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NUCLEAR DIVISIONS IN THE POLLEN MOTHER-CELLS OF CONVALLARIA MAJALIS L.

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The significance which recent investigators have read into the two maturation divisions, as well as the peculiar behavior of the chromatic substance during and after synapsis, make the chromosome the cynosure of all the problems in heredity.

Although the Liliaceae have served as a classical group of angiosperms for this kind of work in botanical research, only twice have their closest allies, the Convallariaceae, received attention. Strasburger ('88) in a very general way, refers to the chromosomes of Convallaria, and Weigand ('99, '00) takes up the development of the microsporangium, and of the embryo sac of Convallaria, but little emphasis is laid on the nuclear divisions in the pollen mother-cells. Weigand holds that the reduced number of chromosomes is eighteen, that he could not determine the plane of the first nuclear division of the mother cell, and that the plane of the second division appears to be transverse. He further states that at several instances the chromatin behaves peculiarly, and that the generative cell of the pollen grain is cut off shortly before the flower opens.

The present writer undertook a careful study of the nuclear phenomena of the pollen mother-cells of Convallaria majalis L, with the hope of making further contribution towards the solution of the problem of heredity and the chromosome. With none of the above mentioned observations of Weigand does the present writer agree. This paper is offered as a preliminary to further work on the chromosome.

To Professor Guyer, at whose suggestion the work was undertaken, the writer is much indebted for valuable assistance.

MATERIAL AND METHODS.

Neubert's "Multibell" brand of German Lily-of-the-Valley pips were "forced" in moist sand, at the temperature of about 20 C., in a green-house, in subdued light. When the sprouting pips were of various lengths stamens were isolated from the flower buds. Pips two to four centimeters in length contained all the stages desired. After killing and fixing the anthers in a chrom-acetic acid solution* they were washed in tap-water, passed through the series of alcohols, and preserved in 85% alcohol. Paraffin (51° C.) sections (3-10**u**), stained on the slide, cleared in xylol, and mounted in balsam, were used for study. Longitudinal and transverse sections of anthers were made. As stains, Heidenhain's iron-alum-haematoxylin with Orange-G, and Delafield's haematoxylin with safranin gave good results. Drawings and observations are based on slides stained chiefly with the former combination. The pollen mother-cells near the apex of the anther are slightly more advanced than those nearer the filament.

OBSERVATIONS.

The nucleus enters a state of rest at the conclusion of the last archesporial division of the sporophyte tissue (Fig. 1). This is of short duration, however, and the chromatin matter soon becomes transformed into a number of fine, delicate threads in the form of a network (Fig. 2). The nucleolus is visible from the beginning, and it, as the cell becomes larger, behaves peculiarly (Figs. 1–8), in that it becomes larger, less chromatic, and shows a clear, vacuole-like area near its center. Occasionally it fragments into micronucleoli. With an increasing volume of the cells the chromatin matter of the nucleus becomes more conspicuously granular, and the linin appears to contract. There seems to be a reciprocal loss of chromatic substance from the nucleolus (Figs. 6, 7, 8). The clear area in the nucleolus makes it resemble in appearance an erythrocyte of man (Fig. 6a). Occasionally two such clear areas are noticeable in the nucleolus (Fig. 8). The cell increases in volume, the spirem continues to contract, and the continuity of the thread becomes more and more apparent (Figs. 3, 3a, 4, 5a).

From now until the apparent climax of the process which ends in synapsis, the contraction of the spireme thread is more rapid, and the network becomes more twisted. The linin is at all times of smaller diameter than the chromatin granules (Figs. 3a, 5a). The continuity of the twisted spireme thread becomes very apparent, and the thread becomes entirely separated from

*Chrom-acetic acid solution: Chromic acid 0.3g., glacial acetic acid 0.7cc., water 99cc.

the wall (Fig. 6). The chromatin granules are now quite uniform in size and shape, and the spireme exists as a single thread throughout its entire length (Figs. 6, 6b).

After synapsis a loosening or unwinding of the thread begins. The linin becomes thicker, the granules elongate, and the spireme becomes shorter, although it again occupies the whole of the nuclear cavity (Fig. 7). Then an apparent division of the granules takes place and a double row can be seen (Fig. 8a). Occasionally a part of the thread appears still single while the rest is double (Fig. 8a). This appearance would be the same if the granules were dividing or conjugating. The fact that the granules lying opposite each other are so much alike in size and shape is all that makes this appear to be a division of the thread. There is quite a marked decrease in chromatin matter, however, and this, at first sight, would favor a conjugation, but it must be remembered that the spireme is rapidly contracting (Figs. 7, 8, 9). After a short time this doubleness is no longer apparent. For the present this will be considered a temporary division of chromatin granules. In later studies the author intends to give this step more consideration.

With continued thickening the ribbon begins to show an arrangement into definite loops (Figs. 9, 10, 11), which later become sixteen chromosomes. Just how these are formed from the thirty-two of the spermatogonia has not yet been determined. They are of various shapes and sizes, but a common thickness. In fact, thickness seems to be the only factor which the sixteen chromosomes have in common. They are apparently twice as thick as the chromosomes of the sporophyte cell. Since the relative stage of development of chromosomes was found to govern thickness, it was thought advisable to compare for this purpose chromosomes of nearly the same stage. Figs. 17, 21, 26, show telophases of cells after the first reduction division, second reduction division, and sporophyte cell division, respectively. The fact that the daughter chromosomes of Fig. 17 are so much thicker than those of Fig. 26 seems to indicate that the phenomenon after synapsis might have been a pairing of granules.

There is, usually, one chromosome which is much longer than the others. In Fig. 13, it is the fourteenth chromosome. It shows a lobing at one end. Many of the chromosomes at this stage show a lobing at either, or both ends. (Fig. 12a). This lobing is either the beginning of the longitudinal division, or else it is a remnant of the double phenomenon noticed shortly after synapsis. The nucleolus now fragments and passes into the cytoplasm. With the ejection of the micronuclei radiations in the cytoplasm appear, and with their polarization the chromosomes assume a median position between the two centers of radiation. No centrosomes are present. This is the prophase of the first reduction division. Fig. 14 is a very late prophase or early metaphase.

THE FIRST NUCLEAR DIVISION OF THE MOTHER-CELL.

All sixteen chromosomes divide almost simultaneously. To determine the plane of this division was no easy task. That it is a transverse division is quite evident from the cells examined. Figs. 14a, 16a, 16b, show some chromosomes dividing transversely, and since this is the only transverse division, it should be considered qualitative. The spindle fibers end near or at the free ends of the chromosomes. The homologous daughter chromosomes show not only a marked correspondence in size, but also in shape (Fig. 14, 14a, 15, 16b). Whether this similarity in shape is inherent, or is the result of a stress brought about by the spindle fibers, or is the result of a repulsion of the daughter chromosomes has not been determined. J, I, V, U, shaped chromosomes are present. I and J shaped predominate (Figs. 15, 16). In rare instances indications of a median cleft throughout the length of the chromosome in metaphase could be noticed (Fig. 14a). This is probably the beginning of the second division. Since the daughter chromosomes show such a marked similarity in shape, the author was at first skeptical about the plane of the division, but enough data are now at hand to prove that the division is transverse. Figs. 14a, 14b, 15a, 16a, 16b, etc., show this. In the migration of the daughter chromosomes to their respective poles, a doubleness is occasionally detectible (Figs. 16, 17a). This is probably the beginning of the second division. A wall now develops and a distinct nuclear plate is seen between the daughter nuclei (Fig. 17). The micronuclei apparently re-enter the nucleus (Figs. 17, 18).

THE SECOND NUCLEAR DIVISION OF THE MOTHER-CELL.

The daughter nuclei do not enter into a definite period of rest, and the chromosomes soon become developed into the mother skein of the second division. The transition is so rapid, and the telophase of the first division and the prophase of the second are so close together, that the individual chromosomes seem to be separate from the beginning. Occasionally, a cell is seen in which the second division has already taken place, while its sister cell is still undivided (Fig. 22). Ordinarily, the sixteen chromosomes of each of the two daughter cells of the first division are almost immediately ready for the second division. Sometimes a cell is seen in which some chromosomes, the double nature of which is apparent, lie in a horizontal position in prophase, while other chromosomes, which are slightly more advanced, are at right angles to the horizontal ones, and are already migrating to their respective poles (Fig. 19). There seems to be little probability that this is a cell in which some chromosomes are dividing longitudinally while others are dividing transversely as McClung

('05) found in certain Orthoptera. Such a pronounced irregularity in appearance as shown in Fig. 19 is of comparatively rare occurrence.

In this division the spindle fibres are not nearly as prominent as in the first division. No centrosomes are present. The fragmented nucleolus, which was in the nucleus during the prophase, is again in the cytoplasm.

That the plane of division is longitudinal is evident (Figs. 19a, 20a). The line of cleavage seems to be that occasionally indicated earlier in the process (Figs. 14a, 16a, 19a, 20a). The homologous daughter chromosomes, as expected, show a marked similarity in shape and size (Figs. 19a, 20a). Although a great variety of shapes were seen the U and I shaped chromosomes predominated. (Figs. 20, 21).

The nuclear membrane disappears during late prophase (Fig. 14), is absent during metaphase and early telophase (Figs. 15, 16, 26), and again appears in late telophase (Fig. 17). It remains present during the subsequent stages (Figs. 18, 22, 23, 24, 25). The breaking down of the membrane is co-incident with the migration of the nucleolus into the cytoplasm as micronucleoli.

THE MICROSPORES.

After the late telophase of the second division (Fig. 21), the chromosomes of the daughter nuclei develop into irregular networks, and, with the appearance of a transverse wall, a cell is formed out of each of the two hemispheres, and thus the spore-tetrad is completed. The formation of the wall is, in Convallaria, as in most of the Monocotyledons, "successive," i. e., begun after the first division, and completed after the second. The young microspores are arranged bilaterally in the spore-tetrad (Fig. 23). Occasionally, as already said, a cell is found in which one of the cells has not yet divided, and then only three cells are enclosed by the wall (Fig. 22). Whether the undivided cell will ultimately divide, could not be determined.

Sometime after the common wall is completed, each microspore develops a delicate wall which seems to be independent of the former. This thin wall grows in thickness and later becomes differentiated into the intine and exine layers (Figs. 23, 24, 25). The tetrad then breaks, and the four microspores become free and separated. All this occurs long before the flower opens.

The next change that the nucleus undergoes is a division, whereby the generative cell is formed. The nucleus of this cell is at first chromatic and so deeply stained that its structure cannot be determined (Fig. 24). After a short time, however, the cell becomes differentiated by a distinct cell wall. Although the generative nucleus is at all times more chromatic than the nucleus from which it arises, its structure becomes discernable sometime before the flower opens. The generative nucleus usu-

ally contains several micronucleoli, and a network of chromatin matter (Fig. 25). The pellucid matter which surrounds the generative nucleus contains many minute granules and is probably the cytoplasm of the new cell. Convallaria agrees with most of the other Monocotyledons in the early separation of its generative cell. No sperm-nuclei could be found, and their formation doubtless takes place during the formation of the pollen-tube, and is, therefore, a part of fertilization, as it is in most of the Monocotyledons.

SUMMARY.

1. The continuous spireme up to and through synapsis is a single row of chromatin granules on a linin thread.

2. The chromatin granules seem to show a temporary division shortly after synapsis.

3. The spireme shortens and thickens, and breaks up into sixteen chromosomes.

4. The first division of the chromosomes in the microsporocyte is transverse, and, therefore, qualitative.

5. The homologous daughter chromosomes show marked similarity in size and shape.

6. The daughter chromosomes of the first division seem to represent the chromosomes of the second.

7. The second nuclear division is longitudinal, and therefore, equational.

8. The spore-tetrad breaks up into four incipient pollen-cells, in each of which a generative cell forms, quite a while before the flower opens.

9. The nucleolus fragments and passes into the cytoplasm just before or during the prophases of the two reduction divisions.

10. The nuclear membrane disappears during late prophase, is absent during metaphase and early telophase, is present in late telophase and remains during subsequent stages.

11. The chromosomes of the generative cells are thicker, but not longer than the chromosomes of the sporophyte cells.

12. The homologous daughter chromosomes of the second division also show a marked similarity in size and shape.

University of Cincinnati, Feb., 1909.

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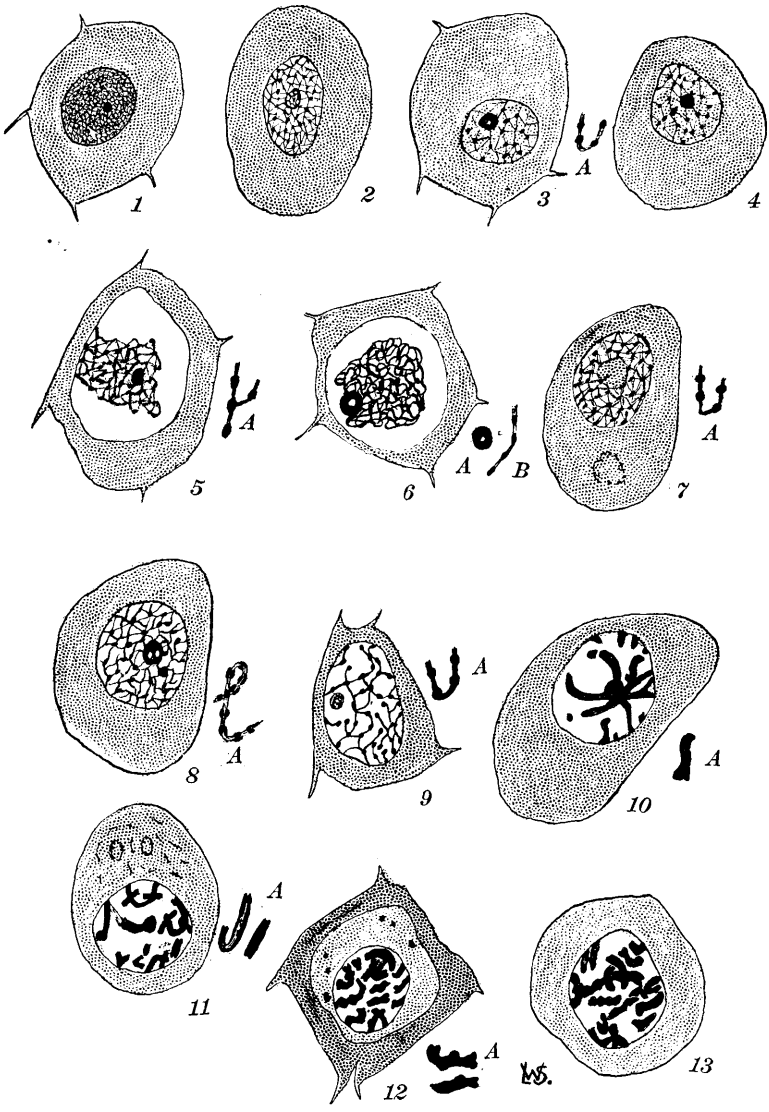
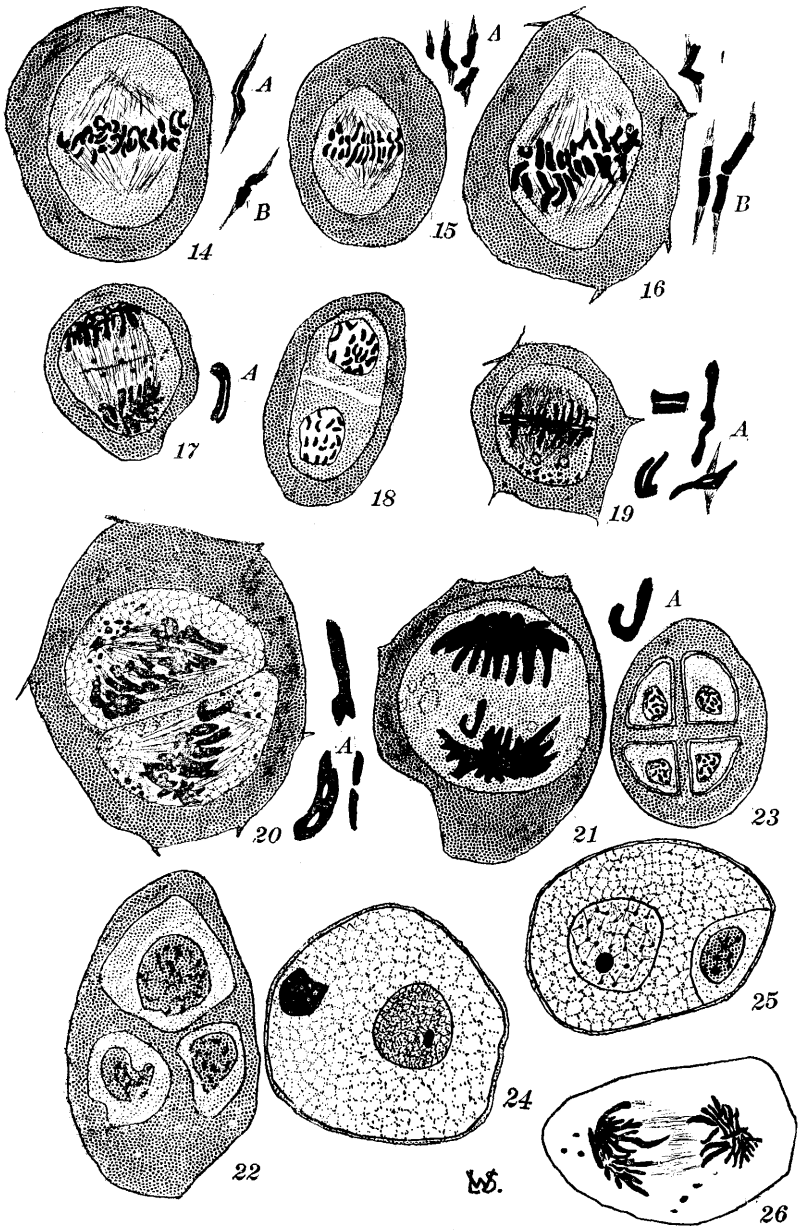


SAUER on "Convallaria."

OHIO NATURALIST. *Plate XXV.*



SAUER on "Convallaria."

EXPLANATION OF PLATES XXIV AND XXV.

All figures were made with the aid of a camera lucida. The figures representing entire cells (excepting Fig. 20) were studied with a B. & L. one-inch ocular and a 1-16 oil immersion objective; Fig. 20, and all sub figures (excepting 20a) with a Zeiss No. 12 ocular and Leitz 2 m. obj.; Fig. 20a with the No. 12 ocular and the 1-16 objective.

Fig. 1. Microsporocyte with a very dense network of chromatin matter; nucleolus visible, nuclear membrane distinct.

Fig. 2. Microsporocyte with chromatin fibrils and definite granules.

Fig. 3. Microsporocyte with thread contracting, the granules spherical, linin definite, nucleolus deeply stained. 3a. A part of the thread.

Fig. 4. More advanced stage: more granules noticeable.

Fig. 5. Early synapsis, nucleus occupies most of the cell. 5a. Granules are more oval.

Fig. 6. Synapsis, thread free and continuous. 6a. Nucleolus shows a clear area. 6b. Granules quite uniform in size, linin distinct.

Fig. 7. Nucleus smaller, nucleolus pale and irregular, chromatin matter contracting. 7a. A part of the thread.

Fig. 8. A later stage. 8a. The thread, some of the granules showing a doubleness.

Fig. 9. The contraction more advanced, the granules single, the nucleus somewhat larger. The nucleolus very pale. 9a. A part of the chromatin ribbon.

Fig. 10. A microsporocyte with loops nearly developed. The nucleolus apparently the center of activity. 10a. A separated, homogeneous band of chromatin matter.

Fig. 11. The chromosomes nearly formed. 11a. Chromosomes showing lobed ends and a differentiation down their median axes.

Fig. 12. The sixteen chromosomes formed, micronucleoli in the cytoplasm. 12a. Two chromosomes.

Fig. 13. A sporocyte showing sixteen chromosomes.

Fig. 14. Metaphase of first maturation division. 14a, 14b, chromosomes showing transverse divisions.

Fig. 15. Late metaphase. 15a. Chromosomes showing transverse divisions.

Fig. 16. Telophase of heterotype division. 16a. Chromosomes dividing.

Fig. 17. Telophase of heterotype division, micronucleoli, wall, plate, etc. 17a. A chromosome with lobed ends.

Fig. 18. Late telophase, the resting chromosomes smaller than in 17.

Fig. 19. Metaphase of second division. Median split more noticeable. 19a. Chromosomes dividing.

Fig. 20. Metaphase of the homotype division, the mitoses simultaneous in the two daughter cells of the first division. 20a. Chromosomes.

Fig. 21. Telophase of second division, chromosomes homogeneous, no lobed ends. 21a. A chromosome.

Fig. 22. A tetrad, the upper cell has not yet undergone a homogeneous division.

Fig. 23. A spore-tetrad with common wall around the four cells.

Fig. 24. An incipient pollen-grain. The dark body is the generative nucleus.

Fig. 25. Mature pollen-grain, generative cell with its clear cytoplasm at margin of the pollen-grain.

Fig. 26. Telophase of a sporophyte division, showing long chromosomes, nucleoli scattered in the cytoplasm.