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Cytological Studies of Pollen Mother Cells of *Rhoeo discolor*, HANCE with special reference to the Question of the Mode of Syndesis

By

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With Plates XIII and XIV and 9 Text-figures

(Received January 18, 1930)

The occurrence of end-to-end linked chromosomes has been recently increasingly reported by many authors in various plants. In the heterotype division of the pollen mother cells of *Rhoeo discolor* the chromosome number of which was determined to be n=6 by the studies of SUSSENGUTH (1921 a, b), TISCHLER (1921)[1], BELLING (1927) and DARLINGTON (1929 b), this peculiar behavior of chromosomes uniting in a ring has been also observed. The point of particular interest in this plant is that there is a difference in the position of spindle fiber insertion among the chromosomes forming the ring, a fact which may have some important bearing upon the question of the mode of syndesis. The results so far obtained will be given in the present paper.

MATERIAL AND METHODS OF FIXATION

Some material was fixed in May from plants grown in the green house and others in September from those grown in the field. For

1) TISCHLER, G. (1921–22) Allgemeine Pflanzenkaryologie. p. 581.

fixation the following two methods were found to give the most satisfactory results :—

1. After having been treated with CARNOY's mixture containing chloroform for three minutes, the Bonn modification of FLEMMING's solution was applied. After 24-hours' fixation, the material was washed in running water for 24 hours, and then was dehydrated with graded alcohols and imbedded in paraffin in the usual manner.

2. The material was fixed simply with CARNOY's mixture containing chloroform for three minutes. It was washed with absolute alcohol, and imbedded in paraffin as usual.

Sections were cut 12 micra thick and stained exclusively with HEIDENHAIN's iron alum haematoxylin. Those from the material fixed by the former method, after having been bleached with peroxide of hydrogen, were placed for 3–5 hours in MINOUCHI's modification of CHURA's bath, which is made up of equal parts of glacial acetic acid and saturated picric acid (MINOUCHI, 1927). This latter method of procedure was used for the purpose of dissolving cytoplasmic inclusions, and at the same time because it is capable of increasing the staining affinity of chromosomes for haematoxylin.

The figures reproduced in the present paper are all drawn from preparations from material fixed by the first method of fixation, but in chromosome counting preparations from materials fixed by both the first and second methods of fixation were employed.

DESCRIPTION

Synizesis. The nucleus at this stage lies eccentrically in the cell as does the contracted mass of nuclear threads in the nucleus. They are both dislocated towards the same side (Fig. 1). This parallel dislocation is of common occurrence in different preparations, and it compels us to the conclusion that the eccentric position of the nucleus and the chromatic mass is a characteristic feature of the synizetic stage. CLELAND (1924) has also observed this parallel dislocation, and regards

it as suggesting the possibility of synizesis to be an artifact due to the influence of fixing fluids. When, however, living pollen mother cells of *R. discolor* and also those of *Lilium speciosum*[1] are pressed out of the pollen sacs on a slide glass and are observed in their pressed-out juice, we can clearly recognize that the parallel dislocation of the nucleus and the mass of nuclear threads is quite the same as that described above in the case of the fixed materials.

Throughout the length of the threads composing the synizetic knot there is no sign of their double nature to be seen (Fig. 1). The nucleolus is a small spherical body at this stage. It appears to be extremely small in its relative size to the nucleus as compared with cases of *Oenothera* and many other plants (Fig. 1).

Open spireme. The synizetic tangle of the nuclear threads become gradually loose and some parts of the threads or loops begin to stretch out in the nuclear cavity, while the central mass of threads is still drawn together in a compact mass (Fig. 2). In this stage some indication of end-to-end conjoining of chromosomes is seen in the loosened out loops (Fig. 2). When the open spireme stage is reached, the loops grow thicker and thicker. They consist of single threads or chromosomes conjoined end-to-end with one another (Fig. 3.)

Second contraction. The second contraction now supervenes, the loops being drawn in towards the center of the nucleus. The aggregation of the loops is not so compact as in the first contraction, but only to such an extent that closer observation can disclose their clear separation from one another. Usually some loops are found radiating outwards from the central mass, and they sometimes show an indication of segmentation into individual chromosomes, thinner portions appearing as fine threads (Fig. 4). In the next stage the individual chromosomes become nore clearly recognizable (Figs. 5 and 6), although they are connected end-to-end by a thin or thick thread.

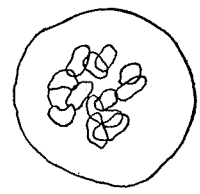
"Diakinesis" The whole length of the chromosome chain, all

[1] In the case of *Lilium* a pollen sac was cut at a tip with a sharp knife and then the contents were genlty pressed out on a slide glass.

the 12 univalents being still connected with one another by thin threads into a ring or sometimes an open chain, becomes distributed irregularly near the periphery of the nucleus. In this plant chromosomes entirely fail to form gemini (Fig. 7).

The thin portions or threads by which the chromosomes are connected with one another stain much less than the chromosome proper. These portions are found sometimes to be made up of double threads, but usually they appear to be single threads.

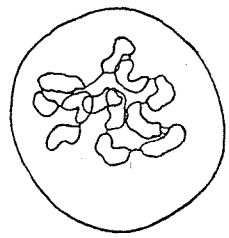
Heterotype metaphase, anaphase and telophase. When the nucleolus and nuclear membrane disappear at the end of the prophase, and the whole length of the chromosome ring or chain is exposed directly to the cytoplasm, the chain is drawn towards the center of the cell, where it frequently forms a mass so closely pressed together in the central region of the cell that the individual chromosomes can not be made out clearly (Text-fig. 1). This contraction has been observed and is called the "third contraction". In this study of *Oenothera sinuata,* SINOTÔ (1927) expresses the view that this contraction may have a certain bearing on the arrangement of the chromosomes in metaphase. Recently MINOUCHI (1929) stated in his study of the albino rat that when the nuclear membrane disappears, the chromosomes gather from all direction towards the center of their distribution in three dimensions, the spherical area occupied by them becoming smaller, and also that they then draw apart again from one another gradually showing a tendency to become arranged in two dimensions. Figures similar to this "third contraction" have been observed by SHINKE (1929) in pollen mother cells of *Lilium tigrinum* in the living state. From these results of observation it seems to be more reasonable to consider that the third contraction is a natural phenomenon in the living cells than to assume that it is a result of inadequate fixation. Fig. 8 shows chromosomes in the stage of unfolding stage from the third contraction to be arranged in the equatorial plane.



Text-fig. 1. The chromosomes occupying the center region of the cell—the "third contraction". Nucleolus no longer to be seen (Zeiss 1/12×K. 12).

In this stage the jaggedness of the chromosome contour becomes pronounced, and the spiral structure of the chromosomes is observable more or less clearly (Fig. 9). The spirals become more distinct in the meta-anaphase. Fig. 10 shows the chromonemata in the anaphase. The doubleness of the chromonema is seen in the left-hand-side one of the lower chromosomes. This doubleness may be regarded as due to the longitudinal splitting for the following mitosis. Such a doubleness of the chromonema has been observed slso by KAUFMANN (1926) in *Tradescantia pilosa.*

The chromosomes are generally arranged so as to form the equatorial plate in the metaphase of heterotype mitosis. In this case, however, just as in *Oenothera*, the chromosomes do not arrange themselves all in one plane but in two planes in a zigzag manner without forming the ordinary separated or individualized gemini. The typical condition of the arrangement in polar view is illustrated in Text-fig. 2. In side view it is clearly seen that the chromosomes are still conjoined in a ring. The individual chromosomes are of the shape of a short, thick V or J, the apices of the V's or J's being directed towards the spindle poles (Fig. 12 a, b).

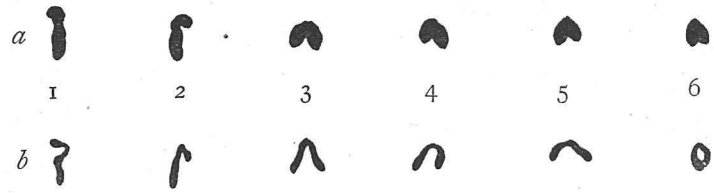


Text-fiig. 2. Metaphase in polar view. The chromosomes arrange themselves taking a zigzag course on two planes (Zeiss 1/12× K. 12).

The spindle fiber insertion is subterminal in 4 chromosomes out of 12 and appears to be median[1] in the remaining 8 (Figs. 12 and 13, and see also Text-fig. 3). These chromosomes assume the shapes of V's or J's according to the position of the point of spindle fiber insertion whether it is median or subterminal. For the sake of convenience in description, the terms, *heterobrachial* and *isobrachial* recently introduced by SOROKIN (1929) will be used in the following description

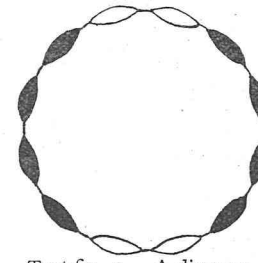
[1] Thanks to DARLINGTON's study (1929 b) of the somatic chromosomes in this plant, which appeared after the present observation was finished, it has been shown that the point of spindle fiber insertion is not exactly median but approximately so.

to express the position of the spindle fiber insertion of both kinds respectively.



Text-fig. 3. Haploid groups of chromosomes in the heterotype anaphase (*a*) and in the homotype anaphase (*b*). The chromosomes 1 and 2 are heterobrachial and 3, 4, 5 and 6 are isobrachial.

If we unfold the folded chromosome ring (such as that shown in Fig. 12 a, b) into a full ring in order to learn the relative position of each individual chromosome, we shall obtain a figure such as the illustration in Text-fig. 4. From this figure, it will be readily seen that the two adjoining heterobrachial chromosomes are situated in the position diagonally opposite to the other two of the some shape and that between these two sets of chromosomes there are 4 isobrachial chromosomes on each side of the sets.



Text-fig. 4. A diagram of the chromosome ring. The chromosomes represented in outline are the heterobrachial chromosomes and those in solid black are the isobrachial ones.

The heterobrachial chromosomes may conjoin either with their proximal ends, the ends near which the spindle fiber insertion is found, or with their distal ends, but never with the proximal end of one chromosome and the distal of the other. This mode of conjoinment with the proximal or distal ends is the same in both pairs of the heterobrachial chromosomes in the same ring. To put it in other words, when the conjoinment is made with the proximal ends in one pair, the same is always the case with the other pair and when it is with the distal ends in one, it is also the case with the other. No case was observed where it was made with the proximal ends in one pair and with the distal ends in the other of the same ring. Both these modes of conjoinment

of the heterobrachial chromosomes are found in about equal frequency. These observations were made in rings which took up a regular arrangement in the metaphase.

The mode of disjoining of the chromosomes is, generally speaking, such as described by many authors in *Oenothera*. The contiguous chromosomes pass to the opposite poles and the alternate ones to the same pole. Careful observation revealed the fact, however, that this mode of disjunction is not true for all the chromosomes. In the case of the heterobrachial chromosomes this is true only when the contiguous ones are conjoined with the distal ends (Fig. 13). When they are conjoined with the proximal ends they always pass to the same pole (Fig. 12 a, b).

Not infrequently, irregular zigzag arrangements such as that illustrated in Fig. 11 occur. In Fig. 11 b a chromosome is found inserted or suspended between the chromosomes which are to pass to the upper and lower poles respectively. In such an irregular arrangement the suspended chromosome may pass to the wrong pole so that the result should be non disjunction, such as 5 chromosomes to one pole and 7 to the other (Fig. 22 a, b). A similar arrangement has been observed by CLELAND (1924) in *Oe. franciscana sulfurea* (see his Fig. 20) and in *Oe. Lamarckiana* in which the spindle fiber insertion is median in all of the chromosomes (1929; his Fig. 26). More irregular distributions are also found in this stage (Fig. 14). No case was, however, observed where the chromosome distribution was 8 : 4, nor any other possible unequal distribution, so far as the present investigation is concerned. The statistical data of the non-disjunction we obtained are given in Table 1. Counting was carried out in the anaphase in the case of the hetertype division and in the metaphasic sister chromosome groups in the case of the homotype division.

From Table 1 we see that the frequency value of the equal distribution is 64.4 % and that of the clear cases of unequal distribution 33.3 %.

TABLE 1

Statistical data obtained from 225 pollen mother cells. The number in brackets indicates the numer of the lagging chromosomes.

Chromosome distribution	6:6	5:7	5:(2):5	5:(1):6	Total
Heterotype anaphase	59	30	2	3	94
Homotype metaphase	86	45	0	0	131
Total	145	75	2	3	225
Percentage	64.4	33.3	0.9	1.3	99.9

The J and V shapes of chromosomes become clearly manifest in the later stage of metaphase, and during the anaphase these V's and J's become double by the premature longitudinal fission for the homotype mitosis (Figs. 16 and 17). The premature fission, which is peculiar to the anaphase, can be most clearly observed when the chromosomes are viewed from the pole (Figs. 21; 22 a, b and 23). The six longitudinally split chromosomes at the poles are mostly so arranged that five of them lie in the form of a circle around the sixth (Fig. 21), though the case was also observed where all the six chromosomes were arranged in a circle having none inside it (Fig. 23). The chromosomes become thinner as the anaphase progresses, but on reaching the pole they become thicker again and then enter upon the next stage of telophase (Fig. 24).

As shown in Fig. 15 a small granule was found at the point of spindle fiber attachment of a chromosome. This granule is deeply stained and is connected with the main body of the chromosome by a chromatic substance. This may be compared with the granule known as the "polar granule" or "proximal granule" observed by WENRICH (1916) in *Phrynotettix,* by *Minouchi* (1927) in the albino rat (*Mus norvegicus albus*), by NAWASCHIN (1927) in *Galtonia,* by NAKAMURA (1929) in *Cycas* and by others especially in animals.

Occasionally some few chromosomes, generally two in number, lag behind the others and remain near the equatorial region (Fig. 18).

These lagging chromosomes do not form dwarf nuclei, unlike the cases of various hybrids observed by many authors. They pass to the poles quite at random. It is obvious that these chromosomes are those that have failed to have spindle fibers inserted in them in the metaphase, and remained suspended by linking between the two adjoining chromosomes destined for the upper and lower poles respectively (Fig. 11 b). Some lagging chromosomes are found clearly separated into two longitudinal halves (Figs. 18 and 19), but no case was observed in the interphase and homotype prophase where the nucleus contained one of the longitudinal halves of these chromosomes. The chromosome bridge between two daughter chromosome groups at the poles is sometimes found even in the stage where the cell plate has been formed (Fig. 20).

Interkinesis. The nucleus undergoes marked changes during the interkinesis in both external shape and inner features. Up to the mid-interkinesis, where the chromosomes have lost their polar orientation, and run irregularly and sinuously as rather long and slender threads, the nucleus, which is of a lenticular shape in side view, becomes ovoidal in this stage, and after this it is spherical. On the basis of this change in shape, figures drawn at different stages in the interkinesis can be arranged in a continuous series in the due order without much difficulty. The stages in the interkinesis proceed at a comparatively rapid pace in this plant, so that transformation of the chromosomes into the anastomosis condition to form a reticulum does not take place.

The split chromosomes or dyads are at first so orientated in the interkinesis nucleus as to have their region of spindle fiber insertion directed towards the poles (Fig. 25). A change in the configuration of the dyads becomes then markedly perceivable. The arms of the dyads become apart from each other until they stretch their arms in the form of X's or widely opened double V's in the case of the isobrachial chromosomes and a cross of J's in that of the hetrobrachial ones, both kinds of chromosomes thinning out and presenting a sinuous appearance to a greater or less extent as they stretch further.

Even at this stage the partners of the dyads or longitudinal halves remain still conjoined with each other at the point of spindle fiber attachment (Fig. 26). In the next stage these arms become elongated more and more, and run sinuously (Fig. 27). This stage is that which we call here " mid-interkinesis". In Fig. 27 a pair of the split chromosomes appears to make a cross at a point marked with the letter *a* but really they do not cross each other. They are only attached at their sides at the point (*a*) of spindle fiber insertion. The chromosomes then become gradually contracted and lose their sinuosity, and the two sister longitudinal halves or partners of the dyad approach each other (Figs. 29 and 30), when they come to form the homotype equatorial plate. No longitudinal split is observed to take place during the interkinesis, but the split that is observed in the anaphase of the heterotype division is the split of the homotype division.

The change in the chromosomes in their morphological features from the mid-interkinesis down to the homotype metaphase is, briefly speaking, a repetition in the inverse order of what has taken place in the stages from the heterotype telophase upwards to the mid-interkinesis. The chromosomes become thicker and thicker as the stage proceeds towards the homotype metaphase (Figs. 28 and 29.)

Homotype mitosis. When the nuclear membrane and nucleolus disappear, the nuclear plates simultaneously appear in the sister cells in the majority of cases, but not seldom they may be formed successively as is indicated in Fig. 33. In this figure one of the divisions is in the stage of metaphase while the other is in the anaphase.

The chromosomes of the homotype metaphase arrange themselves on the equatorial plane so as to have their angles with the spindle fiber insertion directed towards the center of the arrangement and the free ends of the arms towards the periphery of the cell (Figs. 31 and 32). The longitudinal halves are joined together at the points of spinble fiber insertion, and more or less free from each other near their ends. The configuration of the chromosome arrangement presents a striking contrast to the chromosome ring in the heterotype mitosis

(comp, Text-fig. 2 and Fig. 12 a, b with Fig. 31). All the chromosomes are quite free from one another.

Comparing the shape of the homotype chromosomes with that of those in the heterotype mitosis, we find that the former is much more slender than the latter. In his study of *Oenothera*, DAVIS (1909) says, "A comparison of this stage (homotype metaphase)[1] with the metaphase of heterotype mitosis shows that the daughter chromosomes are not so large as those of the latter mitosis and have the form of short and sometimes slightly bent rods rather than of V's."

The number of chromosomes counted in the metaphase was, in most cases, 6, the characteristic number of this plant, but not seldom a case was found where one of the sister equatorial plates showed 5 chromosomes and the other 7 (Fig. 32 and also comp. Table 1). This latter fact is obviously due to the abnormal division of heterotype mitosis in which 5 chromosomes pass to one pole and the remaining 7 to the other (Fig. 22 a, b).

The spindle fiber insertion becomes clearly manifest, when the chromosomes are passing to the poles. Two of the 6 chromosomes show the subterminal insertion and the remaining 4 appear to have median (Fig. 34). The mode of spindle fiber insertion is, therefore, the same in both heterotype and homotype divisions (see Text-fig. 3).

In an early stage of daughter nuclei resulting from the homotype mitosis, the coromosomes take up polarized orientation as is shown in Fig. 35. The change in the morphological features of the chromosomes during further development of the nuclei is similar to that found during interkinesis, except the spiral structure of chromosomes which is clearly observable in these nuclei. As the stage proceeds, the chromosomes enter into a complicated condition of anastomosis. No lagging chromosomes are found in the homotype mitosis and, generally speaking, the division proceeds normally. The resulting pollen tetrads generally appear to be normal, the four nuclei being of nearly the same size

[1] The words in brackets were inserted by the writer.

and shape, notwithstanding the fact that in the heterotype division abnormal distribution of chromosomes may sometimes occur (Fig. 36).

DISCUSSION

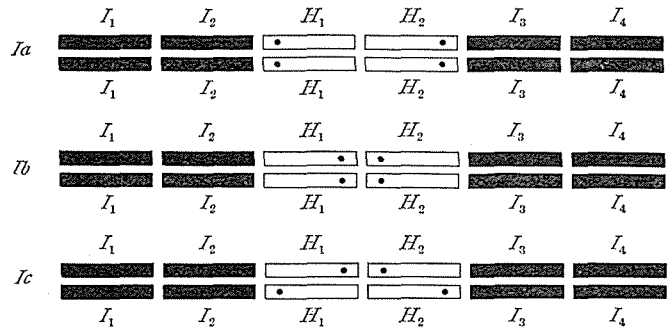
While some authors interpret the mode of syndesis in a plant as parasyndesis, others interpret it as telosyndesis in the same plant as, for instance, in *Lilium, Tradescantia, Allium, Vicia* and *Galtonia.* The critical basis which determines the mode of syndesis rests on the point whether the doubleness of the nuclear threads seen in the early stage of prophase is to be regarded as due to the longitudinal split of a chromosome or to the close approximation of the two homologous chromosomes. While correct judgment on this point is very difficult in most cases, there is a case which appears in a later prophase to be telosyndesis in every respect. This is the case of *Oenothera,* in which the chromosomes appear to have been united end-to-end into a chain or chains from some early stage of prophase to the time of disjunction, and the plant has been long held by the majority of authors as one presenting a remarkable example of telosyndesis. The end-to-end conjoining of some chromosomes in a stage slightly before metaphase has been found by MIYAKE (1905) in *Tradescantia virginica* the mode of syndesis of which has been assumed by him as parasyndesis on the basis of his critical study of the early history of chromosome development. This seems to suggest that there is a certain stage in the early history of *Oenothera* chromosome development which needs closer investigations before the plant is decided to be one whose mode of syndesis is telosyndesis. In his study of *Oenothera,* BOEDYN (1924 and 1925) came to the conclusion that the homologous chromosomes conjugate parasyndetically in the early stage. If in *Oenothera* there is a pair of chromosomes of a particular shape among others, the study of the relative position of these chromosomes in the ring at metaphase should confirm BOEDYN's conclusion. Unfortunately, however, in *Oenothera* all the chromosomes are of the same size and shape, and there is no morphological characteristic which can clearly distinguish one

chromosome from the others.

In *Rhoeo discolor,* 4 chromosomes can be clearly distinguished from the others by the difference in the mode of spindle fiber insertion. This difference will, taking the difference in the mode of conjoinment of the two contiguous heterobrachial or J-shaped chromosomes together into account, allow us, at least to some extent, to discuss the mode of syndesis which takes place in this plant. In the present case it is possible to consider three different original arrangements of chromosomes, from which the chromosome ring in the metaphase is to be derived. They are: I) double chain type, II) radial type and III) ring type.

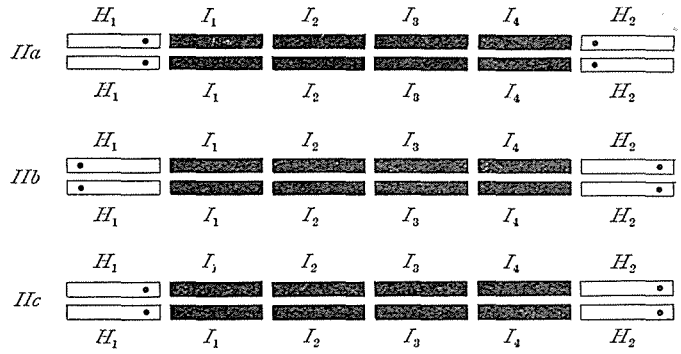
I) *Double chain type.* The double chain type may be divided into two Sub-types 1 and 2 which fulfil the cytological requirement that the 4 isobrachial chromosomes are inserted in the ring on each side of the 2 conjoined heterobrachial chromosomes.

1. The two pairs of heterobrachial chromosomes H_1H_1 and H_2H_2 occupy the middle positions on the double chain I_1I_1, I_2I_2, H_1H_1, H_2H_2, I_3I_3, and I_4I_4 in which each homologous member takes the opposite position to the other. According to the cytological data we obtained, the mode of the conjoinment of the two contiguous pairs of heterobrachial chromosomes, H_1H_1 and H_2H_2 is always the same in the same ring; the chromosomes of both pairs may conjoin with each other at the distal ends (Text-fig. 5. Ia) ar at the proximal ends (Text-fig. 5. Ib).



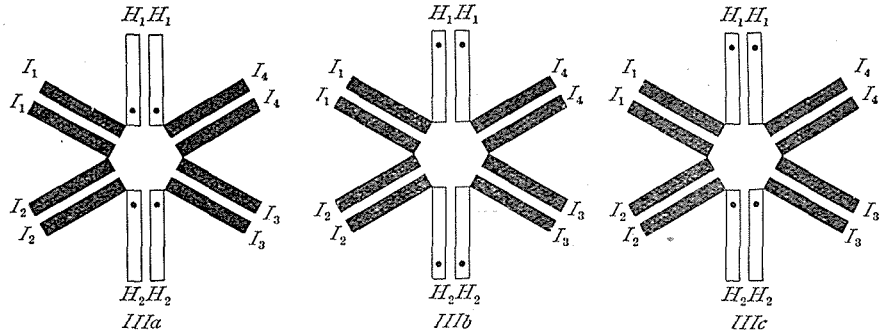
Text-fig. 5. Two possible (*Ia* and *Ib*) and one unrealizable (*Ic*) mode of conjoinment of the heterobrachial pairs H_1H_1 and H_2H_2 in the double chain type 1. A dot in the heterobrachial chromosomes indicates the point of spindle fiber attachment of each chromosome.

2. Instead of taking the middle position in the chain, each pair of the heterobrachial chromosomes takes a side position. Here we have three possible cases of arrangement as illustrated in Text-fig. 6. IIa, IIb and IIc.



Text-fig. 6. Three possible modes of conjoinment of the heterobrachial pairs in the double chain type 2.

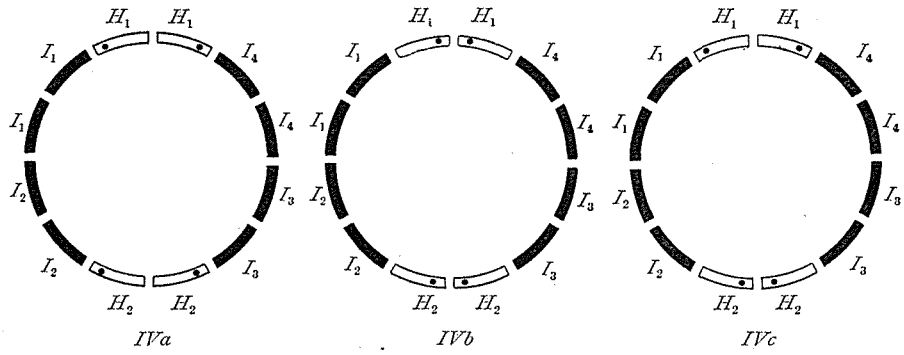
II) *Radial type.* KIHARA (1927) has observed the arrangement of three pairs of prophasic chromosomes in the radial form in *Rumex acetosella* (see his figures 17–19 and 30–32), and has interpreted the chromosome ring found in that plant as derived from this radial tpye. In the present case we have three different possible cases as shown in Text-fig. 7. IIIa, IIIb and IIIc.



Text-fig. 7. Three possible modes of conjoinment of the heterobrachial pairs in the radial type.

III) *Ring type.* In this type we assume that the original form of the chromosome ring is a ring composed of a single chain of

chromosomes conjoining end-to-end after the telosyndetic scheme. Here we have also three different possible cases as seen in Text-fig. 8. IVa, IVb and IVc.



Text-fig. 8. Three possible modes of conjoinment of the heterobrachial pairs in the ring type.

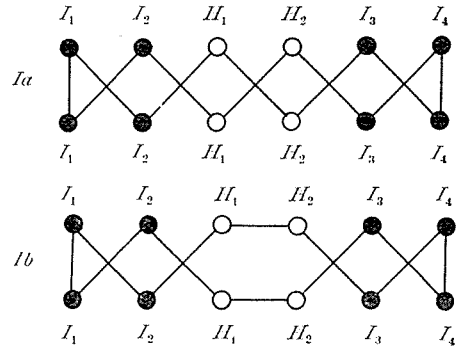
We shall now enter on the question which type of the various forms of chromosome arrangement considered above is real in this plant. In all the types except Sub-type 1 of the double chain type (Text-fig. 3), there are three possible modes of conjoinment of the heterobrachial chromosomes (Text-figs. 6, 7 and 8); each two heterobrachial chromosomes may conjoin in pairs in the same mode in the same ring either at their proximal ends or at their distal ends (IIa, b; IIIa, b and IVa, b), or they may conjoin at the proximal ends in one pair and at the distal ends in the other (IIc, IIIc and IVc). In the first type of the double chain, however, this last mode of conjoinment (Ic) is not to be expected, because all the homologous chromosomes must be arranged symmetrically with the ends of the same sign side-by-side. So far as the present investigation goes, no case was actually observed where in the same ring the heterobrachial chromosomes of one pair were conjoined at their proximal ends and those of the other at their distal ends. Whenever the heterobrachial chromosomes of one pair were found conjoined at the proximal ends, the conjoinment in the other pair was also at the proximal ends, and when at the distal ends, it was also at the distal endsin the other.

This cytologiical datum is fulfilled only by Sub-type 1 of Type I. Thus the writer is inclined at present to regard the chromosome ring in this plant as an outcome of opening out of the double chain or the parasyndetic arrangement of the homologous chromosomes which must have taken place in a certain early stage of prophase.

Recently DARLINGTON (1929 a, b) put forward a hypothesis of segmental interchange of chromosomes through which he interprets the formation of the chromosome ring in *Rhoeo*. If this interpretation is correct the heterobrachial chromosomes must conjoin with each other only in a definite mode at their proximal or their distal ends, and not in both modes sometimes at their proximal ends and sometimes at their distal. Our cytological results show, however, that both these modes actually occur.

We have next to make some remarks on the non-disjuuction of chromosomes which is indicated as taking place by the fact that we have cases where one of the sister groups of the chromosomes in the homotype metaphase shows 5 chromosomes, and the other 7. If we adopt in the present case any other type of the chromosome syndesis considered above than Sub-type 1 of Type I, we expect an occurrence of another type of non-disjunction in which the chromosome number itself remains normal, because we have the peculiar fact of the heterobrachial chromosome pair on disjunction passing to the same pole when they are conjoined with each other at their proximal ends. In so far as the non-disjunction is concerned with the peculiar behavior of the heterobrachial chromosomes, Sub-type 1 of Type I realizes the non-disjunction of this type neither in the case of the chromosomes passing to the same pole nor in that of their passing to different poles, as can be readily seen from Text-fig. 9.

But DARLINGTON (1929b) has observed some complicated types of non-disjunction of the chromosomes in this plant. They may be classified into three types: a) double non-disjunction on the opposite side; b) double non-disjunction on the same side; c) quadruple non-disjunction, three on the same side and one on the opposite. We



Text-fig. 9. Shema showing disjunction of chromosomes arranged according to Sub-type 1, Type I. The upper chromosomes pass to the upper pole and the lower ones to the lower. In both *Ia* and *Ib* all the chromosomes separate disjunctionally.

shall now have to examine these cases from the standpoint that the mode of syndesis in this plant is of the double chain type called above Type I, Sub-type 1.

a. In this case the resulting pollen grains may be all viable (Scheme a_1) or all non-viable (Scheme a_2).[1] In both a_1 and a_2 the resulting pollen grains have the normal number of chromosomes.

a_1 — I_2 II_1 I_3 ⋮ I_4 II_2 I_1 (viable)
I_1 II_2 I_4 ⋮ I_3 II_1 I_2 (viable)

a_2 — I_2 II_2 I_3 ⋮ I_4 II_2 I_1 (non-viable; no II_1)
I_1 II_1 I_4 ⋮ I_3 II_1 I_2 (non-viable; no II_2)

b. In this case the resulting pollen grains with 7 chromosomes may be viable (b_1 and b_2) or non-viable (b_3). In schemes b_1 and b_2 it is shown that in these cases one of the heterobrachial pairs disjoins in the abnormal way, thus resulting in non-disjunction, as in the non-disjunction of the general case.

b_1 — I_1 II_1 II_2 I_4 ⋮ I_3 II_2 I_2 (viable)
I_2 I_3 ⋮ I_4 II_1 I_1 (non-viable; no II_2)

b_2 — I_1 II_2 I_4 ⋮ I_3 I_2 (non-viable; no II_1)
I_2 II_1 I_3 ⋮ I_4 II_2 II_1 I_1 (viable)

b_3 — I_2 II_1 I_3 I_4 ⋮ I_3 II_1 I_1 (non-viable; no II_2)
I_1 II_2 ⋮ I_4 II_2 I_2 (non-viable; no II_1 and I_3)

[1] In these schemes, if the zigzag chain of chromosomes is folded at the point where the dotted line crosses the chain the double chain will be produced.

c. In this case all the pollen grains produced will be non-viable (Scheme *c*),

$$c\ \frac{I_1\ I_2 \qquad\qquad I_3\ I_4 \vdots\ I_3\ H_2 \qquad I_2}{H_1\ H_2 \qquad\quad \vdots\ I_4 \qquad\quad H_1 \qquad I_1}$$

(non-viable; no H_1)
(non-viable; no I_2 and I_3)

To put it briefly, in these complicated types of non-disjunction we may have viable pollen grains with 6 and 7 chromosomes and non-viable ones with 5, 6 and 7 chromosomes. These expected results are in accord with DARLINGTON'S finding that in some of the pollen grains invesetigated 7 chromosomes were counted, and also with his expectation that there may be non-viable pollen grains with the normal number of chromosomes. The number of sterile pollen grains must be greater than we can estimate from the result of non-disjunction give in Table I.

SUMMARY

I. In most cases, the nucleus in the synizetic stage lies eccentrically in the cell, towards the side to which the synizetic knot of the nuclear threads is shifted.

2. The nuclear thread becomes differentiated into thicker and thinner portions, the thicker portions growing single chromosomes conjoined end-to-end into a chain or ring.

3. The 12 univalent chromosomes in the diakinesis make a ring or chain, and there is no tendency towards forming gemini or pairs.

4. The spindle fiber attachment is subterminal in 4 chromosomes of the 12 and seems to be median in the remaining 8.

5. The position of these two kinds of chromosomes in a ring is definite. The 4 heterobrachial chromosomes occupy two by two the diagonally apposite position to each other and on each side of the ring between these two sets of chromosomes 4 isobrachial chromosomes are inserted. The mode of conjoinment of the two contiguous heterobrachial chromosomes is the same in the same ring. They may be conjoined either at their proximal ends or at their distal ends.

6. The chromosomes linked together are arranged in the equatorial

plane so as to present a zigzag appearance in side view. The two contiguous heterobrachial chromosomes pass to different poles or the same pole according to whether they are conjoined with each other at their distal ends or their proximal ends. In both cases the contiguous isobrachial chromosomes pass to the opposite poles alternately.

7. A polar granule is sometimes found in chromosomes at their point of spindle fiber insertion in the meta-anaphase of the heterotype division. It is connected by achromatic substance with the main body of the chromosomes.

8. A certain irregularity which causes non-disjunction is sometimes found in the heterotype metaphase. Some lagging chromosomes are also found in the anaphase.

9. In the heterotype anaphase the chromosomes show a clear longitudinal fission for the homotype division.

10. Individual chromosomes can be followed throughout the interkinesis. No anastomosis takes place among them.

11. The behavior of the chromosomes in the homotype division is normal. All pollen tetrads produced are normal in appearance in spite of the fact that the phenomenon of non-disjunction takes place in ca. 33 % of the cases.

12. On the basis of the particular mode of conjoinment of the two contiguous heterobrachial chromosomes, the mode of syndesis which takes place in this plant is discussed. The conclusion to which we are led is that the mode of syndesis is a sort of parasyndesis in which the homologous mates are prematurely separated from each other, but the segmentation of the spireme thread is postponed, so that a chromosome ring is produced.

In conclusion the writer wishes to express his cordial thanks to Prof. Y. KUWADA for his kind suggestions and criticism throughout the work.

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EXPLANATION OF PLATES

All the figures were drawn with the aid of ABBE'S camera lucida using ZEISS' imm. 1/12 and comp. oc. 12.

Plate XIII

Fig. 1. Synizesis. Loops of nuclear threads emerging from the chromatic knot. The nucleus is found eccentrically in the cell towards the side on which the chromatic mass is dislocated.

Fig. 2. Later stage showing irregular thickening of the threads and their end-to-end conjoining at some points.

Fig. 3. Open spireme stage.

Fig. 4. Second contraction. The nuclear threads are much contracted into thicker ones connected by very thin threads.

Figs. 5 and 6. Advanced stages of the second contraction.

Fig. 7. "Diakinesis". Chromosomes united end-to-end are clearly visible. There is evidently no formation of gemini.

Fig. 8. Unfolding stage from the third contraction, preparing for the formation of heterotype nuclear plate.

Fig. 9. Showing spiral structure of chromosomes in the stage of unfolding from the third contraction.

Fig. 10. Three contiguous chromosomes in the anaphase of the heterotype division, showing the doubleness of a chromonema.

Fig. 11 *a* and *b*. Two optical sections of a chromosome ring in metaphase. In this ring a chromosome is suspended between two chromosomes destined to go to the upper and the lower pole respectively (*b*). Two contiguous chromosomes (the one at upper corner on the right in *a* and the other in the same position in *b*) are destinted to go to the same pole (to the upper in the figure); the others alternately to the upper and the lower pole.

Fig. 12 *a* and *b*. Metaphase reproduced in two optical sections. The heterobrachial chromosomes are found inserted two by two in pairs between two sets of the 4 isobrachial chromosomes. In each pair they are conjoined at their proximal ends and they pass to the same pole.

Fig. 13. Metaphase showing two pairs of the heterobrachial chromosomes conjoined at their distal ends. In each pair the two chromosomes are destined to go to the different poles.

Fig. 14. Irregular distribution of chromosomes in metaphase.

Fig. 15. Showing a chromosome in late metaphase with the median spindle fiber attachment. A small granule is seen at the point of attachment which is connected by achromatic substance with the main body of the chromosome.

Fig. 16. Late anaphase. 6 longitudinally split chromosomes at each pole with an X-shaped appearance.

Fig. 17. An irregular distribution of chromosomes having 7 chromosomes at one pole and 5 at the other.

Plate XIV

Fig. 18. Late anaphase with 2 lagging chromosomes near the equatorial region. These lagging chromosomes are longitudinally divided.

Fig. 19. A split lagging chromosome with spindle fibers inserted in each pair.

Fig. 20. Showing a chromosome bridge between the two chromosome groups.

Fig. 21. Polar view of late anaphase, showing 5 chromosomes arranged in the form of a ring around the sixth.

Fig. 22 *a* and *b*. Two sister chromosome groups at poles, 5 chromosomes at one pole (*a*) and 7 at the other (*b*).

Fig. 23. Polar view of late anaphase, showing 6 chromosomes arranged in the form of a circle.

Fig. 24. Telophase just prior to the beginning of interkinesis.

Fig. 25. Early interkinesis. All the longitudinally split chromosomes are oriented towards the pole.

Fig. 26. Advanced stage. The polar orientation of chromosomes is put out of order.

Fig. 27. Mid-interkinesis. The separated longitudinal halves of each chromosome are much elongated, presenting a coil appearance. They are connected with each other at the point of spindle fiber attachment as marked by the letter *a*.

Fig. 28. Late interkinesis. The coiled chromosomes are thicker.

Fig. 29. Advanced stage. the chromosomes are much thicker and shorter, the coiled appearance being lost.

Fig. 30. The stage just before the homotype metaphase.

Fig. 31. Homotype metaphase in polar view. The chromosomes lie on the equatorial plane, having their points of spindle fiber attachment towards the center of the arrangement.

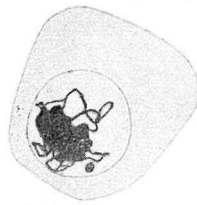
Fig. 32. Two sister nuclear plates having different numbers of chromosomes, one with 7 chromosomes and the other 5.

Fig. 33. Two sister spindles in different stages of mitosis, one in metaphase and the other in anaphase.

Fig. 34. Homotype anaphase showing points of spindle fiber attachment of chromosomes. Two chromosomes out of the 6 in a group are subterminal and the other 4 nearly median.

Fig. 35. A later telophase. The coiled chromosomes are oriented towards the poles.

Fig. 36. 4 pollen tetrads within the wall of mother cell. Chromosomes are coiled.



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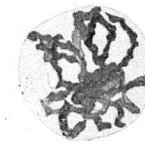

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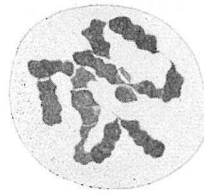

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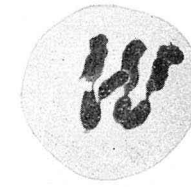

11*b*

12*a*

12*b*

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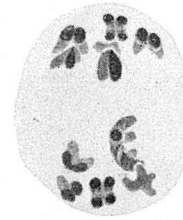

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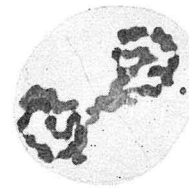

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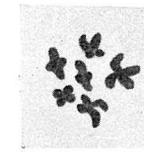

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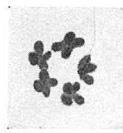

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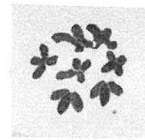

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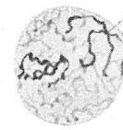

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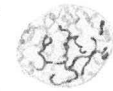

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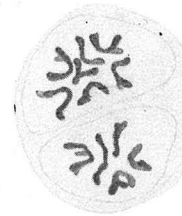

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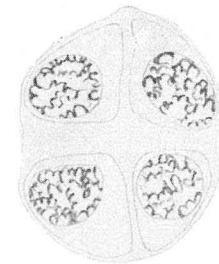

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