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Chromosome Structure and Behaviour in Some Plants I. Chromosomes of *Diphylleia* *Grayi* FR. SCHM.

By

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(With 8 text-figures)

The spiral structure of meiotic chromosomes has been described by many workers in various plants. There are, however, yet many unsolved important problems and considerable difference of opinions among investigators, regarding the origin and nature of spiral structure of chromosomes. To make clear these questions and to confirm the universality of the facts observed by previous workers, further investigation with many suitable materials and excellent method may be demanded.

The present material, *Diphylleia Grayi* FR. SCHM., belonging to *Berberidaceae*, proves to be favorable for karyological studies due to the following reasons: 1) Its low number of large chromosomes, i.e. 6 in haploid. 2) The manifestation of spiral structure of meiotic chromosomes being relatively easy. 3) The morphological differentiation of each chromosome being clear except for one pair.

In the present paper the details of morphological features of chromosomes in this material were made clear and some observations on the structure of meiotic chromosomes were reported preliminarily.

Concerning karyological studies on this plant, there is only one report of MATSUURA and SUTÔ (1935), in which they have made clear the chromosome number of it to be $n=6$, and they have furthermore suggested that the complement of this plant consists of two pairs each of median, submedian and subterminal chromosomes. In other species, *Diphylleia cymosa*, LANGLET (1928) has counted twelve chromosomes in somatic cells.

Before going further, the writer wishes to express his heartiest thanks to Prof. H. MATSUURA for his valuable suggestions and criticism in the course of the present work. His thanks are also due to the Scientific Research Fund of the Department of Education, by a grant from which this work was supported.

Material and Method

The material used in the present study was collected on Mt. Soranuma near Sapporo and it was transplanted in the experimental gardens of our Laboratories for the investigation of meiosis, because the meiosis of this plant usually begins at the end of March or in April under snow, and this renders getting material at such time in natural habitat very difficult.

For the determination of somatic complement the root-tip cells were employed. They were fixed with La Cour 2BE and then sectioned in paraffin at a thickness of 26 microns. The slides were stained with gentian-violet after the NEWTON's schedule.

In some cases chloralization was tried to make clear the morphological characteristics of chromosomes by the following treatment: after rootlets were immersed together with rhizome in 1% chloralhydrate for one hour, rinsed in running water for 30 minutes, and allowed to remain for three or four hours in wetted saw-dust (18° - 20° C), the root-tips were cut and fixed with the described solution. This treatment proved to be very effective for the aim mentioned above.

To observe the morphological influence of low temperature on the chromosomes, several rhizomes were buried in wetted saw-dust and kept in the chamber of 5° C for 7 days.

For the study of structural details of meiotic chromosomes in PMCs, MATSUURA's water pretreatment (MATSUURA, 1938b) was tried. The procedure is as follows. The pollen mother cells are smeared on a slide and then one or two drops of tap water are quickly added on them. The water is left on the material for two minutes (the time needed for treatment varies according to material) and then the excess of water is removed, as far as possible, with blotting paper. The fixing solution, LA COUR 2BE, is dropped quickly on the material pretreated with water to make permanent the preparations which are then very carefully washed in running water. The slides are stained with gentian violet after the NEWTON's schedule. This treatment gives satisfactory result in this material.

Observations

i. Somatic chromosomes. The somatic complement of this plant contains twelve large chromosomes consisting of two pairs of median, submedian and subterminal ones on the position of kinetochore (Fig. 1, 2). This agrees completely with the suggestion of MATSUURA and SUTO (1935)



Fig. 1. Somatic metaphase showing 12 chromosomes. **a**, from the material treated with 1% chloralhydrate. **b**, non-treatment. **c**, from the material cooled in 5°C for 7 days. $\times 2000$.

as mentioned above. The six pairs, furthermore, are able to be classified into five types of A, B, C, D and E according to the relative length¹⁾ of their arms and the other morphological characters (Fig. 2).

Type A, Containig two pairs of chromosomes with median kinetochore and being the longest of the complement. A_1 is very difficult to be distinguished from A_2 morphologically.

Type B, The whole length is shorter than type A, though the long arm is approximately equal to that of A. The kinetic constriction is situated at about a third of whole length.

Type C, Having submedian kinetochore, the ratio of the length of the long arm to that of the short arm is about 7.0 to 4.5. The whole length is about equal to that of B. The long arm is approximately equal to that of E, and it is the shortest long arm in the complement. This type is easily distinguished from B with a clear secondary constriction situated at about central position of the short arm.

Type D, The long arm is the longest, but the short arm is the shortest in the complement.

1) The actual length of each chromosome was not measured in the present study, because the chromosomes of this plant are exceedingly large, consequently it is too difficult to measure their true length accurately. The relative length, however, were determined on the preparations treated with chloralhydrate, as the chromosomes are shortend and straightened by the treatment (Compare Fig. 2a with Fig. 2b).

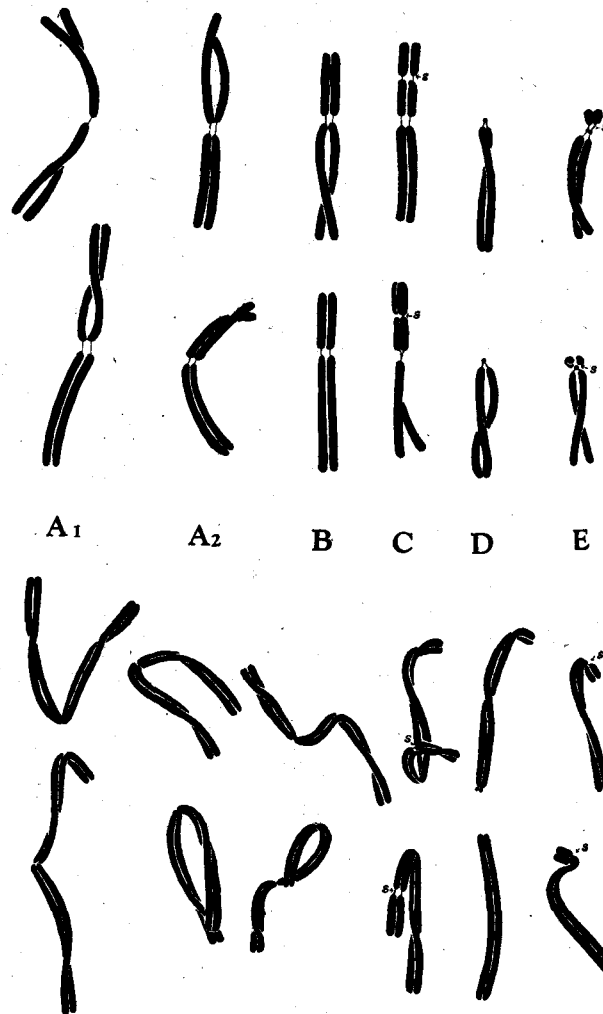


Fig. 2. Chromosomes classified from the Fig. 1, upper group from a, lower group from b. s shows the region of the secondary constriction. $\times 2000$.

Type E, The shortest chromosome in the complement. The prominent mark of this chromosome is the trabant or secondary constriction on the short arm.

ii. **Nucleolus.** In the present material the number of nucleoli in telophase nuclei of somatic cells was two to four (Fig. 3a, b), but a nucleus

having more nucleoli than four was not seen. Considering the number of secondary constriction in the present material, the fact mentioned above, as far as the number of nucleoli is concerned, agrees with HERTZ's theory (1931 a, b) that the nucleoli originated in the satellite stalk of SAT-chromosome or secondary constriction in telophase, and that the number, position and size of the nucleoli correspond to those of these

regions of SAT-chromosome or secondary constriction present in the nuclei. The writer, however, could not completely confirm the HERTZ's theory, as the direct observation on the relationship between nucleoli and chromosomes has not been done in the present study. There are, indeed, some reports

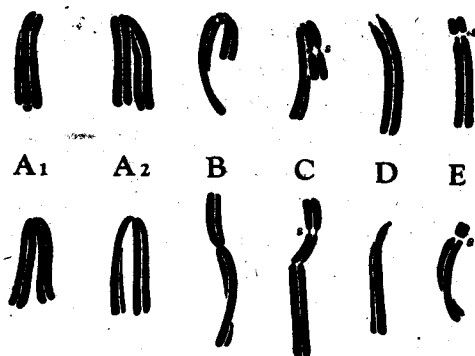


Fig. 3. Chromosomes classified from the Fig. 1c. s shows the region of the secondary constriction. $\times 2000$.

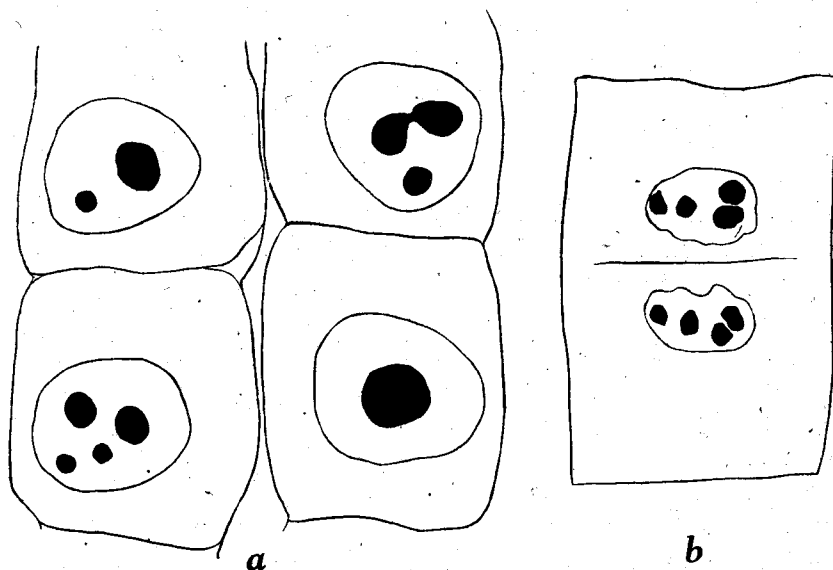


Fig. 4. a shows nuclei having various numbers of nucleoli. Notice that, the more the number of the nucleoli decreases, the larger the volume of nucleoli becomes. b represents telophase nuclei showing four nucleoli. $\times 1500$.

asserting that the secondary constrictions have no connection with the nucleolus (HEITZ, 1933; KAUFMANN, 1934, 1937; FERANDES, 1936; MATSURA, 1938; OKUNO, 1937; RESENDE, 1937; SATO, 1936a, b, 1937). The decrease of the number of nucleoli in the late telophase or resting stage may depend upon the result of their fusion (Fig. 4a).

In meiotic prophase the nucleolus usually was only one, but sometimes three or four nucleoli instead of two were observed in the nuclei of the first telophase (Fig. 5a). This fact may depend upon the reason that the paired chromatids of a chromosome are already separated from each other

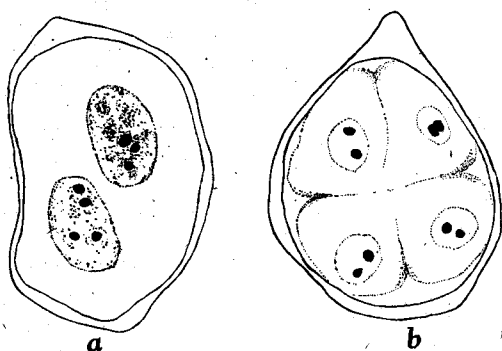


Fig. 5. a is a PMC in first telophase, showing the two nuclei having three and four nucleoli. b is a PMC of tetrad stage. $\times 1200$.

in the first anaphase, attaching only at the position of kinetochore, so the nucleoli originate independently on each separated chromatid of the nucleolar chromosome in the telophase. But it is naturally supposed that the nucleoli formed on each separated chromatid of nucleolar chromosome may be apt to fuse easily, for the sake of the interval of the separated chromatids being very limited. In tetrads stage the

numbers of nucleoli were counted usually one or two per nucleus (Fig. 5b).

iii. The influence of treatments on the chromosome.

a. *The influence of chloralhydrate* (refer. Fig. 1a and 2). Some remarkable influences of chloralhydrate with the method described are as follows: (1) Contraction of the chromosomes and less twisting are evident. (2) The kinetic constriction becomes very pronounced and elongated, but secondary constriction or akinetic constriction remains intact as observed by HAGA (1940). (3) Chromosomes are spread out in cytoplasm. The degree of these influences is different in different cells.

b. *The influence of low temperature* (refer. Fig. 1c and 3). (1) The reaction of "1" in chloralization is also verifiable in this treatment, but the degree of contraction of chromosomes in each cell is more variable than in the case of chloralization, i.e., there are cases which show more contraction than that of chloralization, and at the same time, cases which are not considerably different from the control. The degree of influence,

however, is always constant in one cell. The causes that even the influence under the same treatment shows various degree in each cell may be partly attributed to differences in each cell's stage when the stimulus was initiated. (2) The elongation of kinetic constriction is never seen in this treatment: this shows remarkable contrast to the case of chloralization. (3) The secondary constriction does not show any conspicuous difference from that of the control in this treatment too, although in some cases it contracts very slightly. (4) Chromosomes become rather crowded, instead of spreading as seen in chloralization. (5) The dividing figures are much rarer than in the control.

To give a critical explanation for the facts mentioned above, is beyond the present study, but, at least, the elongation of kinetic constriction seems to occur independent of the contraction of chromosomes. In contrast to the kinetic constriction, it is further interesting to notice that the secondary constriction shows no remarkable difference between the cases treated with chloralhydrate and low temperature and the case of non treatment, although in some cases under low temperature there is a slightly greater construction than in those of the control.

iv. Meiotic chromosomes. Chromosome behavior during the prophase was not studied. Metaphase pairing is usually regular, showing 6 bivalents paired with many chiasmata and sometimes with kinetochores and chiasmata (Fig. 6, 8). The failure of pairing was once observed in each of median and submedian chromosomes (Probably A and B; Fig. 7a, b). MATSUURA and SUTÔ (1935) have frequently observed four univalents in one nucleus, but the occurrence of more univalents than two was not met with in the present study.

The chiasmata were mostly interstitial, the terminal chiasmata being rare (Table 1). The maximum frequency of chiasmata in one chromosome was 5 as observed in A type chromosome.

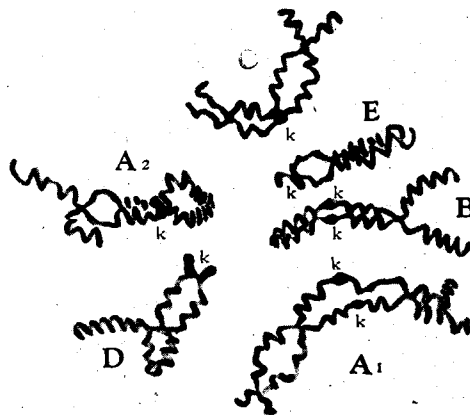


Fig. 6. A first metaphase of PMC, showing 6 bivalents. The spiral structure was demonstrated with MATSUURA's water pretreatment. The direction of coiling is partly indistinct, especially in the chiasma loops. k shows kinetochore. $\times 2450$.

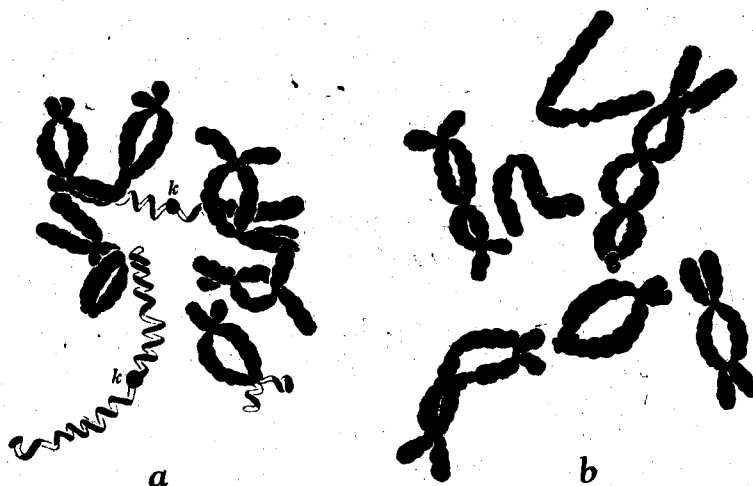


Fig. 7. a and b show the unpairing of median and submedian chromosomes respectively. $\times 2450$.

Table 1. Chiasma frequency in 20 PMCs. The association of the kinetochores is not included. M, SM and ST show median, submedian and subterminal chromosomes respectively.

Type of chromosome	Interstitial chiasmata		Terminal chiasmata		Total	
	actual number	frequency per bivalent	actual number	frequency per bivalent	actual number	frequency per bivalent
M	118	2.95	6	0.15	124	3.10
S. M	73	1.83	8	0.20	81	2.03
S. T	58	1.45	4	0.10	62	1.55
Total	249	2.07	18	0.15	267	2.23

The identification of each chromosome is difficult in the first metaphase of PMC unless the spiral structure of chromonema is demonstrated. The kinetochore becomes recognizable as a somewhat spherical body well stained, when the spiral structure was manifested.

The representation of the kinetochore and the comparison of turn numbers of the spiral of each chromosome help the identification of each chromosome.

The spiral¹⁾ of chromonemata of this material are more slender than those of *Trillium* and *Tradescantia*. It is somewhat difficult to count strictly the turn numbers of each chromosome on account of the existence of numerous chiasmata. The rough numbers of turns in each arm of all chromosomes are shown in Table 2. This data correspond well with the relative length of somatic chromosomes.

Table 2. The number of turns in each arm of all chromosomes.

Type of chromosome	Long arm	Short arm	Distal part from secondary constriction	Total
A ₁	9±	9±		18±
A ₂				
B	9±	4.5-5		14±
C	7.5±	3-	3-	13±
D	10±	0		10±
E	7±	0	1-	8±

Remarks: The numbers of turns of each chromosome is more or less different in cells.

The fact that the direction of major spirals changes frequently along the whole length of the chromonemata within the meiotic chromosomes has been observed by many workers in various materials. Such change in direction of spirals is usually found to occur at the kinetochore as seen in Fig. 8a, b (HUSKINS and SMITH, 1935; HUSKINS and WILSON, 1938; IWATA, 1935, 1941; MATSUURA, 1935b, 1937a, b; NEBEL, 1932; SAX, 1930, 1935; SAX and HUMPHREY, 1934; SHINKE, 1934; TAYLOR, 1931; TOYOHUKU (the present writer who, meanwhile, has changed his name), 1939), and at the point of interstitial chiasmata (Fig. 8a, b, d and 1; HUSKINS and SMITH, 1935; HUSKINS and WILSON, 1938; IWATA, 1941; SAX, 1936), however, statistical investigation was not done in the present study. Besides those cases, reversals in direction of spirals occur also within the whole arm or a part of arm. Within the loop of chiasmata, the spirals frequently take incomplete form, especially in cases where the loops of chiasmata are small, as reported by MATSUURA (1941), but the perfect corrugated form (Fig. 8c) observed in *Trillium* (MATSUURA, 1941) seems to be rare in the present material.

1) As far as no other remark is given, "spiral" means the major spiral of the chromonema of the first metaphase in PMC in the present study.

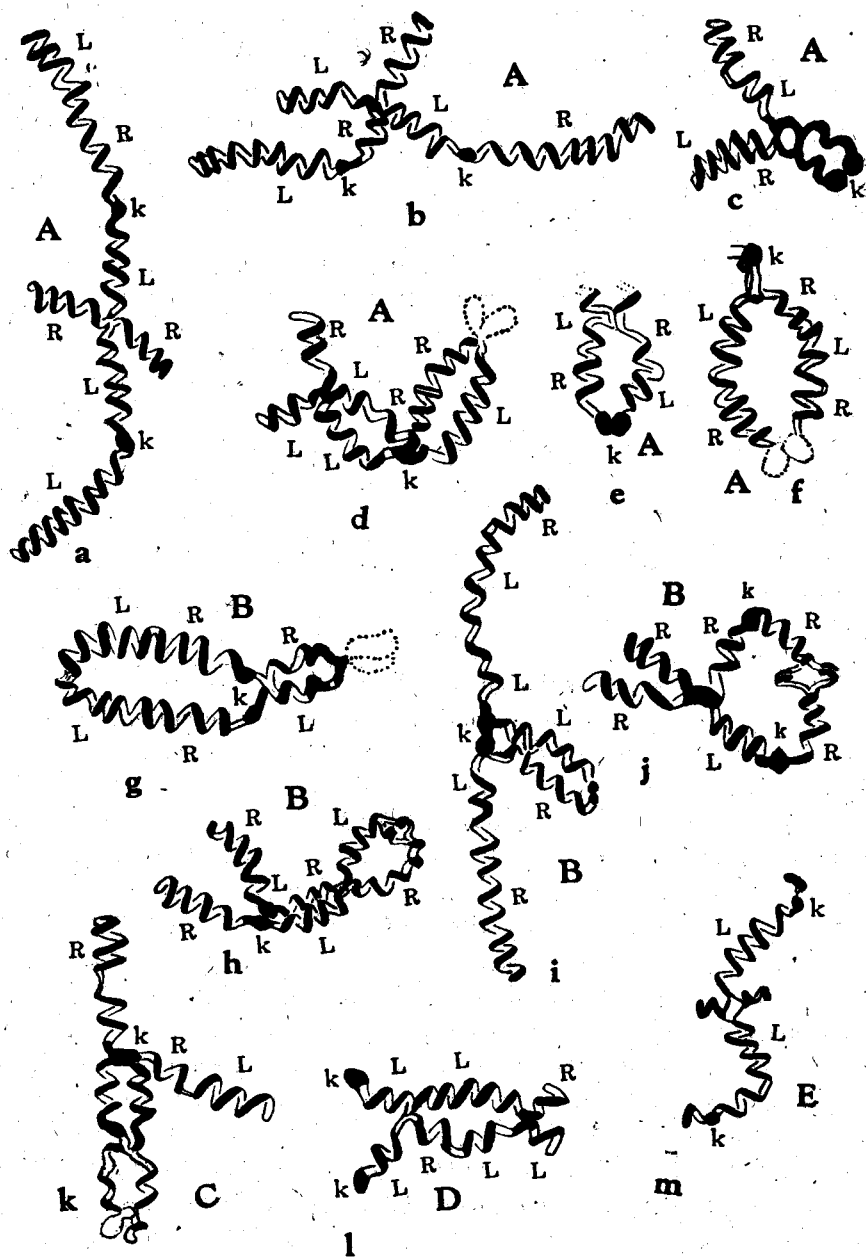


Fig. 8. Chromosomes of the first metaphase drawn from various PMCs. The symbols of R, L, k and A-E show dextrorse, sinistrorse spiral, kinetochores and types of chromosomes respectively. $\times 3550$.

The configuration of spiral structure in the loop of interstitial chiasmata and of free arms is shown in Table 3 and 4 respectively.

Table 3. Structure of loops of interstitial chiasmata.

No. of turns and type of arms	Configuration	No. of loop-arms observed	Freq. of reversal (Per 100 turns)
4 (Bs)	$\frac{R_4}{L_4}$ (Fig. 8i)	10	0
4 (A)	$\frac{L_{1.5}R_{1.5}L_1}{R_4}$		2
4 (A)	$\frac{R_4}{R_4}$		0
4 (A)	$\frac{L_4}{R_2.L_2}$ (Fig. 8d)		1
4 (D1)	$\frac{L_{2.5}R_2}{L_4}$		1
4.5 (A)	$\frac{R_{2.5}L_2}{R_{2.5}L_2}$ (Fig. 8e)	10	2
5 (A)	$\frac{R_{2.5}L_{2.5}}{L_5}$		1
5 (E1)	$\frac{R_1.L_4}{R_3.L_2}$		2
5 (B1)	$\frac{R_5}{L_5}$ (Fig. 8h)		0
5 (D1)	$\frac{L_5}{R_{2.5}L_{2.5}}$ (Fig. 8l)		1
6 (A)	$\frac{L_{0.5}R_4.L_{1.5}}{R_{2.5}L_{3.5}}$	6	3
6 (A)	$\frac{L_{1.5}R_{4.5}}{R_6}$		1
6 (A)	$\frac{R_6}{L_6}$ (Fig. 8d)		0
7.5 (A)	$\frac{L_4.R_{3.5}}{R_{2.5}L_{3.5}R_2}$ (Fig. 8f)	4	3
7.5 (D1)	$\frac{L_{7.5}}{R_4.L_{3.5}}$		1

	Total	Actual ratio	Expected ratio	Dev.
Total of dextrorse spirals	75.5	0.97	1.00	-0.03
„ „ sinistrorse spirals	79.5	1.03	1.00	+0.03

Remarks: In the above table, loops formed between two chiasmata, and, between a kinetochore and a chiasma were dealt with. Loops including the kinetochore in the loops were excluded.

Table 4. Structure of free-arms.

No. of turns and type of arms	Configuration	No. of loop- arms observed	Freq. of reversal (Per 100 turns)
3 (A)	$\frac{R_3}{L_3}$	} 4	0
3 (D1)	$\frac{L_3}{R_3}$		
4 (E1)	$\frac{R_4}{L_4}$	} 20	0
3.5 (A)	$\frac{L_{3.5}}{R_{3.5}}$		0
3.5 (B1)	$\frac{R_{3.5}}{R_{3.5}}$ (Fig. 8j)		0
4 (B1)	$\frac{L_4}{R_4}$		0
3.5 (A)	$\frac{R_{3.5}}{R_2.L_{1.5}}$		1
4 (A)	$\frac{L_4}{L_4}$		0
4 (A)	$\frac{R_1.L_3}{L_3.R_1}$		2
4 (A)	$\frac{L_4}{R_4}$		0
4 (A)	$\frac{R_4}{R_4}$		0
3.5 (B1)	$\frac{R_{3.5}}{L_{3.5}}$		0
5 (A)	$\frac{L_5}{R_{1.5}.L_{3.5}}$	} 18	1
4.5 (Bs)	$\frac{R_2.L_{3.5}}{L_{4.5}}$ (Whole arm)		1
5 (A)	$\frac{R_5}{L_5}$		0
4.5 (A)	$\frac{R_{4.5}}{R_{4.5}}$		0
4.5 (A)	$\frac{R_{4.5}}{R_{4.5}}$		0
5 (B1)	$\frac{L_5}{R_5}$		0
5 (E1)	$\frac{L_5}{R_5}$		0
4.5 (A)	$\frac{L_{4.5}}{R_{4.5}}$		0
4.5 (A)	$\frac{L_2.R_{2.5}}{L_{4.5}}$		1

6 (D1)	$\frac{R_6}{L_{4.5} \cdot R_{1.5}}$	10	1	3 (5%)
6 (D1)	$\frac{L_6}{L_6}$		0	
5.5 (C ₈)	$\frac{L_{2.5} \cdot R_3}{R_{2.5} \cdot L_3}$ (Fig. 8k)		2	
6 (A)	$\frac{L_6}{L_6}$		0	
5.5 (A)	$\frac{R_{5.5}}{L_{5.5}}$		0	
6.5 (C1)	$\frac{R_{6.5}}{R_{6.5}}$	8	0	3 (5.4%)
6.5 (A)	$\frac{R_{6.5}}{L_{6.5}}$		0	
7 (D1)	$\frac{R_5 \cdot L_2}{L_6 \cdot R_1}$		2	
7 (B1)	$\frac{L_7}{R_3 \cdot L_4}$		1	
8.5 (D1)	$\frac{L_{4.5} \cdot R_{4.5}}{R_{6.5} \cdot L_{4.5}}$	14	2	8 (6.35%)
8.5 (A)	$\frac{L_{8.5}}{R_{8.5}}$ (Whole arm) (Fig. 8b)		0	
9 (A)	$\frac{L_9}{R_9}$ (Whole arm)		0	
8.5 (D1)	$\frac{R_{8.5}}{L_{6.5} \cdot R_{2.5}}$		1	
9 (B1)	$\frac{L_3 \cdot R_7}{R_9 \cdot L_3}$ (Whole arm) (Fig. 8i)		2	
8.5 (A)	$\frac{L_{8.5}}{R_{5.5} \cdot L_3}$ (Whole arm) (Fig. 8a)		1	
8.5 (A)	$\frac{L_{3.5} \cdot R_{1.5} \cdot L_{3.5}}{L_{8.5}}$ (Whole arm)		2	

	Total	Actual ratio	Expected ratio	Dev.
Total of dextrorse spirals	197	0.97	1.00	-0.03
„ „ sinistrorse spirals	209	1.03	1.00	+0.03

In those tables the symbols of R and L mean dextrorse and sinistrorse spirals respectively, and the figures of right side of the symbols show the number of turns. The numerator and denominator of the configuration in the Tables represent the configuration of two arms; it was provided that recording always starts from the proximal region and ends at the distal

region. In calculation of the frequency of reversals fractional numbers such as 4.5 or 5.5 are calculated as 5 or 6 respectively.

As seen in the above Tables, the frequency of reversals in the loops of interstitial chiasmata is high as compared with the frequency of reversals in free arms. MATSUURA (1941) has observed the same fact in *Trillium* and has interpreted it as follows: "Free" strands are able to rotate in spiralisation and consequently the parallel arrangement of the paired chromatids can be converted into the twisted orientation in spirals, that is, the relational spiral, while in "fixed" strands their freedom of rotation is prevented and therefore the paired chromatids take more complicated configurations, as the present paper has shown. In the latter, the paired strands will assume either a relational spiral in the form of a balanced spiral or a parallel one in the form of an unbalanced spiral." The data of the present study seem to support MATSUURA's statement, although the clear "balanced type" as observed by him was not met with. The observation in the loops including terminal chiasmata was not done in the present study. The frequency of reversals in free arms shows a clear association with the number of turns, as seen in Table 4 (MATSUURA, 1935b, 1937a, b; HUSKINS and WILSON, 1938). In free arms the ratio of the total number of dextrorse spirals to that of sinistrorse spirals is approximately as 1 to 1 (Table 4), suggesting that the direction of spirals is subjected to the law of chance, as observed by MATSUURA (1935b, 1937b) in *Trillium*.

The present study is not yet enough to give a conclusive view on the structure of the meiotic chromosome of this plant, but, at least, the data of the present study seem to be well in accordance with those observed by MATSUURA (1935, 1937a, b, 1941) in *Trillium kamtschaticum*.

The studies on the structure and behavior of the chromosomes in the succeeding stages are left for near future, when sufficient data may be at the writer's disposal.

Summary

1. The chromosome number of *Diphylleia Grayi* was confirmed to be 6 in PMC and 12 in somatic cells. The chromosomes were classified into five types of A, B, C, D and E, according to their relative length and other morphological characters.
2. The treatments of low temperature and chloralization were tried on the somatic chromosomes. The manner of reaction of the kinetic constriction is different under each treatment.
3. The major spirals of the chromonemata in the first meiotic meta-

phase were studied. Some statistical analyses were made on the direction of spirals in free arms and of the loops of interstitial chiasmata.

4. The reversals of the direction of spiral in free arms in the first meiotic metaphase were confirmed to be proportional to the number of turns and it seems not to be associated with chiasmata.

5. The frequent reversals of the direction of spiral were observed in the loops of interstitial chiasmata. These frequent reversals seem to confirm MATSUURA's statement.

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