161. Somatic Syndesis in *Daphne odora*. I^{*)}

The Chromosome Behavior in Mitosis

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(Comm. by Y. KUWADA, M.J.A., Dec. 12, 1958)

A brief report will be given in the present paper on the behavior in mitosis of the chromosomes in somatic syndesis in *Daphne odora*. The observations were made in different parts of the plant with materials prepared by the acetocarmine smear as well as the paraffin section method. As fixatives, La Cour 2BE or Licent's fixative was used, and sections were stained with Heidenhain's iron haematoxylin or Feulgen's nucleal stain, light green being used as contrast stain in the latter case. In the case of leaf epidermal cells, living material was also observed with liquid paraffin as mounting medium.

The parts of the plant observed are: basal region of young leaves, growing point in shoots and root tips, vascular bundle cells in development in young petals and anthers, shoot cortical and pith cells near the growing point, and tapetum cells. The principal results are the same in these different parts. The following descriptions are based on the observation of the young leaf cells, and for other cases studied only particularities will be mentioned.

Observation. 1) Interphase. The interphase nucleus shows a number of massive chromosomal bodies of some irregular outline (Figs. 1 and 2). They are nearly twice as large as or even appear larger than the chromosomes at metaphase, and in some favourable cases show a light line which may suggest their double nature. The number of these bodies varies to certain extent, and a statistical investigation was made (Table I).

Table I. Interphase

No. of chromosomal bodies	11	12	13	14	15	16	17	18
No. of nuclei observed	5	10	31	77	20	7	0	1
%	3.3	6.6	20.5	51.0	13.2	4.6	0.0	0.7

In this plant, the $2n$ number is 28 (Osawa, 1913), and the statistical investigation shows that 14 is the number of the highest frequency among others. We may thus conclude that in this plant the chromosomes are found in some condensed state in interphase, and have a strong tendency of pairing.

^{*)} Studies on the mechanism of nuclear division, III. This investigation is supported by a Grant from the "Jimbun Kagaku Kenkyuhi" of the Ministry of Education.

2) Prophase. The first visible principal change is a clearer presentation of the chromonema structure in the chromosomal bodies (Fig. 3). As they become diffuse, some of the bodies come in contact with one another to form groupings of higher order, thus in this stage (chromonema stage; Zerstäubungsstadium of Heitz, 1929, 1933) no case is found where the number of chromosomal bodies is greater than the n -number (Table II).

Table II. Chromonema stage

No. of chromosomal bodies	10	11	12	13	14
No. of nuclei observed	2	3	12	10	8
%	5.7	8.6	34.3	28.6	22.9

Then, the so-called spiral stage follows, where the chromonemata reappear in a regular zig-zag or spiral configuration (Fig. 4). In this stage the tendency of chromosomal bodies to join together, recognizable in interphase and the earliest prophase, seems to disappear, and the double nature of chromosomal bodies is often clearly shown by two spirals lying side by side in the body. Table III is the result from counting made at the spiral stage and a somewhat advanced stage.

Table III. Spiral and somewhat advanced stages

No. of chromosomal bodies	14	15	16	17	18	19
No. of nuclei observed	7	4	2	0	1	1
%	46.7	26.7	13.3	0.0	6.7	6.7

The spirals then begin to be drawn out into threads (Figs. 5 and 6). In this stage, the double chromosomes are lessened in number, but can be found in variable numbers in 80.3% cases of the nuclei observed (Table IV), while in metaphase they occur only in 21.8% cases, a fact which indicates that the liberation from the double state is proceeding further to take place as stage advances. At the late prophase (Figs. 7 and 8) the chromosome splitting is often observed in the chromosomes thus liberated.

Table IV. Mid prophase

No. of chromosome threads	23	24	25	26	27	28
No. of nuclei observed	2	10	10	9	6	9
%	4.3	21.7	21.7	19.6	13.0	19.6
80.3%						

3) Metaphase. In this stage, three forms are found in chromosome association. They are: single state (Fig. 9), double state in somatic bivalent (cf. Fig. 8), and paired state in varying degrees. The frequencies of occurrence of these three forms observed in 64 metaphase

plates are shown in Table V. It is seen from this table that only 68 (3.8%) of 1792 (28×64) chromosomes observed form bivalents, and 140 (7.8%) are in paired associations and the remaining 88.4% free and single. The somatic bivalents show a tetrad constitution at metaphase, with its plane of pairing parallel with the spindle axis and the plane of splitting meeting it at right angles.

4) Anaphase and telophase. It seems highly probable that the bivalents of tetrad constitution separate in anaphase through the plane of splitting as in the other cases of chromosomes where they are just only loosely paired or distinctly single. When the chromosomes begin

Figs. 1-12. Photomicrographs of leaf cells of *D. odora*. All the figures were taken with a Zeiss' imm. 1/12 and a Zeiss' oc. 4 except Fig. 1, which was taken with a Zeiss' imm. 1/12 and a Leitz's periplane oc. 8.

Fig. 1. Living leaf cells in interphase. Liquid paraffin medium. Fig. 2. Leaf cells in interphase. Acetocarmine preparation. c, chromosomal body. Figs. 3-12. Leaf cells in mitosis. Acetocarmine preparations. Fig. 3. Chromonema stage. ch, chromonemata seen in chromosomal body. Fig. 4. Spiral stage. sp, chromonemata in spiral configuration. Figs. 5 and 6. Mid prophase. l, showing liberation from paired state of chromosomes. Figs. 7 and 8. Late prophase. Two optical sections of the same cell. b, bivalent chromosome. Fig. 9. Metaphase. sc, single chromosome. Fig. 10. Anaphase. Figs. 11 and 12. Telophase. p, chromosomes in paired association

Table V. Metaphase

Nuclear plates with the chromosomes				
All single (I)	Paired (P) and single (I)	Bivalent (II), paired (P), and single (I)	Bivalent (II) and single (I)	
28 I	1P+ 26 I 3P+ 22 I	1 II+ 3P+ 20 I	1 II+ 26 I	
	1P+ 26 I 3P+ 22 I	2 II+ 3P+ 18 I	1 II+ 26 I	
	1P+ 26 I 3P+ 22 I	2 II+ 3P+ 18 I	2 II+ 24 I	
	1P+ 26 I 3P+ 22 I	2 II+ 4P+ 16 I	2 II+ 24 I	
	2P+ 24 I 4P+ 20 I	2 II+ 4P+ 16 I	3 II+ 22 I	
	2P+ 24 I 4P+ 20 I	3 II+ 5P+ 12 I	4 II+ 20 I	
	3P+ 22 I 4P+ 20 I	4 II+ 2P+ 16 I	5 II+ 18 I	
	3P+ 22 I 5P+ 18 I			
	3P+ 22 I			
Total:	924 I	46P+384 I	16 II+24P+116 I	18 II+160 I
No. of nuclear plates observed:	33	17	7	7
%	51.6	26.6	10.9	10.9
			21.8%	

Note: Total no. of chromosomes in pair (P): $70 \times 2 = 140$ (7.8%).
 Total no. of chromosomes in bivalent (II): $34 \times 2 = 68$ (3.8%).
 Total no. of single chromosomes (I): 1584 (88.4%).
 Total no. of chromosomes (P+II+I) observed: 1792.

to separate to poles, more chromosomes come to be found in paired arrangement than at metaphase (Fig. 10). This pairing tendency grows more conspicuous as the chromosomes approach to the pole where they appear in figures of X, ||, (), or | (Figs. 11 and 12). Some chromosomes remain, however, still single or in a loosely paired state (Table VI). When the nuclear membrane is formed, the number of chromosomal bodies is almost reduced to the haploid number in the majority of cases (Table VII).

The same results were obtained with the shoot growing points and root tips.

In young vascular cells, too, the result obtained was generally speaking the same, but correlated with the elongated ellipsoidal form

Table VI. Anaphase

No. of chromosomal bodies at pole	28-26	25-23	22-20	19-16
No. of chromosome groups observed	9	13	15	33
%	12.9	18.6	21.4	47.1

Table VII. Telophase

No. of chromosomal bodies	20-17	16-14	13-11
No. of nuclei observed	11	46	3
%	18.3	76.7	5.0

Figs. 13-16. Camera lucida drawings of vascular and pith cells. Fig. 13. Interphase nucleus in vascular cell. Fig. 14. Metaphase plate with 13 bivalents and 2 single chromosomes. Vascular cell. Fig. 15. Diploid nucleus in interphase. Pith cell. Fig. 16. Syndiploid nucleus in interphase. Pith cell

of the nucleus in this case (Fig. 13), the bivalent form in metaphase was found in a greater number than we have given above (Table VIII). In an extreme case, 13 bivalents and 2 single chromosomes were counted (Fig. 14).

Table VIII. Metaphase chromosome association in leaf and vascular cells

	Leaf cells	Vascular cells
Relative nuclear volume	140	100
Nuclear plate with chromosomes of: I form only	51.6%	16.1%
I and P forms	26.6%	38.7%
I and II forms	21.8%	45.1%
I, P, and II forms		
No. of cells observed	64	31

In cortical and pith cells of shoots in which mitotic figures are occasionally found, the chromosome behavior is also the same in essential feature as observed in leaf cells. In these tissues, there are found cells with syndiploid nucleus in scattered localization. It is a very significant fact that in the case of the syndiploid nuclei, too, the somatic syndesis pairing is recognizable to occur between single chromosomes, and no further pairing between bivalents (Figs. 15 and 16).

In young tapetum tissues the nucleus is diploid, and in fully developed tissues it is syndiploid or di-syndiploid. In interphase, most of the chromosomal bodies are bivalents in constitution. The syndetic behavior of the chromosomes in mitosis shows no particularity from those cases described above.

Conclusion. The somatic syndesis has been studied by Stevens (1910), Metz (1916, 1927), Metz and Nonidez (1921), Guyènot and Naville (1929), Naville (1932), and Kaufmann (1934) in *Diptera* in animal, and by Němec (1910) in *Ricinus* in plant. In *Diptera*, according to some of these authors, pairing becomes looser as the chromosome condensation advances, the liberation degree being maximum at

metaphase, and is brought to a close association again at anaphase and telophase; according to some others, the syndetic pairing takes place twice in a mitotic cycle, first at prophase with the liberation at late prophase, and second at anaphase with the liberation at late telophase; and according to still others, the paired arrangement is looser at metaphase than in prophase. These results may be summarized that in *Diptera* somatic syndesis takes place at anaphase and telophase, and is cancelled at prophase, though the paired arrangement may remain as such still at prophase or metaphase. In *Ricinus*, according to Němec, pairing takes place at prometaphase and anaphase. Taking together all these results of observation into consideration, it seems likely that the active pairing takes place at anaphase or telophase, though it may remain even at metaphase as a passive form. The results of the present investigation by statistical method confirm this conclusion, and support Darlington's opinion that a pair can associate but a pair of pairs can not (Darlington, 1937).

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