Chromosome Studies on *Trillium kamschaticum* Pall.**XIV. Primary and secondary chiasmata¹⁾**

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The results from an experimental study on the chiasma formation in *Trillium kamschaticum* undertaken by the present writer and T. Haga were interpreted as favoring the two-plane theory of its origin (No. 10 of this series). The writer considers from this and other unpublished sources of evidence that the mode of chiasma development is as follows. The synaptic association of the homologous chromosomes at zygotene *usually* begins at their distal ends and proceeds towards the proximal ends. Thus the pairing process terminates at the kinetochores. Likewise the opening-out of the chromatids at diplotene *usually* originates at the distal ends and proceeds proximally. Thus the separation of the paired kinetochores takes place as the final event. With respect to these behaviors, the kinetochore is thus always later in time than the rest of the chromonema (cf. No. 13 of this series). In such *normal* case the plane of chromatid opening-out which was first determined at the distal ends by the repulsion between the pair of paired chromatids will continue throughout the paired arms and consequently no chiasma will be formed, the pair of paired chromatids being associated only at the kinetochore region and forming characteristic cross-shaped figures. On the other hand, when the meiotic division is accelerated by e.g. heat, certain discrepancies may occur in the mode of pairing and subsequent repulsion; repulsion may set in interstitially.

When repulsion begins at two different intercalary points and the planes of opening-out of the chromatids are different in these two sections, the result is the formation of one interstitial chiasma. Thus the frequency of chiasmata per arm pair is taken as a function of these beginning points of repulsion.

Supporting the above view of chiasma development, an interesting fact has been revealed this time from observations on PMCs which were taken from plants placed in an incubator of 20°C for about a week till meiotic metaphase began. In the present study La Cour 2BE-Gentian violet smears, pretreated with water according to the writer's method (No. 11 of this series), were used.

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In this material the association of the kinetochores is complete and the frequency of chiasmata is rather high, like the t_1 type described in a previous paper (No. 10 of this series). Very remarkable is the very frequent occurrence of small chiasma loops which are located either proximally neighboring the kinetochore or interstitially close to a larger loop. These situations are illustrated in Figs. 2-4. Likewise close to the distal ends of paired arms when associated together, a chiasma as shown in these figures is very frequently found. These small chiasma loops are discernible only when the chromonema structure of chromosomes is quite obvious as in these photos, and this is the reason why no particular description of such chiasmata has been hitherto made.¹⁾ Now how these small chiasma loops were brought about is the subject matter to be considered here.

In the writer's view of chiasma development, these small loops must have been caused by different openings-out independent of one in neighboring larger loops, but to what should the peculiarity that these loops are always so small be attributed? The writer believes it reasonable to ascribe this peculiarity to the equilibrium between the strength of repulsion at the time of opening-out on the one hand and the surface tension and the viscous resistance set up by the matrix substance on the other. This implies that if the chromatid opening-out takes place after the matrix became viscous to such a degree that certain accumulation of the matrix substance will occur at these junctions—a condition supposed to be analogous to the case when two appressed fingers are dipped into mucilage and then separated slowly so that the aggregation of mucilage will occur at the root-junction of the fingers—the portion of the chromatids lying within such unseparated matrix will constitute a system independent with respect to chromatid opening-out of the neighboring loop.

This relation of repulsion to matrix resistance is diagrammatically represented in Fig. 5. When repulsion occurs at two different interstitial points, A and B, within an arm-pair (Fig. a), two corresponding principal loops, A and B, will be formed at diplotene and accompanying these loops the accumulation of the matrix at the junctions will bring forth three small regions, proximal (x), interstitial (y) and distal (z), which are no longer under the effect of the

1) In the study on meiotic chromosome structure in *Trillium erectum*, Huskins and Smith ('35) state: "it is noteworthy that chiasmata very frequently occur immediately adjacent to and on both sides of the attachment." Most probably these chiasmata correspond with those in the present question. Moreover in some of their figures, e. g., Figs. 11 and 12, Pl. II, interstitial small loops are clearly shown, though they made no remark on this point.

Figs. 1-3. For explanation, see p. 383.

Figs. 1-4. Representing various configurations of bivalents; 1 showing no formation of chiasmata, 2-4 showing various types of formation of proximal, interstitial or terminal chiasmata. Chromosome type: 1, 3e, B; 2, 3d and 4, A. Figs. 3a, b and c and Figs. 4a and b were taken at different successive foci. K indicates the kinetochore. $\times$ ca. 2420.

Fig. 5. Representing the development of proximal (x), interstitial (y) and distal (z) junctions (a and b) and resulting figures of opening-out of chromatids in these regions (c). In this diagram only one pair of arms is represented. k represents the kinetochore.

original repulsion forces (Fig. b). Within these junctions, the chromatids will then take their own ways of opening-out, as illustrated in Fig. c. Based on the two-plane theory of chiasma development, the formation or non-formation of chiasmata in these regions can be statistically treated. The numerical data obtained will be given in the following under separate headings, viz., (i) proximal, (ii) interstitial and (iii) distal junctions.

i) Proximal junction. The probability that the plane of opening-out differs in the two regions, x and A, thus resulting in the formation of a chiasma at the border of them, is calculated as $\left(\frac{1}{3} \times \frac{2}{3}\right) \times 3$, that is, $\frac{2}{3}$. Accordingly with respect to bivalents, those having chiasmata on both sides of the kinetochore (+ +), those having a chiasma on one side of the kinetochore (+ -), and those having no such a chiasma (- -) are expected to occur respectively in a 4:4:1 ratio. Since it was quite difficult to analyze all the members of the complement, the writer selected at random favorable chromosomes of the A, B, C and E types in which both arms of a bivalent are analyzable, and careful records were made on them. Observations on a total of 114 bivalents showed the following results:

The results indicate that the observed ratio per 9 for the + +, + - and - - classes is 3.95 : 3.47 : 1.58, the deviation from the expected ratio being ± 0.58 . When individual arms are separately treated, the frequency of the two classes, + and -, is 94 and 64 respectively, i.e., a ratio of 2.78:1.22 per 3. Such a rather large discrepancy of the actual data from the expectation will be ascribed to a considerable excess of the - - class in the present records. Most probably this is due to greater ease in analysis and consequently more frequent picking up of the bivalents of this type.	Chromosome type	+ +	+ -	- -
	A	14	19	4
	B	20	12	6
	C	8	4	5
	E	8	9	5
	Total	50	44	20

ii) Interstitial junction. The probability that the three regions, A, y and B, all open out in the same plane, is calculated as $\left(\frac{1}{3}\right)^3 \times 3$, that is, $\frac{1}{9}$. This is a case of non-formation of chiasmata, and in the remaining cases of $\frac{8}{9}$ either one chiasma or two are expected to occur. The formation of one chiasma is expected either from the opening-out of A and y in the same plane and of B in a different plane, or from that of y and B in the same plane and of A in a different plane. Thus the chiasma is formed at the border of y and

B in the former case and at the border of A and y in the latter. In each case its occurrence is calculated as $3\left(\frac{1}{3} \times \frac{1}{3} \times \frac{2}{3}\right)$, that is, $\frac{2}{9}$. Therefore pairs of arms of $\frac{4}{9}$ are expected to have one chiasma in the y region. The formation of two chiasmata in this region, namely, at both the borders of A and y and of y and B, will result either from the opening-out of A and B in the same plane and of y in a different plane or from that of all these three regions in different planes. In each case its occurrence is likewise calculated as $\frac{2}{9}$. Therefore cases of $\frac{4}{9}$ must have two chiasmata. Accordingly pairs of arms having such one interstitial chiasma (—) and those having two contiguous chiasmata (+) are expected to occur in a 1:1 ratio. The records from various bivalents selected at random showed: $\frac{+}{19} \frac{-}{20}$. Though the number of observations is very small, owing to rather low frequency of interstitial chiasmata in this material, the data accord well with the expectation.

iii) Terminal junction. To this case also the same principles based on the two-plane theory can be applied. Cases of chiasma formation (+) at the boundary of the two regions, B and z, are expected to be twice as many as those having no chiasma (—). The former results in a characteristic double-crossed figure in which the pair of small limbs lies symmetrically within the common matrix (Fig. 3e). In the latter the chromatids come to show the end-to-end association, their free separation being arrested by the materical substance (Figs. 2 and 4).¹⁾ Records of these two types indicated: $\frac{+}{26} \frac{-}{19}$. Thus the observed ratio per 3 is 1.73:1.27 for the + class and the — one, the deviation from the expected 2:1 ratio being ± 0.27 . This deficiency of the + type may be attributed to greater ease in the judgment of the — type. In fact there is a certain degree of variation in the length of the open limbs, as in the proximal and interstitial junctions, and some cases were recorded in which the open limbs protrude slightly beyond the limit of the chromosome contour. These might have been such of terminal origin as described above but were excluded from the + type in recording, by reason of ambiguity in demarcation of such one from the true interstitial chiasma type.

1) The establishment of these two types of terminal chiasmata, viz., true terminal and apparent terminal, is further justified by the finding that the relation of them to the mechanism of chromonema spiralisation is entirely different from each other (see No. 15 of this series).

The configurations of bivalents can therefore be given in terms of these variants, x, y and z. For instance, one arm pair in the bivalent represented in Fig. 4 may be shown as being of the constitution of x (+). y (+). z (-). Were there no interrelation of one situation to others, the eight possible combinations of xyz, viz., + + +, + - +, + + -, + - -, - + +, - - +, - + -, and - - -, can be expected to occur in the ratio of 4:4:2:2:2:2:1:1. In the present study however the paucity of data did not allow to verify this point.

From the above considerations it may be said that it is possible to distinguish two types of chiasmata with respect to their mode of origin. The first may be called "*primary chiasmata*" because they are related directly to the development of interstitial repulsion. The second will be referred to as "*secondary chiasmata*"; they are controlled by the relation of the strength of the repulsion force to the surface tension and the viscous resistance set up by the matrix substance. Thus the physical nature of the matrix at the time of chromatid opening-out is to have an important bearing upon the formation of such secondary chiasmata. Since the secondary chiasma is thus subjected to two sources of variation, viz., (i) repulsion and (ii) matrical resistance, a certain variation in the length of the secondary loops may be expected under different conditions. In cases when the chromatids open out at a time when the matrix is in a less viscous and perhaps less elastic state such secondary loops may be much smaller. In preparations on which the third paper of this series was based, no such remarkable loops were noted; most probably this may bespeak such a sort of difference in the nature of the matrix between the material employed at that time and that in the present observations. This assumption is supported by the fact that even in the same anther different pollen mother cells may differ slightly in the length of secondary loops, as will be seen when one compares Fig. 2 with Fig. 4. When the repulsion force overcomes the matrical resistance, the chromatids will open out to the final point, that is, the kinetochore. The precocious type of bivalents (cf. No. 5 of this series) will be taken as affording the indisputable case of this condition.

It is evident that the present findings and interpretations are at variance with the following hypotheses of Darlington and his school, (i) of chiasma terminalisation, (ii) of chiasma interference in relation to crossing over interference and (iii) of terminal affinity (cf. Darlington '37). Full discussion on these subjects will be presented together with additional data on another occasion.

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