TETRAPLOIDY IN RHOEO DISCOLOR

By

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INTRODUCTION

The presence of rings or chains of chromosomes during the meiotic divisions of plants has been known for nearly forty years. Gates (1907a) first observed chromosomes attached end to end in hybrids of Oenothera lata, deVries and Oenothera lamarckiana, Hort. He did not realize, however, that a regular arrangement of the chromosomes was present. In these same hybrids, Gates (1907b) described the chromosomes as joined together to form rings. He thought the end to end union was the result of an upset condition resulting from a state of mutability. Davis (1909) observed in Oenothera grandiflora, Ait. that the chromosomes became associated in seven ring-shaped pairs. They remained intact as rings until separation of their halves at early anaphase. In Oenothera franciscana, Bartlett., Cleland (1922) found four of the fourteen chromosomes attached end to end in a ring. He also observed that these rings were of a given size and that such chromosomes configurations were regular.

Later, Cleland (1923) compared several species of Oenothera and each of these showed a constant arrangement of the chromosomes in rings and pairs bearing out his previous observations on Oenothera franciscana, Bartlett. Each displayed at least one large circle consisting of a definite number of chromosomes united end to end. He also observed that contiguous chromosomes were attached to spindle fibers leading to opposite poles. In more detail, Cleland (1924) discussed the chromosomes of Oenothera franciscana sulfurea. In this plant of hybrid origin he observed a closed circle of twelve chromosomes and a single pair. Sometimes this pair was linked with the chain but later it became separated. This chain formation persisted through the first meiotic metaphase. During the anaphase alternate chromosomes went to opposite poles resulting in a zig-zag separation

of the chromosomes in the chain. This distributed the chromosomes equally at the two poles. In about sixteen percent of the cases the metaphase arrangement was upset. In such cases six went to one pole while eight went to the opposite pole and he observed nuclei with these numbers instead of the normal seven. The constant grouping of the chromosomes in late prophase into groups of twelve and two as well as their distribution during anaphase suggested that the chromosomes had definite positions with reference to one another and were not scattered in the nucleus without order. This failure of all but one pair of chromosomes actually to pair suggested that this species of Oenothera was largely heterozygous. Five additional species of Oenothera were reported by Cleland in 1925. They also showed this end to end arrangement of chromosomes which resulted in rings of chromosomes of various sizes in the different species.

Later, in more detail Cleland (1926a) studied Oenothera muricata, L. He found that a typical diakiensis stage was absent in this plant. All fourteen of the univalent chromosomes were united end to end to form a closed circle or an open chain. Later, in Oenothera biennis, L., Cleland (1926b) found that the circle of six and the circle of eight previously reported for this plant remained throughout metaphase. By 1929 many configurations in a large number of races and hybrids of Oenothera had been reported. These configurations confirmed the first observations of Gates, Davis, Cleland and others that some of the chromosomes in Oenothera were joined end to end and that a regular arrangement of the chromosomes was present.

A satisfactory explanation of this end to end arrangement of the chromosomes was suggested by Belling (1927). At this time Belling (1927) suggested that segmental interchange between non-homologous chromosomes would account for the end to end arrangement of the chromosomes as found by Gates, Davis,

Cleland and others. The possible explanation of this end to end arrangement of the chromosomes was furnished by Blakeslee and Cleland (1930) who showed by a comparison of the chromosomal phenomena in Datura and Oenothera that segmental interchange was a possible basis for circle formation in both genera. Immediately after this Cleland and Blakeslee (1930) offered a specific line of evidence that segmental interchange has been responsible for the evolution of circles in Oenothera. Cleland and Blakeslee (1930) were able to predict on the basis of the hypothesis of segmental interchange what the chromosomal configuration ought to be in certain complexes. From this evidence it was concluded that segmental interchange was probably the basis of circle formation in Oenothera. Hakansson (1930) offered further evidence supporting the view that the rings of Oenothera had arisen through interchange between nonhomologous chromosomes in a study of several mutants of Oenothera Lamarckian. He also observed that alternate chromosomes passed to opposite poles. In addition Emerson and Sturtevant (1931) were able to predict what configurations were possible when certain combinations of Oenothera were made. They tested these complexes by an application of the translocation hypothesis. Sturtevant and Dobzhansky (1930) had studied four cases of translocations involving two large v-shaped pairs of chromosomes in Drosophila melanogaster. As a result somatic pairing was upset and the translocated arms of the chromosomes paired with an arm belonging to another chromosome. At this same time McClintock (1930) showed that a ring was formed by a reciprocal translocation or segmental interchange between the 2nd and 3rd smallest chromosomes in a semi-sterile stock of Zea. This further substantiated the work of Belling, Cleland, Blakeslee, Hakansson and others in explaining the ring formations in Oenotheras.

Observations of the end to end arrangement of the chromosomes was not restricted to Oenothera. Other plants were found to form chains or circles of chromosomes. As early as 1912, Digby illustrated this end to end arrangement of chromosomes in a study of the cytology of Primula. In a study of triploid maize Randolph and McClintock (1926) found that trivalents of the metaphase complement occasionally appeared as three chromosomes attached end to end. Newton (1926), also observed in Tulipa linifolia Regel that all the chromosomes were terminally attached. End to end attachment of chromosomes were observed by Belling and Blakeslee (1926) in trivalents of Datura. In addition to these Hakansson (1929a) and Sansome (1932) observed rings in Pisum sativum L. Rings or chains of five or more chromosomes were found in Aucuba japonica Thunb. by Heurman (1929). This plant proved to be a tetraploid having four sets of eight chromosomes. Darlington (1929b) supplemented the list of ring forming plants while observing this end to end attachment of chromosomes in Tradescantia and Zebrina pendula Schnizl. Again in 1931, Darlington showed that terminal pairing occurred in a hybrid of Triticum. The tendency for alternating chromosomes to pass to opposite poles as first reported by Cleland (1924) in Oenothera was also observed by Hoar (1931) in Hypericum punctatum, Lam. in which he also reported the chain formation of the chromosomes. Certain Avena hybrids were found to show a ring of 4 chromosomes at first metaphase (Ellison, 1940). Finally, chromosome configurations in Paeonia californica Nutt. included all combinations of pairs or rings possible with 10 chromosomes (Walters, 1942).

It may be seen, therefore, that the terminal attraction of chromosomes was not restricted to any one plant. It was also established that this end to end attachment of the chromosomes was due to a reciprocal translocation or segmental interchange between non-homologous chromosomes and that this phenomena was responsible for the formation of the rings or chains observed.

Pairing of entire chromosomes occurred only when the chromosomes were homologous throughout their entire length. When such rings or chains occurred it was found that the chromosomes were regularly arranged and that alternate chromosomes passed to opposite poles.

The first observations on the end to end arrangement of the chromosomes in Rhoeo discolor were reported by Belling (1927), but he did not make a detailed study of chromosome behaviour. He found that univalent chromosomes of Rhoeo discolor were sometimes united at reduction division metaphase into a chain of V's. Several years later Darlington (1929b) made a more complete study of the chromosomes of Rhoeo. He found in the majority of cases a ring of 12 chromosomes at metaphase. Frequently, however, Darlington (1929b) observed a chain or chains of chromosomes. This occurrence was explained as a result of one or more chiasmata between chromosomes which terminalized before metaphase. The approximate distribution of chromosomes was as follows: ring, 30 percent; one chain, 45 percent; two chains, 20 percent; three chains, 5 percent. The 12 chromosomes were divided into the several chains in different proportions; where two chains were present the combinations of 11 and 1, 10 and 2, 9 and 3, 8 and 4, 7 and 5 were observed and where three chains were present, the combinations 7 and3 and 2 and 6 and 4 and 2 were observed. At early metaphase the ring or chain of chromosomes was found to arrange itself with reference to the spindle. Successive chromosomes in the chain or ring were so oriented that alternate chromosomes passed to opposite poles. Lagging chromosomes were noted and these occasionally divided at the first division.

Kato (1930) also made a cytological study of meiosis in the pollen mother cells of Rhoeo discolor. He also found that the chromosomes were joined end to end. The contiguous chromosomes passed to opposite poles as previously described for Oenothera. Kato (1930) also observed that the

chromosomes were arranged in definite order: 2 heterobrachial chromosomes, 4 isobrachial chromosomes, 2 heterobrachial chromosomes and 4 isobrachial chromosomes. The heterobrachialchromosomes were found to pair at either their proximal or their distal ends. At anaphase some few chromosomes (usually two) lagged behind the others. These lagging chromosomes did not form micronuclei but passed to the poles at random. Interkinesis progressed so rapidly that no reticulum was observed. During the second division chromosome rings or chains did not appear and this division was regular.

Sax (1931) in a similar study of Rhoeo discolor observed that 12 chromosomes usually were arranged in a ring, or one or two chains at 'diakinesis' of the first meiotic division. Chromosome chains were found more frequently than rings. In both rings and chains the chromosome frequency was found to be the same. He observed that alternate chromosomes passed to opposite poles in about half the first meiotic divisions. The chromosomes retained their individuality during interkinesis and again the second division was observed to be normal. Pollen grains were found to be 80 to 90 percent imperfect morphologically so that the plants only were partially fertile.

Haselwarter (1937) described meiosis in the pollen mother cells of 2N Rhoeo plants which were treated by temperature shock. Upon studying the divisions of the pollen mother cells he observed that they contained 24 chromosomes arranged in a closed ring or in 2 rings of 12 each.

Dermen (1938) was the first to observe the effect of colchicine on the pollen mother cells of Rhoeo discolor and (p. 221) his photomicrographs indicated then that chains or rings of chromosomes rather than paired chromosomes, resulted from such treatment. Later, he treated seedlings of Rhoeo and six of these were made available to the author in 1940.

Akemine (1940) also treated Rhoeo to a low temperature for several weeks and found that the anthers contained a considerable number of 4N pollen mother cells among the normal diploid ones. He observed univalents, ring bivalents,

chains of three or more chromosomes, rings of 4 chromosomes, and polyvalents bearing triple terminal chiasmata during metaphase I.

According to the theory of segmental interchange we should expect that the number and position of chromosomes in a diploid would be constant in rings or chains only because two segments would be present with the same homologous attraction. This is apparently the case in diploid Rhoeo discolor as previously reported by Sax, Darlington, Kato, and others. In such a plant the 12 chromosomes could be represented by the following scheme:

AB CD EF GH IJ KL

BC DE FG HI JK LA

The chromosome complement of a tetraploid plant originated through a duplication of the above chromosomes should accordingly be:

AB BC CD DE EF FG GH HI IJ JK KL LA
AB BC CD DE EF FG GH HI IJ JK KL LA

Every chromosome should thus have one entirely identical mate and the possibility of forming bivalents is thus evident. At the same time four terminal segments are present with the same homologous attraction. This, then, affords the possibility of forming rings or chains as well as bivalents.

When some colchicine induced tetraploid plants were made available by Dermen, this presented an excellent opportunity to further study the situation which had been observed by him in 1938 and to compare these with the observations of both Haselwarter (1937) and Akemine (1940). After this study had been completed Walters and Gerstel (1948) reported on a tetraploid Rhoeo which appeared spontaneously in a population of seedlings. They observed that chains, rings, and 'ring pairs' occurred with varying frequency during metaphase I. These observations afforded a further opportunity to compare the behaviour of the chromosomes of this natural tetraploid with those of the colchicine induced tetraploid and others mentioned above.

MATERIALS AND METHODS

Prior to 1940 Dermen treated seedlings of Rhoeo discolor with colchicine for varying lengths of time by the shallow immersion methods. From these there eventually developed six mature plants which were transferred to the writer for investigation.

Root tips were fixed in Carnoy's solution, macerated and stained in 45 percent proprio-carmine. The anthers were used fresh and were crushed and stained in 45 percent proprio-carmine.

Temporary slides observed to be of value were made permanent. Each temporary slide was flooded with 95 percent alcohol until the stain appeared to be replaced with the alcohol under the cover glass. The cover glass, while still flooded with alcohol, was carefully removed with forceps and the slide drained of any excess alcohol. A drop of Diaphane was placed over the materials and the cover glass carefully pressed into its original position.

Photomicrographs were taken of all desirable material with a Bausch and Lomb Type K photomicrographic camera. A Zeiss microscope equipped with a 90X oil immersion objective, n.a. 1.30, was used in conjunction with 10x and 7.5X oculars. The magnification of all photomicrographs was X1500 and the drawings were made to correspond to the magnification of the photomicrographs.

RESULTS

Somatic chromosome number

Soon after the six colchicine treated plants were obtained it was evident that they varied with respect to their chromosome number. All were taller and more robust than the diploids. Of the 6 plants only two produced flowers and each of these had the 4N number of chromosomes as determined

from root tip smears. The other four had 22 chromosomes rather than the expected number of 24. Only one of the 6 plants produced side shoots and that one was a true tetraploid. Viable seed was never found in either of the flowering tetraploid plants in over 3 years observed growth.

Meiosis

A total of 200 pollen mother cells was studied in detail. Early prophase stages are not favorable for detailed analysis. The observations reported were obtained by a careful study of the late prophase and metaphase of M1. It was found that the chromosomes were not uniform in their behaviour in each of the cells studied. Certain associations of chromosomes were observed to occur more frequently than others. In Table 1, it can be seen that short chains appeared more frequently than longer chains. Chains of 2 chromosomes were most numerous. Pairs of chromosomes were found in almost one half of the cells studied. In each cell, however, these pairs never exceeded three. Single, unattached chromosomes were found in 85 of the 200 cells and the number of unattached chromosomes varied somewhat in different cells. Closed rings occurred infrequently. The number of chromosomes in these closed rings varied from 3 to 6. However, rings of 4 were most numerous. A few chains of 24 chromosomes were observed but a complete and closed ring of 24 chromosomes was never found. Eleven cells showed the pairing of 3 chromosomes at one end. However, no pairing of 4 chromosomes at one end was observed. It was possible to determine the exact associations of only 11 of the cells reported in Table I. A more detailed account of these 11 cells is given in Table II. Four of these cells showed chains of chromosomes longer than 8 but again the shorter chains were still more numerous. Pairs occurred in about one half of these cells also.

Figures 1 - 10 illustrate by diagram the typical associations of the chromosomes in those cells in which the entire complement could be determined

with accuracy. A complete open chain of 24 chromosomes is illustrated in Figure 1. Cells showing the varying length of the chains found in these cells may be seen in Figures 2, 3, 4, 5. Shorter chains were more numerous and this was illustrated by all the figures with the exception of Figure 1. Chromosome pairs, associated by chiasmata in their terminal regions, can be seen in Figures 4, 5, 6, 8 and 10. In no case were more than 3 pairs observed in any single cell (Figure 6).

The diagrams in Figures 11, 12, 13 and 14 illustrate the closed rings which included 3, 4, 5, and 6 chromosomes in the ring. Figure 13 is a diagrammatic representation of the cell photographed in Figure 15. Figures 16, 18 and 19 are photographs of cells showing chains of various lengths. Metaphase association is illustrated in Figure 20. The photograph in Figure 17 showing the attachment of 3 chromosomes at one point is illustrated in the diagram in Figure 12.

Positive identification of chromosomes in the chains and rings was not attempted due to the small size of the chromosomes. It was observed that alternate chromosomes in the chains passed to opposite poles although no determination of the frequency of their occurrence could be made. (Figure 21). Lagging chromosomes were also observed as the chromosomes moved to opposite sides of the cells. (Figure 22). During interkinesis the chromosomes did not retain their individuality (Figure 24). The second divisions were normal and resulted in regular tetrads (Figure 2). Pollen grains with 11, 12, and 13 chromosomes were observed (Figures 25, 26, 27).

It was evident from the observations that pairing may occur but was not detected due to the localization of chiasma formation. The terminalization of chiasmata was observed in the figures illustrated during M1. Apparently pairing was restricted to subterminal regions or else pairing occurred throughout the chromosome but chiasma formation was localized in

subterminal regions. It was not possible to distinguish between the two alternatives due to the poor meiotic prophases.

DISCUSSION

Complete rings involving allthe meiotic chromosomes have been described in diploid species of Rhoeo and Oenothera. Rings involving some of the chromosomes have been found in other genera among them Datura and Zea. In 1930 Sturtevant and Dobzhansky presented genetic evidence for segmental interchange between non-homologous chromosomes of Drosophila which resulted in a ring of four chromosomes at meiosis of the translocation heterozygote. In all instances the end to end arrangement of the chromosomes at diakinesis and metaphase 1 has been explained on the basis of segmental interchange.

Although homologous chromosome sectors were present in duplicate in diploid Rhoeo discolor, naturally occurring reciprocal translocations have rearranged the diploid complement to the extent that no individual chromosome had a structurally identical homologue. The minute size of the chromosomes and the absence of favourable meiotic prophases excluded the possibility of a precise analysis of the length of the individual translocated segments. Meiotic metaphase 1 configurations showed rings or chains of 12 chromosomes indicating the formation of chiasmata within the homologous chromosome segments.

It is accepted in typical diploid organisms that each chromosome has a structurally similar homologue. At prophase of meiosis the two homologues pair as bivalents and the association of homologous chromosomes in pairs, initiated by pairing and maintained by chiasmata, continues through metaphase 1. Since each chromosome in diploid Rhoeo is structurally unique, the possibility for the chromosome complement to pair in homologous bivalents would exist only in the tetraploid where each chromosome would have a structurally identical homologue.

In the typical diploid organism, each pair of homologous chromosomes is essentially genetically and structurally distinct from every other pair. The fact that chromosomes are present in similar pairs, with each pair genetically and structurally isolated from every other pair insures the regular formation of bivalents in meiosis. If one of the chromosomes of the complement is present in triplicate, as in trisomes, trivalent formation of homologous chromosomes is usually apparent. Regular bivalent formation in typical diploid organisms is due to the fact that homologous chromosomes and homologous chromosome segments are present only in duplicate.

In diploid Rhoeo, homologous chromosome segments are present in duplicate, but complete homologous pairs of chromosomes are absent due to reciprocal translocations involving each chromosome in the complement. Rings and chains rather than bivalents are present in meiosis. In tetraploid Rhoeo, each chromosome is present in duplicate, as in the typical diploids of other organisms, and the possibility for bivalent formation is evident. However, unlike typical diploid organisms, homologous chromosome segments are present in quadruplicate. The regular formation of bivalents would indicate that the force of attraction between homologous chromosomes is sufficient to prohibit pairing with other homologous segments in the tetraploid complement. If bivalent formation is the exception rather than the rule, it would indicate that attraction between homologous parts is more frequent rather than attraction between homologous chromosomes.

The present study revealed that all kinds of associations from pairing to complete chains had occurred in the 4N Rhoeo induced by colchicine. Chains and pairs occurred in greatest numbers in the present study. This observation conformed with those of Walters and Gerstel (1948). However, in the present study of colchicine induced tetraploid Rhoeo chains of 2

were most numerous while in the spontaneous tetraploid of Walters and Gerstel (1946) 'ring pairs' were found to be most abundant.

The 'ring pairs' of Walter and Gerstel (1948) and the 'ring bivalents' of Akemine (1940) fit the description of the chromosome pairs of the present study. These pairs were found to occur in 81 out of the 200 cells studied and in no case were more than 3 pairs observed in any one cell. Walters and Gerstel (1948), on the other hand, described as many as 8 'ring pairs' in one cell.

The present study revealed closed rings involving 3, 4, 5, or 6 chromosomes. The rings of 4 chromosomes which were most numerous in the present study bear out the observations of Akemine (1940) who reported only rings of 4 chromosomes in the 10 cells reported upon. The work of Walters and Gerstel (1948) revealed rings of 4, 6 and 8 chromosomes. Their observations did not include rings of 3 or 5 as found in the present study of the induced tetraploid. Walters and Gerstel (1948), however, did find rings of 8 which were never observed in the present work.

Akemine (1940) and Walters and Gerstel (1948) both reported triple terminal chiasmata but no more than a double terminal chiasmata was found in the present study of the induced tetraploid of Rhoeo. Three chromosomes were found associated by terminal chiasmata in 11 out of the 200 cells studied. Akemine (1940) described triple chiasmata but no quadruple chiasmata as reported by Walters and Gerstel (1948). These observations are similar to those reported by Seitz (1935) for Oenothera.

As many as 85 cells out of 200 showed univalent chromosomes and the number of univalents varied in each cell of this induced tetraploid. Akemine (1940) and Walters and Gerstel (1948) also reported many univalent chromosomes in their 4N plants.

Lagging chromosomes were observed by the present author in the induced tetraploid and also by Walters and Gerstel (1948) in their spontaneously derived tetraploid but Akemine (1940) did not mention seeing these in his treated plants. The observance of lagging chromosomes in the 4N Rhoeo parallels what Darlington (1929) reported in 2N Rhoeo.

The nuclei of the induced tetraploid became reticular during interkinesis which was in accord with the observations of Simmonds (1945) in which he reported an interphase in a 2N Rhoeo found growing in nature. The second division appeared normal and resulted in regular tetrads being formed agreeing with all other workers for 4N Rhoeo with the exception of Walters and Gerstel (1948) who found that their tetraploid exhibited more irregularity at first telophase and in the tetrad formation than did the diploid Rhoeo. However, some few tetrads of the colchicine induced 4N Rhoeo showed micronuclei which Walters and Gerstel (1948) also reported in their spontaneously derived tetraploid.

It was assumed that 2N Rhoeo was a plant in which translocations had occurred so that now each member of its complement of 12 chromosomes was unlike every other one except for a small portion at the end of the chromosome. In the 2N plant there were only two terminal segments which were identical as illustrated below: A ———— B, B ———— C, A ———— D, D ———— C. Since only two terminal segments of two different chromosomes were alike only those 2 segments could pair and for this reason it was found that the chromosomes formed rings or chains. The conjugation of these segments was due to the fact that the segments were homologous and the chromosomes formed only terminal or subterminal chiasma. No interstitial chiasmata were ever found in this tetraploid Rhoeo bearing out the observations of Bhaduri (1942); although Walters and Gerstel (1948) reported 2 cases of interstitial chiasmata. Complete terminalization of such chiasma

appeared to be the normal procedure since all associations of chromosomes gave the appearance of being attached end to end during late prophase and during metaphase resulting in ring formation as illustrated in Figure 27. This end to end attachment of the chromosomes in 2N Rhoeo had been described by Darlington (1929) and by Sax (1931) and in 4N Rhoeo by Walters and Gerstel (1948). Davis (1943) observed numerous pairs and chains in a tetraploid Oenothera obtained from a hybrid whose diploid ancestor had a single ring of 10 and 2 pairs.

In 4N Rhoeo where the complement of chromosomes has been duplicated there were 4 terminal segments whose ends are designated by letters:

A——————B B——————C
A——————B B——————C

A——————D D——————C
A——————D D——————C

The pairing of the chromosomes again took place only between homologous regions and there was no necessity of supposing that pairing was possible between non-homologous regions. It was logical to assume that at least the parts of the chromosomes which showed connections were homologous in plants like Oenothera, Rhoeo, Aucuba, Tulipa, and others, even though the associations existed in these plants between chromosomes which could not be homologous in the same way as in ordinary plants where two structurally similar homologues were present.

According to the theory of segmental interchange the number and position of the chromosomes in rings in diploid plants should be constant since only 2 segments with similar attraction would be present. This was apparently the case in 2N Rhoeo as reported by Darlington, Sax, and others. In 4N Rhoeo, however, where the tetraploid plants had apparently arisen from a duplication of the diploid chromosome complement the condition was very different. Since the diploid contained all the chromosomes in a single

chain or ring, multiple chiasmata and complete terminalization meets all the requirements for the ring or chain formation. If the present tetraploid plant originated through duplication of the diploid complement every chromosome should have one quite identical mate, therefore, the possibility of forming bivalents was given. There were present, however, at the same time 4 similar segments with the same attraction. Thus, this affords the possibility of rings or chains of chromosomes of any length being formed. This condition was found to be the case in the present study and has been reported by Akemine (1940) and Walters and Gerstel (1948). All possible combinations of chromosomes were found from chains of two up to chains of 24. Most frequently there were found chains of chromosomes and pairs of chromosomes. The association of three ends at one point was rarely found while the association of four ends at one point was not observed in this material. The formation of pairs and chains could be explained here as in the diploid plants on the assumption that chiasmata were possible only at subterminal regions on the chromosomes. Apparently in Rhoeo the region for the formation of chiasmata was so small that one chiasma inhibited to some extent the formation of two and completely eliminated the formation of three chiasmata. The possibilities of one chiasma occurring was physically more probable since each end of the chromosome had three other homologous mates with which it could pair. This then accounted for the chains of various lengths and the large number of pairs that were observed in this material. More associations were rare for two reasons, primarily, the interferences of the 1st chiasma; and secondly, spatial separations of the like ends of more than two chromosomes at the time of conjugation was likely to inhibit their pairing. Thus, there were found few associations where three ends of the chromosomes were involved and two chiasmata were formed. Also the possibility of three chiasmata forming would be completely inhibited

by the presence of the 1st two chiasmata.

If we assumed the following chromosome complement in 4N Rhoeo:

then the scheme of chiasmata formation would follow:

1. Considering only the B segment, chromosome AB may find a pair with either A'B', BC or B'C' which allows for 3 possible combinations (Figure 29).

2. Considering both terminal segments of chromosome AB, chromosome AB could pair with A'B', BC or B'C' and then one of the ends of the other chromosome may pair with another like end (Figure 30).

3. Chromosomes AB, A'B', B'C' and BC must be spatially located so that all four ends may pair providing the region for formation of terminal chiasmata was great enough to allow for three chiasmata to take place (Figure 31). This was not observed, however, in the present study.

Walters and Gerstel (1948) reported that some of the aneuploid seedlings obtained from their spontaneous tetraploid did flower and most of the true tetraploids also produced flowers. In the present study four aneuploids were observed having twenty-two chromosomes but none of these ever produced flowers. The two tetraploids did flower but never produced viable seeds. However, they did grow taller and more robust. One of these true tetraploids produced many side shoots which also flowered and these were found to have twenty-four chromosomes also. The other tetraploid never produced any side shoots during four years observed growth.

SUMMARY

A cytological study of a colchicine induced tetraploid plant of Rhoeo discolor, Hance was made. It was observed that the 4N Rhoeo was a more robust plant than the diploid. An examination of the root tips by the smear technique revealed that the chromosome number was 24 in 2 flowering plants and 22 in 4 non-flowering ones. Examinations of 200 pollen mother cell divisions showed that no bivalent formation occurred. The chromosomes were arranged as univalents, chains of 2 or more, pairs, rings of 3, 4, 5 or 6 chromosomes and polyvalent chromosomes showing a maximum of 2 terminal chiasmata. An explanation of the chromosome behavior is attempted on the basis of terminal chiasmata formation.

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Table I

Chromosome configuration in 200 MI cells of the tetraploid.

TABLE I

Chromosome configuration	Pairs	Chains of													Rings of				2 chiasmata	3 chiasmata	Univalents
		2	3	4	5	6	7	8	9	10	11	12	24	Misc.	3	4	5	6			
Number of cells	91	102	63	41	23	13	16	7	2	3	1	4	1	8	1	6	2	1	11	0	85

TABLE I

Table II

Complete configuration in 11 MI cells of the tetraploid.

TABLE 2

TABLE II

Cell	Pairs	Chains of												No. in Chain	Rings of				2 chiasmata	3 chiasmata	Univalents
		2	3	4	5	6	7	8	9	10	11	12	24	Misc.	3	4	5	6			
1	2	1												18							
2		1	1	1		1															7
3				1										20							
4	2	3	2					1													
5													1								
6	2		2			1												1			2
7	1	3		1		1															6
8	3	1	1	1		1															3
9		2	1	3	1																
10												2									
11		1												22							

Plate I

Fig. 1. A complete chain of 24 chromosomes.

Fig. 2. Chains of 22 and 2.

Fig. 3. Chains of 21 and 3.

Fig. 4. Chains of 18 and 2; and 2 pairs.

Fig. 5. Chain of 7; and 2 chains of 2; 2 pairs; 2 univalents; and a ring of 6.

Fig. 6. Chains of 6, 4, 3, and 2; 3 pairs; and 3 univalents.

Fig. 7. Chains of 4; 3 chains of 3; 3 chains of 2; and 5 univalents.

Fig. 8. Chains of 8; 2 chains of 3; 3 chains of 2; and 2 pairs.

Fig. 9. Chains of 6 and 4; 2 chains of 2; and 10 univalents.

Fig. 10. Chains of 6 and 4; 3 chains of 2; 1 pair; and 6 univalents.

PLATE I

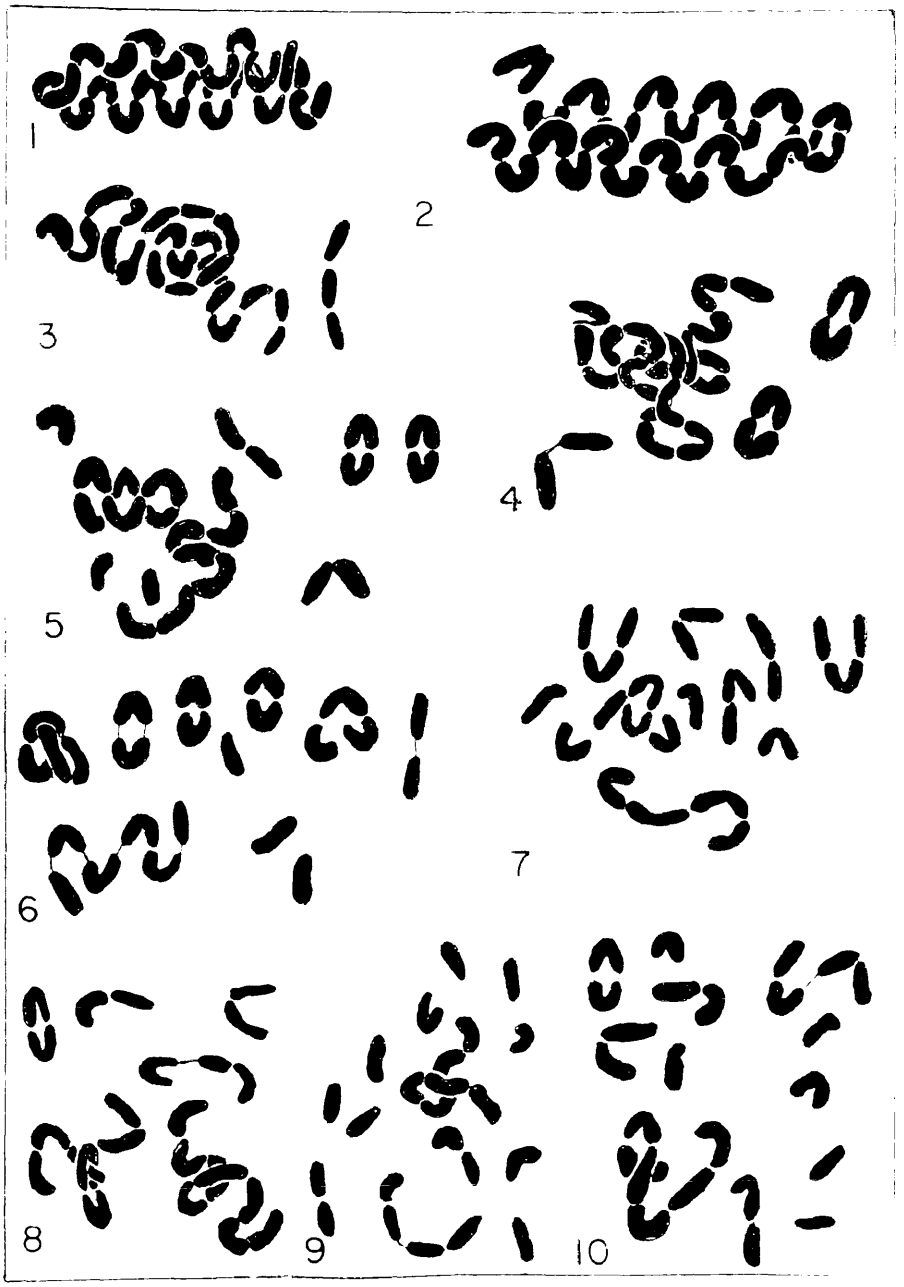


Plate II

Fig. 11. Ring of 3; chains of 5, 3, and 2; 1 pair; and 4 univalents.

Fig. 12. Ring of 5; chains of 9 and 2; 1 pair; 2 univalents and a double chiasmata.

Fig. 13. Ring of 4; chain of 7; 3 chains of 2; and 1 univalent.

Fig. 14. Ring of 6; chains of 7 and 2; 2 pairs; and 1 univalent.

Fig. 15. Shows ring of 4.

Fig. 16. Shows chains of various sizes and univalents.

Fig. 17. Shows the double chiasmata illustrated in Fig. 12.

Fig. 18. Shows chain of 2 and some pairs.

Fig. 19. Shows chains and pairs.

Fig. 20. Shows pairs and univalents.

PLATE 2

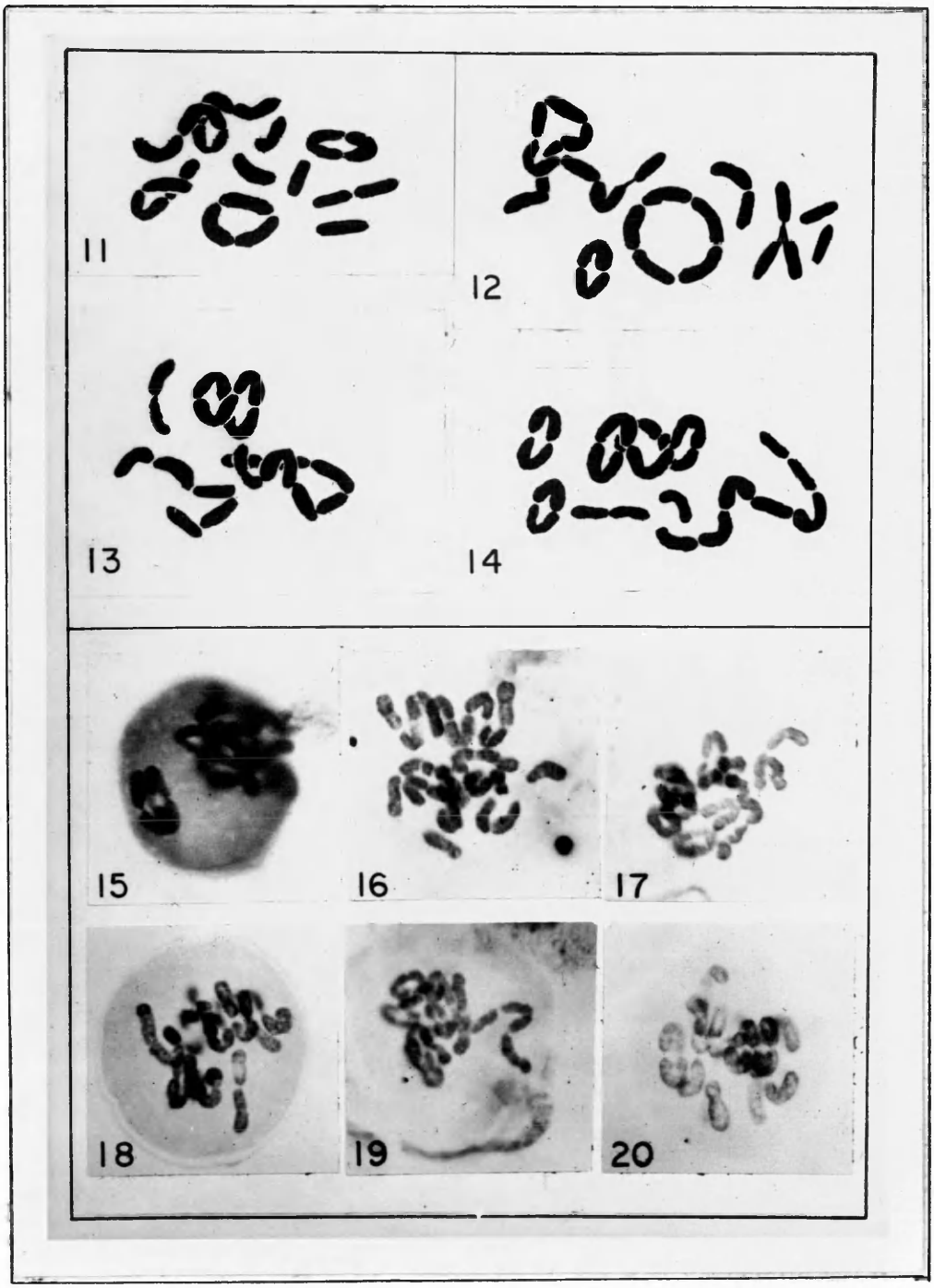


Plate III

Fig. 21. Photo micrograph shows alternate movement of the chromosomes and a chain of 2.

Fig. 22. Photo micrograph shows lagging chromosome already divided at telophase I.

Fig. 23. A tetrad with 1 micromucleus.

Fig. 24. Interphase.

Fig. 25. Pollen cell with 11 chromosomes.

Fig. 26. Pollen cell with 12 chromosomes.

Fig. 27. Pollen cell with 13 chromosomes.

PLATE 3

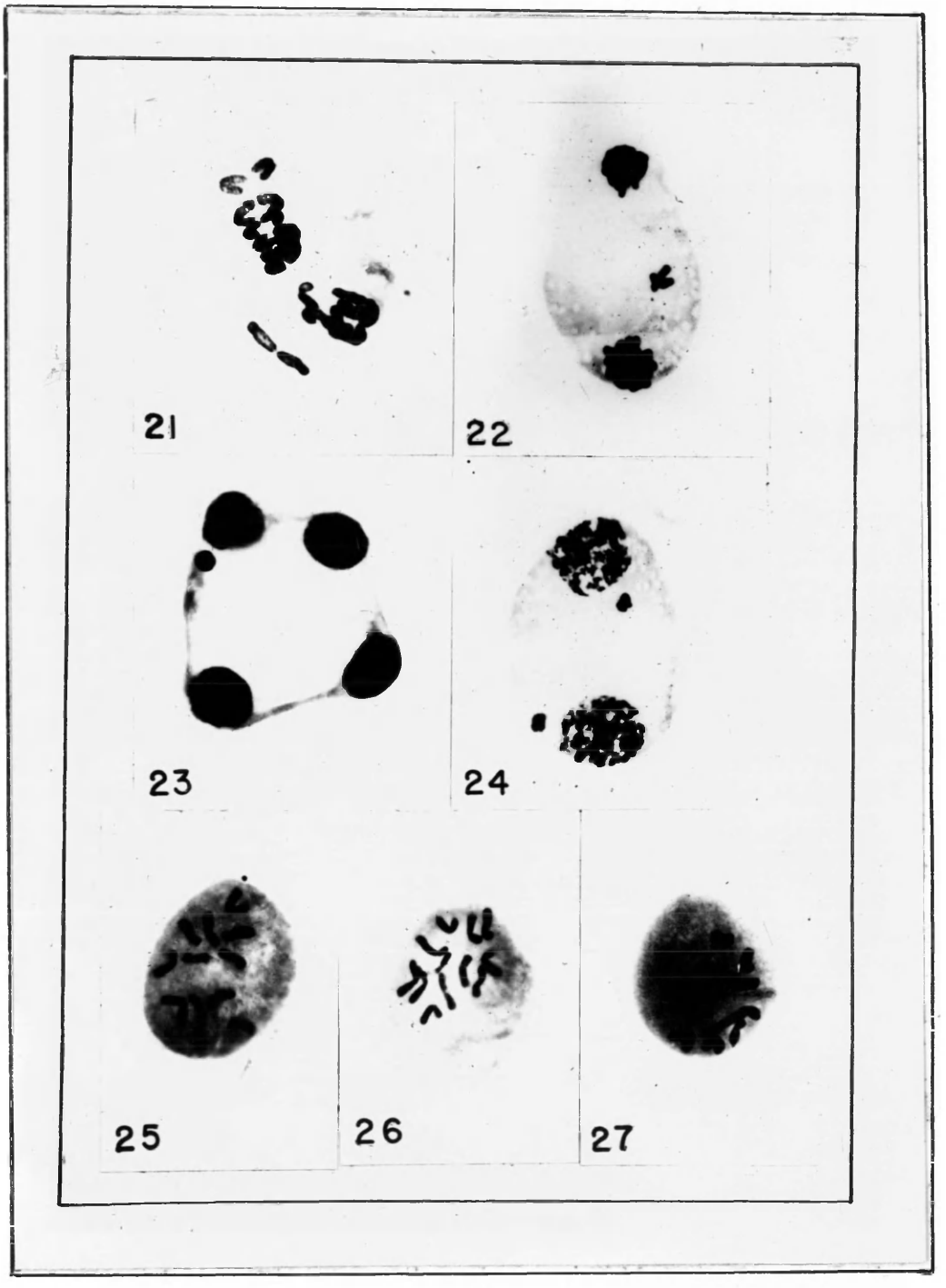


Plate IV

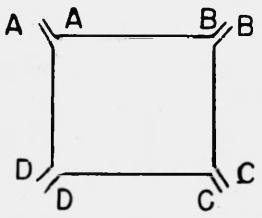
Fig. 28. Diagramatic representation of single chiasma in the diploid showing mechanism of ring formation.

Fig. 29. Single chiasma in the tetraploid between different chromosomes illustrating the formation of chains of 2; and single chiasma at each end of 2 like chromosomes illustrating the formation of pairs.

Fig. 30. Diagramatic representation of a double chiasmata.

Fig. 31. Diagramatic representation of a triple chiasmata.

PLATE 4



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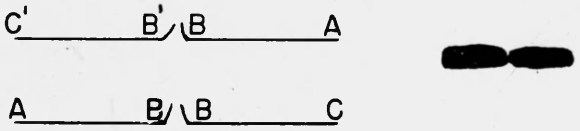

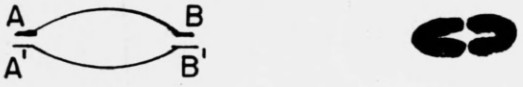

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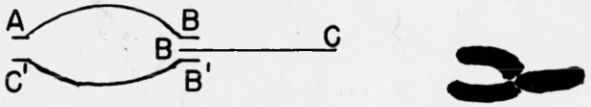
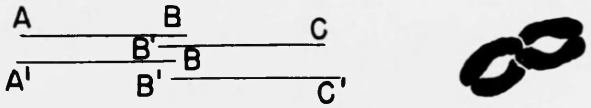


30



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