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Chromosome Arrangement.

V. Pollen Mother Cells in *Torilis Anthriscus*, BERNH. and *Peucedanum japonicum*, THUNB.

By

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With Plate XXIX and 6 Text-figures.

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The Umbelliferae is one of the greatest families in Angiospermae, and contains many useful plants for food and medicinal purposes. Few plants belonging to this family, nevertheless, have been cytologically studied. In 1917, WINGE stated that "Strange to say, no chromosome number has up to the present been given with certainty for the great family of the *Umbelliferæ*." Plants in this family the chromosome numbers of which are given in TISCHLER's "Pflanzliche Chromosomenzahlen" (1927) are only those given below:

Myrrhis odorata,	n=11, EM. MARCHAL, 1920
Anthriscus silvester,	n=8, H.E. PETERSON, 1914; WINGE, 1917
Anthriscus cerefolium,	n=9, EM. MARCHAL, 1920
Aegopodium Podagraria,	n=ca. 20, WINGE, 1917
Pastinaca sativa,	n=ca. 8, BEGHTEL, 1925

In carrying out some cytological investigations of several plants belonging to the Umbelliferae, I was able to determine the chromosome numbers of these plants in pollen mother cells with great accuracy.[1] The results obtained are as listed in Table I.

[1] For fixing material, several fixing mixtures were employed. Among them, BOUIN's, NAWASCHIN's, and LICENT's mixtures gave the best results. Each fixative was used after the material had been treated with CARNOY's mixture for about two minutes. FLEMMING's solution produced dark granules in the cytoplasm and made observations difficult. Sections were cut 8-12μ thick, and stained with HAIDENHAIN's iron-alum haematoxylin.

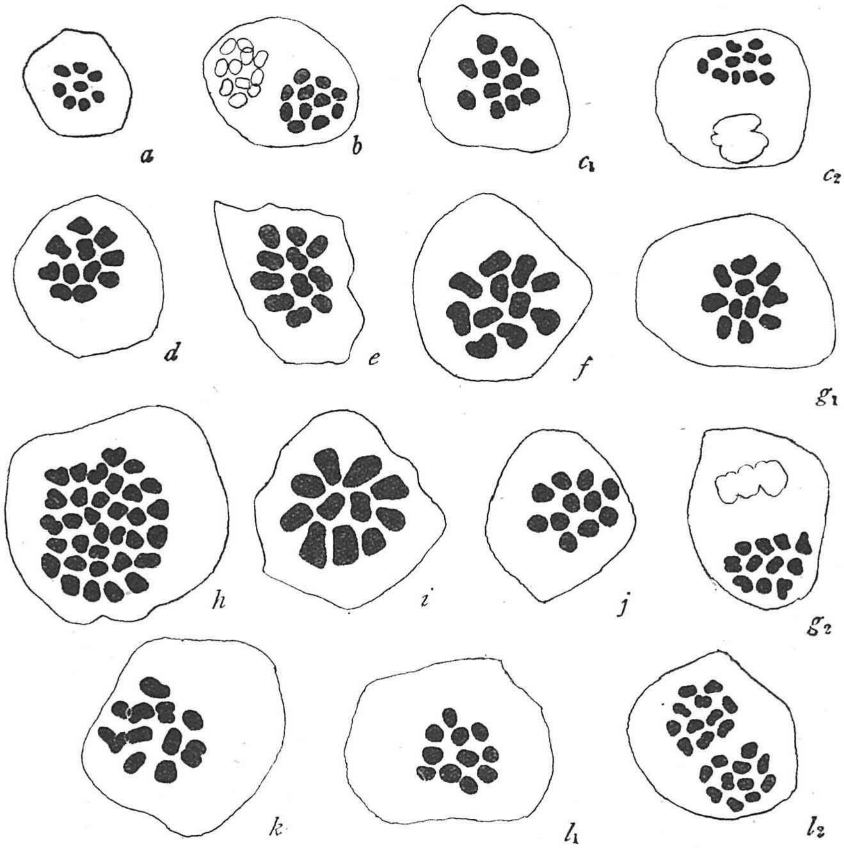
TABLE I.

Plant name	n-chromosome no.	Camera drawing
Torilis Anthriscus, BERNH.	8	Text-fig. 1*a*
Petroselinum sativum, HOFFM.	11	" 1*b*
Cicuta virosa, L.	11	" $1c_{1-2}$
Foeniculum vulgare, GAERTN.	11	" 1*d*
Ligusticum acutilobum, SIEB. et ZUCC.	11	" 1*e*
Angelica pubescens MAXIM.	11	" 1*f*
Angelica sylvestris, L.	11	" $1g_{1-2}$
Angelica, sp.	33	" 1*h*
Phellopterus littorialis, FR. SCHM.	11	" 1*i*
Peucedanum japonicum, THUNB.	11	" 1*j*
Peucedanum decursivum, MAXIM.	11	" 1*k*
Pastinaca sativa, L.[1]	11	" $1l_{1-2}$

Considering from the results given above, the number 11 may be taken as the base number of the Umbelliferae, as we have found this number in ten different species examined which belong to eight different genera, and $3 \times 11 = 33$ chromosomes in a species of *Angelica*. On the other hand we have found eight chromosomes in *Torilis Anthriscus*. WINGE has observed the same number of chromosomes in *Anthriscus silvester*, an allied genus to *Torilis*. These facts seem to suggest that there are at least two different series of plants in the Umbelliferae in respect of the number of chromosomes.

As the plants examined present very clear figures of chromosomes arranged in the equatorial plates, some closer observations on the chromosome arrangements were attempted especially in *Torilis Anthriscus* and *Peucedanum japonicum*.

[1] The chromosome number of this species has been reported by FLOYD E. BEGHTEL (1925) to be about eight in his study of "the Embryogeny of *Pastinaca sativa*." But I observed many clear equatorial plates with eleven chromosomes in both heterotype and homotype spindles.



Text-fig. 1_{a-l}. Camera drawings of chromosomes in equatorial plates in meiotic divisions of the plants listed in Table I. ×2100.

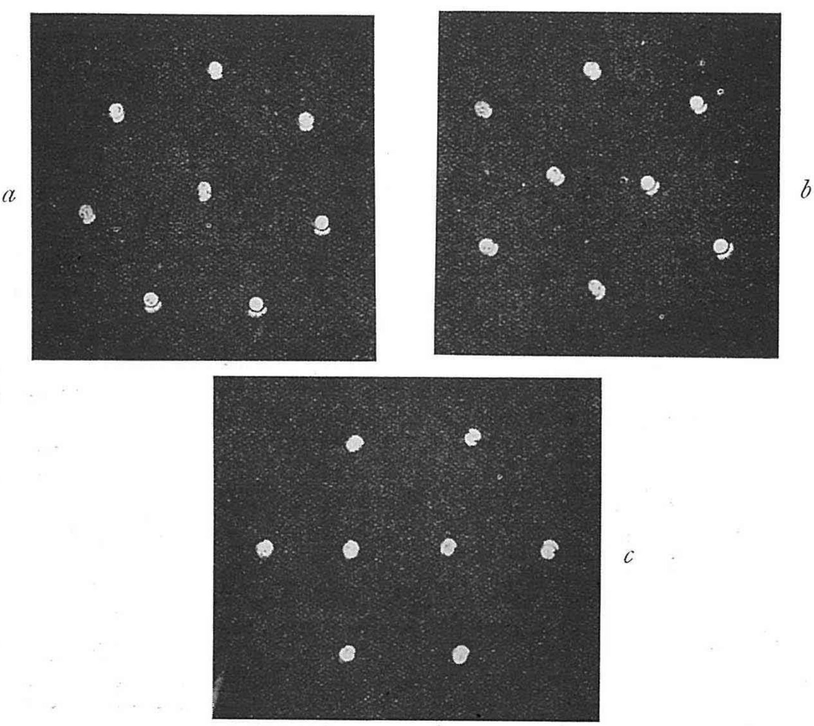
a. Torilis Anthriscus, heterotype division; *b. Petroselinum sativum*, homotype division; c_{1-2}. *Cicuta virosa*, c_1 heterotype division, c_2 homotype division; *d. Foeniculum vulgare*, heterotype division; *e. Ligusticum acutilobum*, heterotype division; *f. Angelica pubescens*, heterotype division; g_{1-2}. *Angelica sylvestris*, g_1 heterotype division, g_2 homotype division; *h. Angelica*, sp., heterotype division; *i. Phellopterus littoralis*, heterotype division; *j. Peucedanum japonicum*, heterotype division; *k. Peucedanum decursivum* heterotype division: l_{1-2}. *Pastinaca sativa*, l_1 heterotype division, l_2 homotype division.

Since model experiments to compare the chromosomes with MAYER's floating magnets were attempted first by R. S. LILLIE (1905), they have attracted the attention of some authors to this field of inquiry (DONCASTER, 1920; CANNON, 1923). Recently KUWADA (1928) has compared the arrangements of chromosomes of the same size as well as of different sizes with those of the floating magnets and has explained the reasons why we often meet arrangements which hardly show a resemblance to the stable form of arrangement of the floating magnets.

OBSERVATION.

1. Torilis Anthriscus.

As mentioned above, the chromosome number in the meiotic divisions in pollen mother cells of this plant is eight, and all chromosomes are round and of nearly uniform size in polar view. DONCASTER (1920) has pointed out the fact that "Especially when the chromosomes are short and of nearly uniform size, the figures of equatorial plates have



Text-fig. 2_{a-c}. Photographs of eight floating magnets arranged as in GANOT'S Fig. 697, 8*a*, *c*, *b*, respectively. (See foot-note on page 313).

a remarkable resemblance to those given by physicists of the groupings of various numbers of magnets floating in a confined space,......".

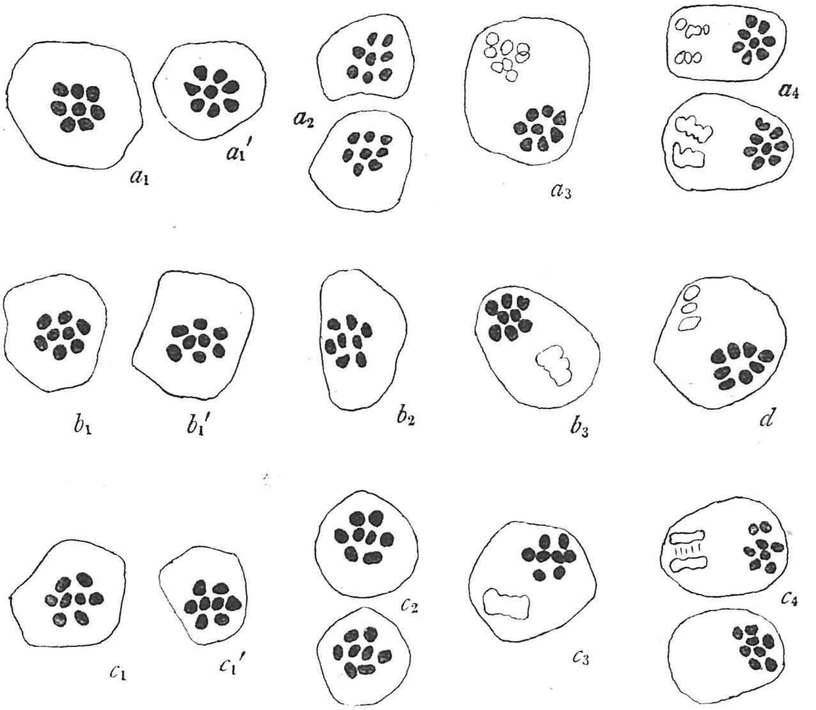
To analize a phenomenon, we should begin first with a simpler case, and from this point of view this plant may be taken as a good material which answers our purpose. In this plant, the chromosome arrangements in the equatorial plate are mostly regular in both heterotype and homotype divisions, and can be classified very clearly, as shown in Table II, into four classes, Forms A (Text-fig. $3a_{1-4}$ and Fig. $1a_{1-4}$, Pl. XXIX), B (Text-fig. $3b_{1-3}$ and Fig. $1b_{1-2}$, Pl. XXIX), and C (Text-fig. $3c_{1-4}$ and Fig. $1c_{1-4}$, Pl. XXIX), which correspond respectively to GANOT's Fig. 697, 8*a*, *c* and *b*[1] (cp. Text-fig. 2), and Form D (Text-fig. 3*d* and Fig. $1d_{1-2}$, Pl. XXIX) in which the chromosomes are arranged in a ring having none of them inside it. Irregular arrangements which do not fall into any of these classes were rather low in frequency of occurrence, there being only 45 cases out of 419 cases observed.

TABLE II.

Number of cases of different forms of chromosome arrangements.

Stage \ Form		Regular arrangement				Irregular arrangement	Total
		Form D	Form A	Form B	Form C		
Heterotp. division	Metaph.	——	64(51.6%)	16(12.9%)	23(18.5%)	21(16.9%)	124
	Anaph.	——	22(55.0%)	6(15.0%)	5(12.5%)	7(17.5%)	40
Homotp. division	Metaph.	21(9.0%)	176(75.5%)	8(3.4%)	11(4.7%)	17(7.3%)	233
	Anaph.	——	20(90.9%)	——	2(9.1%)	——	22
Total		21(5.0%)	282(67.3%)	30(7.2%)	41(9.8%)	45(10.7%)	419(100%)

[1] In GANOT'S Fig. 697, 8*a*, eight floating magnets are arranged in the form of a heptagon having one in its centre; in *b*, in the form of a hexagon having two in the central region which lie on a line connecting two opposite angles of the hexagon, or floating magnets lying at these angles, at about equal distances from one another; in *c* these two central floating magnets lie on a line connecting the middle points of opposite sides of the hexagon. Compare our Text-fig. 2 *a*, *c*, and *b* respectively.



Text-fig. $3a$–d. *Torilis Anthriscus.* Camera drawings corresponding to various forms of chromosome arrangement given in Table II. ×2100.

a_{1-4}. Chromosomes arranged as in Form A in the table: a_1, a'_1. heterotype metaphase, a_2. two sister groups in heterotype anaphase, a_3. homotype metaphase, a_4. two sister groups in homotype anaphase.

b_{1-3}. Chromosomes arranged as in Form B: b_1, b'_1. heterotype metaphase, b_2. heterotype anaphase, b_3. homotype metaphase.

c_{1-4}. Chromosomes arranged as in Form C: c_1, c'_1. heterotype metaphase, c_2. two sister groups in heterotype anaphase, c_3. homotype metaphase, c_4. two sister groups in homotype anaphase.

d. Chromosomes arranged as in Form D, homotype metaphase.

From Table II, we see that Form A is the most numerous, being more than 67% of all the cases observed including cases of irregular arrangements, and that putting the irregular cases aside for a moment, Forms C, B, and D come next in frequency of occurrence in the order named. Form A is the form which is taken by floating magnets in their stable state of distribution, and forms B and C are forms which we often meet also in the case of model experiments with floating magnets. The latter two are unstable forms which pass into the stable Form A. Form D was found only in the homotype metaphase and it needs perhaps a special explanation, but we shall not go far into speculation on this point at present.

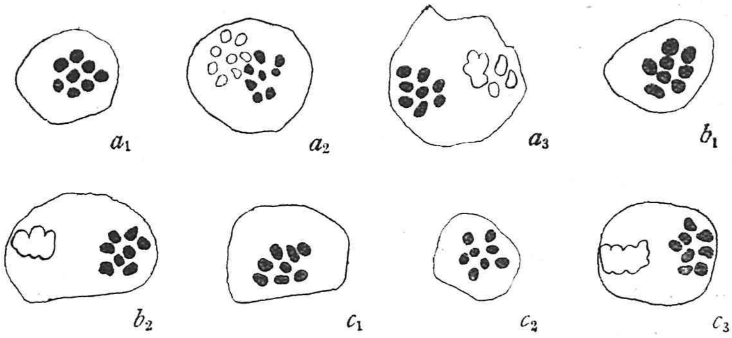
Though the irregular cases cannot be classified into these different types of form, the majority of them can be grouped into types if only the number of chromosomes occupying inner positions is regarded. Text-fig.4a_{1-3} and Fig.2a_{1-2}, Pl. XXIX, represent some of these types in different phases where only one chromosome occupies the inner position, and Text-fig.4b_{1-2}, the second type where there are found two of these chromosomes, and Text-fig.4c_{1-3} and Fig.2b, Pl. XXIX, other extremely irregular cases where a clear classification is hardly possible. In Table III, the frequency of occurrence of these three types is given.

TABLE III.

Number of cases of different types of irregular arrangements, with regard to the number of chromosomes occupying the inner positions.

Stage \ Form		Number of chromosomes inside the chromosome ring		Extremely irregular arrangement	Total
		1	2		
Heterotp. division	Metaphase	14	5	2	21
	Anaphase	5	—	2	7
Homotp. division	Metaphase	12	3	2	17
	Anaphase	—	—	—	—
Total		31	8	6	45

In this table we see again that the cases where the number of chromosomes found inside the arrangement ring is one are the most numerous, and that the cases where it is two come next in frequency, and extremely irregular cases which we can not group together with any of these two types of arrangement occur most seldom. If we classify



Text-fig. 4*a–c*. *Torilis Anthriscus*. Camera drawings corresponding to Table III.

a_{1-3}. A case where one chromosome may be regarded as occupying the central position : a_1. heterotype metaphase, a_2. heterotype anaphase, a_3. homotype metaphase.

b_{1-2}. A case where two chromosomes may be regarded as occupying the central positions b_1. heterotype metaphase, b_2. homotype metaphase.

c_{1-3}. Examples of the "Extremely irregular arrangement" given in the table: c_1. heterotype metaphase, c_2. heterotype anaphase, c_3. homotype metaphase.

all the cases observed only with regard to the number of the chromosomes distributed inside the arrangement ring we obtain the result shown in Table IV.

TABLE IV.

Number of cases of different types classified with regard to the number of chromosomes occupying inner positions.

No. of inside chromosomes	0	1	2	Extrem. irr. arr.	Total
No. of cases	21	313	79	6	419

This table shows that in this plant the chromosome arrangements present a remarkable resemblance to the arrangement of MAYER's floating magnets. In Table II we see that the cases resembling the stable form of arrangement of floating magnets are higher in frequency of occurrence in anaphase than in metaphase in both heterotype and homotype divisions. This fact is very interesting and suggestive from the point of view of comparing the chromosome arrangement with that of floating magnets, but the number of cases observed in the anaphase is too small for any definite conclusion to be drawn from it.

2. *Peucedanum japonicum.*

The haploid chromosome number of this plant is eleven and the chromosomes are all of nearly uniform shape and size as in *Torilis Anthriscus.* The chromosome arrangement was observed in the heterotype and homotype divisions of pollen mother cells. The results obtained are as given in Tables V and VI.

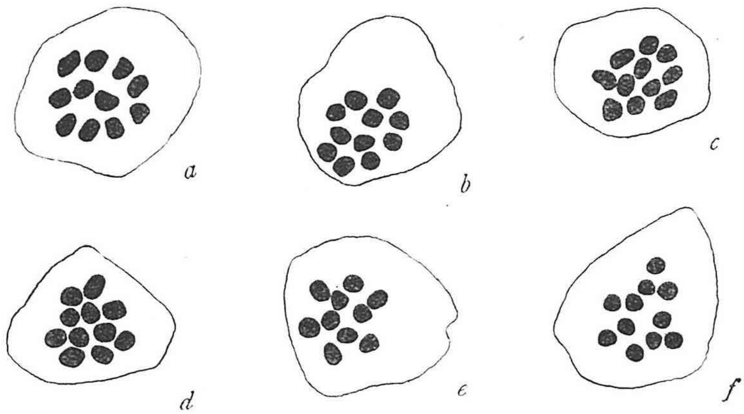
TABLE V.

Number of cases of different forms of chromosome arrangements in the heterotype division. Corresponding figures are given in Text-fig. 5_{a-f} and Fig. 3_{a-c}, Pl. XXIX.

No. of chromosomes inside the chromosome ring	1	1–2	2	2–3	3	3–4	4	4–5	Total
No. of cases	—	—	21	17	115	13	4	1	171
%	—	—	12.3	9.9	67.3	7.6	2.3	0.6	100

From this table, it is obvious that in the heterotype metaphase, the cases where three chromosomes are found inside the chromosome ring (Text-fig.5*c* and Fig.3*b*, Pl. XXIX), that is, the cases which resemble the stable form of arrangement of eleven floating magnets[1] are the most numerous and those where there are two inside or central chromosomes (Text-fig.5*a* and Fig.3*a*, Pl. XXIX), and cases intermediate between 2–3 (Text-fig.5*b*), and those between 3–4 (Text-fig.5*d*), come next in frequency of occurrence in the order named, other forms of arrangement being of relatively rare occurrence. The frequency of the first case is 67.3% of all the cases observed. Experience acquired from model experiments

[1] See MIZUNO (1916) and also CANNON (1923).



Text-fig. 5*a–f*. *Peucedanum japonicum*. Camera drawings of chromosomes in heterotype equatorial plates corresponding to Table V. ×2100.

a. A case where two chromosomes are found in the centre.

b. An intermediate case between *a* and *c*, or so to speak, between 2-3 in the centre.

c. A case where three chromosomes are in the centre.

d. An intermediate case between 3-4 in the centre.

e. A case where four chromosomes are in the centre.

f. An intermediate case between 4-5 in the centre.

with floating magnets[1] bring us to the idea, that cases intermediate between 2–3, and between 3–4 may represent stages just before the chromosomes attain their final stable positions in the arrangement having three inside the ring. If, for this reason, we sum up the numbers for these three cases, we shall see that the resemblance to the arrangement of floating magnets becomes more remarkable. If we again take into consideration the fact that in the case of eleven floating magnets we have, not infrequently, a temporarily stable form in which two are inside the ring, we learn that the resemblance in arrangement between chromosomes and floating magnets exsists in almost all the cases observed.

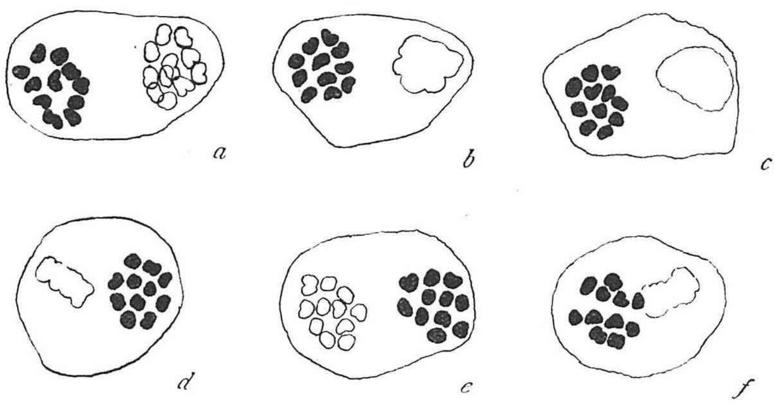
The results obtained in the homotype metaphase are, however, somewhat different from those found in the heterotype metaphase as shown in Table VI.

1) In model experiments we have, not infrequently, a transitory form in which two or four are inside the ring.

TABLE VI.

Number of cases of different forms of chromosome arrangements in the homotype division. Corresponding figures are given in Text-fig. 6_{a-f} and Fig. 4_{a-d}, Pl. XXIX.

No. of chromosomes inside the chromosome ring	1	1–2	2	2-3	3	3–4	4	4-5	Total
No. of cases	2	—	89	40	99	10	7	—	247
%	0.8	—	36.0	16.1	40.1	4.0	2.8	—	100



Text-fig. 6_{a-f}. *Peucedanum japonicum.* Camera drawings of chromosomes in homotype equatorial plates corresponding to Table VI. ×2100.

a. A case where one chromosome is found in the centre.
b. A case where two chromosomes are in the centre.
c. An intermediate case between 2-3 in the centre.
d. A case where three chromosomes are in the centre.
e. An intermediate case between 3-4 in the centre.
f. A case where four chromosomes are in the centre.

In this table, we see that the cases where three chromosomes are found inside the arrangement ring (Text-fig.6*d* and Fig.4*c*, Pl. XXIX) are the most numerous in the case of homotype division too, but the percentage of frequency is here rather low (40.1%) as compared with that obtained in the case of the heterotype division, and, on the other hand, that the frequency of the cases where two chromosomes take the central positions (Text-fig.6*b* and Fig.4*b*, Pl. XXIX) rises to 36% of all the cases observed.

We are quite ignorant at present of the reason why there is such a difference between the cases of heterotype and homotype divisions. But we have two points which must be emphasized in this connection :—1) The formation of two spindles in a mother cell without any partitions between them. The two nuclear plates are here often found not in the round shape as in the cases of heterotype division. If this is not an artifact, there may be a certain causal relation between a certain stress exerted upon the two nuclear plates and the difficulty of simultaneous occupation of the central positions by three chromosomes. 2) In the homotype metaphase, the chromosomes are not all round in shape as in the heterotype metaphase, but here they are of dumb-bell-, short rod-, v-, or other irregular shapes, the longitudinal split being clearly recognizable in them. Though we are not in a position at present to give an adequate explanation of the question at issue, it seems to be rather proper to regard the chromosome arrangement in the homotype division at least in this plant as a special case. In *Torilis Anthriscus* there is no such difference in the frequency curve of various chromosome arrangements between the heterotype division and the homotype division, this probably being due to its low number of chromosomes, but it may be pointed out here that in the homotype division, a form of arrangement in which the number of chromosomes occupying the central positions is less by one, as in *Peucedanum japonicum*, than that of the form of the highest frequency, (in *Torilis Anthriscus* it is zero, because the number in the latter here is one) was observed in 9% of the cases, while in the heterotype division no such form was observed either in the metaphase or in the anaphase.

SUMMARY.

1) Both 8 and 11 may be regarded as the basic chromosome numbers in the Umbelliferae.

2) In meiotic divisions of *Torilis Anthriscus* and *Peucedanum japonicum*, the arrangement of the chromosomes resembles that of MAYER's floating magnets in a large measure, except the case of the

homotype division of the latter plant, where the resemblance is less remarkable.

In conclusion, I wish to express my cordial thanks to Prof. Y. KUWADA, for his kind direction throughout the investigation.

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EXPLANATION OF PLATE XXIX.

All figures are microphotographs taken with ZEISS' apochrom. imm. 2mm. and comp. oc. 18.

Fig. 1_{a-d}. *Torilis Anthriscus*. Chromosome arrangements corresponding to Table II.

a_{1-4}. Figures corresponding to Form A in the table: a_1. heterotype metaphase from the same equatorial plate as that reproducted in Text-fig. 3. a'_1; a_2. heterotype anaphase; a_3. homotype metaphase; a_4. homotype anaphase.

b_{1-2}. Figures corresponding to Form B: b_1. heterotype metaphase from the same equatorial plate as in Text-fig. 3. b'_1; b_2. heterotype anaphase.

c_{1-4}. Figures corresponding to Form C: c_1. heterotype metaphase from the same equatorial plate as in Text-fig. 3. c'_1; c_2. heterotype anaphase; c_3. homotype metaphase; c_4. homotype anaphase.

d_{1-2}. Figures corresponding to Form D: d_1. homotype metaphase; d_2. A case in the same phase as in d_1, but here the chromosomes are arranged in two rows, an arrangement which may be regarded as having been derived from such an arrangement as shown in d_1.

Fig. 2_{a-b}. *Torilis Anthriscus.* Chromosome arrangements corresponding to Table III.

a_{1-2}. A case where one chromosome may be regarded as occupying the central position: a_1. heterotype metaphase; a_2. homotype metaphase.

b. A figure belonging to the "Extremely irregular arrangement" of Table III, taken from the same equatorial plate as the drawing in Text-fig. 4. c_3.

Fig. 3_{a-c}. *Peucedanum japonicum.* Figures of chromosome arrangements in heterotype metaphase corresponding to Table V.

a. A case where two chromosomes are in the centre.

b. Cases where three are in the centre.

c. A case where four are in the centre.

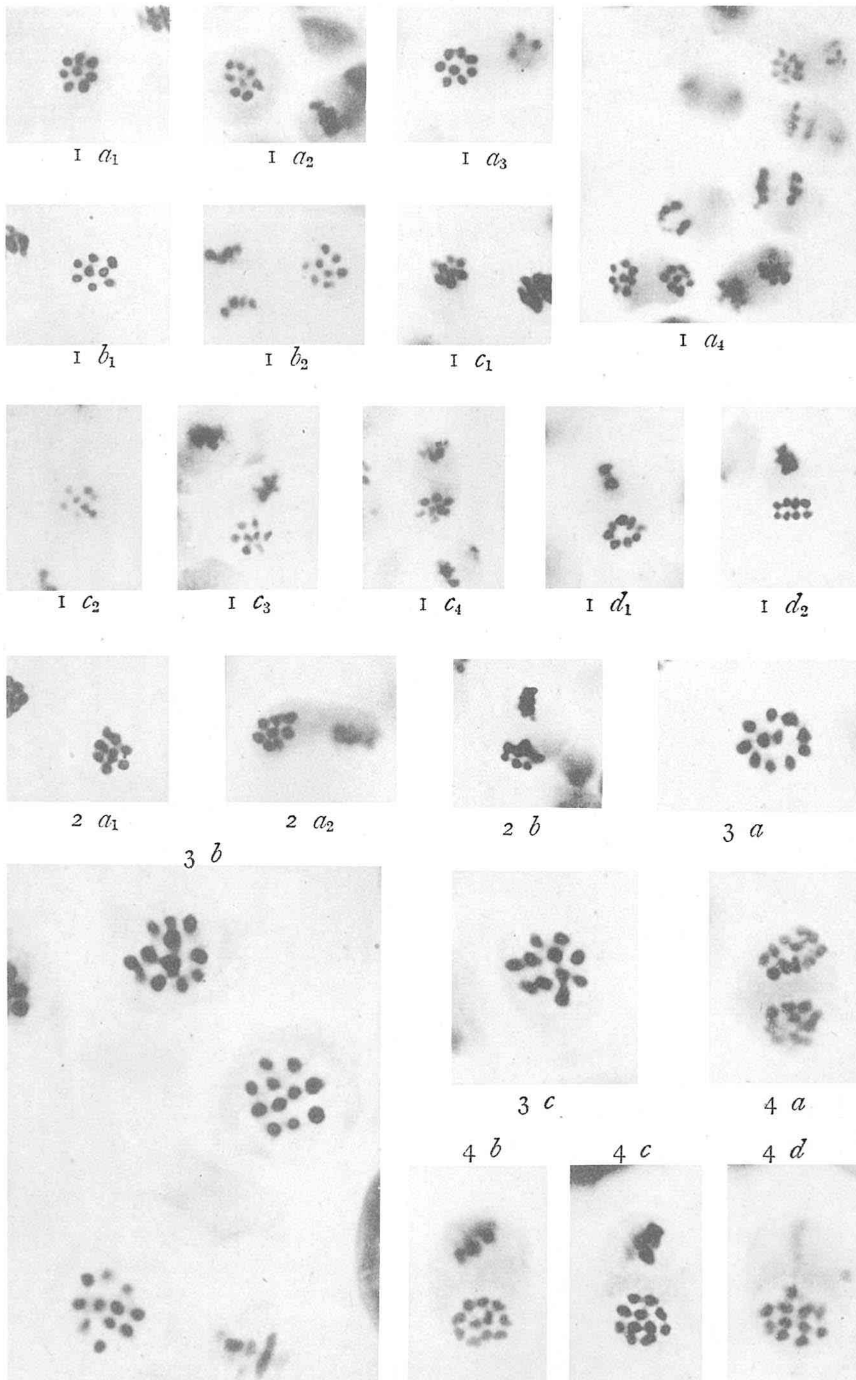
Fig. 4_{a-d}. *Peucedanum japonicum.* Figures of chromosome arrangements in homotype metaphase corresponding to Table VI.

a. A case where one chromosome is in the centre.

b. A case with two chromosomes in the centre.

c. A case with three in the centre.

d. A case with four in the centre.



1 a_1 1 a_2 1 a_3 1 a_4

1 b_1 1 b_2 1 c_1

1 c_2 1 c_3 1 c_4 1 d_1 1 d_2

2 a_1 2 a_2 2 b 3 a

3 b 3 c 4 a

4 b 4 c 4 d

OGAWA phot.