Cytological Studies on Nemalion¹.

BY

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With Plates XL and XLI and a Figure in the Text.

WITHIN the past half-century only four papers of broad scope bearing on the Florideae have appeared. The work of Bornet and Thuret ('67), and that of Janczewski ('76), both models of their kind, presented clearly and exhaustively the external morphology of the sexual organs and the cystocarp. Antedating, however, critical cytological methods they left much to be desired. Schmitz ('83) attacked the subject in somewhat greater detail, but was misled by the cytoplasmic fusions so characteristic of the group. Attaching a deeper significance to this than the facts warranted, he believed that those fusions actually involved the nuclei, and that here we have a sort of 'double fertilization.' This was, of course, confusing, as it left the group presenting anomalous conditions. Oltmanns ('98), reinvestigating the subject, reached the conclusion that only the cytoplasm is involved, and that the ooblastema-filaments of *Dudresnaya*, for example, simply graft a cell derived from the fertilized egg upon the auxiliary cell. The original nucleus of the auxiliary cell disintegrates, leaving the nucleus derived from the zygote, which by repeated division gives rise to the favella of spores. This condition of affairs is entirely in harmony with current views as to the significance of the sexual act. Sachs ('74) had already extended the theory of 'Alternation of Generations' to the Ascomycetes, the higher Chlorophyceae, and the Florideae. The results of Oltmanns go far toward establishing the correctness of this view, which holds that in these forms what may conveniently be termed the interval between the spore and the fertilized egg is homologous with the sporophyte in higher plants. Before this can be regarded as established, however, a comparative investigation of the chromosome-relations in the nuclei of this interval and of the vegetative cells remains to be made.

It is not clear why, in view of the great number of cytological studies that have appeared in recent years, the Florideae have received so little

¹ Contributions from the Cryptogamic Laboratory of Harvard University.—LIX.

attention. The minuteness of the cell and cell structures, together with the complexities attendant upon the act of fertilization, may be in a measure responsible for this. Perhaps also the group has been less attractive owing to the fact that it appears to be modern, highly specialized and terminal, hence not in itself a factor in the evolution of the plant series. Nevertheless, it has seemed to the writer that it would be interesting to know how far the nuclear phenomena in this side line parallel those in the direct line of evolution.

With a view to filling this gap in our knowledge a large supply of *Nematium multifidum*, Ag., was collected at Woods Holl, Massachusetts, at different times during the summer of 1901. The structure of the frond and the simple sexual relations make this a favourable type for such a study. Consisting of a central axis of intertwining filaments from which branches radiate perpendicularly, and, in addition, having apical growth, a longitudinal section presents vegetative cells in division at the growing point, and all stages from the youngest procarp backward to the mature cystocarp. Other demands upon the writer's time have delayed the completion of this work. It was begun in the Botanical Laboratories of the University of Chicago and continued at the Marine Biological Laboratory at Woods Holl, under the direction of Dr. B. M. Davis, and brought to completion under the supervision of Professor Roland Thaxter at Harvard University. The writer wishes here to express his thanks both to Professor Thaxter and to Dr. Davis for constant suggestion and criticism.

Aside from phenomena related to chromosome-reduction, which was the primary purpose of this investigation, during its progress a considerable body of information has been gathered bearing on other matters of cytological interest. For convenience these results will be discussed under the following heads:—

A. METHODS.

B. THE CELL.

(a) *Structure of the Chromatophore.*

(b) *Division of the Chromatophore.*

C. MATURATION AND SEXUAL REPRODUCTION.

(a) *Oogenesis.*

(b) *Spermatogenesis.*

(c) *Fertilization and Development of the Cystocarp.*

D. MITOSIS (*centrosome, spindle, &c.*).

(a) *The Nucleolus.*

(b) *Reduction.*

METHODS.

The chrom-acetic mixtures gave entire satisfaction as killing fluids. Material fixed when collected was suitable for most purposes, but a greater abundance of dividing nuclei was seen in that kept in running water in the laboratory and killed at various times during the night. Material killed in 2 % formaldehyde in sea-water and gradually transferred to pure glycerine kept its colour perfectly, and, except for a slight shrinkage and the loss of the small amount of chlorophyll, presented much the appearance of the living plant.

In connexion with the study of fertilization a method was employed which made it possible to examine a great number of procarps at all stages of development with a much smaller expenditure of time and labour than would have been the case if sections had been used. Young tips were crushed in water under a cover-glass and on a slide that had previously been treated with fixative; the cover was then removed and the water on the slide allowed to evaporate. The gelatinous nature of the wall prevents the contents of the cell from being affected by this treatment even when the albumen has hardened sufficiently to hold the filaments firmly in place. Preparations made in this way were then double stained in safranin and gentian-violet by the usual method and mounted in balsam. This was usually accomplished without any shrinkage whatever; and, as the decolorization could be controlled with the aid of the microscope, a finer differentiation was secured than is possible by staining in bulk.

For the study of nuclear detail iron-alum-haematoxylin after the method of Heidenhain gave excellent definition, and, in addition, was easy to control. Erythrosin, or eosin, was used where a plasma stain was desired. Flemming's triple stain was repeatedly and persistently tried, but in no case were results secured in any way approaching those obtained with the Heidenhain.

Sections $3\ \mu$ in thickness were used in most cases, but in order to establish certain points it was occasionally found to be necessary to cut them as thin as $1\ \mu$.

THE CELL.

The cell-wall is relatively thick, and under high powers is evidently lamellate in structure. It has been figured by Wille ('94) as a double wall, and certainly such an appearance may be seen in certain cases (Pl. XL, Fig. 24). It is by no means easy, however, to determine whether this appearance is due to the actual presence of a double wall, or whether it may not more probably be a misleading appearance resulting from its often very distinctly lamellate structure. The usual tests produce a characteristic though delicate cellulose reaction, the weakness of which is evidently due to the partially gelatinized condition of the walls.

The contents of adjacent cells are united by means of the characteristic protoplasmic connexions usually conspicuous in these plants, and in each a chromatophore, surrounding a body which has been called a pyrenoid, and a well-defined nucleus are present.

The nucleus occupies a characteristic position in the basal portion of the cell just below the chromatophore. Its structure is a matter of considerable interest, inasmuch as not the slightest indication of a reticulum has ever been seen, the chromatin being collected in a single, large, densely staining, centrally placed 'nucleolus.' The nuclear wall is somewhat remote from this body, extremely thin and usually difficult to differentiate, and, moreover, connected with the nucleolus by means of delicate radiating fibrillae.

The cytoplasm forms a finely granular layer closely applied to the inner surface of the wall, and extending throughout the cell, envelops the nucleus together with the intricately constructed chromatophore, between the processes of which are left vacuoles of irregular contour (Fig. 25).

Structure of the Chromatophore.

By far the most striking member of the cell in both living and prepared specimens is a highly complex structure, the chromatophore. In the living condition the chromatophore is seen to consist of a centrally placed ellipsoidal body more deeply pigmented than the numerous strands which radiate from it in all directions. These strands, which are of the same material as the central body and continuous with it, as they approach the cell-wall flatten out gradually on all sides, forming a continuous membrane which lies just within the peripheral layer of cytoplasm, and is clathrate through the presence of numerous openings, as seen in Fig. 24. These unpigmented areas thus represent breaks in the continuity of this peripheral portion of the chromatophore, the substance of which is not sharply delimited from the surrounding cytoplasm, and the transition from the one to the other is thus somewhat gradual, the distinction in nature being less abrupt than is represented in the figure. Nothing was observed which appears to indicate that there was a difference either in composition or structure between the various portions of this complex organ other than that of relative density, the material being denser in the central body and gradually becoming less so toward the periphery.

Material crushed on the slide and stained in safranin and gentian-violet shows that this central body is not a homogeneous solid. Sections reveal more clearly a wall layer of the same material as the rest of the chromatophore, surrounding what appears to be a mere vacuolar cavity. This vacuole represents the so-called pyrenoid, which earlier writers have described as characteristic of the vegetative cells of this plant. In the effort to differentiate the substance of this 'pyrenoid' a great number

of stains and other reagents were thoroughly tested, but in no instance was it found possible to demonstrate the presence of any organized material in this central region. Since this cavity is only about 3μ in diameter its absolute section is secured only in the thinnest sections, and not in such as were chiefly used in this investigation. In these preparations the central body of the chromatophore is seen as a hollow ellipsoid, from the upper portion of which a slice has been removed in another section; therefore, even at the median focus, the concave wall always appears, and being out of focus, presents a granular appearance as is shown in most of the figures. The fact, however, that such confusing appearances are not seen in the absolute median section, like that represented in Fig. 25, which is drawn from a section 1μ in thickness, demonstrates, to the satisfaction of the writer at least, the absence of any body of such organization as is assumed to be characteristic of the pyrenoid by all recent investigators. It may be objected that the conditions just mentioned might be due to the fact that the contents had dropped out of such a thin section; the essential agreement, however, seen in thicker sections and even in material which has not been sectioned, it would seem, make this supposition, to say the least, in the highest degree improbable.

It should be mentioned in this connexion that Schmitz ('82) in speaking of the pyrenoid in the Bangiaceae and Nemaliae remarks (p. 52, l. c.): 'Bei diesen Algen nämlich quellen die Pyrenoide bei Einwirkung von süßem Wasser, Spiritus, verdünnter Essigsäure u. s. w. auf und vertheilen sich schliesslich vollständig in dem umgebenden Lösungsmittel.' The possession of such remarkable powers of solubility by a distinct organ of the cell would certainly seem to be anomalous; and, although in the somewhat extensive literature of the subject there is so little agreement as to the nature of the pyrenoid, it is perhaps fair to assume that we are here dealing with a wholly different structure. To indicate this diversity of opinion: Meyer ('83), for example, representing perhaps an extreme view, holds that the pyrenoid is of a crystalloidal nature and has no function other than that of serving as reserve material; Boubier ('99), on the other hand, finds it to be a crystalloid, but with a characteristic reaction to stains and surrounded by a membrane of distinct and definite structure; and further, Timberlake ('01) has given a most detailed and admirable description of the structure and function of this body in *Hydrodictyon*, regarding it as proteid in nature and manifesting its activity by cutting off segments which are directly converted into starch. Whatever may be the true nature of the bodies investigated by these writers, it is very evident that in *Nemalion* there is no structure present which could be said even remotely to approach the conditions described.

The chromatophore alone thus appears to be responsible for the constructive metabolism of the plant. No starch, however, could be

demonstrated, although with several other Florideae entirely satisfactory tests were obtained with iodine. The inner surface of the chromatophore surrounding the vacuole presents a granular appearance (see Figs. 24 and 25), but the nature of these granules was not further investigated.

Assuming, then, that the views above expressed are correct, the chromatophore may be described as consisting of a hollow ellipsoid with vacuolar contents, the thick wall of which, granular on its inner surface, becomes gradually less dense in its peripheral region, whence numerous diverticula radiate to the cell-wall, within which they form a continuous, thin, clathrate membrane.

Division of the Chromatophore.

The accounts that have been given of this process in other plants suggest a considerable degree of variability in the process; Davis, for example ('99), has figured a simple elongation and constriction as accompanying the division of the single chromatophore in the spore-mother-cell of *Anthoceros*. Chmilowsky ('97), investigating the mode of multiplication in several green Algae, finds that contemporaneously with the division of that body the chromatophore splits from centre to circumference.

The process in *Nematium* is somewhat different, and recalls in appearance at least the method of cell-formation by gemmation so characteristic of this plant. In this connexion it should be noted that cell-formation precedes nuclear division by a considerable period. When the daughter-cell has very nearly reached its adult size, the thick wall of the central body of the chromatophore bulges out toward the young cell as if pushed from within (Figs. 26, 27, and 29). The bulge increases in size until the two together present the appearance of one elongated chromatophore (Fig. 30). Soon after this a slight constriction may be observed (Fig. 31), which rapidly increases until the two walls of the central body are in contact, when the two daughter chromatophores begin to separate from each other (Fig. 32).

At about the time when this separation occurs, the cytoplasm loses its granular character, and becomes absolutely hyaline in the region that marks the position of the wall which is to divide the two cells (Figs. 32 and 33). The appearances presented in these figures, and in Fig. 34, suggest that the cytoplasm in this region is transformed into the substance of the future wall.

As can be seen from the figures, there is no exact correspondence in time between the events of nuclear and chromatophore divisions, although in general the nucleus divides contemporaneously with the chromatophore. The conditions seen in Fig. 28, which represents a terminal cell of a filament of the young cystocarp, suggest that the process may be very

transient and entirely independent of nuclear activity in rapidly growing cells.

Although this is the normal process of chromatophore division, in exceptional cases it seems to take place in a different manner; a new chromatophore being organized from one of the coarser strands of the other. This condition, which was described by Schmitz ('82), and has been seen by the writer, may perhaps account for the not infrequent presence of two chromatophores in old cells.

This body is constantly present in all phases of the life history of the plant, and in all cases arises from a pre-existing body of the same nature. The fact that in sperm-cells it disappears in a manner that will be hereafter described, and that, for a short time after the *second* division of the zygote, it has been impossible to demonstrate it, owing no doubt to the dense surrounding cytoplasm, does not militate against the accuracy of this statement.

MATURATION AND SEXUAL REPRODUCTION.

Oogenesis.

The carpogonium, as is well known, is borne at the end of a short branch, developed in connexion with sterile branchlets at right angles to the longitudinal axis of the frond. This carpogenic branch is composed usually of three cells; since, however, the number varies from two, in the simplest noted, to as many as five, it cannot be considered as in any way significant. It can be readily recognized even when, as in its youngest stage, it consists of but a single cell. Its peculiar shape (Fig. 1), as well as its capacity for absorbing dyes, serves to differentiate it at once in stained preparations. In life its almost colourless condition renders it equally conspicuous. The members of this branch arise by the divisions of the primary carpogonial cell, which acts as an apical cell by cutting off successive basal segments.

The cells of the branch, excepting in shape and colour, are organized practically just as are the ordinary thallus-cells (Fig. 2). Janczewski ('76) describes the cells of which it is composed as being without nuclei and chromatophores; a manifestly erroneous statement, since there is present a well-defined nucleus differing in no way from the usual type. The chromatophore is not conspicuous in the living cell, because of the small amount of pigment which it contains, but staining brings it out readily. In some cases, at least, a distinctly appreciable amount of colour is present even in the living condition. The cells are short cylindrical blocks contrasting rather sharply in form with the usually ellipsoid cells, which are characteristic of vegetative filaments (Fig. 24). The lowest cell of the series partakes to some extent of the characters of both, and thus lessens the abruptness of the transition between the two types.

As the carpogenic branch becomes differentiated its terminal cell

begins to develop into a procarp by sending out the trichogyne, which consists of a small finger-like protoplasmic process surrounded by a distinct wall; while the chromatophore and the primary egg-nucleus, both normally organized, assume their characteristic positions in the basal portion as indicated in Fig. 3. A little later the tip of the trichogyne becomes much swollen, and in this swollen portion an unmistakable nucleus is always present, which, although the mitotic figure has not been seen, must be assumed to have resulted from the division of the primary nucleus of the procarp. After this division the egg-nucleus, as it may now be called, lies in that portion of the procarp immediately above the chromatophore. Very probably it migrates to this position as a preparation for this mitosis, but such procedure is unusual, and it is but fair to call attention to the fact that it is not a phenomenon usually accompanying mitosis in this plant. While actual division of the primary nucleus was not observed, these conditions presented in Fig. 4, which were seen in several cases, can hardly be explained on any other supposition. Figs. 5 and 6, in which the egg-nucleus has returned to its characteristic position, make clear the fact that this occurrence is not to be explained upon the assumption of an artifact. At all events a well-marked nucleus is now present in the trichogyne. The wall of the trichogyne, which has hitherto been relatively thick, now becomes extremely thin and delicate throughout the terminal receptive portion, and fusion with the spermatium is thus accomplished without difficulty (Figs. 4-11).

Soon after this nucleus has entered the trichogyne it begins to break up into a variable number of nuclear fragments which retain their staining power for a considerable period (Figs. 8 and 9), and become gradually smaller and less markedly differentiated as the trichogyne approaches maturity (Fig. 10). At the period just preceding fertilization inconspicuous aggregations of slightly staining material may often be distinguished, which probably represent the last stages in this process of nuclear dissolution. These facts are of importance, since were such differentiated nuclear-like masses present, it would prove difficult or even impossible to distinguish them from the sperm-nuclei after the entrance of the latter. While this might be regarded as negative evidence, the writer feels reasonably certain of the facts, as a very careful examination was made of a large number of well-stained trichogynes before reaching this conclusion.

So far as the writer is aware, the earliest detailed account of a nucleated trichogyne is that of Davis ('96) in his paper on *Batrachospermum*. It is true, of course, that Schmitz ('83), together with some of the earlier observers, found certain differentiated granules present in the unfertilized trichogyne. Schmitz, however (p. 12, l. c.), dismisses them with the statement that they react towards stains like chromatin. Further, Schmitz noted the presence of chromatic bodies in the trichogyne after fertilization,

which he regarded as 'Richtungskörper.' These have, however, been correctly explained by Schmidle ('99) as supernumerary male nuclei discharged into the trichogyne. Oltmanns ('98) also noted the granules, previously observed by Schmitz, in the trichogyne of *Gloeosiphonia* (p. 110, l. c.), but concludes that they have absolutely no relation to nuclei. Schmidle, reinvestigating *Batrachospermum*, failed to find the nucleus described by Davis, and Osterhaut, in his paper on the same genus ('00), remarks (p. 113, l. c.): 'Ich habe in meinen Präparaten nicht die geringste Andeutung eines Kernes im Trichogyne gesehen vor Eintritt des Spermatiumkernes.' In view of these conflicting statements, the evidence in the present case was examined with extreme precaution; but the conditions were so evident that the writer has been forced to conclude with Davis that the trichogyne (in *Nemalion* at least), instead of being a mere hairlike outgrowth from a cell, is at first a cell in the strictest sense of the word, which only later is specially modified in connexion with the reproductive processes.

For purposes of comparison, it may be instructive to note that a corresponding condition of the female sexual apparatus is described in the case of the Laboulbeniaceae by Thaxter ('96). In these plants, which closely resemble the Florideae in their sexual processes, the procarp consists of a carpogenic cell and trichogyne connected by an intermediate cell, and the trichogyne presents every degree of complexity from a unicellular vesicular prominence to a highly developed branching system of nucleated cells, the terminal cells in these structures constituting the receptive portion, and being often specially modified by spiral twisting, or otherwise, as, for example, in the genus *Compsomyces*.

It is also of interest to note, as a further indication of the probable differentiation of the trichogyne as an independent cell, that in two cases at least conditions were observed which strongly suggest that a chromatophore derived from that body in the procarp also passes into the young trichogyne. This, of course, is indeed the more probable from the fact that the chromatophore is clearly present in the trichogyne of the nearly related *Batrachospermum*. However, the protoplasm of the procarp stains so heavily at this time that a satisfactory demonstration of this point was not secured.

Spermatogenesis.

The general morphology of the antheridial branch has already been figured in the works of Bornet and Thuret ('76, '78, and '80), Guignard ('89), and others. In *Nemalion* this branch arises from what appears to be an ordinary vegetative cell, and like the carpogenic branch is remarkable for the variability in the number of cells of which it is composed. Similarly, too, it can be distinguished when very young by the absence of pigment and the short cylindrical form of its cells (Figs. 35 and 36).

Each cell of the antheridial branchlet usually gives rise to four antheridia which bud out radially, although even here the number is not constant. Fig. 37 represents a median section of such a branch in which only those antheridia developed laterally are shown.

The divisions of the nucleus of the mother-cell from which these antheridia arise correspond to those of other vegetative cells in all respects, and the chromosomes, as usual, are about eight in number. The minuteness of the figure necessarily renders accurate counting a difficult matter, but so many were seen in both longitudinal and polar view, and the cytoplasm is so clear at this time, that there is little reason to doubt that this estimate is approximately correct. The spindle is in no way peculiar, except that it is smaller and details are relatively even more difficult to differentiate than in the case of other cells of the plant. The spindle appears to be intranuclear, as is certainly the case in other mitoses. A centrosome was occasionally distinct at the poles of the spindle at metaphase (Fig. 39), but could not be made out in the majority of the cases examined (Fig. 38).

The chromatophore divides after the nucleus (Fig. 42), and for a short time can be recognized as a faintly staining body, with a not very definite outline in the antheridium (Fig. 43). Soon, however, the contents of the cell undergo a remarkable reorganization, after which it is no longer possible to recognize any differentiated bodies in the protoplasm, except the nucleus itself (Fig. 47). In this reorganization the chromatophore disappears and a mass of densely staining matter is seen at the distal end of the antheridium (Fig. 44), a portion of which seems certainly to be derived from the disorganization of the chromatophore. For a time the nucleus is seen lying below this mass, but very soon is indistinctly visible in its midst (Fig. 45). This matter begins to stain less deeply, and well-marked, deeply staining, fairly uniform granules, twenty to thirty in number, appear on the nuclear wall. These granules suggest those bodies figured as chromatophore-derivatives by earlier writers (Figs. 44-46). Although the phenomenon bears a striking resemblance to the conditions seen in the early prophase of ordinary mitoses (see Figs. 54 and 55), it is evident that it cannot be thus interpreted as a prophase character, from the fact that the nucleus resumes again its normal organization preparatory to the succeeding mitoses (Fig. 16, the spermatium at the left). As to the origin of these granules, it seems more reasonable to suppose that they represent food material, a part of which at least is derived from the chromatophore, and is now passing into the nucleolus. It should here be stated as further evidence in support of this interpretation that preparations of the cell at this stage (Fig. 47 in median section, and Fig. 48 in surface view), as well as in the stages immediately preceding, show that the nucleolus retains its normal size and appearance (Figs.

37-44), while after the disappearance of the granules in question its size becomes greatly increased. The condition just described, in which the nucleus is surrounded by granules (Figs. 46-48), is seen at the time when the contents of the antheridium are discharged into the water as a naked or thin-walled mass of protoplasm; and although the terms antherozoid, pollinoid, and spermatium, have been variously applied to it, the subsequent history seems to indicate that it may more properly be termed an antheridium. Since hundreds of instances of such antheridia showing these conditions are presented in almost any well-stained preparation, it would seem that the arrangement persists for a considerable period. At the time, however, when the spermatium has reached the trichogyne these granules have usually disappeared (Fig. 16) and the nucleus has resumed its normal appearance, although, as already mentioned, the nucleolus has undergone a conspicuous increase in size. That the so-called spermatium cannot be regarded as a sexual element, but, as just noted, is to be homologized with the antheridium, is shown by the fact that a rapid division into two sperm-cells now takes place (Figs. 49, 50), which, so far as it has been possible to determine, is an invariable preliminary to actual fecundation, and takes place shortly after conjugation with the trichogyne. The figure in this mitosis is of the type already described, and, so far as could be certainly determined, presents no unusual features. In several cases the number of chromosomes could be fairly well estimated, and is certainly very close to the number eight, which is seen in the preceding mitosis. From the above facts it would appear that, although the term spermatium might be conveniently retained in conformity with previous usage, it should be distinctly understood that it is not the homologue of a sexual element.

It should be mentioned that a binucleate spermatium has been described by Schmidle ('99) in his paper on *Batrachospermum*, but he leaves us somewhat in doubt as to when and how this division occurs, which is of importance, as Osterhout ('00) found this body in the same genus to be uninucleate and suggests that a binucleate appearance is probably due to disorganization. Further, Davis ('96) noted the rare occurrence of a binucleate appearance in the spermatium of this plant, assuming, however, that they were fragmentation-products. In presenting the mitotic figures from which these two nuclei arise, and which are of very common occurrence, the writer believes that he has established the fact that this binucleate condition is a normal phenomenon.

Fertilization and Development of the Cystocarp.

The trichogyne, as has been stated above, contains at this time no organized nucleus. Both the male cells, as we have already seen, seemed normally to be discharged into the trichogyne. The processes of ferti-

lization, as they have been observed by the writer, correspond to the account given by Wille ('94) with the following exception. This author states (p. 59, l. c.): 'Wenn der Spermakern sich der Verengung des Carpogoniums nähert, wandert der Eikern diesem entgegen.' In the numerous preparations of this stage in the writer's material, the male nucleus is present in the upper part of the carpogonium, while the female still occupies its usual position (Fig. 12).

Soon after, however, the female passes into the upper part of the cell, and there fusion takes place (Figs. 13-15). The male nucleus is often somewhat smaller than the female, although more frequently it is very nearly of the same size. Differences in staining power, such as were noted by Wille, were not observed. The staining of the protoplasm, according to the method employed to demonstrate these stages, somewhat veiled the processes, rendering an accurate examination of the finer details in fusion of the chromatic masses unsatisfactory. Actual fusion is evidently a very rapid process. In the scores of cases examined, no stages intermediate between these presented in Figs. 13 and 16 were observed.

Just about the time of fusion, the protoplasm of the trichogyne separates from that of the carpogonium (Figs. 13-16). No cellulose plug appears to be formed, as stated by the earlier observers, but a distinct wall is thrown around the zygote within the original wall of the carpogenic cell, in much the same manner as has been described for the fertilized egg-cell of the Bryophytes. This wall, however, does not sever the original protoplasmic connexion with the cell immediately below (Figs. 17 and 20), which Oltmanns ('98) has characterized as the hypogynous cell; and soon after it is laid down, the fusion-nucleus passes from its position in the upper portion of the carpogonium to its normal one below the chromatophore. These characteristic positions of the nuclei are of importance, since they eliminate all possibility of mistaking late stages of zygote-division for fusion.

As seen in Fig. 16, many spermatia may conjugate with the same trichogyne. Sperm-nuclei in all stages of dissolution can be noted at different points in this organ. Nuclei thus discharged appear to disintegrate almost immediately. The protoplasm of the trichogyne contracts away from the delicate wall soon after fertilization, and the structure as a whole withers very rapidly. Small bodies resembling male nuclei (Fig. 14) were noted in several cases, indicating, perhaps, that more than one male nucleus may enter the carpogonium. This is, of course, not impossible, since the protoplasmic passage between the egg and the trichogyne is not broken until fusion is practically complete. Further information, however, concerning the origin and fate of these bodies was not obtained.

When the fusion-nucleus returns to the basal portion of the carpegonium, division begins at once, and, as stated by both Wille and Janczewski, a basal cell is first cut off by a transverse wall. This cell usually undergoes no further division, but a single case was observed in which it divided once longitudinally; suggesting that although it normally functions as a sterile 'stalk-cell,' it may be considered as potentially similar to the primary sporogenous cell above it, from which the sporogenous filaments, or gonimoblasts, are developed.

Wille has already noted that at the time of the division of the zygote into these two superposed cells, the chromatophore also divides (Figs. 13 and 19). The history of this body, and of the chromatic elements as well, could not be satisfactorily followed through the two or three immediately succeeding divisions, owing to the intense staining of the surrounding cytoplasm. At the period, however, when the young cysto-

FIG. 51. Mother-cells of carpospores proliferating from a subterminal cell. The walls of their predecessors persistent. (Optical section. $\times 1500$, reduced in reproduction.)

carp consists of six or eight cells the divisions of nucleus and chromatophore can be followed with certainty. These divisions present double the number of chromosomes characteristic of the thallus-cells. This phase of the subject will be more thoroughly presented in a subsequent portion of this paper. The details of the structure of the chromatophore can be seen at this period of development and its presence proved in every cell of the cystocarp, from such stages as are illustrated by Fig. 22, to the mature condition (Fig. 23). Its demonstration in the stages intervening between the two and the six-celled condition will no doubt be possible with the development of special technique.

The gonimoblasts when fully developed are more or less copiously and repeatedly branched, and the cells in the peripheral region of the resultant structure ultimately give rise distally to numerous subterminal and terminal mother-cells, the contents of which, severing their protoplasmic

connexions, escape as carpospores through a terminal break in the mother-cell wall, which is left behind as an empty adherent sheath, and within which, by further successive proliferations, several more mother-cells of carpospores may be produced, from which a corresponding number of spores are successively discharged (Text-fig. 51).

The function of the stalk-cells of the carpogenic branch can be stated in brief to be that of furnishing organized material to the rapidly growing cystocarp. Whether or not the heavily staining masses appearing in these cells (Figs. 21 and 22) represent food material, as is suggested by Wille and as further indicated by their disappearance as the cystocarp develops, is a matter of some uncertainty. At least a portion of this material, in the writer's opinion, results from the disorganization of the chromatophores which disappear during the early development of the cystocarp (Figs. 21 and 22).

MITOSIS (Centrosome, Spindle, &c.).

In the mitoses of this plant two heavily staining masses invariably appear at the poles of the spindle at metaphase, with the possible exception of the antheridial mitoses already mentioned, which, it would seem, are certainly to be identified with the structures usually described as centrosomes. In *Nematode* this body is of relatively large size and always surrounded by a delicately outlined hyaline area (Pl. XLI, Figs. 63, 67, &c.), although within this region no fibrous radiations could be satisfactorily observed. The occurrence of instances in which it appeared to divide (Figs. 63 and 70), together with such conditions as are illustrated in Fig. 53, make it probable that the centrosome is always present even in the resting condition, and corresponds to the bodies thus designated in many plant and animal-cells. While its size alone would make it a favourable subject for research, the fact that it has not been shown to be accompanied by an aster, or any other very characteristic structure which might serve to differentiate it clearly from the granules of food and other material which react to stain in much the same manner, has led the writer to refrain from attempting at this time to examine it in more detail.

The spindle is organized within the original nuclear cavity, and possibly from a residual non-chromatic nucleolar substance which may be seen as a lightly staining mass, apparently fibrous in character, occupying the central region of the nucleolus after the chromatic substance has all passed to the nuclear wall (Figs. 56 and 60). No indication whatever of an extra-nuclear origin for the achromatic structure was ever observed. The suspending fibrillae already mentioned (Fig. 54) may of course co-operate with this nucleolar matter. The spindle may be

best observed at metaphase, at which stage the shape of this structure in mitoses occurring in the early development of the gonimoblasts presents a strong contrast to that assumed by it in the vegetative phase, since in the latter it is relatively long and narrow, with the fibres meeting at the poles at a fairly acute angle, whereas, on the other hand, in the cells of the gonimoblasts its length remains about the same, while its width has become nearly doubled, and consequently the angle at the poles is now markedly obtuse (cf. Fig. 75 with 65, for example). It should be mentioned, perhaps, that the formation of the cell-wall which has been described above as occurring after mitosis is quite independent of these fibres. The central portion of the spindle could be observed for a short time after the separation of the daughter-chromosomes (Fig. 66), while its cones remain intact, somewhat shortened perhaps, for a considerable time (Fig. 69).

Nucleolus.

The nucleus, as has been already briefly pointed out, consists of a relatively large cavity bounded by a delicate wall and filled with nuclear sap, in the centre of which appears a single, large, heavily staining 'nucleolus,' attached to the wall by a considerable number of radiating fibrillae (see figs.). The dimensions of the nucleus, and nucleolus as well, vary to some extent, as may be seen from the figures, its increase in size being apparently connected with preparations for division. The nucleolus is not homogeneous in structure, and consists of a peripheral region of denser material surrounding a central substance which shows a much less marked capacity for absorbing stains. This condition of affairs is represented in Fig. 51, which illustrates a favourable section passing through the body in question. As mitosis approaches, this peripheral substance aggregates in heaps, leaving thinner areas between (Fig. 52); and in some cases these aggregations are of such uniform character as to suggest that they may be the units from which the chromosomes are to be formed, their number being decidedly in excess of, and probably about double that of the chromosomes (Fig. 57). At this period the nucleolus in many cases assumes a lenticular shape, its short axis probably coincident with the long axis of the future spindle. The correctness of this assumption is indicated by the conditions present in later stages (Figs. 59, 61, and 62), in which the chromosomes are gathered chiefly in the equatorial region of the dividing nucleus.

At this period the radiating fibrillae stain somewhat more conspicuously, the nucleolus becomes irregular in outline and often slightly drawn out along them, while distinct, deeply staining masses appear at the points where they intersect the nuclear wall (Figs. 54-56). Inasmuch as previously no stainable material whatever could be detected in this

region, there is, it would seem, little reason to doubt that a substance originally stored in the nucleolus is passing in the manner indicated to this position, and further, that this substance is, from its subsequent history, certainly to be identified with chromatin.

These chromatic masses are now directly organized into chromosomes without, so far as could be certainly determined, the intervention of a spireme stage (Figs. 58–60), and very soon are apparently drawn from a general equatorial position to the nuclear plate (Figs. 61–65). After splitting has occurred, the daughter-chromosomes remain separate for a considerable time (Figs. 66 and 69), eventually, however, fusing into two or three masses (Figs. 70 and 71), which further fuse into the single centrally placed nucleolus (Fig. 72), finally assuming the appearances already described as characteristic of the resting nucleus.

In the development of the gonimoblasts, but not elsewhere, conditions were observed which cannot, it would seem, be explained upon any other assumption than that a portion of the nucleolar substance is expelled from the nucleus in the prophases of division (Figs. 56 and 68). It could not be determined whether or not such extruded masses represent a substance other than chromatin located in the nucleolus, and the homologue of the so-called plasmosomes, or true nucleoli. Inasmuch, however, as its reaction to stain, as well as its mode of origin, appears to be entirely similar to that of the chromatin, the presumption is in favour of identifying it with that substance.

In a recent paper on the nucleolar conditions of *Phaseolus* ('04), Wager has shown that the nucleolus in this plant is simply a storehouse for the greater portion of the chromatin, which, as division approaches, passes out of that body into the spireme-thread through fibrous connexions. Although in *Nemalion* no reticulum is present and no spireme is formed, the chromatin, which, as we have seen, is in this instance wholly confined to the nucleolus, passes out from it along fibrillae in much the same manner. The nucleolus of *Spirogyra*, according to numerous investigators, is also concerned to a greater or less extent in the formation of the chromosomes; Moll ('93), for instance, regarding all the chromatin as derived from that body, while Van Wisseling ('98) finds that only portions of two chromosomes owe their origin to the nucleolus. Golenkin ('00), in his paper on *Sphaeroplea*, describes conditions according to which the nucleolus breaks up directly into morphological chromosomes. This author goes further in stating that nuclei organized upon this plan are characteristic of many green Algae and Musci. In view of the fact that Golenkin did not section his material, it would seem entirely possible that the fibrillae above described might easily escape such an examination, and altogether likely that a method better adapted to reveal such detail would show conditions which would bring these forms more nearly into harmony with the accounts

of these phenomena as described in *Nemalion*, *Phaseolus*, and many other plants.

Such nucleoli have been variously characterized as false nucleoli, chromatin nucleoli, or karyosomes, in contradistinction from the true nucleoli, or plasmosomes, which appear to contain no chromatin, and are supposed by some to be cast out of the nucleus at mitosis. Since, however, they disappear immediately before the formation of the achromatic spindle, they are held by other observers to be concerned in its formation. In general, the former have been supposed to be confined to lower plants, while the so-called plasmosomes were believed to be characteristic of vascular plants. Evidence is accumulating, however, which appears to indicate that even in the Phanerogams the body hitherto distinguished as the plasmosome is also concerned in the formation of the chromosomes, and suggests that further research in this direction is likely to show that similar conditions are, at least, of very general occurrence throughout the plant kingdom. The data at present available would thus seem to indicate that the differences existing between the various structures which in the nuclei of plants have been termed nucleoli are not such as would justify their separation under two distinct categories; and that, although forms may exist which contain little or no chromatin, every gradation may be observed through a gradual increase of the chromatin content, to such conditions as are illustrated by *Nemalion* or *Sphaeroplea*, in which the nucleolus represents the entire chromatin content; and therefore the writer would agree with Wager in the conclusion (p. 50, l.c.) that 'the nucleolus is intimately bound up with the formation of the chromosomes, and Strasburger's contention that it is only concerned in spindle or kinoplasmic formation does not hold good, although it is not impossible that a portion of it—the plastin or pyrenin of Zacharias and Schwarz—may be used up in this way.'

Reduction.

As has already been mentioned in the introduction, the primary object for which the present investigation was undertaken was to determine whether in the plant under consideration a differentiation into gametophyte and sporophyte could be proved, and, if so, to what extent the limits of these generations could be fixed by such cytological evidence as might be obtained. As will be seen by the following account, we may assume the existence of such an alternation, and hence with propriety characterize the period comprising the earlier divisions of the gonimoblasts as the sporophyte, and as the gametophyte that beginning with spore-formation and ending with the differentiation of the sexual elements.

As previously stated in the description of the behaviour of the nucleolus, the number of chromatic masses appearing upon the nuclear wall in

prophase is far in excess of the chromosome-number characteristic of that generation, sporophyte, or gametophyte, respectively, in which the division occurs. Since the diameter of the nucleus does not exceed $3\ \mu$, it is evident that an exact enumeration is a very difficult matter, and, in fact, in the earlier divisions of the gonimoblasts estimates of the number of these masses varied from twenty to thirty, and appeared to be correspondingly less in the gametophyte. Inasmuch as the number of chromosomes eventually appearing is less than the number of granules by about half, it is not impossible that these granules may be chromatic units which may fuse two and two at the time of their organization into morphological chromosomes, although the details of this process could not be determined.

The chromosomes are rounded bodies which stain intensely, and are thus, notwithstanding their small size, fairly distinct. From a number of estimates very carefully made and repeatedly confirmed, the writer feels entirely safe in stating that in the divisions of vegetative nuclei they are about eight in number; certainly not less than seven, nor more than nine (Figs. 29 and 75).

In the cystocarp the details of mitosis could not be followed for the first two or three divisions of the zygote, owing to the fact, previously mentioned, that the cytoplasm at this time stains so intensely. In all divisions subsequent to this period, however, mitotic figures were observed in a variety of stages and in great abundance. In the earlier divisions in which the mitotic figures could be clearly differentiated, the chromosomes, owing to their crowded position upon the nuclear plate, form at times an almost continuous band (Fig. 65), and in general their number could not be ascertained with the same degree of certainty as in the vegetative phase. Owing to the fact that these preliminary aggregations of chromatin, illustrated in Fig. 56 in the prophase stages, might possibly be mistaken for polar views of the spindle at metaphase, polar views were taken as corroborative only, and such approximations alone relied upon as could be obtained from longitudinal and oblique views in which, since both centrosomes were present and the spindle clearly differentiated, it could be definitely determined that the bodies in question were morphologically chromosomes. A great number of estimates were made, based upon such stages, and certainly the number is greatly in excess of that characteristic of the vegetative phase, approximations varying from twelve to sixteen, with the weight of probability in favour of the latter number (Figs. 61-65). The decided increase in breadth of the spindle, which has previously been noted as characteristic of the earlier mitoses in the cystocarp, further indicates the presence of a greater number of chromosomes in these divisions. In this connexion, perhaps, it should be stated that the phenomenon of 'splitting' presents characteristic features and cannot be

easily mistaken for the conditions above described, since the daughter-chromosomes are at this period, the early anaphase, approximately thirty-two in number, and are, in addition, arranged uniformly in two receding circles (Fig. 66).

Since, as we have seen, the number of chromosomes in the earlier dividing nuclei of the cystocarp are approximately double that seen in the vegetative cells, a reduction must be assumed to occur at some point in this life history. By analogy with the conditions seen in higher plants, it might be expected that this reduction would be associated with the formation of spores; and such, in fact, has been found to be the case. At a more advanced stage in the development of the cystocarp (Figs. 67 and 68), mitotic figures were seen in terminal cells, which present a marked contrast to those previously occurring in these divisions (Figs. 61-65), but agreeing absolutely in number of chromosomes, shape of spindle, and all other details, so far as could be seen, with the figures observed in vegetative cells (Figs. 29 and 75). Moreover, it seems certain that the new cell resulting from this reduction division is destined to become a spore. The further history of the sister-nucleus which remains within the cell in which this reduction division occurs was, however, not followed, owing to the great difficulties of orientation involved. The habit of proliferation, already briefly described, apparently admits of a rather wide variability in the number of spores that may be formed from such a cell, inasmuch as it gives rise terminally and laterally to spores as well as to short branches bearing spores. The number is further multiplied by proliferation, within the mother-cell, more than once repeated after the carpospores have been successively discharged (Text-fig. 51). Although it has been impossible to follow out this matter in detail and with certainty, owing to the complicated conditions just mentioned, it is entirely probable that the reduced number of chromosomes once established in a given cell persists throughout its further divisions, and is continued in all its products. Since, however, the reduction division in this plant is not associated with the formation of a tetrad of spores, a parallelism with higher plants does not exist beyond the phenomenon of actual reduction.

The process by which the chromatin is reorganized into the reduced number of chromosomes in the prophases of this reduction division could not be followed, owing, as may be readily seen, to the fact that in the reconstruction of the daughter-nuclei resulting from the preceding division, these structures necessarily lose all morphological identity, since it is from their fusion that the nucleoli are formed. Inasmuch as an extrusion of chromatic substance has in some cases been described as being associated with the reduction division, it might perhaps be well to mention in this connexion the fact that the chromatic extrusion previously referred to in this paper, while seen to occur in connexion with reduction (Fig. 68),

does not appear to be confined to such divisions alone, being seen in connexion with what appeared to be a much larger number of chromosomes.

The events above described constitute, in the opinion of the writer, conclusive evidence in support of a view long maintained, according to which the cystocarp of the red Algae is held to be the homologue of the sporophyte in higher plants. Resulting from a sexual act, intervening between that sexual act and the formation of spores, bearing a spore which gives rise to an individual unlike that upon which it is borne, and finally presenting double the number of chromosomes seen in the vegetative phase, it possesses the essential characters of such a sporophyte.

SUMMARY.

Since it has seemed to the writer that the present condition of our information concerning the phenomena of reduction in Thallophytes is far too meagre and conflicting to render any further discussion of the subject at the present time of value, he has chosen to content himself with the presentation of such matters of fact as appear to have been developed in the course of this investigation. It may, however, be pointed out that in the Florideae, a family usually regarded as an independent and terminal phylum, or at least in *Nemalion*, which is not considered as an illustration of their highest development, a condition of alternation is present which, in so far as the cytological phenomena accompanying it are concerned, is very closely comparable with that which is familiar in the archegoniate series—the double number of chromosomes appearing after the union of the gametes and continuing in the sporophyte to the point at which spore-mother-cells are formed. Regarding, then, the present paper as a contribution which may be of value when a sufficient body of information concerning such cytological conditions in the lower plants renders their co-ordination possible, the general results obtained may be summarized as follows:—

(1) The chromatophore of *Nemalion* is in the form of a hollow ellipsoid from which processes radiate to the periphery of the cell, and there flatten out to form a clathrate membrane.

(2) The region surrounded by the ellipsoid portion of the chromatophore, and generally regarded as a pyrenoid, consists entirely of vacuolar material, and hence cannot be considered as constituting any such definite organ of the cell.

(3) The chromatophore is present in all cells of the plant, with the exception of the mature antheridium and the two sperm-cells to which it gives rise; and in all cases originates from a pre-existing body of the same nature.

(4) The sex-organs cannot be regarded as unicellular structures: since

(5) In earlier stages the trichogyne possesses a well-organized nucleus, which fragments as that organ matures; and, the egg-cell thus becoming an intercalary cell, the trichogyne must be regarded as a cell which has been specialized in connexion with the reproductive processes, and further,

(6) The contents of the spermatium normally divide into two fertilizing elements which are discharged into the trichogyne: these events constitute this so-called spermatium an antheridium.

(7) Fertilization consists essentially in the union of a male and a female gamete, and from the further divisions of the resultant fusion-nucleus the cystocarp arises after the separation of the stalk-cell.

(8) In the mature cystocarp the ultimate cells give rise terminally, as well as by subterminal proliferation, to a variable number of carpospores, which is further augmented by repeated proliferation within the successively formed mother-cell walls.

(9) In the nucleus the entire chromatin content is stored in the nucleolus, and in the prophases of division passes to the nuclear wall along delicate fibrillae.

(10) The spindle is intra-nuclear, and centrosomes are distinctly visible at the poles at metaphase.

(11) The conclusion that *Nemalion* presents the essentials of an antithetic alternation of generations, and that the cystocarp is, therefore, the homologue of the sporophyte in higher plants, is indicated by the cytological evidence—since

(12) Approximately sixteen chromosomes are present in the divisions of the cells of the cystocarp up to the period of spore-formation, and approximately eight in those of the thallus; the reduction division being immediately associated with the production of the carpospores.

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EXPLANATION OF FIGURES IN PLATES XL AND XLI.

Illustrating Dr. Wolfe's Cytological Studies on *Nemalion*.

All figures are drawn at the level of the stage with the aid of a camera lucida, and using the apochromatic 2.0 mm. objective of Zeiss with compensating ocular 18, giving an approximate magnification of 2250 diameters. Plate XL is reduced about four-sevenths in reproduction, the other is reproduced without reduction. Figs. 2-19, 49 and 50 are drawn from specimens crushed and stained on the slide in safranin and gentian-violet; 24, from material preserved in formalin, and mounted in glycerine; 25 from a section 1 μ , 22, 35, and 44-48 from sections 3 μ in thickness, and stained in safranin and gentian-violet; all others are from 3 μ preparations stained in haematoxylin after Heidenhain.

Oogenesis.

Fig. 1. Young carpogenic branch, consisting of the primary carpogonial cell borne on a vegetative cell. A nucleus and chromatophore are present in each.

Fig. 2. Older. The procarp is supported upon three modified cells. The branch arises from a normal vegetative cell. The procarp is bulging out to form the trichogyne.

Fig. 3. The trichogyne is now a narrow protoplasmic process surrounded by a heavy wall. The primary egg-nucleus is in its characteristic position below the chromatophore.

Fig. 4. The egg-nucleus is now above the chromatophore—an unusual position. The primary egg-nucleus has probably divided, giving rise to the egg-nucleus, and to that of the trichogyne. The wall of the trichogyne is now, and also in later stages, very thin at the tip.

Fig. 5. The egg-nucleus has resumed its normal position below the chromatophore and in the basal portion of the cell.

Fig. 6. The trichogyne is elongating, and the nucleus is still intact.

Fig. 7. Fragmentation of the nucleus of the trichogyne is beginning to take place.

Figs. 8-10. Later stages in the process of fragmentation. The fragments gradually stain less deeply. A thin wall surrounds the entire receptive portion of the trichogyne.

Fig. 11. The carpogonium is now ready for fertilization, and shows what are probably the last traces of a disorganizing nucleus.

Fertilization and Development of the Cystocarp.

Fig. 12. An early stage in fertilization. Residual protoplasm and fragments, probably, of the second male nucleus, are seen in the spermatium, which has conjugated with the trichogyne. The male nucleus is in the top of the carpogonium, while the egg-nucleus is still in the basal portion. The protoplasm of the trichogyne has shrunk away from the thin wall, and is beginning to disorganize.

Fig. 13. Actual fusion of the male and female nuclei, the male the smaller of the two. Its nucleolus is at one side. The nuclear walls are more distinct than usual.

Fig. 14. A similar stage. Apparently a second nuclear body lying beside the chromatophore.

Fig. 15. The same stage.

Fig. 16. The fusion-nucleus now lies in the top of the carpogonium. The separation of the trichogyne is beginning to take place. Three spermatia are in contact. The one at the left has not fused, and its nucleus has not divided. The empty one has probably given rise to two sperms, one of which has fused with the egg-nucleus, and the other may still be seen midway the trichogyne. The third spermatium has just discharged one of its sperms, and the other is still within the mother-cell.

Fig. 17. A wall is now formed around the zygote, and the fusion-nucleus has resumed its usual position at the base.

Fig. 18. Division of the zygote into a stalk and a sporogenous cell. The chromatophore has already divided.

Fig. 19. Somewhat later.

Fig. 20. Similar to 17. Granular masses appear in the hypogynous cell.

Fig. 21. First division of the sporogenous cell. The nucleus of the cell to the right is in a lower focus. The dark mass is probably the chromatophore. The chromatophores of the stalk and hypogynous cells are disintegrating.

Fig. 22. A section of a young cystocarp at about the six-celled stage. The chromatophores are now distinctly differentiated.

Fig. 23. Gonimoblasts. The terminal cells are separating as spores. Chromatophores are present in all the cells.

Structure of the Chromatophore.

Fig. 24. The chromatophore may be seen to consist of a central ellipsoid, from which processes radiate to the periphery of the cell, and there flatten out into a clathrate membrane.

Fig. 25. An absolute median section through the chromatophore, showing the interior of the ellipsoidal body to be devoid of protoplasmic contents.

Division of the Chromatophore.

Figs. 26 and 27. Processes extending into the new cell.

Fig. 28. Illustrating a rapid division of the chromatophore independent of the nucleus.

Fig. 29. The central body is bulging out into the daughter-cell.

Fig. 30. The bulge has increased until the two together present the appearance of a single elongated chromatophore.

Fig. 31. Constriction beginning.

Fig. 32. Division almost complete. A hyaline region of the protoplasm marks the position of the future wall.

Fig. 33. The wall forming.

Fig. 34. Cell-division complete.

Spermatogenesis.

Fig. 35. A young antheridial branch.

Fig. 36. An antheridium being formed. The nucleus in anaphase. About eight chromosomes are present.

Fig. 37. Antheridia in various stages.

Fig. 38. Nuclear division in the antheridial branch. No centrosome apparent.

Figs. 39 and 40. The centrosome is seen in some cases.

Fig. 41. Nuclear reorganization.

Fig. 42. The chromatophore is protruding into the antheridial mother-cell, and about to divide.

Fig. 43. A chromatophore in the antheridium.

Fig. 44. Granular material appears at the distal end of the antheridium. No chromatophore can now be differentiated.

Fig. 45. The nucleus appearing within this material.

Fig. 46. The nucleus is now greatly increased in size, and shows definite aggregations of material on the nuclear wall. The antheridium is now mature.

Fig. 47. The antheridium after it has been discharged into the water. A median section.

Fig. 48. The same in surface view.

Fig. 49. An antheridium conjugating with the trichogyne, and dividing into two sperm-cells.

Fig. 50. A similar stage.

Mitosis.

Fig. 51. Illustrating the structure of the nucleolus, with the chromatin at the periphery and surrounding a lightly staining substance. Radiating fibrillae are present.

Fig. 52. The chromatic material gathering into clumps.

Fig. 53. Probably centrosomes.

Figs. 54 and 55. The chromatin is passing along fibrillae out of the nucleolus to the nuclear wall. Prophase.

Fig. 56. Somewhat later. Also an extrusion of nucleolar substance.

Fig. 57. The nucleolus at this stage is somewhat lenticular in shape.

Fig. 58. The chromatin granules are now aggregated into morphological chromosomes.

Fig. 59. The chromosomes are grouped in the equatorial region of the nucleus.

Fig. 60. A similar stage.

Figs. 61-63. The chromosomes are gathering at the nuclear plate. The spindle is now present with distinct centrosomes at the poles.

Figs. 64 and 65. Metaphase in the sporophyte. About sixteen chromosomes present.

Fig. 66. Anaphase.

Fig. 67. The reduction division, showing about eight chromosomes.

Fig. 68. The same stage, but showing in addition an extrusion of nucleolar material.

Fig. 69. The anaphase of the reduction division.

Figs. 70-72. Stages in the fusion of the chromosomes to form the daughter-nucleoli.

Figs. 73 and 74. Vegetative prophase corresponding to 54 and 60, respectively, in the cystocarp.

Fig. 75. Vegetative metaphase corresponding to 64 in the sporophyte, and to 67 of the reduction stages.

Vaer.

Huth lith. et imp.

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