THE CHROMOSOMES OF TRILLIUM

BY EDWARD C. JEFFREY AND EDWIN J. HAERTL
(U. S. A.)

RESUMEN

Los Cromosomas de Trillium. — El autor estudia los cromosomas en el género *Trillium*, que es, quizás, el sujeto más favorable entre plantas y animales, debido al gran tamaño de sus elementos. Demuestra que el concepto que se tiene con respecto a la organización de los cromosomas, no es comprobado en la estructura del género *Trillium*. Los cromomeros, que se supone son los representantes morfológicos de los genes, resultan ser solamente una ilusión óptica y no tienen, en realidad, una existencia estructural. Además, desecha la hipótesis de la reducción o de la división reproductora por no encontrarla fundada. Estas conclusiones, de ser ciertas, modificarían profundamente los conceptos citológicos sostenidos hasta el presente.

Although known for many years chromosomes still continue to provide a basis for strong differences of opinion in regard to their organization and relation to heredity. It is now generally agreed that they are not homogeneous structures, but consist of a ground substance and a complex iron-containing substance chromatin. In recent years the technique of chromosomal investigation has shown marked improvement as a consequence views which have been held for half a century have undergone fundamental modification. It has been assumed more or less generally since the important investigations of Balbiani that the chromatin occurs in the chromosomes in the form of particles, often designated chromomeres. Many geneticists have considered these particles to be the actual genes or carriers of hereditary qualities. It is obviously desirable to arrive, if possible, at a clearer conception of the real organization of chromosomes, particularly as a number of cytologists in recent years have expressed doubt as to the existence of chromomeres.

The genus *Trillium* of North America and Asia has proved itself to be particularly favorable on account of the large size and ready fixation of its chromosomes. This genus has been investigated by Matsuura ('35, '37), Huskins ('35, '37, '38) and Nebel ('36). These authors have confined their attention almost exclusively to the large reproductive chromosomes. While these are favorable by reason of their phenomenal size, they present serious abnormalities and in many cases undergo many abortive changes. The cause of these abnormalities the present authors hope to make clear in a subsequent investigation. The somatic chromosomes, however (it is usually the case where reproductive degeneracy is present) are quite normal and in spite of the fact that they have less than half the diameter of the chromosomes of the reproductive elements, constitute material of the greatest importance for the resolution of the general problems of chromosomal organization.

MATERIAL

Four species of *Trillium* have been examined in the present connection, namely *T. grandiflorum, T. erectum, T. undulatum* and *T. stylosum*. The material was derived from the states of Massachusetts and North Carolina. In the case of the latter locality we are indebtet to Dr. Henry W. Jensen, who has sent us abundant vegetative and reproductive parts preserved in accordance with our directions. In Massachusetts material was gathered in the vicinity of Boston and also in the southwestern part of the state.

METHODS

At first the parts were preserved by the methods described by one of us (Jeffrey, '37). In accordance with these the material was cut into almost microscopically thin slices and inmediately dropped into Benda's fluid and exhausted of air at once by an automobile pump with reversed piston. Even with the greatest care, however, the penetration of the preservative was unsatisfactory sometimes and although at its best this method gave very satisfactory results, it was found more advantageous on the whole to use either Carnoy's fluid or equal parts of absolute alcohol and glacial acetic acid. The former was used on the aerial parts of the plants while the latter proved extremely advantageous in the case of the tips of the roots which suffer serious shrinkage when fixed with Carnoy's fluid. The advantage of the two fluids indicated in their reliability in the matter of penetration. After washing with repeated changes of strong alcohol, the parts were imbedded in nitrocellulose. Sections were generally cut at the thickness of five micromillimeters. Thick sections recommended by many in the study of chromosomes were not found to be advantageous. The fuming methods recently advised for te study of chromosomal organization seem to offer no advantages over Carnoy alone or the mixture of equal parts of absolute alcohol and glacial acetic acid.

It has been found extremely advantageous to bleach the sections with from three to ten *per centum* hydrogen peroxide. They are often advantageously left over night in the bleaching fluid which causes no damage even after forty-eight hours of immersion. After washing prolonged treatment with iron alumn was found to give clearer images. Generally the sections were allowed to remain over night in either so-called white hematoxylin (one-eighty of one *per centum)* or a solution of similar strength of hematein in distilled water. Decoloration was carried on slowly with iron alumn solution of one *per centum* or less. The prolonged staining gave rich details of structure particularly when th edecoloration was gradual. As an alternative to hematoxylin acetocarmine was used and frequently gave excellent results although the details of structure were not so well brought out as with the formed reagent. Crystal violet, a reagent praised by many investigators, has not proved sufficiently selective for our purpose on account of its tendency to produce a diffuse stain which fails to reveal important details of structure.

OBSERVATIONS

For reasons described at the outset, the somatic chromosomes receive first consideration. It will be convenient to begin with the early prophase. In fig. 1, plate I is shown the earlier prophase of mitosis in the root-tip of *Trillium grandiflorum.* At this stage the spireme is apparently continuous except where it has been severed in preparing the sections. The figure shows that the as yet undivided nuclear filament is characterized by two intertwined strands which rum spirally in opposite directions. As a consequence the gyres or turns of the spirals cross one another at intervals. When less well preserved these areas of intersection present the appearance of necklacelike rows of spherules, the chromomeres (genes!). In fig. 2 a somewhat denser and more advanced prophase is represented in which the spirally wound strands, the chromatids, are more closely twisted. In fig. 3 the spireme is clearly segmented into chromosomes and each of the two chromatids shown in figs. 1 and 2 has now become an autonomous chromosome, and like the mother chromosome has developed a pair of gyrating chromatids. This phase shortly precedes the metaphase. This stage is shown in polar view in fig. 4. As the metaphase develops the daughter chromosomes begin to unwind and straighten out in anticipation ot the anaphase shown in fig. 5. The anaphase chromosomes now clearly show their spiralized chromatids. These, however, were present long before the appearance of the anaphase or even the metaphase. It has been impossible to follow satisfactorily the mode in which the young chromatids appear in what later became the anaphase chromosomes in the ordinary somatic cells. It appears probable, however, that some light may be thrown on this important subject later by the investigation of the cytological developments in the tapetal cells of the anthers. As the anaphase passes into the telophase and later into the resting or interphase, the spirals of the chromatids become much broader and more lax. We incline to the view that they pass through the resting stage in this condition. Fig. 6 represents the earlier resting stage. Nucleoli have made their appearance and the chromatidal gyres have become extremely lax. As they loosen the chromatids of the resting nucleus become more and more slender. With the resumption of activity the nuclear filament or spireme shows the same two chromatids which were present as the nucleus passed into the condition of quiescence. It is thus apparent that the double organization of the chromosomes is present long before the anaphase of the division. In other words the two spiralized chromatids present in the metaphase and anaphase of the chromosomes of one division are perpetuated in the prophases of the next division.

It will be well at this stage to turn our attention to the reproductive or meiotic divisions of the genus Trillium, particularly as the investigations of the past few years have been confined almost entirely to this category, in view of the relatively large size of the chromosomes involved. When the nuclear filament first clearly emerges in the genus under discussion, it is found to be as in comparable cases, it is seen to represent a very fine and apparently continuous spireme. This is the stage known as the leptotene. Huskins *(opera cit. supra)* and others who have investigated in recent years the organization of the leptotene in this and other Liliaceous genera (e. g. Hermans, C. La Prophase meiotique chez le *Lilium Martagon*, La Cellule 45, fasc. I, pps. 45-57, 1936) have noted again what has often been observed in the past, namely a somewhat moniliform or segmented appearance. We have found it possible, by special methods of fixation and staining, as well as by the use of the very best available optical equipment (for example *new* 2 and 3 millimeter apochromatic objectives of Zeiss and Leitz of 1.4 angle of aperture and also the Zeiss 1.5 millimeter apochromatic immersion) to resolve the structure of the so-called leptotene. Fig. 7, plate 2, represents the course and organization of this stage in a part of the younger spore mother cell of *T. erectum,* which may be profitably compared with Huskin's representation of the same stage *(op. cit.* 35, fig. 1, plate I). Our observations show it to be organized as a filament in which two chromatids occur, precisely as have been described for the somatic elements in earlier paragraphs. As we have found invariably in the somatic stages in a large number of plants and animals, the double spirals revolve in opposite directions in such a manner that their gyres or coils cross one another. A similar condition has been less clearly observed by Koshy ('37) in the leptotene filaments of *Aloe littoralis* (pl. I, fig. 8). It is paralleled in a photomicrograph of a somatic anaphase in *Hyacinthus orientalis* recently published by Naithani ('37) (pl. 4, fig. 2). We have observed similar conditions in the leptotene of other species of Trillium and also in Lilium, Tradescantia, Rhoeo, Pinus, etc., to be published elsewhere. Passing over the subsequent stages, which will receive attention elsewhere, it will suffice in the present connection to mention that the originally continuous leptotene becomes segmented into five portions. (For this stage we propose the designation arthrotene). The segments of the leptotene now begin to shorten and thicken, still retaining their double complement of contrarywise wound spirals. The spirals begin to unwind and straighten and in this process become separate chromosomes, each of which in turn shows the pair of spiral chromatids, apparently characteristic of all stages of active chromosomes. In fig. 8, plate 2, the nuclear spindle of the first meiotic division is established and the daughter chromosomes (the apparently erroneously designated synaptic mates) move in a somewhat lagging fashion towards the poles of the spindle. In fig. 9, plate 2, the so-called synaptic mates have undergone further thickening and contraction. Fig. 10, plate 2, takes us back to the stage preceding the anaphase, represented in figs. 8 and 9. Here the chromosomes are grouped more or less in the equatorial plane of the nuclear spindle to form the metaphase. It is clear that at this stage as in the following anaphase described above the chromosomes are already double, that is contain each a pair of oppositely wound chromosomes. In fig. 11 is shown the polar view of the two metaphase plates. It is clear that not only are the metaphase chromosomes double, but that each of their two component loosely wound chromosomes is likewise double, thus anticipating the next or second meiotic division. There are, as in other species of Thillium, five reproductive chromosomes. In fig. 12, a not uncommon appearance is presented where instead of five chromosomes appearing in the metaphase group, there are six. This is not unusual in the genus and has been observed in other species by other investigators. It is caused by the premature separation of one pair of chromosomes. In fig. 13 the details of organization of three of the first meiotic chromosomes are seen. A photomicrograph of these same chromosomes will be described at a later stage. In fig. 14 is shown a polar view of the late anaphase of the first reproductive division. Here can be seen that the five chromosomes have already anticipated the second metaphase by appearing clearly separated in pairs. In fig. 15, the same stage is represented in lateral view to show the two daughter groups of clearly separated chromosomal pairs. In fig. 16 appears the late anaphases of the two daughter groups shown in fig. 15. It is clear that all the chromosomes present exhibit two diversely spiralling chromatids precisely as in the other figures of active somatic or reproductive chromosomes. In fig. 18 the nucleus of the young pollen grain is shown passing into the resting stage. This condition can appropriately be compared with that shown for the sporophytic somatic cells, shown in fig. 6, plate I. It may summarily be stated that the essential organization of the somatic and reproductive chromosomes is identical as is likewise their conduct in division. They only important difference between them as has recently been indicated by Jeffrey ('36, '37) is in their number.

Fig. 17, plate 2, shows the metaphase chromosomes of *Trillium erectum.* Here the so-called synaptic or syndetic mates are spirally wound around one another in opposite directions. In this representation of the metaphase chromosomes we are in agreement with Koshy *(op. cit.)* in the case of Aloe and in opposition to the results recently published by Matsuura *(op. cit.)*, Huskins *(op. cit.)*, Nebel *(op. cit.)*, and Sax. The explanation of this radical difference of opinion will be elucidated later. The earlier and later stages of meiosis in *T. erectum* agree closely with those described above for *T. grandiflorum.* The same statement holds for two other species, *T. undulatum* and *T. stylosum*, in which by contrast to the two earlier mentioned species, we have had extremely inadequate material. It may be added that in our experience the species of Trillium supply rather unsatisfactory material for the study of meiotic phenomena, in spite of the large size of their chromosomes, owing to the large amount of degeneracy and abortion present, which indeed has been noted by several of the authors cited above. The allied Asiatic and European genus Paris seems to present to a less extent similar abnormalities, together with nuclear conditions otherwise favorable.

In view of the contradictory results obtained by recent competent authors in describing the chromosomes of Trillium and other liliaceous genera, it will be desirable at this stage to consider the objective evidence supplied by photomicrographs. Text Fig. A shows the chromosomes of the somatic (sporophytic) anaphase of the root of *T. grandiflorum* very highly magnified. As a result of the great magnification only a part of the photograph is in such sharp focus as to show the details of structure. In the chromosome on the upper right hand of the figure, the presence of two chromatids in the chromosome can be made out. It is further obvious that the chromatids or paired elements of the chromosome are spiralled in opposite directions in such a manner that their gyres or turns cross one another. Similar conditions have been clearly observed by the present authors not only in several species of Trillium, but also in te somatic divisions of numerous species of Lilium and Tradescantia. One of us (Jeffrey '37) has recently described them in Trillium with drawings and photographic illustrations. Naithani *(op. cit.)* has shown photomicrographs of this condition in Hyacinthus. Very recently Mensinkai ('37) and Gates and Mensinkai ('38) have photographed and described them in the root of Trillium. Too little attention has been given of late to the organization of somatic chromosomes in plants. All the recent evidence indicates clearly and with substantial unanimity that the somatic chromosomes are characterized in the active and also indeed in the resting condition by the presence of a pair of chromatids, the spirals of which invariably cross in opposite directions.

Gates and Mensinkai *(op. cit.)* point out as one of us had done at an earlier date ('37, *op. cit.)* that crossing gyres of these spirals are responsible for the optical illusions, which have been designated chromomeres and interpreted as the morphological representatives of the genes.

Turning our attention now to item B or the text figure, we see represented a very highly magnified image of the reproductive (meiotic) chromosomes of *Trillium grandiflorum.* The organization can best be seen in the long chromosome appearing on the left of the photomicrograph. It is obvious that this chromosome contains two oppositely wound spirals. The photomicrographic illustration is in pointed agreement with the drawing of it made independently by the junior author, shown in fig. 13, plate 2, and not influenced in any way by the photomicrograph, which was actually taken sometime after the drawing had been made. Neither in the drawing nor the photomicrographs can there be any doubt that the chromatids (chromonemata) are revolved in opposite directions. This is clear not only from their relations to one another in the body of the chromosomes, but also by the divergent position or their free ends. There is further no possibility of the coils being merely superposed and not intertwined as the chromosomes are in this case so large that it is quite easy to follow the diversely intercoiling gyres of the chromatids (chromosomes at a later stage).

In the above account the older literature has not been considered because it is only in the past three or four years that any adequate knowledge of the integral organization of the chromosome has been reached. A common defect in the material studied in this connection is inadequate fixation or faulty staining or both. A convenient illustration of defects is presented by drawings of the mitotic chromosomes of *Lilium pyreniaeum, L. Henryi, L. japonicum* and *L. tigrinum,* shown in fig. I of an article entitled *Meiosis in Lilium* by K. Mather, published in *Cytologia* 6, p. 354, May, 1935. Here the chromosomes are represented as hollow cylinders of stainable substance (chromatin?) surrouding a light center. Adequate fixation followed by proper staining would reveal the fact that the envelope of chromatin is not a continuous cylinder, but is composed of two spiral chromatids revolving in opposite directions. The same criticism apparently applies to the meiotic chromosomes represented in the same article. This article is chosen merely because it is the product of a well-known establishment for research and also because it typifies apparently a very common error in the conception of organization of chromosomes. Obviously too much attention has been devoted until quite recently to the silhouettes rather than to the internal organization of chromosomal structures. Doubtless many of the often weird and certainly frequently highly contradictory hypotheses as to the genetical significance of chromosomes are with a high degree of probability due to this cause.

However beyond question the highly contradictory conceptions as to the organization of reproductive and somatic chromosomes are not entirely or at the present time, at any rate, due to bad technical treatment of the material. For example in the case of the reproductive chromosomes, the prevailing view at the present time is that the two spiral chromatids present revolve in parallel gyres, which are only interrupted by such more or less incidental disturbances as spindle attachments or so-called chiasmata. This view is held by literally a host of genetical cytologists. In spite of the fact that there are in existence many excellent figures, in the case of *Lilium* for example, showing the reversely coiling chromatids in the meiotic chromosomes, it is maintained by such reputable cytologists as Sax, Matsuura and Huskins, to mention only examples, that in Tradescantia and Trillium the gyres of the chromatids belong to parallel spiral chromatids. The present authors are in a position to maintain the exact opposite for the genera named.

In the case of the less well known somatic chromosomes, there is greater agreement probably because reliable observations are much fewer on account of the smaller size of the structures and the consequently greater difficulties of observation. It appears to be established on the basis of the observations Gates, Koshy and Mensinkai and the present authors supported in a number of instances by objetive photomicrographs, that the chromatids of the somatic chromosomes of *Trillium* are two in number under the simplest conditions and contain chromatid spirals running in contrarywise directions. Our text fig. IA for example appears to establish the correctness of this view beyond any reasonable doubt. On general grounds it appears highly improbable that the somatic and reproductive chromosomes should be characterized by such fundamentally different organizations. Further our text figure IB apparently makes it clear that the essential organization of both is the same and at the same time confirms the accuracy of our drawings shown in plate 2, fig. 13, for *T. grandiflorum* and plate 2, fig. 17, which are contradictory to the structural organization shown in the illustrations of Matsuura and Huskins.

Obviously these extreme differences cannot be set down to inadequacies of technique, but most have another explanation. It is an interesting and apparently significant fact that there is general agreement as to the organization of somatic chromosomes in recent investigations. The disagreements present themselves in the case of the much larger, thicker and more dense reproductive chromosomes. The whole situation is apparently explainable in a very simple manner. On account of their great length and comparative slenderness, the coils of the somatic chromosomes are in general much more lax than are those of the reproductive cycle. The result of this fundamental difference can apparently be shown quite clearly by an objective diagram such as that supplied by text fig. 2. Here we have strands of woven cotton rope twisted around one another. In A the coils are loose and it is clear that the gyres run in in opposite directions and consequently cross one another. In B the coils are tightly twisted, as a consequence the lower turns are nearly entirely obscured and as a necessary consequence the gyres, in appearance only, seemingly are parallel. Applying this objective diagram to the elucidation of the somatic and reproductive chromosomes respectively, the loosely coiled strands correspond to the conditions found in the somatic chromatids, while the tightly coiled condition has its counterpart often but by no means invariably in the shorter, thicker and more tightly coiled reproductive chromatids.

In support of this explanation may be adduced the fact that it is very easy in properly fixed and stained material to provide photomicrographs of reproductive chromosomes in which the gyres unquestionably cross one another in reverse direction. This is particularly true of such classic objects of cytological research as *Allium, Lilium, Trillium, Tradescantia* and even *Vicia faba*. It thus appears clear that the contradictory results reached in the case ef reproductive and somatic chromosomes are based on an error of interpretation, which apparently now illuminated and elucidated.

CONCLUSIONS

If the facts and interpretations of them described in the foregoing paragraphs are as objective as we feel them to be, a new general view of the organization of chromosomes and their relation to heredity must be adopted. It is clear that the active chromosome contains two or a multiple of two chromatids. These are spiral in their nature and their coiled character is apparently the natural result of the shortening and thickening of the chromosomes as the processes of mitosis and meiosis progress. Where the coiling is relatively lax, as is generally the case with the somatic chromosomes, the gyres are distinctly observable as crossing in opposite directions. In the often more strictly wound thicker and more voluminous reproductive chromosomes, as a result of an optical illusion, the gyres are sometimes seemingly parallel although a more critical examination shows that their relations are exactly similar to those of the somatic chromosomes. If we take such an example as *Vicia faba* where the somatic elements are relatively small, it is not easy always to distinguish the oppositely coiled chromatids except in the telophase of mitosis. Roy has pointed this out in the genus Lathyrus and it has been confirmed by one of us in the species named above. The oppositely orientated gyres are however easily seen in the metaphase and later stages of meiosis and have been illustrated photographically by as vigorous a representative of the hypothesis of parallel gyres as Sax ('37). The views here developed put forward a consistent and uniform explanation of the organization of active chromosomes in both somatic and reproductive cells. If they are adopted, obviously many of the existing often highly contradictory and even obscure cytogenetic hypotheses must be consigned to the limbo which awaits the great majority of scientific theories. Obviously the hypothesis of genes so far as it has any morphological foundation must be interred without undue grief. The same fate apparently awaits synapsis and chiasmotypy. It would, however be going beyond the scope of the present article to make detailed statement in regard to these and many other matters which are tied up in the existing apparently insecurely developed plexus of cyto-genetics.

Summary. 1. In all active chromosomes the chromatin is present in the form of spiral chromatids, which are basically two in number but at times are multiples of that number;

2. These coil about the outside of the core provided by the so-called linin axis of the chromosomes;

3. The spirals are coiled in opposite directions;

4. The crossing areas of the spirals, in inadequately prepared material, present the artefacts which have been recognized in the past as chromomeres (genetically supposed to represent the genes);

5. The organization of reproductive and somatic chromasemata is identical;

6. As a consequence the leptotene, so-called of meiosis, has its exact counterpart in the early stages of development of the mitotic spireme;

7. The view that the chromatids of the reproductive chromosomes are parallel spirals is based on an optical illusion;

8. The hypothesis of synapsis or syndesis appears to rest at the present time on an extremely insecure foundation.

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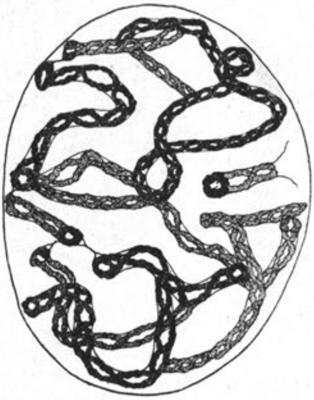

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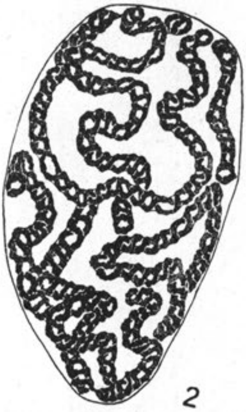

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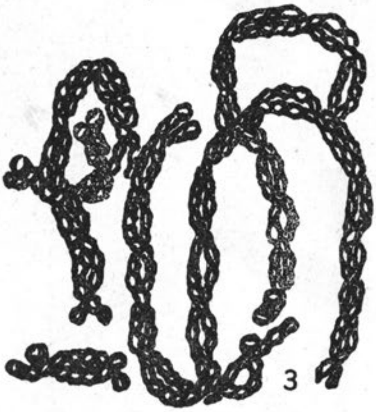

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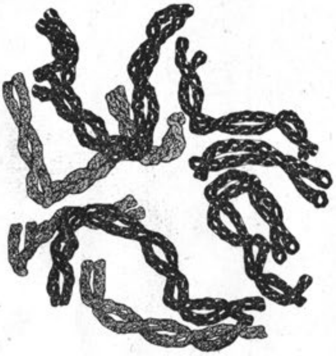

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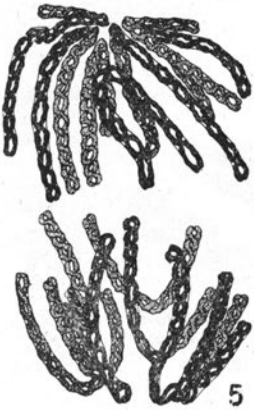

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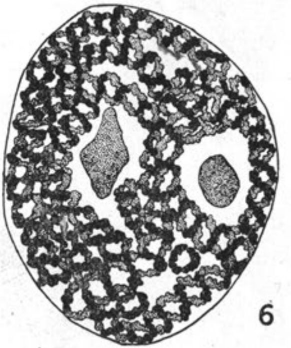

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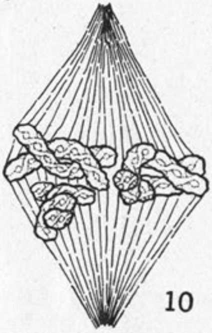

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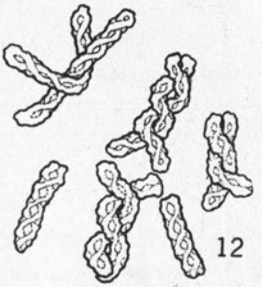

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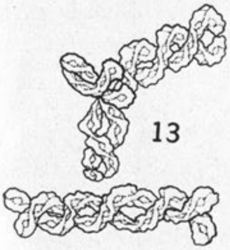

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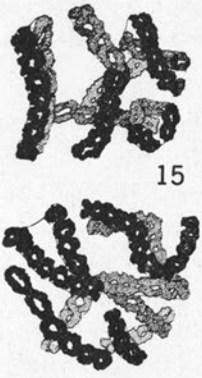

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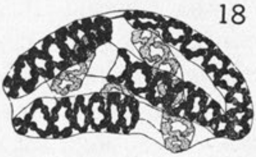

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