

The Behaviour of the Chromatin in the Meiotic Divisions of *Vicia Faba*.

BY

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With Plates **XLIII** and **XLIV**.

IN 1911 an account was published of the vegetative mitoses in *Vicia Faba* (4), in which it was shown that, alike in the sporophytic and gametophytic divisions, longitudinal fission takes place in the telophase and persists through the resting stages till it is completed on the spindle of the following division. In the last premeiotic telophase (that is to say, in the initiation of the spore mother nucleus), as in those of other vegetative divisions, longitudinal fission of the daughter chromosomes occurs, and it has appeared worth while to connect this division with the mitoses already studied in the pollen grain, by tracing the longitudinal fission through the meiotic phase. The great simplicity of some of the stages in the Bean, serving, as it does, as clear and easily obtainable demonstration material, may further justify an account of this often investigated stage.

Plants of Carter's 'Monarch' were grown in the summer of 1910 by my former colleague, Mr. Snell, in a garden at Penge, and in 1911 at the Chelsea Physic Garden, under the direction of Mr. W. Hales, whom I desire to thank for his care in this connexion.

The buds were fixed between 11 a.m. and 2.30 p.m., mainly on sunny days. Various media were used, of which Flemming's strong fluid, diluted with an equal quantity of water, proved the most successful. Sections were cut from 5μ to 15μ in thickness, and were stained for the most part with the combination of Breinl, which gives excellent results on this material.

Development was studied both in the micro- and in the megasporangium, and while, for the sake of uniformity, it has seemed preferable to restrict the figures and description to the former, it may be noted that the main facts have been confirmed in the latter also.

EARLY PROPHASES.

The pollen mother-cells, before meiosis begins, are packed with a dense, finely granular cytoplasm. The nuclei are large (Pl. XLIII, Fig. 1); they generally contain a single nucleolus, lying in a more or less central position, and the reticulum is spread just inside the membrane, so that it forms a hollow sphere. It consists of an irregularly diamond-shaped mesh, the origin of which may be traced to the longitudinal fission and cross connexion of the chromosomes of the preceding telophase. The spaces in the network are thus alternatively due to the pulling apart of the sides of the chromosome where fission has taken place, and to cross attachment between neighbouring chromosomes which, it must be kept in mind, are viscous structures.

The first evidence that reduction is about to take place is given by the partial separation of the reticulum from the nuclear membrane (Fig. 2). The enlargement of the membrane has been recorded at different times by a number of authors, and has been specially emphasized by Lawson (5) in 1911. While it is taking place the chromatin mass in *Vicia* contracts (Figs. 2, 3), still retaining its reticulate character, till it closes in about the nucleolus and the synaptic stage is entered. The nucleolus is sometimes squeezed out (Fig. 4) and lies at the periphery of the nuclear vacuole, where it may be recognized during the succeeding stages.

It is very difficult to gauge the amount of contraction occurring at this period, since nuclei at the same stage of development are by no means necessarily of the same size, but measurements of neighbouring nuclei show that whereas, in a particular case, the diameter of the presynaptic nucleus in which the chromatin lay in contact with the membrane was 12.5μ , the membrane after synapsis had begun was 15μ in diameter, and the contracting reticulum 9 by 10μ , while the chromatin mass in full synapsis, in a vacuole of similar dimensions, measured only 7.5μ across. These differences are too constant to be accounted for by the inadvertent selection of tangential sections, too great, in otherwise uncontracted material, to be due to artifact, and it seems impossible to doubt that in *Vicia Faba* a definite first or synaptic contraction of the chromatin occurs, in addition to the enlargement of the nuclear membrane.

During the earlier stages of synapsis fine threads may be observed bridging across the space between the nuclear membrane and the reticulum (Pl. XLIII, Fig. 2). As contraction increases these are broken and finally disappear, being no doubt absorbed either into the chromatin network or into the cytoplasm. This connexion between the membrane and the reticulum accounts for the fact that the presynaptic chromatin is held around the outer limits of the nuclear vacuole, and it may be surmised that a similar provision exists in vegetative nuclei.

The synaptic mass is dense and impenetrable when ordinarily stained,

but a light stain reveals that the chromatin retains its reticulate arrangement during the period of maximum contraction (Fig. 3). Later the cross connexions begin to break down (Fig. 4), and the formation of the free spireme is initiated.

POST-SYNAPTIC PROPHASES.

As the nucleus passes out of synapsis loops of free spireme are thrown into the nuclear vacuole (Figs. 5-9), the whole area of which, as the loosening proceeds, becomes occupied by the chromatin thread; this is not always wholly in the form of a simple spireme, but may still show the diamond-shaped mesh of the reticulum (Fig. 10) to a greater or less extent. We have thus clear evidence of the persistence of a longitudinal split, which can be traced back through synapsis to the preceding telophase; quite similar fissions may be recognized (Fig. 11) even where the cross attachments have already disappeared.

Subsequently, after the chromatin has been spread through the nuclear vacuole, a second contraction begins, and, with the shortening and thickening of the thread, the longitudinal fission becomes difficult or impossible to trace. It is rendered clearly recognizable again only with the onset of the metaphase.

The synaptic stages are frequently found, and usually extend throughout the whole anther; they may be judged, therefore, to be somewhat prolonged. The same criteria indicate that the loosening and formation of the spireme take place slowly, but that the second contraction is a much more rapid process. The subsequent metaphases and anaphases, as well as the homotype division, appear to be swiftly accomplished.

At all stages in meiosis, disintegration of the contents of the anther may set in. This is a not uncommon phenomenon among cultivated plants, and is indicated by contraction and abnormal appearance both of the cytoplasm and the nuclear contents. At an early stage, when the cell as a whole still presents a healthy appearance, globules of chromatin are extruded from the nucleus and pass into the cytoplasm, frequently invading a neighbouring cell (Pl. XLIV, Fig. 33). They are for a time attached by fine threads to the parent nucleus, and closely resemble the 'chromatic bodies' described by Digby (1) as normally present in *Galtonia candicans*. The bodies observed in *Vicia Faba*, however, seem clearly related to the incidence of an abnormal condition.

THE FORMATION OF THE GEMINI.

The first indications of pairing appear among the free strands of spireme which have already been described as thrown out across the nuclear vacuole when the loosening of the synaptic mass begins. This occurs prior to the temporary obscuration of the longitudinal split, which is still quite clear some time after pairing has begun. Loops of similar form are observed lying side by side in pairs (Pl. XLIII, Figs. 5, 7, 8) in more or less close

relation. At other points isolated loops occur with markedly similar sides, united by a swollen dot (Figs. 6, 8). A little later straight segments of the spireme may be found lying parallel one to another (Fig. 8), or again curved filaments connected by their longer limbs while their shorter limbs lie free (Fig. 9). These and other figures, in which the associated strands become intertwined and more closely approximated, are found after the loosening of synapsis is complete (Fig. 11) and, still more frequently, throughout the second contraction phase (Figs. 12–16), until the appearance of the mature gemini. As development proceeds, one end of a double loop is frequently set free (Fig. 13). The long twisted structures thus produced are the precursors of the figure-of-eight-shaped gemini on the heterotype spindle.

The earliest recognizable approximations thus occur immediately on the loosening of synapsis, when portions of the spireme (which is not necessarily continuous) are first set free from the reticulum. The threads, paired and unpaired, soon extend through the whole of the nuclear area, but the increasing association between separate filaments no doubt entails some pulling about and rearrangement of the whole, till, as pairing becomes more frequent and the thread begins to thicken, the chromatin is drawn into the loose, irregular knot which constitutes the second contraction. The chromosomes, or their precursors on the spireme, run out to the limits of the nuclear vacuole but merge into a central tangle, so that each chromosome is pretty sure to lie in contact with several others, and attractions between allelomorphs as yet unpaired are thus facilitated. A similar arrangement has been observed by several investigators, and its interest lies in the comparatively simple opportunity it affords for the association between homologous paternal and maternal segments.

The spireme of the second contraction breaks up to form seven gemini; some of these are at first of considerable length (Pl. XLIV, Fig. 16), and are twisted hither and thither in the nuclear areas; they undergo the usual shortening and thickening (Fig. 17), and, by the time that they are arranged on the spindle, they are relatively small and stout, and of more or less uniform size (Fig. 18).

THE NUCLEAR MEMBRANE AND NUCLEOLUS.

Until the formation of the gemini the nuclear membrane shows no marked alteration in appearance, while the cytoplasm, which is at first a uniform, finely granular mass, increases in density around the nucleus and leaves the periphery of the cell relatively empty. By these changes, and by the subsequent rounding off of the pollen mother-cell, the succession of stages in the nuclear history can be roughly gauged.

As the gemini shorten and thicken, the cytoplasm encroaches on the nuclear area and the limiting layer loses its definite character, till, on the

equatorial plate, a nuclear membrane cannot be identified, and the vacuole is represented only by a light area around the chromosomes (Fig. 18). Meantime the encroaching cytoplasm gives rise to the spindle.

Lawson (6, 7) has regarded similar phenomena as evidence that the nuclear membrane becomes 'closely applied'¹ to each of the gemini, so that there are formed 'as many osmotic systems as there are bivalent chromosomes'.² For him the nuclear membrane never disappears, and the spindle fibres do not intrude into the nuclear system but are attached to the nuclear membrane where it is wrapped around each chromosome.

In *Vicia Faba* no evidence could be obtained that the nuclear membrane as a definite entity 'completely envelops'³ each of the gemini. If we choose to define the nuclear membrane as the inner limit of the cytoplasm wherever it abuts on nuclear material, we may be justified in using this term to indicate the area of contact between the surrounding cytoplasm and each individual chromosome, when at last the nuclear vacuole has begun to disappear. On such an interpretation, Lawson's description may be applied to this plant, but it is impossible to regard the area of contact of the chromosomes and cytoplasm as in any special way related to the limiting layer of the vacuole, much less as identical with it. The term nuclear membrane seems more appropriately restricted to the latter.

At about this time also the nucleolus disappears. In the early prophase it is in close relation to the developing chromosomes, to which it apparently gives up a part of its contents. On the heterotype spindle, and during the homotype division, a number of deeply staining granules are present, and these, no doubt, represent the remains of the nucleolus. A nucleolus is not formed in the brief resting stage between the heterotype and the homotype divisions, but nucleoli again appear (Pl. XLIV, Fig. 31) as soon as the latter mitosis is complete.

THE CHROMOSOMES ON THE SPINDLE.

The gemini, after they are set free, assume the familiar oval and twisted forms. Single and double figures of eight apparently occur in every nucleus (e. g. Figs. 18, 19), and various more or less elongated loops may also be observed. There is considerable regularity in the appearance of the same figures in different nuclei, but there is not enough variety of form to repay a special study of this point.

The gemini are always attached to the spindle by one end (never by the middle), in such a way that the two sides of the loop or twist—that is to say, the two homologous chromosomes—are both in contact with the spindle and both stand right out from it more or less coiled round one another (Fig. 19). Very soon they begin to move apart, producing a U-shaped

¹ Lawson (7), p. 616.

² Lawson (6), p. 144.

³ Lawson (7), p. 616.

figure (Fig. 20); and as they move, each arm of the U is seen to be longitudinally split (Fig. 21), so that the bivalent chromosome, which, viewed laterally, appears as a U form, is revealed when examined from the front, as two V's set base to base. Thus the longitudinal fission, lost sight of at the beginning of the second contraction, reappears upon the spindle. The chromosomes are attached to the spindle by their apices, and somewhat bulged out from it; where they are much twisted, figures somewhat more complicated than those described above may be observed.

The next stage is the separation of the two V-shaped sister chromosomes. Generally one pair of ends is freed before the other (Fig. 22); the free limbs appear to contract somewhat, suggesting that the chromosomes are being pulled apart. Later, in the anaphase (Fig. 23), the limbs are usually of the same length. The longitudinal fission is always recognizable first at the end of the chromosome not attached to the spindle, and is never seen, at this stage, to reach quite to the attached end.

It is instructive to compare the state of affairs on the heterotype spindle with that in the vegetative division. There also in *Vicia Faba* the chromosomes of the metaphase are attached to the spindle by one end, and there also the two halves of the chromosome move apart, so that a U-shaped or penthouse-shaped figure is formed. Only, in the vegetative division, this arrangement is due to the longitudinal split, and not, as in the heterotype, to the separation of whole premeiotic chromosomes. Consequently no further fission takes place on the vegetative spindle, and the chromosomes of the anaphase have the form of rods and not of V's.¹ Otherwise the mechanism of the two types of division is strikingly similar.

THE HOMOTYPE DIVISION.

When the chromosomes reach the pole of the spindle they become united one to another laterally (Figs. 25, 26), and sometimes also by their ends (Fig. 26), as in the somatic telophases, but without losing their V-shape. They do not produce a reticulum, and no satisfactory evidence of a new fission in their limbs could be obtained. After a brief resting period the cross attachments disappear, the chromosomes elongate considerably (Fig. 27), extending through the vacuole which has developed about them, and reach a stage which may be compared to the spireme of the vegetative prophase. Contraction then takes place, and they pass on to the homotype spindle, still retaining their individuality and their characteristic form (Fig. 28).

The two homotype spindles may lie parallel (Fig. 29) or at an angle (Fig. 30) one to another, often one or both are curved (Fig. 30), and they

¹ A V-shaped chromosome in vegetative mitosis is due to the attachment of a bent rod by its middle, and is represented in the meiotic anaphase by a double V such as is found in *Lilium*.

may be laterally compressed so that one diameter of the equatorial plate is much longer than the other. The broad aspect of such a spindle is shown in Fig. 28.

On the spindle the two arms of the V separate, so that the longitudinal fission, begun in the last premeiotic telophase, is here completed, and the daughter chromosomes pass as rods (Fig. 29) to the poles of the spindle (Fig. 30). Cross connexions appear between them, longitudinal fission in preparation for the first mitosis in the pollen-grain occurs (Fig. 31), and a spireme is produced (Fig. 32), the diamond-shaped mesh of which, as already shown (Fraser and Snell, '11), is quite similar to that of the diploid nuclei.

CONCLUSIONS.

The points of special importance emerging from this series of events are the persistence in *Vicia Faba* of the longitudinal fission from the last sporophytic telophase to the metaphase of the homotype division, and the fact that, as far as any indication can be obtained, the association of the allelomorphs begins after synapsis, taking place in the course of the formation of the heterotype spireme, when the cross connexions of the reticulum break down.

The relative unimportance of the position which the chromosomes of a pair assume in relation one to another has been recently emphasized by Farmer (1912), and attention may be called to the fact that in *Vicia Faba* lateral and end-to-end approximations take place in the same nucleus, and become recognizable at the same late stage of development, nor is either type related to the appearance of a double thread, either in the presynaptic prophases or in the premeiotic divisions.

The chromosomes of the vegetative division are seen to be double structures, not only in the premeiotic telophases, but in the postmeiotic also (Fraser and Snell, '11), so that, unless a different interpretation be placed upon the duplication at different stages, it is clear that it cannot be ascribed in the former, since it is impossible to ascribe it in the latter, to an approximation of paternal and maternal segments. Rather it is due in both alike to longitudinal fission or vacuolization. As cross connexions appear, and as the nuclear area enlarges, the diamond-shaped mesh of the reticulum is formed by the pulling apart of the split portions of the chromosomes. In the meiotic prophase this mesh can be traced unbroken into full synapsis, and may be recognized in places at a considerably later stage. After the synaptic phase, as the cross connexions break down, the sides of the split strands tend to approximate, and the fission is only made clear here and there by fortunate accident.

Having regard to the origin of the duplication, it is impossible to relate it to the formation of the gemini, and the confusion which has so frequently

arisen is probably due to the fact that the very severance of cross connexions which tends to obscure the split, makes possible the approximation of the first strands of the now free spireme, and the consequent pulling apart and rearrangement by which fresh attractions come into play, and new pairs are produced.

Where a presynaptic association of paternal and maternal segments occurs, evidence of its existence must be looked for, not in the double thread, but rather in the pairs of chromosomes of similar form which have been recorded by Müller (8, 9) for the sporophyte of *Yucca* and other plants, and by Reed (10) for that of *Allium*. Pairing of this type has not been recognized in *Vicia*.

SUMMARY.

1. The spore mother-cell shows a dense, finely granular cytoplasm, and a large nucleus. The nuclear reticulum is spread just inside the membrane, and shows an irregularly diamond-shaped mesh such as is found, both in other diploid, and in haploid nuclei (Pl. XLIII, Fig. 1).

2. The first indication that reduction is about to take place, consists in the separation of the reticulum from the nuclear membrane (Fig. 2). The membrane enlarges and a considerable contraction of the chromatin mass occurs. During synapsis the chromatin retains its reticulate character (Fig. 3).

3. In the loosening of synapsis some of the cross connexions of the reticulum break down (Fig. 4), and free lengths of spireme appear (Figs. 5-8). Some of these form pairs of similar loops, and other paired figures between the sides of which approximation takes place (Figs. 5-9).

4. The loosened spireme extends throughout the nuclear vacuole; in its threads the longitudinal fission, initiated in the preceding telophase, and recognizable in the diamond-shaped mesh of the reticulum, can still be traced (Figs. 10, 11).

5. Pairing between independent portions of the spireme becomes more frequent, and the rearrangement entailed pulls the thread into a loose knot (Fig. 12).

6. The thread becomes shorter and thicker, and the second contraction figure is produced (Figs. 13, 14).

7. The spireme breaks up into seven paired segments, the gemini (Pl. XLIV, Figs. 16, 17), which are thus produced by the association of different parts of the spireme. It is indifferent whether this association takes place laterally (Pl. XLIII, Figs. 7, 12) or end to end (Figs. 6, 8, 14).

8. The nuclear membrane disappears, the cytoplasm invades the nuclear area, forming the spindle, the gemini shorten and thicken, and are arranged on the equatorial plate (Pl. XLIV, Fig. 18).

9. Each of the gemini is attached to the spindle by one end (Fig. 19). The two constituent chromosomes move apart along the spindle fibres, but remain for a while in contact at their free end (Figs. 19, 20).

10. As they separate the longitudinal fission in each becomes distinct (Figs. 21, 22). The chromosomes travel up the spindle as V-shaped structures attached by the apex of the V (Figs. 23, 24).

11. At the pole of the heterotype spindle the chromosomes become united laterally, and sometimes end to end, by cross connexions (Figs. 25, 26).

12. When these break down they elongate; they retain their individuality and their V-form (Fig. 27).

13. They pass as V's on to the homotype spindle (Fig. 28), and there the limbs of the V separate, going to different poles, so that the longitudinal fission, begun in the last premeiotic telophase, is at last completed; the daughter chromosomes have the form of rods as in a vegetative division (Fig. 29).

14. At the pole of the homotype spindle the chromosomes become united (Figs. 30, 31), both laterally and end to end. Longitudinal fission takes place in them, and the reticulum of the spore nucleus is produced, and shows a diamond-shaped mesh similar to that of other vegetative nuclei (Fig. 32).

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EXPLANATION OF PLATES XLIII AND XLIV.

Illustrating Dr. H. I. C. Gwynne-Vaughan's paper on the Meiotic Divisions in *Vicia Faba*.

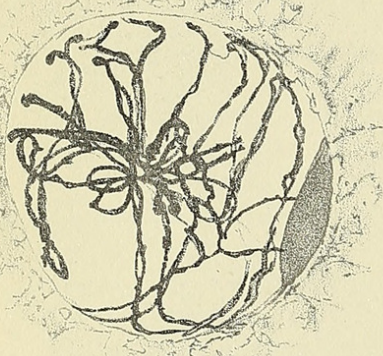
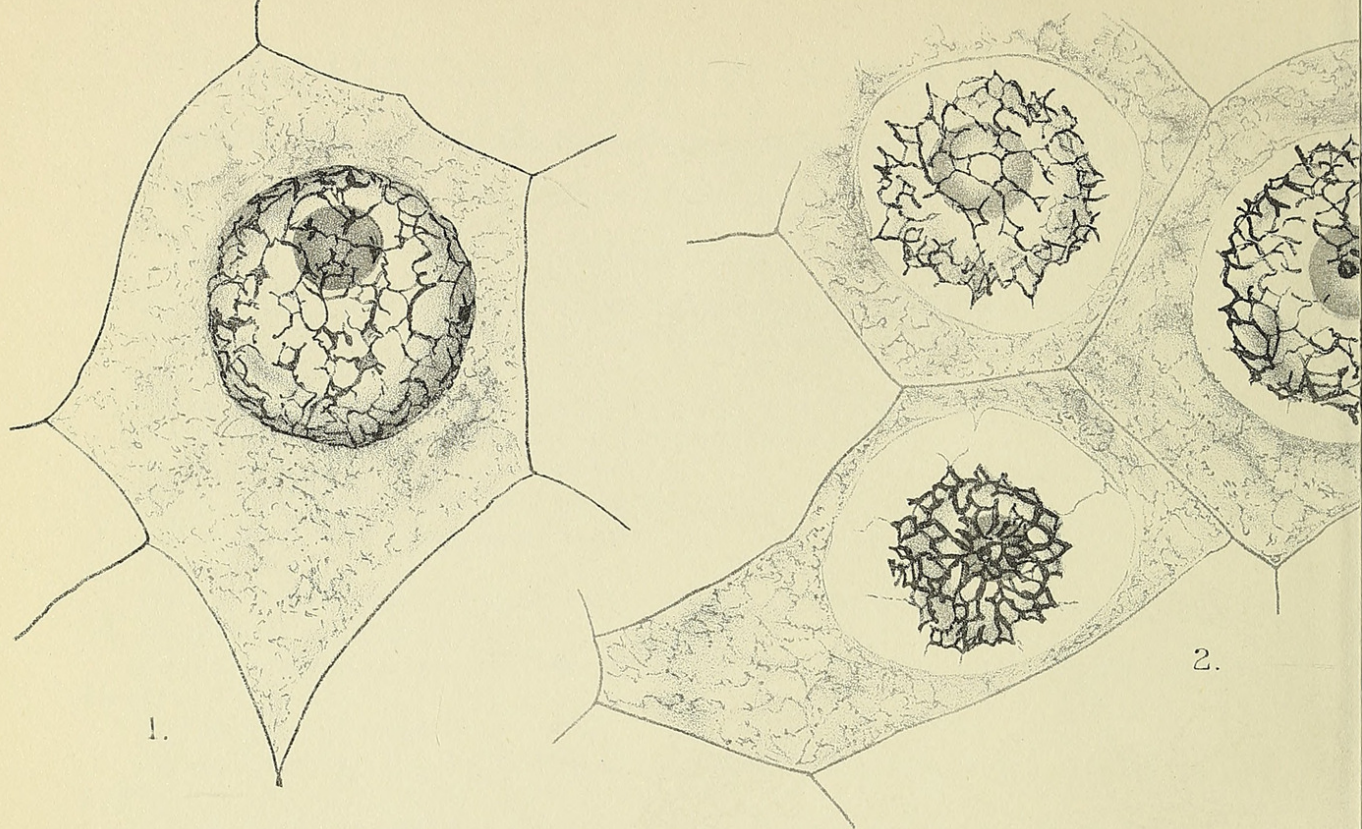
Magnification $\times 2,000$ throughout.

PLATE XLIII.

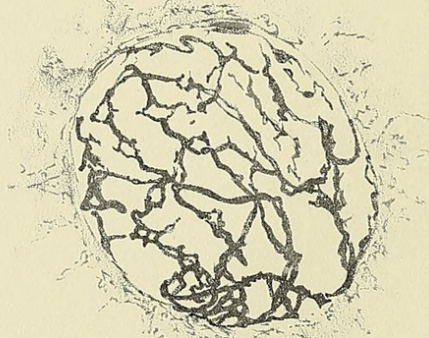
- Fig. 1. Pollen mother-cell with resting nucleus showing diamond-shaped mesh of reticulum.
- Fig. 2. Three stages in the separation of the reticulum and nuclear membrane.
- Fig. 3. Full synapsis; surface view.
- Fig. 4. Rather later stage, showing extrusion of nucleolus and beginning of formation of spireme.
- Fig. 5. Loosening of synapsis; two similar loops thrown out.
- Fig. 6. Same; threads united end to end.
- Fig. 7. Same; parallel association of threads.
- Fig. 8. Same, rather later; three distinct pairs.
- Fig. 9. Same.
- Fig. 10. Nuclear area filled by postsynaptic spireme showing fission; cross connexions have in this case persisted longer than usual.
- Fig. 11. Postsynaptic spireme; some paired strands recognizable, also fission of chromatin thread.
- Fig. 12. Second contraction.
- Fig. 13. Later stage of same, showing a conspicuous double loop, one end of which has been set free.
- Fig. 14. Second contraction; several pairs of associated chromosomes may be recognized.
- Fig. 15. Same; longitudinal fission indicated in places.

PLATE XLIV.

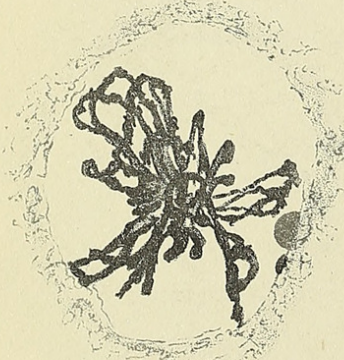
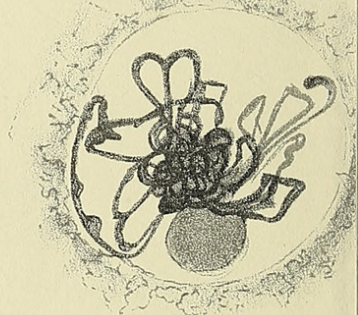
- Fig. 16. Five newly separated gemini.
- Fig. 17. Gemini, rather later stage.
- Fig. 18. The seven gemini on the equatorial plate.
- Fig. 19. Metaphase of first meiotic division.
- Fig. 20. U-shaped gemini of metaphase.
- Fig. 21. First meiotic division, showing longitudinal fission in the sister chromosomes.
- Fig. 22. Same; separation of the V-shaped sister chromosomes.
- Fig. 23. First meiotic division; anaphase.
- Fig. 24. Same; telophase.
- Fig. 25. Cross attachment of chromosomes after first meiotic division.
- Fig. 26. Same; showing both lateral and terminal attachments.
- Fig. 27. Elongation of V-shaped chromosomes.
- Fig. 28. V-shaped chromosomes on second meiotic spindle.
- Fig. 29. Second meiotic division; anaphase.
- Fig. 30. Same; telophase.
- Fig. 31. Very late telophase, showing fission and attachment of chromosomes.
- Fig. 32. Resting nuclei of young pollen grains, showing diamond-shaped mesh of reticulum.
- Fig. 33. Extrusion of chromatic bodies from nucleus of pollen mother-cell.



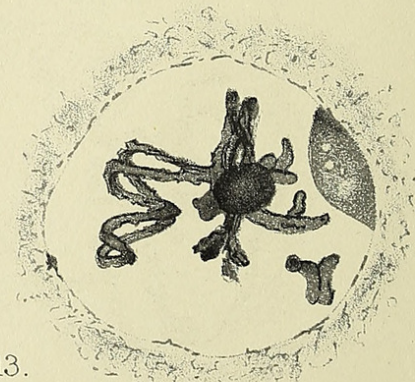
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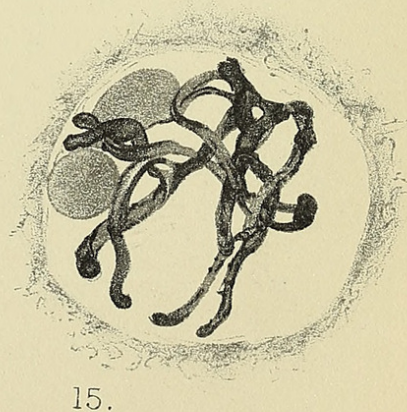
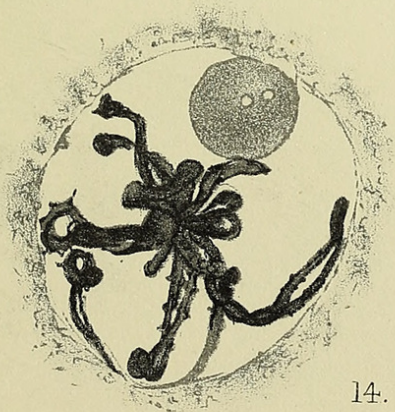
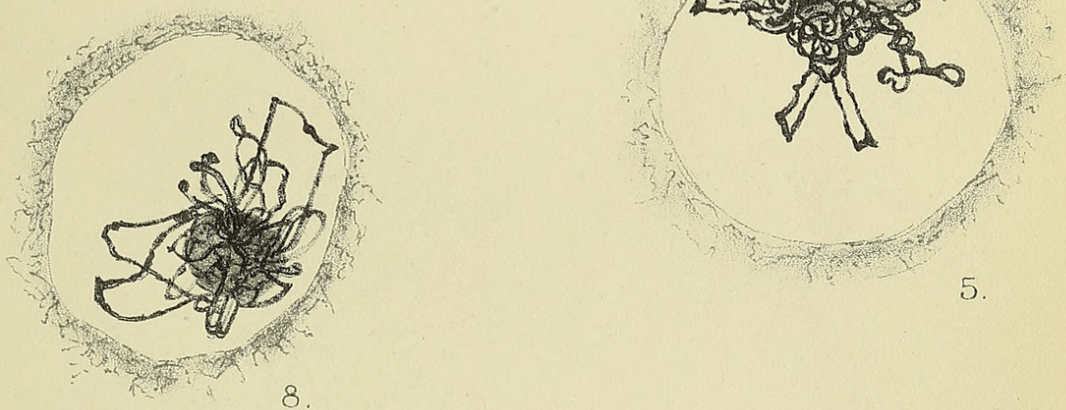
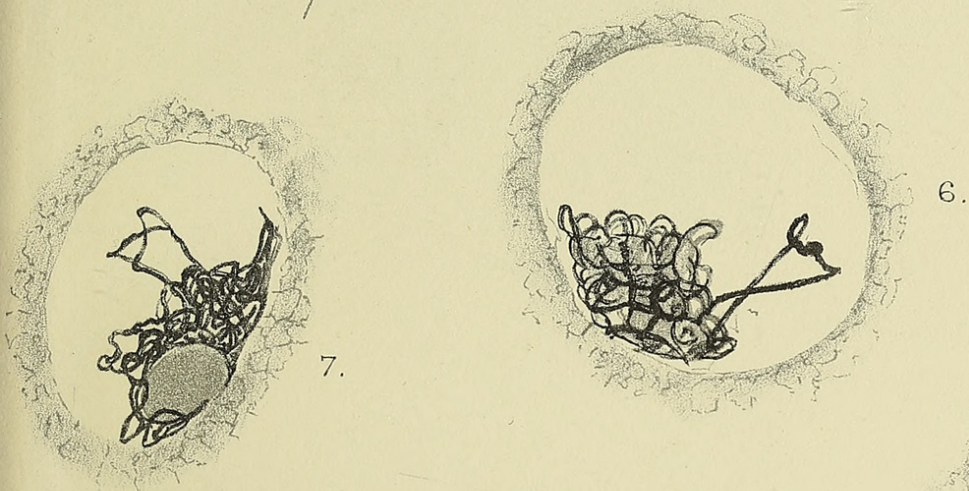
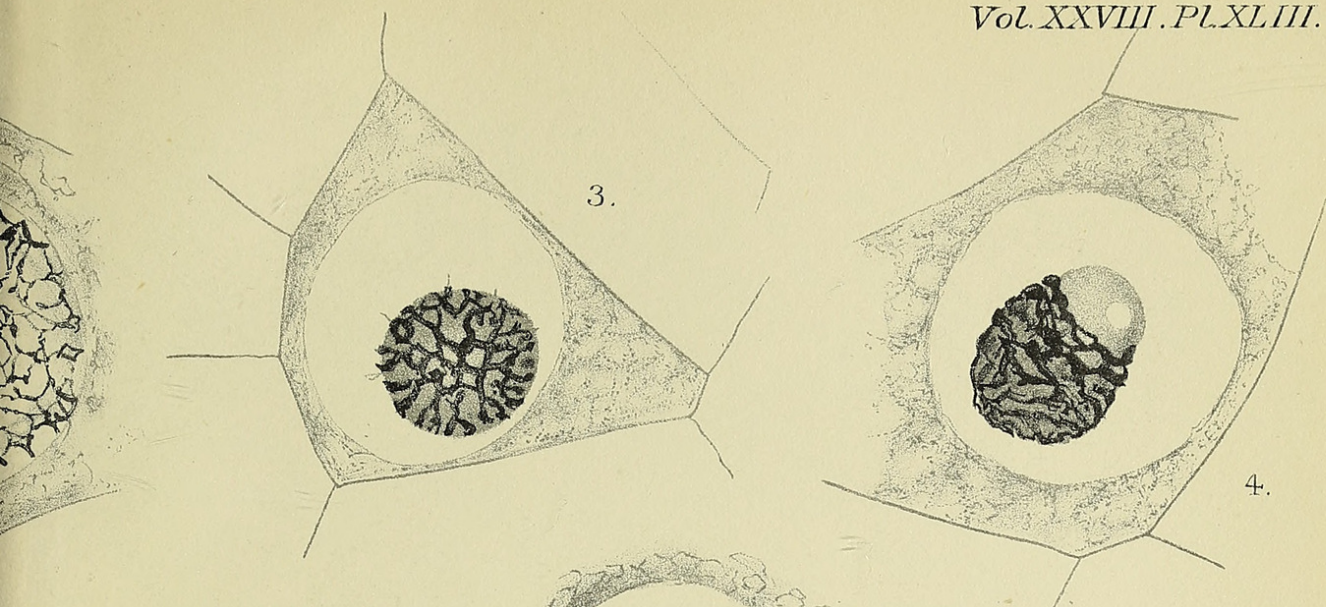
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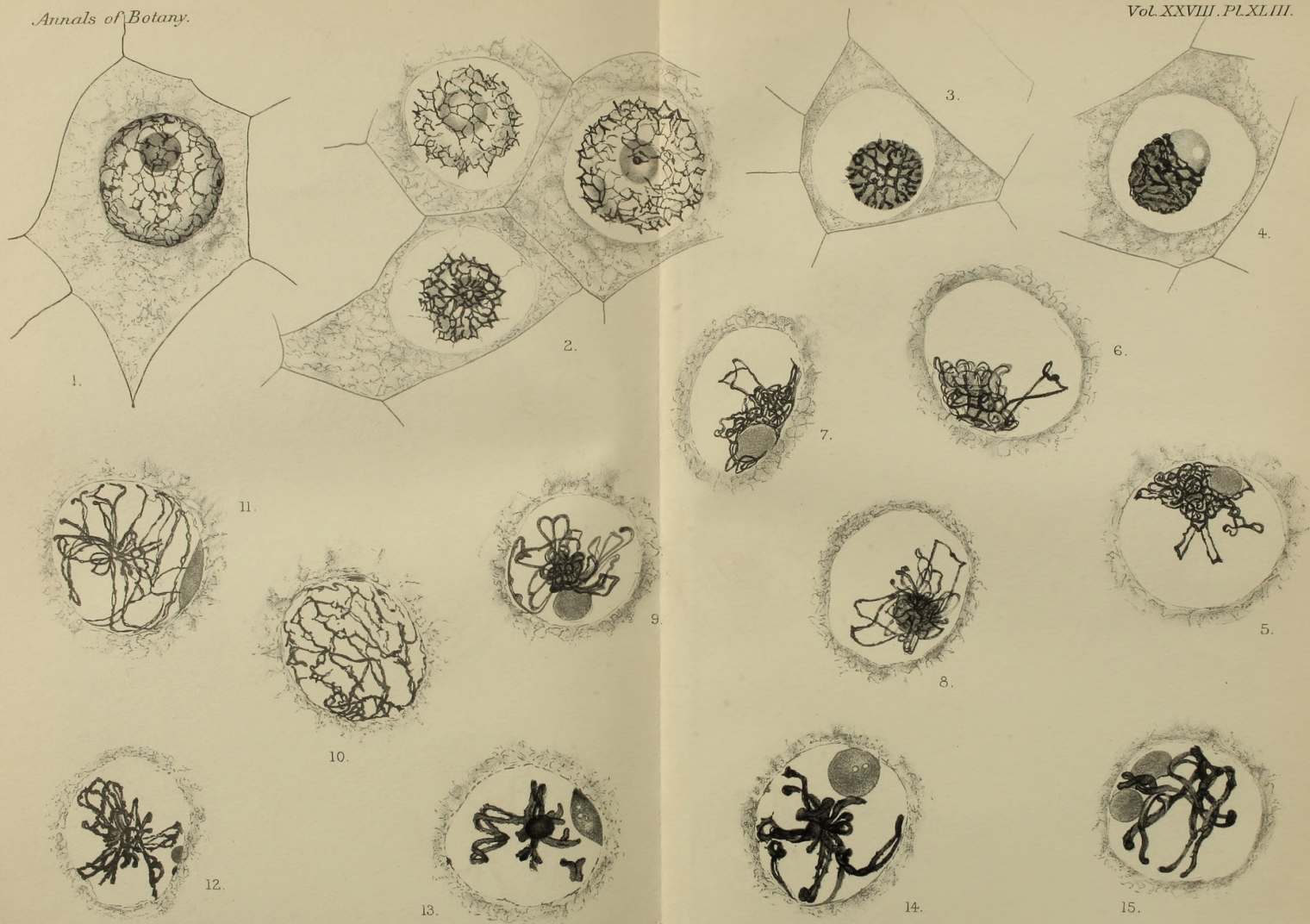


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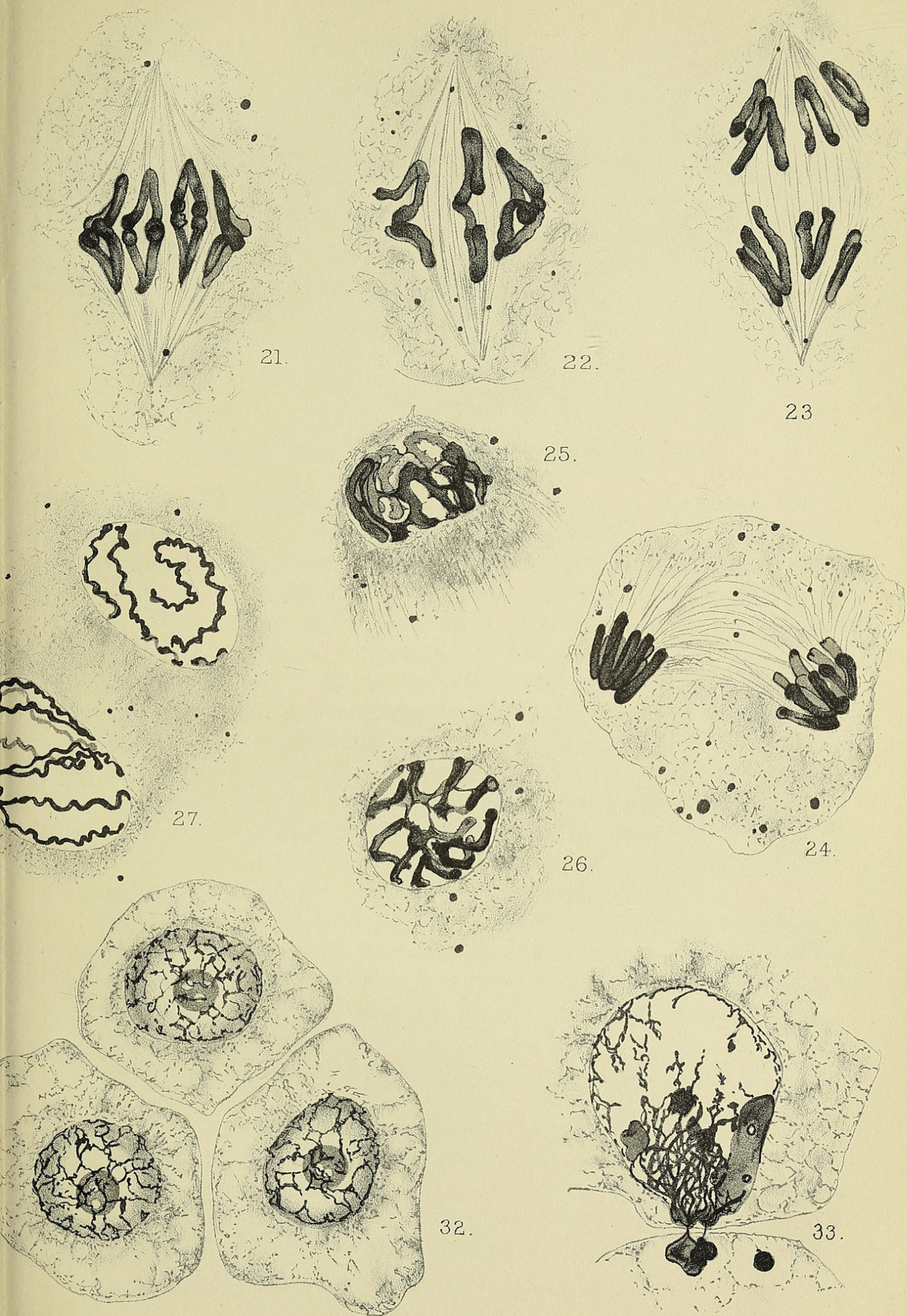


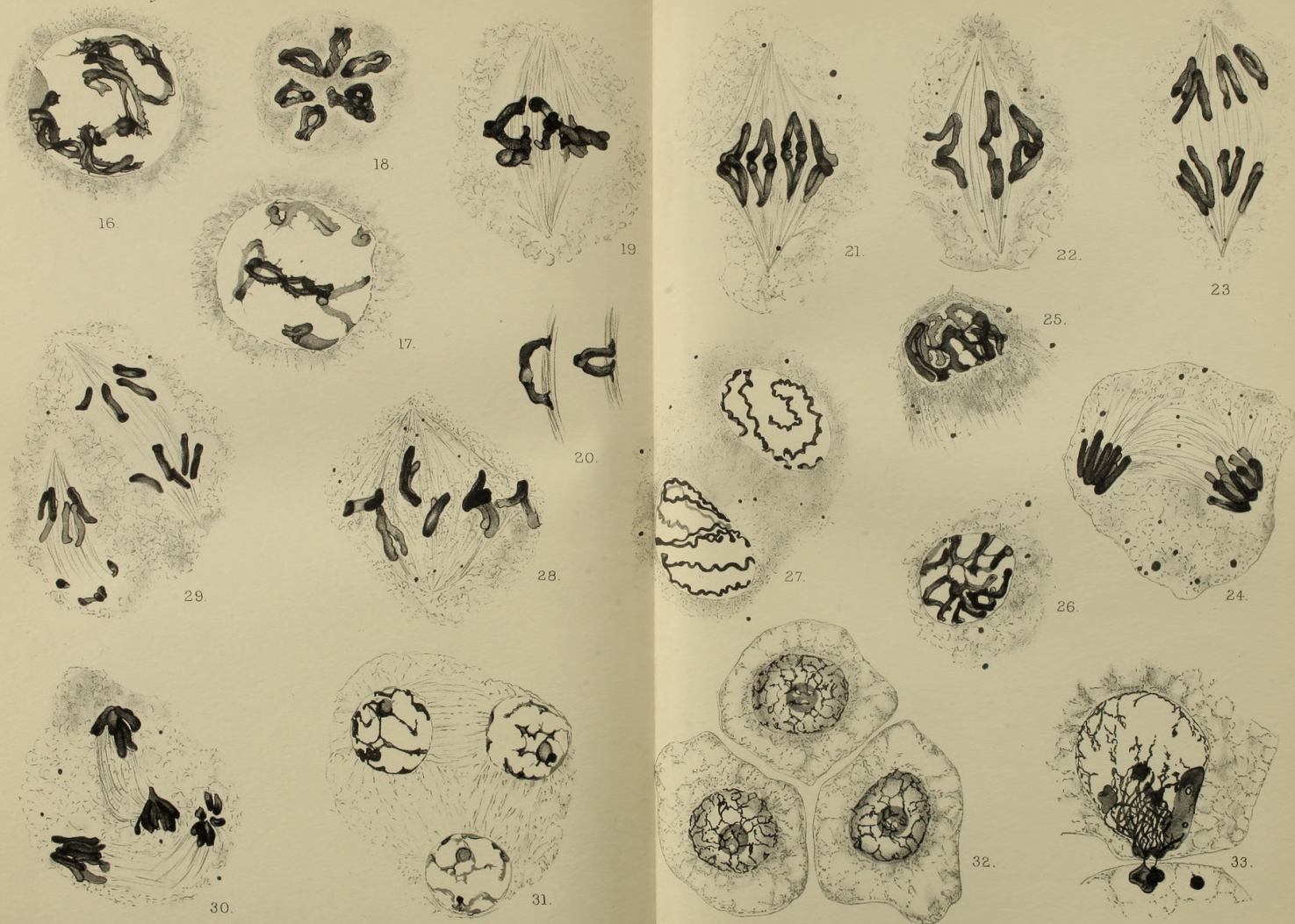
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