Chromosome Studies on *Trillium kamtschaticum* PALL. II. The Direction of Coiling of the Chromonema within the First Meiotic Chromosomes in the PMC

By

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(With Plates XV–XVII and 4 Text-figures)

In the first paper of this series (MATSUURA '35), the writer has made a comparison between the normal meiotic chromosome type of *Trillium kamtschaticum* and the two abnormal types, called 'abnormal I' and 'abnormal II', which resulted from the effects of abnormally high temperature in which the plants grew, in respect to the coil number in the chromonema of meiotic metaphase chromosomes and the relationship of it to the volume of chromosomes. The conclusion was drawn that the coil-inclination angle of the chromonema remains unaffected throughout these three types, despite their possible alternation in chromosome volume.

In the present study special attention has been devoted to find out certain characteristics of the direction of coiling of the chromonema within the chromosomes at the same stage and also those at first anaphase. Observations were made mainly on the normal type; a few of abnormal I type were however supplemented to them, as the bivalent formation in the latter is principally the same with that in the normal ones. The spiral situation in abnormal II type will be described in another paper.

The problems principally involved in the present study are as follows:

i) Is the direction of coiling a stable character of a given chromosome, and if so, to what extent?

ii) Is there any relation between the two homologues at metaphase as to this feature?

iii) Is it possible that the chromonema reverses its direction of coiling in any part of a chromosome?

iv) Is there any characteristic difference as to this feature between different cells or different individuals?

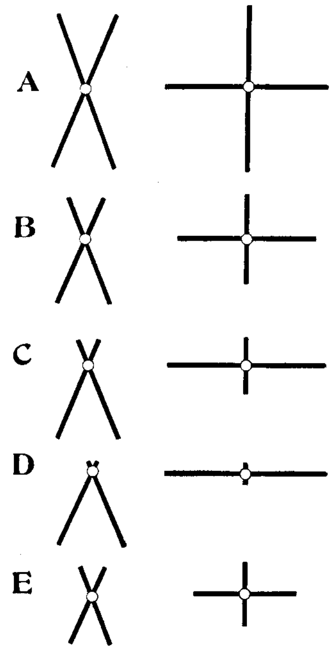
Up to the present, not many observations have been made on these features, and most of them were even very fragmental. To the first question, no definite answer has been given; there has been no detailed study in which reference to individual chromosomes was made. On the direction of coiling as a whole, NEBEL ('32b) states however that "the total number of dextrorse and senistrorse coils in a cell tends to be equal" but "may be modified by a characteristic mode of coiling in certain chrososomes". SHINKE ('34) likewise observed an approximately equal frequency of occurrence of dextrorse and senistrorse spirals. As to the second question, ISHII ('31) maintained the opinion that the direction of coiling in gemini is always the same. According to NEBEL (*id.*), on the contrary, there is no such rule, both kind of gemini, 'symmetrical' and 'asymmetrical', having been observed, although the direction of coiling in gemini appeared to have somewhat characteristic features in different species studied, for in one species asymmetrical gemini outnumbered symmetrical ones, whereas in another the opposite condition proved true. On the third question, SAX ('30), TAYLOR ('31) and ISHII ('31) observed the actual reversal of coiling direction in a part of a chromosome. NEBEL (*id.*) confirmed these observations with a certain reservation that the change in coiling direction may take place only at the insertion region of a chromosome, and does not occur between it and the end of a chromosome. No observation has been made on the last question.

Methods and General Explanations

Practically all the observations were made on the same permanent smear preparations as employed in the previous study. Photomicrographs of Plate XV and XVI were secured from material fixed by TAYLER's solution; those of Plate XVII were taken from material fixed by LA COUR 2BE. The preparations were throughly stained with gentian-violet according to NEWTON's method.

As previously described (MATSUURA, '35), each of the five bivalents in a complement of this plant always takes at metaphase a cruciferous form, being attached only at the insertion region. There are two types of bivalents generally distinguishable at this stage: type $\times$ and type $+$, the former being a transitional form to the latter, the final form. Diagrammatic representation of the five bivalents in a complement, A, B, C, D and E, is given in Text-fig. 1. These characteristic configurations of metaphase chromosomes render it impossible to identify each member of the homologues, that is, which short arm, together with which long arm, is to constitute a component member of the bivalent. Consequently a certain degree of limitation is placed on the present analytic study on the chromonema situation of meiotic chromosomes.

In order to facilitate the understanding of configurations, the writer introduced the following diagrammatic signs and modes of representation. (i) The dextrorse coiling of the chromonemata in 'dyads' is expressed by a capital "R", and that in 'half dyads' at first anaphase by a small latter, "r"; "L" and "l" likewise stand for the senistrorse coiling in dyads and half dyads respectively. (ii) In cases where the identification of the two arms of a chromosome is possible, the configuration of a bivalent is given by a scheme such as "$\frac{\text{R–L}}{\text{L–R}}$," the dash, "–", indicating the insertion region and the latters ahead of it representing the coiling situation in the short arm and those behind it that in the long one. Owing to the impossibility of identifying each member of the bivalent, $\frac{\text{R–L}}{\text{L–R}}$ can not be distinguished from $\frac{\text{R–R}}{\text{L–L}}$. This mode of expression is applied to the B, E, C and D chromosomes, although in the latter two the direction of coiling in the short arm is very often scarcely determinable and they are then shown by a scheme such as $\frac{\text{–L}}{\text{–R}}$. (iii) In chromosome type A, where the two arms are of nearly equal length and are not usually distinguishable from one another, the coiling situation of a vivalent is given *e. g.* by $\frac{\text{R.L}}{\text{L.R}}$, the point "." representing here the insertion region. The $\frac{\text{R.L}}{\text{L.R}}$ form is to include then the following three forms to be classified according to (ii): $\frac{\text{R–L}}{\text{L–R}}$ $\left(\text{or } \frac{\text{R–R}}{\text{L–L}}\right)$, $\frac{\text{L–R}}{\text{L–R}}$ and $\frac{\text{R–L}}{\text{R–L}}$. (iv) In cases where the chromonema reverses its direction between the insertion region and the distal end of a chromosome, the situation is represented as *e. g.* –LR for 'single' reversal, –LRL for 'double' reversal, etc.



Text-fig. 1. Explanation in text.

For convenience sake, the description of data will be given first as to the direction of coiling in the part of the chromonema proximate to the insertion region, and the occurrence of the reversal of direction will be dealt with in a separate chapter. The former will be justified on the basis that the differential condensation or 'spiralization' of the chromonema begins at a certain stage of prophase at the point of attachment.

Description of Data

i) *Metaphase Chromosomes*

a) A chromosome type.

TABLE I
Direction of coiling in the A chromosomes

Classes	Observed	Expected
1) L.L / L.L	4	*1.5*
2) L.R / L.L	2	*3.0*
3) R.R / L.L	4	*6.0*
4) R.R / R.L	3	*3.0*
5) R.R / R.R	2	*1.5*
Total	15	*15.0*

A total of fifteen bivalents were analyzed as to the direction of coiling of the chromonema. Their distribution into the five classes to be distinguished is tabulated in TABLE I, together with the theoretical values which are expected from the assumption that the direction of coiling in the chromonema is subjected to mere chance.

Considering the chromosome arms separately, we get:

Classes	(1)	(2)	(3)	(4)	(5)	Total
L	16	6	8	3	0	33
R	0	2	8	9	8	27

b) B and E chromosome types.

In these chromosome types, nine classes are distinguishable, as to the coiling situations in the long and the short arms. Fifteen bivalents of the B type and twelve of the E type were analyzed. Their distribution is given in TABLE II.

The proportion of L arms to R arms is calculated as follows:

Classes		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	Total
B	L	0	6	6	3	8	0	0	0	0	23
	R	0	2	6	1	8	0	0	0	20	37
E	L	8	3	0	6	8	2	2	0	0	29
	R	0	1	0	2	8	6	2	0	0	19

TABLE II
Direction of coiling in the B and E chromosomes

Classes	B	E	Total	Expected
1) L–L / L–L	0	2	2	*2.7*
2) L–L / R–L	2	1	3	*2.7*
3) R–L / R–L	3	0	3	*2.7*
4) L–L / L–R	1	2	3	*2.7*
5) L–L / R–R or L–R / R–L	4	4	8	*5.4*
6) R–L / R–R	0	2	2	*2.7*
7) L–R / L–R	0	1	1	*2.7*
8) L–R / R–R	0	0	0	*2.7*
9) R–R / R–R	5	0	5	*2.7*
Total	15	12	27	*27.0*

c) C and D chromosome types.

Here only three classes are to be distinguished, as to the direction of coiling in their long arms. Some of the short arms were actually analyzed, but they were neglected in order to simplify the matter. The results of analysis on thirteen bivalents of the C type and sixteen of the D type are given in TABLE III.

TABLE III
Direction of coiling in the C and D chromosomes

Classes	C	D	Total	Expected
1) –L / –L	4	9	13	*7.25*
2) –R / –L	8	2	10	*14.50*
3) –R / –R	1	5	6	*7.25*
Total	13	16	29	*29.00*

The proportion of L's to R's in these types will be given as:

Classes		(1)	(2)	(3)	Total
C	L	8	8	0	16
	R	0	8	2	10
D	L	18	2	0	20
	R	0	2	10	12

It will be obvious from these data that each chromosome type may take all possible forms with respect to the coil direction in the chromonema presenting no relation between the two homologues as to this feature. Some classes were actually missing in certain types (as in B and E, TABLE II) and further certain great quantity of discrepancies of frequency from the theoretical expectation was frequently met with, but these may be due to the rather small number of observations and probably are not to be considered as statistically significant. In reality, if the data from the A, B and E types are put together and arranged into the same five classes as in TABLE I, one can get a closer agreement of the results with the expected values (see TABLE IV).

TABLE IV
Summing up TABLES I and II

Classes	A	B	E	Total	Expected
1) 4L (or 0R)	4	0	2	6	*4.2*
2) 3L (or 1R)	2	3	3	8	*8.4*
3) 2L (or 2R)	4	7	5	16	*16.8*
4) 1L (or 3R)	3	0	2	5	*8.4*
5) 0L (or 4R)	2	5	0	7	*4.2*
Total	15	15	12	42	

The proportion of L arms to R ones is summarized in the following table (TABLE V).

TABLE V
The proportion of L's to R's

	A	B	C	D	E	Total	Average	Deviation
L	23	23	16	20	20	121	113	+8
R	27	37	10	12	19	105	113	−8

Of the total 226 arms observed, 121 are L's and 105 R's, the deviation from the average amounting only to ± 8. This will be taken as a further confirmative evidence that there is no correlation as to this feature between the two arms composing one chromosome, that is, each arm behaves independently of the other.

As might be expected from the data given above concerning individual chromosomes, it is shown by TABLE VI that there is also no definite rule on the coil direction of the chromonemata within the five bivalents in an entire cell. While in cases, Nos. 1, 3, 4, 5, 6 and 7 the L arms outnumbered the R ones, cases Nos. 2, 8 and 9 were found to be the opposite. It must be added that all cells of No. 1 to No. 7 were taken from one and the same plant. This will be then taken to be positive in denying a tendency for one certain condition of coiling to appear more than another, either in the individual cell or in the individual plant. Based on the theoretical expectation, No. 1 cell must be of a very rare type, as nearly all the arms (except 2 of the total 16 which were analyzed) showed the same senistrorse coiling.

ii) *Reversal of Direction of Coiling*

In most cases, the reversal in the direction of coiling of the chromonema occurs 'singly', that is, one time between the insertion region and the distal end of a chromosome. The double reversal takes place with less frequency, but no case was observed to change its direction more than two times. In this connection it should be noted that in the distal ends of the chromonemata sometimes the last small portion of the coil appears to reverse its direction, as observed in other plants by TAYLOR ('31), TUAN ('31) and NEBEL ('32 a). In the following description, the reversal in this region was neglected, because this is probably to be ascribed to some certain spacial relation between the chromonema proper and its surrounding matrix, and hence is of a nature, from the view of development of spirals, to be distinguished from the primary reversal.

The reversal of the coil direction may take place at any point of the chromonema and its position does not correspond in the homologues. On account of these facts and in order to avoid complication of description, data concerning the position of reversal are not given in the following account, except in case of necessity.

1) A chromosome type. Of 15 bivalents (30 chromosomes) observed, 7 showed no reversal; the others were of the following reversal forms:

$$\frac{\mathrm{R.RL}}{\mathrm{L.L}},\ \frac{\mathrm{LR.LR}}{\mathrm{L.LR}},\ \frac{\mathrm{R.RLR}}{\mathrm{LR.R}},\ \frac{\mathrm{LR.L}}{\mathrm{R.R}},\ \frac{\mathrm{RL.LR}}{\mathrm{RL.LR}},\ \frac{\mathrm{L.LR}}{\mathrm{R.LR}},\ \frac{\mathrm{R.L}}{\mathrm{RL.R}} \text{ and } \frac{\mathrm{R.LR}}{\mathrm{L.RL}}.$$

It follows then that of the total 60 chromosome arms, 16 were of the

TABLE VI
Direction of coiling in the chromonema of the five chromosome members in nine complete cells

No. of cells	A	B	C	D	E	The proportion of L : R
(1)[1]	L.L L.L	L–L R–L	–L –LRL	–L –LR	L–L R–LR	14 : 2
(2)	R.R LR.R	R–R L–L	LR–L R–L	–R –L	L–L R–R	7 : 11
(3)	L.R L.R	L–L L–R	–R –L	–R –R	L–R L–L	9 : 7
(4)	L.L L.L	R–R R–R	–R –?	–L –L	L–R R–L	8 : 7
(5)	LR.L R.R	R–LR LR–L	L–L L–L	–L –L	L–R L–R	11 : 7
(6)	RL.LR RL.LR	LR–LRL RL–RL	–RL –LRL	–LR –LR	R–LR L–R	11 : 5
(7)	L.LR R.LR	L–L R–R	R–R L–L	–RLR –RLR	L–R?[2] L–L?	10 : 8
(8)[3]	R.L RL.R	R–RL R–R	R–L L–RLR	–R –R	L–L L–L	8 : 10
(9)[4]	R.LR L.RL	R–LR L–R	–R –LR	LR L	–R –L	8 : 6

1) Refer to Plate XV and Text-fig. 2.
2) It was impossible to ascertain whether the reversal of coiling occurs in the long arms or not.
3) Refer to Plate XVI and Text-fig. 3.
4) This cell was taken from a plant of abnormal I type.

reversal type, only one of which was doubly reversed.

2) B chromosome type. Of 15 bivalents (30 chromosomes) observed, 8 showed no reversal; the others were of the following forms: $\frac{\text{R–L}}{\text{R–LR}}$, $\frac{\text{R–RL}}{\text{R–R}}$, $\frac{\text{R–LR}}{\text{LR–L}}$, $\frac{\text{LR–LRL}}{\text{RL–RL}}$, $\frac{\text{R–RL}}{\text{R–R}}$, $\frac{\text{R–L}}{\text{RL–LR}}$ and $\frac{\text{R–LR}}{\text{L–R}}$. It may be seen then that of the total 60 arms, 12 showed reversal, only one of which was doubly reversed.

3) C chromosome type. Of 13 bivalents (26 chromosomes) observed, 5 showed no reversal; the others were: $\frac{\text{–R}}{\text{–LR}}$ (in two cases), $\frac{\text{–L}}{\text{–LRL}}$, $\frac{\text{LR–L}}{\text{R–L}}$, $\frac{\text{–RL}}{\text{–LRL}}$, $\frac{\text{–L}}{\text{–RLR}}$, $\frac{\text{–LR}}{\text{–L}}$, and $\frac{\text{–LR}}{\text{–RL}}$. Hence 10 of the total 36 arms (some of the short arms having not been observed) were of the reversal type, 3 of which were doubly reversed.

4) D chromosome type. Of 16 bivalents (32 chromosomes) observed,

10 showed no reversal; the others were: $\frac{\text{-R}}{\text{-RL}}$, $\frac{\text{-LR}}{\text{-LR}}$ and $\frac{\text{-RLR}}{\text{-RLR}}$. It follows then that of the total 32 arms (the short arms having been entirely neglected here), 8 were of the reversal type, 2 of which were double-reversed ones.

5) E chromosome type. Eleven bivalents (22 chromosomes) were analyzable as to this point. Of them, 9 showed no reveral; the other two were: $\frac{\text{L–L}}{\text{R–LR}}$ and $\frac{\text{R–LR}}{\text{L–R}}$. Of the total 44 arms, 2 were thus singly reversed.

From these data, it will be possible to measure the percentage of reversal in the direction of coiling per single chromosome arm for each of the five chromosome types (*v*. TABLE VII).

TABLE VII

Percentage of reversal of coiling per single arm

Chromosome type	Total arms observed	Frequency of reversal	Percentage of reversal
		(Cases of double reversal are shown in parentheses)	
A	60	16 (1)	26.6 (1.7)
B	60.	12 (1)	20.0 (1.7)
C	36	10 (3)	27.8 (8.3)
D	32	8 (2)	25.0 (6.3)
E	44	2 (0)	4.5 (0)
			Averages: *20.8* (*3.6*)

TABLE VII indicates that the percentage of reversal per single chromosome arm is highest in C chromosome type and lowest in E type. If the reversal of coiling is taken to be caused by chance only, the consequence must be that its percentage should be proportionate to the length of the chromosomes. In this respect, it must be taken into consideration that the data on the C and D chromosomes were principally taken from their longer arms.

In order to determine then to what extent the percentage of reversal is related to the chromosome length, TABLE VIII was prepared, in which the percentage of reversal per single coil was calculated for each of the five chromosome types and compared with each other. Such means of calculation will be justified, since, as previously described (MATSUURA, '34), the gyre number for each chromosome type is proportional in exact manner with the chromosome length.

TABLE VIII
Percentage of reversal of coiling per single coil

Chromosome type	Gyre number in each arm	Reversal % per single arm (Percentages of double reversal are shown in parentheses)	Reversal % per single coil
A	11-11[1]	8/30[2] = 26.6 (0.5/30 = 1.7)	ca. 2.4 (0.15)
B	9	8/30 = 26.6 (1/30 = 3.4)	ca. 2.9 (0.38)
	5	4/30 = 13.3 (–)	ca. 2.6 (–)
C	11	10/36 = 27.8 (3/36 = 8.3)	ca. 2.5 (0.75)
D	12	8/32 = 25.0 (2/32 = 6.3)	ca. 2.1 (0.52)
E	7	2/22 = 9.0 (–)	ca. 1.3 (–)
	4	0/22 = 0 (–)	0 (–)
			Average: ca. *2.0* (*0.23*)

1) *cf.* MATSUURA ('34, '35).
2) The numerators indicate the frequency of reversal and the denominators the total number of arms under observation.

It will been seen from this table that the reversal percentage fluctuates within a rather small range, that is, from 2.1 to 2.9, for the four chromosome types, A, B, C and D. This seems to imply that there is no speciality to be ascribed to these chromosomes as to this feature and the occurrence of reversal in the direction of coiling is principally due to chance. And the very low percentage of reversal shown by the E chromosomes may be reasonably interpreted in either of the following possible manners: (i) it is due to the rather small number of observations, (ii) it is due to personal error which is necessarily greater when the chromosome is shorter, or (iii) it is due to a certain limitation that for the occurrence of reversal coils more than a certain range is necessary. The possibility of the last interpretation seems to be somewhat supported by the findings by NEBEL ('32 b) on the meiotic chromosomes in certain plants of *Tradescantiae,* in which the chromosomes are very short, no one possessing more than four coils, and no reversal of the direction of coiling is observed. The last interpretation may also be available for the case of double reversal. It will be seen from TABLE VIII that the occurrence of double reversal appears to be possible only in rather long chromosome arms, such as ones the coil number of which amounts to about 9 (the long arm of the B type). It remains however as an open question why the percentage of double reversal is so high in the C and D chromosomes and so low in the A chromosome, despite the fact that these three chromosome arms under observations are of nearly equal length.

The next question to be raised is whether the frequency of reversal is equally distributed in individual cells or not; in other words, whether the tendency of reversal is not different in different cells. A compilation of all the data on nine complete cells analyzed, TABLE IX, will serve for the elucidation of this point.

TABLE IX
Distribution of reversal frequency in nine complete cells

No. of cells (*v.* TABLE VI)	Frequency of reversal (Double reversals in parentheses)	Total number of arms observed
(1)	3 (1)	16
(2)	2 (0)	18
(3)	0 (0)	16
(4)	0 (0)	15
(5)	3 (0)	18
(6)	12 (2)	16
(7)	4 (2)	18 (2 uncertain)
(8)	3 (1)	18
(9)	5 (0)[1]	14

1) This is a cell of abnormal I type.

If one takes the average percentage of reversal per single chromosome arm, 20.8% (*cf.* TABLE VII), then the theoretical frequency of reversal for each case from 14 arms to 18 ones may be calculated as:

No. of arms:	14	15	16	17	18
Frequency of reversal:	2.9	3.1	3.3	3.5	3.7

With the exception of one cell, No. 6, all the others appear not to diverge very far from these theoretical values. This will be taken to indicate that there is no general tendency in the chromosomes, the chromonemata of which more frequently reverse their direction of coiling in particular cells. The cell, No. 6, is particularly of interest in its high percentage of reversal, which is expected theoretically to occur very rarely. The configurations of the five chromosome types in this cell were as follows:

$$A = \frac{\text{5R6L.4L7R}}{\text{6R5L.5L6R}}$$

$$B = \frac{\text{5L1R–2L1R6L}}{\text{4R2L–3R6R}}$$

$$C = \frac{\text{–2R9L}}{\text{–3L2R5L}}$$

$$D = \frac{-3L8R}{-5L6R}$$

$$E = \frac{4R\text{–}2L4R}{4L\text{–}6R}$$

In the above, the figures indicate the coil number roughly estimated. As will be obviously seen from these configurations, the point of reversal does not exactly correspond in the homologues, even in some bivalents (as in A and D) where the reversal appears to be exceedingly balanced in them, a matter naturally suggested from the previous conclusion that in the direction of coiling the individual homologues are independent of one another.

iii) *First Anaphase Chromosomes*

Owing to insufficiency of preparations giving clear images of anaphase chromonemata, only one complete cell was available for analysis of their direction of coiling. Despite that the present observations are thus of a preliminary nature, a brief note will be made here, since some interesting situations of coiling were actually revealed.

The reduction to a certain extent in the number of coils of the chromonema is a remarkable feature of anaphase chromosomes. It was observed that the number of coils is approximately the same in each of the corresponding halves of a geminus and is represented, though roughly, as follows:

a	9–9
b	4–7
c	2–9
d	. –9
e	3–5

Compared with the number of coils within the previous metaphase chromosomes, these values indicate the reduction of about 26% in each chromosome type. This implies that the anaphase chromonemata go toward the poles unravelling their coils. The elongation of chromosomes at anaphase will be the natural consequence of this. The unravelling process appears to initiate at the distal end of the chromosome, as may be suggested from the frequent occurrence of certain irregularities in the direction of coiling at the last one or two coils, sometimes giving the impression that the chromonema entirely reverses its direction at this region. That such irregularities are secondary consequences due to unravelling of coils is obvious from the non-occurrence of the same in the corresponding sister chromatid. For the description of the direction of coiling in half dyads, these irregular regions were then neglected.

The half dyads separated shortly after first metaphase take the same configuration as the dyads, they remaining attached only at the insertion region.[1] In the consideration of coiling direction in them, attention should be paid to the following facts: (i) the sister chromatids are always characterized by parallel coiling, and (ii) the direction of coiling in the chromatids remains unaffected during their migration to the poles, except for the possible change at their distal ends. These facts make it possible to reconstruct the original dyad condition of coiling from those in the half dyads, and offer direct evidence concerning the mode of reduction, as utilized by NEBEL ('32 b) in *Tradescantia reflexa* and by SHINKE ('34) in *Sagittaria Aginashi.*

The analysis of direction of coiling in a complete cell represented in Pl. XVII, Figs. 1–7, and schematically in Text-fig. 4, showed the following chromonema configurations for each half of the gemini.

$$
\begin{array}{ll}
a_1 = \dfrac{l.r}{l.r} & a_2 = \dfrac{l.r}{l.r} \\[2ex]
b_1 = \dfrac{l\text{–}l}{r\text{–}lr} & b_2 = \dfrac{l\text{–}l}{r\text{–}lr} \\[2ex]
c_1 = \dfrac{\text{–}lr}{\text{–}lr} & c_2 = \dfrac{\text{–}l}{\text{–}l} \\[2ex]
d_1 = \dfrac{\text{–}l}{\text{–}lr} & d_2 = \dfrac{\text{–}l(?)}{\text{–}l(?)} \\[2ex]
e_1 = \dfrac{l\text{–}l}{r\text{–}l} & e_2 = \dfrac{(?)\text{–}l}{(?)\text{–}l}
\end{array}
$$

It should be noted that the point of reversal in the direction of coiling is also correspondent in the sister chromatids, as indicated by arrows in Pl. XVII, Figs. 1 and 3 for each one of the long arms of b_1 and b_2 and Figs. 7 and 8 for the two long arms of c_1.

A normal reduction division should result in symmetrical forms as to the direction of coiling in each half of a geminus. But the reverse is not always true. Consequently it is not possible to determine whether a geminus $\frac{L.R}{L.R}$ yielded a_1 and a_2 through the reduction division or through the non-reduction division, i.e., exchange of homologous chromatids. On the other hand, the configurations in c_1 and c_2 affords a decisive evidence

1) As seen in Plate XVII, Figs. 3 and 4, one of the half dyads of the type D shows the complete separation of its insertion region. This probably indicates that the second division of the kinetic body initiates at these stages.

of the reductional first division (at least for their longer arms) of a geminus $\frac{-\mathrm{L}}{-\mathrm{LR}}$. The other three types of chromosomes are characterized by asymmetrical forms of coiling and must have arisen as results of the non-reductional first division. The original D geminus would have probably been $\frac{-\mathrm{L}}{-\mathrm{LR}}$, though in one of the half gemini (d_2) the corresponding reversal of direction of coiling has not been ascertained. The e_1 and e_2 configurations are supposed to have resulted from a form $\frac{\mathrm{L-L}}{\mathrm{R-L}}$ through a non-reduction division, at least for the chromatids constituting their short arms. A more complete evidence of the non-reduction mode of division is offered by b_1 and b_2, which are asymmetrical as to coiling situations both in the short and the long arms. It is clear that the original geminus was $\frac{\mathrm{L-L}}{\mathrm{R-LR}}$ or $\frac{\begin{matrix}\mathrm{1-1}\\ \mathrm{1-1}\end{matrix}}{\begin{matrix}\mathrm{r-1r}\\ \mathrm{r-1r}\end{matrix}}$ and the change of one of the entire chromatids gave the forms $\frac{\mathrm{1-1}}{\mathrm{r-1r}}$ and $\frac{\mathrm{1-1}}{\mathrm{r-1r}}$.

Thus the present study on anaphase chromonemata, though it was carried out on a very small scale, points to more frequent occurrence of the non-reduction mode of the first division than would be expected. A more detailed study would reveal quantitative relations of these two modes of division.

Conclusion

In answer to the question set forth on page 233, it may be said that:

i) The direction of coiling is not a stable character for a given chromosome, since it can take all possible forms in this respect.

ii) There is no definite relation as to the direction of coiling between the two homologues. They behave independently from one another.

iii) The chromonema may reverse its direction of coiling at any point, although there may be some certain restriction for the occurrence of the reversal, in such a way that a certain number of coils is at least necessary for it.

iv) No characteristic difference appears to exist in the coiling situations in different cells of the same plant and probably in different individuals.

All these findings seem to point to the fact that the process of coiling is purely mechanical and is subject to the law of chance. The possibility is excluded then that the direction of coiling remains stable throughout the life cycle of a given chromosome.

Furthermore attention must be paid to the importance of the kinetic body bearing on the behaviors of chromonemata, as substantiated by the following findings: (i) the development of coiled chromonemata is controlled from the kinetic body, and (ii) there is no relation in regard to coiling between the two sides of the kinetic body. It follows then that the condensation of chromosomes is conditioned independently on the two arms; in other words, the kinetic body is a neutral one, that is, its constitution differs materially from that of the chromonema, offering no direct connection of the latter on the two side of it. Additional items of evidence for this have been obtained from the behaviors of the kinetic body at meiotic divisions. Although a full discussion on these points, together with evidences from other sources, will be given in another paper, suffice it to point out here that the division of the kinetic body is different in its time and independent in its mode from that of the chromonema. The first division of the chromonema takes place at an early prophase stage, and the second division (separation of chromatids) at metaphase, whereas the kinetic body comes to divide first shortly after metaphase and next at the stage previons to interkinesis. Its first division may take place at either of the two planes, one separating homologous chromatids, the other separating sister chromatids.[1] The former mode of division leads to the reductional one and the latter to the non-reductional one. Confirmative evidence for this was presented by the present observations on the direction of coiling in anaphase chromosomes. It may be added also that the possibility of another mode of division which involves an exchange of one arm of the chromatids, thus resulting in partial non-reduction, is not excluded here, as pointed out by NEBEL ('32 b).

DARLINGTON ('32, p. 290) considers that the chromosome thread commences its spiralization at the attachment constriction, turning in the opposite direction on its two sides. A similar conjecture has been made

1) HUSKINS and SPIER ('34) conclude from behaviors of heteromorphic bivalents in *Triticum vulgare* that sister chromatids are always associated at the spindle attachment and non-reductional first divisions may take place as results of crossing over between homologous chromatids. This interpretation however can not be applied to the present case, because in *Trillium* the four strands of a bivalent at metaphase are all associated only at the spindle attachment, decidedly excluding the possibility of such crossing over, as their interpretation demands.

by CATCHESIDE ('32, p. 109). The present findings offer however no positive evidence for it; on the contrary it was decisively demonstrated that the chromonema behaves in its condensation not as one entity but as two independent segments parted by the kinetic body.

Summary

The present paper deals with the direction of coiling in the chromonema within the first metaphase and anaphase chromosomes in the PMC of *Trillium kamtschaticum* PALL. The five individual types of chromosomes were analyzed as to this feature. The main results obtained are summarized below.

1) No evidence was found for any characteristic direction of coiling in the chromonema of a given chromosome nor for any definite relation as to this feature between the two homologues.

2) On the contrary it was demonstrated that the two arms of a chromosome behave independently as to this feature and the total number of dextrorse and senistrorse coils in them tends to be equal, suggesting that the direction of coiling is strictly subject to the law of chance.

3) The chromonema may reverse its direction of coiling at any point of it. The reversal of direction appears to follow chance, as no definite evidence is obtained indicating that any one certain type of chromosomes is more liable to be subjected to it. There is some evidence however that the change in the direction occurs only in chromonemata of more than a certain number of coils.

4) The two types of division, reductional and non-reductional, were directly demonstrated by analytic observations on the direction of coiling in the first anaphase chromosomes.

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Explanation of Plates

All the figures are photomicrographs taken from the same material and under the same condition as those described in the previous paper. The lens combination was a Zeiss apoch. immersion objective 1.4 mm. and Leitz Homal IV, giving a magnification of 1660.

Plate XV, Figs. 1 to 7. Seven successive levels of a PMC (labelled No. 1, *v.* p. 240) at metaphase.

Plate XVI, Figs. 1 to 8. Eight successive levels of a PMC (labelled No. 8, *v.* p. 240) at metaphase.

Plate XVII, Figs. 1 to 8. Eight successive levels of a PMC at anaphase.

It must be mentioned that these figures do not pretend to present exhaustive evidence of all chromosomes; to give it far more pictures would have to be made to meet all requirements of focal levels. In order to facilitate the understanding of configurations in these cells, the following schematic figures were prepared, which were made after prolonged visual study of the original slide. In these figures, the black part of the chromosomes represents coils of senistrorse twist of the chromonemata, the patched part coils of dextrorse twist, and the circle stands for the insertion region. The blank part of the chromosomes is that in which the direction of coiling could not be ascertained.

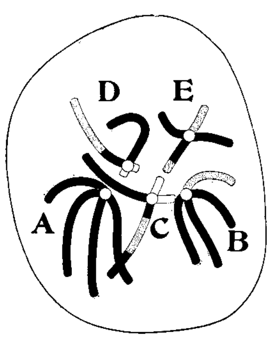

Fig. 2.

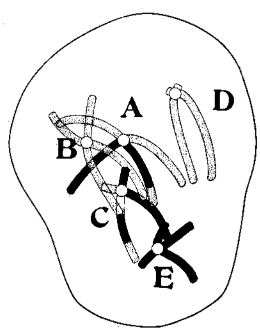

Fig. 3.

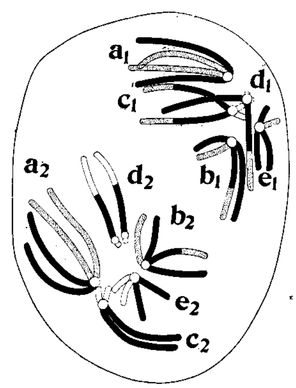

Fig. 4.

(Refer to Pl. XV, XVI and XVII respectively.)

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2

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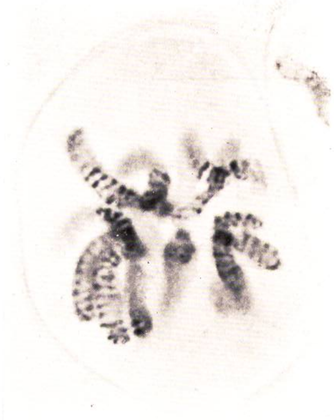
4



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H. Matsuura photo.

H. MATSUURA: Chromosome Studies on *Trillium kamtschaticum* PALL. II.

Journ. Fac. Sci., Hokkaido Imp. Univ., Ser. V, Vol. III, No. 5.

Pl. XVI

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H. Matsuura photo.

H. MATSUURA: Chromosome Studies on *Trillium kamtschaticum* PALL. II.

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H. Matsuura photo.

H. MATSUURA: Chromosome Studies on *Trillium kamtschaticum* PALL. II.