Chromosome Studies on *Trillium kamtschaticum* PALL.
I. The number of coils in the chromonema of the normal and
abnormal meiotic chromosomes and its relation
to the volume of chromosomes¹⁾

By

Hajime Matsuura

Hokkaido Imperial University, Sapporo

Received May 1, 1934

Of several hypotheses on the chromosome structure, the chromonema theory certainly covers all others. It fulfills the cytological requirement for the continuity and individuality of chromosomes as well as the genetic one that the genes must be located on the chromosome in the linear arrangement. In this view there can be no doubt that the structure and behavior of the chromonema serve as valuable means for the elucidation of some fundamental problems in the fields both of karyology and of genetics. As a part of my investigation on these lines, are reported here some of the data so far accumulated concerning the gyre number of chromonemata within the metaphasic chromosomes at the first meiotic division and the relation of it to the volume of the chromosomes. There is no reference to this feature in the literature, except some brief statements on the gyre number in the chromonemata made by ISHII (1931) and NEBEL (1932).

The present material, *Trillium kamtschaticum* PALL., proves to be very favorable for karyological studies, due to its low chromosome number, i.e., 5 in haploid, and, which is more advantageous, due to the extremely clear morphological features of the chromosomes, that is, the distinct differences in their relative size and in the position of the constriction, making the identification of each chromosome individual very easy.

The number and outer morphological characteristics of the chromosomes of this plant has been previously investigated by three Japanese workers, GOTOH & STOW (1930), GOTOH (1933), and HAGA (1934). The last author has carried out detailed measurements of the chromosomes in a complement and designated the five chromosome pair as A, B, C, D and E, according to the order of their size, the first mentioned being the largest. The same designation has also been adopted in the present paper.

1) An outline of the present paper was given at the Annual Congress of the Botanical Society of Japan held in April, 1934.

All the observations were based on preparations which had been made by the smear method of fixing the PMC, applying the fixatives to the slide. The fixatives employed were TAYLOR's solution and La COUR 2BE, both having given satisfactory results. For the comparison of chromosome volume, only the preparations treated with the former fixative were used, in order to avoid the occurrence of possible variations due to different treatments. The fixation for less than 30 seconds proved to be sufficient; the slides were then rinsed in water for one or two minutes and directly stained in gentian violet after NEWTON's method. Thus the preparations were completed, from fixation to mounting in Canada balsam, in less than 5 minutes. Plants numbering over four hundred individuals were used in the two years during which the present study has been carried out.

i. The number of coils in the chromonema

In a previous note (1934), the writer has shown that (i) the number of coils within a chromosome at the first metaphase is of a stable character, and (ii) the pitch of coils in the chromonema is exactly the same throughout all five chromosome types, A, B, C, D and E, in a complement. It follows then that the length of chromosomes is expressed as a function of the number of spiral gyres.

In contrast with these observations on the normal type, the writer has found among plants treated by high temperature two interesting cases characterized by chromosomes of abnormal structures. For convenience sake, the two types are here called 'abnormal I' and 'abnormal II' respectively (*cf.* Text-fig. 1).

Text-fig. 1. Three PMCs of different types at MI. Slightly schematized.

Abnormal I. This type was shown by plants brought into a greenhouse of an average temperature of 16°C two weeks before the meiotic division takes place. In this type, the chromosome configuration at

the meiotic metaphase is apparently normal, that is, all five gemini are always of the cruciform type being paired only at one point, *viz.*, the point of the attachment constriction,¹⁾ but it is distinguishable from the normal type by (i) greater dimension of the mother-cell (*cf.* Pl. 6 Figs. 1A and 2D), and (ii) shorter and broader chromosomes. The gyre number in the chromonema was found to be about 64% of that in the normal cases, as is shown in Table 1.

Table 1. Comparison of gyre numbers in chromonemata of the three types.
cf. Plate 1.

Chromosomes	Normal	Abnormal I	Abnormal II
A	11 ₊ —11 ₊ (23)	15 _—	10 _—
B	9 ₊ — 5 ₊ (15)	10 _—	7 _—
C	11 ₊ — 3 ₊ (15)	9 ₊	6 ₊
D	12 ₊ — 1 _— (13)	8 ₊	6 _—
E	7 _— — 4 ₊ (11)	7 ₊	5 _—
Total	77	49 ₊	34 _—
Percentages	100	64	44

Note: In the above table, the coil number in each one dyad is given, because as previously found by SAX (1930), TAYLOR (1931), NEBEL (1932), etc., each homologue is characterized by the same number of coils. In the second column of the table, a dash, —, denotes the insertion region and the suffixes + and — indicate that the coil number is more or less, respectively, than the given figure by less than one half coil. The figures in brackets represent the approximate total number of coils in each dyad of the normal type. The meaning of suffixes, + and —, is the same in the abnormal types, but here no attention was paid as to the insertion region.

It must be mentioned here also that counting the coil number in the chromonema is not an easy task, owing to extreme difficulties in determining the coils at the region adjacent to the attachment constriction, at the region where the twist reverses its direction and also at the chromosome ends. The above given figures for the coil numbers are accordingly based upon many trials on chromosomes which were found to be favorable for counting. In so far as the writer's observations have gone, no case was found to be recognizable as a real variation in the coil number, except only one case in abnormal I, in which only the A chromosomes are provided with a much reduced number of coils (about 12₊ coils), the other members, however, remaining quite unaffected as to this point (Pl. 7 Fig. 7). Although the actual cause for this variation in structure is not known, it is probably concerned with certain differences of the A chromosomes in question from the others in the length-breadth ratio, as compared with normal chromosomes (*v. infra*).

Abnormal II. This type was shown by all the plants which were placed in the green-house two months before the meiotic division begins. The influences of high temperature manifest themselves at earlier stages than in abnormal I type. The process of division has

1) This mode of pairing of chromosomes at MI is analogous with the case described in the spermatogenesis of *Mecostethus* and allied genera of Orthoptera by JANSSENS (1924), MCCLUNG (1927) and others. In plants, a somewhat similar condition has been found in *Fritillaria Meleagris* (NEWTON & DARLINGTON 1930, DARLINGTON 1932) and in *Allium fistulosum* (LEVAN 1933).

certain resemblance to the asynaptic maize reported by BEADLE (1933), but differs from the latter in the earlier occurrence of the division of the spindle attachments, thus causing the complete separation of chromatids of chromosomes already at diplotene. Thus five groups of four chromatids, instead of five bivalents, usually appear at MI (Text-fig. 1). The four chromatids constituting a group, two of them being 'homologous' chromatids and the other two their 'sister' ones, take a radial arrangement at this stage, and are materially independent from one another, no chiasmata existing between them.¹⁾ The size of mother-cells of this type is also distinctly greater than that of the normal type, but the more remarkable characteristic of this type is the conspicuous reduction in the chromatid bulk and in the spiral number. As shown in Table 1, the number of coils in this type was calculated as about 44% of the normal one.

ii. Comparison of chromosome volume in the three types

A comparison of the chromosome dimensions in the three meiotic chromosome types, normal, abnormal I and abnormal II, was carried out by measuring chromosomes from smear preparations made in the same manner. Table 2 represents the results of measurements of two

Table 2. Comparison of chromosome lengths in the three types.

Chromosomes	Normal	Abnormal I	Abnormal II
A	19.5 μ	15.2 μ	9.5 μ
B	13.8	8.9	6.9
C	11.6	9.1	6.5
D	11.8	8.8	5.4
E	9.3	6.9	4.7
Tot.	66.0	48.9	33.0
Exp.	66.0	49.5	33.0

or three bivalents (4 or 6 chromosomes) for each chromosome type in a complement which were found to lie flat. Though these values should not be considered as absolutely decisive, owing to the small number of observations and a certain variation in chromosome dimension which necessarily results from different states of fixation in different cells, the total values indicate that the chromosomes of abnormal I amount to nearly 3/4 of the normal ones in length (the deviation of the actual data from the expectation being only -0.6μ), and the length of the abnormal II chromosomes is just half that of the normal ones.

Table 3 represents the comparison of diameter of chromosomes and chromatids in these three types. The measurements were also based

1) Full descriptions of these features will be given in another paper.

on preparations of the same treatment and carried out by choosing at random 5 to 10 chromosomes and chromatids.¹⁾ In normal and abnormal I types, the chromatids to be measured were taken from those at

Table 3. Diameter of chromosomes and chromatids.

	Normal	Abnormal I	Abnormal II	Nor./Abn. I
Chromosomes	1.86 μ	2.14 μ	—	0.87
Chromatids	1.22	1.43	1.49	0.85
Chromatids Chromosomes	0.66	0.67	—	—

very early anaphasic stage where the separation of chromatids is just completed (Pl. 7, Figs. 4 & 5). In abnormal II, however, there is no stage of 'dyad' chromosomes as in the other types, and hence only the chromatid was measured. Moreover, at MI of this type, the chromatids are not strictly cylindrical rods, but oval in section. The pairs of chromatids are very narrow in their diameter when they are seen sideways-on, but much wider when they are seen endways-on. The difference in chromatid diameter in different lines of vision seems to have relation with the forces operating between the four strands which are associated together (*cf.* Text-fig. 1). The measurement of the diameter of the abnormal II chromatids was then made on those at late anaphase when they are sufficiently separated from one another and take a complete cylindrical form (Pl. 7, Fig. 6).

The results of measurements in normal and abnormal I indicate that the ratio of the average diameter of the former chromosomes to that of the latter is very close to their chromatid ratio (0.87 and 0.85 respectively). It is also to be noted here that the ratio of chromatid diameter to chromosome is nearly the same in both the types (0.66 in normal and 0.67 in abnormal I), and agrees with the theoretical expectation that two chromatids are equivalent in volume to one chromosome ('dyad') from which they came; the diameter of chromatids should be $1/\sqrt{2}$, that is, 0.69, of that of chromosomes, if the difference in their lengths is neglected.

These results from measuring suggest that the chromosome (or chromatid) bulk is the same in both the types, normal and abnormal I, because the actual data seem to fulfill the following relation: $\frac{r_n}{r_{ai}} = \sqrt{\frac{l_{ai}}{l_n}}$. where r_n and l_n represent the diameter and length, respec-

1) Owing to the extreme difficulty in measuring directly the chromosome or chromatid diameters, the measurements were carried out on several photomicrographs, the negatives of which were enlarged at the exact magnification of 3700.

tively of normal chromosomes (or chromatids), and correspondingly r_{ai} and l_{ai} those of abnormal I chromosomes (or chromatids); thus:

$$\frac{r_n}{r_{ai}} = 0.85-0.87,$$

$$\sqrt{\frac{l_{ai}}{l_n}} = \sqrt{\frac{3}{4}} = 0.86.$$

The diameter of abnormal II chromatids is slightly greater than that of abnormal I ones. But the difference 0.06μ may be too small to be at all significant or it may be due to the difference in stages at which the measurements were carried out (*v. supra*). Since the length of chromatids of this type is half that of the normal ones and their diameter is nearly equal to that of abnormal I chromatids, their volume is much reduced, as compared with other types. In this connection, it may be noteworthy that in contrast with abnormal I in which the subsequent meiotic divisions take place in the normal fashion, resulting in the production of apparently good pollen grains, all mother-cells of abnormal II are destroyed before the second division takes place. The discrepancy or unharmony between the chromatid volume and the chromonema proper in abnormal II may have some connection with this prevention of the further normal divisions.

iii. The relation of coil number to chromosome or chromatid volume

a) Comparison of abnormal I with normal

The average pitch values of the coiled chromonemata in normal and abnormal I are calculated as follows:

$$\begin{array}{ll} \text{normal:} & 66.0 \div 77 = 0.85 \mu, \\ \text{abnormal I:} & 48.9 \div 49.4 = 1.00 \mu. \end{array}$$

Text-fig. 2. Diagram representing the geometrical relation of an abnormal I chromonema to a normal one.

Thus the pitch ratio of normal to abnormal I, $0.85/1.00$, is very close to the chromosome (or chromatid) diameter ratio ($0.85-0.87$). This finding clearly indicates that the inclination of coiling in the chromonema is the same in both the types.

These results lead us to a further impor-

tant consideration on the relationship between coil number and chromosome length in these types. Let us assume that axb and ayc in Text-fig. 2 be one coil each within normal and abnormal I chromosomes respectively. Then ab' and ac' will be their actual lengths respectively and form a straight line, owing to the same inclination angle of these chromonemata. Then as already stated, the relation of the diameter ratio to the length ratio in the two types is expressed by :

$$\frac{r_n}{r_{ai}} = \sqrt{\frac{l_{ai}}{l_n}} \dots\dots\dots (1)$$

Whereas the diameter ratio, $\frac{r_n}{r_{ai}}$, is in positive proportionality to the pitch ratio, $\frac{p_n}{p_{ai}}$, where p_n and p_{ai} denote the pitches of the spiral in normal and abnormal I chromosomes respectively. Hence we get the following relation :

$$\frac{p_n}{p_{ai}} = \sqrt{\frac{l_{ai}}{l_n}} \dots\dots\dots (2)$$

Now let the coil number in normal chromosomes be designated as s_n and that in abnormal I ones as s_{ai} . Naturally the relation between the pitch, length and coil number is as follows :

$$\left. \begin{aligned} p_n &= \frac{l_n}{s_n} \\ p_{ai} &= \frac{l_{ai}}{s_{ai}} \end{aligned} \right\} \dots\dots\dots (3)$$

From equations, (2) and (3), the following formula is obtained :

$$\frac{s_{ai}}{s_n} = \frac{l_{ai}}{l_n} \sqrt{\frac{l_{ai}}{l_n}} \dots\dots\dots (4)$$

Thus the coil number ratio can be expressed as a function of the length ratio. The actual coil number ratio was observed as 0.65 which is very close to the ratio calculated from the length ratio, 0.65 (from $3/4 \sqrt{3/4}$).

Table 4. Comparison of gyre numbers in the three types.

Chromosomes	Normal	Abnormal I		Abnormal II	
		Obs.	Cal.	Obs.	Cal.
A	23	15 ₋	14.9	10 ₊	10.1
B	15	10 ₋	9.7	7 ₋	6.5
C	15	9 ₊	9.7	6 ₊	6.5
D	13	8 ₊	8.4	6 ₋	5.6
E	11	7 ₊	7.1	5 ₋	4.7
Total	77	49 ₊	49.8	34 ₋	33.4
%	100	64	65	44	43

Taking the latter value as the basis, the coil number in each chromosome individual type in the complement of abnormal I is calculated as in Table 4. It is obvious that there is close agreement between the actual data and the expected numbers.

b) Comparison of abnormal II with abnormal I

On the same principles, namely, by assuming that the inclination of the chromonemata remains unaffected also in abnormal II, the number of coils of this type is deduced from that of abnormal I. Since the chromatid diameter of Abnormal II is considered as equivalent to that of abnormal I, the ratio of coil numbers is to be proportional to the length ratio. If s_{ai} and s_{aII} represent the coil numbers, and l_{ai} and l_{aII} the chromatid lengths, in abnormal I and II respectively, the relation between them is expressed as :

$$\frac{s_{ai}}{s_{aII}} = \frac{l_{ai}}{l_{aII}} \dots\dots\dots (5)$$

Thus s_{aII} is calculated as $\frac{65}{75} \times 50$, namely, 43% which is approximately close to the observed value, 44%.

Taking the theoretical value, 43%, as the basis, the coil numbers in the five chromosome individual types of the complement in abnormal II are calculated and compared with the actual data in Table 4. Here also a close agreement between them will be obvious.

iv. Conclusions and further problems

The relation of chromosome or chromatid volume to gyre number in the chromonema in the three types dealt with in the present paper may be summarized by the term of percentage as in the following table (Table 5). The conclusion which is deduced from this table is that the inclination angle of the chromonema is the same throughout the three types.

Table 5. Relation between chromatid volume and gyre number in the three types.

	Normal	Abnormal I	Abnormal II
Chromatid length	100	75	50
Chromatid diameter	100	$100\sqrt{\frac{4}{3}}$	$100\sqrt{\frac{4}{3}}$
Chromatid bulk	100	100	$100 \times \frac{2}{3}$
Gyre number	100	65 (= $100 \times \frac{3}{4}\sqrt{\frac{3}{4}}$)	43 (= $65/75 \times 50$)

From these results, it is possible to give the following general statement: When the chromonemata of given two chromosome types coil with the same inclination angle, the relation of gyre number to chromosome bulk in these types is expressed as follows: $\frac{s_1}{s_2} = \frac{l_1 r_2}{l_2 r_1}$.

where s_1 , r_1 and l_1 are respectively the number of coils, the diameter and the length of the chromosomes of one type, and s_2 , r_2 and l_2 are correspondingly those of the other type. This is a formula applicable to the relation between normal and abnormal I in the present study. From the above formula, if the diameter remains unaffected, we get:

$\frac{s_1}{s_2} = \frac{l_1}{l_2}$, a formula which explains the relation between abnormal I and abnormal II. If the chromosome bulk is constant and the diameter changes, the general formula given above is expressed as: $\frac{s_1}{s_2} = \frac{l_1}{l_2} \sqrt{\frac{l_1}{l_2}}$, because $\frac{r_1}{r_2}$ is equal to $\frac{l_1}{l_2}$. The case is the relation between normal and abnormal I chromosomes.

In connection with the present findings, several other points of particular interest should be investigated in further studies. It is important to solve whether the total length of the chromonema proper, that is, the total length of the threads when the secondary spiral and the primary one (*cf.* Fujii, 1927) as well were completely uncoiled, remains constant throughout the three types described in the present paper. Furthermore it must be studied whether the chromonema of a given type does actually maintain the same inclination angle of coiling throughout all the cyclic changes of cell development, viz., in meiotic as well as in somatic chromosomes. On the other hand, it is necessary to study how these abnormal types of chromosomes have arisen. The significance of the present findings will be given at the time when these studies were brought to completion.

v. Summary

The present paper deals with three types of meiotic chromosomes in *Trillium kamtschaticum*: normal, abnormal I and abnormal II (*cf.* Text-fig. 1), the latter two having arisen through the effects of abnormally high temperature. In abnormal I, the chromosomes are shorter but broader than those of normal, their volume however remaining unaffected (*cf.* Tables 2 and 3). Abnormal II type is characterized by the appearance at MI of four chromatid strands associating together in place of a geminus. The volume of chromatids of this type is much reduced (about 66% of the normal), their length being half that of normal chromatids and their diameter nearly equal to that of abnormal I ones (*cf.* Tables 2 and 3).

The coil numbers in these three types were studied and compared with one another in connection with the volume of chromosomes or chromatids (*cf.* Table 1), and were explained by assuming that the inclination angle of the chromonema remain constant throughout the three types (*cf.* formulae (4) and (5), and Tables 4 and 5).

April 18, 1934

Botanical Institute, Faculty of Science,
Hokkaido Imperial University, Sapporo

Literature cited

- Beadle, G. W., 1933. Further studies of asynaptic maize. *Cytologia* 4: 269-287.
 Darlington, C. D., 1932. *Recent Advances in Cytology*. London: Churchill and Co.
 Fujii, K., 1927. Recent progress in cytology and its method of investigation. (in Japanese). *Rept. Jap. Assoc. Adv. Sci.* 2 (Rept. of Kyoto-Meeting, 1926): 373-415.
 Gotoh, K., 1933. Karyologische Studien an *Paris* und *Trillium*. *Jap. Jour. Genet.* 8: 197-203.
 Gotoh, K., & I. Stow, 1930. Karyologische Studien an *Paris* und *Trillium* (Vorl. Mitt.). (in Japanese). *Jap. Jour. Genet.* 5: 114-117.
 Haga, T., 1934. The comparative morphology of the chromosome complement in the tribe *Parideae*. *Jour. Fac. Sci., Hokkaido Imp. Univ.*, S. V, 3: 1-32.
 Ishii, T., 1931. On the structure of chromosomes (preliminary note). (in Japanese) *Jap. Jour. Genet.* 7: 128-139.
 Janssens, F. A., 1924. Chiasmotypie dans les Insectes. *La Cellule* 34: 135-359.
 Levan, A., 1933. Cytological studies in *Allium*, IV. *Svensk Bot. Tidsk.* 27: 211-232.
 Matsuura, H., 1934. On the gyre number in the chromonema. (A preliminary note). (in Japanese). *Jap. Jour. Genet.* 9 (in press).
 McClung, C. E., 1927. Synapsis and related phenomena in *Mecostethus* and *Leptysmia* (Orthoptera). *Jour. Morphol. Physiol.* 43: 181-265.
 Nebel, B. R., 1932. Chromosome structure in *Tradescantia* I. *Zts.f. Zellforsch. u. mikr. Anat.* 16: 251-284.
 Nebel, B. R., 1932. Chromosome structure in *Tradescantia* II. *Zts. f. Zellforsch. u. mikr. Anat.* 16: 285-304.
 Newton, W. C. F. & C. D. Darlington, 1930. *Fritillaria Meleagris*: chiasma formation and distribution. *Jour. Genet.* 22: 1-14.
 Sax, K., 1930. Chromosome structure and the mechanism of crossing over. *Jour. Arnold Arbor.* 11: 193-220.
 Taylor, W. R., 1931. Chromosome studies on *Gasteria* III. *Amer. Jour. Bot.* 18: 367-387.

Explanation of plates

All the photomicrographs were taken with the aid of a LEITZ' microphotographic apparatus MAII equipped with a Compur shutter, under 5 amp. D.C. arc light, with a combination of Wratten filters B and E interposed.

In figs. 1-6, the lens combination was a ZEISS' apoch. immersion objective 1.4 mm. and LEITZ' Homal IV, giving a magnification of 1660.

In figs. 7 and 8, the same objective was used in conjunction with ZEISS' comp. oc. 7×, the magnification being ca. 550 diameters.

In order to avoid variable effects, if any, by different treatments, all the photos were made from the preparations of the same treatment, that is, those all fixed in TAYLOR's solution and stained by NEWTON's gentian-violet method.

Plate 6

Figs. 1 A-E, 2 A-E, and 3 A-E. The five types of chromosome individuals (A, B, C, D and E) in normal, abnormal I and abnormal II respectively. Fig. 1 A repre-

sents a whole mother-cell, for convenience of comparison of cell size with abnormal I, a whole cell of which is represented in fig. 2 D. It is to be noted that these figures serve only for rough realization on the gyre number, some parts of chromosomes being necessarily out of focus (especially in fig. 2 B). Fig. 3 D is side view, only two of the four chromatids being represented.

Plate 7

- Figs. 4, 5 and 6. These figures represent anaphasic chromatids in normal (fig. 4), abnormal I (fig. 5) and abnormal II (fig. 6). They are representative of photos on which the chromatid diameter was measured. Note also that the width of the 'primary' spiral is much greater in abnormal I and II than in normal.
- Figs. 7a and b. A mother-cell of an exceptional type of abnormal I, taken under two different foci. Explanation in text (p. 272).
- Figs. 8a and b. A mother-cell of typical abnormal II, taken at two different levels. Note the difference in its cell size from abnormal I.
-

Matsuura photo.

Matsuura: Chromosome Studies on *Trillium kamtschaticum* PALL. I

Matsuura photo.

Matsuura: Chromosome Studies on *Trillium kamtschaticum* PALL. I