

CHROMOSOME STRUCTURE IN THE MEIOTIC CHROMOSOMES OF *RHOEO DISCOLOR* HANCE

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With plates 130 and 131

COILED CHROMONEMATA have been observed in both mitotic and meiotic chromosomes in various species of plants. The degree of coiling in mitotic chromosomes may vary from an irregular corrugation or loose spiral to a rather compact regular coil. At meiosis in certain plants the coils are much larger and can be analyzed in more detail.

The behavior of the meiotic chromosomes of *Rhoeo discolor* provides additional information regarding the nature of the coiled chromonemata. The observations are based on both aceto-carmin preparations and permanent smears fixed in Flemming's solution. In both cases the microsporocytes, after smearing, were usually pretreated with alcohol and ammonia before fixing. Immersion in tap water for a few seconds and a brief exposure to ammonia fumes also gave good results. This is essentially the method used by Kuwada. The ammonia vapor seems to dissolve the chromosome matrix and permit the spiral chromonemata to expand.

The chromosomes of *Rhoeo* are arranged in a ring or one or more chains at the first meiotic division. At early metaphase each chromosome contains a coiled chromonema consisting of two closely associated chromatids. The general appearance of these rings is shown in the photographs from aceto-carmin preparations (Figs. 1 and 2), but the finer details are best observed in permanent smears (Figs. 3 and 4). The number of coils is four or five per chromosome. At this stage the two chromatids are so closely associated that the coils appear to be single, but their double nature can be observed at certain loci. The diameter of the coil decreases gradually before the separation of the chromatids.

At late metaphase the coiled chromatids separate. The number of coils in each chromatid remains the same, but they are much smaller, even though there is little or no elongation of the chromosome as a whole (Figs. 5 and 6). At this stage both the terminal chiasmata and the fiber constrictions are very conspicuous, so that the order of the individual chromosomes in the ring can be determined. As observed earlier (Sax 1931), the order of the twelve chromosomes is always the same, thus supporting Belling's suggestion that such rings are the result of

segmental interchange. Six of the twelve chromosomes are distinctly heterobrachial, and the short arms are paired.

The meiotic chromosomes are always paired by terminal "chiasmata" and, in the chromatids at late metaphase, most of the chiasmata appear to be symmetrical. The chromatids are often parallel in several successive chromosomes or even in most of the chromosomes in the ring.

As the chromosomes pass to the poles at the first meiotic division, the chromatids separate except at the fiber constriction and become shorter (Fig. 7). During interphase the chromatids elongate considerably. At the second meiotic division they are about twice as long as they were at the first division. At this time the minor or somatic type of coiling can be observed. The coils are only about half as wide as the major coils found in the first division, and the number of coils is about 12 per chromatid (Fig. 8). These coils appear to be single at metaphase. There is some evidence of a split at late anaphase, as indicated by narrow regions at certain loci. If the chromatids are split at second metaphase, the two halves must be coiled together. At late anaphase they may tend to separate, but the slipping apart of the coils is difficult to detect except where there is a twist which appears to constrict the chromosome at such loci. More definite evidence of split chromatids has been obtained from microsporocytes which were subjected to low temperatures during development.

Under normal conditions the microspores receive six chromosomes, but occasionally there are seven, owing to irregularities in the first meiotic division. About 80 per cent of the microspores fail to develop, owing to segmental non-disjunction. The normal fertile microspores undergo a single nuclear division, followed by the differentiation of the daughter nuclei into the large and more or less degenerate tube nucleus and the compact elongated generative nucleus.

When the plants are kept at a temperature of about 6° C., two kinds of abnormalities appear. The chromosomes of the one nucleate microspore may divide but do not form daughter nuclei. The 12 chromosomes pass back into the resting stage and divide regularly at the next division to form diploid gametes. The other type of abnormal development begins when the low temperature inhibits chromosome pairing at meiosis. The twelve univalent chromosomes pass into the resting stage without nuclear division. They come out of the resting stage, divide without nuclear division and form a giant nucleus with 24 chromosomes, each of which consists of two coiled chromatids held together only at the fiber constriction (Fig. 9). Throughout this process the chromosomes never pass through the contraction characteristic of normal telophase stages.

The chromatid spirals are much looser than those found in the chromatids of the normal chromosomes at the second meiotic division, and it is perfectly clear that many of these chromatids are split. The splits are especially clear near the ends of the chromatids. At a somewhat later stage the two chromatids become completely separated, the chromatids elongate, and their structure is very clear. In the same chromatid one can observe the transition from a single coil to two parallel finer coils (Fig. 10). The mechanism of separation of daughter chromatids in these chromosomes, which are essentially of the somatic type, is similar to that found in the coiled chromatids in the first meiotic division.

The structure of the meiotic chromosomes in the permanent preparations was clear enough to permit an analysis of direction of coiling in the spiral chromonemata. We were able to determine the direction of coiling at all loci in each of the twelve chromosomes in 14 cells with complete chromosome rings. The classification of right- or left-handed spirals is purely arbitrary, since the direction depends on the sequence of determination in the ring. There is a strong tendency for the direction of coiling to be in the same direction in both arms of a chromosome. Of the 168 chromosomes examined, 50 had a right-handed spiral in both arms, 52 a left-handed spiral, and 66 showed a reversal of coiling, presumably at the fiber attachment. In only two chromosomes was there a second change of direction of coiling. Individual chromosomes could not be identified consistently, so that the direction of coiling could not be established for any one chromosome in all the different cells, but there is good evidence that direction of coiling is not a stable character. The number of chromosomes with left-handed coils ranged from 1 to 5, with reversed coils from 2 to 8, and with right-handed coils from 1 to 7, in different cells. The direction of coiling of chromonemata of paired adjacent chromosome arms is at random, with 85 coiling in the same direction and 83 coiling in reverse directions.

Both rings and chains of chromosomes are found at meiosis. If one or more chains are formed, the breaks may occur between either the long segments or the short segments. Three of the terminal chiasmata are formed between short segments, and eight of the terminal chiasmata are formed between long segments. The extra chiasma is between a long and a relatively short segment. The position of the breaks, or failure of chiasma formation, was obtained for 20 cells containing one or more chains. There were 9 breaks between the short segments and 16 between the long segments. These results suggest that chiasma formation is somewhat less likely to occur, or less likely to persist until late metaphase, between the short segments. The latter possibility is more probable

because we have found chromosome rings in practically all cells in some preparations.

The lengths of the chromosomes at different stages in meiosis were obtained to aid in the analysis of factors involved in chromosome contraction. It was not possible to get an accurate measurement of the length of the pachytene spireme in *Rhoeo*, but the total length is approximately 700 microns or an average of somewhat more than 100 microns per chromosome. The approximate average chromosome length at meiotic metaphase is 5–6 microns, and is about 9 microns at the second meiotic division.

THE MECHANISM OF CHROMOSOME CONTRACTION

The great contraction in chromosome length between pachytene and meiotic metaphase stages in *Rhoeo* is associated with the coiling of the chromonema. This coiling may not be the only factor involved. Belling (1928) believed that the approximation of chromosomes caused about one-third of the contraction in the chromosomes of *Lilium*, and that the coiling of chromonema effected the final shortening to give approximately a 10 to 1 reduction in length of the meiotic chromosome. Bridges (Alexander, 1928), on the other hand, assumed that coiling is the primary factor in chromosome contraction, and that the gene string maintains approximately the same length at all stages in the chromosome cycle. Another factor in chromosome contraction is the secondary or minor coils within the primary or major coils as described in *Tradescantia* by Fujii, Kuwada and Nakamura (1933) and found in *Sagittaria* by Shinke (1934).

We believe that three factors are involved in the great decrease in length of the meiotic chromosomes of *Rhoeo*; first, a linear contraction of the gene string; second, the major coiling of the chromonema; and third, the formation of minor spirals within the major spiral. The minor coils are not clearly differentiated at the first meiotic division in *Rhoeo*, but there is some evidence of loose coiling. The contraction of the chromonema and reduction in the width of the major coils between early and late metaphase are attributed to the further coiling of the minor spirals in each chromatid. A similar reduction in the major coils with no increase in chromosome length is found in *Secale* (Sax, 1930).

The coiled chromonema at early metaphase consists of two chromatids coiled together so that the chromonema often appears as a single coil, as is the case in *Tradescantia*, *Secale*, *Lilium*, and *Vicia*. The free separation of coiled chromatids has been explained by Kuwada (1927).

The reverse twists postulated by Kuwada can be observed in *Tradescantia* (Sax and Humphrey, 1934) and in *Trillium* (Huskins and Smith, 1935).

The direction of coiling in the chromonema spirals is more or less at random in *Rhoeo* and in *Tradescantia* (Nebel, 1932, Sax and Humphrey, 1934). According to Huskins and Smith, the paired meiotic chromosomes of *Trillium* usually coil in opposite directions, although no statistical evidence is presented, nor is there any adequate explanation for such behavior. Within a single meiotic chromosome the direction of coiling may change at the spindle fiber point, but is seldom reversed at other loci in *Rhoeo*, *Secale*, *Gasteria* (Taylor, 1931), *Tradescantia*, and *Sagittaria* (Shinke, 1934). Huskins and Smith find frequent changes in direction of coiling of anaphase chromosomes in *Trillium*. These changes in direction of coiling are usually associated with chiasmata. We have found changes in direction of coiling at chiasmata in the meiotic chromosomes of *Vicia*. If the chromonema coiling is caused by a contraction of the matrix, as suggested by Kuwada, the fiber attachment points and the chiasmata would tend to break any continuity of stress on the chromonema and changes in direction of coiling would be expected to be more or less at random at these points. There is a strong tendency, in both *Rhoeo* and *Tradescantia*, for the direction of coiling to be the same on both sides of the spindle fiber attachment, and only about one-third of the chromosomes show reversal of coiling at this locus. Huskins and Smith find that the reversals in direction of coiling between the fiber attachment and the distal ends of the chromatids at first anaphase is about twice the chiasma frequency at metaphase. This relation would be expected if the direction of coiling in homologous chromosomes is at random, and if reversals in coiling occur at random at the chiasmata.

In *Rhoeo* all chiasmata are terminal, and most of them appear to be symmetrical. The short chromosome arms are paired almost as frequently as the long arms. Changes in direction of coiling are rarely observed between the fiber and the distal end of the chromosome. These observations seem to indicate that chromosome pairing in *Rhoeo* is not dependent on the formation of interstitial chiasmata, but is dependent on a terminal association of homologous chromosome segments. This terminal association in certain rod bivalents in *Tradescantia* seems to involve the chromosome pellicle or matrix, but in *Rhoeo* there is evidence of fine chromatic connecting fibers.

During interphase the meiotic chromosomes of *Rhoeo* elongate but maintain some evidence of loose coils during the resting stage. At the

second meiotic division, a new spiral appears which has finer and more numerous spirals than the major coils at the first meiotic division. These minor spirals have been described in *Lilium*, *Rhoeo*, *Allium*, *Tricyrtis*, *Najas*, and *Hosta* by Shinke (1930), and in *Tradescantia* by Nebel (1932), Kuwada and Nakamura (1933) and Sax and Humphrey (1934). These coils in *Rhoeo* are wider than the minor spiral within the major spiral at the first meiotic division and presumably are formed independently during the prophase of the second meiotic division. These spirals are much like those found in certain somatic chromosomes. The transition from about five major spirals to 20–25 minor spirals in the successive meiotic divisions in *Tradescantia* and a similar behavior in *Rhoeo* is difficult to reconcile with the “heterogonic growth” hypothesis of spiralization suggested by Huskins and Smith.

The anaphase chromosomes at the second meiotic division have been described as two-parted in a number of plants (*Gasteria*, Taylor, 1931; *Galtonia*, Smith, 1932; *Tradescantia*, Nebel, 1932; *Trillium*, Huskins and Smith, 1935; et al.; Cf. Sharp, 1934). Both Kuwada and Nakamura, and Sax and Humphrey found only single coils in the second anaphase chromosomes of *Tradescantia*. In *Rhoeo* there is evidence that the anaphase coil is double, but that the two half-chromatids are coiled together so intimately that they appear as a single coil at early anaphase. As the coils begin to separate the gyres are matched so closely that the dual structure is not clear, but where a twist occurs there is a narrow region in the chromosome. The abnormal “microspores” of *Rhoeo* show the chromatid splits clearly in various stages of separation. The minor coils, characteristic of somatic chromosomes, are similar in structure to the major coils; the two chromatids (or half-chromatids) are coiled together in parallel in such a manner that they can separate freely without entangling. If the split occurs while the chromosome is coiled, there must be some lateral polarity so that the division occurs in only one plane parallel to the axis of the chromosome, as Nebel (1933) has suggested.

If there is a chromatid split in the anaphase chromosomes of the second meiotic division in *Tradescantia*, the chromatid must behave as a single unit until midprophase of the microspore division. *Tradescantia* microspores subjected to x-rays show chromatid breaks for about two days after raying, but after three or four days only chromosome breaks are observed at metaphase (Riley—unpublished).

We find that either abnormally low or high temperatures will cause nuclear irregularities. These include failure of chromosome pairing at

meiosis, chromosome division without nuclear division, and failure of normal differentiation of nuclei. Since these temperatures are within the range occasionally experienced in nature, it is probable that temperature fluctuations have played an important part in chromosome changes in nature (Cf. Randolph, 1932). This work with temperature effects has been aided by a grant from the American Academy of Arts and Sciences.

SUMMARY

The twelve chromosomes of *Rhoeo discolor* are arranged in a segmental interchange ring at the first meiotic division. Each chromosome contains a spiral chromonema consisting of two chromatids coiled together. There is some evidence, both direct and indirect, that there is a minor spiral within the chromatids of the major spiral. During metaphase the major spirals become smaller, and the two chromatids separate. The chromosomes elongate greatly during interphase, but there is evidence of a spiral structure during the resting stage. At the second meiotic division, new minor spirals are formed which are smaller and more numerous than the major spirals of the first division. The chromatids at anaphase of the second meiotic division are split, but the two half-chromatids are so closely coiled together that they are not easily observed. They can be differentiated easily in cells where the normal chromosome cycle is disturbed by subjection to low temperatures.

The reduction in the length of the meiotic chromosomes of *Rhoeo*, between prophase and first metaphase is attributed to three factors:—a linear contraction of the gene string, the coiling of the chromonema into major coils, and the development of minor coils in the chromatids of the major spirals. The direction of coiling in the major spirals seems to be at random. In a single chromosome the direction of coiling may change at the fiber attachment point, but it is seldom reversed at other loci.

The meiotic chromosomes are paired at the ends, apparently without the formation of interstitial chiasmata.

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EXPLANATION OF PLATES

PLATE 130

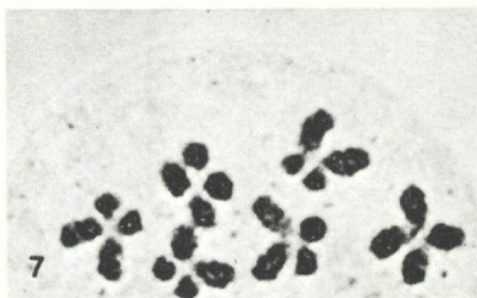
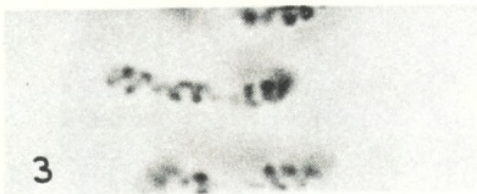
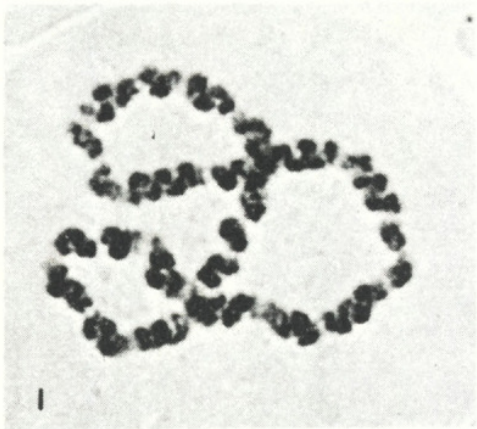
- Meiotic chromosomes of *Rhoeo discolor*. Figs. 3 and 4 from permanent smears. All others from aceto-carmin preparations. $\times 2000$.
- Figs. 1 and 2. The coiled chromonemata in the chromosome ring at early metaphase.
- Figs. 3 and 4. Coiled chromonemata showing reversal of coiling and reduction in width of coiling at metaphase.
- Figs. 5 and 6. Separation of coiled chromatids at late metaphase. The same number of coils are found in the coiled chromatids as in the coiled chromonemata, but the spirals are smaller.
- Fig. 7. Telophase of first meiotic division.
- Fig. 8. Chromosomes at the second meiotic division showing minor spirals which appear to be single.

PLATE 131

Chromosomes from abnormal "microspores" produced by chromosome multiplication induced by cold treatment. Magnification $\times 2000$.

- Fig. 9. A giant cell derived from a microsporocyte by chromosome division without nuclear division. Each of the 24 chromosomes consists of two chromatids held together at the fiber attachment. Many of the chromatids are split.
- Fig. 10. Chromatids at a somewhat later stage showing the transition from a single coiled chromatid to two coiled daughter chromatids.

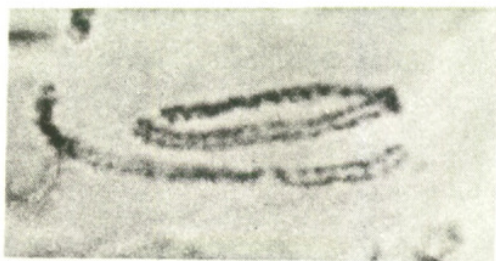
ARNOLD ARBORETUM,
HARVARD UNIVERSITY.



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OF *RHOEO DISCOLOR*



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