

## Chromosome Arrangement.<sup>1)</sup>

### I. Model Experiments with Floating Magnets and Some Theoretical Considerations on the Problem.

By

**YOSHINARI KUWADA.**

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*With 18 Text-figures.*

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<sup>1)</sup>A brief discussion of the problem has been made in Japanese under the title "A comparison of the chromosome arrangements with those of floating magnets." "Tōyō Gakugei Zasshi" Vol. 44. 1928.

### INTRODUCTORY.

If there is a certain law governing the various arrangements of chromosomes, this law will suggest some idea as to the mechanism by which these arrangements are brought about.

A comparison of the chromosome arrangement with that of MAYER'S floating magnets has been attempted by several investigators. R. S. LILLIE (1905) is perhaps the first who made experiments with the floating magnets with the view of comparing them with the chromosomes. DONCASTER (1920) points out the fact that especially when the chromosomes are short and of nearly uniform size, the figures of equatorial plates have a remarkable resemblance to those given by physicists of the groupings of various numbers of magnets floating in a confined space, and that this fact may have some bearing on the theories concerning the mechanism of nuclear division (p. 36). In view of this phenomenon capable of supporting LAMB'S theory of the mechanism of mitosis, CANNON (1923) gives in his paper various figures of chromosome arrangements which resemble those of corresponding numbers of floating magnets. While some of figures of the chromosome arrangements show such a resemblance to the groupings of the floating magnets as shown in CANNON'S Fig. 2, we often meet, on the other hand, with cases where the arrangement of the chromosomes differs very much from those of the corresponding numbers of floating magnets. This latter fact demands a closer investigation as to whether the resemblance between the arrangement of chromosomes and that of the floating magnets is only an accidental phenomenon, or whether it has actually some bearing upon the mechanism of mitosis as suggested by the earlier authors. To make clear this point of inquiry, some model experiments were attempted with floating magnets on the one hand, and statistical investigations into the chromosome arrangements on the other hand. The latter section of investigation was carried out mainly by the author's collaborators. The results so far obtained will be found in subsequent papers on the same subject published together

with this paper, each being written by the collaborator in charge of his own branch. Further investigations are now being under taken.

## MODEL EXPERIMENTS AND THE CHROMOSOME ARRANGEMENTS.

### *1. Arrangement of Experiments.*

For the general method of the arrangement of the experiments the writer is indebted to Dr. KIMURA, Professor of Physics in our University, to whom the writer wishes to take this opportunity of expressing his sincere thanks for his kind suggestions. In these experiments we have, of course, not attempted to repeat the physicists' experiments, but only intend to obtain from the knowledge acquired in our own experiments some idea about the origin of the various configurations which we have found in the case of chromosome arrangement.

As the vessel containing water as a floating medium, a wooden jar of about 25 cm in diameter for relatively small numbers of floating magnets, and of about 32 cm for larger numbers, was used. Five and seven turns respectively of a conducting wire were made around the vessels and both ends were connected with terminals of the 110 volt direct current circuit, and electric lamps were inserted in the circuit as resistance, so as to have a current intensity of 3-5 amperes passed. As floating magnets, magnetized gramophone needles stuck in small pieces of cork were used. They were floated on the water surface of the vessel, each having the magnetic pole of the same sign at the same end, upper or lower, and the current was passed so as to make the upper and lower electromagnetic poles be opposite in sign to the upper and lower poles respectively of the floating magnets.

### *2. Some General Aspects of Chromosome Arrangement, especially in Cases where All the Chromosomes in a Group are of nearly the Same Size and Shape.*

*A. The arrangement configuration of floating magnets. Accord-*

ing to MAYER (1879), when the number of floating magnets is 3, the magnets are distributed in the stable form in a triangle; when 4, a square<sup>1)</sup>; and when 6, a pentagon. When the number is 6 or more than 6, one or more of the magnets is distributed in a definite position or order inside the supposed ring, on the circumference of which the rest of the magnets are distributed in a polygonal shape. When the number

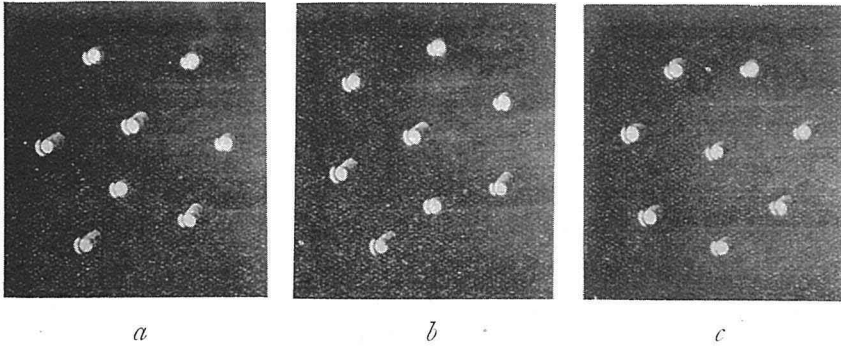


Fig. 1 *a-c.* *a.* An unstable arrangement form of eight floating magnets with two inner magnets. *b.* A form in transition to the stable form, *c.* with one inner magnet.

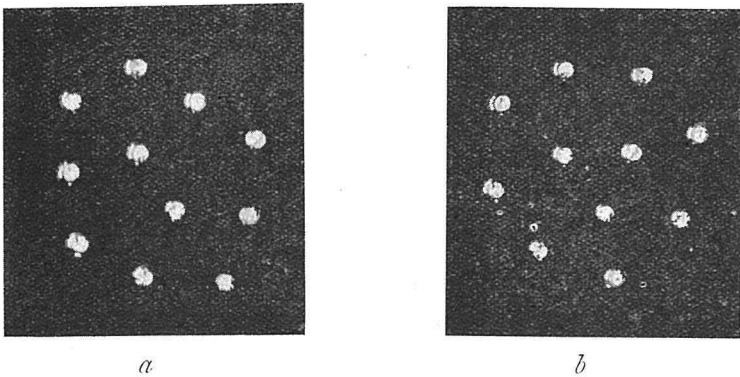


Fig. 2 *a-b.* *a.* The same of eleven floating magnets with two inner magnets. *b.* Stable form with three inner magnets.

of the magnets is from 6 to 8, the number of these magnets is one;

<sup>1)</sup>We find in MELLOR's book (1923) another form of arrangement, in which three of the four make the angles of a triangle, while the remaining one occupies the central position.

when 9<sup>1)</sup>, it is two; and when 10<sup>2)</sup> and 11, there are 3 inside. For cases of greater numbers of floating magnets we refer to MAYER's original paper (1879) or to MIZUNO's book, "Theory of the Atom," Part II (1916).

In our experiments, at the commencement of the experiments a definite number of floating magnets consisting of pieces of cork each with one magnetized needle stuck in it were arranged near the periphery of the vessel roughly in the form of a ring, and then the circuit was closed. When the current is passed, the floating magnets begin to move towards the center of the ring taking irregular shapes which change every second until they come to a stable state of arrangement. In this state of the arrangement the floating magnets take up definite positions forming figures just the same as those given by the physicists. When the number of floating magnets is relatively large, the number of the magnets found inside the ring may be greater or less before the final distribution is attained than the number found in the final stable form of arrangement. When it is greater, some of the magnets occupying inner positions are driven out to take a position on the circumference (Fig. 1), and when less, some of the magnets on the circumference are driven inside the ring (Fig. 2). The movement of these magnets is very slow, and it takes much more time to reach the final state of distribution than in cases where a definite number of floating magnets corresponding to the number found inside the ring of the stable form has occupied the inner positions already before the others are more or less regularly distributed. If the distribution of chromosomes on the equatorial plate is comparable with that of the floating magnets, there must be also such cases in the cases

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<sup>1)</sup>According to CANNON 8 floating magnets make a ring having one inside it. In the cases of 12 and 13 floating magnets, there are found 3 inside according to CANNON, and 4 according to MAYER and MIZUNO. In the writer's experiments, in the cases of 9 and 13 the number of floating magnets distributed inside the ring was the same as the number given in MAYER's figures or MIZUNO's photographs and in the case of 12, it was the same as that mentioned by CANNON.

<sup>2)</sup>According to MIZUNO and CANNON there are two inside the ring. According to MAYER this form is less stable.

of the chromosome distribution as we meet with in the case of floating magnets.

*B. The chromosome arrangements.* In the cell the movement of chromosomes must be far slower than that of the floating magnets. Moreover, there must be in the case of chromosomes some complications which are connected with the original positions of the chromosomes in the nucleus, all not being kept afloat on one plane as in the case of the floating magnets, but being distributed in a spherical area and capable of moving in all the three dimensions as pointed out by LILLIE (1905). Thus the natural conclusion is that in microtome sections we have cells fixed in various stages of chromosome movement from the very commencement up to the final distribution. According to KARLING (1928), in the nuclear division in the antheridial filaments of the Characeae, "the early and late telophases are the most numerous and spireme stages, resting nuclei, equatorial plate, and dispireme stages come next in frequency in the order named." This fact shows that the stage of the equatorial plate is of relatively long duration, and that there is needed a considerable time before the final distribution of the chromosomes is attained. The fact that we find various kind of irregular arrangements of chromosomes can, therefore, not be a proof for an argument that the chromosome arrangements are not comparable with the regular distribution configurations of the floating magnets.

From what has been stated above, it is clear that we have to choose for the comparison with the arrangement of the floating magnets only those chromosome groups in which the chromosomes are arranged all on one plane. The statistical results we have obtained in two cases where the chromosomes have all been distributed in one plane and where some of them are found still below or above the plane show that this is really true. They are summarized in Table I, the two cases being designated A and B respectively.

The details peculiar to each plant named in the table will be found in the paper written by the respective author himself, but some remarks may be made here. In *Vitis* the different types of chromosome arrange-

TABLE I.

Frequency values of the forms of chromosome arrangement resembling the stable forms of floating magnets.

Observer	Plant	Case A	Case B
HIRAYANAGI (C.A.III)	<i>Vitis</i>	66.66%	3.64%
MUTO (C.A.II)	<i>Phaseolus</i>	63.2%	52.6%
"	<i>Cassia</i>	58.8%	56.5%
SHINKE (C.A.IV)	<i>Iythium</i> (case of 15 elements)	35.5%	28.9%

C.A. = "Chromosome Arrangement."

ments are less in number in case A than in B. This is a very interesting and noteworthy fact from such a view point as that given above concerning the origin of the irregular arrangements. It must, however, be pointed out that in the majority of cases where the chromosome arrangement does not resemble the stable form of floating magnets, the author's classification into these different types seems largely to depend on the judgement of the figures based on his subjective view. The arrangements in these figures are very irregular at the first glance, and it seems to be rather rational to conclude that they cannot be classified into different types having such regular configurations as shown by his schematic representations. In these cases there is perhaps no regular configuration. The regular configurations would be found only in the arrangement which resembles the stable form of floating magnets or its less stable modifications, as is really seen from the author's Table II (HIRAYANAGI, "Chromosome Arrangement," III).

In 1927, the writer of the present paper himself observed in aceto-carmine preparations of fresh pollen mother cells in *Cycas revoluta* that at least in a large number of cases the chromosome arrangement resembles the case of the floating magnets, when the chromosomes are arranged all on one plane. Figs. 3*a*, *b*, *c* show camera drawings and

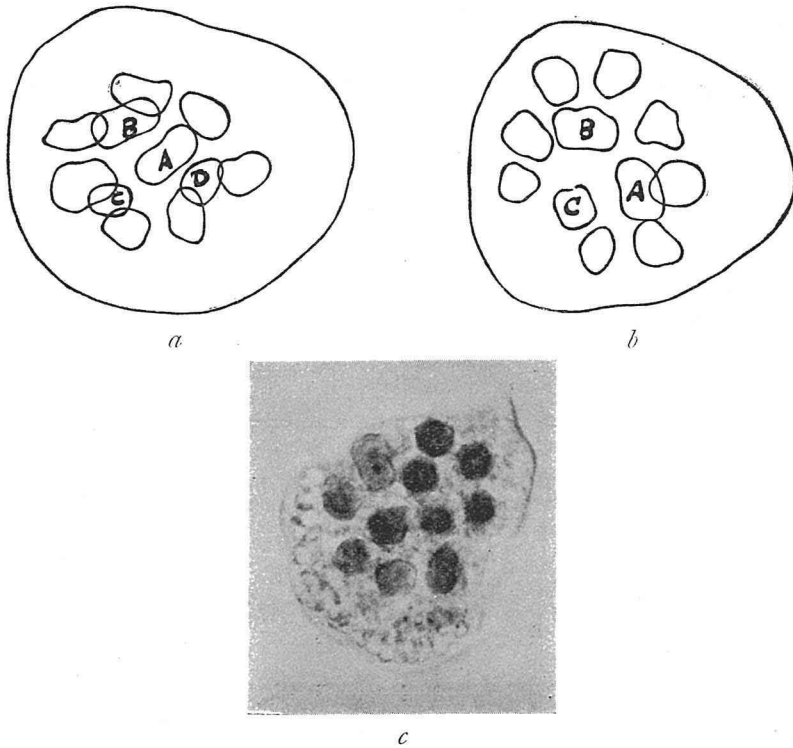


Fig. 3 *a-b*. Three nuclear plates in heterotype division in pollen mother cells of *Cycas revoluta* from aceto-carmin preparations. *a* and *b* are camera drawings and *c* is a micro-photograph.

a micro-photograph from different pollen mother cells. Figs. 3*a* and 3*b* show stages in transition to the final distribution.<sup>1)</sup> It may be inferred from the knowledge acquired in the model experiments with floating magnets, that chromosomes A and B, and either C or D in Fig. 3*a* will occupy the inner positions, the remaining one, D or C, being expelled to help to form a ring with the rest of the chromosomes. In Fig. 3*b*, chromosome C will occupy the inner position with A and B, both of which are found already quite inside the chromosome ring. Fig. 3*c* shows the final distribution figure, in which all the 11 chromosomes are

<sup>1)</sup> The predominant number of gemini in pollen mother cells in *Cycas revoluta* is 12, while in the female gametophyte (endosperm) the number of chromosome is 12, as expected from ISHIKAWA's result. For closer description, see p. 222, and also "Chromosome Arrangement" IX.



arranged on one plane as is shown to be the case by the fact that they are all photographed at one focussing.

In microtome sections we found later that there were a large number of cases where two chromosomes are found inside the chromosome ring instead of three. In *Cycas revoluta* there are marked size difference among chromosomes. It seemed to us that a certain deviation in arrangement might be possible owing to this, and a statistical investigation with microtome sections was undertaken by Mr. NAKAMURA at the writer's request ("Chromosome Arrangement" IX). The results obtained show that cases where two chromosomes occupy the inner positions are more numerous than cases where there are found three chromosome inside, although the latter may also occur quite frequently. This case of *Cycas* will be discussed in the next chapter.

TABLE II.

Cases where all the chromosomes are of nearly the same size and shape.

Observer	Plant	Stage	Frequency expressed in per cent.	Total number of cases observed
HIRAYANAGI (C. A. III)	<i>Vitis</i>	Heterotype metaphase	66.66	63
MUTO (C. A. II)	<i>Phaseolus</i>	"	63.2	144
"	<i>Cassia</i>	"	58.8	80
OJAWA (C. A. V)	<i>Torilis</i>	"	51.6	124
"	"	Heterotype anaphase	55.0	40
"	"	Homotype metaphase	75.5	233
"	"	Homotype anaphase	90.9	22
"	<i>Peucedanum</i>	Heterotype metaphase	67.3	171
"	"	Homotype* metaphase	40.1	247
NAGAO (C. A. VIII)	Triploid <i>Narcissus</i> (trivalent)	Heterotype metaphase	65.3	147

\* This makes an exception, having another form of such a high frequency as 36.9%. The discussion will be seen later.

In cases where all the chromosomes are of nearly the same size and massive in shape, and all of the chromosomes are arranged in one plane, the frequency curve of the various forms is generally leptokurtic in nature. The same is also true in the case of trivalent chromosomes. The maximum values so far obtained are summarized in Table II. They are the frequency values of the forms of chromosome arrangement resembling the stable forms of floating magnets.

According to MAYER (1879) besides the stable configurations mentioned above there are some modifications which are less stable than the former. They may be transformed by a slight shock from one form into another and more stable form (GANOT, 1898). For instance, in the case of 6 floating magnets they may be so arranged that all take the peripheral positions, the form of arrangement being roughly a ring having none of the magnets inside it. In the case of 8 magnets, we have two modifications, in both of which two magnets occupy the central positions instead of one, and in the case of 10, two instead of three. In the case of chromosome arrangements the floating medium, or the cytoplasm, is of course more viscous than that in the case of the floating magnets (cf. HEILBRONN, 1914), and hence these less stable forms of arrangement must be less easily transformable. From this state of affairs we may expect that more than one arrangement of chromosomes may be obtained even in the case where all the chromosomes are found on one plane. But we should expect at the same time also that the cases resembling the stable form of floating magnets must be more numerous than those resembling the less stable modifications of the form. This is beautifully shown by OGAWA in *Torilis Anthriscus* and by MAEDA and KATO in *Spinacia oleracea* actually to be the case. The numerical results obtained by them are given in Table III (cf. also their own papers, "Chromosome Arrangement." V and VII).

TABLE III.

Observer	Plant	Stage	Cases resembling stable form of Fl. Mg.	Cases resembling less stable form of Fl. Mg.	More or less irr. form of arrangt.	Total no. of cases observed
MAEDA and KATO (C.A.VII)	<i>Spinacia oleracea</i>	Hetero. metaph.	107 (Cases I+II)	19 (Case III)	8	134
		Hetero. anaph.	76 (Cases I+II)	25 (Case III)	12	113
		Homo. metaph.	98 (Cases I+II)	41 (Case III)	17	156
Total			281	85	37	403
OGAWA (C.A.V)	<i>Torilis</i> <i>Anthriscus</i>	Hetero. metaph.	64 (Form A)	39 (Forms B+C)	21	124
		Hetero. anaph.	22 (Form A)	11 (Forms B+C)	7	40
		Homo. metaph.	176 (Form A)	19 (Forms B+C)	17 21*	233
		Homo. anaph.	20 (Form A)	2 (Forms B+C)	0	22
Total			282	71	66	419

\* The number marked with an asterisk is the number of those cases where the eight chromosomes are arranged in the form of a ring having none of the chromosomes inside it (Form D).

In case the investigation is carried out with fixed materials, it must be borne in mind also that the fixative causes shrinkage of the protoplasmic contents, so that the original position of each chromosome may be altered to a greater or less extent. It is highly probable that in the cases denoted in Table III as "irregular" there are at least some which really resemble the stable or less stable form of floating magnets, but have been rearranged into irregular forms by the action of the fixing agents.

As has been seen in Table II, if the chromosomes are arranged all in one plane, and if there is no special obstacle, the cases where the chromosome arrangements show a marked resemblance to the stable

forms of floating magnets are the most numerous, generally being more than 50% of all the cases observed. The resemblance is more marked when the chromosome number is relatively small. This is very beautifully illustrated by OGAWA in *Torilis* and by MAEDA and KATO in *Spinacia*. In *Torilis*, the cases where the chromosome arrangement resembles neither the stable form of floating magnets nor its modifications (less stable forms) are 66 in number out of 419 cases observed, and in *Spinacia* they are only 37 out of 403. If in these cases displacement of chromosomes due to fixation is taken into consideration, the number of cases where the resemblance to the forms of floating magnets is not to be seen can be estimated to be less than the numbers given above, and conversely the number of cases resembling the forms of floating magnets must be greater in reality than the numbers we obtained. In

TABLE IV.

Frequency of the form resembling the stable form of floating magnets expressed in per cent.

Stage	<i>Torilis Anthriscus</i> (OGAWA, C.A.V)	<i>Spinacia oleracea</i> (MAEDA and KATO, C.A.VII)	Total number of cases obsd.
Heterotype metaphase	51.6	79.9	124 134
anaphase	55.0	67.3	40 113
Homotype metaphase	75.5		233
anaphase	90.9		22

the case of *Torilis* we have 39 cases of irregular arrangements which may be regarded as due to fixation (*vide* OGAWA, "Chromosome Arrangement," V, Table 3), and thus we may say that in this plant the forms of chromosome arrangement which show no resemblance to the arrangement forms of floating magnets are only 28 in number out of 419 cases observed.

If the chromosomes are comparable with the floating magnets, the arrangements we meet with in the former resembling those of the latter

seem to be higher in frequency in the anaphase than in the nuclear plate. But the results obtained by OGAWA and by MAEDA and KATO show that this is not the case, although in *Torilis Anthriscus* studied by OGAWA there may be recognizable a certain tendency towards higher frequency in the anaphase. But in this case the number of cases observed in the anaphase is too small for any definite conclusion to be drawn from them (cf. Table IV). This fact shows that in the cell the chromosomes may disjoin or separate before they attain their most stable position in the equatorial plane, their movement being hindered by the viscous nature of the cytoplasm through which they move, and that the rearrangement does not proceed further in the anaphase.

3. *Chromosome Arrangements in the Cases where the Chromosomes are not of the Same Size and Shape.*

A. *Model experiments with floating magnets.* We have now other cases which must be considered here. They are cases where some of the chromosome present a marked difference in size to the other chromosomes in the same group. To compare with these cases, floating magnets with different numbers of magnetized needles stuck in correspondingly large pieces of cork were taken for the experiments. This idea originated in the suggestion of Dr. KIMURA, Professor of Physics. In these experiments it was found that the final distribution of the floating magnets may not be of a regular polygonal shape, but of an irregular or malformed one, its shape being determined by the number of the magnets that consist of a different number of magnetized needles from the others (cf. Figs. 4 *a*, *b*, and *c*), and that the large floating magnets or those having more needles showed a stronger tendency to occupy the central positions than the small ones, or those having a smaller number of needles (cf. Figs. 5 *a*, and *b*). Some of the latter may occupy the positions inside the ring too, if they find themselves by chance inside a not definitely formed polygon at the beginning of the experiment. Such a chance for the small floating magnets is greater when the total number

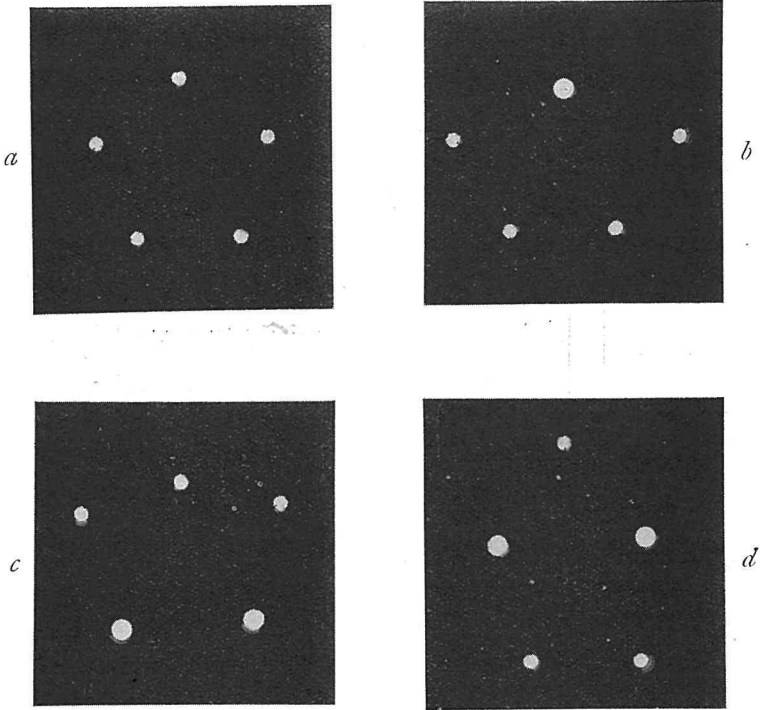


Fig. 4 *a-d*. Stable form of arrangement of five floating magnets. In *a* every magnet consists of a small piece of cork with one magnetized needle; in *b* the large cork carries three magnetized needles, the rest, one each; and in *c* and *d* there are two of these large magnets carrying three needles.

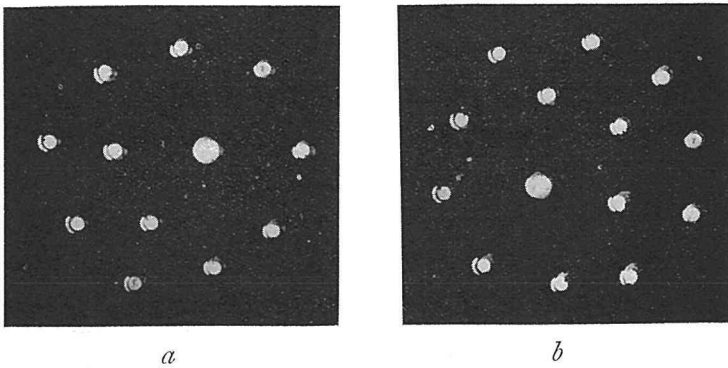


Fig. 5 *a-b*. *a*. A stable configuration of 12 floating magnets. The large magnet carries nine magnetized needles and the small ones each one needle. *b*. The same of 14 floating magnets. The large magnet has nine needles and the other small ones each one needle.

of the floating magnets is large than when it is small<sup>1)</sup>. When these are not found inside the polygon, however, the floating magnets with more needles generally enter in and occupy the final positions inside the polygon. In the case of chromosomes, various figures drawn by various authors from plant as well as animal cells generally show that small ones have a very strong tendency to occupy the inner or central positions in direct opposition to the case of the floating magnets. This contradiction appears to mar at the first glance the conformity of the cases of the floating magnets and the chromosomes. How can this disconformity be explained? If really the small chromosomes have a greater tendency, and the larger chromosomes a smaller, to occupy the central positions, the following facts seem to furnish us with an idea of what the explanation may be.

a) The viscous nature of the cytoplasm as compared with water which is used as the floating medium in the case of the model experiments with the floating magnets. A higher viscosity will cause a difference in the migration velocity of chromosomes according to their size, if the strength of the attracting force is uniform for all the chromosomes, or if there is at least no marked difference in the force exerted on them. Here we must naturally consider change in viscosity taking place in the dividing cells, which has been observed by CHAMBERS (1919), HEILBRUNN (1921) and others (WILSON, 1925, p. 197).

b) The long chromosomes radiate outwards, having the small round ones in the central region of the distribution figure, or at least show such a tendency, when they are straight and of a rod shape. When they are of V-shape, the apex of the V is found towards the center of the distribution figure. As examples, STRASBURGER's figure from *Galtonia* in the Bonn Text-Book of Botany (1911) and METZ's figures on p. 591 in his chromosome studies in the Diptera may be cited (METZ, 1916). According to METZ the long, V-shaped chromosomes attach to the spindle at the median portion (apex of the V), and rod-like,

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<sup>1)</sup> When the total number of floating magnets is large, the number of magnets occupying the central positions becomes larger than when it is small.

straight chromosomes attach to the spindle at one end and are radially arranged in the metaphase. MAEDA and KATO observed the same arrangement of chromosomes in pollen mother cells of *Vicia Faba*. The details of the observations will be found in the accompanying paper written by themselves ("Chromosome Arrangement" VII). At disjunction, component chromosomes of gemini or the longitudinal halves of chromosomes begin to separate towards the poles first at the point of spindle fiber attachment irrespective of whether it is terminal, or non-terminal.

The facts mentioned in (b) go to show that the chromosomes are pulled towards the center of the group at the point of the spindle fiber attachment. If we compare these chromosomes with the floating magnets, they may be regarded as those floating magnets in which the magnetized needles are stuck in at the point of the spindle fiber attachment. If we look upon the chromosomes in this way, the longer ones must have more resistance to the viscous cytoplasm in migrating through it than the small round ones, and hence may lag behind the latter and will be arranged radially having the small round ones at the center.

In such an explanation as that just put forward, we must suppose that there is one electrically charged center in the chromosomes and there may be not a very marked difference in the magnitude of the charge between chromosomes of different sizes. OSAWA's figures drawn from pollen mother cells in *Morus* seem to sustain this view, if, as is shown in those figures, one large round chromosome is always found in the peripheral ring of the chromosome arrangement (OSAWA, 1916).

To test the view stated above, the following experiments were made. Two kinds of floating magnets in size and weight were prepared. By a large floating magnet is meant here a relatively large piece of cork with one magnetized needle in its center and with four non-magnetized needles in its periphery with an approximately equal distance between each needle, magnetized or non-magnetized. The small floating magnet consisted of a smaller piece of cork carrying only one magnetized needle stuck in its center. The difference in size between these two kinds of corks was so arranged that needles of the two kinds of floating magnets,



large and small, should sink to the same depth in water, so that their corresponding ends might be found all at the same level.

In order to have the floating magnets arranged near the periphery of the jar form a ring as regular as possible, so that all the floating magnets might receive an equal chance of occupying the central positions, the following method of arrangement was adopted:—At first the floating magnets were arranged roughly in the form of a ring near the periphery of the jar and the current was passed. The floating magnets were drawn towards the center of the water-surface area to make the final distribution figure. Before they took up their final positions, the circuit was opened.

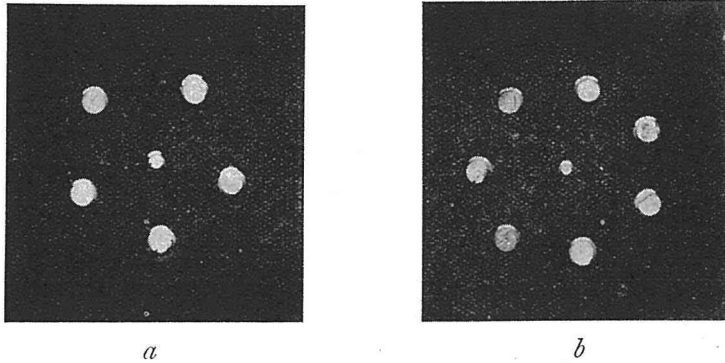


Fig. 6 *a-b*. *a*. Stable configuration of six floating magnets. The large magnets consist of one magnetized needle stuck in the center of a cork and four non-magnetized needles stuck in its periphery, the small magnet carries only one magnetized needle. *b*. The same. Seven large magnets and one small one.

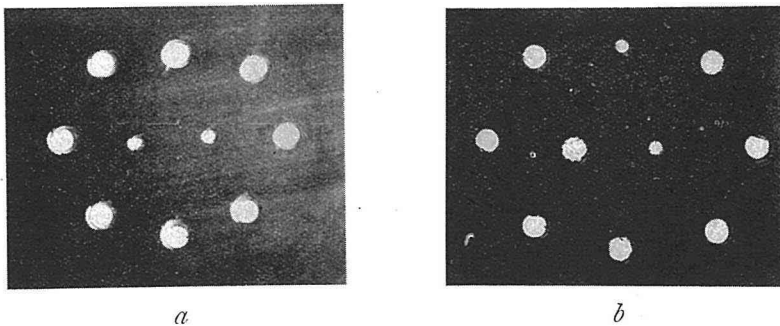


Fig. 7 *a-b*. Two different stable configurations of 10 floating magnets. Eight large and two small. These two kinds of magnets are prepared like those in Fig. 6.

The floating magnets were driven then back to the periphery of the jar by their mutual repelling force. When they were near the periphery, the circuit was again closed. These processes were repeated two or three times, and we could have the floating magnets arranged near the periphery of the jar nearly in the form of a regular circle or ring, or a polygon with a magnet at each angle of it.

Experiment 1. A set of floating magnets consisting of five large and one small and another set of seven large and one small were used for the experiments. In both these cases the small floating magnets always occupied the inner position, all the large ones forming a ring surrounding it (Figs. 6, *a* and *b*).

Experiment 2. In this experiment 8 large floating magnets and two small ones were taken. In this case the result was not so simple as in the former experiments. When the two small floating magnets are opposite to each other at the beginning of the experiments, they both always enter inside the ring, which is composed merely of the large floating magnets (Fig. 7, *a*). But as their relative position changes from this extreme to the other in which the one stands next the other, the chance for these small ones both to enter inside the ring becomes less and less, and at the other extreme it is zero; but even in this relative position one of the two magnets that find themselves inside the ring is always the small one (Fig. 7, *b*). When the circuit is closed with the small floating magnets in this relative position, they both are pulled at first towards the center of the water-surface area faster than the large ones, but when the repellent action of each magnet becomes effective, as they come nearer each other with the advance towards the center, one of the two which goes ahead of the other is pushed forwards by the mutual repellent force so as to occupy the central position while the latter is driven backwards to join the ring. While one of the small floating magnets is being pushed back, one of the large floating magnets on the side opposite to it enters the ring.

Experiment 3. To compare the distribution configuration of the floating magnets with that of *Morus* chromosomes, the following ex-

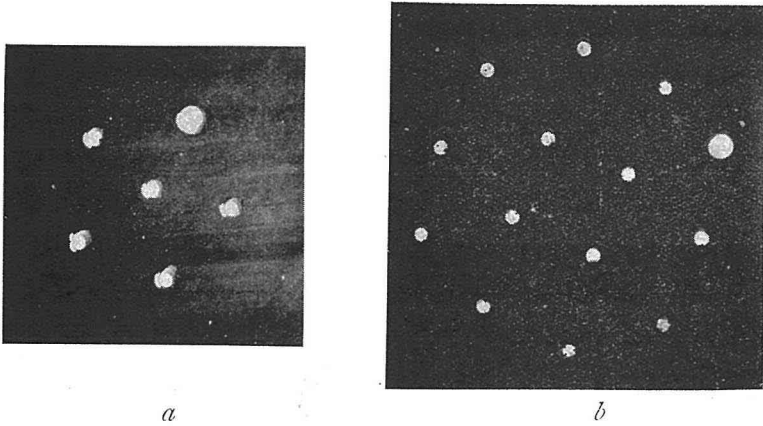


Fig. 8 *a-b*. Stable configurations of six (*a*) and 14 floating magnets (*b*). The large one carries one magnetized needle and four non-magnetized needles and the small ones each one magnetized needle only.

periments were made. Five small floating magnets and one large magnet in one case and thirteen small and one large in another were taken. The results were very simple. The large magnet always occupies a position on the circumference of the ring (Fig. 8, *a* and *b*), as in the case of *Morus* chromosomes.

The results of the experiments mentioned above largely show that the chromosome arrangements can be imitated by the floating magnets, but at the same time we feel that statistical investigations into the chromosome arrangements must precede any attempt to imitate them.

*B. The chromosome arrangements.* The model experiments just mentioned were carried out on the supposition that the magnitude of the electrical charge carried by chromosomes is nearly the same even in chromosomes of different sizes. But we not infrequently come across figures in which a large rod-shaped chromosome is found in the center of the chromosome group surrounded by smaller chromosomes (AGAR, 1920, Fig. 49, D and H; DONCASTER, 1920, Pl. XVII, Figs. 1 and 2, and Pl. XVIII, Fig. h), an arrangement which leads us to the conclusion that the magnitude of the charge carried by chromosomes may be at least proportional to their size. This probably shows that even

in the cases we have discussed above the magnitude of the charge is not the same in chromosomes of different sizes. The larger chromosomes may have a larger charge, though it may not be so large as to cause the chromosomes to be pulled towards the center of the equatorial plate faster than the smaller chromosomes. In fact we see in the model experiments too that in cases where some of the floating magnets slightly differ from the others in the number of magnetized needles, the tendency to take up inner positions is not so well marked in the floating magnets carrying the larger number of needles. The numerical results we obtained in the cases where the large chromosome took up the peripheral position and in those where it occupied the inner are summarized in Table Va, and those of the small chromosome in Table Vb. In these tables, besides the observed numbers, the numbers to be expected from the purely mathematical point of view on the supposition that each chromosome has an equal chance of occupying the central position are given.

TABLE Va.

Observer	Plant	Peripheral position		Inner position	
		Observed number	Expected number	Observed number	Expected number
MAEDA and KATO (C. H. VII)	<i>Spinacia</i>	90	89.17	17	17.83
"	<i>Vicia</i>	87	122.5	60	24.5
SHINKE (C. A. IV)	<i>Sagittaria</i>	149	113.4	7	42.5

TABLE Vb.

Observer	Plant	Stage	Periph. position		Inner position	
			Obs. number	Exp. number	Obs. number	Exp. number
SHINKE (C. A. VI)	<i>Sagittaria</i>	Heterotp. metaph.	23	113.4	133	42.5
NAKAMURA (C. A. IX)	<i>Cycas</i>	"	16	28.36	23	10.63

From the tables we see that the tendency of the large chromosome to occupy the central position is different in different cases. In *Spinacia* the chance of the large chromosome to occupy the central position is nearly equal to that of the small chromosomes. This means that the large chromosome has so large an electrical charge that it is strong enough to make the chromosome move through the cytoplasm with a velocity about equal to that of the small chromosomes, which have a lesser mechanical resistance to the cytoplasm than the large one, and hence that the large chromosome carries a larger electrical charge than the small chromosome. In the case of *Vicia*, this tendency of the large chromosome appears to be remarkably great, and in *Sagittaria* it seems to be small. For the small chromosomes the tendency is clearly shown in the case of *Sagittaria* to be very great.

TABLE VI.

Cases where all the chromosomes are not of uniform size and shape.

Observer	Plant	Stage	Frequency value expressed in %	Total number of cases observed
MAEDA and KATO (C. A. VII)	<i>Spinacia</i>	Heterotp. metaph.	79.9	134
		Heterotp. anaph.	67.3	113
		Homotp. metaph.	62.8	156
"	<i>Vicia</i>	Heterotp. metaph.	71.0	207
SHINKE (C. A. IV)	<i>Sagittaria</i>	Heterotp.* metaph.	42.1	76
		"	53.8	262
		Homotp. anaph.	6.6	61
NAKAMUKA (C. A. IX)	<i>Cycas</i>	Heterotp. metaph.	32.5	120

\* In this case the chromosomes are not arranged all in one plane.

In Table VI, frequency values of the form of chromosome arrangement resembling the corresponding stable configurations of floating magnets are shown. As is seen from the table, it is a very interesting fact that while in the cases of *Spinacia* and *Vicia* where the chromosomes are of nearly the same size and shape except the large chromosome, the frequency value of the arrangement form resembling that of the floating magnets is as high as in the case where all the chromosomes are of nearly the same size and shape, in the case of *Cycas* where the chromosome of the group make nearly a graded series in regard to size, it is markedly low, and moreover, does not make the first maximum, but the second (*vide* "Chromosome Arrangement" IX.) We have analogous cases in a plant having a tetrapartite chromosome (*Lythrum*) and in the homotype division of some other normal plants too (cf. *Sagittaria*, Table VI). They are summarized in Table VII.

TABLE VII.

Observer	Plant	Stage	1st. maximum	2nd. maximum	Total number of cases observed
OGAWA (C.A.V)	<i>Peucedanum japonicum</i>	Homotype metaphase.	40.1% (Form 3)	36.0% (Form 2)	247
SHINKE (C.A.IV)	<i>Sagittaria Aginashi</i>	Homotype anaphase	57.4% (Form 2)	23.0% (Form 1)	61
"	<i>Lythrum salicaria</i> Case of 15 bivalents	Hetero. & homo. meta.	35.5% (Form 5)	30.8% (Form 4)	107
"	Do. (Cases where some of chromosomes lye out of equatorial plate)	Do.	28.9% (Form 5)	26.7% (Form 4)	135
"	Case of 14 elements. (One of the elements is tetrapartite)	Heterotype metaphase.	33.3% (Form 4)	18.8% (Form 5)	48
NAKA-MURA (C.A.IX)	<i>Cycas revoluta</i>	Heterotype metaphase	44.2% (Form 2)	32.5% (Form 3)	120

"Full-faced Form" is the form which resembles the stable form of floating magnets.

While, in the majority of the cases we have observed, the frequency curve of different forms of chromosome arrangement is leptokurtic in nature, it has the second maximum in those cases given in Table VII. This second maximum is generally found in the form, the number of the inner chromosomes of which is less by one than the number found in the form resembling the stable form of floating magnets. To put it in other words, the maximum shows a tendency to be shifted towards the form having the smaller number of inner chromosomes. As an extreme case we have *Sagittaria*. In the homotype division of this plant Form 3, which resembles the stable form of floating magnets, no longer shows the maximum frequency, Form 2 presenting the first maximum and Form 1 the second. It is noteworthy in this connection that in *Sagittaria* there are marked differences in size among the chromosomes. While it is the general rule so far as the observations we have hitherto made are concerned, that in case the maximum frequency is not found in the form resembling the stable form of floating magnets, it is found in the form having the smaller number of inner chromosomes, the case of the 14 elements in *Lythrum* forms an exception to this rule. In this latter case, one of the elements is tetrapartite, and the second maximum is found in the form, the number of inner chromosomes of which is the same as that in the form resembling the stable form of floating magnets in the case of 15 elements or the case where the tetrapartite element has been disunited into two independent bivalents.

It is very interesting that when all the chromosomes of a group are of nearly the same size and shape, this characteristic feature is found only in the homotype division, and when there are marked differences in size among the chromosomes as in *Sagittaria*, it is generally very manifest. In this division we have another peculiarity in *Torilis Anthriscus*. While in the heterotype division no case belonging to Form D, i. e. the form in which all the chromosomes are arranged in the form of a ring having none of them inside it, has been found, in the homotype division this form has been observed as frequently as in 9% of all the cases observed. In *Spinacia* such a hollow ring arrangement of chromo-

somes is found also in the heterotype division, but is more frequent in the homotype division. These peculiarities in the homotype division go to show that there is in this division a certain disturbance in the chromosome arrangement. In the heterotype division no longitudinal division of chromosomes takes place, but simply disjunction of the two mating chromosomes, while in the homotype division it is the essential feature of the division. This latter fact may have a certain bearing upon the peculiarities of the chromosome arrangement in the homotype division. In this connection, statistical studies of chromosome arrangement in somatic cells are very desirable. These are now being undertaken.

If the longitudinal division of chromosomes is controlled by some electrical phenomena, and if they have some effect on the magnitude of the electrical charge carried by each chromosome, the peculiarities may be regarded as having a certain common origin in both the heterotype and the homotype division, because we have in the cases of the heterotype division showing these peculiarities, data which show that the magnitudes of the electrical charge carried by chromosomes are not all alike, but recognizably different in different chromosomes. It is in fact very difficult thoroughly to explain the mechanism by which the peculiarities are brought about, but we shall attempt some imitation of them by model experiments with floating magnets. In this attempt we feel that knowledge of the morphological characteristics of every chromosome is of primary importance. As the writer has been interested in the chromosomes of *Cycas revoluta* for some years and has a certain knowledge of their morphological features, the case of *Cycas* has been taken as an example in the following explanation,

a) Cytological data<sup>1)</sup>:—In the heterotype nuclear plates of pollen mother cells there are countable 11 double chromosomes or gemini in the majority of cases<sup>2)</sup>, of which two are of about the same size and shape, and easily distinguishable from the other chromosomes. These

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<sup>1)</sup> Cf. camera drawings reproduced in "Chromosome Arrangement" IX.

<sup>2)</sup> For a more detailed description of the chromosome number in pollen mother cells and in the other phases in the life cycle, see the same paper, "C. A." IX.



two chromosomes are designated here  $I_1$  and  $I_2$  respectively. They are both telomitic elements roughly speaking<sup>1)</sup> and composed of two rod-shaped univalents in contact along their long axis without twisting about each other. They are very large in size and lie horizontally in the equatorial plate, and generally do not yet present any sign of separation into the component univalents, even when this is recognizable in the other chromosomes at the proximal ends attached by the spindle fibers. Six of the remaining 9 are also telomitic, and the proximal ends have generally been separated earlier than in the case of  $I_1$  and  $I_2$ . The component univalents are pear-shaped, the proximal ends being drawn into a slender, pointed form. Some of these six elements are slightly smaller in size than  $I_1$  and  $I_2$ , and the others much smaller. While in  $I_1$  and  $I_2$  the upper component univalent goes to the upper pole, and the lower to the lower pole without twisting, in some of the pear-shaped chromosomes it has been sometimes clearly observed that the proximal end of the upper univalent is directed towards the lower pole and that of the lower towards the upper pole, thus making a twist. The remaining three are of massive form and at least one of them is clearly atelomitic. They are slightly different in size from one another, but all smaller than the other eight chromosomes described above. Generally speaking, there is a serial gradation in the size of the chromosomes, so that it is often hardly possible to distinguish one chromosome from the others, except  $I_1$  and  $I_2$ . These two chromosomes have the peculiarities in behaviour described above, and are generally easily distinguishable. A small chromosome is also distinguishable in the majority of cases, but it has no characteristic feature except its small size, so that we can not determine with certainty which of the small chromosomes described above it is.

b) Model experiments with floating magnets :—To imitate the *Cyrcas* chromosomes three kinds of floating magnets, two large ones each with 5 magnetized needles, four medium-sized ones each with 3 magnetized

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<sup>1)</sup> Strictly speaking, their spindle fiber attachment may be said to be subterminal.

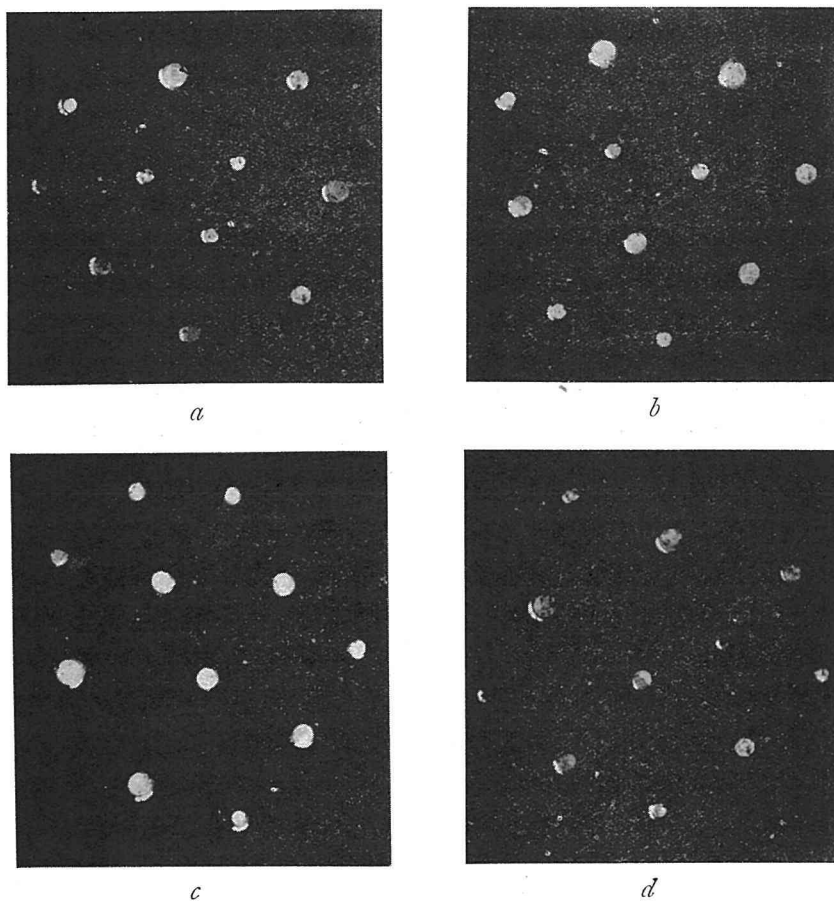


Fig. 9 *a-d*. Detailed explanation in the text. *a*. Three small magnets in the central region. *b*. One medium-sized magnet and two small ones in the central region. *c*. Two medium sized magnets in the central region. *d*. One medium-sized and one small magnet in the central region.

needles, and five small ones each with one magnetized needle, were prepared. In the practically stable configuration of these floating magnets we have sometimes three magnets in the inner region of the configuration, and sometimes two. Rarely we have one floating magnet in the central region, but in this case the whole configuration is very irregular. If all the inner three magnets or at least two of them are small ones, the configuration is practically the stable one, (Fig. 9, *a* and *b*) but if only

one of them is a small magnet, the other two being both large, or both medium-sized, or one large and the other medium, the configuration is unstable, and any of the central three, the small, the medium or the large, is driven out to the circumference of the peripheral ring of arrangement, thus transforming it to a stable configuration having two inner magnets. These inner magnets may present all the possible combinations of the different kinds of magnets, (Fig. 9, *c* and *d*), except the combination of two large ones alone. In this latter case the configuration is generally unstable and one of the large ones is apt to be replaced by a medium-sized or small one.<sup>1)</sup> If there is a set of 11 chromosomes, of which two are large, five small, and the remaining four of medium size, and if all the chromosomes have an equal chance of occupying the central positions in the arrangement,<sup>2)</sup> we shall have the following different combinations of the three central chromosomes and their frequencies (Table VIII).

TABLE VIII.

Combination*	Frequency
I. No small chromosome.	$(2+1)C_3 = 20$
II. All small chromosomes.	${}_5C_3 = 10$
III. Two small chromosomes, the third one large or medium.	$({}_3C_2)(2+1)C_1 = 60$
IV. One small chromosome, the other two being both medium, or one large and the other medium.	$({}_1C_1)(2+4)C_2 = 75$
Total	$(2+4+5)C_3 = 165$

\* Since the large and the medium floating magnets are the same so far as their behaviour in determining the number of the inner floating magnets is concerned, the difference in size between the large and the medium ones is disregarded in the table.

<sup>1)</sup> This is very interesting in view of the fact that microscopically no case has been found where both  $L_1$  and  $L_2$  occupy the inner positions in the arrangement having two inner chromosomes.

<sup>2)</sup> This assumption is possible, because in the nucleus just before the disappearance of its membrane the chromosomes may be distributed near the periphery of the nucleus without any regular order as regards the positions of particular chromosomes, so that any chromosome can

From the model experiments we have seen that in the case of combinations I and IV, the number of the central chromosomes is transformed into two in any combination of the chromosomes, large, medium or small, as the case may be. Thus we must expect in the final stable arrangement two different configurations, one with two inner chromosomes, and the other with three. The frequency values of these two configurations are readily obtained by adding the frequencies given in Table VIII for combinations I and IV on one hand, and II and III on the other.

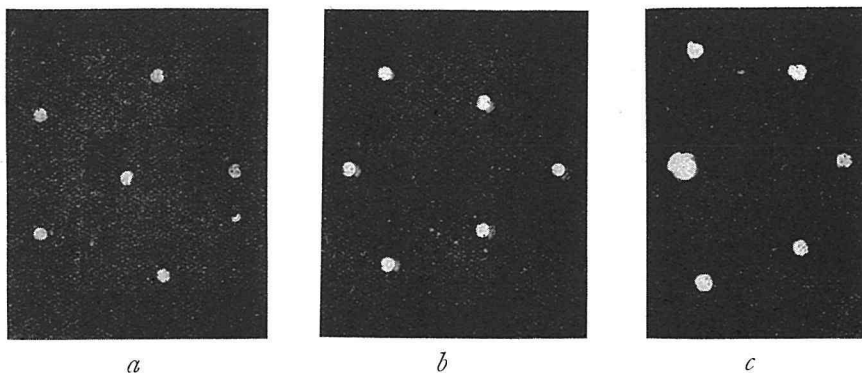


Fig. 10 *a-c*. More or less stable configurations of six floating magnets. *a*. Stable form. Every magnet carries one magnetized needle. *b*. A less stable form of the same. In *c*, the large magnet carries five needles, the other small magnets carrying only one needle (comp. C. A. VII, Text-fig. 6).

They are 95 and 70 respectively. The actual numbers obtained in *Cycas* by NAKAMURA are 53 and 39. The ratios between 95 and 70 is about 1.357, and between 53 and 39 it is also about 1.359. Thus we see a close approximation between the results obtained microscopically and those calculated on the basis of the results of the model experiments.

The model experiments mentioned above seem to show, therefore, that the peculiarities in the chromosome arrangements are largely due to inequality in the magnitude of the electrical charge carried by the

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occupy the position having its plan on the equatorial plane near its center, or the position in which it can have more chance of occupying a central position on the equatorial plane than the chromosomes that have their plans on the equatorial plane near its periphery.

chromosomes. It may be a noteworthy in this connection that in *Spinacia* the empty ring arrangement of chromosomes is more frequently found in the homotype division than in the heterotype division. While in the heterotype division the ratio between the frequency of occurrence of the typical arrangement with one chromosome in the center and of the empty ring arrangement without any of the chromosomes inside it is 5.6 : 1, it is 2.4 : 1 in the homotype division. From the model experiments we see that while the arrangement form is generally typical when the number of magnetized needles is the same for all six floating magnets (Fig. 10, *a*), if none of the magnets receives a special handicap in occupying an inner position, it is a hollow malformed hexagon,<sup>1)</sup> when one of them has been furnished with a certain larger number of magnetized needles than the other five. In the experiments the former was furnished with 5 needles and the latter with one needle each. The magnets were so arranged in the beginning of the experiments as to form an empty ring. Then the circuit is closed, the magnets come nearer one another to take the minimum size of a circular arrangement, on which rearrangement begins, caused by the tendency of a magnet to enter the circle, the more or less regular hexagonal form being thus pressed inwards at one corner, where the magnet showing this tendency occupies its position. When there is one large magnet among the others, this magnet is the large one in most cases. It does not for a time proceed further inwards, however, but the configuration comes to a practically stable form—an empty ring (Fig. 10, *c*). When there is on the other hand no large magnet, the magnet showing a tendency to enter the circle generally proceeds farther, and occupies the central position to form the typical configuration.

In the majority of the cases observed the peculiarity in the arrangement is a shifting of the frequency maximum towards the form

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<sup>1)</sup> This may not be the stable form in the strict sense, but it is practically stable at least for a time.

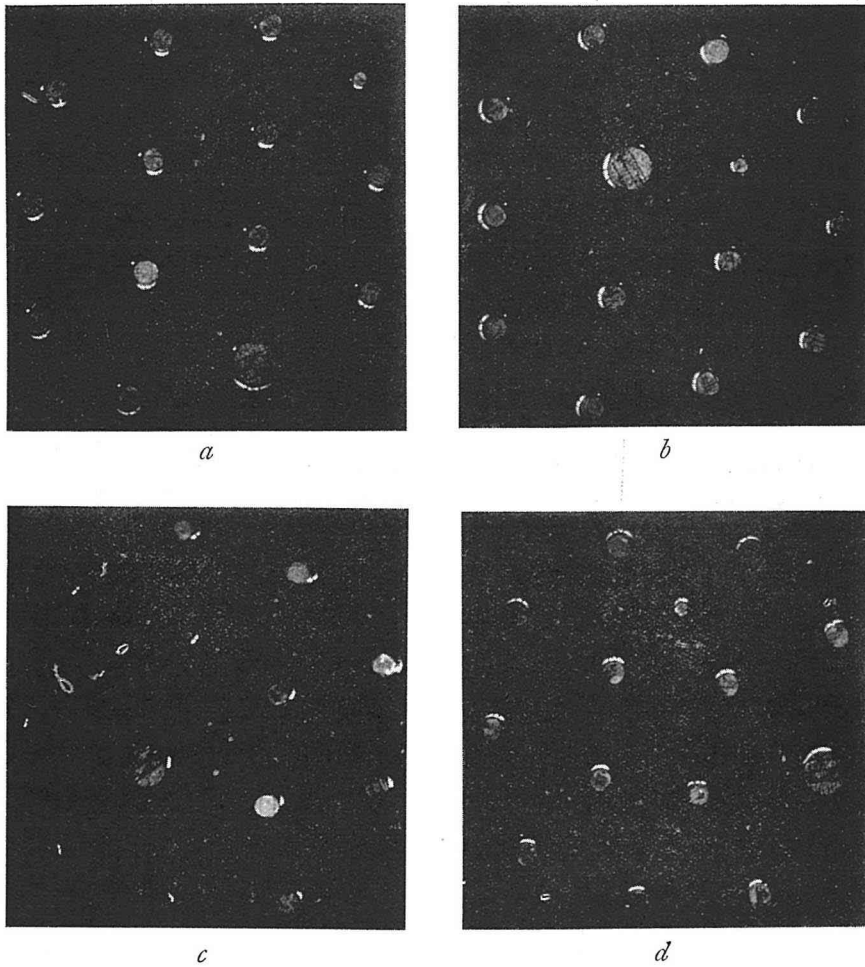
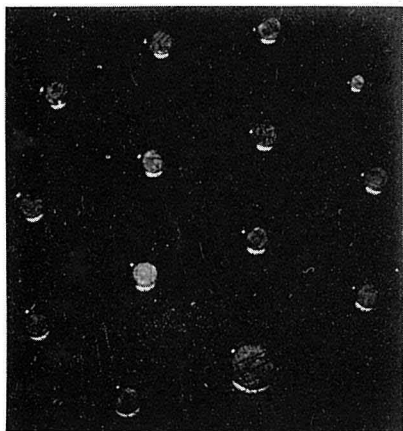


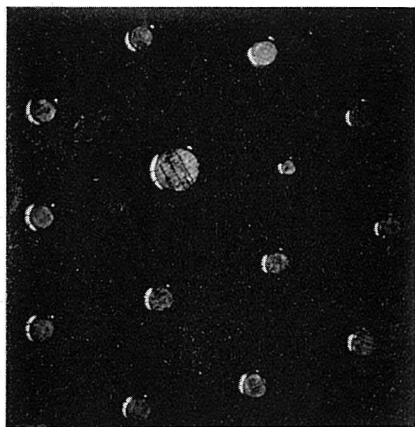
Fig. 11 *a-d*. Fourteen floating magnets. Detailed explanation in the text. *a*. Both large and small in the peripheral region. Four inner magnets. *b*. Both large and small in the central region. Four inner magnets. *c*. The same. Five inner magnets. *d*. The large in the peripheral and small in the central region. Five inner magnets.

of arrangement having a smaller number of central chromosomes, only the case of *Lythrum salicaria*, with a tetrapartite chromosome complex, forming an exception, as was pointed out above. The thorough interpretation of this latter case here also difficult, but we can imitate this with floating magnets too.

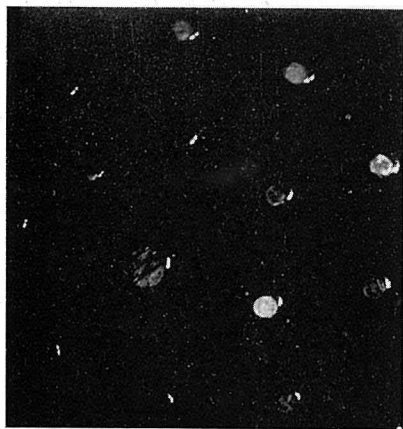
Fig. 11



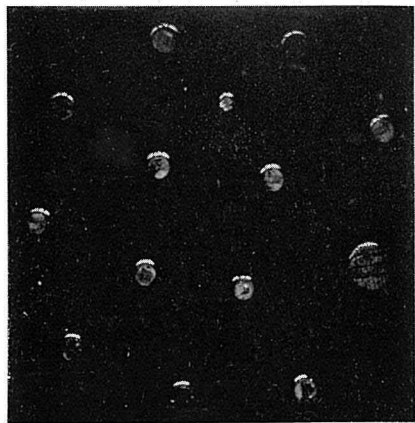
*a*



*b*



*c*



*d*

As will be seen in "Chromosome Arrangement" IV, SHINKE's experiments show that in the case of 15 chromosomes, that is, the case where there is no tetrapartite chromosome, the imitation can be made by assuming that one of the chromosomes carries a larger electrical charge than the others. We assume here that this is the element which forms a tetrapartite chromosome with any one of the others. That the tetrapartite chromosome carries a large charge is inferrable from the fact that notwithstanding its distinctly large size this chromosome can occupy the central position at least so frequently as might be expected for the other small chromosomes from the view point of probability.

Now if we assume that besides this particular chromosome there is one chromosome which carries an electrical charge smaller in magnitude than the other ordinary chromosomes, we can imitate to some extent with floating magnets the peculiarities we have found in the cases of both 14 and 15 chromosomal elements in *Lythrum*. We shall describe first the experiments for the case of 14 elements. One large floating magnet with twenty magnetized needles, twelve medium sized ones each with five magnetized needles, and one small magnet with only one needle were prepared. Generally speaking, the number of the central magnets in the arrangement is four, but when the small one occupies the inner position and at the same time the large one takes its position on the circumference of the peripheral ring of arrangement, it is five (Fig. 11, *d*). In all other cases except one, where both large and small are inside, it is four (Fig. 11, *a* and *b*). In the latter named case the number may be five, though it is rather a rare occurrence (Fig. 11, *c*). The expectable frequency of occurrence of different cases of arrangement with respect to the large and small magnets on the supposition that all the magnets of different kinds have an equal chance of occupying the central position is as shown in Table IX.



TABLE IX.

Combination	Number of inner magnets	Frequency
I. Both large and small inside	4 (rarely 5)	${}_{12}C_2 = 66$
II. Both large and small outside	4	${}_{12}C_4 = 495$
III. Large inside and small outside	4	$({}_{13}C_4) - ({}_{12}C_4) = 220$
IV. Small inside and large outside	5	$({}_{13}C_3) - ({}_{12}C_2) = 220$
Total		${}_{14}C_4 = 1001$

From the table we have the frequency value  $781^{\text{D}}$  for the case of 4 central magnets and 220 for the case of 5. The corresponding observed values given by SHINKE are 33.3 and 18.8 respectively. These values are so far in accord with the calculated values that the form of arrangement having 4 inner chromosomes occurs far more frequently than the form having 5 inner chromosomes. It is also interesting in this connection that no instance where the large chromosome is inside has been observed in the case of the 5 inner chromosomes, but only in the case of the 4 inner chromosomes, and also that in this latter case the large chromosome occupies the central position much less often than it takes up the peripheral position, as is seen also to be the case in our calculated results (cf. SHINKE's Table VIII).

In the case of 15 floating magnets, one medium-sized magnet was added, according to our assumption mentioned above, to the set of floating magnets used in the case of 14. The general results are the same as given by SHINKE. When the large floating magnet occupies the inner position, and at the same time the small one is found in the circumference of the peripheral ring, the number of the inner magnets is 4 (Fig. 12, a), and when both these magnets take the inner positions or

<sup>D</sup> It is assumed here that in combination I (Table IX) the number of the inner magnets is always 4. In reality it may sometimes be 5 in this combination, and therefore the ratio between the frequency of cases of 4 and that of 5 inner magnets must become more nearly approximate to that in the case of chromosome arrangement.

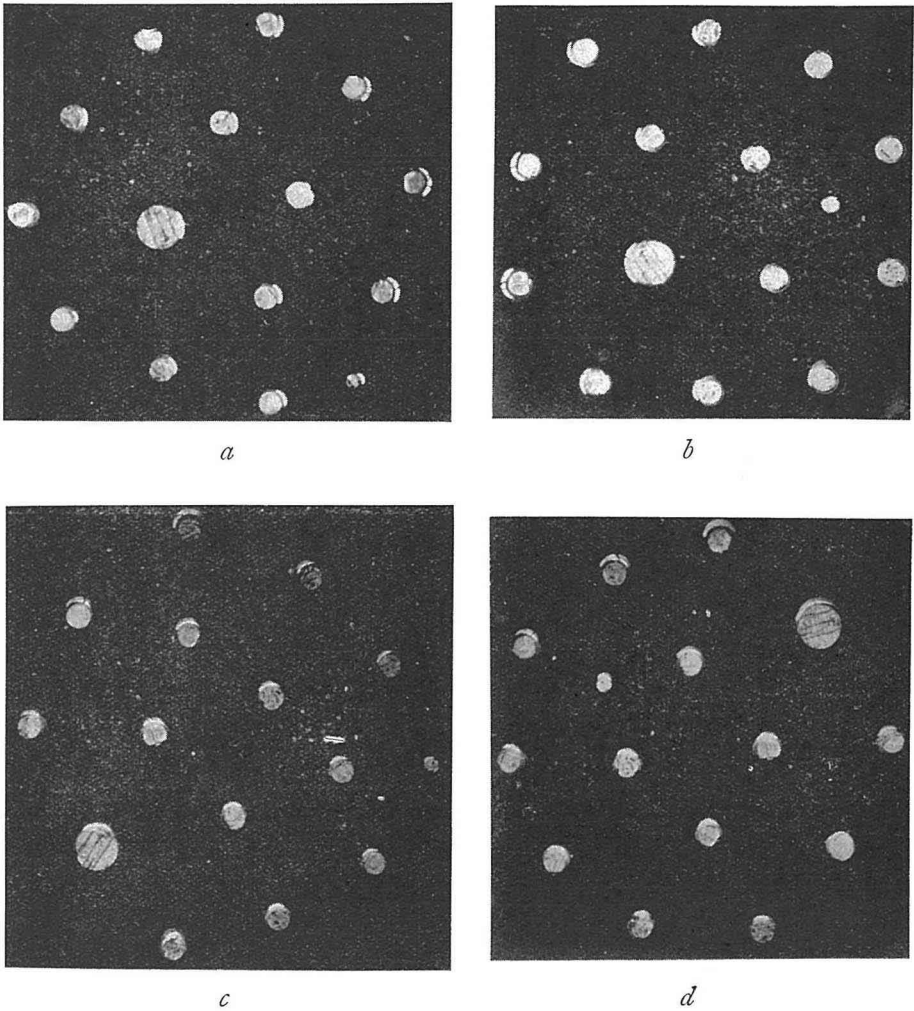


Fig. 12 *a-d*. Fifteen floating magnets. Detailed explanation in the text. *a*. Large in the inner and small in the peripheral region. Four inner magnets. *b*. Both large and small in the inner region. Five inner magnets. *c*. Both large and small in the peripheral region. Five inner magnets. *d*. Large in the peripheral and small in the inner region. Five inner magnets.

when the large one is on the periphery of the arrangement configuration, it is 5 (Fig. 12, *b*, *c*, *d*). The expectable frequencies of these two cases are shown in Table X.

TABLE X.

Combination	Number of inner magnets	Frequency
I. Large outside (Small in or out)	5	${}_{14}C_5 = 2002$
II. Both large and small inside.	5	${}_{13}C_3 = 286$
III. Large inside and small outside	4	${}_{13}C_4 = 715$
Total		${}_{15}C_5 = 3003$

The observed frequency values given by SHINKE for the two cases having 5 and 4 inner chromosomes are 35.5 and 30.8 respectively. The ratio between these values differs very much from the corresponding ratio obtainable from the calculated results, but it is so far in accord with the latter that the cases having 5 inner chromosomes are more numerous than those having 4 inner chromosomes. There seems to be some possibility of having 4 central magnets in the case of combination II in Table X. If it is really so, the results of the model experiments will become more nearly approximate to those obtained microscopically.

The experiments mentioned above are of course only attempts at making imitations and the results are only of an analogous nature, but they seem to throw some light on the explanation of the mechanism of chromosome arrangement.

Before concluding this chapter we may make some remarks on the tetrapartite chromosome. This chromosome consists of two ordinary chromosomes united. Therefore the magnitude of its charge, like its size, seems to be about twice as large as that of the other chromosomes. To put it in other words, it is larger than the other chromosomes in size, and at the same time is correspondingly larger in the magnitude of the charge which it carries. If it is considered in this way, it seems to be not improbable that it may occupy the central position by chance, although owing to its larger size it has a greater mechanical resistance to the cytoplasm through which it moves than the ordinary smaller chromosomes. According to SHINKE's results this tetrapartite chromo-

some is found in the central region of the chromosome distribution figure in a frequency of about  $\frac{1}{5}$ . This fact perhaps implies that it carries at least so a larger electrical charge as the sum of the charges carried by two ordinary chromosomes.

We are quite ignorant about the mechanism of the formation of the tetrapartite chromosome and of its resolution into the component elements, but we may imagine that there are certain peculiarities in the chromosomes or in one of those forming the tetrapartite complex. The results of SHINKE'S observations of the chromosome arrangement in the case of the 15 chromosomal elements and his model experiments with floating magnets imitating the arrangement of these 15 chromosomes seem to show that one of the 15 chromosomes carries a larger electrical charge than the other 14 chromosomes. This means that if all the chromosomes carry a charge of a certain magnitude the chromosome union will not take place, and perhaps suggests that the chromosome fusion may be due to some other than electrical phenomena.

We have some reason to believe that the chromosomes are of a compound nature. The breaking-up behaviour of somatic chromosomes into many small mero-chromosomes in *Ascaris megalocephala* (Boveri, 1904) may be taken as an example showing such a nature of the chromosomes. In some plants and animals (in *Zea Mays* by KUWADA, 1919; in *Secale cereale* by GOTO, 1924; and in certain insects by SEILER, 1922, 1925) the transversal segmentation of chromosomes into two mero-chromosomes has been also observed. From these facts we may regard the tetrapartite chromosomes as representing a transitory stage of some kind between the simple and compound state of the chromosomes, and we expect that the behaviour of the tetrapartite chromosome in the arrangement on the equatorial plate will be the same as ordinary larger chromosomes which are of compound structure in higher orders than smaller chromosomes.

Further investigations may furnish us with some idea of the mechanism of the chromosome fusion, and also of the origin of the compound nature of chromosomes, but we shall content ourselves at

present with pointing out the fact that the separated chromosomes now repel each other like ordinary chromosomes, just as do the mero-chromosomes in somatic cells of *Ascaris megalocéphala*. In this connection investigations on the point of spindle fiber attachment of the holo- and mero-chromosomes are very desirable.

#### 4. *Chromosome Arrangement in the Cases of Hybrids.*

Now we shall look at the cases of hybrids for a moment. In certain insect hybrids where there are differences between the parents in the size of their chromosomes we find no regularity in the arrangement of chromosomes of different sizes (DONCASTER, 1920, Fig. 11). This seems to show that there is a certain disturbance in the mechanism of chromosome arrangement. In the heterotype division of triploid hybrids the disturbance is seen to exist markedly only in the arrangement of univalent or unpaired chromosomes. They are commonly distributed rather at random between the poles, while the bivalents or paired chromosomes form the equatorial plate. Some may remain in the cytoplasm and form micronuclei, while the others may join, the chromosomes originating from the bivalents to contribute to the formation of daughter nuclei. This behaviour of the unpaired chromosomes seems to show that there is a certain lack in the magnitude of the charge of the chromosomes.

In cases where the unpaired chromosomes are longitudinally split and each half is drawn to one of the poles, a more regular distribution is seen. We may take for example *Rosa* hybrids studied by TÆCKHOLM (1922). He describes the behaviour of the unpaired chromosomes as follows :—“Die Fig. 27 *b-f* stellen alle die frühe Metaphase dar. In *b* liegen sowohl die bivalenten, als auch die univalenten Chromosomen zerstreut in der Spindel, aber bald wandern die Gemini nach dem Äquator und bilden dort eine regelmässige Platte (*c* und *b*). Die Mehrzahl der Einzelchromosomen bleiben aber noch ziemlich lange in den peripheren Teilen der Spindelfigur zurück.....Auch die ungepaarten

Chromosomen nähern sich nach und nach dem Äquator (Fig. 27 *e* und *f*) und schliesslich sind sämtliche Chromosomen dort angesammelt und bilden eine regelmässige Platte.....In Fig. 22 sind sechs andere Platten pentaploider Spezies abgebildet. Sie sind alle von demselben eigentümlichen Typ. Im Zentrum befinden sich die 7 Gemini und rings um dieselben herum die ungepaarten Chromosomen." (p. 193-195). As is seen from this quotation, even in the relatively regular case the univalent chromosomes remain still scattered for a time, while the bivalent are already arranged in the equatorial plate. This difference in behaviour between the univalents and bivalents goes to show that while the mechanism of chromosome arrangement is normal for the latter, there is something lacking in it for the former. The fact that while the bivalents are grouped in the central region of the distribution figure, the univalents are arranged in the area surrounding the bivalent group seems to show that this difference is to be found in the difference between the charges carried by the univalents and the bivalents, and that the univalents only later attain the normal state of the charge.

It is an interesting fact that while in *Rosa*, where micronuclei are often formed by scattered univalent chromosomes, the univalents are generally arranged in the area surrounding the central group of the bivalents, in the triploid plant of *Narcissus* studied by NAGAO, where the formation of micronuclei is rather of rare occurrence, the behaviour of the univalents in the arrangement is not markedly different from that of the bivalents or trivalents ("Chromosome Arrangement." VIII).

In *Drosera obovata*, a hybrid between *D. longifolia* and *D. rotundifolia*, studied by ROSENBERG (1909) and in *Nicotiana* hybrid studied by BRIEGER (1928), it has been observed that the two homotype nuclear plates are sometimes in contact or almost fused with each other. This seems to indicate that owing to a certain lack in the magnitude of the repelling force keeping the two nuclear plates separated from each other, the two plates are pressed together by a force which is similar to the force that brings the nucleus into the central region of the cell. The peculiar behaviour of chromosomes seen in the formation of what is

called "Restitutionskerne" by ROSENBERG may also be explained as being due to a certain lack in the electrical system.

### CHROMOSOMES COMPARED TO FLOATING MAGNETS.

The comparison of the chromosome behaviour with that of the floating magnets brings us to the view that chromosomes are comparable to those floating magnets into which a magnetized needle is stuck at a position corresponding to the point of spindle fiber attachment of the chromosomes. MAEDA and KATO ("Chromosome Arrangement" VII) have beautifully demonstrated in *Vicia* that if only the points of spindle fiber attachment are considered, the arrangement of the chromosomes is very similar to that in *Spinacia*. Is there any differential organization in

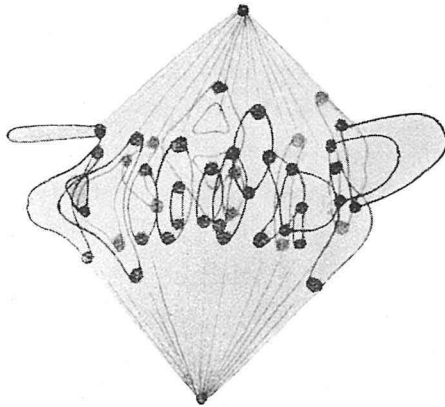


Fig. 13. First spermatocyte division of the albino rat showing polar granules. Drawn by Mr. MINOUCHI.

the chromosome for such a thing as an electrically charged center corresponding to the magnetized needle of the floating magnets? The so-called polar granules described in animal chromosomes may be taken as such, because these are typically found at the proximal end or the end to which the spindle fiber is attached (WILSON, 1925, p. 911). Mr. MINOUCHI, Lecturer in the Zoological Institute of our University

kindly showed to the writer one of his figures drawn very carefully from a preparation of the first spermatocytes of the white rat (Fig. 13<sup>1</sup>), in which all the chromosomes had a deeply stained granule at the point of the spindle fiber attachment. Mr. NAKAMURA of the same Institute has found in the spermatogenesis of a snake, *Natrix tigrina*, BOIE, that when the chromosomes are of the V-shape, the granules are found at their apex, that is, at the point of the spindle fiber attachment<sup>2</sup>). In plants the point of spindle fiber attachment is often found at the constricted portion of the chromosomes (for instance, see SAKAMURA, 1915, Fig. 4, and NAWASCHIN, 1927, Fig. 4), and it has been sometimes clearly observed that this point to which the spindle fibers are attached is connected with a small portion which is comparable in its staining reaction to the polar granules (cf. NAWASCHIN, 1927). In pollen mother cells of *Cycas revoluta* the ends of chromosomes to which the spindle fibers are attached are generally deeply stained with HEIDENHAIN'S haematoxylin, while the remaining larger portion of the chromosome is much bleached by alum. Recently Mr. KATO in our laboratory has found in the heterotype division of pollen mother cells in *Rhoeo discolor* that the deeply stained, pointed portion of the atelomitic chromosomes (median attachment) attached to the spindle fiber is connected with the main body of the chromosomes, which is also deeply stained, by an unstained, hyaline appearing small portion which appears to be comparable with the constricted portion of telomitic chromosomes<sup>3</sup>). Why does this portion of a chromosome adsorb dyes more firmly than the other portion?

We are now dealing with the center of the electrical charge of chromosomes. According to the electrical theory of staining, a staining test will tell us something about what we are trying to learn. But these

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<sup>1</sup>) This figure has been borrowed from Mr. MINOUCHI, to whom the writer wishes to take this opportunity of expressing his thanks for the kindness extended to him.

<sup>2</sup>) Verbal information.

<sup>3</sup>) Unpublished.



observations just mentioned above have been made with fixed materials, and hence, there is too much complication to allow of any conclusion being drawn from the results of staining. On the other hand the kinoplasmic differentiation as "spindle fibers" at or near these points seems to indicate more expressly the existence of such a center in chromosomes.

In the living state of the cell the spindle fibers are generally indistinguishable from the rest of the kinoplasm, but at a certain concentration of hydrogen ions in the medium in which the cell is observed, they may be distinguishable (W. H. and M. R. LEWIS, 1924, p. 415). The phenomenon is reversible and it is comparable with that taking place in chromosomes which swell in the medium of a low hydrogen ion concentration and contract in its high concentration (KUWADA and SAKAMURA, 1926). This fact seems to show that there is a differentiation in the kinoplasm to a certain degree already in the living state, although it is not to so high a degree that we can observe the fibers distinctly. In fixed materials the fibers are more sharply manifested at the point of attachment, and become less pronounced towards the poles. Thus we see a certain parallelism between the morphological aspect of the spindle fibers and of the astral rays. According to the micro-dissection study of CHAMBERS (1917) the rays of the centrosome are more rigid near the centrosome and less rigid towards the periphery of the cell. In this case, there seems to be, in their formation, some causal relation to the physiological phenomena connected with the centrosomes (cf. KUWADA and MAEDA, 1929). In our opinion the rays radiating from the blepharoplasts in *Cycas revoluta* and *Ginkgo biloba* are quite different. In the living state of the body cells of these plants no radiating figure can be observed. It is only visible in fixed material and the structure is different with different fixatives. They are only comparable with BÜTSCHLI's artificial radiation from an air bubble. The formation of the astral rays seems, on the other hand, if BERNSTEIN's theory of electrokinesis is accepted (BERNSTEIN, 1912), to find its causal relation with ions migrating from the centrosome to the periphery

of the cell, accompanying physical phenomena, such as convection current and some others co-operating. In the case of the spindle fiber formation certain plasma colloids may become aggregate at the region of the spindle fiber attachment more densely than the other part of the chromosomes, probably at or near the polar granules. These colloid particles may migrate towards the pole carrying an electrical charge of the opposite sign. They may have a tendency to appear as fibers on being fixed. The density of the colloid particles will become less towards the poles, and thus the fibers become less manifest the farther apart they are from the chromosomes. It is usual for the fibers to be very clearly recognizable only relatively near the chromosomes. Being explained in this way, the spindle fibers are a morphological expression of the migration lines of certain colloid particles from the chromosomes to the poles. The fact that in "lagging" chromosomes, which may be regarded as carrying an insufficient electrical charge, the spindle fiber is generally very inconspicuous, seems to support this view. ROSENBERG (1909) states in his study of *Drosera obovata*, a species hybrid:—"Es scheint, als wenn die Anheftung der Spindelfasern in regelmässiger Weise nur an den Doppelchromosomen stattfände, während die Einzelchromosomen nur zufälligerweise nach dem einen oder anderen Pol transportiert würden..." (p. 32). In this connection it is perhaps worth mentioning that when the mitosis is rendered abnormal by treating material with cold or narcotics or other physical means such as X-rays or radium rays, one of the peculiarities generally observed is the failure of the spindle fiber formation.

#### MECHANISM OF CHROMOSOME ARRANGEMENT.

1. *Normal mitosis.* We shall now proceed to the problem of the mechanism of chromosome arrangement. CANNON (1923) explains the mechanism by comparing the chromosome arrangements with those of the floating magnets on the basis of LAMB'S hydrodynamic theory of mitosis, which rests on the work of BJERKNES. In this explanation it is assumed that chromosomes attract one another, but are repelled

by the centrosomes. In trying to explain the mechanism provisionally by the hypothesis concerning the rôle of electrical phenomena, on the other hand, we seem to find a simpler explanation, not only for the mechanism of the arrangement itself, but also for various accompanying phenomena such as for instance the behaviour of chromosomes having their point of spindle fiber attachment directed towards the center of the chromosome group in the stage of the nuclear plate, &c., although we must here admit, of course, with CONKLIN that "mitotic phenomena are complex and they are doubtless due to several factors, rather than to a single one." (CONKLIN, 1912, p. 520).

Cataphoresis experiments with nuclei show that the nuclear substance is on the whole electro-negative to the cytoplasm (WILSON, 1925, p. 189). Spermatozoa, the large part of which consists of the nucleus, have been shown to have the same sign of charge as the nucleus. These facts seem to show that chromosomes are also electro-negatively charged in the nucleus. But what sign of charge do they carry, when they are directly exposed to the cytoplasm as the result of disappearance of the nuclear membrane?

Experiments with chromosomes free from the nuclear membrane, i. e. those in the metaphase and anaphase, by placing them in the electric current, were tried by some authors, such as HARDY, PENTIMALI and McCLENDON. In all these experiments, however, the results were either negative or not convincing, probably due to the increased viscosity of the cytoplasm under the influence of the electric current (cf. BERSA and WEBER, 1922), and we have at present no direct evidence as to the sign of charge carried by these "free" chromosome exactly. We have on the other hand some reason to suppose that the sign of charge of chromosomes may be reversed when they are exposed directly to the cytoplasm.

Although there is some controversy as to its existence, it is generally accepted that the nucleus has a limiting membrane, whatever its origin may be, and therefore the physical and chemical conditions in the nucleus may be different from those in the cytoplasm. When the chromosomes

are exposed to different conditions, they may carry a different sign of charge. This has been demonstrated actually to occur by cataphoresis experiments with unicellular materials such as blood-corpuscles. BAYLISS (1921), in explaining the mechanism of staining filter paper with congo-red, cites PERRIN's work saying:—"From the work of PERRIN we know that an ion may be adsorbed on an oppositely charged surface to so great an extent that the charge on this surface may actually be reversed in sign."

Neutral violet extra, a dye mixture which stains, according to KELLER (1920), the cathode of a tissue blue and the anode red, shows a distinct change in reaction according to whether the chromosomes are confined within the nucleus or free in the cytoplasm (KUWADA and SUGIMOTO, 1928). In diakinesis the chromosomes are stained blue and in the nuclear plate they are red. This difference in reaction seems to show the change in the sign of the charge carried by the chromosomes.

Now we assume provisionally that all chromosomes are electro-negative in the nucleus. Thus they repel each other. The peripherally arranged position of all the chromosomes in diakinesis — a characteristic feature in this stage — is easily comprehensible by such an interpretation. In the typical mitosis, chromosomes are generally much longer than in the heterotype division, and the mutual repulsion of chromosomes appears less manifest than in the diakinesis, but a similar state of the chromosome arrangement to that in the diakinesis is also recognizable. When the nuclear membrane disappears at the end of the prophase and the chromosomes are exposed directly to the cytoplasm, the electro-negative chromosomes are attracted to the electro-positive equatorial region of the cell, — so assumed here on the basis of BERNSTEIN's opinion (1912), — and at the same time they are repelled by the poles of the same sign of charge as their own, — according to BERNSTEIN, — so that they may be driven to a tendency to form the nuclear plate. Through the repellent action exerted between the chromosomes and both poles of the spindle the chromosome arrangement will tend to a simple form of ring having

none or a smaller number of chromosome inside the ring than that found in the final and stable form of arrangement.

Meanwhile the chromosomes may be reversed in sign by having been exposed to the cytoplasm directly. On reversal in sign the chromosomes repel one another and must get farther apart, so that the area occupied by the chromosomes also becomes larger. According to MINOUCHI ("Chromosome Arrangement" VI), in the first spermatocyte of the albino rat the area occupied by chromosomes becomes smaller when the nuclear membrane disappears. The same phenomenon has been observed in a plant, *Lythrum salicaria*, by SHINKE ("Chromosome Arrangement" IV) in the fixed as well as the living state of the cells. This contraction (the "third contraction") seems probably to be due to loss of the turgid state of the nucleus by disappearance of its membrane<sup>1)</sup> and to inward

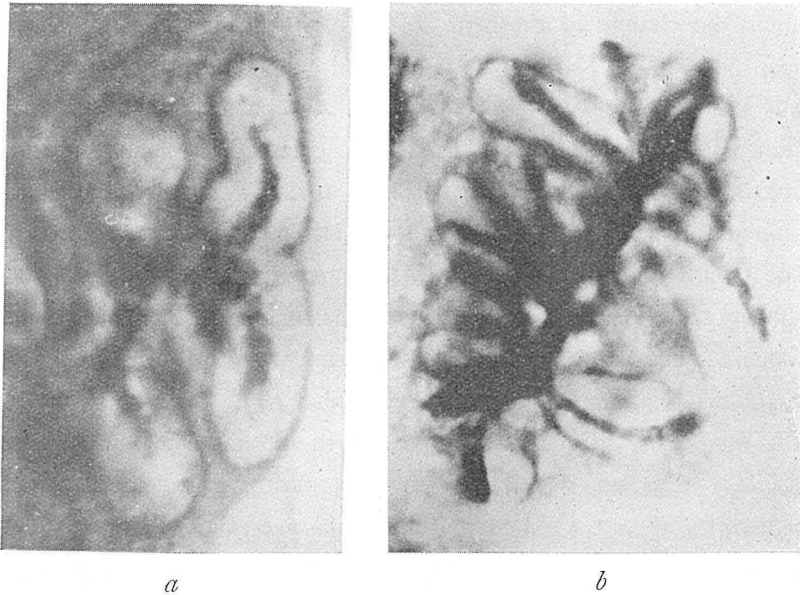


Fig. 14 *a-b*. Chromosomes in metaphase in root-tips treated with boiling water. *a*. *Vicia Faba*. *b*. A garden variety of the tulip.

<sup>1)</sup> That the nucleus is turgid in its living state is shown by the fact that it loses the smooth spherical nature of its surface when it dies (KUWADA and MAEDA, 1929).

pressure of the cytoplasm of denser consistency than the nuclear fluid, rather than due to loss of the mutual repelling action of the chromosomes. In the albino rat the chromosomes then tend to be arranged in two dimensions to form the equatorial plate, the area occupied by them becoming larger again. This phenomenon is in accord with our assumption that the chromosomes are brought to the equatorial plane at least in part by the pushing force of the poles. When the chromosomes form a beautiful equatorial plate, the chromosome area is again smaller, and this diminution in area is accompanied by the change in the capacity of the chromosomes to be stained by HEIDENHAIN'S hæmatoxylin. The chromosomes which had hitherto been stained in their peripheral part only, become solid black in this stage, the central portion being stained as well. This central portion represents perhaps the ground substance of the chromosomes. According to NĚMEC (1910) it swells and at last dissolves when the cell is treated with boiling water. In repeating the experiment with root-tips of *Vicia Faba* and a garden variety of the tulip, we obtained the same result with the exception that the ground substance did not dissolve at all. In its swelling, the spiral chromatic part imbedded in it is widened out into a large spiral still maintaining its peripheral position in the matrix, or may sometimes remain in its original size occupying the central or axial portion of the swollen matrix (Fig. 14, *a* and *b*). The latter did not show any affinity for dyes either acidic or basic, while the spiral part was stained intensely with basic dyes<sup>1)</sup>. In material fixed by the usual methods the spiral chromatic portion of chromosomes is found in their peripheral region and the achromatic portion or the ground substance in the central, thus the whole chromosomes appearing as a hollow cylinder in side-view and a ring in end-view. The ring appearance of the albino rat chromosomes in the early equatorial plate stage is probably due to the fact that only the peripheral region of the chromosomes is here stained, and it would not mean that the chromosomes are

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<sup>1)</sup> The material was fixed with BENDA'S fixing mixture. As acid dyes, congo-red, light-green, eosin, erythrocin, and acid fuchsin were used, and as basic dyes, methylgreen, safranin, cyanin, neutral red, and neutral violet extra were employed.

really hollow. When the stage proceeds, the central portion of the chromosomes too becomes furnished with an affinity for dyes, thus the whole chromosome now appearing to be a solid mass. Such difference in staining between the chromosomes in the early and the late equatorial plate stage is observable, according to MINOUCHI, only in those materials which are fixed with fixatives containing no trace of acetic acid. There is much complication in this, and we can hardly draw a conclusion from this fact alone, but it seems to be a fact which is favourable to our opinion that the electrical sign of the chromosomes becomes reversed in the stage between the early and the late equatorial plate. If this is true, it can be easily understood that in the late equatorial plate stage the area occupied by the chromosomes becomes smaller again, because the chromosomes are now electrically charged opposite in sign to the poles, and they are attracted from both poles.

Explaining the sign of electrical charge carried by chromosomes in the later stage of equatorial plate in this way, we may briefly say that in the metaphase the chromosomes repel one another and at the same time are attracted by both poles of the spindle. In the case of the floating magnets too, each magnet repels the others, and at the same time it is attracted by both electro-magnetic poles. The attraction-repulsion relation is quite the same in these two cases. Thus the final distribution of the chromosomes on the equatorial plate must present the same figure as those obtained in the case of the floating magnets, stable or less stable. Practically we can imitate the various forms of arrangement of chromosomes with floating magnets by changing the number of magnetized needles in them so as to correspond to the size of the chromosomes.

As a matter of fact we meet, however, a relatively small number of irregular figures of chromosome distribution even where in the majority of cases the figures resemble the arrangement forms of floating magnets. These figures are to be regarded as representing transitory stages on the way to the final stable form of distribution or as due to the action of fixation, as already stated in the former part of this paper. We have here, however, a fact which seems to need a special explanation. This

is the arrangement of chromosomes into a simpler form of ring having none or only a smaller number of chromosomes inside the ring as compared with the stable form of the floating magnets. These forms of arrangement of chromosomes seem to represent the early stage of the equatorial plate. If chromosomes distributed in a space of three dimensions are driven by the repellent force of the spindle pole to the formation of the equatorial plate, there may be a certain tendency for all or the majority of the chromosomes to be distributed on the equatorial plane in the form of a circle. Figures such as shown in OGAWA's Text-fig. 3*d* look as if they represent this stage of the equatorial plate.

There may be, besides, some complications in the case of chromosome arrangement as compared with the arrangement of floating magnets. These are those which are due to the original position of the chromosomes distributed in a space of three dimensions on the one hand, and those due to non-synchronous reversion of the sign of charge of chromosomes on the other hand.

In the foregoing interpretation of the mechanism of chromosome arrangement, we assumed that the region of the centrosome is electro-negative and the region of the equatorial plate is electro-positive. Now we shall proceed for a moment to the microscopical basis of these distributions of the electrical tension in the cell in mitosis.

According to BERNSTEIN's electrokinesis theory of mitosis, there are in the cell in mitosis two maxima of negative electrical tension, each in the region of the centrosome, which fall towards the equatorial plate and the periphery of the cell. Thus the equatorial plate receives a minimum of negative electrical tension, or a maximum of positive electrical tension. Is there any microscopical expression revealing such a distribution of electrical tension in the cell?

It is a well-known fact that in the typical mitosis the blepharoplasts, chromatophores, and other formed structures which may be called collectively cytosomes may regularly take their position at the spindle poles like the centrosomes.

In his study of root-tubercles in *Lupinus*, MILOVIDOV (1926) has



demonstrated that bacteria are distributed without any polarized localization in the cell, when the nucleus is in the resting stage, but that in the prophase, especially in the metaphase and anaphase they are beautifully localized in both poles of the spindle figure, and that in a later telophase they are distributed more or less evenly in the whole cell again.

This behaviour of the cytosomes, pole-plasm, and bacteria seems to suggest that the so-called "mitokinetic" force (HARTOG, 1905) may be electrical in nature. Bacteria are known to be electro-negative in charge in a certain condition (HÖBER, 1914, p. 300), and therefore we may conclude from this fact that both polar regions are electrically charged centers. It seems to be also probable from the results of SAKAMURA'S experiments, where cytosomes crowded together under the influence of CO<sub>2</sub> gas (SAKAMURA, 1927), that the cytosomes which may be included in what is called collectively the "pole-plasma," are also electrically charged structures, and thus we may understand the reason why they are gathered in the polar regions. There is, however, a certain diversity between the behaviour of the cytosomes in typical and in heterotypical mitosis. According to LEWITSKY (1910, p. 541), while in mitosis in the somatic cells the chondriosomes seem to be distributed "ziemlich unregelmässig, höchstens mit einer Tendenz, an den Polen sich zu häufen," they enclose in pollen mother cells of *Asparagus officinalis* "dicht mantelförmig die Teilungsfigur fast ringsherum; die Spindelpole aber bleiben hier von jenen frei" (p. 541). A similar behaviour of chondriosomes during the meiosis is seen also in spore mother cells of *Equisetum palustre* L. studied by the same author (1925). In animal spermatogenesis, the same is also the case (WILSON, 1925, p. 358). How can we, then, interpret this diversity in the behaviour of the cytosomes, crowding sometimes at the poles and sometimes about the chromosome group in the equatorial plane? As seen from SAKAMURA'S observations and experiments on the aggregation of cytosomes in relation to mitosis (1927) which perhaps implies their being isoelectric in HARDY'S sense, there seem to take place certain complex phenomena in the dividing cells, and at present we are not in

a position to give any clear interpretation to this diversity between the typical and the heterotypical nuclear division, but at least we may say that there is a certain phenomenon, probably electrical in its nature, taking place in connection with the aggregation or non-aggregation of the cytosomes about the spindle-poles.

Somewhat the same behaviour as that of chondriosomes may be seen in starch grains during the meiotic divisions. Since it was observed by DU BOIS-REYMOND that starch grains in the cells of a section of living potato tuber move towards the anode, and a reversal of current brings the starch grains to the opposite wall<sup>1)</sup>, the behaviour of starch grains in the nuclear division attracts our special interest in connection with the question at issue. Some observations<sup>2)</sup> were made with fresh pollen mother cells of *Cycas revoluta*, stained with acetocarmine. The behaviour of starch grains in the prophase was not observed owing to want of sufficient material in these stages. In the late prophase about diakinesis starch grains are often found in two groups, although we have not determined whether the regions occupied by the groups are the cell poles or not. In metaphase, in the majority of cases the groups of starch grains are found near the equatorial plate generally in the form of a mantel as in the case of chondriosomes in pollen mother cells of *Asparagus officinalis* (Fig. 15, *a, b, c, d*<sub>1</sub>). In the anaphase some of the starch grains are drawn near the chromosome groups which are migrating towards the poles (Fig. 15, *e, d*<sub>2</sub>). In the latter stages the starch grains are often very regularly arranged in rows. In the interkinesis almost all the starch grains are pushed back to the equatorial plane and beautifully arranged on that plane (Fig. 15, *f*). This latter behaviour of the starch grains is very conspicuous, and there is not a single exception at all in this stage. In the homotype division such behaviour of the starch grains as observed in the heterotype division is not to be seen. In the metaphase and anaphase the majority of the starch grains are still found regularly arranged in the equatorial plane of the mother

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<sup>1)</sup> Cited from MEIER (1921).

<sup>2)</sup> Closer observations are left for further researches.

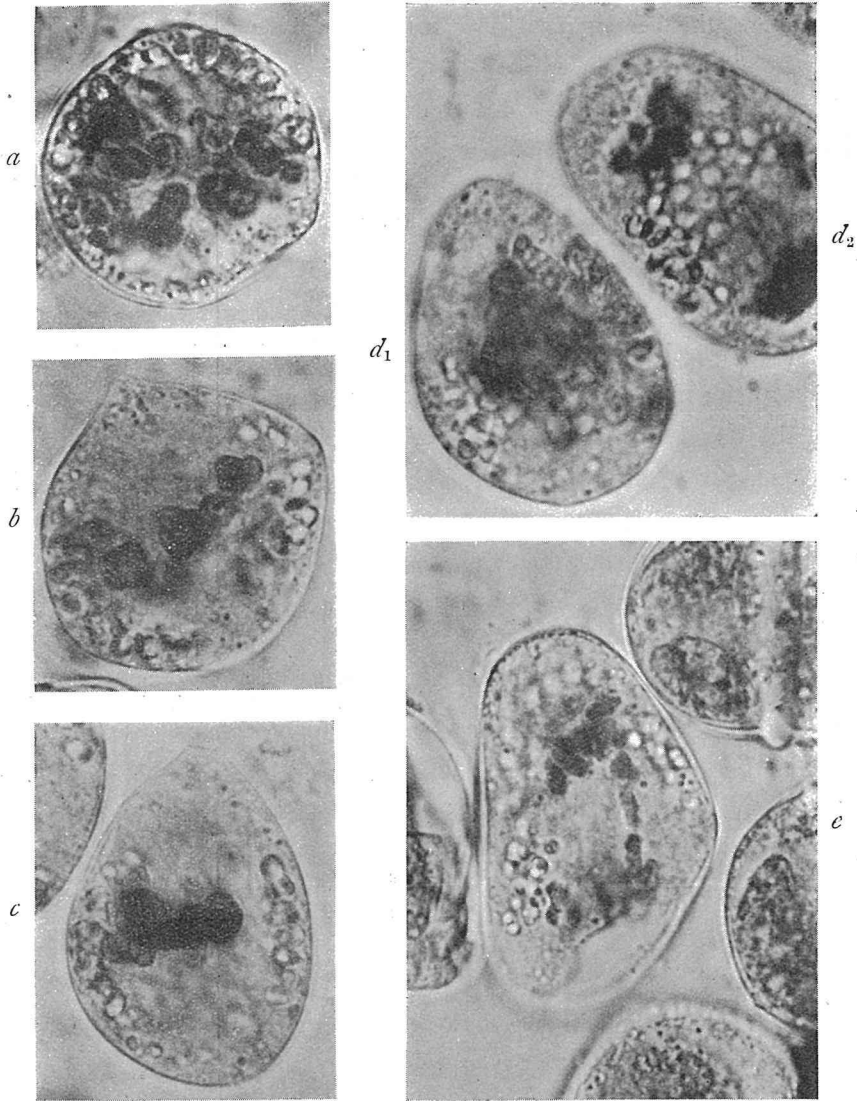


Fig. 15. Meiotic divisions in pollen mother cells of *Cycas revoluta*. All figures are microphotographs from acetocarmine preparations. *a-e*. Heterotype division. *a*. Nuclear plate nearly in polar view. *b*. The same nearly in side view. *c*. Early metaphase in side view. *d*<sub>1</sub>. Meta-anaphase. In some of the chromosomes the spiral structure is seen. *d*<sub>2</sub>. Early telophase. *e*. Anaphase.

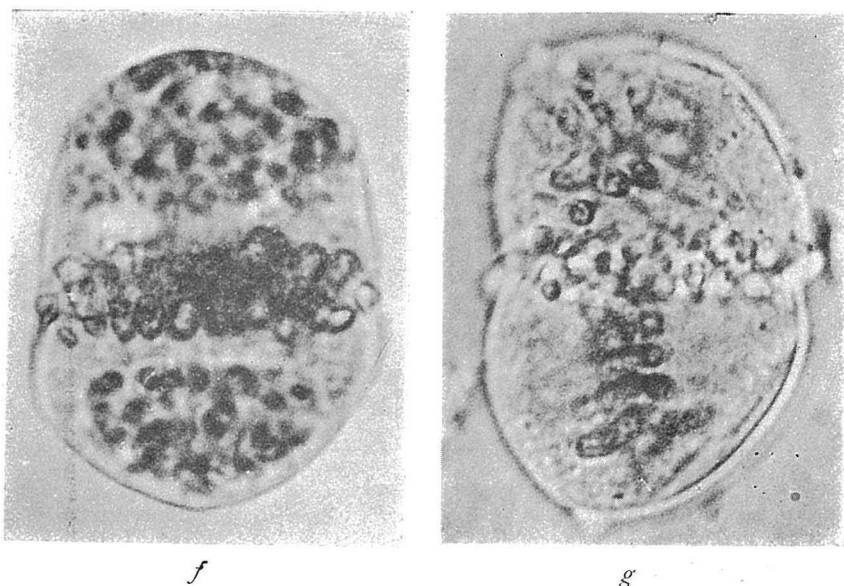


Fig. 15. *f-g*. Enlarged from the original microphotographs. *f*: Interkinesis *g*: Homotype division.

cell (Fig. 15, *g*), except only a few grains which are irregularly scattered in the cytoplasm. Mr. Muro has found the same behaviour of starch grains as in *Cycas* in the interkinesis stage of the meiotic division of pollen mother cells in *Cryptomeria japonica*, although in this plant the behaviour is not so pronounced especially in both meta- and anaphase as in *Cycas revoluta*. The present writer has convinced himself in Mr. Muro's preparations by working very carefully at the microscrew that in the late anaphase or early telophase the starch grains are found distributed anywhere between both chromosome groups.

Closer observations of the behaviour of starch grains during the meiotic divisions have been made by FITTING (1900) in macrospore mother cells of some *Isoetes* species. Here the behaviour seems to have its ground on such a complex phenomenon taking place during the divisions, that a simple explanation is not sufficient for the whole of the behaviour, but it is very significant that while the heterotype spindle poles lie "in der Mitte zwischen je zwei der vier tetraëdrisch angeordneten

Stärkeklumpen," i. e., in a position apart from the groups of starch

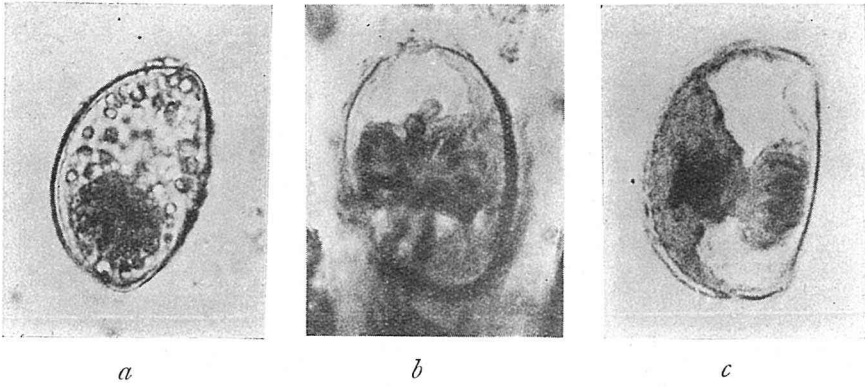


Fig. 16 *a-c.* Young pollen grains of *Tradescantia virginica*. Acetocarmine preparation. Enlarged. *a.* Before division. *b.* In metaphase (polar view). *c.* In anaphase (side view).

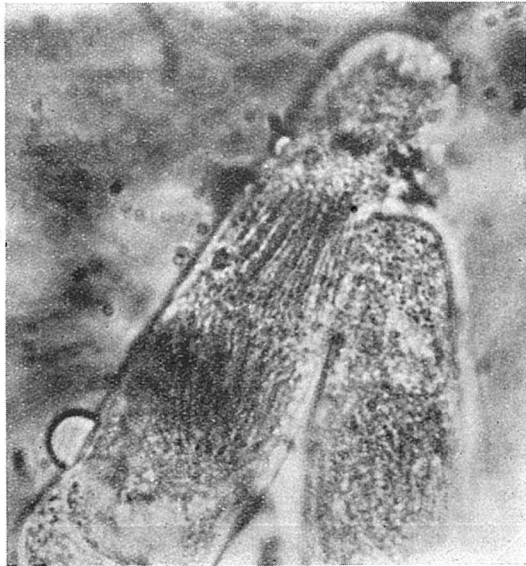


Fig. 17. Staminate hair of *Tradescantia virginica* in division (anaphase). Acetocarmine preparation

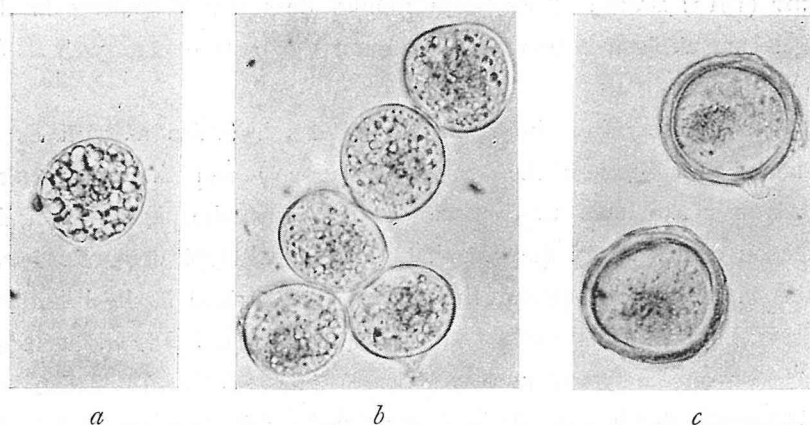


Fig. 18 *a-c*. Pollen grains of *Cryptomeria japonica*. *a* and *b*. Young pollen grains. *c*. Older pollen grains.

grains, the homotype spindle poles come to lie “dicht seitlich an—aber nie in—je einen der Stärkeklumpen.” Thus we see here again a marked difference in the relative position of the spindle poles and the groups of starch grains between the heterotype and the homotype—perhaps here an equational—division, just as found by LEWITZKY in the case of chondriosomes between the heterotypical and the typical division.

The writer has not observed this behaviour of starch grains in the typical mitosis. In *Tradescantia virginica* similar behaviour of starch grains is observable in the meiosis in pollen mother cells<sup>1)</sup> and we find many starch grains in young pollen grains (Fig. 16, *a*). But in the typical mitosis in the pollen grains cutting off the generative nucleus, the starch grains all disappear (Fig. 16, *b, c*). In the mitosis taking place in staminate hairs of the same plant we find also no starch grains (Fig. 17). In *Cryptomeria japonica*, though we had no chance to observe the division figure in pollen grains, we could ascertain that when the pollen grains are older, the starch grains become less abundant (comp. Fig. 18, *a-c*). In his physiological studies of cell division, HABER-

<sup>1)</sup> Kindly suggested by Prof. FUJII, to whom the writer's thanks are due.

LANDT (1913) states: "Es ist ja richtig, dass der Zellteilung in der Regel eine wenigstens teilweise Auflösung der Stärke vorausgeht" (p. 331).

The difference in the behaviour of starch grains as well as of chondriosomes between the typical (equational) and the heterotypical mitosis seems to have an important bearing upon the physiology of the reduction division, and the fact that while in the typical mitosis starch grains in the cell disappear, they remain undissolved in the heterotype division, is very interesting in view of A. and L. GURWITSCH'S conclusion on the typical nuclear division, "dass der in den Zellen sich anreichernde, durch seine Aufspaltung für die Zellteilung energieliefernde Stoff ein Kohlenhydrat ist" (1928). Mr. SHINKE in our laboratory has found starch grains in the heterotype division of pollen mother cells also in *Oenothera biennis*, *Najas major*, *Rhoeo discolor* and *Veronica* sp. and fat-like drops which present fat reaction with sudan III and osmium in *Lilium figrinum*, *L. speciosum*, *L. Hansonii* and *L. longiflorum*.

Coming back to our present problem, we are driven to the conclusion, from the facts mentioned above, that there is a certain characteristic of electrical nature at each pole of the dividing cells, and perhaps also in the region of the equatorial plane. Aggregation of starch grains or cytosomes may mean that they have lost their electrical charge, but if they aggregate always at a certain definite region, we may perhaps conclude that this region must have played a rôle in causing the aggregation.

We have another cytological fact which may be mentioned in this connection. This is the so-called "tassement polaire" or a close crowding of chromosomes at a stage of the telophase. When they are in metaphase and anaphase, they are kept apart from one another. Near the poles they draw nearer, but are still not in contact. At the poles, however, they suddenly come closely in contact and after a while, when a new nuclear membrane begins to be formed, they become again separate from one another, and finally are distributed at the periphery of the daughter nuclei. These phenomena seem to show that the

chromosomes become discharged at the poles, so that they no longer repel one another, but come closely in contact with each other. When the nuclear membrane begins to be formed, the chromosomes become charged again their sign being reversed, and thus they repel one another again, so that they take peripheral positions in the new daughter nuclei. This interpretation of the phenomenon is in accord with the staining reaction of chromosomes to neutral violet extra (KUWADA and SUGIMOTO, 1928).

2. *Abnormal mitosis.* Before concluding this chapter, we may consider the irregular arrangements of chromosomes in abnormal mitosis. According to CHAMBERS (1919) and HEILBRUNN (1921) the viscosity of the cytoplasm increases during the mitosis. HEILBRUNN (1920 b) has found that when the viscosity is sufficiently decreased by antigelatinizing agents or by other means such as cold, the spindle fibers are prevented from forming. Non-formation of the spindle-fibers has been also reported by SAKAMURA (1920) in root-tips of *Vicia Faba* treated with some anaesthetics, chloral hydrate and other drugs. With the view of finding out whether this is also connected with decrease in the viscosity of cytoplasm, Mr. KATO has made some experiments at the writer's suggestion by the centrifuge methode, with root-tips of *Vicia Faba* treated with a 0.75% solution of chloral hydrate, as directed by SAKAMURA in his paper. Mr. KATO's results are yet insufficient to allow of any definite conclusion being drawn, but one case shows that the viscosity of the cytoplasm is markedly decreased in the root-tips treated with chloral hydrate, as will be seen from the results summarized in Tables XI and XII. Mr. KATO noticed the non-formation of the spindle fiber in materials No. III and No. IV.<sup>1)</sup>

The non-formation of the spindle fiber is always associated with an abnormal chromosome distribution. As means or agents to induce the change of viscosity of the cytoplasm or protein we may mention anaesthetics (WEBER, 1922; HEILBRUNN, 1920 a), cold (HEILBRUNN,

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<sup>1)</sup> Unpublished.



TABLE XI.

Treatment of material. (June 5, 1926)

Expt. no.	Placed in a 0.75% chloral hydrate soln. at (Room temp.: 21°C.)	Washed in water at (Water temp.: 15°C.)	Placed in saw-dust at (Temp.: 18°C)	Centrifuged at	Fixed at
I.	9A.M.—10A.M.			10 A.M.	Immediately after centrifuged
II.	" "	10A.M.—11A.M.		11 A.M.	"
III.	" "	" "	11A.M.—noon	noon	"
IV.	" "	" "	" -1.P.M.	1.P.M.	"
V.	" "	" "	" -2 P.M.	2 P.M.	"
VI.	" "	" "	" -3 P.M.	3 P.M.	"
VII.	" "	" "	" -4 P.M.	4 P.M.	"

TABLE XII.

Results of the experiments.

Expt. no.	No. of revolutions per minute	Time for centrifuging in minutes	Dislocation of nuclei
I.	2000	3	+
II.	"	"	+++
III.	"	"	+
IV.	"	"	+
V.	"	"	+
VI.	"	"	+
VII.	"	"	+

1920 a, WEBER and HOHENEGGER, 1923), electricity (BERSA and WEBER, 1922), X-rays (COLWELL and RUSS, 1924, p. 106, protein<sup>1)</sup>), radium

1) WEBER has reported, on the other hand, as the result of his experiments on plant cells, "dass in lebenden pflanzlichen Zytoplasma als primäre Röntgenwirkung keine Viskositätsänderung auftritt". (Cited from E. BRIEGER'S review in Bot. Centralbl. N. F. Bd. 3, 1924).

rays (WILLIAMS, 1925; COLWELL and RUSS, 1924, p. 99, protein) and ultra-violet rays (ELLIS and WELLS, 1925, p. 273, protein). Cells treated with any of these means show in almost all cases abnormal mitosis, in which suppression of the spindle-fiber formation or irregular distribution of chromosomes is characteristic, as shown by NĚMEC (1904) and SAKAMURA (1920) in the case of chloralization, by HEILBRUNN (1920 b), MICHAELIS (1925), SHIMOTOMAI (1927) and others in the case of cold, by SAKAMURA and SUTOW (1926) in the case of a higher temperature than that to which the plant in question is normally subjected, by KOMURO (1924) and PEKAREK (1927) with X-rays, by GAGER (1908) with radium rays, and TAKAMINE (1923) with ultra violet rays<sup>1)</sup>.

In such a view of the nature of the spindle fiber as discussed in the former chapter, this phenomenon of non-formation of the spindle fiber seems to be due to a certain lack in the system of electrical phenomena rather than a decreased viscosity of the cytoplasm. To put it in other words, though the decrease in viscosity is incidental to the affection by which the formation of spindle is prevented, it must have little to do with the abnormal chromosome behaviour, except the passive movement of chromosomes due to convection current in the cell. On the other hand, if the viscosity of the cytoplasm is increased by some external agencies or conditions to such an extent that it is still reversible, it will play an important rôle in inducing abnormal behaviour of chromosomes making it difficult for them to migrate.

Inability of the chromosomes to migrate towards the poles is often found in pollen mother cells of certain hybrids, while in the somatic cells of the same plants the normal migration takes place (for instance, in the case of pollen sterility). SAKAMURA (1920) has found that in *Vicia Faba* meiotic mitosis is much more sensitive to external agencies than somatic mitosis. Hybrids consist of two different genotypes, and

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<sup>1)</sup> We have no convincing results of abnormal distribution of chromosomes induced by electrical treatments of a tissue or cells. CONKLIN (1912) has observed in eggs of *Crepidula* that the chromosomes disappeared from the amphiaser as an effect of electrical current.

thus the normal physiological equilibrium may be disturbed, so that the plants are easily affected, especially in the sporogenesis, by external agencies or a slight change in their environmental conditions, perhaps resulting in an insufficiency in the electrical system.

In LAMB's theory, to explain the migration of chromosomes towards the poles in anaphase, it must be assumed that the density of the chromosomes is changed in this stage of division. CANNON explains this change in chromosome density by its relative change due to the change in density of the continuous medium in which the chromosomes are suspended. This explanation seems, however, to meet with a difficulty on its being applied to the cases of hybrids where some of the chromosomes lag behind the others in division.

There should be gradation in sensitiveness to external conditions according to remoteness in blood-relationship between the parents. And accordingly there should be different types in the abnormal mitosis. There are known several main types, but adequate explanations of the mechanism of these different types are left for further researches.

#### SUMMARY.

1) The chromosome arrangements are compared with the results of experiments made with floating magnets and discussed in respect to the following four cases :—

- a.* Cases where all the chromosomes of one group are nearly of the same size and shape.
- b.* Cases where some of the chromosomes are different in size and shape from the other chromosomes in the same group, and also where one of the chromosomes is tetrapartite.
- c.* Cases of hybrids.

The final distribution figures seem to be determined mainly by the magnitude of the electrical charge which the chromosomes carry.

- 2) As the causes of the irregular chromosome arrangements which

we meet with in fresh as well as in fixed materials, the following factors are pointed out:—

- a.* The original orientation of the chromosomes distributed in a space of three dimensions. This state of orientation presents a marked contrast to the case of floating magnets set afloat on a water surface.
  - b.* Viscous nature of the cytoplasm which makes the movement of the chromosomes much harder than the movement of the floating magnets in water.
  - c.* Relatively long duration of time taken from the moment of disappearance of the nuclear membrane up to the completion of the nuclear plate formation. This state of affairs gives us a chance of meeting with every transitory stage of arrangement up to the final distribution. The regular distribution figures are, therefore, theoretically only to be sought in the stage where all the chromosomes are arranged on one plane.
  - d.* The fact that even in the case of floating magnets more than one arrangement not equally stable may be produced. This fact explains certain variations found in the case where all the chromosomes are distributed strictly on one plane.
  - e.* Effect of fixatives and other artificial causes.
- 3) A differential organization of chromosomes in connection with their movement is suggested and an attempt is made to explain the mechanism of the chromosome arrangement.

The microscopical results so far obtained, the details of which will be found in the following papers, "Chromosome Arrangement" II—IX, each written by the observer himself, are summarized as follows:—

- 1) The frequency of occurrence of the form of chromosome arrangement which resembles the stable form of the corresponding number of floating magnets is higher in the case where all the chromosomes of the group are distributed in the equatorial plate than in the case where some of them lie out of the plate, below or above.
- 2) There seems to be no marked difference between metaphase

(equatorial plate) and anaphase in the frequency of occurrence of the arrangement form resembling the stable form of floating magnets.

3) The resemblance of the chromosome arrangement to the stable form of floating magnets is marked when the number of chromosomes is relatively small and when the chromosomes present no marked size difference between one another. Not only the arrangement form resembling the stable form of floating magnets, but also those resembling the less stable forms given by the physicists are found. This has been beautifully illustrated in *Torilis Anthriscus* and *Spinacia oleracea*.

4) The frequency with which the large chromosome occupies the central position of the arrangement is different in different cases; it may be nearly equal to (*Spinacia, Cycas*), or greater (*Vicia*) or less (*Sagittaria*, some of S-chromosomes in *Cycas*) than the frequency expectable from the view point of probability. The small chromosomes show on the other hand a high tendency to take the central position so far as the present results of our observations are concerned.

5) The fact that if only the points of spindle fiber attachment are considered, the arrangement form becomes equal to that of massive, round chromosomes, even when the chromosomes are of rod or V shape has been found in *Vicia Faba*.

6) In the heterotype division :—

a. When the chromosomes are all of nearly the same size and shape, the frequency curve of the different forms of chromosome arrangement is leptokurtic in nature, the maximum being presented by the form resembling the stable form of the corresponding number of floating magnets.

b. When there are size differences in the chromosomes, i) the maximum frequency value tends to become low, and ii) in an extreme case we have found that this value is shifted to that form of arrangement the number of central chromosomes of which is less by one than in the form resembling the stable form of floating magnets, although the latter form maintains still a high value, making a second maximum.

7) In the homotype division:—

*a.* Even in the case where the chromosomes appear all to be of the same size and shape in the heterotype division, the maximum frequency value may be markedly low in the homotype division, a second maximum being found in the form in which the number of central chromosomes is less by one than in the form presenting the first maximum or the form which resembles the stable form of floating magnets.

*b.* When there are size differences among the chromosomes and the maximum frequency value is markedly low in the heterotype division, shifting of the maximum towards the forms having a smaller number of central chromosomes than the form resembling the stable form of floating magnets becomes marked.

8) In only one case the shifting of the number of frequencies towards the form having a larger number of central chromosomes, or a number which is equal to that of the form showing the maximum in the case of a chromosome number more by one than in this case, was found in the case of the 14 chromosomal elements of *Lythrum salicaria*. In this case one of the 14 elements is a tetrapartite chromosome which may be broken into two ordinary bivalent chromosomes, thus making the total number of chromosomes 15.

9) In a triploid plant (*Narcissus*) the arrangement of trivalent chromosomes has been found to be the same as in the cases of bivalents.

The present paper is an introduction with working hypotheses to our further investigations into the chromosome arrangement and its mechanism. The results of the microscopical observations go to show that the comparison of the chromosome arrangements with those of the floating magnets is worth proceeding with in as many different cases as possible. They call our attention especially to those cases where there are marked size differences among the chromosomes in one group, and where there are some multipartite chromosomes. Com-

parison of the cases of homotype division having the separating wall between two sister spindles with those having no wall seems to be very desirable. As to the mechanism of the arrangement we feel keenly from the view of the working hypotheses presented above that microscopical data which form the basis of discussion of the problem are yet lacking in a large measure.

#### POSTSCRIPT.

The writer wishes to express his sincerest thanks to Prof. FUJII for his kindness in showing him the manuscripts, as yet unpublished, of the results of investigations carried out by Mr. WADA (1924-'25, 1926-'27), Mr. TAKAMINE (1926), and Mr. MORINAGA (1926-'27) on the same subject as the present paper. The writer feels very sorry that he could not refer to the results of these investigations, which are very instructive to us, in appropriate places in the present paper owing to the fact that the paper was in the press when he was kindly given the opportunity of reading those manuscripts.

One more remark may be added here. After this manuscript had been sent to the press, the December number (1928) of the Botanical Gazette, containing ZIRKLE's paper, "Nucleolus in Root Tip Mitosis in *Zea Mays*", came to hand. In this paper, ZIRKLE states: "The poles of the spindle form and the electro-negative spireme retreats to the equatorial plane, the maximum distance from the two poles. There it is permeated by the electro-positive nucleolar material until it is mostly electro-positive; whereupon it fragments into chromosomes which split. The chromatin, now bearing a different charge, reverses its previous motion and migrates to the two poles". This interpretation of the formation of the equatorial plate and the separation of the longitudinal halves of chromosomes towards the poles coincides largely with the view we have presented above, except that ZIRKLE regards the reversal of the sign of the electrical charge carried by the chromosomes as due to the permeation of the chromosomes by the electro-positive nucleolar material.

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