MEMOIRS OF THE COLLEGE OF SCIENCE, KYOTO IMPERIAL UNIVERSITY, SERIES B.
VOL. IV. NO. 3, ARTICLE 12, 1929.

Chromosome Arrangement.

IV. The Meiotic Divisions in Pollen Mother Cells of *Sagittaria Aginashi*, MAKINO, and *Lythrum salicaria*, L. var. *vulgare*, DC., subvar. *genuina*, KOEHNE.

By

NAMIO SHINKE.

With Plate XXVIII and 9 Text-figures.

(Received January 26, 1929)

A resemblance between the arrangement of floating magnets in a magnetic field and that of the chromosomes in the equatorial plate, has been pointed out by R. S. LILLIE (1905) and illustrated by H. G. CANNON (1923). But this resemblance seems to be found, as pointed out by L. DONCASTER (1920), only when all the chromosomes are practically of the same size and shape; and therefore, it is a matter of interest to make closer investigations into cases, where one or more of the chromosomes are of different sizes from the others.

In the heterotype mataphase in pollen mother cells of *Sagittaria Aginashi*, MAKINO, and *Lythrum salicaria* L. var. *vulgare*, DC., subvar. *genuina*, KOEHNE, there is found one large chromosomal element among the others though it is not always in the latter plant. This fact led the writer to investigations into the arrangement of chromosomes in the nuclear plate in pollen mother cells of these plants.

A. OBSERVATION OF THE MEIOTIC DIVISION IN POLLEN MOTHER CELLS OF *Sagittaria Aginashi*, MAKINO[1].

For fixing material, BOUIN'S solution, the Bonn modification of FLEMMING'S, and FLEMMING'S stronger solutions were employed, a previous treatment with CARNOY'S fluid for a few minutes having been applied. Of these fixatives, the last one gave the best results.

Sections were made generally 10–14 μ thick, and stained with HEIDENHAIN'S haematoxylin.

The Number and the Size of Chromosomes.

The number of double chromosomes in the heterotype metaphase of this plant are eleven, while in some other species of *Sagittaria* the diploid chromosome number has been reported by W. R. TAYLOR (1925) to be about twenty and by N. NAWA (1928) just twenty.

In *Sagittaria Aginashi*, some variations in the shape of the chromosomes, are recognizable already in the late diakinesis. When they are viewed from the pole in the metaphase, we can usually distinguish one large and one small chromosome from the remaining nine. (Fig. 1). The former represents itself as 8–, a long rod or various other shapes, while in the side view, it often comes out as a tangential ring tetrad with the non-terminal spindle fiber attachment as frequently reported in insects. (Fig. 2). In the anaphase, its separating halves present a double V-shape. (Fig. 3). We designate this large chromosome the "*M*-chromosome" in the present paper. The small one which is designated here as the "*a*-chromosome," presents already in the metaphase a longitudinal split for the homotype division which is usually somewhat obscure in the polar view. The chromosome can be discriminated from the other chromosomes by its short rod-like or spherical form, though sometimes it is hardly distinguishable from some other small elements. The other

[1] Closer descriptions of the observations made in the meiotic divisions in this plant will be given in another paper.

nine chromosomes usually appear as horizontal rings or V-shaped tetrads as shown in Fig. 1.

In the early heterotype anaphase, when the univalent components of bivalents begin to migrate towards the poles, it is often noticeable that in one or two of these univalents, the halves due to the longitudinal split are entirely separated from, but remain near each other, as reported by W. R. TAYLOR (1924) in *Gasteria*. In Fig. 4, this peculiar behaviour of the *M*-chromosome is shown. Here the halves are so distinctly separated from each other that they may be counted as two independent chromosomes.

It must be added here that separation of the univalent components of the bivalents towards the poles does not take place simultaneously in all the gemini, and accordingly one or some remain still in the equatorial region, while the others have already reached the poles. (Fig. 5). It is left undecided whether or not only some definite gemini show such a particular behaviour, but so far the present observations are concerned, it seems that any of the gemini may behave in this manner.

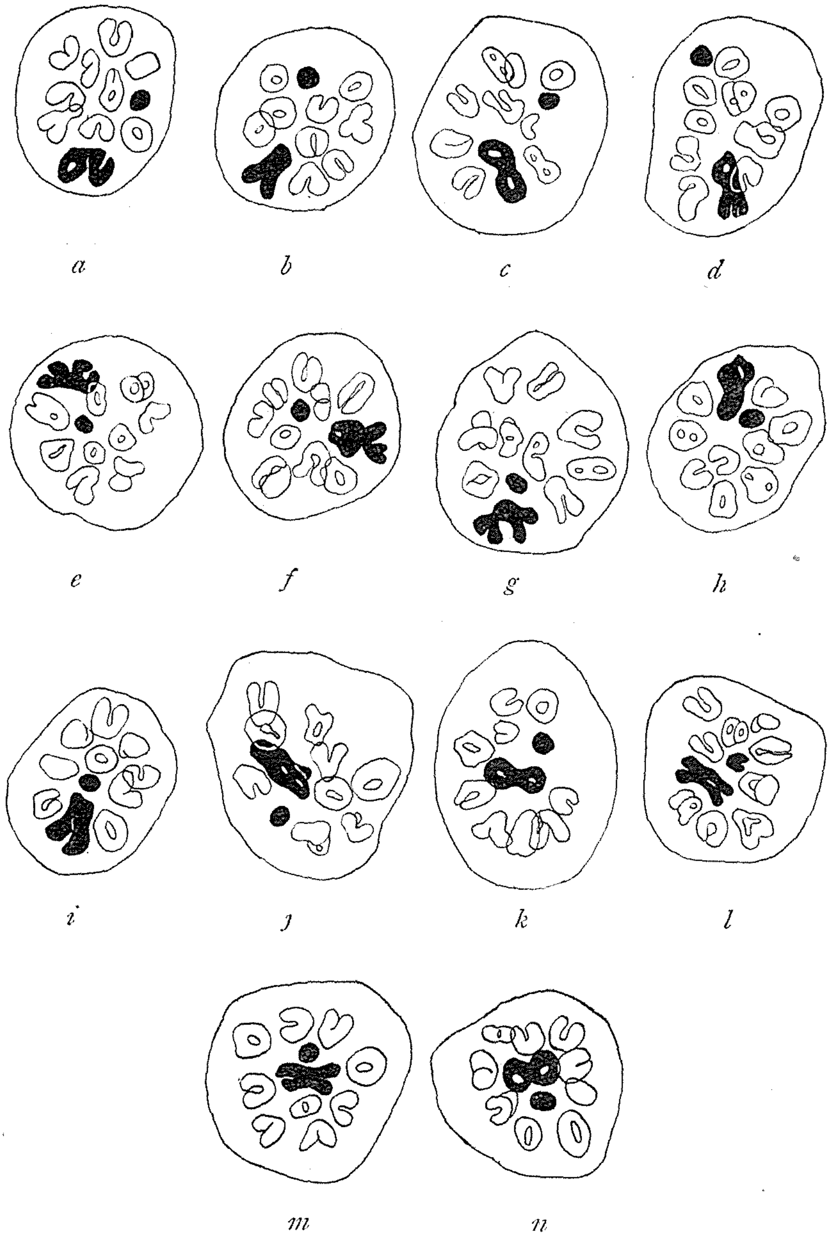
Arrangement of Chromosomes in the Heterotype Metaphase.

In the polar view of the heterotype mataphase, the *a*-chromosomes is usually found in the central part of the chromosome arrangement, while the *M*-chromosome takes the peripheral position (Fig. 1). When we consider the relative position of these two elements, we can expect various kinds of configuration, of which only the following six cases are considered in the present paper.

Case I. The cases where both the *a*- and *M*-chromosomes take up their positions on the peripheral ring of the chromosome arrangement (Text-fig. 1, *a–d*).

Case II. The case where the *M*-chromosome is found on the peripheral ring, while the *a*-chromosome takes up its position in the central part of the configuration (Text-fig. 1, *e–i*).

Case III. The case where the *M*-chromosome is placed inside the



Text-fig. 1 *a–n*. Polar views of heterotype metaphase of *Sagittaria Aginashi*, MAKINO, showing various kinds of chromosome arrangement, corresponding to the various cases given in Table II. × 1800.

peripheral ring, while the *a*-chromosome is found on its circumference (Text-fig. 1, *j* and *k*).

Case IV. The case where both the *a*- and *M*-chromosomes take up positions inside the ring of the arrangement (Text-fig. 1, *l–n*).

Case V. The case where the *a*- or the *M*-chromosomes takes up an intermediate position between the central and the peripheral.

Case VI. The case where the *a*-chromosome can not be distinguished from the other chromosomes distinctly.

The numerical results obtained are given in Tables I and II, being classified into these six different cases. In Table I, cases where one or more of the chromosomes are found below or above the nuclear plate are grouped together, and in Table II, only cases where all the chromosomes are arranged in the nuclear plate.

TABLE I[1].

Case	Form of arrangement								Total
	5-4	4	4-3	3	3-2	2	2-1	1	
I	1	0	1	8	1	6	0	1	18
II	0	3	6	22	6	7	0	0	44
III	0	0	0	0	0	0	0	0	0
IV	0	0	1	0	1	0	0	0	2
V	0	0	2	1	3	0	0	0	6
VI	0	1	1	1	1	2	0	0	6
Total	1	4	11	32	12	15	0	1	76
Percentage	1.3	5.3	14.5	42.1	15.8	19.7	0	1.3	100

[1] In this and all the following tables, the forms of arrangement are represented by a single figure or two figures connected with a dash, which correspond to the number of chromosomes inside the ring of arrangement. The notation 4-3, for instance, indicates a case where the number of chromosomes occupying inner positions, may be counted as being either 4 or 3, according to different views of interpreting the arrangement configuration, and the letters in brackets indicate drawings marked with the corresponding letters in the Text-figures.

TABLE II.

(Comp. Text-fig. 1 *a*—*n*)

Case	Form of arrangement								Total
	5-4	4	4-3	3	3-2	2	2-1	1	
I	0	0	0	14(*a*)	4(*b*)	6(*c*)	0	1(*d*)	25
II	1	16(*e*)	18(*f*)	105(*g*)	16(*h*)	8(*i*)	0	0	164
III	0	0	1(*j*)	1	1	3(*k*)	0	0	6
IV	0	0	1(*l*)	6(*m*)	0	4(*n*)	0	0	11
V	0	0	7	0	9	1	0	0	17
VI	1	6	7	15	3	5	1	1	39
Total	2	22	34	141	33	27	1	2	262
Precentage	0.7	8.4	12.9	53.8	12.5	10.3	0.4	0.7	99.7

In both Tables I and II, we see that the configurations with three chromosomes inside the peripheral ring of the arrangement are the most numerous and that this tendency becomes more evident when the chromosomes are all arranged in the nuclear plate (Text-fig. 1), the frequency being here more than 50% of all the configurations observed.

According to MIZUNO (1916) and CANNON (1923), when we have eleven equally magnetised floating magnets, exposed in a magnetic field, the number of magnets which occupy the inner positions of the arrangement is always three in the stable form of arrangement. This and our cytological results show that in *Sagittaria*, there is a marked resemblance between the arrangement of the chromosomes and that of the floating magnets, though in it we must bear in mind that there is a certain disturbance in the arrangement which may be due to the unequal sizes of the chromosomes.

In Tables I and II, we find another peculiarity in the arrangement of the chromosomes, namely, the strikingly high frequency of configurations grouped in Case II, where the large *M*-chromosome takes up a position on the peripheral ring and the small *a*-chromosome inside the ring of arrangement. To learn what is meant by this peculiarity, we shall examine the results obtained for the four cases I, II, III, and IV purely from the mathematical point of view. We assume, in the first place, that any three chromosomes out of eleven have an equal chance to occupy the central positions. Then, we shall have as many different combinations of these chromosomes as ${}_{11}C_3=165$. If both the *a*- and *M*-chromosomes always take the peripheral positions (Case I), the number of such combinations will be ${}_9C_3=84$, and if either of the *a*- (Case II) or the *M*-chromosome (Case III) finds its position always in the peripheral region of the arrangement, it will be ${}_9C_2=36$, and thirdly if both the *a*- and *M*-chromosomes occupy the central positions (Case IV), it will amount to ${}_9C_1=9$. Now the cases having the larger number of combinations should have a higher frequency value, and thus, on the basis of the number of combinations expected we can calculate frequency values for the four cases respectively, which amount in all to the total observed number 156. In Table III, both these numbers, expected and observed, are compared.

TABLE III.

Cases	Combinations	Expected number	Observed number
I	${}_9C_3=84$	79.4	22
II	${}_9C_2=36$	34.0	127
III	${}_9C_2=36$	34.0	1
IV	${}_9C_1=9$	8.5	6
Total	${}_{11}C_3=165$	155.9	156

In this table we see that in cases where the *a*-chromosome takes the peripheral position (Cases I and III), we have only 23 examples while we can expect 113.4 of these cases. In the case where the *a*-chromosomes enters into the central region of the arrangemeet (Cases II and IV) we obtain, on the other hand, a far greater number (Case II) of observed cases than, or nearly as large as the expected number (Case IV). This shows that the *a*-chromosome has a very strong tendency to take the central position. In respect to the *M*-chromosome, we see a reverse of the matter. Comparing the observed number for Case III with the corresponding expected number, we learn that this large chromosome very rarely takes a position inside the peripheral ring of the arrangement, and when we compare further Case II with Case III, we become more impressed by the fact that there is a remarkable contrast, in the matter between the *a*- and *M*-chromosomes. A similar tendency in regard to the position of the *a*- and *M*-chromosomes is recognizable to a greater or less extent in those cases, too, where the forms of arrangement of the chromosomes do not resemble those of the floating magnets. If we assume that there are normally several different forms of arrangement in one organism, each being independent of the others, and that each of Forms 4, 3 and 2 represents one of these forms, we may divide the number of observed cases in Form 4–3 into two groups proportionally to the number of cases in Forms 4 and 3, and add one of the groups to the number given under Form 3 and the other group to Form 4, regarding them as representing Form 3 and 4 respectively. The number of cases in Form 3–2 is divided into two groups in the same manner, and the corresponding groups are grouped together with the numbers given under Forms 3 and 2 respectively. In Table IV, the numbers of observed cases are grouped together in this manner, and the resulting numbers are given under corresponding Forms.

TABLE IV.

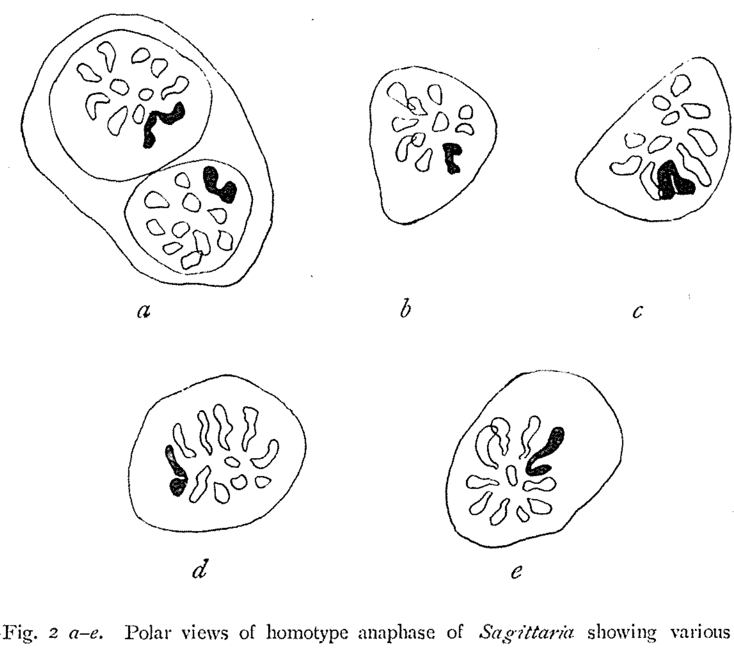
Case	Form 4			Form 3			Form 2		
	Combination	Expected number	Observed number	Combination	Expected number	Observed number	Combination	Expected number	Observed number
I	${}_9C_4=126$	9.0	0.5	${}_9C_3=84$	104.2	26.2	${}_9C_2=36$	25.7	13.8
II	${}_9C_3=84$	6.0	23.1	${}_9C_2=36$	44.6	167.6	${}_9C_1=9$	6.4	17.3
III	${}_9C_3=84$	6.0	0	${}_9C_2=36$	44.6	2.3	${}_9C_1=9$	6.4	3.8
IV	${}_9C_2=36$	2.6	0	${}_9C_1=9$	11.2	8.6	1	0.7	4.4
Total	${}_{11}C_4=330$	23.6	23.6	${}_{11}C_3=165$	204.6	204.7	${}_{11}C_2=55$	39.2	39.3

In this table too, we can notice that in all Forms 4, 3 and 2, Case II occurs far more frequently than we expect, while Cases I and III are less frequent.

If we leave out of consideration insufficiency in the number of cases observed, we may conclude from Tables III and IV that the small *a*-chromosome is found far more, and the large *M*-chromosome far less frequently in the central position of the arrangement, than we can expect.

Arrangement of Chromosomes in the Homoeotype Anaphase.

In the homotype division, the chromosomes become more elongated and show a special structure (Fig. 6), the report on which will be made later in another paper. When they are arranged in the nuclear plate, the longitudinal halves appear very clearly, usually only attached to each other at one point. Thus precise observation of the chromosome arrangement was difficult. But when they move towards the poles, the observa-



Text-Fig. 2 *a–e*. Polar views of homotype anaphase of *Sagittaria* showing various kinds of configurations of chromosomes. Comp. Table V. × 1800.

tion becomes easier. In the polar view, the *M*-chromosome can be distinguished from the others by its V-shape, usually being found on the peripheral ring of arrangement, while the *a*-chromosome, now being longer and more slender, is hardly distinguishable from the other small chromosomes.

In the anaphase, contrary to the case of the heterotype division, we usualy find only one or two chromosomes instead of three in the central region of arrangement as shown in Table V (Comp. Text-fig. 2).

TABLE V.

(Comp. Text-fig. 2 *a*—*c*)

	Form of arrangement						Total
	4—3	3	3—2	2	2—1	1	
Number of observed figures.	2(*a*)	4(*b*)	4(*a*)	35(*c*)	2(*d*)	14(*e*)	61
Percentage.	3.2	6.5	6.5	57.3	3.2	22.9	99.6

In Table V, we find only four cases where three chromosomes occupy the central positions, while there are 35 and 14 cases having two or only one chromosome within respectively. Comparing these data with those of the heterotype division, in which we found cases conformable with the arrangement of the floating magnets in so a high frequency as 53.8%, we may conclude that in a homoeotype division there is a certain disturbance the attainment of such a state of chromosome arrangement as in the heterotype division. Although we do not know whether such is a universal phenomenon or not, we find it very distinctly in *Sagittaria* where the chromosomes are by far greater in length in the homotype division than in the heterotype division. In *Lythrum* where the chromosomes are dumb-bell shaped both in the heterotype and in the homotype division, we have not found such a difference in the mode of chromosome arrangement between these two divisions, as will be seen in Table VII *a*.

B. OBSERVATION OF THE MEIOTIC DIVISIONS IN POLLEN MOTHER CELLS OF *Lythrum salicaria*, var. *vulgare*, DC., subvar. *genuina*, KÖHNE[1].

In the heterotype metaphase of this plant, we have two sorts of pollen mother cells, one with fifteen chromosomes[2] and the other with only fourteen chromosomal elements, one of which consists of two bivalent chromosomes joined end-to-end.

Materials were taken mainly from middle-styled plants and fixed in various fixing fluids, of which BOUIN's and NAWASCHIN's gave the best results.

Sections were cut in thicknesses varying from 7 to 13 μ and stained with HEIDENHAIN's haematoxylin.

Formation and Behaviour of the Tetrapartite Chromosome.

Before we enter on our main subject, it may be necessary to give a brief description of the formation and behaviour of the tetrapartite chromosome.

The end-to-end conjoining of chromosomes in the metaphase has been reported by various authors, in various plants.[3] Among these, the works of L. DIGBY (1912) on *Primula Kewensis* (seedling form) and H. KIHARA (1927) on *Rumex* and *Oenothera*, may especially be mentioned here. In the present observations, the tetrapartite (or quadrivalent) chromosome complex is generally recognizable already in the late prophase, when the segmentation of the spireme has completely finished. Gradual condensation of it follows, then, to form at last a large ring, consisting of four parts, although, it often comes out in other shapes such as shown in Fig. 7. In case there is not found the tetrapartite chromosome, we

---

1) Similar to *Lythrum salicaria*, L., the flowers of this plant are trimorpho-heterostyled.

2) G. TISCHLER (1918) has reported the haploid number of chromosomes of *L. salicaria*, L. to be about 24.

3) K. MIYAKE (1905), L. DIGBY (1912), A. B. STOUT (1913), H. KIHARA and T. ONO (1923), O. HEILBORN (1924), O. STOUT (1927), H. KIHARA (1928), and others.

count, in the diakinesis, distinctly fifteen bivalents as in Fig. 8. They can be arranged on a line according to their shapes and sizes to form a graded series, but may be classified practically into three groups. Of the fifteen chromosomes, three or four are of a ring shape, other four of a dumb-bell shape, the remaining ones being V-, U-, or rod-shaped, as shown in Fig. 8 and Text-fig. 3.

Text-fig. 3 *a–c*. Three sets of diakinetic chromosomes taken from three different pollen mother cells, arranged in a line according to size and shape.
a. Without the tetrapartite chromosome.
b. With the tetrapartite chromosome of ring shape.
c. With the tetrapartite chromosome of L-shape. × 1800

In comparing Text-fig. 3 *a* with *b* and *c*, each of which represents a set of diakinetic chromosomes arranged in the way mentioned above, the writer is driven to the view that some two of the dumb-bell shaped bivalents in Text-fig. 3 *a*, have sometimes a certain affinity between each other to conjoin end-to-end, and thus form a large tetrapartite element. O. HEILBORN (1924) has also observed a similar tendency of two peculiar bivalent chromosomes to conjoin in *Carex*.

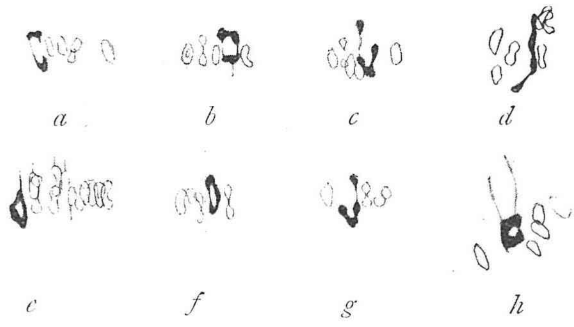
When the nuclear membrane disappears, contraction or aggregation of all the chromosomes towards the central part of the cell gradually follows. (Figs. 9 to 11). Figs. 10 and 11, which were drawn from pollen mother cells of the same loculus, show this process of contraction. Fig. 10 presents an earlier stage and Fig. 11 a later. It seems to be more reasonable to regard this aggregation of chromosomes as a consequence of certain sudden physical or physico-chemical changes in the protoplasm due to, or accompanying the disappearance of the nuclear membrane, than to regard it as being merely caused by inperfect fixation. As evidence supporting this view, it may be mentioned that the writer has observed similar figures also in pollen mother cells in the living state in *Lilium tigrinum*. Y. SINOTÔ (1927) has suggested that the contraction may have a certain bearing upon the arrangement of the chromosomes in the metaphase.

The chromosomes then gradually migrate towards the equatorial plate of the cell to form the nuclear plate. In Fig. 12, the tetrapartite chromosome in the tripolar spindle stage is shown.

A typical polar view of the nuclear plate with the ring-shaped tetrapartite element is reproduced in Fig. 13. In this stage, as will be seen later more in detail, if the cells are properly fixed, this quadrivalent element seems generally to be found on the outer chromosome ring of arrangement, though sometimes it may occupy a position inside the ring too (Text-fig. 9, *h*).

The grade of union of the two bivalents of which the tetrapartite complex consists, is different in different cells in a certain measure. Sometimes, the coalescence into one occurs so intensely that the two bivalents look apparently as if they were only one large chromosome, while, in other cases fifteen elements can be clearly counted without difficulty (Text-fig, 6). Besides these two cases, we often observe such a figure as Fig. 14, which can be regarded as belonging neither to the former case nor to the latter. These figures are denoted as intermediate figures in the present paper.

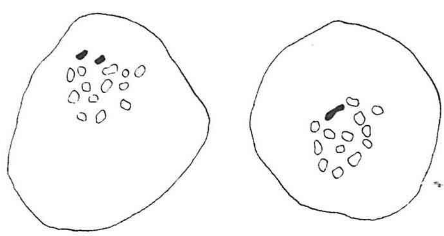
In the side view, the metaphasic tetrapartite complex presents the appearance of a ring, the constituent elements conjoining to one other at their ends, while sometimes, it takes V-, C- or various other shapes



Text-Fig. 4 *a–h*. Lateral views of the tetrapartite chromosome in late metaphase in *Lythrum*. ×1800.

as shown in Text-fig. 4, the end-to-end union being broken at a joint. Usually the complex is attached by the spindle fiber at one point in each side of the complex, upper or lower, as is usually the case with the ordinary bivalent chromosomes or gemini (Fig. 15), but in one case it was observed, as might have been expected, that the complex was attached by the spindle fiber at two points (Text-fig. 4, *h*). This mode of spindle fiber attachment was especially clearly observed in the upper side of the complex in the figure.

In the anaphase, the tetrapartite element divides into two component complexes, each of which consists of two univalents. The two univalents are then found usually separated from each other (Fig. 16), but sometimes they remain still united, so that we find one large element in one of the chromosome groups of the anaphase, while, in the other, the homologous elements are completely separated into two independent chromosomes. To put it in other words, we can find, in this case, fifteen chromosomes in one of the anaphasic chromosome groups, and fourteen in the other (comp. Text-fig. 5 with Fig. 16.). Unfortunately,



Text-Fig. 5. Two anaphasic chromosome groups in heterotype division in *Lythrum*, drawn from one and the same pollen mother cell. ×1800.

the writer was not able to determine whether this non-separating element may be found also in the homotype metaphase or not, but so far as the present observations are concerned, both sister homotypic nuclear plates were always found consisting of fifteen distinctly individualised chromosomes (Fig. 17).

Although it has not been adequately investigated as yet, we have some facts which suggest further reduction in the number of chromosomes in the heterotype metaphase by conjoining of other chromosomes. (cf. DIGBY, 1912).

It may be added here also that the results of the present observations have shown that generally the pollen mother cells, pollen grains and their nuclei, are larger in the long stamen than in the short stamen of the same flower, and that those of the long styled plant are smaller than those of the middle and short styled plants, and also that no data were obtained in the present investigation, positively supporting N. E. STEVENS' (1912) statement as to the definite correlation between the size of chromosomes and the heterostylism in *Fagopyrum*.

Arrangement of Chromosomes in the Meta- and Anaphase.

As already mentioned above, in this plant, there are two kinds of pollen mother cells, namely, the one with fifteen chromosomal elements in the heterotype metaphase, and the other with only fourteen of these, two of the fifteen temporarily conjoining into one. Naturally, there are many intermediate forms which belong neither to the former kind nor to the latter. Table VI shows the frequency of occurrence of these three classes of pollen mother cells, observed in the heterotype metaphase.

TABLE VI

Style	Stage	Number of chromosomal elements.			Total
		15 (No tetrapartite element.)	Intermediate (The tetrapartite element is not of the typical shape)	14 (One element is tetrapartite)	
Middle	Early Metaph.	14	6	*2+**12	34
Middle	Metaph.	51	13	*8+**30	102
Long	Metaph.	21	3	**10	34
Total		86	22	*10+**52	170

* Number of pollen mother cells with the tetrapartite chromosome inside the chromosome ring of arrangement.

** The same with the tetrapartite chromosome on the circumference of the ring.

From Table VI, we see that of all the pollen mother cells observed, difference in stage at which the observations were made being neglected, about one half are those with fifteen elements and the other half with fourteen. The chromosomes are arranged generally in the form of a regular or irregular ring having some number of chromosomes inside it.

i) Arrangement of 15 Elements.

The results obtained were examined, being distinguished into two cases according to whether the chromosomes are arranged all in the equatorial plate regularly or not. They are summarised in Table VII, *a* and *b*, in which the figures show the number of pollen mother cells observed.

From these two tables, we can see that there is in both cases a strong tendency for five or four chromosomes to be inside the chromosome ring of arrangement, and that this tendency becomes more pronounced

TABLE VII, *a*.

Cases where the 15 elements are all arranged strictly in the equatorial plate. (Comp. Text-fig. 6 *a*—*h*)

Style	Stage	Form of arrangement											Total
		8	8-7	7	7-6	6	6-5	5	5-4	4	4-3	3	
Middle	Heterotp. Metaph.	0	0	2(*a*)	0	1(*b*)	0	17(*d*)	8(*e*)	14	7(*g*)	2	51
Middle	Homotp. Metaph.	0	0	0	0	0	2(*c*)	15	4	11	1	2	35
Long	Heterotp. Metaph.	0	0	0	0	2	1	6	3	8(*f*)	1	0	21
Total		0	0	2	0	3	3	38	15	33	9	4	107
Percentage		0	0	1.9	0	2.8	2.8	35.5	14.0	30.8	8.4	3.7	99.9

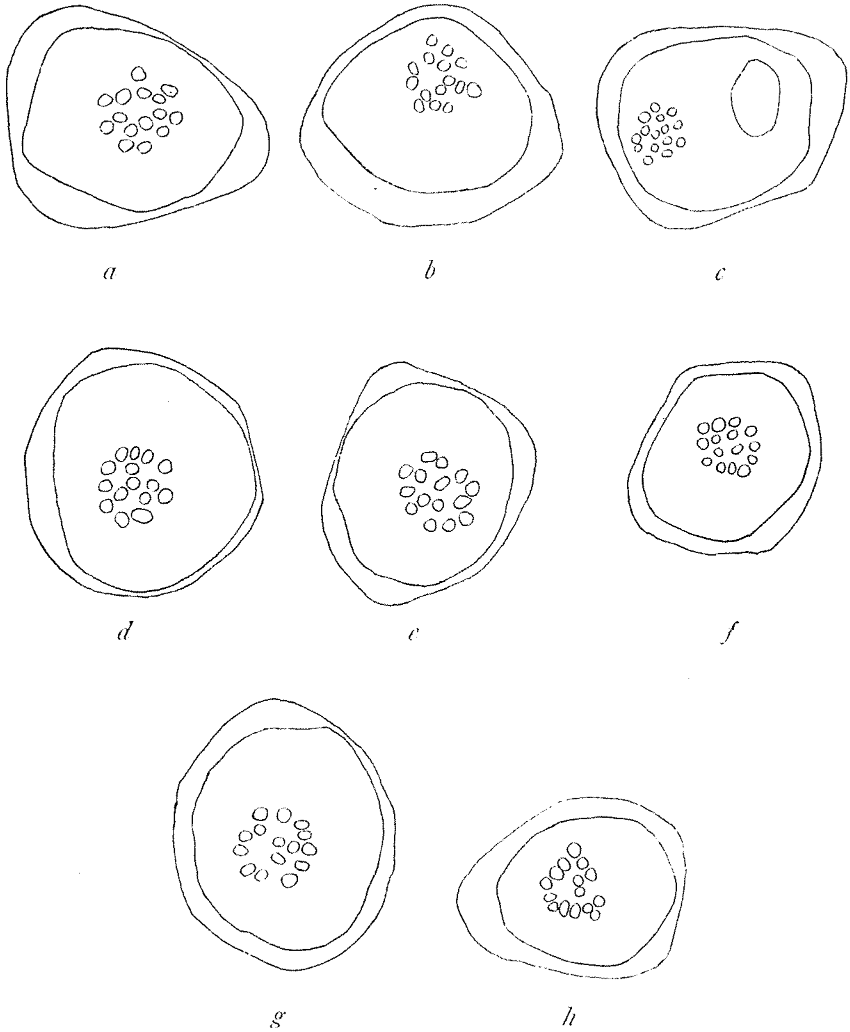
TABLE VII, *b*.

Cases where some of the 15 elements are found somewhat above or below the equatorial plate.

Style	Stage	Fom of arrangement											Total
		8	8–7	7	7–6	6	6–5	5	5–4	4	4–3	3	
Middle	Heterotp. Early Metaph.	1	0	0	1	4	1	1	1	4	1	0	14
Middle	Homotp. Early Metaph.	0	0	0	0	1	0	7	0	6	0	1	15
Middle	Heterotp. Anaph.	0	0	2	0	15	9	27	15	24	2	3	97
Long	Heterotp. Anaph.	0	0	0	0	1	0	4	2	2	0	0	9
Total		1	0	2	1	21	10	39	18	36	3	4	135
Percentage		0.7	0	1.5	0.7	15.6	7.4	28.9	13.3	26.7	2.2	3.0	100

when all the chromosomes are arranged strictly in the nuclear plate, as is readily seen by comparing Table VII, *a* and *b*.

According to CANNON and MIZUNO, when we have fifteen floating magnets, exposed in a magnetic field, the number of magnets which occupy the inner position is five in the stable form of arrangement (comp. Text-fig. 7.). Thus we may say that the chromosome arrangement is at least so far comparable with the arrangement of floating magnets, that cases where it resembles the latter (Form 5) are the most numerous in frequency of occurrence. But in this case, we have another maximum frequency in Form 4 which does not resemble the stable form of arrangement of 15 floating magnets, but resembles that of 14 magnets. This presents a marked contrast to the results obtained in *Sagittaria* in which the frequency of occurrence of the chromosome arrangement resembling the stable form of floating magnet arrangement is distinctly higher than that of the other forms which do not resemble it.

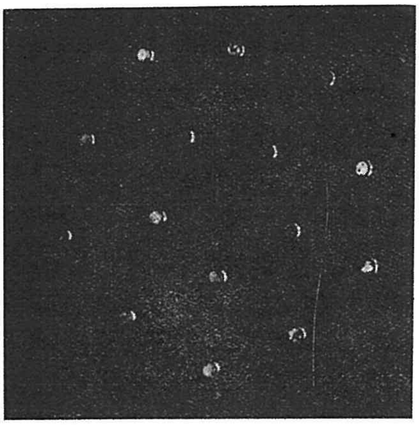


Text-Fig. 6 *a–h*. Polar views of heterotype and homotype metaphase in *Lythrum* with fifteen chromosomes which are arranged strictly in the nuclear plate. Comp. Table VII, *a*. ×1800

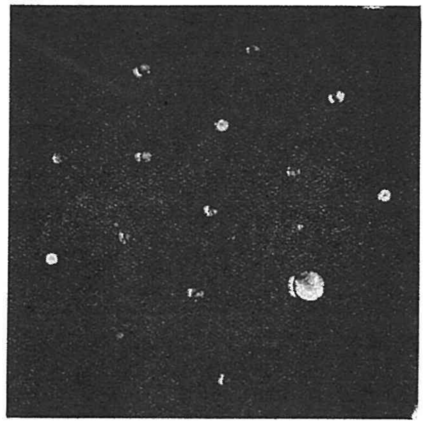
We have, then, a question why in *Lythrum* Form 4, or the form having four chromosomes inside the ring, occurs in so high a frequency as stated above, while other forms which, like the form in question do not resemble the stable arrangement of the floating magnets, are of far less frequent occurrence. This question is unexplainable at present, but as will be seen later (comp. Table VIII), we have a very interesting and suggestive fact in the case of the 14 chromosomal elements, of which one is tetrapartite. In this case we have found again a large number of cases in a form which does not correspond to the stable arrangement of the floating magnets. In the case of 15 chromosomes such a form was a form where the number of chromosomes occupying the inner position was less by one than the number we find in the stable arrangement of the floating magnets, that is, the number which corresponds to that which we have in the stable form of arrangement of 14 floating magnets. In the case of 14 chromosomes where one chromosome is tetrapartite while the others are bivalents the matter appears to be quite the reverse. In this case the number of the inside chromosomes in the form which does not correspond to the stable form of arrangement of floating magnets, but is of very frequent occurrence, is not less than the number found in the stable form of the floating magnets,[1] but more than it by one, that is, the number which we find in the stable form of arrangement of 15 floating magnets, or the number which we have found most frequently in the case of chromosomes where the tetrapartite chromosome complex has separated into two independent chromosomes, thus making the total number of chromosomes 15.

To imitate Form 4 with fifteen floating magnets, the following experiment was undertaken at the suggestion of Prof. KUWADA. 15 floating magnets were prepared, of which one consisted of a large cork float with several magnetised gramophone needles stuck in it, and the other 14 of only one magnetised needle. They are placed afloat on the water between the electromagnetic poles. In case the large one occupies a peripheral position in the arrangement, five, or not infrequently six, small magnets enter the peripheral ring of arrangement as is shown in Text-fig. 8, *a* and 8, *b*, but if the large one happens to enter the peripheral ring the arrangement becomes as shown in Fig. 8, *c*, three small and

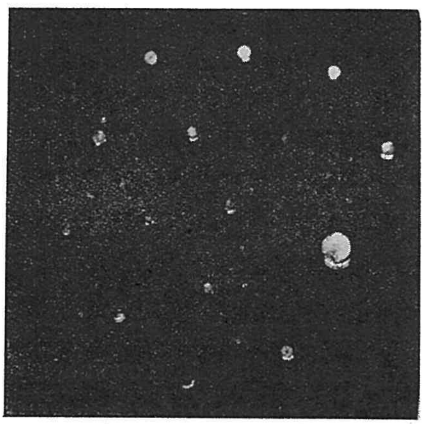
[1] In the case of 14 floating magnets, the number of floating magnets found inside the ring of the stable form is 4 (comp. MIZUNO 1916).



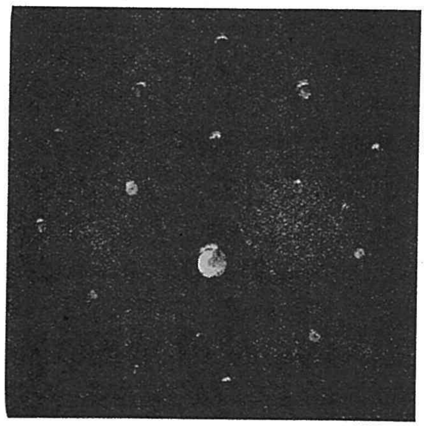
7



8 *a*



8 *b*



8 *c*

Text-Fig. 7. Photograph of the stable form of arrangement of 15 floating magnets exposed between the electromagnetic poles. Each floating magnet consists of a piece of cork with an equally magnetised gramophone needle stuck in it.

Text-Fig. 8*a*–*c*. Photographs of practically stable forms of arrangement of 15 floating magnets, one of which onsists of a large cork float with more magnetised needles in it than in the small ones. *a*. With six small floating magnets inside the peripheral ring of the arrangement. *b*. With five small ones. *c*. With one large and three small ones.

one large occupying the central region of the practically stable form of the arrangement (Text-fig. 8, *c*). This behaviour of the floating magnets may give some idea about the origin of the deviation in the chromosome arrangement.

ii) *Arrangement of 14–15 Elements.*

As the writer was not able to discriminate whether these figures are those representing the natural state of chromosome arrangements or those produced by incomplete fixation, these intermediate cases have not been considered here.

iii) *Arrangement of 14 Elements.*

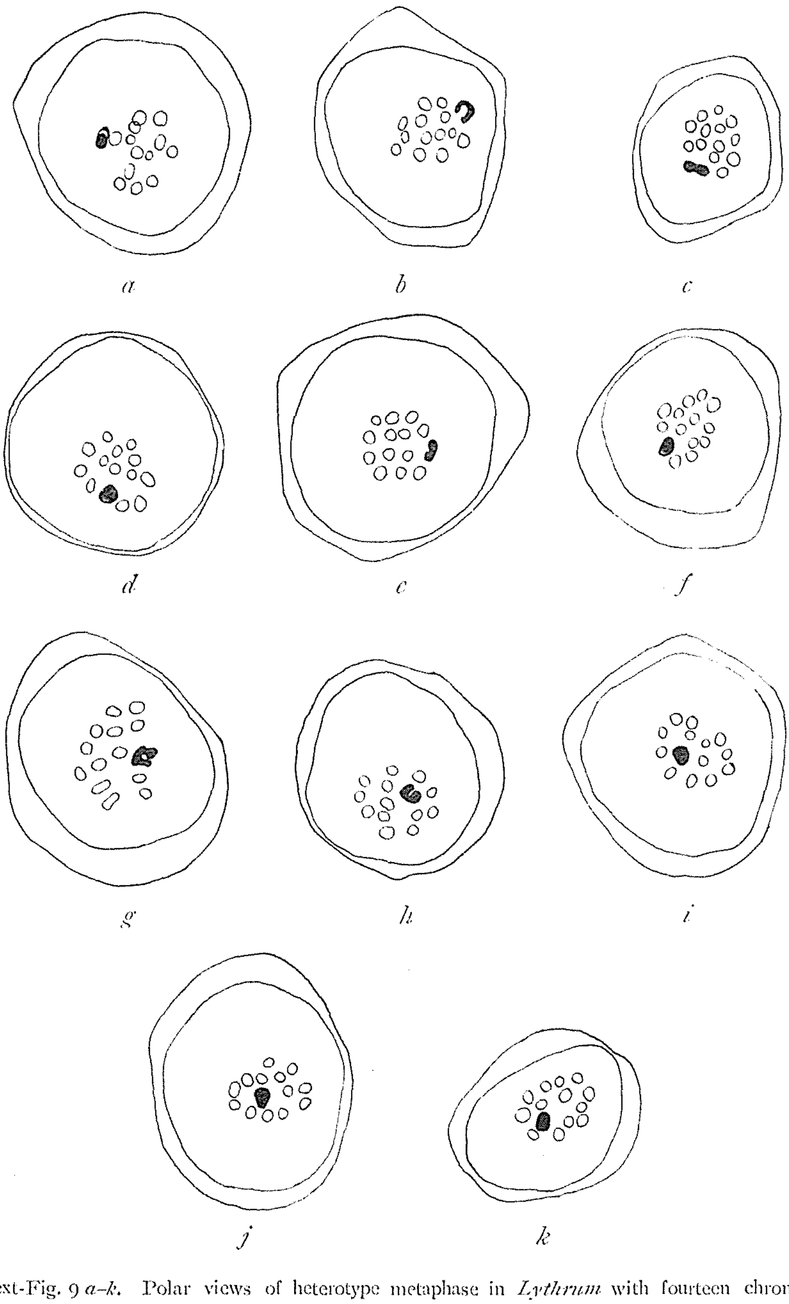
In this case, one element is the tetrapartite complex. The numbers of cases of different forms of chromosome arrangement observed are given in Table VIII. In Case A of the table, those cases where all the chromosomes are arranged strictly in the equatorial plate are grouped together, and in Case B, those where some chromosomes are found

TABLE VIII.

(Comp. Text-fig. 9)

Case	Style	Stage	Form of arrangement											Total
			8	8–7	7	7–6	6	6–5	5	5–4	4	4–3	3	
A	Middle	Heterotp. Metaph.	0	0	0	2(*a*)	0	1(*b*)	7	*2(*h*) 3(*d*)	*2(*i*) 11(*e*)	3(*j*) 1(*f*)	*1(*k*) 5(*g*)	*8 30
	Long	Heterotp. Metaph.	0	0	0	0	0	0	2(*c*)	3	3	2	0	10
	Total		0	0	0	2	0	1	9	8	16	6	6	48
	Percentage		0	0	0	4.2	0	2.1	18.7	16.6	33.3	12.5	12.5	99.9
B	Middle	Heterotp. Early Metaph.	0	0	0	0	0	0	4	4	*1 3	0	*1 1	*2 12

* Figures with an asterisk denote the number of cases where the tetrapartite chromosome is found inside the chromosome ring of arrangement. In others, the large chromosome occupies a position on the circumference of the ring.



Text-Fig. 9 *a–k*. Polar views of heterotype metaphase in *Lythrum* with fourteen chromosomal elements, one of which is the tetrapartite chromosome (solid black). Comp. Table VIII. $\times 1800$

slightly above or below the equatorial plate (comp. Text-fig. 9.) In Case A, we see that cases which belong to Form 4 are the most numerous. This form has an arrangement that resembles the stable arrangement form of floating magnets.

Besides Form 4, Form 5 has a relatively high frequency of occurrence as compared with other forms which likewise do not resemble the stable form of arrangement of floating magnets, and the frequency curve obtainable from these results is nearly the same as that from the case of 15 chromosomes, although the number of cases examined is here far smaller than in the latter case.

Now we shall consider the behaviour of the tetrapartite complex in the arrangement. It generally occupies a position on the circumference of the chromosome ring of arrangement, but is sometimes found inside the ring in a frequency such as is shown in Table VIII. Although the latter case may be partly due to the action of the fixing fluids, we become furnished with a more accurate knowledge about the origin of these figures, when we consider them from the mathematical point of view. If we assume that any three, four, or five elements of a total of fourteen can go inside the ring of arrangement quite at random, we shall have as many different configurations as ${}_{14}C_3=364$, ${}_{14}C_4=1001$ or ${}_{14}C_5=2002$ respectively. In these three cases, the numbers of cases where a definite element can occupy the inner position are ${}_{13}C_2=78$, ${}_{13}C_3=286$, and ${}_{13}C_4=715$ respectively. Therefore this definite element has as many chances to occupy the inner position as $78/364(21\%)$, $286/1001(29\%)$ or $715/2002(36\%)$ respectively. The results given in Table VIII are too meagre to compare with those calculated results, but on the ground of the latter results they seem to allow us to infer that the tetrapartite element takes the central position merely by chance just as might be expected for the other thirteen small elements.

CONCLUSION AND SUMMARY.

From the data presented above we learn the following :—

1) In the heterotype metaphase of *Sagittaria*, in spite of the various sizes and shapes of the chromosomes, the cases where the chromosome arrangement resembles the stables form of arrangement of floating magnets are the most numerous, being 53.8% in frequency of occurrence. The small *a*-chromosome takes the central position more frequently and the large *M*-chromosome less frequently than we might expect from the view point of probability. Between the heterotype metaphase and the homotype anaphase, there is a certain difference in the form of the arrangement.

2) In *Lythrum*, in both cases where the number of chromosomal elements is 15 and 14, the form of chromosome arrangement resembling the arrangement of the floating magnets occurs most frequently, but there is, contrary to the case of *Sagittaria*, among the other forms of arrangement which do not resemble that of the floating magnets, another maximum in frequency which marks itself distinctly from the frequencies of the other forms of arrangement. This second maximum is found in the case of 15 elements in that arrangement which affords the first maximum in the case of 14 elements, and in the latter case it is found in the arrangement that gives the first maximum in the case of 15 elements.

When there exists a tetrapartite chromosome among the others, we find usually this large element in a peripheral position, and there seems to be no special tendency for the quadrivalent element to occupy the central position, at least so far as the present observations are concerned.

In conclusion, the writer wishes to express his cordial thanks to Prof. Y. KUWADA under whose directions the present investigations were carried out.

LITERATURE CITED.

DIGBY, L. (1912): The cytology of *Primula Kewensis* and of other related *Primula* hybrids. Ann. of Bot. Vol. 26.

HEILBORN, O. (1924): Chromosome numbers and dimensions, species formation and phylogeny in the genus CAREX. Hereditas. Bd. 5.

KIHARA, H. (1927): Ueber das Verhalten der "end-to-end" gebundenen Chromosomen von *Rumex acetosella* und *Oenothera biennis* während der heterotypischen Kernteilung. Jahrb. für wiss. Bot. Bd. 66.

NAWA, N. (1928): Some Cytological Observations in *Tricyrtis*, *Sagittaria* and *Lilium*. Bot. Mag. Tokyo. Vol. 42.

SINOTO, Y. (1927): Mikrosporogenesis in *Oenothera sinuata*, L. Bot. Mag. Tokyo. Vol. 41.

STEVENS, N. E. (1912): Observations on heterostylous plants. Bot. Gaz. Vol. 53.

TAYLOR, W. R. (1924): Cytological studies on *Gasteria*. I. Chromosome shape and individuality. Amer. Journ. of Bot. Vol. 11.

——(1925): Chromosome constrictions as distinguishing characteristics in plants. Amer. Journ. of Bot. Vol. 12.

TISCHLER, G. (1918): Untersuchungen ueber den anatomischen Bau der Staub- und Fruchtblätter bei *Lythrum salicaria* mit Beziehung auf das "Illegitimitätsproblem." Flora. Bd. 111.

EXPLANATION OF PLATE XXVIII.

All figures were drawn with the aid of an Abbe's camera lucida using ZEISS' apochr. imm. 2mm. (Figs. 1 to 6) and achrom. homog. imm. 1/12 (Figs. 7 to 17) with comp. oc. 18.

M-, *a*- and tetrapartite chromosomes, when present, are marked in the figures with letters *M*, *a* and *T* respectively. Figs. 1–6 represent *Sagittaria Aginashi*, MAKINO and Figs. 7–17, *Lythrum salicaria*, L. var. *vulgare*, DC., subvar. *genuina*, KÖHNE.

Fig. 1. Heterotype metaphase in polar view.

Fig. 2. Heterotype metaphase in side view.

Fig. 3. Heterotype anaphase in polar view.

Fig. 4. The same stage, where the *M*-chromosome is completely divided into two longitudinal halves.

Fig. 5. The same stage, showing the univalent components of gemini separating not simultaneously.

Fig. 6. Homotype anaphase in polar view with elongated chromosomes, indicating the spiral structure.

Fig. 7. Various forms of the tetrapartite chromosome in the diakinesis.

Fig. 8. The same with fifteen chromosomes.

Fig. 9. Slightly later stage than Fig. 8. Fourteen chromosomal elements with the tetrapartite chromosome.

Fig. 10. Disappearance of the nuclear menbrane. Chromosomes are distributed rather loosely.

Fig. 11. Later stage than Fig. 10. Chromosome distribution area is contracted.

Fig. 12. Tripolar stage with the tetrapartite complex.

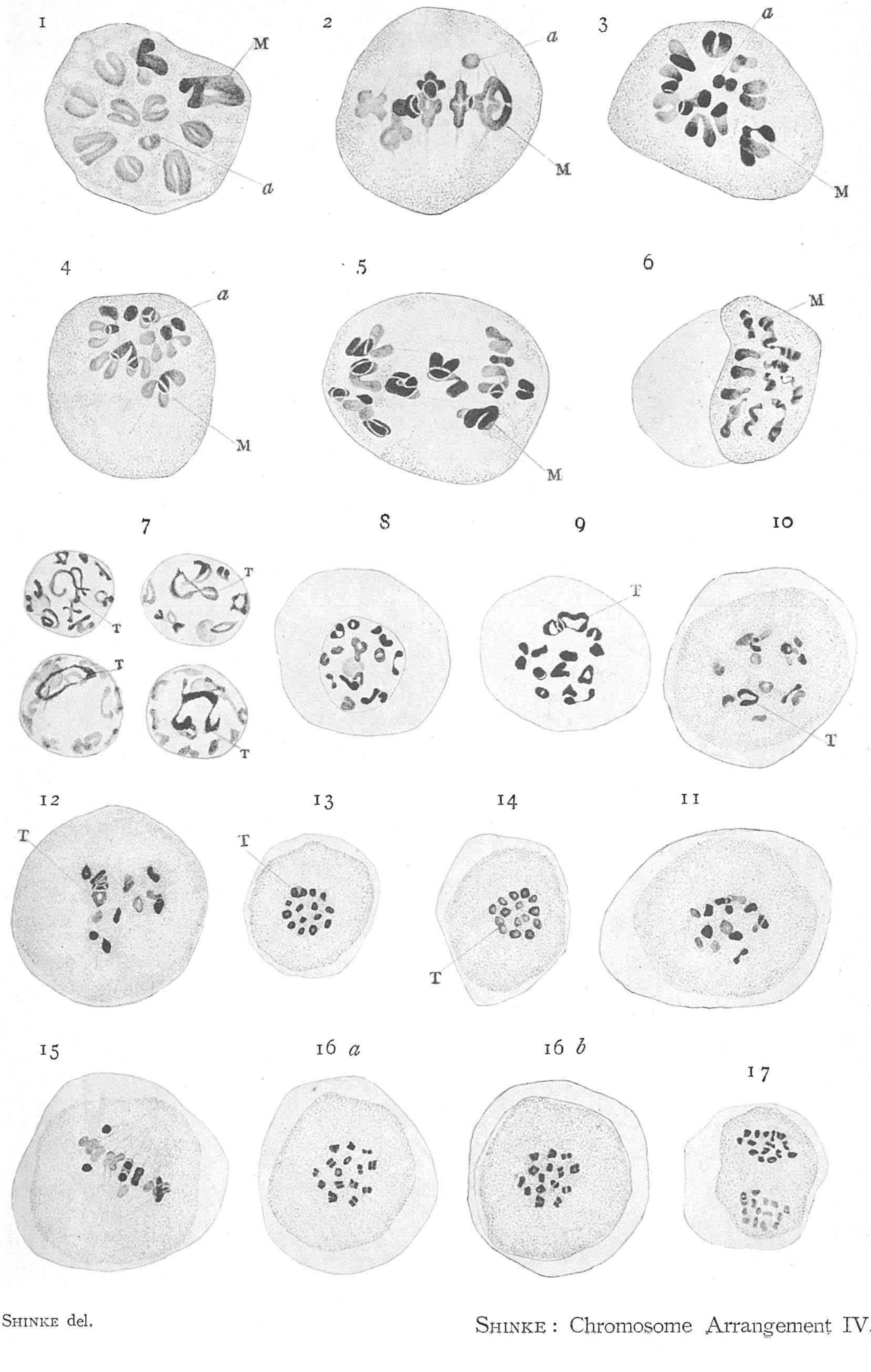
Fig. 13. Heterotype metaphase in polar view with fourteen chromosomal elements. The tetrapartite complex presents its typical shape of ring.

Fig. 14. The same stage with the tetrapartite chromosome of intermediate form.

Fig. 15. Heterotype metaphase in side view.

Fig. 16. Heterotype anaphase in polar view. *a* and *b* present the sister chromosome groups both with fifteen chromosomes. The longitudinal split is visible in all the chromosomes.

Fig. 17. Homotype metaphase in polar view. Fifteen chromosomes in both groups.



SHINKE del.

SHINKE: Chromosome Arrangement IV.