C	Oct.12,1939 - Jan. 15,1940.
66-T-89-1		refrigerator	3°- 4° C	Sept.1939 - Jan.11,1940

IV. Observations.

General Appearance of Interlocked Bivalents.

Early prophase stages were not examined for interlocking, but interlocked bivalents were observed at diplotene, diakinesis, and metaphase. These interlocks were of two main types, namely true interlocks and false interlocks.

True interlocks are those in which one pair of chromatids of one bivalent pass between the pairs of chromatids of another bivalent, the interlock being retained by the presence of chiasmata on both sides of it in both of the bivalents involved (see Text Fig.1).

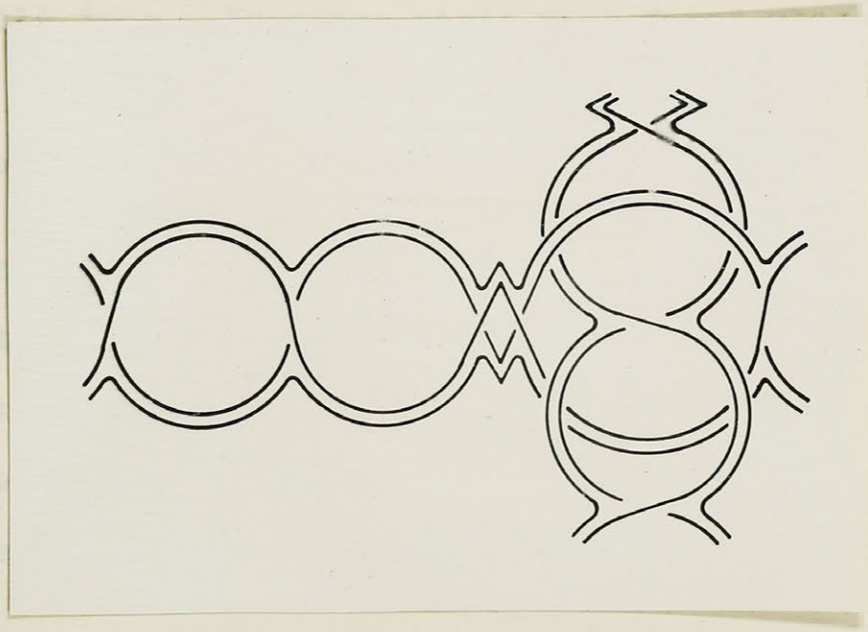


Text Fig. 1. - Diagram of the relationship of the chromatids in a true interlock.

Incomplete true interlocks were also found, in which one of the bivalents involved had failed to form one of the necessary distal

chiasmata. There is, therefore, the possibility that such interlocks might slip apart before metaphase.

False interlocks are those in which all four chromatids of one bivalent pass between the chromosomes of another, with chiasmata in the outer bivalent on either side of the interlocked bivalent (see Text fig. 2). Since false interlocks are not held in place by



Text Fig. 2. - Diagram of the relationship of the chromatids in a false interlock.

chiasmata in both bivalents it should be possible for them to slip apart before metaphase.

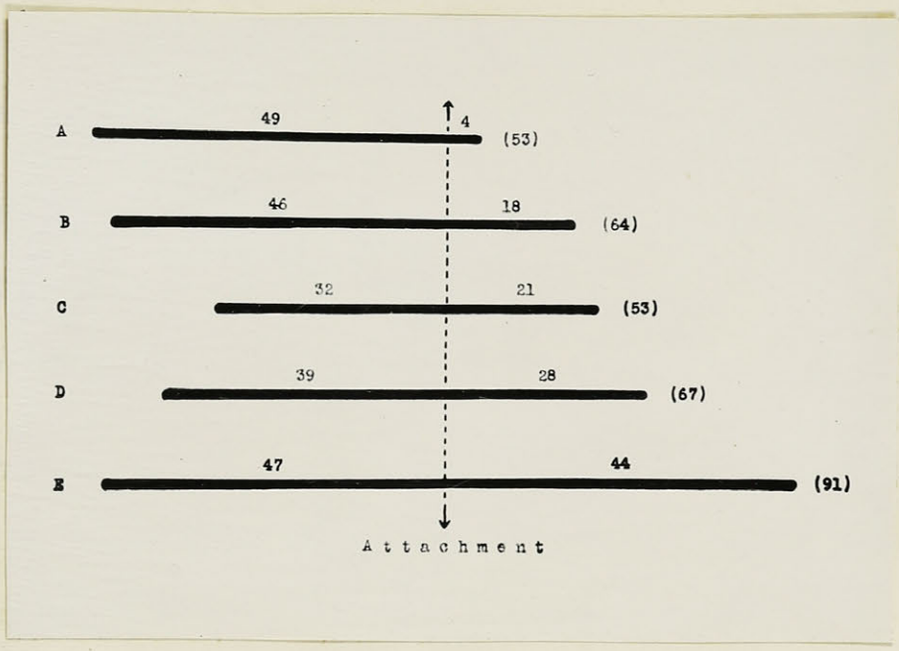
In the case of diplotene interlocks the bivalents concerned show no definite orientation with regard to each other, frequently

lying parallel, with even the interlocked loops nearly parallel. By diakinesis, however, the bivalents generally lie at right angles to each other. In cases where the bodies of the bivalents lie nearly parallel, the interlocked loops, at least, tend to be nearly at right angles to each other. This orientation of the loops appears to be an equilibrium position attained as a result of the mutual repulsion of the chromosomes. The loops of the bivalents involved in interlocking are frequently enlarged, but this is not universally true. Numerous cases were found where the interlocked loop was as small as the adjacent loops, or even smaller. The enlarged condition appeared to be more common.

Unlike the condition reported by Mather (1935) in Lilium, and Upcott (1936) in Eremurus, where the outer bivalent of a false interlock is always around a chiasma in the inner one, in Trillium it was found that the outer bivalent was as frequently between chiasmata in the inner one as it was around a chiasma.

In cells containing interlocked bivalents the number of bivalents involved varied from one locked on itself to all five involved in interlocks. In the case where all five bivalents were involved three were locked together to form a chain, while the other two formed a separate interlock. There were, therefore, only three interlocks in the cell. More than three interlocks were never observed in one cell.

The three bivalents with submedian attachments, the C, D, and E (see Text fig. 3) were all found locked on themselves, the



Text Fig. 3. - Diagram of the relative lengths of the chromosomes and chromosome arms.

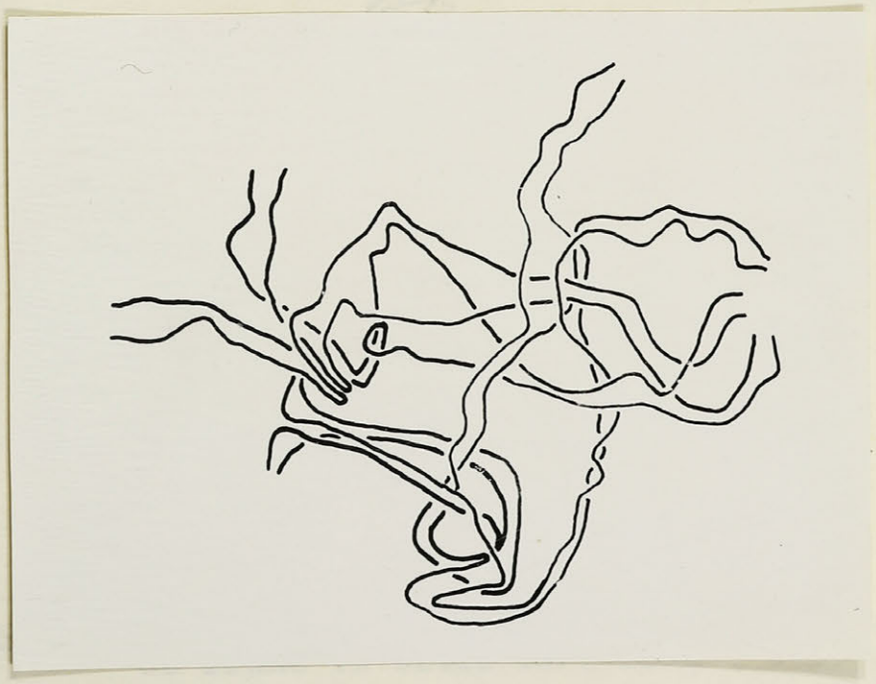
E eleven times, the C twice, and the D once. In these cases of bivalents locked on themselves the two arms were interlocked with each other in either a true or false interlock. One arm of a bivalent was never found locked on itself.

Two cases were found in which two bivalents were locked together in two separate places. In slide 65-M-9a the D and E bivalents were locked together in a true interlock, and also one chromosome of the D passed through another loop of the E in an incomplete true interlock. In slide 65-M-9c the A bivalent was falsely locked around the B, and another loop of the A was also around one chromosome of the B forming an incomplete true interlock. Also

in slide 65 - M - 9c were two cases where the E bivalent was locked with itself and also with the A. In one case both the interlocks were true, in the other both were false.

Three bivalents interlocked to form a chain were found frequently in all slides except 66-T-81-c, in which only one cell was seen containing more than one interlock.

One case of double interlocking was observed in slide 65-M-9c (see Text fig. 4.). One loop of the B bivalent was

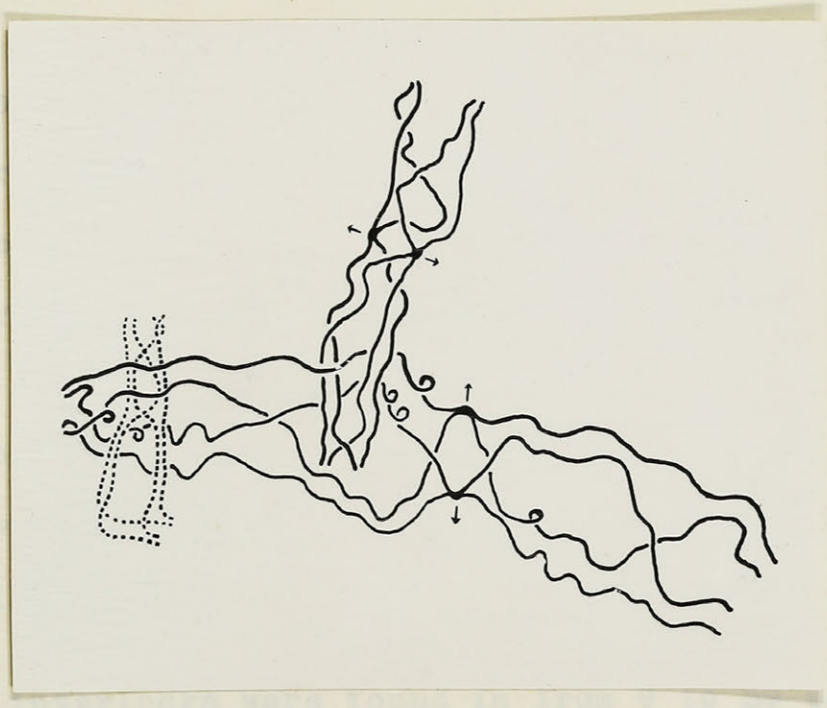


Text Fig. 4.- Camera lucida drawing of the B bivalent doubly interlocked with the D, from slide 65-M-9c.

locked with two adjacent loops of the D, and around the chiasma between them. This is the only case of double interlocking so

far reported in which the chromatids could be clearly traced. It could be plainly seen that there was only one chiasma in the D bivalent between the pairs of chromatids of the B.

In slide 66-T-85-d one case of chromatid interlocking was found (see Text fig.5). In the loop next to the attachment loop



Text Fig. 5.- The C bivalent locked around one chromatid of the E as found in slide 66-T-85-d (semi-diagramatic).

of the E bivalent one of its chromatids passed between the chromosomes of the C. If this chromatid had been outside the C instead of through it the configuration would have been a normal false interlock of E around C. This was the only case of chromatid interlocking found.

Anaphase bridges, indicating inversions, were found to be very common in all the slides.

Frequency of Interlocking.

Interlocked bivalents were seen in all Trillium erectum slides observed. The frequency of this interlocking in the slides studied was found to be rather high (see Table II). All percentages were obtained from counts of one hundred cells per slide with the exception of slide 65-M-9a in which only forty cells could be found which were clear enough for analysis. From 18 to 54 percent of the pollen mother-cells contained interlocked bivalents, the mean frequency being 35.3 percent. This is considerably higher than has been reported in most other materials, with the possible exception of ring-forming organisms, such as Oenothera and Campanula, in which 50 percent or more of the cells have been found to contain interlocks.

True interlocks were found in from 9 to 39 percent of the pollen mother-cells, the mean being 19.7 percent, while false interlocks varied in frequency from 11 to 31 percent of the pollen mother-cells, with a mean frequency of 17.7 percent. The ratio of cells containing true interlocks to those containing false interlocks (19.7 : 17.7) is approximately 1:1. The actual numbers of the two types of interlocking found (137 true and 123 false) are also approximately in the ratio of 1:1. The values of true interlocks, both here and elsewhere, include, as well as ordinary true interlocks, any incomplete true interlocks found. These incomplete true interlocks form about 24.1 percent of the

TABLE II.

Frequency of Interlocking

Slide	Temperature	Percent of cells with interlocks	Percent of cells with true interlocks	Percent of cells with false interlocks	Percent of bivalents involved in interlocks.
66-T-89-l	3° - 4° C	18	9	11	7.8
66-T-85-d	4° - 6° C	30	16	15	12.6
66-T-85-f	4° - 6° C	36	20	19	15.8
66-T-81-c	8° - 10° C	26	9	18	10.2
58-5d3	16° C	21	10	12	9.2
65-M-9a	22° - 24° C	42.2	35	15	20.5
65-M-9c	22° - 24° C	54	39	31	30.0
Mean		35.3	19.7	17.7	15.2

total number of true interlocks.

The percentage of bivalents involved in interlocking ranged from 7.8 to 30.0, with a mean value of 15.2 percent (see Table II).

As shown in Tables I and II the slides were made from plants which underwent meiosis at temperatures ranging from 3°- 4° C to 22°- 24° C. Plant 66-T-89, grown at 3°- 4° C contained the least interlocking, namely 18 percent of the pollen mother-cells, while plant 65-M-9, grown at 22°- 24° C, showed the highest value of 54 percent. These two values would seem to indicate a direct correlation between temperature and the amount of interlocking. Plants grown at intermediate temperatures, however, show a negative correlation between temperature and interlocking. Plant 66-T-85, at 4°- 6° C, contained interlocked bivalents in 30-36 percent of the pollen mother-cells, 66-T-81, at 8°- 10° C in 26 percent, and 58-5d, at 16° C, in only 21 percent. It seems evident, therefore, that there is no direct correlation, either positive or negative, between the temperature at which the plants underwent meiosis and the amount of interlocking.

Bivalent Ratios.

The respective frequencies with which the five bivalents were involved in interlocking were determined (see Table III). These values were subdivided into four separate groups. The first group, total interlocks, shows the frequency with which the bivalents were involved in interlocks of any kind. The second group true interlocks, shows the frequency with which they were involved in true interlocks. The third and fourth groups are composed of

the bivalents involved in false interlocks, these being divided into the encircling and the encircled bivalents.

The relative frequencies of the five bivalents in the total interlocks, true interlocks, and the encircling bivalents of the false interlocks were found to be nearly the same. In the case of the encircled bivalents in false interlocks, however, this ratio is upset by the excess of A and the scarcity of E bivalents. It is to be noted that the excess of A bivalents approximately equals the deficiency in the E bivalents.

The frequencies observed for individual slides are too small for their variations to have much significance, but in the case of the encircled bivalents of the false interlocks it is noteworthy that in slide 65-M-9c which has the largest number of interlocks and in which variations should, therefore, be more significant than in other slides, the value of the A is more than twice that for the E. Excluding slide 65-M-9c from the totals for encircled bivalents the remaining values are A 21 (23.3%), B 18 (20.0%), C 6 (6.7%), D 17 (18.9%), and E 28 (31.1%). These percentages correspond quite closely to those for the other groups, suggesting that some unusual condition in slide 65-M-9c may be responsible for the difference between the encircled bivalent group and the other three groups.

In the hopes of finding whether this difference might not be due, in part at least, to special affinities between certain pairs of chromosomes, the false interlocks were analysed to

TABLE III.

Bivalents Involved in Interlocks.

	Total Interlocks					True Interlocks				
Slide	A	B	C	D	E	A	B	C	D	E
66-T-81-c	12	10	1	8	23	4	3	0	2	9
66-T-85-d	18	13	3	13	19	9	7	2	6	12
66-T-85-f	17	20	6	10	39	10	8	2	4	18
66-T-89-1	6	8	2	9	17	2	6	1	4	7
65-M-9a	9	8	3	10	16	6	5	2	7	10
65-M-9c	39	32	25	31	56	18	21	13	20	36
58-5d3	12	10	3	5	18	5	5	2	1	9
Total	113	101	43	86	188	54	55	22	44	101
Percent	21.3	19.2	8.1	16.2	35.4	19.5	19.9	8.0	15.9	36.6

TABLE III. cont.

False Interlocks.

	Encircling Bivalents					Encircled Bivalents				
Slide	A	B	C	D	E	A	B	C	D	E
66-T-81-c	4	3	0	5	6	4	4	1	1	8
66-T-85-d	5	3	1	2	4	4	3	0	5	3
66-T-85-f	1	6	2	3	13	6	6	2	3	8
66-T-89-1	3	1	0	2	5	1	1	1	3	5
65-M-9a	0	2	0	1	5	3	1	1	2	1
65-M-9c	5	6	8	6	13	16	5	4	5	7
58-5d3	4	2	0	1	6	3	3	1	3	3
Total	22	23	11	20	52	37	23	10	22	35
Percent	17.2	17.9	8.6	13.6	40.6	29.1	18.1	7.9	17.3	27.6

TABLE IV.

False Interlocks.

Encircled Bivalents.	Encircling Bivalents. A	B	C	D	E	Total.
A	0	9	4	7	18	38
B	6	0	1	2	14	23
C	3	3	1	0	3	10
D	3	3	2	0	14	22
E	10	8	3	11	3	35
total	22	23	11	20	52	

determine the relationship between the encircling and the encircled bivalents (see Table IV). As may be seen, however, the numbers were too small to be significant. A further investigation, with larger numbers of interlocks, might provide an answer to this question.

Bivalent Pairs.

If Sax and Anderson's suggestion (1933) that interlocking is the result of intertwining of the threads due to the interchange of very short chromosome segments, is correct, then it might be

expected that certain chromosomes would tend to be locked with each other in a disproportionately high number of cases.

In order to test this possibility the interlocks were analysed with respect to the pairs of bivalents involved (see Table V), the total values being split up into those for true and for false interlocks. The false interlock values were taken regardless of which of the bivalents was the encircled and which the encircling one.

The relative percentages of the ten types of bivalent pairs are quite similar in true, false, and total interlocks, in spite of the very small numbers in certain of the categories. This similarity would seem to indicate that in this respect, unlike the individual bivalent values, the false interlocks behave similarly to the true ones. It also appears to indicate that this ratio must be quite constant, since if it were subject to many fluctuations these variations would be expected to be very noticeable in the small number groups of this table.

Position of Interlocking.

Early analyses of the position of interlocking were mainly concerned with whether the interlock occurred in the attachment loop (proximal interlocking) or in some other loop (distal interlocking). As evidence for distinguishing between the classical and partial chiasmatype theories of chiasma formation the importance of the ratio between proximal and distal interlocking is slight, since it may be affected by other factors. More import-

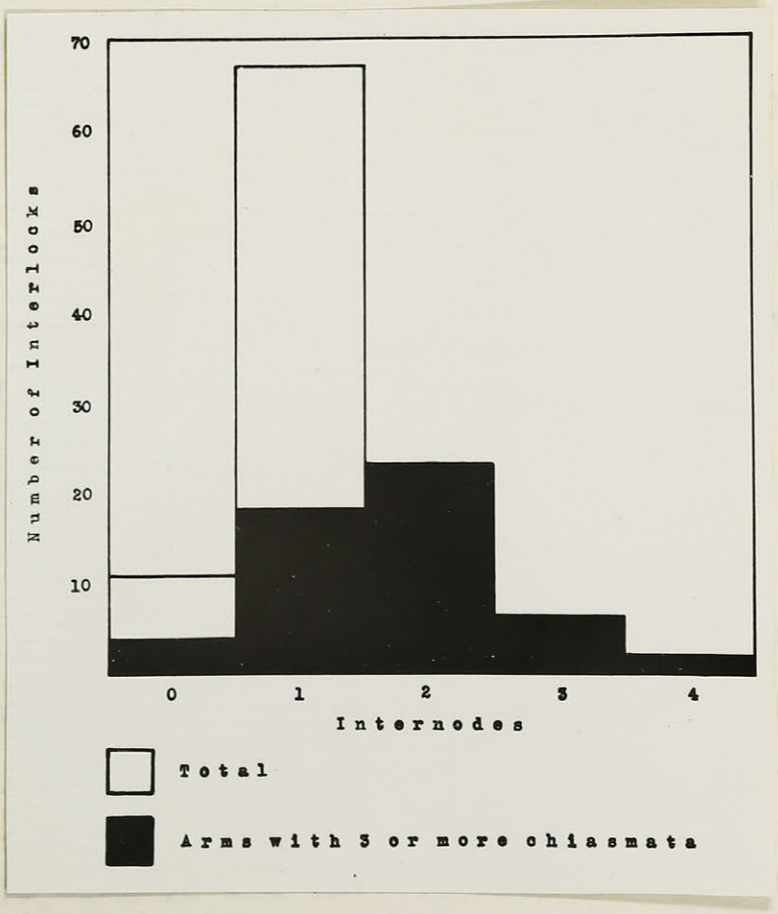
TABLE V.

Pairs of Bivalents Involved in Interlocking.

Total Interlocks	Bivalent Pairs.									
Slide	A & B	A & C	A & D	A & E	B & C	B & D	B & E	C & D	C & E	D & E.
66-T-81-c	4	0	2	6	0	1	5	0	1	5
66-T-85-d	6	1	4	7	0	3	4	0	2	6
66-T-85-f	3	1	1	11	1	3	12	0	4	5
66-T-89-1	0	1	0	5	1	2	5	0	0	7
65-M-9a	1	0	3	5	1	3	3	0	2	4
65-M-9c	11	4	6	16	3	6	12	4	9	13
58-5d3	3	1	1	7	1	1	5	0	1	3
Total	28	8	17	57	7	19	46	4	19	43
Percent	11.3	3.2	6.9	23.0	2.8	7.7	18.5	1.6	7.7	17.3
True Interlocks										
Total	15	2	7	30	3	14	24	2	12	19
Percent	11.8	1.6	5.5	23.4	2.3	10.9	18.7	1.6	9.4	14.8
False Interlocks										
Total	13	6	10	27	4	5	22	2	7	24
Percent	11.8	5.4	9.1	24.5	3.6	4.5	20	1.8	6.4	22.4

-ant is the consideration of whether the interlocking occurs in odd or even internodes.

In Table VI the position of interlocking is analysed. The internodes are numbered outwards from the attachment, the attachment loop being labelled 0, the next loop 1, and so on, irrespective of which arms of the chromosomes are involved. From Table VI and the graph (Text fig. 6) it may be seen that the majority of



Text Fig. 6. - Graph of internodes where interlocking occurs.

the interlocks (61.5%) occur in the loop next to the attachment loop, where interlocking is impossible according to the classical theory of chiasma formation. On the partial chiasmatype theory this would be expected in bivalents with not more than two

chiasmata per arm, since the attachment loop is almost invariably small in Trillium, indicating early pairing of the attachment regions and thus reducing the chances of proximal interlocking . For this reason the position of interlocking was analysed separately in bivalents having three or more chiasmata in the arms involved. In these cases the highest percentage of interlocking was found to be in internode number 2 with approximately a 1:1 ratio between even and odd internodes (29:24), while in arms with less than three chiasmata this ratio was 1:7. For the total interlocks the ratio of even to odd internodes was 1:2. In bivalents with four chiasmata in the arm involved in interlocking the ratio of even to odd internodes is 1:4, with the majority of the interlocking in internode number 3, yet in these arms there are equal numbers of odd and even internodes.

With two chiasmata per arm most interlocking is in internode number 1, with three chiasmata - in internode 2, and with four chiasmata - in internode 3. That is, in all these cases the greatest amount of interlocking was found to be in the terminal internode.

Slide 65-M-9a was omitted from Table VI since the chromatids were not differentiated clearly enough for reliable analysis of structure. Inability to trace the chromatids might allow two chiasmata to be mistaken for one.

Chiasma Frequency.

To test the possibility of a relationship between the frequency of interlocking and the frequency of chiasmata, chiasma

TABLE VI.

Internodes at Which Interlocking Occurs.

	Internodes (Total)					Internodes (3 or More Chiasmata per Arm)				
Slide	0	1	2	3	4	0	1	2	3	4
66-T-81-c	2	6	5	2	0	1	2	5	2	0
66-T-85-d	0	17	2	1	0	0	4	2	1	0
66-T-85-f	2	18	4	1	0	0	4	4	1	0
66-T-89-1	1	8	2	0	0	0	0	2	0	0
65-M-9c	4	10	5	1	2	2	3	5	1	2
58-5d3	2	8	5	1	0	1	5	5	1	0
Totals	11	67	23	6	2	4	18	23	6	2
Percent	10.1	61.5	22.0	5.5	1.8	7.5	33.9	43.4	11.3	3.8

	Less than three chiasmata per arm	Three or more chiasmata per arm	Total
Even Internodes	7	29	36
Odd Internodes	49	24	73

frequencies were determined from slides with a wide variation in interlocking frequency (see Table VII). In Table VII effective chiasma frequency is that obtained from quick counts, and is

similar to that which would be obtained from mass stained material, while the true chiasma frequency is obtained by tracing the

TABLE VII.

Interlocking and Chiasma Frequency

Slide	Percent of Inter-Locking.	Chiasma Frequency			
		Effective		True	
		Total	Interlock	Total	Interlock
66-T-89-1	18	14.1			
58-5d3	21	17.5		21.3	
66-T-85-f	36	15.4	14.2		
65-M-9c	54			22.9	22.4

chromatids. Slides containing interlocking frequencies of 18,21, and 36 percent showed chiasma frequencies of 14.1, 17.5 and 15.4 per cell respectively. This indicates that there is no direct correlation between interlocking and chiasma frequencies.

In slides 66-T-85-f and 65-M-9c the chiasma frequency of cells containing interlocks was taken separately. These values were slightly lower than the values for all the cells, suggesting that interlocking may slightly reduce the chiasma frequency. These few values indicate that further investigation of this question, in which not only cells containing interlocks but the interlocked bivalents themselves were analysed separately, might prove profitable.

V. Discussion and Conclusions.

Most of the data presented may be divided into two classes (1) those concerned with the mechanics of interlocking, how interlocking occurs and the forces governing the behaviour of interlocked bivalents, and (2) those which provide evidence concerning the method of chiasma formation. There is, of course, some overlapping between these two classes, since certain data have a bearing on both questions, but as far as possible they will be discussed separately.

Mechanics of Interlocking.

The very variety of types of interlocks found indicate a certain randomness in their formation. All combinations of pairs of bivalents were found, true and false interlocking occurred in equal numbers, and bivalents with submedian attachments were locked on themselves.

(a) Bivalent Ratios. If interlocking is purely a matter of the chance relationships of the chromosomes to each other at the time of zygotene pairing it seems reasonable to suppose that the ratio of the percentages with which the five bivalents are involved in interlocks would be directly proportional to the total lengths of the chromosomes. To test this possibility the percentages which would be expected for each bivalent were calculated, the lengths used for these calculations being those shown in Text figure 3. These lengths were those found by Dr. S. G. Smith in mitotic anaphase stages. Since only relative lengths and not absolute lengths were needed for these calculations it was thought

TABLE VIII.

Percentage of Interlocking in Relation to Chromosome Length.

Total Interlocks	A	B	C	D	E	χ^2	D.F.	P
Percentage Found	21.3	19.2	8.1	16.2	35.4			
Percentage Expected	15.5	20.0	16.4	20.2	27.8			
Difference	5.8	0.8	8.3	4.0	7.6			
$\frac{x^2}{m}$	1.72	0.03	4.20	0.79	1.65	8.39	4	0.08
True Interlocks								
Percentage Found	19.5	19.9	8.0	15.9	36.6			
Percentage Expected	15.5	20.0	16.4	20.2	27.8			
Difference	4.0	0.1	8.4	4.3	8.8			
$\frac{x^2}{m}$	1.03	0.01	4.30	0.91	2.78	9.03	4	0.05

TABLE VIII. cont.

Percentage of Interlocking in Relation to Chromosome Length

False Interlocks	A	B	C	D	E	χ^2	D.F.	P
Encircling Bivalents								
Percentage Found	17.2	17.9	8.6	13.6	40.6			
Percentage Expected	15.5	20.0	16.4	20.2	27.8			
Difference	1.7	2.1	7.8	6.6	12.8			
$\frac{x^2}{m}$	0.19	0.22	3.71	2.16	5.97	12.25	4	0.01 -0.02
Encircled Bivalents								
Percentage Found	29.1	18.1	7.9	17.3	27.6			
Percentage Expected	15.5	20.0	16.4	20.2	27.8			
Difference	13.6	1.9	8.5	2.9	0.2			
$\frac{x^2}{m}$	11.92	0.18	4.40	0.42	0.00	16.92	4	Less than 0.01

that these mitotic lengths would be sufficiently accurate. In the case of the A chromosome the length used was that of the long arm only, since the attachment is so nearly terminal that it may be considered to be so in effect.

The calculated values were compared with the values actually found (Table III), and χ^2 tests made to determine whether or not the values found fitted the hypothesis (see Table VIII). The tests were made separately for the total interlocks, true interlocks, and two types of bivalents involved in false interlocks.

As may be seen from Table VIII the values of χ^2 varied from 8.39 for the total up to 16.92 for the encircled bivalents of the false interlocks. With four degrees of freedom these give probability values (P) from 0.08 down to less than 0.01. It is obvious from this that the relative frequencies of the bivalents involved in interlocking do not fit the hypothesis, although the total interlocks are a borderline case, thus, <u>the bivalents are not involved in interlocks in proportion to their relative lengths.</u>

It was noticed that in the 101 cases in which B bivalents were involved in interlocking the short arm was involved only once. This suggested the possibility that a minimum length of chromosome arm might be necessary for interlocking. If this were so it seemed reasonable to suppose that since the short arm of the B was only once found to be involved in an interlock its length must approximate very closely to this minimum. On this assumption it is not the total length of the chromosomes which should be used

TABLE IX.

Percentage of Interlocking in Relation to the Length of Chromosome Arms Minus a Minimum Value.

Total Interlocks	A	B	C	D	E	χ^2	D.F.	P
Percentage Found	21.3	19.2	8.1	16.2	35.4			
Percentage Expected	19.1	17.3	10.5	19.1	34.0			
Difference	2.2	1.9	2.4	2.9	1.4			
$\frac{x^2}{m}$	0.25	0.21	0.55	0.44	0.06	1.51	4	0.8
True Interlocks								
Percentage Found	19.5	19.9	8.0	15.9	36.6			
Percentage Expected	19.1	17.3	10.5	19.1	34.0			
Difference	0.4	2.6	2.5	3.2	2.6			
$\frac{x^2}{m}$	0.08	0.39	0.60	0.54	0.20	1.81	4	0.8

TABLE IX. cont.

Percentage of Interlocking in Relation to the Length of Chromosome Arms Minus a Minimum Value.

False Interlocks	A	B	C	D	E	χ^2	D.F.	P
Encircling Bivalents								
Percentage Found	17.2	17.9	8.6	13.6	40.6			
Percentage Expected	19.1	17.3	10.5	19.1	34.0			
Difference	1.9	0.6	1.9	5.5	6.6			
$\frac{x^2}{m}$	0.19	0.02	0.34	1.58	1.28	3.41	4	0.45
Encircled Bivalents								
Percentage Found	29.1	18.1	7.9	17.3	27.6			
Percentage Expected	19.1	17.3	10.5	19.1	34.0			
Difference	10.0	0.8	2.6	1.8	6.4			
$\frac{x^2}{m}$	5.24	0.04	0.64	0.17	1.20	7.29	4	0.14

to calculate the expected percentages of each bivalent, but the total length with the minimum value subtracted from each arm. Therefore instead of 49, 64, 53, 67, and 91 for the A, B, C, D, and E chromosomes respectively, 31, 28, 17, 31, and 55 should be used. In Table IX the percentages found for each bivalent are compared with the expected percentages calculated from the corrected chromosome lengths above.

The χ^2 values for the total interlocks, true interlocks, and encircling bivalents of the false interlocks were 1.51, 1.81, and 3.41 respectively. With four degrees of freedom these gave values of P of 0.8, 0.8, and 0.45. These three types, therefore, fit the minimum value hypothesis. In the case of the encircled bivalents of the false interlocks, however, χ^2 was 7.29, giving a value of 0.14 for P. This category did not fit the hypothesis as closely as did the others, due to the very high value for the A bivalent and low value for the E. From Table III it may be seen that in the case of the encircled bivalents slide 65-M-9c shows a ratio of A to E of more than 2:1. When the 65-M-9c values for all five bivalents are subtracted the remainder fit the minimum value hypothesis closely (see Table X), with a χ^2 of 2.97 giving P = 0.6. Further investigation would be necessary before it would be possible to offer any explanation of the unusual behaviour of the encircled bivalents in slide 65-M-9c.

From the results of the above tests it may be stated that, except in the case of the encircled bivalents of false interlocks in slide 65-M-9c, the frequency with which bivalents are involved

TABLE X.

Percentage of Interlocking in Relation to the Length of Chromosome Arms Minus a Minimum Value.

Encircled Bivalents Minus 65-M-9c Values	A	B	C	D	E	χ^2	D.F.	P
Percentage Found	23.3	20.0	6.7	18.9	31.1			
Percentage Expected	19.1	17.3	10.5	19.1	34.0			
Difference	4.2	2.7	3.8	0.2	2.9			
$\frac{x^2}{m}$	0.92	0.42	1.38	0.00	0.25	2.97	4	0.6

in interlocking is proportional to the relative lengths of their arms minus the length of the short arm of the B chromosome, which is the minimum arm length which will permit interlocking to occur.

(b) Bivalent Pairs. Having considered the bivalents individually the next consideration is pairs of interlocked bivalents. Do the ten types of bivalent pairs occur in the ratio expected from the frequency of interlocking of the individual bivalents ? This would indicate randomness. Or do certain pairs occur with greater frequency than expected ? This would indicate some specific attraction between the pairs such as might result from segmental interchange between them.

To answer these questions the percentages expected for the various bivalent pairs were calculated from the percentages of the individual bivalents minus those which were locked on themselves. In Table XI the percentages found, separated into total, true, and false interlocks, are compared with the expected values, calculated from the frequency of interlocking of the individual bivalents as stated above. These three comparisons give χ^2 values of 4.482, 6.617 and 8.459, which, with nine degrees of freedom, give values for P of 0.82, 0.7 and 0.5. Clearly, the percentages found correspond closely to the calculated ones. It may be concluded from this that a specific attraction does not exist between any of the pairs of non-homologous bivalents, the interlocking of these pairs of bivalents

TABLE XI.

Total Interlocks	A&B	A&C	A&D	A&E	B&C	B&D	B&E	C&D	C&E	D&E	χ^2	D.F	P
Percentage Found	11.3	3.2	6.9	23.0	2.8	7.7	18.5	1.6	7.7	17.3			
Percentage Expected	11.5	4.4	9.6	19.2	4.1	8.8	17.6	3.4	6.7	14.6			
Difference	0.2	1.2	2.7	3.8	1.3	1.1	0.9	1.8	1.0	2.7			
$\frac{x^2}{m}$	0.035	0.327	0.759	0.752	0.412	0.137	0.460	0.952	0.149	0.499	4.482	9	0.82
True Interlocks													
Percentage Found	11.8	1.6	5.5	23.4	2.3	10.9	18.7	1.6	9.4	14.8			
Percentage Expected	10.3	4.1	8.3	18.9	4.2	8.4	19.3	3.3	7.7	15.4			
Difference	1.5	2.5	2.8	4.5	1.9	2.5	0.6	1.7	1.7	0.6			
$\frac{x^2}{m}$	0.218	1.530	0.940	1.070	0.860	0.740	0.002	0.880	0.375	0.002	6.617	9	0.7
False Interlocks													
Percentage Found	11.8	5.4	9.1	24.5	3.6	4.5	20.0	1.8	6.4	22.8			
Percentage Expected	10.9	5.0	10.0	20.7	3.9	7.8	16.1	3.6	7.3	14.7			
Difference	0.9	0.4	0.9	3.8	0.3	3.3	3.9	1.8	0.9	8.1			
$\frac{x^2}{m}$	0.007	0.003	0.008	0.700	0.000	1.40	0.94	0.89	0.011	4.50	8.459	9	0.5

being entirely random. This evidence is contrary to Sax and Anderson's proposal that interlocking is the result of the exchange of very short chromosome segments which causes the attraction and consequent interlocking of non-homologous chromosome pairs.

(c) Position of Interlocking. Interlocking was found in every internode of the bivalents along their entire length. It tended to be most frequent in the internodes most distal from the attachment, that is, it was not found to be entirely at random along the length of the bivalent. This greater frequency of interlocking in the most distal internode possible may be the result of the attachment regions pairing before the rest of the chromosomes, which presumably would tend to reduce proximal interlocking. Since the chiasmata of Trillium are not terminalised it is reasonable to suppose that in the case of true interlocks, at least, the position in which interlocking is observed is approximately the position in which it occurred. It may be concluded, therefore, that interlocking may occur in any internode along the entire length of the bivalents, but tends to occur most frequently in that internode most distal from the attachment.

(d) Orientation of Interlocked Bivalents. At diplotene interlocked bivalents were not found to take up any definite positions with regard to each other. By late diakinesis or metaphase, however, they tend to lie at right angles to one another. It was also noted that the internodes involved in

interlocking were frequently larger than the other internodes.

These two observations indicate that by diakinesis there is developed a repulsion not only between chromosomes of a bivalent, but also between non-homologous chromosomes. It may reasonably be supposed that these are not two separate forces, but one non-specific repulsion.

The enlarged interlocking internodes suggest two possibilities. The first is that the presence of another chromosome between a pair of homologous chromosomes prevents the formation of chiasmata close to it; the second is that the development of the body repulsions of the chromosomes causes the interlocked internodes to become enlarged by the movement of chiasmata away from the interlock. Either of these possibilities might explain the minimum length found necessary for interlocking. According to the first a subterminal interlock would be prevented by the inhibition of the chiasma distal to it necessary for it retention. According to the second the chiasma distal to the interlock might be forced off the end of the bivalent, thus allowing the two bivalents to slip apart.

It might be possible to check these two possibilities to find which, if either, is correct. If the first were correct, and a subterminal interlock prevented a chiasma from being formed distal to it, then incomplete true interlocks should be far more common in early prophase stages than they are at diakinesis and metaphase. If the second possibility were correct,

however, and the chiasma distal to a subterminal interlock became eliminated, then true interlocking should be more common in early than in late stages, while incomplete true interlocks might increase from diplotene to metaphase.

(e) The Interlocking of Non-homologous Bivalents. The observations made on Trillium erectum support the hypothesis, proposed by many workers, that bivalent interlocking results from a mechanical accident occurring during zygotene pairing. When homologous chromosomes pair at zygotene one or both chromosomes of another pair may be caught between them. With the formation of the necessary chiasmata these interlocks are retained as true and false interlocks respectively.

This theory of interlocking as a matter of the chance distribution of the chromosomes would explain the occurrence of true and false interlocking with equal frequency. It would also explain the random interlocking of the five bivalents in relation to the relative lengths of their arms minus a minimum length, and the random association of pairs of bivalents in the ratio expected from their individual frequencies.

The early pairing of the attachment regions would explain the excess of distal interlocking over proximal.

The minimum length required for interlocking may be explained by interference, either purely mechanical, or as the result of mutual repulsions.

Chiasma Formation.

(a) Position of Interlocking. Interlocking was found in both odd and even internodes, in every internode, in fact, from the attachment loop to the most distal (see Table VI and Text fig. 6). According to the classical theory of chiasma formation it should only occur in the even internodes, except for occasional cases where a chiasma had broken. It was found, however, that odd internodes were involved in interlocking twice as frequently as even internodes. This evidence is directly contrary to the classical theory, and can not be made to fit it even by assuming that homologous rather than sister chromatids are associated at the attachment.

(b) Double Interlocking. The one case of double interlocking found in slide 65-M-9c is the first case reported where the chromatids could be traced (see Text fig. 4). It is perfectly clear that there is only a single chiasma in the D bivalent between the chromosomes of the B. Interpreted on the classical theory of chiasma formation this would mean that sister chromatids were separated, passing on either side of one chromosome of the B, in one internode, or that there had been two chiasmata in the D bivalent between the chromosomes of the B, one of these chiasmata subsequently having broken. The explanation on the partial chiasmatype theory of chiasma formation is far simpler. It is unnecessary to assume any abnormal condition; sister chromatids are paired in both the internodes of the D bivalent concerned, and the chiasma arose by crossing-over.

It is to be noted that in this configuration the two arms of the D bivalent are locked together in a true interlock.

(c) Chromatid Interlocking. One case of chromatid interlocking was observed in slide 66-T-85-d (see Text fig.5). Since all the chromatids could be traced there was no doubt that the C bivalent was locked around a single chromatid of the E. Gairdner and Darlington (1931) state that chromatid interlocking should occur if the chromatids ever separate equationally. Neither theory of chiasma formation, however, will explain the configuration seen. If crossing-over is conditioned by torsion, as suggested by Darlington (1935, and 1937 p..483), the most reasonable explanation is that one of the chromatids of the E (separated by the C) broke and rejoined to form a chiasma on the other side of the C chromosome. The fact that the interlock as observed is some distance from the chiasma may possibly be due to repulsion having shifted the C bivalent proximally relative to the E bivalent.

The evidence obtained from the study of interlocked bivalents in Trillium strongly supports the partial chiasmatype theory of chiasma formation, while no evidence was found in favour of the classical theory.

VI. Summary.

1. Interlocked bivalents were studied at diplotene, diakinesis, and metaphase in Trillium erectum L. Two main types of interlocking were found, namely true interlocking and false interlocking. These primary types appeared in many forms - one bivalent locked on itself, three bivalents forming a chain, etc.. One case of double interlocking was found, the first reported where the chromatids could be traced. A clear case of chromatid interlocking was also found. By diakinesis the interlocked bivalents were usually orientated at right angles to each other, with the interlocked loops frequently enlarged.

2. The frequency of interlocking was found to be high, occurring in from 18 to 54 percent of the pollen mother-cells. No correlation was found between the frequency of interlocking and the temperature at which meiosis occurred. True and false interlocks occurred with equal frequency indicating randomness of formation.

3. The ratio of the frequencies with which the individual bivalents are involved in interlocking is not proportional to the total length of the bivalents, but is proportional to the relative lengths of the chromosome arms minus a minimum length approximately equal to the length of the short arm of the B chromosome. This suggests that pairing is initiated at the attachment.

4. The ten types of bivalent pairs were found in the ratio expected from the frequency of interlocking of the individual

bivalents, indicating the absence of any specific attraction between certain pairs of bivalents.

5. Interlocking was found in all internodes of the bivalents, the ratio of the number of odd internodes interlocking to the number of even internodes interlocking being 2:1. Interlocking was most frequent in the most distal internodes, again indicating that pairing is initiated at the attachment.

6. No correlation was found between chiasma and interlocking frequencies. There was some indication that interlocking may cause a reduction in the chiasma frequency in the cells in which it occurs.

7. The evidence of the observations made indicates that in Trillium erectum L. interlocking is the result of a mechanical accident occurring during zygotene pairing, and is dependent upon the chance distribution of the chromosomes throughout the nucleus at that time.

8. The evidence from double interlocking and the internodes in which interlocking occurs supports the partial chiasmatype theory of chiasma formation. No evidence was found in support of the classical theory.

VII. Acknowledgments.

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VIII. Bibliography.

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