MEIOTIC CYTOKINESIS OF CANNABIS

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(WITH PLATE VII)

During the summer of 1922, the writer investigated the meiotic divisions in the pollen mother cells of ***Cannabis*** *sativa*, for the purpose of throwing some light upon the cause of the variability of pollen grain size which occurs in this species. Although the results obtained were not conclusive, so far as the solution of the problems at hand were concerned, it seems desirable to publish a short account of meiosis in this plant, and to add another to the several already reported cases of cytokinesis by furrowing in the cells of higher plants. Since an excellent review of the literature dealing with this interesting phase of plant cytology has been given by FARR (2), it does not seem necessary to take space for literature review here.

STRASBURGER (6) published a few figures of meiotic divisions in ***Cannabis,*** mostly of the anaphase of the heterotypic division, showing ten chromosomes as the reduced number. In the root tips of the staminate and of the pistillate plants, he found that the chromosomes apparently were identical in size and shape, and no indication of a sex chromosome was observed. The results of recent research, however, have shown that so many irregularities occur in the development of the staminate buds, that a more complete account of chromosome behavior during the reduction division in this plant is desirable. The series of drawings in pl. VII show the changes which take place from the time of the archesporial cell to the liberation of the pollen grains.

Materials and methods

The material for this study consisted of staminate buds fixed whole in ALLEN'S (1) modification of Bouin's fluid. This reagent was found to be very satisfactory for fixing and preserving both the chromatic and the achromatic figures, although there is a slight tendency to cause shrinkage of the tissue. Considerable time and

[1] Contribution from Bussey Institution of Harvard University.

labor were saved by examining one anther from a bud while in the fresh condition, after staining and fixing with aceto-carmine. If the material proved to be in the right stage for study, the remainder of the bud was fixed at once. It was found that the best time to collect buds for studying the reduction division was between 7 : 00 A.M. and 11 : 30 A.M. Attempts to obtain satisfactory material in the late afternoon were practically without success.

The examination of large numbers of cells is facilitated by the fact that in a longitudinal section of a staminate bud many pollen mother cells are to be seen within a single loculus. In the premeiotic conditions the archesporial cells are crowded closely together, and occupy the whole space inclosed by the lining of tapetal cells. The greater part of each cell is filled with a rather dense cytoplasm, homogeneous in appearance. In the center or slightly to one side can be seen the large, nearly spherical nucleus, with a well defined membrane, and containing the deeply stained nucleolus. During the premeiotic period the chromatin is in such a fine state of division that no definite units can be observed, and it is not until the formation of chromatin threads during the early stages of synapsis that definite chromatin elements become visible.

The tapetal cells are conspicuous during all stages of meiosis as a ring of closely packed cells, lining the loculus of the anther. During the early synapsis stages of the mother cells, the nuclei of the tapetal cells divide mitotically, but the cells do not divide. These binucleate cells then remain in this condition until the rupture of the mother cell wall, when they begin to disintegrate and soon disappear.

Heterotypic division

The first indication of preparation for the heterotypic division is a slight enlargement of the nucleus and the appearance of a fine network pervading the space inclosed by the nuclear membrane. This fine mesh of threadlike elements appears to extend in all directions in a criss-cross manner, completely filling the nucleus and usually enveloping the nucleolus; but the threads are so fine and cross each other so frequently that it is impossible to make accurate counts of the number present. The next step is a gradual contraction of the chromatin material away from the nuclear membrane.

The condensation is not uniform at all points, and frequently the intertwining mass is found at one side of the nuclear cavity (fig. 4). The continued withdrawal from the periphery of the nuclear cavity causes the individual elements to become very closely massed together. The nucleolus at this time is usually found surrounded by chromatin, although cases have been observed where it was almost free and lay between the chromatin complex and the nuclear membrane. That this period of the nuclear reticulum and chromatin contraction is a relatively long one, is indicated by the fact that much of the material examined was in this stage of development, and seldom did the mother cells at the opposite ends of a loculus show appreciable differences. In some of the later stages, where development is more rapid, it is possible to observe synapsis stages in one end of the loculus and mother cells with two nuclei in the opposite end.

The relatively long period of contraction which passes under the name of synapsis is one during which the chromatin threads are in such close association with one another that little can be learned from observing the closely contracted mass. Fig. 5 shows the appearance of a typical cell at this time. When the chromatin complex begins to expand, the threads spread out in all directions until they reach the limiting membrane. As the threads emerge from the synaptic knot they are thicker, fewer in number, and more twisted about one another than in the early prophase. As the threads reach the membrane they contract more and more, until short, rod-shaped chromosomes are formed. The paired chromosomes which result from this contraction are shown in the region of the nuclear membrane in fig. 8.

From the end of the synaptic period, when the chromosomes are found in the region of the nuclear membrane, to the time when they are arranged in the center of the cell preparatory to being pulled apart to the opposite poles, is a period of many and rapid changes in the cell. The nucleolus, which up to this time has been very conspicuous, begins to fade and finally disappears. At about the same time the nuclear membrane very suddenly disappears. The chromosomes migrate to the center of the cell, the spindle fibers radiate into this mass from the poles of the bipolar cell, and the

chromatin units again become indeterminate. The daughter chromosomes remain in this position only a short time before they begin to move apart to the opposite poles of the cell. A polar view of the chromosomes just after they have left the plate shows them well separated and ten in number. The unusual tangential view shown in fig. 12 shows the whole twenty chromosomes as they are leaving the plate, and gives a good demonstration of their morphological identity. As the two groups of daughter chromosomes approach the opposite poles of the cell each begins to contract, and passes through a series of obscure changes, the net result of which is the formation of a complete nucleus. The heterotypic division is now complete, and the binucleate cell awaits the beginning of the homotypic division, which starts almost immediately.

Homotypic division

The homotypic division is characterized by greater rapidity than the heterotypic division, and therefore is less easily observed. At the beginning of this period the chromatin is usually found in a very fine state of division, although it is not uncommon to see small pieces of chromatin which have not undergone complete transformation in the region of the nuclear membrane. The general course of events is much the same as in the heterotypic division, but from the interkinesis condition shown in fig. 14 to the formation of the spindles is an exceedingly obscure period. The threadlike elements of each nucleus contract to form masses of chromosomes, which become arranged in the center of the nuclear cavity; the nuclear membranes and the nucleoli disappear, and spindle fibers appear. The details of these events are difficult to observe. There seems to be much less separation of the chromatin elements during this division than during the heterotypic, and as a result it is practically impossible to follow individual chromosomes. Sometimes both nuclei divide at about the same time, but in other cases one nucleus may be seen in the telophase when the other is just starting to form the mitotic figure. The divisions may both occur in the same plane, or at right angles to each other, or in various planes at different angles to one another. Fig. 15 shows both division figures parallel and in the same plane. The usual result of such an oc-

currence is that all four nuclei can be seen in one section of the mother cell, a rather unusual condition in ***Cannabis.*** The most usual condition is to see three of the four nuclei (fig. 17). As the chromosomes leave the equatorial plate in the homotypic division they are much massed together, and only in exceptional preparations can the chromosome number be determined. The final stages which follow are much the same as in the heterotypic division; the four chromosome groups become rounded off, a membrane forms about each, the spindle fibers disappear, and there results a cell with four nuclei. The cytoplasm is homogeneous in appearance throughout the cell, no connecting fibers are to be seen between the nuclei, and traces of a cell plate are entirely absent. The condition of the mother cell at this stage is represented in fig. 17.

Cytokinesis

After the meiotic divisions have been completed there is a relatively long period of apparent inactivity within the mother cell. Sections of whole buds frequently show all of the mother cells in this condition, with no indications of further development. The first sign of further change is the appearance of constrictions in the cytoplasm, at four points on the periphery, but inside the mother cell wall and at equal distances from the nuclei. The first visible effect of these constrictions, as seen in a section of the cell, is a change from the nearly spherical to a triangular form. The constrictions become very sharp and extend to the center of the mother cell, cutting it into four parts. Many mother cells can be found which show the completed process, but due to its extreme rapidity, cells which show the constrictions only part way to the center are more rare (fig. **19).** The writer has observed a similar process in the mother cells of *Nicotiana*, where it is much less rapid in its development and correspondingly easier to follow.

Until recent years the method of cytokinesis in the dicotyledonous plants was supposed to be by the formation of a cell plate, and this feature was one which constituted a distinction between plant and animal cells. FARR (**2, 3**), however, has shown that cytokinesis by furrowing occurs in the dicotyledons *Nicotiana*, ***Primula, Helianthus, Ambrosia,*** *Tropaeolum*, ***Chrysanthemum,*** *Magnolia*, and

in the monocotyledons *Sisyrinchium* and *Nelumbo*. Mrs. W. K. **FARR (4)** has found the same process in *Cobaea*. **GATES** and **REES** (5) reported a similar process occurring in the pollen mother cells of *Lactuca*. It is evident, therefore, that the occurrence of cytokinesis by furrowing is not as rare among plants as it was thought to be a few years ago, and it is probable that more examples will be found as more plants are investigated. **FARR (3)** reports that the division of the mother cells in *Nelumbo* is accomplished in part by furrowing and in part by the formation of a cell plate. A cell plate starts to form at the periphery of the cell, and after extending in for a short distance disappears. The process is then completed by the formation of furrows which divide the cell into four parts.

After the formation of the pollen grains as a result of the rapid extension of the furrows to the center of the mother cell, each grows somewhat and rounds off, although the shape of the pollen grains is never exactly spherical while they are retained within the mother cell wall. Finally, the wall of the mother cell is ruptured, the pollen grains pass through a period of growth and assume a spherical form, filling the loculus and awaiting the dehiscence of the anther.

Summary

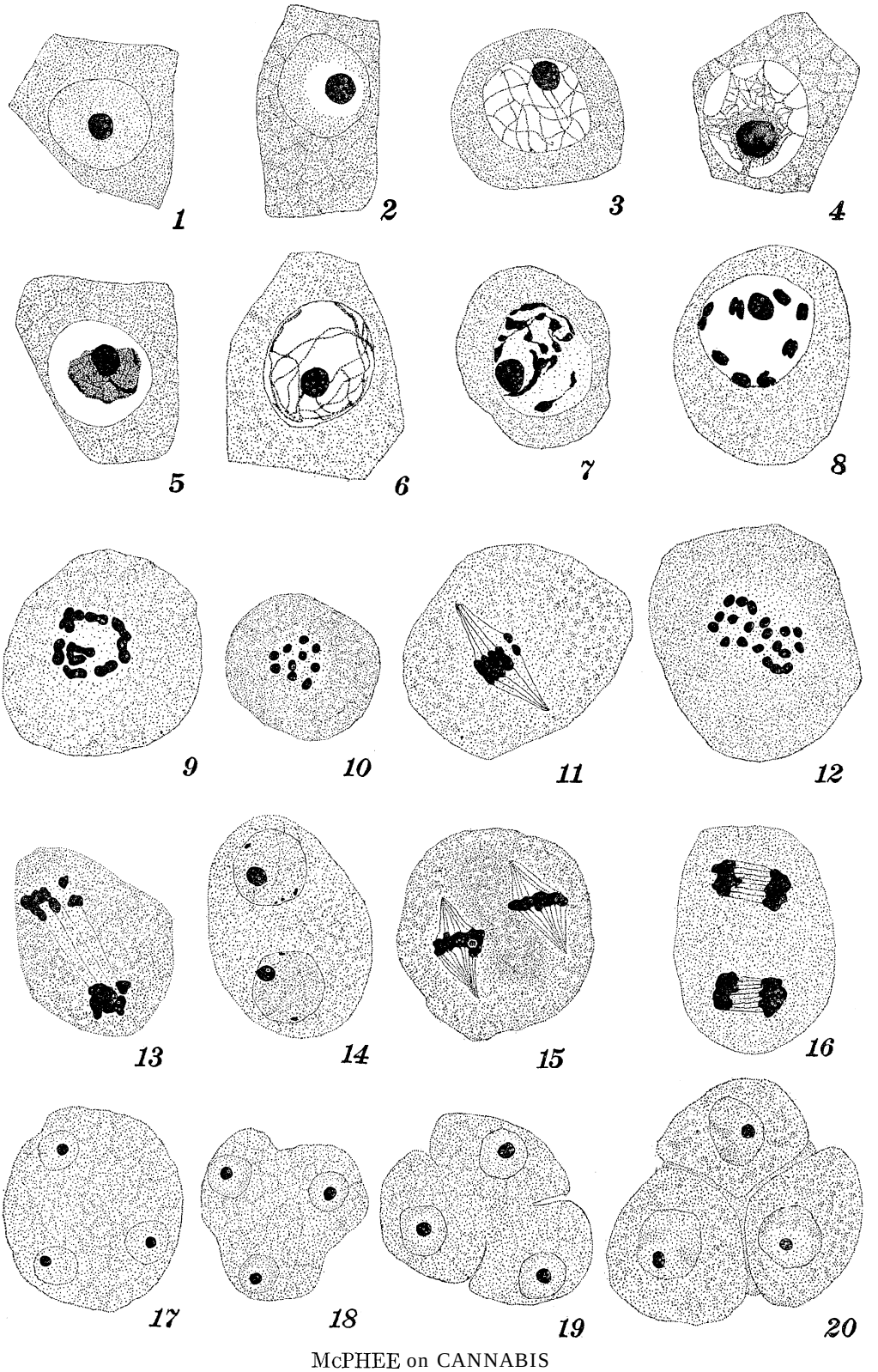
1. Counts made in the pollen mother cells of *Cannabis sativa* show that the reduced number of chromosomes in this species is ten.

2. The reduction division commonly occurs during the period from sunrise to noon. Although mitotic figures can be found in material collected during the afternoon or evening, they are comparatively rare.

3. The heterotypic division is easily studied, and chromosome counts are most easily made during the anaphase of this division. The behavior of the chromosomes is normal, and no unusual features were noted.

4. Little can be learned concerning the individual chromosomes during the homotypic division, because of the massing of cell elements and the rapidity of the division.

5. There is no morphological evidence that any one of the ten chromosomes is a sex chromosome.



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6. The method of cytokinesis is by furrowing. The furrows appear at the periphery of the cytoplasm'and at points equidistant from the mother cell nuclei. These furrows extend rapidly to the center of the mother cell, cutting it into four parts.

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EXPLANATION OF PLATE VII

All drawings were made with the aid of a camera lucida, and all figures were drawn from single sections; magnification 1800 diameters.

FIG. 1 .-Archesporial cell.

FIG. 2 .-Archesporial cell showing shrinkage of cytoplasm.

FIG. 3 .-Parasynapsis.

FIG. 4.-Contraction of chromatin.

FIG. 5 .-Synizesis.

FIG. 6.—"Open spireme" showing conjugation of threads.

FIG. 7.—Condensation of threads to form chromosomes.

FIG. 8.—Diakinesis.

FIG. 9.-Metaphase of heterotypic division, polar view.

FIG. 10.—Anaphase, polar view, showing ten chromosomes.

FIG. 11 .-Metaphase, side view.

FIG. 12.—Tangential view, late metaphase.

FIG. 13.—Daughter chromosomes at poles.

FIG. 14.—Interkinesis, nuclei in resting condition.

FIG. 15.—Metaphase of homotypic division, side view.

FIG. 16.—Anaphase of homotypic division, side view.

FIG. 17.—Mother cell showing three resting nuclei.

FIG. 18.—Mother cell showing beginning of furrowing.

FIG. 19.—Constrictions extending to center of cell.

FIG. 20.—Four pollen grains before rupture of mother cell wall; mother cell wall not shown.