Chromosome Studies on *Trillium kamtschaticum* Pall. and Its Allies.¹
XVII. A study of chromosome interlocking in
***T. Tschonoskii* Maxim.**

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Interlocking of bivalents will depend upon two processes, viz., (i) the mode of chromosome pairing at zygotene, which determines its occurrence and (ii) the mode of chromatid opening-out at diplotene, which determines its retention. Therefore the analysis of the types of interlocking will be significant in that it may offer a clue for understanding how these causal processes of meiosis take place at its prophase where exact analysis of these is quite difficult by means of direct observations.

It is evident that in these bivalents the interlocked internodes are those opened out along the reductional plane at diplotene. Hence if chiasmata are caused by the alternative opening-out of sister and non-sister chromatids, as the present writer claims, interlocking can occur at the reductional internodes only. On the contrary, if chiasmata are the result of previous crossing-overs and only sister chromatids are associated at diplotene, as the chiasmotype theory claims, then interlocking could occur at any internode. The case of double interlocking has been thus taken as a demonstration of the latter theory (Mather '33).

The aim of the present study is then in making decisive answer to this critical point on the basis of more advanced concept of bivalent constitution.

Material and Methods

Several corms of *T. Tschonoskii* were transplanted in our green house early December 1943. The meiotic divisions took place about three weeks later. Since the control usually begins its division in the middle of February under snow, the division in the present material has been accelerated nearly a month and a half earlier. Such acceleration of division may be responsible for the occurrence of interlocked bivalents (vide infra).

The PMCs at MI were smeared, pretreated with 0.28 mol solution of KCl for one minute and stained with acetocarmine. This pretreatment

¹ The main title of this series has been altered as above, "and its allies" being added to the previous title. This is because of necessity of comparative studies with other species of *Trillium*.

gave results very favorable for revealing chromatid relationship which is primarily necessary for the analysis of such complicated configurations as interlocked bivalents. A total of 29 preparations were brought under close observations, the result of which constitutes the principal part of this paper.

Observations

i) **Bivalent constitution.** Before going further, it will be necessary to give a description of the constitution of bivalents in the present material. As already described by Haga ('34), its complement consists of each two members of bivalents A, B, C, D and E, totaling 10 bivalents, each of which constitutes the fundamental component in the family *Trilliaceae*. As to the constitution of bivalents no essential difference exists between *T. Tschonoskii* and *T. kamtschaticum*. This will be clear when one compares Fig. 1 of the present paper with Fig. 4 in the

Fig. 1. Representing a configuration of a bivalent A of *T. Tschonoskii*. On one side (the left arms) of the paired kinetochores (K), there is a secondary chiasma, while on the other side it is lacking. Note that in the upper arm of the chiasma internode a reversal of coiling direction has occurred exactly at its middle point, as indicated by an arrow, whereas there is no such reversal in the corresponding lower arm, a condition quite similar with the bivalent of *T. kamtschaticum* represented by Fig. 6 of No. 15 of this series. $\times 2020$.

14th report of this series. The pairing of the kinetochores is complete (the K-type of bivalents, v. No. 10 of this series), and secondary chiasmata may or may not be formed in the vicinity of the kinetochore and of primary chiasmata (v. No. 14 of this series). These structures may become

ambiguous in usual "bulk" fixation; the paired kinetochores would be confused with a chiasma, as most of cytologists have done, and a chiasma associated with a secondary one with single chiasma. These points have an important bearing on consideration of interlocking. First, when a question arises as to whether an interlocked internode is the first one from the kinetochore or the second or far more, such a clear manifestation of chromatid relationship is indispensable; otherwise conclusions drawn from vague figures would bring nothing but confusion. Secondly, the previous classification of interlocking types by Gairdner and Darlington ('31) is not applicable to the present case. They distinguished two kinds of interlocking, viz., proximal interlocking, involving the internode containing the kinetochores, and distal one, involving the internode next the kinetochore loop. However in the K-type of bivalents, there is no such kinetochore loop. Their classification is only applicable to the k-type of bivalents, where the kinetochores remain unpaired at metaphase and chiasmata only are responsible for the retention of bivalents. Earlier observations on interlocking were made on plants which are characterized by such a type of bivalents, viz., *Oenothera* (Catcheside '31), *Campanula* (Gairdner and Darlington '31) and *Tradescantia* (Sax and Anderson '33, '34). These plants are those suggestive of structural hybridity and hence Sax and Anderson ('34) said: "Interlocking may be associated with segmental interchange, since interlocking has been found only in genera where segmental interchange is commonly found". There is no sound foundation of this statement however.

ii) **Single interlocking.** The types of interlocking are due to certain entanglements in the arrangement of the chromosomes concerned at the time of pairing (Fig. 2). There are two kinds of entanglements distinguishable, either both chromosomes of one pair pass between the chromosomes of the other, or one chromosome of each pair passes between the chromosomes of the other pair. These two kinds of arrangements are expected to occur on equal chance, because no peculiarity is attributed to either case. The retention of such entanglements until metaphase, however, is due to another factor, that is, chiasma formation at the region distal to the entanglements. If in the former type of arrangement no chiasma occurs at this region of the surrounding bivalent, the two resulting bivalents will be free from one another and no trace of entanglement may be found at metaphase. However if chiasmata are formed at this region, the two bivalents will be falsely interlocked, with two arms of the other bivalent being caught within the loop. On the other hand, in the latter type of arrangement, the situation will be somewhat different. Here free bivalents result only when no distal chiasmata are formed in both the pair of paired arms, involved in the entanglement, of the two bivalents. When such distal chiasmata occur only in one of the bivalents, the result-

ing configuration will be another kind of false interlocking, with one arm of a bivalent being caught within the loop. And when both the arm pairs are provided with distal chiasmata, this gives rise to true interlocking. The bivalents of true interlocking remain entangled until metaphase, while falsely interlocked ones may slip free of one another before metaphase. Figs. 3-8 illustrate these types of interlocking at metaphase. Compound

Fig. 2. Diagrammatically illustrating how and what kind of interlocking is caused. a and b, two kinds of entanglements at pachytene. 1-4, resulting bivalents; 1, free bivalents; 2 and 3, false-interlocked bivalents; 4, true-interlocked bivalents.

forms of interlocking, as represented by Fig. 6, are not rare in the present material.

The occurrence of interlocking will depend upon two variable conditions. First, as pointed out by Upcott ('36), the variation in rapidity of the early prophase changes has some effect on its occurrence. If the early stages, especially the stage of chromosome pairing, are rapid, the homologous chromosomes will have not enough time to approach their respective partners at zygotene and some entanglements in the chromosome arrangement, as described above, will happen to occur. Secondly, the frequency of chiasmata, especially of terminal ones, has an important bearing on the retention of interlocking. Both these factors are variable under different conditions. It is also probable that both are dependent with one another in certain degree, the shorter the preceding stages of prophase are, the more frequently chiasmata arise (v. No. 10 of this series). These considerations will explain some considerable local variations in the frequency of interlocking recorded by Sax and Anderson ('33) in *Tradescantia* and by Upcott ('36) in *Eremurus*. In several *Tradescantia*, Sax and Ander-

Figs. 3-8. Representing various types of interlocking. 3, 5, 6 and 8, from *T. Tschonoskii*; 4 and 7 from *T. Hageae*. 3, false interlocking, a pair of arms of bivalent A being intruded in a loop of bivalent B. 4, true interlocking; in one bivalent involved (E) the kinetochores are in the asynaptic state, a condition frequently found in interlocked bivalents (see also Figs. 7 and 11). 5, true interlocking; an instance where a terminal loop of a bivalent (A) is involved in interlocking. 6, an instance of compound interlocking. 7, false interlocking, where all three homologous arms of trivalent A have been caught by a loop of bivalent D. 8, two cases of another type of false interlocking in one and the same cell; in each, one of the arms of A is intruded in a loop of another bivalent. $\times 1010$.

son ('34) showed also that the correlation between chiasma formation and the percentage of interlocking is positive. In the present material, such local variation of interlocking seemed to be true, although no statistical treatment was not undertaken, because of frequent occurrence of too complicated configurations to be analyzed. Generally speaking, it seems to be possible to say that within the genus *Trillium*, species in which chiasmata are usually formed rather frequently, such as *T. Tschonoskii*, *T. Smallii* and *T. Hagae*, are also provided with interlocked bivalents more frequently than *T. kamtschaticum* where chiasmata are extremely rare under usual conditions. Furthermore in the present material, the not seldom occurrence of compound forms of interlocking and the simultaneous occurrence of two pairs of interlocked bivalents or more in one and the same cell (see Figs. 8, 9, 10 and 12) suggest this possibility.

On the basis of above considerations it will be inferred that chromosome or arm length is related with the occurrence of interlocking. The longer the chromosomes or arms are, the more frequently the entanglement in their arrangement at the time of pairing and the subsequent chiasma formation will take place. In order to test this point, a total of 50 cases of single, true interlocking in the present material were analyzed as to chromosome type (Table 1).

Table 1. Analysis of interlocked bivalents

Bivalent combination	Frequency	Arms involved (arranged in the order of length)					
		DI	CI	A ₁ , A ₂	BI	EI	BS
A—D	13	13		13			
A—C	9		9	9			
A—B	7			7	6		1
B—C	4		4		2		2
B—D	2	2			2		
C—C	1		2				
C—D	9	9					
C—E	1		1			1	
D—D	3	6					
E—E	1					2	
Total	50	30	25	14.5 (29/2)	10	3	3

From the above data, when arranged according to the length of whole chromosomes, it follows: A 29, B 13, C 25, D 30 and E 3, indicating no direct relationship of interlocking to chromosome length, but as shown in Table 1, when arranged in the order of the length of separate arms, certain proportionality is revealed between these two variables. That the length of chromosome arms, not that of whole chromosomes, is involved in the relationship with interlocking frequency, is able to understand by considering that although the occurrence of interlocking is determined by chance at the time of chromosome pairing and hence the total length of

the chromosomes should be involved in this relationship at the time of its occurrence, its retention will be conditioned by the chiasma formation at the part distal to it, especially by the formation of terminal chiasmata and since these chiasmata are further conditioned by the length of arms in the case of K-bivalents (No. 10 of this series), the frequency of resultant interlocking remaining up to metaphase will be proportional to the arm length, and not to the total length of the bivalents. In this connection it will be noticed that in the arms shorter than the short arm of bivalent E, inclusive, viz., Es, Cs, and Ds where no chiasmata are usually formed, no interlocking was observed—a matter of course.^{1,2}

iii) **Double interlocking.** By double interlocking a configuration is meant that two successive loops of a bivalent have chromosomes interlocked with them. Such configurations must be of very seldom occurrence and up to the present time these have been described but three times, each once in *Lilium regale* (Mather '33), *Lilium elegans* (Beal '36) and *Eremurus spectabilis* (Upcott '36)³ Mather and others took this as a decisive evidence favoring the chiasmotype theory of crossing-over, on the reasoning that the successive loops of a bivalent invaded by other chromosomes must have opened out in the reductional plane at diplotene and therefore the central chiasma must be the consequence of a crossing-over (Fig. 13a). On the classical theory it would be only possible to explain such configurations when we assume that two central chiasmata had existed and one broke and resolved (Sax '34)—a remote possibility.

The above discussion on the chiasmotype theory will be indisputable if the central connection of the successive loops were a real, single chiasma. However, with the advance in our concept of bivalent constitution, there comes several other possibilities. First, the central connection may be the paired kinetochores, as the opening-out of chromatids can take place along the reductional plane on both sides of the paired kinetochores (Fig. 13b). This suggestion was put forth by the writer early as 1937 at the regular monthly meeting of the Genetics Association of Sapporo held May 27, that year. Secondly, it may be merely a twist between the homologous arms of a bivalent (Fig. 13c).

¹ In *T. Smallii*, Mr. Amano of our Laboratory (unpublished) analyzed 8 cases of interlocking, which consisted of the following bivalent combinations: twice of A-D, each once of A-A, A-B, A-C, B-B, D-D and D-E. Though the data are small, this will be taken as parallel to the present case.

² Similarly Boothroyd (Abstract, '40) found in *T. erectum* that "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 length of the chromosome arms minus a minimum length." And he infers from this that "pairing is initiated at the attachment," but the writer's alternative interpretation as stated in the text seems to be more plausible in view of his retardation hypothesis of the kinetochore cycle (No. 13 of this series).

³ Another case of double interlocking "around a chiasma" is reported by Boothroyd (Abstract, '40) in *T. erectum*. At present nothing can be said on his case until more definite information becomes accessible to the writer.

Figs. 9-12. Representing double interlocking. In these four cells, all members of the complement were analyzable. $\times 1010$.

Such an overlapping would be taken as a single chiasma in "bulk" fixation. Thirdly, it may be two chiasmata closely lying together—secondary chiasmata, which might be equally mistaken as a chisma when chromatid relationship was masked by the coagulation of chromosome matrix (Fig. 13 d).

In order to test which of these possibilities is the case, efforts were especially concentrated on finding double interlocked bivalents in this material and four cases were possible to record (Figs. 9-12). All of the

11

12

cases indicate the first possibility, that is, the central connection is the paired kinetochores and not a chiasma. Figs. 9-11 represent that two successive loops, each one on both sides of the kinetochore, of bivalent **A** are interlocked by a loop of bivalent **D**. In Fig. 9, the configuration is further complicated by another bivalent being also interlocked with the **A** bivalent. In Fig. 12, both the loops of bivalent **A** are separately interlocked by other bivalents.

Throughout these cases, two characteristic features may be recognized; first, the interlocked bivalent is always of chromosome type **A**, and secondly, the invading bivalent involved in double interlocking is always of chromosome type **D**. Such a limitation of occurrence of double interlocking will not be strange when we recall a fact that within the comple-

ment, only in bivalent A large loops can be made on both sides of the kinetochore, and furthermore one that has so long arms as to permit to pass through these loops is most probably only bivalent D whose long arm is the longest one of the complement.

Fig. 13. Diagrammatic representation of chromatid relationship in double-interlocked bivalents. a, the interpretation adopted by Mather on the chiasmotype theory. b-d, those suggested by the present writer. None was actually demonstrated as fitted the type represented by Fig. a. All cases observed in *T. Tschonoskii* were of the type as in Fig. b.

Figs. 14-16. Configurations of double interlocking figured by earlier workers. 14, reproduced from Mather ('38); 15, from Beal ('36); 16, from Upcott ('36).

It seems to be most probable that the configurations observed by Mather, Meal and Upcott, as reproduced by Figs. 14-16, are of the same nature as in the present case which might have been manifested when the chromatid relationship was revealed in more proper fixation.

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Summary

The present paper deals with the analysis of interlocking of bivalents in PMCs of *Trillium Tschonoskii*. A total of 50 cases of single interlocking at MI were analyzed as to chromosome type. The result indicated that the frequency of interlocking is proportional to the length of the chromosome arm. This finding can be interpreted by reasoning that in the present material the bivalent constitution is of the K-type (see No. 10 of this series) and hence the arm, not directly the whole chromosome, is involved in the frequency of interlocking.

As to double interlocking, four cases were analyzable. In all of them, the central connection of the two successive loops of the bivalent at question was the paired kinetochores and not a real chiasma. Therefore one of the "evidences" favoring the chiasmotype theory of crossing-over has entirely lost its force.

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