Nuclear Studies on Pellia¹.

BY

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With Plates X and XI.
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THE Hepaticae present an exceedingly attractive field for research to the student of the plant-cell. There is much of interest in the special peculiarities exhibited in the arrangement and organization of the elements in the sporogenous and vegetative tissues and in the details of mitoses, which have many distinctive features. However, to the writer's mind, the Liverworts are most interesting in this connexion because they offer the possibility of solving certain problems of great importance to our understanding of the morphology of the plant-cell, and particularly the conditions characteristic of nuclear divisions in the higher plants, Spermatophytes and Pteridophytes.

Since 1897 a number of contributions have appeared, dealing with karyokinesis in many types from these two groups, and considering a variety of tissues both reproductive and vegetative². Naturally the most striking results have

¹ Contributions from the Hull Botanical Laboratory, No. 25.

² The papers have considered such a variety of forms that a brief list of the most important contributions is worth a moment's notice. Arranged approximately in order of publication we have Osterhout ('97) on *Equisetum*; Mottier

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come from studies upon the spore-mother-cell, pollen-mother-cell, and the embryo-sac. But more recently several detailed investigations have treated of vegetative tissue. It is not necessary to review this literature at this time. The discussion of the centrosome problem is fresh in the minds of all interested in plant-cytology, and, besides, the subject has recently been treated in great fullness by Strasburger ('99). In spite of contradictory reports, the writer feels that there is sufficient accord among investigators to justify some general statements of certain events of mitosis.

It appears probable for the Spermatophytes and Pteridophytes that the spindles in the spore-mother-cell and its homologues arise from fibrillae developed in the kinoplasm around the nucleus. In early stages of mitosis the fibrillae may radiate from the nucleus, and later converge into brush-like groups. Or the fibrillae may surround the nucleus as a closely packed felt with a general parallelism of direction; such that an axis may be early recognized. When the fibrillae are in brush-like bundles they sooner or later arrange themselves to form two general groups, that come to lie on opposite sides of the nucleus, and determine the poles of the spindle.

The behaviour of kinoplasm in vegetative tissue at the time of mitosis is more difficult to follow, but in the examples best known from the studies of Hof ('98) and Nemec ('99) the spindle-fibres are preceded by accumulations of kinoplasm

('97 and '98) on *Lilium*, *Podophyllum*, and *Helleborus*; Juel ('97) on *Hemerocallis*; Ishikawa ('97) on *Allium*; Sargent ('96 and '97) on *Lilium*; Calkins ('97) on *Pteris* and *Adiantum*; Guignard ('97) on *Nymphaea*, *Nuphar*, *Limodorum*, and *Magnolia*; Coulter, Chamberlain, and Schaffner ('97) on *Lilium*; Lawson ('98) on *Cobaea*; W. C. Stevens ('98a and '98b) on *Scolopendrium* and *Asclepias*; Schaffner ('98) on *Allium*; Fulmer ('98) on *Pinus*; Cavara ('98) studies chiefly upon nucleoli and chromatin in a variety of forms; Hof ('98) on *Pteris*, *Aspidium*, *Ephedra*, and *Vicia*; Belajeff ('97 and '98) on chromosome-reductions in several forms; Blackman ('98) on *Pinus sylvestris*; Chamberlain ('98) on *Pinus Laricio*; Nemec ('99a and '99b) on *Allium* and *Solanum*; Atkinson ('99) on *Arisaema* and *Trillium*; Duggar ('99) on *Bignonia*; Wiegand ('99) on *Convallaria* and *Potamogeton*; Grégoire ('99) on *Lilium* and *Fritillaria*; Guignard ('99) on *Naias*; Strasburger ('99) on *Tradescantia*, *Iris*, *Larix*, *Osmunda*, *Nymphaea*, *Lilium*, and other types; Williams ('99) on *Passiflora*; Duggar ('00) on *Symplocarpus* and *Peltandra*; Lawson ('00) on *Gladiolus*; Smith ('00) on *Osmunda*.

on opposite sides of the nucleus, forming two caps extending over the ends of that structure. The kinoplasm composing the caps is at first granular, but finally the greater part of the substance is changed into fibrillae.

It has not been possible to establish centrosomes either giving rise to or accompanying the activities of the kinoplasm, and the close study of the development of the spindle, together with the absence of the structure known as the aster, has led to a retreat from the views very generally held previous to 1896 respecting the presence of centrosomes in the cells of Pteridophytes and Spermatophytes. It is probably the belief of most plant-cytologists that neither the centrosome nor the aster is present in the cells of vegetative tissue in these plants or in that phase of ontogeny when the sporophyte passes over to the gametophyte.

The most interesting problem in spindle-formation, apart from the very difficult physiological explanation of the behaviour of fibrillae, is perhaps the question of phylogenetic evolution. How is the condition in the Pteridophytes and Spermatophytes, where the fibrillae are largely free and distinct from one another and acting independently of a fixed centre, to be related to centrosomes and asters present in certain Thallophytes? The problem is one of very broad interest, as it involves a form of protoplasmic differentiation apparently restricted to the higher plants, and not found in the animal kingdom.

Investigations among the Thallophytes have proceeded sufficiently far to establish the presence of beautiful asters for the several groups, in certain instances with definite centrosomes. The most notable examples are presented by the Phaeophyceae in *Fucus* (Farmer and Williams, '96 and '98, Strasburger, '97); *Sphacelaria*, studied with the greatest detail by Swingle ('97), and *Dictyota* (Mottier, '98 and '00). From the accounts of Lauterborn, '93 and '98, we may expect these structures in the Diatoms, but other groups of Algae are less promising, although they have been very little studied. *Corallina*, a member of the Rhodophyceae (Davis, '98), has

remarkable centrosphere-like aggregations of kinoplasm at the poles of the spindles in the tetrasporangium, but these structures are not permanently present in the protoplasm. We know almost nothing of spindle-formation in the Chlorophyceae, but observations on the development of zoospores indicate that kinoplasm may there take form and appear as blepharoplast-like bodies (Strasburger, '99). The conditions in the Fungi of course only indirectly affect the problem of phylogeny concerned with the Hepaticae. However, especially well-defined centrospheres and conspicuous asters occur in the ascus (Harper, '97), and centrosomes have been reported in several other groups.

One more class of plants, the Bryophytes, remains for our consideration in this brief statement of our present knowledge respecting the aster. Of spindle-formation in the Musci nothing has been published, and the members of this group are certainly not promising subjects for study, the cells and nuclei being relatively small. But among the Hepaticae we meet with forms whose cells are admirably adapted to a minute examination. Farmer ('94 and '95) studied a number of genera, reporting conditions of great interest. He described asters and centrosomes (Farmer and Reeves, '94) for the early mitoses in the spores of *Pellia* as they germinate in the sporangium, and his results were confirmed by Strasburger ('95) from Farmer's own preparation. Schottländer ('92), before Farmer, had noted centrosomes in the sperm-mother-cells of *Marchantia*, but without attendant radiations. His conclusions, however, are open to criticism on account of the methods employed, and further study of this point is much to be desired.

Of greater interest than the aster in *Pellia* is Farmer's account ('94) of a quadripolar spindle in the spore-mother-cell of *Pallavicinia*. This unique condition is said to arise from a special investing zone of 'archoplasm' around the nucleus. The kinoplasmic material extends into the four lobes of the spore-mother-cell, forming a four-rayed star with the nucleus in the centre, each ray becoming a pole of the spindle. The

chromosomes, four in number, are organized in the nucleus, and by a doubling division are increased to sixteen as mitosis proceeds. Four chromosomes then travel along each division of the four-poled spindle. Finally the four rays of the star-like figure break apart at the centre, the kinoplasm ('archoplasm') in each contracting, with its group of chromosomes, and shortly afterwards the four daughter-nuclei are organized.

In a note and an extensive paper which appeared in the following year Farmer ('95*a* and '96*b*) considers a number of species of Hepaticae distributed through several genera. With respect to the quadripolar spindle he did not find it very generally present. There are two successive divisions closely following one another in the spore-mother-cells of the Marchantiales (*Fegatella*, *Fimbriaria*, and *Plagiochasma*) and the Ricciales. Each mitosis is entirely distinct, but in some instances the first presented the arrangement of the chromosomes known as heterotype. Among the Jungermanniales there was some diversity. *Pallavicinia*, *Fossombronia*, *Pellia*, *Scapania*, *Cephalozia*, *Lophocolea*, *Frullania*, and *Aneura* have, according to Farmer, quadripolar spindles at least in the prophase of mitosis, but sometimes bipolar spindles result from the apparent coalescence of the four original centres. In *Riella* there is never any indication of a quadripolar spindle, but instead, two mitoses following one another, with spindles of the usual bipolar type. However, the latter condition, identical with the usual order of mitoses in the spore-mother-cell, is believed by Farmer to have arisen from the four-poled spindle. He considers certain species of *Pellia*, *Aneura*, *Scapania*, and *Cephalozia* as presenting transitional stages in the process whereby the four centres of the quadripolar spindle, fusing in pairs, form a spindle of the bipolar type.

The quadripolar spindle is then regarded by Farmer as well established for the spore-mother-cell of *Pallavicinia*, and as present but not permanent in the prophase of the homologous mitoses in many other Jungermanniales. In the latter forms it is replaced by the two successive mitoses almost universally present in spore-mother-cells, but Farmer believes these to

have been derived from the quadripolar spindle through the convergence and fusion of the four centres in pairs. Farmer also believes that the quadripolar spindle is associated with and conditioned by the peculiar character of the four-lobed spore-mother-cell. These are very fundamental and interesting suggestions whose establishment would be important contributions to plant-cytology.

A quadripolar spindle, such as Farmer has described for *Pallavicinia*, whose four centres act simultaneously so that the chromosomes separate into four groups, is quite unknown in other plants or in animals. It should be investigated with great care, for upon the fact may depend much of theoretical interest in the problem of the evolution of mitotic phenomena in the spore-mother-cell. If the quadripolar spindle merely accompanies the curious four-lobed spore-mother-cell of the Jungermanniales it may be entirely without morphological significance. But the theoretical considerations involved take on new interest from the facts of double longitudinal splitting of the chromosomes during the first mitosis in the spore-mother-cell, described for a number of forms by Guignard ('99) (*Naias*) and Strasburger ('99).

The writer has not been able to examine much material of *Pallavicinia*, and wishes only to express his fear that Farmer may have misinterpreted his preparations. As will appear from the present investigation of *Pellia*, as well as the examination of *Anthoceros* (Davis '99), the writer holds views on the method of spindle-formation in the Hepaticae very different from those of Farmer. But results in studies of this character come so slowly that one may well hesitate to advance a theory covering an entire group. However, after the account of this investigation, the reader will find some suggestions in that portion of the paper headed 'General Considerations.'

The material, *Pellia epiphylla*, was collected during the summer and autumn of 1899 from several localities, but chiefly from rocky gorges near Starved Rock, Illinois. The mitoses of the spore-mother-cell take place early in October so that the spores are fully developed before frost. Indeed

the earlier nuclear divisions of the gametophyte phase occur in the spore before winter, so that the spore in the following spring is multinucleate. Therefore material collected during October is almost sure to furnish a considerable variety of stages. There is a period of several days when the spore-mother-cells are, one may say, ripe for division, and such plants brought from the cold rocks to a warm room will immediately develop spores. Thus in a relatively short time complete sets of stages may easily be obtained.

A number of fixing agents were employed, but 1 % chrom-acetic acid and the weak formula of Flemming proved to be the most satisfactory. In this case there seems to be little choice between the two killing fluids when one understands certain peculiarities of the safranin and gentian violet stains. These stains hold more readily in Flemming-fixed material, but the manipulation may be regulated to give very clear results after chrom-acetic acid. Material fixed in corrosive sublimate and picro-sulphuric acid proved thoroughly unsatisfactory, that in absolute alcohol, sublimate-acetic and Carnoy's fluid was somewhat better, and Hermann's and von Rath's formulae approached most nearly the excellence of weak Flemming and chrom-acetic acid. The sections were stained on the slide with safranin and gentian violet or with haematoxylin after the method of Heidenhain.

Mitosis was studied at three periods of ontogeny, viz., in the seta of young sporophytes, in the spore-mother-cell and at the beginning of the gametophyte period in the germinating spores. It is most convenient to begin our account with the events of sporogenesis, as the spore-mother-cell so generally presents cytological phenomena most favourably.

The spore-mother-cells of *Pellia* are conspicuously four-lobed, a fact that seems to be generally true of the Jungermanniales. This character appears several weeks before the spores are formed. The spherical cells of the archesporium come to lie freely in a mucilaginous matrix, and develop these lobes simultaneously and symmetrically in four divergent directions. The spherical nucleus lies in one of the lobes

until shortly before the time of sporogenesis, when it may be found, irregular in outline, in positions nearer to the centre of the cell (see Pl. X, Fig. 1), and evidently being carried to that situation by movements of the cytoplasm. The evidences in fixed material of such cytoplasmic activity are dense areas, strands and stream-like structures such as are found in cells where similar activities are known to occur. The nucleus is mobile, changing its form when necessary as it is carried to its final resting-place in the geometrical centre of the cell. As is shown in Figs. 2 and 3, the nucleus almost completely fills the space at the point of junction of the four lobes.

There now follows a period of rest for several days, during which two events happen. Previous to this time the linin network has not been very conspicuous in the nucleus, but it now begins to stain deeply. At this time synapsis occurs in a marked and unmistakable form (see Fig. 3).

Farmer recognized and described the event in detail. The linin material gathers in a close granular tangle at the side of the nucleolus, and all the rest of the interior is quite free from chromatin. Synapsis appears with perfect regularity at this period of ontogeny, agreeing with the writer's observations on *Anthoceros* ('99, p. 97) and the general results of studies in sporogenesis for the higher plants. There is no more reason for regarding the phenomenon as an artefact than in *Anthoceros*, where the conditions for a comparative study of the effects of fixing fluids on the cells were most favourable.

The nucleus, on emerging from synapsis, presents a delicate, closely wound spirem-thread with a single row of deeply staining granules imbedded in the linin material. The segmentation of the spirem-thread and formation of the chromosomes proceed immediately, but we shall defer for the present the discussion of those phenomena.

With the spirem-condition, as is shown in Fig. 4, we find the nucleus ready to enter the early prophase of the first mitosis. The nucleolus becomes vacuolate, and finally frag-

ments and dissolves, but not until the chromosomes are organized and mitosis has nearly reached the metaphase. The changes that most interest us are, however, those of the kinoplasm. The homogeneous granular zone becomes differentiated by irregular lines plainly made up of rows of granules. These soon take the definite form of fibrillae distributed irregularly around the nuclear membrane. But the fibrillae develop most numerous in the four regions where the lobes of the cell diverge from the centrally placed nucleus, as is shown in Fig. 5. Farmer reported the presence of four centrosomes at the points corresponding with the lobes of the spore-mother-cell, but I have failed to find such structures in my preparations.

The nuclear membrane becomes faint and then irregular in outline (Fig. 4), finally disappearing, whereupon the fibrillar kinoplasm immediately begins to occupy the nuclear space, previously clear except for the chromosomes, nucleoli and some granular material. Interesting stages in this process of dissolution are shown in Figs. 6 and 7. In the former case the fibrillae have begun to enter the nuclear space on two sides. In the latter we find the remnant of the nuclear membrane at one point, all the rest of the area being filled with delicate fibrillar kinoplasm.

It is at this period that we have appearances most similar to the quadripolar spindle reported by Farmer ('94) for *Pallavicinia*, and likewise described and figured for *Pellia*. The nucleus may become four-pointed and each point capped with fibrillar kinoplasm actually extending into the four lobes of the spore-mother-cell which are to become the spores. Fig. 5 shows such a condition previous to the dissolution of the nuclear membrane, and the appearance becomes far more marked later (see Figs. 6 and 7), when the kinoplasm increases in quantity. This condition is apparently the stage believed by Farmer to be related to a quadripolar spindle, but the writer cannot attach to it the same importance as that author.

It is true that preparations may be so deeply stained that the nucleus has the clear outline of a four-pointed star, but

such appearances give very little idea of the real structure. The four fibrillar cones are derived from and lie in the zone of kinoplasm which at this period is irregularly distributed, extending into the lobes of the spore-mother-cell. Although the fibrillae are most conspicuously massed in the cone-like bundles, they also completely envelop the nucleus, as may be plainly seen in sections that show a surface view (Fig. 8). The fibrillar cones have no bodies at their tips that might be considered centrosomes. On the contrary, they are generally sheathed by kinoplasm distinguished from the neighbouring cytoplasm by its freedom from granular matter and plastids. The larger number of fibrillae at these points may readily be explained by the accumulations of kinoplasm, which precede the development of the cones, and become evident before the fibrillae themselves appear.

Morphology, as far as the writer's studies are concerned, presents no evidence that the accumulations of kinoplasm and the accompanying cones of fibrillae are developed or controlled by any protoplasmic elements in the cells, such as centrospheres. The preparations of the spore-mother-cells have never presented such appearances as are found with centrospheres and asters. When first visible the fibrillae are distributed all around the nucleus; but shortly afterwards the cone-like bundles develop sometimes simultaneously, but not infrequently in such a manner that one or two are far more prominent than the others.

It is plain that the cones of fibrillae result from accumulations of kinoplasm that precede them in position around the nucleus. Why the kinoplasm should occupy so conspicuously the regions opening into the lobes of the spore-mother-cell seems obvious when we note the position of the nucleus. The nucleus, as may be seen from the figures, quite fills up the space common to the four lobes, and almost touches the cell-wall along the lines of constriction. The accumulations of kinoplasm most naturally then take positions of least resistance, and extend into the lobes of the spore-mother-cell. The four-rayed star is a transitory condition of prophase.

The cones of fibrillae are not spindles, and the figure presented is very far from the metaphase of mitosis. Farmer holds that the arms of the quadripolar body represent the astral portions of incomplete spindles, but he makes it plain that these arms in *Pellia* act in pairs, so that nuclear division is after the normal type, with two successive mitoses in place of a simultaneous division of the nuclear into four, as in *Pallavicinia*. It is a phenomenon of prophase, and the true spindle has a very different structure.

The mitoses in the spore-mother-cell are two in number and successive, with a short period of rest between the first and second division, during which the two daughter-nuclei pass into a resting condition. The spindle presented at the metaphase of the first mitosis is remarkable for the great breadth of the poles and the very large number and delicacy of the fibres that traverse its axis. Not infrequently one of the poles will be so broad that it actually forks (see Fig. 9), part of the fibres lying chiefly in one lobe and the remainder chiefly in another. In other cases the spindle will present the appearance shown in detail in Fig. 10 and characterized by the broad rounded poles. It should be noted that the delicate spindle fibres end in a region of protoplasm rather free from large granules, which is bordered by cytoplasm containing plastids and having a spongy structure. The writer's preparations gave no hint that these poles were ever occupied by bodies comparable to centrospheres or centrosomes. On the contrary the broad, flat, rounded or concave poles indicated that the spindle-fibres were not influenced by a well-defined centre. While the spindle is not multipolar in the manner so clearly shown in certain Spermatophytes, there being no conspicuous separate bundles of fibres, nevertheless the conditions are essentially similar. The parallel spindle-fibres are not gathered to a point but are distributed over a very broad area.

While the daughter-nuclei are being organized, the spindle-fibres grow fainter at the poles, and very shortly a line of granules appears in the equatorial zone (see Fig. 11) marking

the position of the cell-plate. The facts agree well with the theory recently advanced by Timberlake ('00) that kinoplasm is actually drawn to the cell-plate by the contraction and transformation of the spindle-fibres. According to this view Fig. 11 presents a spindle shortened to the form of a barrel between the daughter-nuclei. It is important to note that the cell-plate is laid down in *Pellia* after the usual manner in the Pteridophytes and Spermatophytes.

The writer's studies on *Anthoceros* (Davis '99, p. 103), led him to the conclusion that the cell-walls dividing the spore-mother-cell bore no relation to spindle-fibres, but were formed from films of protoplasm that developed between the pairs of nuclei and chloroplasts. The nuclear figures of the second mitosis in *Anthoceros* are so small that the difficulty in following the fate of the spindle-fibres was frankly acknowledged. They seem to entirely disappear before the cell-plate is laid down, the latter being first a delicate film held in place by numerous strands of cytoplasm. It is of course possible that this film is composed, at least in part, of material derived from the spindle-fibres, but such a relation could not be traced. The writer suggested that the film might be independent of the spindle-fibres and related to the plasmic membrane (Hautschicht). Perhaps more favourable species of *Anthoceros* may make clear the difficulty¹. It is certainly important that we should emphasize the fact that the process of cell-formation in *Pellia* agrees in all essentials with that found in the higher plants.

The daughter-nuclei following the first mitosis pass quickly into a condition in which the chromosomes break up into

¹ Since the above was written observations have been published upon a species of *Anthoceros* more favourable for study (Botanical Gazette, xxx. 395, 1900). Mr. J. M. Van Hook was fortunate in having material of an Italian form whose cells are larger than in our native species and yield more clearly the details of structure. He has established the presence of a small cell-plate between the daughter-nuclei after the second mitosis with the usual relation to connecting spindle-fibres. This cell-plate determines the position of the conspicuous film of protoplasm that later becomes transformed into the cell-wall. Thus *Anthoceros* presents no obstacle to extending through the Liverworts the general rule of cell-formation exemplified in higher plants.

fine granular material that becomes distributed over a linen network. Nucleoli are also developed, and the nucleus finally has every appearance of being completely in a state of rest (see Fig. 12). These facts are of some importance in view of the opinions generally held that the chromosomes maintain their individuality in the daughter-nuclei after the first mitosis of the pollen-mother-cell. This conception is intimately concerned with, and necessarily a part of, the recent studies of Guignard ('99) and Strasburger ('99), on the double longitudinal splitting of the mother-chromosomes. The appearance of the daughter-nuclei following the first mitosis in Spermatophytes is not suggestive of a resting nucleus, and yet it is very difficult to trace individual chromosomes in the irregular network of deeply staining chromatic material in the interior. However, some types are more favourable than others, and Guignard ('99) was fortunate in finding such an example in *Naias*. Strasburger has studied several forms (especially *Tradescantia*, *Larix*, *Allium*, and *Podophyllum*) in which the chromosomes may be followed with certainty through the period between the first and second mitosis. The peculiar difficulties of investigations upon this point have been due in part to the interesting double longitudinal splitting, by which the grand-daughter-chromosomes of the second mitosis are prematurely organized during the first. The daughter-nucleus, following the first mitosis, contains the grand-daughter-chromosomes usually grouped with some definiteness in pairs, and such pairs stand for the daughter-chromosomes of the first mitosis.

In *Pellia* we have a type where the chromosomes cannot be followed after the first mitosis, probably for the reason that they are relatively small. Whatever may be the significance of the deeply stained granules, one cannot discover any regularity in their arrangement or number. The chromosomes seem to be completely lost, as is usually the case in successive nuclei when there is a well-defined period of rest between the mitoses.

The cell-wall laid down after the first mitosis separates the

lobes of the spore-mother-cell into pairs which lie at an angle to one another if not actually perpendicular. The two spindles of the second mitosis are therefore not in the same plane, and a side view of one necessitates an oblique view of the other (see Fig. 14). These spindles present the peculiarity of bending into the form of a bow so that the poles may extend well into the lobes of the spore-mother-cell. This feature is shown in Fig. 13 as well as other characters. The spindle is entirely similar to that of the first mitosis, only smaller. It has the broad rounded poles ending in clear kinoplasm, and is composed of a dense mass of very delicate fibrillae.

Stages of prophase are very much less conspicuous than those of the first mitosis, but there is a well-defined condition when the zone of kinoplasm surrounding the daughter-nucleus becomes differentiated into a mass of fibrillae.

Following the metaphase (Fig. 13) the two sets of daughter-chromosomes come to lie in a region of kinoplasm clearly separated from the surrounding cytoplasm, at the poles of the spindle (see Fig. 14). In this area the daughter-nuclei are organized. A cell-plate appears in the equatorial region of the spindle, is then shortly replaced by a cell-wall, and the division of the spore-mother-cell is completed.

Because of the order in which these cell-walls are laid down the arrangement of the spores is not tripartite. However, the four portions diverge in such a symmetrical manner that sections frequently give the appearance shown in Fig. 15, but in this case it is plain that the first wall laid down runs from *a* to *b*. The nucleus in each spore immediately passes into a resting condition in which the chromosomes break up into granules that become distributed through the interior. The linin network does not appear until the nucleus becomes much larger and has taken its final position near the centre of the cell.

We will now consider the structure and behaviour of the chromosomes. They are eight in number in the mitoses of the spore-mother-cell and in the germinating spore, and sixteen

in all regions of the sporophyte that have been examined. The chromosomes are quite small in all mitoses except those of the spore-mother-cell and the first two divisions in the spore, which are consequently most favourable for study. When the nucleus of the spore-mother-cell emerges from synapsis (Fig. 3) there is presented a narrow much-coiled spirem-thread, bearing a line of granules that seem for the most part to be in a single row. The spirem shortens very much, and at the time of segmentation presents a very different structure. It is much thicker, and in side view appears as a band whose surface bears two rows of minute droplets (see Figs. 7 and 8). However, cross-sections of the thread or the chromosome-segments show that the form is not that of a band but rather of an angled bar, and that the chromatin-droplets are distributed with much irregularity. In Fig. 17 we have presented a segment of the spirem whose ends are bent towards the observer, and in Fig. 18 we have a selected group of chromosomes as seen in cross-section. The angled rod-like form with the seemingly irregular distribution of the granules is apparent from these sections and views. While it is quite possible that cross-sections may present groups of chromatin droplets in fours, as Guignard ('99) reports for *Naias* and Strasburger ('99) for a number of forms, the writer is convinced that no importance can be attached to such conditions in *Pellia*. There is no double longitudinal splitting of the mother-chromosome in *Pellia*, and the conclusions of Guignard ('99) and Strasburger ('99) cannot perhaps be applied as generally as we might desire. In this particular the present investigation conflicts with Farmer's ('94) account of *Pallavicinia*, where four chromatin droplets, by twice doubling, are said to give rise to sixteen chromosomes that then pass in groups of four to the poles of the quadripolar spindle. However, Farmer has shown that the mitoses in the spore-mother-cell of Hepaticae are usually successive, and he regards *Pallavicinia* as an exception to the general rule and an illustration of a process 'very much crowded up' ('94, p. 49).

The division of the chromosomes and their arrangement during metaphase of the two mitoses in the spore-mother-cell presents features well known to cytologists. Farmer described the first mitosis in several *Hepaticae* as heterotypic. The writer found no evidence of such conditions in *Pellia*. The chromosomes split longitudinally and, as they separate, present various figures in both mitoses according to the position at which the fibrillae are attached. Figs. 19 and 10 show the commonest manner of attachment and separation, the twisted daughter-chromosomes being pulled apart from their ends. Very much less frequently the fibrillae are attached in the middle region of the daughter-chromosome as in Fig. 20, and the elements might then present the form of a ring as they are drawn apart, which arrangement would be heterotypic. But the daughter-chromosomes are so much like short rods that there is little opportunity for complexity of arrangement at the time of separation, and the V figure prevails.

The chromosomes at the metaphase of mitosis are homogeneous in their make up, no granules being distinguishable in the structure, but a change comes over the elements during anaphase, and while the daughter-nuclei are being organized. The chromatin-droplets appear again in an irregular row (see Fig. 21), on the variously bent rods which shortly begin to fragment. In this manner the droplets of various sizes are set free in the interior of the daughter-nuclei (see Figs. 12 and 16).

We now pass to the consideration of the mitoses found in the germinating spore. This is a most interesting period in the life-history of *Pellia*, one very favourable for the study of nuclear phenomena, and most so during the first and second mitoses. The nuclei presented in later stages of germination are very much smaller, and peculiarities found at first are inconspicuous then. A paper by Farmer and Reeves ('94), 'On the occurrence of Centrospheres in *Pellia epiphylla*,' dealt with this period of ontogeny. They found centrospheres with radiations outside of and touching the

nuclear membrane. In some instances this structure appeared at a distance from the nucleus, but it was suggested that such examples resulted from sections across an unsymmetrical nucleus. Their material was killed in strong spirit.

These discoveries were well founded. There are aster-like structures in the cytoplasm, and they are frequently associated with a differentiated body that very strongly suggests a centrosphere. To the writer the problem of the significance of this manifestation is of some importance. A form which may have asters and centrospheres at one phase of ontogeny and entirely lack them at another, viz. in the spore-mother-cell, is deserving of careful study.

The nuclei that pass into the lobes of the spore-mother-cell are relatively small. They come to lie near the centre of the spore, and increase rapidly in size until they have attained three or four times their previous diameter, as may be seen by comparing Fig. 16 (Pl. X) with Figs. 22–25 (Pl. XI). At first there is little evidence of a linin network, and the chromatin lies in droplets or granules irregularly distributed in the nuclear cavity. Later, however, the amount of chromatin is very greatly increased, taking the form of large irregular bodies, which finally become arranged in a spirem. These characters of the nucleus are chiefly interesting to us at present as they may be correlated with the development and differentiation of the aster-like structures.

Whether these are true asters or not is a problem far too complicated for our present study, as it involves evidence from phylogeny. Certainly they have every outward appearance of typical asters, and it is only when we examine their origin, behaviour, and fate that we may feel justified in withdrawing such a designation. The writer does not believe that these structures remain permanently in the cell through successive mitoses, nor is he satisfied that they divide to determine the poles of nuclear figures. Such behaviour is generally expected of asters chiefly through investigations on animal forms, but botanists as well have reported such activities in detail, notably Swingle ('97) in *Sphacelaria* and

Mottier ('00) for *Dictyota*. Nevertheless, the writer holds the view that these structures in *Pellia* have a relation, although perhaps very distant, through phylogeny to typical asters, and they will be called such in this paper.

The first indication of an aster in the spore of *Pellia* is a differentiated area of kinoplasm near the nucleus, distinguished from the surrounding cytoplasm by its dense granular character, which stains deeply, its freedom from plastids, and the absence of alveolar structure. The writer believes that the asters arise independently of one another in two distinct regions of kinoplasm. They would not then be related as asters, although it is quite possible that their substance, the kinoplasm, may have come from the same source.

In justice, however, we should first note some facts that to many will seem strong evidence of behaviour quite the opposite to what has been suggested, and fully in accord with the activities expected of typical asters. It is not unusual to find the kinoplasm around a nucleus massed at one side in a very conspicuous manner. Such an example is shown in Fig. 22, a nucleus in synapsis just previous to division. However, the writer was never able to differentiate an aster in such a mass of kinoplasm, and in his studies has never found a nucleus with a clearly-defined solitary aster beside it. This is a very important point, and the search was persistent. The region of kinoplasm at the side of the nucleus may be large, but at this time it is homogeneously finely granular. The asters appear on opposite sides of the nucleus, sometimes applied to the nuclear membrane, sometimes at a distance from it.

Only one example was observed where the asters were separated by much less than 180° , and this specimen is shown in Fig. 23. In this case the distance between the asters is hardly more than 90° . To many the discovery of one such instance will entirely invalidate negative evidence, and they will hold that here is presented a stage in the separation of two daughter-asters that were derived from one. One

must frankly admit the justice of such criticism, and recognize the value of this cautious attitude. It is possible that the asters divide, and the evidence against it is purely negative, but the writer's disbelief is not founded on a hasty examination. There are also some peculiarities that should be noted in this connexion. The kinoplasm displays a remarkable degree of mobility in the various positions which it takes around the nucleus. We have noted the condition where it is massed on one side (Fig. 22), but usually the material is distributed more evenly. If a nucleus be elongated ever so little in any direction one may expect to find accumulations of kinoplasm at the poles. It is not necessary that such a nucleus be preparing for mitosis, although such accumulations of kinoplasm are associated with conditions of prophase. The kinoplasm usually lies close to the nuclear membrane, but it may sometimes be found in masses somewhat removed, and when such accumulations organize asters (see Fig. 25) they are at a distance from the nucleus. As a rule, however, the former conditions prevail, and kinoplasm before the differentiation of the aster appears as in Fig. 24, and after the development of these elements presents the structures shown in Figs. 26 and 27.

In the mobility of the kinoplasm we may have the explanation of the apparent development of two asters independently, and as far remote as possible from one another, sometimes touching the nucleus, and sometimes at a considerable distance from it (Fig. 25). Portions of the kinoplasm seem able to act in a great degree independently of other parts to accumulate material, and at the proper time organize asters.

Certain peculiarities of the aster should be noted before attempting to discuss its origin. The writer has preparations and is familiar with the asters of *Fucus*, *Sphacelaria*, and *Dictyota*, structures more clearly differentiated than the asters of *Pellia*. Of these three the aster of *Fucus* is perhaps the least sharply defined in its minute structure, although most conspicuous as a whole. The aster of *Pellia* resembles that of *Fucus* in that the radiations proceed from a rather vague

centrosphere-like region. But the radiations in *Pellia* are very irregular in distribution, and variable in number. They are also sometimes so coarse as to resemble strands of protoplasm rather than fibrillae. The centre of the aster is most clearly differentiated at periods previous to metaphase. At best it is a region of dense protoplasm, whose margin may stain somewhat deeply where the radiations have their origin. The interior of the structure is frequently granular, but generally homogeneously so, and there is no centrosome. These characters are shown in Figs. 28 and 29, the former being a view from above. Some granules are larger than others, but there is certainly no rule as to number or position.

But the degree of differentiation described above is frequently vague, and sometimes entirely lacking. The centre of the aster may be merely a region where a number of radiations converge, and without further form (Figs. 27 and 30).

As mitosis proceeds, the cytoplasmic radiations become less and less conspicuous, while the spindle-fibres extend and thicken. The former are generally absent at the metaphase, and the poles of the spindles (see Fig. 31) have a broad oval outline, and are usually entirely free from bodies that could suggest centrospheres or centrosomes. But it sometimes happens that the cytoplasmic radiations persist and form a bushy crown of fibrillae at the pole of the spindle, as is shown in Fig. 32. Such conditions remind one strongly of the aster in *Fucus*, but they are exceptional. Broad-poled spindles, quite lacking cytoplasmic radiations or other differentiations of kinoplasm, are the rule in the mitoses of the germinating spore.

With the anaphase the pole of the spindle becomes more pointed (see Fig. 33), the fibres apparently drawing away from the neighbouring cytoplasm so that there may be a clear area around the pole. This is the period when the pole is most pointed, but cytoplasmic radiations seem never to be present. All trace of the aster is lost.

The daughter-nuclei, when organized, lie surrounded by a zone of dense granular protoplasm, separating them from the

plastids and the usual cytoplasm with its alveolar structure. This zone of granular protoplasm is probably kinoplasm, but it fails to show further morphological differentiation. The writer searched in vain for the aster or centrosome that might be expected to accompany these resting nuclei.

As has been said, the asters are most conspicuous at the first mitosis in the spore. They are usually also prominent at the time of the second mitosis, but are not as large. Fig. 35 illustrates the appearance of these structures at the prophase of the second mitosis, when the amount of kinoplasm has been so much reduced that the asters would hardly be recognized were it not for the advantage of their positions. The condition here illustrated is rather exceptional for the second mitosis, as the appearance of the aster is generally more like Fig. 30, but it shows excellently the conditions that appear shortly, as nuclear division proceeds in the spore. As a rule, the aster disappears after the third mitosis. The nuclei decrease in size, becoming perhaps only one-third of their former diameter, and while this naturally makes the examination with respect to asters more difficult, nevertheless the conditions seem clear. The writer found no asters with the prophase of the later mitoses. The spindle seems to arise from an accumulation of kinoplasm that forms a cap over the end of the nucleus, a condition that will be described in greater detail in the account of mitosis in the stalk of the sporophyte.

Certain facts concerning the behaviour of the chromosomes in the mitoses of the spore are worth noting. The increase in the amount of the chromatin after the division of the spore-mother-cell is conspicuous, and results in the appearance of a linin network with deeply stained granules distributed over it. After a synapsis the linin and chromatin material takes the form of a broad spirem-thread. Synapsis is a phenomenon appearing with great regularity in the writer's preparations, not only before the first, but also before the second and later mitoses. It is difficult to think of it as an artifact when found in different phases of ontogeny side by side with other stages that seem well fixed.

At the time when the spirem-thread divides to form the chromosomes, the droplets of chromatin appear clearly in two rows along the side of the segments (see Figs. 26 and 27). The segments shorten up into rods, which clearly split longitudinally a little before their arrangement at the nuclear plate, and generally separate first at one end, where they are drawn apart, making a V-shaped figure. The dissolution of the nuclear membrane takes place after the spindle-fibres from the aster have grown down over it so as to form a sort of cap, as is shown in Fig. 30. When dissolved the fibres immediately enter the nuclear space (see Fig. 29), gathering the chromosomes together at the equator. There are no mantle-fibres, and generally very few radiations at the poles. The nucleolus disappears shortly before the metaphase, after developing a vacuolate structure and fragmenting. A new nucleolus is formed in the daughter-nucleus very shortly after mitosis.

The thin and delicate cell-walls dividing the spore are plainly derived from a cell-plate of the usual structure, formed between the daughter-nuclei.

There remain for our consideration the nuclear activities in another phase in the life-history—the vegetative cells in the stalk of the sporophyte. We shall thus have examined the nucleus in most of the important periods of growth and development, excepting, however, at the time when the gametes are formed. The activities of the cells in the seta remind one of the conditions in root-tips. We find a tissue with very little differentiation, almost all of whose cells divide frequently and irregularly.

The first indication of approaching mitosis is the elongation of a nucleus and an increase in the amount of chromatin, which takes the form of elongated bodies (see Fig. 38) that later unite into a much-coiled spirem. But this nucleus is not favourable for studies upon chromosomes, and its chief interest lies for us in the development of the spindle and the possibilities of centrosomes and asters.

One may frequently find structures that have the appearance

of asters, and such an example is shown in Fig. 36. The two bodies there figured were conspicuous in a cell that contained very little cytoplasm, so that the interior was chiefly given up to vacuoles. The arrangement of the radiating strands of protoplasm is very suggestive of an aster, and the association with an elongated nucleus about to enter prophase lends favour to such a possibility. But the writer does not believe that these structures have anything to do with asters. They are merely an exceptionally regular and delicate arrangement of strands of protoplasm passing from the periphery of the cell to the interior and converging to points near the ends of the elongated nucleus. Such arrangements are very frequent in the cells of the seta, and are generally so well defined that their character is unmistakable (see Fig. 37). They are the strands of protoplasm that swing the nucleus in the centre of the cell. Although usually broad and distributed irregularly as in Fig. 37, the strands may be so delicate and symmetrically disposed as to resemble an aster.

When the spindle is to be developed the protoplasm begins to collect at the poles of the elongated nucleus. There is little in the structure or morphology of these accumulations to indicate their kinoplasmic nature. Like the rest of the cytoplasm they are rather free from granules, and do not stain deeply. That these arrangements are kinoplasmic is shown by their further history. They form caps over the ends of the elongated nucleus, as is shown in Fig. 38. By changing from a granular to a fibrous condition the caps become the poles of the spindle. This history is then in essential agreement with the conclusions of Hof ('98) and Nemec ('99), who have so thoroughly studied spindle-formation in vegetative tissue, and especially root-tips, in a variety of forms.

After the dissolution of the nuclear membrane the spindle-fibres grow rapidly into the nuclear cavity, carrying the segments of the spirem-thread to the centre of the nuclear figure (Fig. 39). There the chromosomes, probably always sixteen in number, are found at the nuclear plate (Fig. 40).

The poles of the spindles, as shown in Figs. 39 and 40, are entirely free from radiations or bodies resembling centrosomes, and they may be broad so that the bundles of fibrillae stand out clearly from one another. The writer can find nothing in the events above described to justify a belief that an aster is really present in the vegetative cells of the seta. It cannot be found with the resting nucleus or at the poles of the spindle, and certain appearances during early prophase are readily explained by the peculiar arrangement of the protoplasm in the cell¹.

GENERAL CONSIDERATIONS.

We are hardly prepared as yet to draw general conclusions on the cytological conditions in the Hepaticae, but some peculiarities appear conspicuously, and certain possibilities may be noted.

It would seem, from the studies on *Pellia*, that the morphological manifestations of kinoplasm may be remarkably various in the same life-history. Indeed there seems to be no fixity of structure apart from the finely granular condition that precedes mitosis. It is true that we have in the delicate granules (microsomes) of this protoplasm the forerunners of fibrillae, and the latter in turn organize the asters, but it has been generally considered that the asters are to a great degree permanent in the cell.

We have examined three important phases in ontogeny of *Pellia*, (1) sporogenesis, (2) the germination of the spore, and (3) vegetative activities in the seta, but there has been no agreement in the form assumed by the kinoplasm during mitosis. In the first instance fibrillae, acting largely inde-

¹ Mr. Van Hook ('00) reports a centrosome with radiations in the archegoniophore of *Marchantia*. This structure was first visible in the prophase at the poles of the elongating nucleus, and was not observed after the radiations disappeared. The suggestion is presented that it is 'a temporary body.' In this respect it agrees with the centrospheres in the gametophyte of *Pellia* and in the kinoplasmic caps in the seta of the sporophyte. It seems probable that in Hepaticae centrospheres and kinoplasmic caps will be found to be related structures, the former but a more highly differentiated condition of the latter.

pendently of one another, organized the spindles in the manner characteristic of Pteridophytes and Spermatophytes. In the second an aster with a centrosphere is developed during the prophase, but becomes less prominent at the metaphase, and disappears before the daughter-nuclei are formed. In the third phase the kinoplasm forms two caps fitting closely over the ends of the elongated nucleus, and these are changed into the poles of the spindle. Fibrillae, centrospheres, and the kinoplasmic caps, however they may be arranged, are all secondary developments from the primal granular protoplasm which is the only form of kinoplasm in any sense permanent in the cell.

Arising from accumulations of granular kinoplasm during the prophase, the substance of the fibrillae, centrospheres, or caps returns to a similar condition at the completion of mitosis, or is lost in the general cytoplasm of the cell. We do not yet know the structure of the nuclear figures when sexual cells are organized, or during the later periods of the gametophyte's history, but it is not unreasonable to expect a blepharoplast at the time of spermatogenesis. However, as nuclear division proceeds in the spore the centrosphere becomes less conspicuous until it cannot be distinguished, and the kinoplasm takes the form of a cap. It seems probable, therefore, that mitosis throughout most of the period of the gametophyte takes place, with spindles organized from caps of granular kinoplasm, as is the case in the seta.

The bearing of these data on the probable evolution of mitotic phenomena in higher plants from conditions in the Thallophytes is most interesting. We cannot trace the centrosphere or the aster phylogenetically back to the Thallophytes, for neither remains throughout the ontogeny of *Pellia*. We do not know the facts for the Algae most closely related to the Hepaticae (higher types of the Chlorophyceae, such as *Coleochaete*), but even if it be found that these forms have centrospheres and asters we shall still have a puzzling problem before us. For a connexion between such structures and the conditions in the Hepaticae may only be followed through

the undifferentiated granular kinoplasm, whose accumulation is the first indication of the approaching development of a spindle in this group.

In this connexion it should be noted that the relation of the aster to ontogeny is variously reported in the Thallophytes. Swingle ('97) was able to follow the aster from cell to cell in *Sphacelaria* with the probability of its being a permanent organ. Similarly, Mottier ('00) has recently traced the division of the aster in the tetrasporangium and vegetative cells of *Dictyota*. Strasburger ('97) has described the division of the aster in the germinating oospore of *Fucus*, and Harper ('97) followed the division of the centrosphere and aster during mitosis in the ascus. However, Farmer and Williams ('96 and '98) believe that the aster disappears with the anaphase of each mitosis in the oogonium of *Fucus*, and that a new structure is organized for the next division. My own examination of *Corallina* ('98) led me to similar views for the centrosphere-like aggregations present during mitosis in the tetrasporangium. In our ignorance of other Thallophytes, we are left in the perplexing situation now presented in zoology, where an increasing number of investigations have thrown considerable doubt on the older view of the permanence of the centrosome and aster in the cell.

It seems quite possible that we shall be forced to the conclusion that centrosomes, centrospheres, and asters are but secondary developments of the granular kinoplasm from which they sometimes, and perhaps frequently, arise. In such case they cannot be considered as strictly homologous structures, even in closely related plants, for phylogenetic connexions may be traced only through the granular kinoplasm.

The conditions in *Pellia* strongly support the celebrated archoplasm-hypothesis of Boveri, and accord well with Strasburger's conception of the structure and behaviour of kinoplasm. In *Pellia* the kinoplasm (archoplasm) consists of minute granules (microsomes), and this structure presents the possibilities of such fibrillar differentiation as may appear during mitosis in various phases of ontogeny. But the fibrillar

condition is nowhere permanent, but constantly returns to the granular state. The fibres then break down or are gathered up into some region of the cytoplasm which may have form, as at the cell-plate, but more frequently is a vague area near the nucleus.

It is interesting to consider the bearing of these studies on theories of the physiology of mitotic phenomena. The facts seem to support the view of fibrillar growth and contractility. The spindle-fibres certainly enter the nuclear cavity, and the nuclear plate takes its form in consequence. Groups of fibres are plainly attached to the daughter-chromosomes, and probably contract, but it is not easy to demonstrate this point. However, the formation of the cell-plate gives strong evidence of contraction, as the spindle shortens and the fibres, drawing away from the poles, contribute their substance to the protoplasmic membrane that foreshadows the cell-wall.

While growth and contractility are characteristic activities of the fibrillae, one must be impressed with the lack of evidence that these functions in *Pellia* are in any way controlled by morphological centres. Indeed it is even hard to believe in dynamic centres, so variable is the form of the kinoplasm. Yet dynamic centres may of course be conceived whose areas are comparatively ill-defined, and such may be presented by the centrospheres and by the vaguer kinoplasmic caps.

But these are mere conjectures, and it is best to face the fact that we have little else upon which to construct our idea of the physiology of the achromatic portion of the nuclear figure than the conception of kinoplasm with its power of forming contractile fibrillae.

SUMMARY.

At the time of sporogenesis the nucleus comes to occupy the geometrical centre of the four-lobed spore-mother-cell, and there passes through synapsis previous to the first mitosis.

Emerging from synapsis the nucleus presents a delicate

spirem-thread, which soon segments into the chromosomes. The thread bears granules irregularly disposed in two rows.

The first indication of spindle-development appears in a zone of granular kinoplasm closely investing the nucleus. Fibrillae are developed in this zone, appearing most numerous in the four regions where the lobes of the spore-mother-cell diverge from the centre. As the nuclear membrane fades the fibrillae enter the cavity, usually from more than two points.

At this period of mitosis an appearance is frequently presented similar to Farmer's ('94) account of a four-poled spindle, there being four conspicuous sets of fibrillae running into each lobe of the spore-mother-cell. This is however a prophase of mitosis, and all trace of these four regions of fibrillae is lost with the metaphase. There is no four-poled spindle.

The mitoses in the spore-mother-cell are two in number, and successive, with a short period of rest between the first and the second. The spindles are remarkable for the great breadth of their poles and the number and delicacy of the fibrillae that traverse the axes. The poles of the spindles are never occupied by bodies comparable to centrospheres or centrosomes. On the contrary the development of the spindle follows closely the plan generally presented by Spermatophytes and Pteridophytes, the fibrillae acting individually or in groups or bundles, to a great degree independently of one another and uncontrolled by fixed centres.

The rod-shaped chromosomes split longitudinally in each mitosis. There is no premature division of the mother-chromosome by double longitudinal splitting to give four daughter-chromosomes, such as has been reported by Guignard ('99) and Strasburger ('99) for several Spermatophytes. On the contrary the daughter-nuclei, following the first mitosis, pass into a resting condition, and the chromosomes, breaking up into granules, seem to lose their form and cannot be distinguished.

The cell-walls separating the spores are laid down from cell-plates formed with each nuclear figure in the manner

usual in Pteridophytes and Spermatophytes, and bear no analogy to the conditions reported by myself for *Anthoceros* (Davis '99) which have recently been explained by Van Hook ('00).

Following the mitoses in the spore-mother-cell, each nucleus takes a position in the centre of the spore, assuming a resting condition. It increases in size and soon prepares for division, passing through synapsis.

The kinoplasmic activities associated with the first two or three mitoses in the spore are very conspicuous. Two centrospheres with radiations resembling asters are organized during the prophase, and these develop the poles of the spindle. It is the writer's opinion that these structures are developed *de novo* from accumulations of granular kinoplasm. He was not able to satisfy himself that they remained permanently through successive mitoses or ever divided. They appear on opposite sides of the nucleus, are most prominent during early prophase, and frequently disappear before the metaphase, leaving a blunt-pointed spindle ending in a region of granular protoplasm. There is no trace of the centrosphere and aster at the anaphase or beside the resting nucleus. The substance of the aster not used in forming the spindle returns to the granular condition. The aster becomes small after the second mitosis in the spore, and cannot be clearly distinguished in later divisions.

The aster in the spore of *Pellia* resembles that of *Fucus* in that the radiations proceed from a vague centrosphere-like region, but they are not as numerous nor as regular in their distribution, and are sometimes so coarse as to resemble strands of protoplasm rather than fibrillae. The interior of the centrosphere is generally homogeneous, containing no centrosome.

The relation of the centrosphere to the spindle is clear. The membrane breaks down at the ends of the nucleus, the spindle-fibres growing into the nuclear space from the centrospheres, gathering the chromosomes at the equatorial plate.

Mitosis in the seta of the sporophyte recalls the nuclear

activities reported by Hof ('98) and Nemec ('99) in root-tips. The first sign of approaching division is the elongation of the nucleus and development of the spirem-thread. Kinoplasmic accumulations then appear, forming two caps over the ends of the nucleus, and these organize two sets of fibrillae which grow into the nuclear cavity carrying the chromosomes to the nuclear plate. Certain arrangements of the cytoplasm sometimes suggest asters, for the nucleus is swung in the centre of the cell by strands of protoplasm, but such appearances are deceptive. The poles of the spindles are entirely free from radiations or bodies comparable to centrosomes.

There are eight chromosomes in the gametophyte and sixteen in the sporophyte.

The most interesting results of these studies are perhaps the evidences of the various morphological manifestations of kinoplasm possible in the same life-history. The granular kinoplasm organizes (1) independently-acting fibrillae in the spore-mother-cell, after the manner in Pteridophytes and Spermatophytes, (2) a centrosphere and aster in the germinating spore which (3) give place to caps in older periods of the gametophyte, and (4) caps are developed in the tissue of the seta similar to those in root-tips. It is probable, of course, that there is likewise a blepharoplast at the time of spermatogenesis.

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EXPLANATION OF FIGURES IN PLATES X AND XI.

Illustrating Dr. Davis's *Nuclear Studies on Pellia*.

The preparations were studied with a Zeiss oil immersion 2 mm. (aper. 1.30) with compensating oculars. The figures were sketched under an Abbé camera with the following magnification: Figs. 1-3, 9, and 15, 500 diameters; Figs. 4-8, 10-14, 16, and 22-40, 1000 diameters; Figs. 17-21, 1500 diameters. Material

shown in Figs. 3-22 and 33 was fixed by the weak formula of Flemming; that in Figs. 1, 2, 23-32, and 34-40 by 1 % chrom-acetic acid. Figs. 1-6, 8, 10, 14-16, 18-20, 22-25, 29, 31-33, 35, 36, and 38-40 from sections $7.5\ \mu$ thick, and stained on the slide with safranin and gentian violet; Figs. 7, 9, 11-13, 17, 21, 26-28, 30, 34, and 37 stained with iron-alum haematoxylin after the method of Heidenhain.

PLATE X.

Figures 1-21, from the spore-mother-cell.

Fig. 1. Portion of spore-mother-cell, nucleus passing to the centre of convergence of the four lobes.

Fig. 2. Nucleus taking position at the junction of the four lobes.

Fig. 3. Synapsis occurring shortly before the first mitosis. Granular protoplasm (kinoplasm) lying next the nuclear membrane.

Fig. 4. Spireme-thread, nuclear membrane faint, granular kinoplasm around the exterior.

Fig. 5. Prophase: kinoplasm developing fibrillae which are converging towards the four lobes of the cell, making the nucleus four-angled and giving an appearance suggestive of a four-poled spindle.

Fig. 6. Prophase: fibrillae growing into the nuclear space; chromosomes organizing.

Fig. 7. Late prophase: nuclear membrane almost entirely dissolved, and nuclear space largely filled with developing fibrillae; chromosomes with two rows of granules.

Fig. 8. Surface view of nucleus in prophase, showing fibrillae extending into one of the lobes of the cell; chromosomes below with two rows of granules.

Fig. 9. Spindle at first mitosis showing broad poles; one of them concave so that part of the spindle-fibres lies in one lobe and part in another.

Fig. 10. First mitosis: daughter-chromosomes about to separate at the nuclear plate; broad spindles with rounded poles, the fibrillae ending in granular kinoplasm.

Fig. 11. Formation of cell-plate after first mitosis; spindle-fibres appearing to contract from the region of the daughter-nuclei; no aster or radiations beside the daughter-nuclei.

Fig. 12. Daughter-nuclei after first mitosis; chromosomes no longer present as organized bodies, but in their place a linen network with numerous granules; several nucleoli; nuclei lying in a bed of granular protoplasm free from plastids, similar to that which formerly surrounded the mother-nucleus as in Figs. 3 and 4.

Fig. 13. Side view of spindle during second mitosis; slightly bent so that the poles enter the two lobes of the spore-mother-cell; poles broad and ending in granular kinoplasm without further differentiation.

Fig. 14. Anaphase of second mitosis, one spindle seen from the side, the other viewed obliquely from above; chromosomes passing into an area of granular kinoplasm.

Fig. 15. Nuclei in spores after the second mitosis; clear protoplasm at base of the spore derived from material associated with the two spindles.

Fig. 16. Nucleus in spore shortly after the second mitosis; chromosomes fragmented into granules; early appearance of a nucleolus.

Fig. 17. Chromosomes at beginning of prophase; granules arranged irregularly in two or more rows.

Fig. 18. Chromosomes at time of prophase, in section showing irregular distribution of granules.

Fig. 19. Daughter-chromosomes pulled apart from the ends.

Fig. 20. Daughter-chromosomes pulled apart from the middle region.

Fig. 21. Chromosomes breaking up in the daughter-nucleus after the first mitosis.

PLATE XI.

Figures 22–35, from germinating spores.

Fig. 22. Nucleus in spore; synapsis previous to first mitosis; aggregations of kinoplasm at one side of nucleus suggests an aster, but is without characteristic differentiation.

Fig. 23. Two asters closely applied to the nucleus, the only example observed where such structures were much nearer than 180° .

Fig. 24. Kinoplasmic differentiation at poles of an elongated nucleus entering prophase, as yet scarcely having the characters of asters.

Fig. 25. Two asters at a distance from the nucleus, the latter not yet in prophase.

Fig. 26. Asters developing from aggregations of kinoplasm; spirem-thread segmented.

Fig. 27. Fibrillae from aster growing around the end of the nucleus.

Fig. 28. Aster viewed from above, showing insertion of fibrillae in an undifferentiated centrosphere-like body.

Fig. 29. Spindle-fibres growing into the nuclear space, centrosphere-like mass of kinoplasm at the poles.

Fig. 30. Spindle-fibres arising from a region of kinoplasm without evidence of an aster-like structure.

Fig. 31. Spindle at metaphase with no trace of aster at the poles, fibrillae running into granular kinoplasm.

Fig. 32. Pole of spindle at metaphase with bushy aggregation of fibrillae.

Fig. 33. Pole of spindle at anaphase, fibres coming to a point in a region of finely granular kinoplasm.

Fig. 34. Organization of daughter-nuclei, no aster present.

Fig. 35. Synapsis and prophase of a later mitosis in the germinating spore, showing the small asters and delicate radiations in a less amount of kinoplasm.

Figures 36–40, from seta of a young sporophyte.

Fig. 36. Arrangement of delicate strands of cytoplasm resembling an aster.

Fig. 37. Kinoplasm assembling at the ends of the nucleus; stellate arrangement of the cytoplasm; strands much broader than in Fig. 36.

Fig. 38. Kinoplasm forming a cap at the poles of the elongated nucleus that is entering prophase.

Fig. 39. Spindle developed from the caps of kinoplasm; chromosomes being carried to the nuclear plate.

Fig. 40. Spindle at metaphase, showing poles formed by convergence of bundles of fibres and without a centre.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 7.

Fig. 6.

Fig. 12.

Fig. 11.

Fig. 16.

Fig. 17.

Fig. 18.

Davis del.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 9.

Fig. 6.

Fig. 7.

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Davis del.

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Fig. 39.

Fig. 40.

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