Chromosome Studies on *Trillium kamtschaticum* Pall.

XII. The mechanism of crossing-over

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Of various hypotheses on the cytological mechanism of crossing-over, Darlington's interpretation ('35) seems to be most logically elaborate. He assumes that the cause of the formation of chromonema spirals is in an internal twist due to a rearrangement of the constituent particles, either between molecules, or within molecules. This molecular spiral leads the thread to coil in an internal spiral in the direction opposite to that of the major and minor spirals, and its direction is subject to unitary control in each of the arms of the chromosome; it is reversed at the kinetochore, so that the two arms of a single chromosome always coil in opposite directions. At the end of pachytene each particle undergoes division; the two threads are twisted round one another by relational coiling. Thus the daughter chromatids are relationally coiled, the direction of coiling being the same in each homologue, but just opposite to that in which the chromosomes are relationally coiled. The four chromatids are thus in a state of tension resulting from the conflict of lateral attraction and longitudinal cohesion. This torsional strain may induce a chromatid break in one of the four chromatids; if one breaks, all the stress will fall on the other chromosome, leading to the break of one of its chromatids. This double break will allow the release of the stress by rotation, after which the reunion of the broken ends may take place, thus resulting in crossing-over in the genetical sense and in a chiasma in the cytological sense. Thus the relational coiling in zygotene is supposed to be replaced by chiasmata in diplotene.

This hypothesis seems to be logical, but unfortunately several assumptions on which this hypothesis rests are at variance with the observed cytological facts. Especially the writer must emphasize that evidences were obtained from the present series of investigations on *Trillium kamtschaticum* which are entirely incompatible with these assumptions. First, it is difficult to understand how the broken ends of the two chromatids *always* rejoin in such a way as to give rise to two new chromatids. Darlington merely says ('37,

p. 548): "This rejoining presents no new problem, for as we have seen, ends of different chromosomes, free and unpaired, are capable of rejoining after X-ray breakage." However the X-ray experiments have showed that the reunion of the broken ends takes place at random (*cf.* No. 9 of this series) and hence it is likewise probable that it gives rise to a dikinetid chromatid and a fragment instead of the formation of two new complete chromatids. Such is not involved in crossing over. Secondly, his precocity theory of meiosis has been invalidated by the evidence that leptotene threads are composed of multiple chromonemata as in mitotic chromosomes (*cf.* No. 8 of this series). There is no reason therefore for assuming that the chromosome division takes place at the onset of diplotene and that this causes the upset of the equilibrium which leads to chromatid breakage. Thirdly, recent studies on the direction of chromonema spirals have indicated, in opposition to Darlington's assumptions, that there is no regularity in direction between two homologues nor between the two arms of a chromosome, and changes in direction which are independent of chiasmata in their origin occur in chromosome arms (*cf.* Nos. 2 and 4 of this series).

It should also be mentioned that our critical analysis of the chiasma and experiments on its origin gave evidence clearly contradicting Darlington's idea of chiasma localisation (No. 10 of this series). Furthermore, statistical study on the tetrad configurations and on the behavior of an heteromorphic pair of chromosomes gave results which strongly support the writer's Neo-two-plane theory (No. 7 of this series). Such accumulation of data against the chiasma type theory of Darlington led the writer believe that the "evidences" taken by him and his school as favoring his theory should be critically reinvestigated on a more scientific basis. Studies on this line are now in progress in our Laboratory.

One of the most important aims of this series of investigations has been the elucidation of crossing-over mechanism. The writer has failed to find it out at meiotic prophase and also to get any conclusive evidence that the chiasma is in its origin related to crossing-over. During the course of his study on the chromonema structure of meiotic chromosomes in *Trillium kamtschaticum*, he came to the conclusion that crossing-over *must* take place at the first meiotic metaphase. The data on which this conclusion rests are given in the present paper. The proposal of such an interpretation of crossing-over mechanism may be rather surprising to those who consider that it must take place at meiotic prophase, but scientists must resort to *verae causae* and avoid the formulation of *fictae causae*.

Terminology

In order to avoid confusion in the terminology, it will be desirable to give here definitions of some spiral forms. The formation of an apparent single spiral by two parallel threads which will be referred to as a "double-thread spiral" is carried out in one of two ways. (1) The spiral can arise by the rotation of both the ends of the threads. If such a spiral is made with a copper wire by wind-

Fig. 1. Models of double-thread spirals, relational (A) and parallel (B).

ing them round a rod, keeping the two threads always parallel, and they are pulled out straight without allowing any relative rotation of the ends, it will be found that the copper wires are relationally twisted with one another in the same direction as the spiral was wound, the number of such half-twists being equal to the number of half gyres of the spiral (Fig. 1A). This type of spiral, parallel in three dimensions but twisted in two dimensions, may be termed a "relational double-thread spiral." (2) The second type of spiral can be formed by winding the wires round a rod without allowing any rotation of the ends. Then in the course of spiralisation a reversed twisting takes place, the number of such half-twists being equal to the number of half-gyres of the spiral. When such spiral is pulled out straight without any relative rotation of the ends, one finds that the two threads are parallel throughout their length, for the twisting produced by spiralisation is here compensated by a twist in the opposite direction at each gyre of the spiral (Fig. 1B). Such a type of spiral, twisted in three dimensions but parallel in two may be termed a "parallel double-thread spiral."

These double spirals should not be confused with the double-coiled spiral (spiral-within-spiral), because very often the latter is also referred to as a double spiral.

Observations

What spatial relationship within a spiral system is taken by the paired chromatids at meiotic metaphase is the subject matter of the present observations. Usual cytological techniques proved to give

no satisfactory result, for the two chromatids of early metaphase are tightly appressed into one another by forming minor coils and hence their ease of sticking together by fixatives tends to give them the appearance of being a single-thread spiral. To overcome that tendency the writer's water-pretreatment method (No. 11 of this series) was found to be exceedingly effectual. By this treatment the two chromatids can be traced without much difficulty, especially in permanent smear preparations. In those treated by aceto-carmines after this pretreatment, the minor spirals are well preserved; although such a state will be more natural, it imposes certain difficulty on the observation and it is only by careful focussing that the duality

Fig. 2. The five bivalents at early metaphase of a PMC. The microphoto was taken from an aceto-carmines preparation pretreated with water. The pressure which was given on the cover-glass made some portions of chromosomes exceedingly stretched out. The relational relationship of the paired chromatids are revealed in these uncoiled portions, as shown by attended explanatory figures. $\times$ ca. 1300.

of the spiral can be traced. When however the major coils are artificially uncoiled by gentle pressure on the cover-glass, the two chromatids are clearly discernible (Fig. 2). Most of the present observations were then made on permanent smear preparations, in which the chromonemata are considerably contracted by the fixation so that the minor spiral is obscured, yielding a favorable condition

Figs. 3-24. Illustrating various internal relationships between the paired chromatids. All the figures were made from permanent smear preparations pretreated with water, the fixative being La Cour 2BE and the stain gentian violet. The magnification of the photos is ca. 1300 and that of the sketches ca. 4050. The appended sketches in Figs. 3-6 are those of the regions indicated by the arrows in the corresponding photos. In Figs. 8-24 (excl. Fig. 20), the upper part in each figure is the distal end of the arm. The region marked by *k* is the kinetochore. The identity of these chromosomes are as follows (for denotation, see the previous papers): 3, dl; 4, bs; 5, dl; 6, a; 7, dsl; 8, es; 9, bl; 10, dl; 11, bl; 12, cl; 13, cl; 14, a; 15, cl; 16, cl; 17, a; 18, bl; 19, a; 20, a whole bivalent B; 21, a; 22, bl; 23, a; 24, a.

For explanation, see p. 394.

for disclosing the internal relationship of the two threads. The material was taken from normal sporocytes.

Some earlier workers maintain that the two chromatids of each chromosome are coiled together in single spirals at early metaphase, *e.g.* in *Secale* (Sax '30), *Tradescantia* (Sax and Humphrey '34), *Rhoeo* (Sax '35), *Vicia* (Sax and Sax '35, Sax '36), while others hold that two parallel-coiled chromatids are found at this stage, *e.g.* in *Gasteria* (Taylor '31), *Trillium* (Huskins and Smith '35), *Fritillaria* (Darlington '35). These opposing views seem to the present writer merely to be due to the difference of stages at which the observations were made, for in the present material it is convincingly clear that at early metaphase the two chromatids take the form of single spirals, while at late metaphase they show separate parallel coils.

The nature of this apparent single spiral at early metaphase was found to be that of the relational double-thread spiral system. This is clearly represented in Fig. 2 at the portions of the chromosome arms which were mechanically uncoiled. In order to meet the doubt that such interlacings of the chromatids might be induced by mechanical uncoiling of the major spiral, closer observations were made in uninjured cells of permanent preparations, and it was found that the same structure also holds true, as illustrated by several microphotos, Figs. 3-6.

Then a question can be naturally raised how a relational spiral at early stage is convertible later into a parallel spiral, that is to say, how the process of parallelisation takes place. From numerous observations, the writer finds that there are many intergrading conditions which can be arranged as follows:

- 1) The two chromatids are relationally coiled throughout the entire length of the arm except a certain portion where the direction of coiling is reversed (Figs. 8, 14 and probably 9).

- 2) The proximal part of an arm is parallel and the residual part relational (Figs. 10, 11, 12, 13, 16, and the left short arm of Fig. 20).

- 3) Both the end regions of an arm are parallel, while the intercalary part is still relational (Figs. 19, 21, 22 and probably 6, 15, 17, 18).

- 4) The two chromatids are parallel with the exception of only one intercalary half-twist (Figs. 23 and 24).

- 5) The two spirals are completely parallel throughout their entire lengths (Fig. 6; see also many figures in No. 3 of this series).

Such a series of events must be taken as indicating the direction of parallelisation. As a corollary it is concluded that the process of parallelisation begins first at the kinetochore region, then independently at the distal end of the arm, and that these two processes advance inwards along the arm (see Fig. 25).

During the course of the present observations the following two facts were noticed which may be worthy of mention: (1) that very often the paired chromatids are attached together at an exactly corresponding point so that it is impossible to identify which are the original threads, as shown in the regions indicated by the arrows in Figs. 19 and 20; (2) that the original spiral takes a cylindrical form, while in the parted spiral the dimension along the cleavage surface is unaltered, but when viewed from the plane orthogonal to it the spiral is of about a half width (Figs. 10, 11, 13, 15 etc.). These points are of significance in considering the mechanism of parallelisation (*v. infra*).

The present observations were restricted to the relationship between the two chromatids. The chromatid however is not structurally the final unit; it consists of at least two visible chromonemata. However, since the chromatid may be taken functionally as an unit, the relationship between these half-chromatid chromonemata has been left untouched in the present study. Furthermore the chromosome arms dealt with in the present study are only those bearing no chiasma. How the chiasma is related to the spiral structure is another problem. Findings on these points will be published in further papers of this series.

Fig. 25. A schematic illustration of the orientation of parallelisation of the spiral system. The circles represent kinetochores, and the arrows the direction of parallelisation. D and E are different aspects of the same spirals viewed from different angles.

Discussion

An hypothesis of the mechanism of crossing-over

That the paired chromatids of a metaphasic chromosome constitute the relational spiral system was first described by Kuwada ('38) in *Tradescantia*.¹⁾ In this paper he says (p. 19): "In *Trillium* such a case would not be expected to occur. If in *Trillium* in which the chromosomes in the second division are of the double coiled structure as in the first division and 'the first anaphase chromosomes pass without material change of structure to the second metaphase' (Huskins and Smith, 1935) such relational coils are formed between the sister chromatids,²⁾ the chromosomes can not separate in the second division. In *Tradescantia*, on the other hand, this twisting around of the sister chromatids would afford no obstacle to separation in the second division, because in this plant the interkinesis exists in which the chromosome spirals are uncoiled or made loosely coiled, and in the second division the major spirals have been uncoiled." This statement is however at variance with the observed fact that in *Trillium* too its early metaphasic chromosomes show the same relational spiral relation of the paired chromatids. Furthermore Dr. Toyohuku of the writer's Laboratory has shown from careful observations on meiotic chromosome structure in *Tradescantia* that, although the paired chromatids of a metaphasic chromosome are relationally coiled except at the region of reversal in coiling direction they are already at early anaphase usually freely separated from one another except at the kinetochore region, thus giving half-bivalents of a cruciferous form, a condition entirely parallel to the case of *Trillium*. This finding leads to the implication that in *Tradescantia* the parallelisation process is retarded somewhat as compared with *Trillium*, that is, to early anaphase. He noticed also in *Tradescantia* the occurrence, though rarely, of chromosomes in the second metaphase in which the double-coiled structure still remains. It may accordingly be concluded that there is no essential difference in the parallelisation of spirals between *Trillium* and *Tradescantia*, except the time of its operation.

The mechanism of this parallelisation constitutes an important subject of the present discussion.³⁾ The conversion of the relational

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1) Darlington ('37) gives a figure clearly indicating the relational spiral in a bivalent of *Tradescantia* (Fig. 137 D), without making any reference to this point.

2) Kuwada employs the term "sister chromatids" in the place of "paired chromatids" in the present usage.

3) The bearing of the fact that the paired chromatids are relationally coiled at early metaphase on the mechanism of spiralisation must be another important subject of discussion. This will be presented later elsewhere.

spiral system into the parallel one must take place under one of two conditions. Either it takes place by rotating the distal end of a chromosome in the direction opposite to that of the spiral, as many times as the number of twists, or else by the occurrence of segmental exchanges at twisting points without rotating the end. The first possibility can be excluded by the following considerations. First, the cause for such a rotation cannot be imagined, since it is considered as nothing but a teleological movement with the aim of parallelisation itself. Secondly, rotation is inconsistent with the fact that the parallelisation initiates also from the proximal end which is a fixed point and hence at which rotation is impossible.

It must therefore be concluded that the parallelisation process is associated with segmental exchanges between the two chromatids. How this is carried out is schematically illustrated in Fig. 26. Here the writer assumes that the major and the minor spirals respectively have their own matrices, adopting the view of Kuwada and Nakamura ('34). These may be termed respectively major and minor matrices.

The minor matrix is supposed to undergo division prior to the division of the major matrix. This division is most probably associated with the initiation of development of repulsions between the paired kinetochores and between the distal end regions of the chromatids, and with their advance towards the middle of the arm.

The division of the minor matrix (in the direction indicated by the arrow in Fig. 26A) will alter the three-dimensionally parallel condition of the two threads, a-a and b-b, into the two-dimensionally twisted condition at a certain point (Fig. 26B). At this cross-point the torsion stress due to the progress of matrix division will cause simultaneous breaks of the two chromatids which will be followed by reunions (Fig. 26C); such reunion following breakage is a common phenomenon in X-ray experiments. The reunion will so take place as to give rise to new chromatids, only in a longitudinal way (a1'-b2' and b1'-a2'), because the transversal join (a1'-b1' and b2'-a2') is rendered impossible by the cleavage of the matrix. The two chromatids, before the breakage, are intimately associated together by the minor coils which constitute a parallel spiral system from the beginning of their development.

This intimate association of the two strands will cause the break *accurately at corresponding points* in each of them. The resulting new chromatids, a-b and b-a, are then in a parallel spiral system (Fig. 26D), and this alternation of the system will cause the two strands to fall apart as far as possible within the major matrix. Thus the resulting contour of the spirals may be altered in their dimension

along the direction of the repulsion, as represented in Figs. 25D and E. The exertion of the repulsion between the two chromatids along their entire lengths will next cause the division of the major matrix,

Fig. 26. Illustrating the mode of break and reunion between the paired chromatids. In this diagram only a half-coil is represented. The two chromatids are enveloped by the minor matrix (pale black) which is further covered by the major matrix (hatched). In order to simplify the diagram, the minor spiral is omitted.

and in this way the half-bivalents of early anaphase come to take a cruciferous form, the chromatids falling apart and associating only at the kinetochore.

According to the writer's Neo-two-plane theory of bivalent constitution (see No. 4 of this series), the paired chromatids of each arm are non-sisters in two-thirds of the cases. Therefore in such cases, the segmental exchange between them results in detectable crossing-over.

Crossing-over is thus necessary consequence following the conversion of the three-dimensionally parallel (but two-dimensionally twisted) spiral system of the two strands into the two-dimensionally parallel (but three-dimensionally twisted) system.

In somatic mitosis each of the two chromatids of a chromosome usually takes an independent spiral system from the beginning of its development. Accordingly there will usually be no chance in mitosis of exchanges between them.

This interpretation of the crossing-over mechanism makes a marked contrast with all other earlier hypotheses which have considered it at early prophase of meiosis. It must be noticed however that although it was genetically proved that crossing-over takes place somewhere during the first reduction division, there is no definite

proof that it should be at early prophase. If the present interpretation be adopted, the time of crossing-over is early metaphase in *Trillium* and early anaphase in *Tradescantia*. Probably certain other shifting of the time (*e.g.* to diakinesis, as supposed to be the case in *Zea* cf. Creighton and McClintock '32) may be shown in different organisms or under different environmental conditions even within the same species, according to different behaviors of the kinetochore in relation to the spiralisation of chromonemata.

The frequency and distribution of segmental exchanges between the two chromatids is the next problem to be dealt with in connection with their genetical consequences. Here there are many items of subjects to be discussed. Although full consideration on a purely theoretical basis will be presented elsewhere, some brief comments may be given here to show how justifiable explanations of the essential phenomena of crossing-over may be put forward in terms of the present theory.

1) The present theory explains as a matter of course a characteristic of crossing-over, that it takes place in the diploid at the four-strand stage of meiosis between only two of the four participating chromatids at any one point.

2) The present interpretation of the mechanism of break and reunion stands for the accurate correspondence in the exchange points between the two strands, which has been always genetically proved, with one exception in *Drosophila*.

3) In short chromosome arms less than the length of a half-coil, the chromatids can separate freely without any breakage. Probably the fourth chromosome of *Drosophila melanogaster* in which no cross-over is usually detectable will be an example of this

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case.

4) Generally speaking the break occurs once in each half-coil; therefore the frequency of exchanges is proportional to the number of spiral gyres (not strictly, *vide* item 5). Furthermore the number of spiral gyres per chromosome arm is exactly proportional to arm length (No. 1 of this series). Genetically these facts may imply that the relative distance between the genes is a function of the cross-over values and there is a certain correspondence of genetical map length with arm length (*vide* also item 6).

5) In the regions of the proximal and distal ends of an arm, one coil length of the chromatids may sometimes be freely separated without any breakage. The number of exchanges per chromosome arm must be between n and $n-2$, where n denotes the number of half-coils per chromosome arm. (The reduction of breaks due to the occurrence of reversals in coiling direction is here left out of

consideration.) This frequent failure of exchanges in both the end regions is parallel to the fact known in *Drosophila* that there is a greater amount of crossing-over per unit of material length in the middle of the X chromosome and also in the middle of the autosomes as compared with crossing-over frequency in the end regions.

6) Where n is small, the effect of the failure of exchanges in the end regions will be relatively greater than in cases where n is larger. Genetically this means that where the size difference is greater, the lack of proportionality between the genetical length and the actual chromosome length is more serious, a condition demonstrated in *Drosophila*.

7) The chromonema coiling is independent in the two arms of a chromosome (see Nos. 2 and 4 of this series). This may be taken as parallel with the fact that there is no interference between cross-overs in different sides of the kinetochore, as shown in the V-shaped autosomes of *Drosophila*.

8) Within a half-coil distance, two breaks will generally occur only rarely but beyond this limit one break will strongly tend to follow another. Genetically this means that a half-coil distance is the average interference distance and explains why interference is strong at adjacent regions but it vanishes and becomes even negative suddenly at a certain distance from the original point of crossing-over, as Kikkawa ('35) demonstrated in *Drosophila virilis*.

9) The process of parallelisation of the spirals which initiates at both ends of an arm and proceeds towards the middle will tend to shift the existing twists of the chromatids towards the central region. Thus the distance between two twists will be shorter in the central region than near the ends (see Figs. 5, 10, 21, and 22). Genetically it was demonstrated in *Drosophila* that the coincidence greatly increases near the middle region of the chromosome arm.

10) The priority of behavior in both the proximal and distal ends is entirely parallel to the conclusion drawn from *Drosophila* experiments by Charles ('38) that "crossing-over may be controlled jointly from the attachment and from the free end."

11) From the writer's Neo-two-plane theory it follows that the paired chromatids are non-sisters in 66% of cases. Therefore the highest recombination value to be expected will be 66%, though such a constant exchange at a definite region will be very rare. This seems to stand for rare cases of the occurrence of crossing-over more than 50%, e.g. about 60% cross-overs between G and S in *Pisum* (Wellensiek '29), which the other current theories of crossing-over fail to explain.

12) Since the length of a half-coil is the same in both the arms

of a chromosome and the force causing chromatid which is initiated first at the kinetochore works equally at both sides of the kinetochore, the frequency of breakage will tend to be high in regions symmetrically placed across the kinetochore. This seems to explain the case found in *Neurospora* by Lindegrens ('37).

13) The form of the spiral is greatly affected by environmental conditions such as temperature (see Nos. 1, 5 and 6 of this series). A fundamental difference in chromosome structure was shown in the chromosome type referred to as 'precocious' in the previous papers, in which the paired chromatids are coiled independently from the beginning of spiralisation as in mitotic chromosomes. Since there is no stage of the relational spiral in these chromosomes, there is no chance of material exchanges. Probably such is the case of *Drosophila* male or *Bombyx* female in which crossing-over is usually completely suppressed.

14) The mode of spiralisation under normal conditions is different in different organisms, even in allied species. The writer has shown that the meiotic chromosomes of *Paris quadrifolia* have more slender minor coils and more compact major coils than *Trillium kamtschaticum* (No. 11 of this series). Thus the absolute length of a half-coil is considerably less in *Paris*. Such structural difference may be related to the difference in the percentage of crossing-over in allied species, such as between *Drosophila melanogaster* and *D. virilis* (cf. Chino and Kikkawa '33).

15) If similar variation in chromosome structure is caused to occur in one organism by external agencies or in different sex, this will explain certain variations in crossing-over percentage shown under different environment conditions or by sex, e.g. *Primula*, *Zea*, *Apottetix*.

16) Under certain conditions the number of twists per chromosome arm is nearly constant (see item 5). It follows then that if some external factors happen to cause more frequent exchanges in a certain part of the chromosome, this will be naturally attended by a reduction of exchanges in the other part. This offers an explanation for the fact known in *Drosophila* and *Primula* that changes in crossing-over percentage are differential with regard to different parts of a chromosome, that is to say, weakening or strengthening of the linkage between two genes of a group is accompanied by correspondent strengthening or weakening of the linkage between other genes of the group.

17) Such changes in exchange frequency may be attributed to certain timing unbalance in the parallelisation process, that is, between the behavior of the kinetochore and that of the distal end.

Abnormal external conditions may cause retardation in the separation of the associated kinetochores. If their separation is so retarded that the parallelisation is completed only in the direction from the distal end, the exchanges will naturally be increased in the neighbourhood of the kinetochore. Really it was found that crossing-over is increased in the kinetochore region of the X chromosome and of the autosomes of *Drosophila* by changes in temperature and by X-rays.

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Summary

1) The paired chromatids of each meiotic chromosome constitute at early metaphase the relational spiral system (Fig. 1A), while those at late metaphase take the parallel system (Fig. 1B).

2) This conversion of the spiral system takes place first from the proximal region and then independently from the distal end. These processes proceed inwards along the arm and when they meet one another the parallelisation is completed.

3) Such an orientation of the parallelisation excludes a possibility that the system is altered by rotation of the free end. The other possibility must, therefore, be admitted that the parallelisation is associated with breaks and reunions between the chromatids at twisting points existing in the spiral system. This breakage is supposed to be caused by the cleavage of the matrix enveloping the minor spiral, which at the same time plays a rôle for preventing the transversal reunions of the broken ends (Fig. 26).

4) According to the writer's Neo-two-plane theory, the paired chromatids are non-sisters in two-thirds of the cases. Therefore in these cases such breaks and reunions should result in detectable cross-overs.

5) This new hypothesis of crossing-over mechanism presents reasonable explanations for many essential characteristics hitherto known in the crossing-over phenomena.

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