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## STUDIES ON THE PLANT CELL.—I.

BRADLEY MOORE DAVIS.

### INTRODUCTION.

THIS is the first of a series of papers that will follow one another in the pages of the *American Naturalist*. They will describe the chief structures in plant cells and the most important events in their life histories, largely from the point of view of the morphologist and student of developmental processes. Research upon the plant cell has entirely outrun the general accounts that may be found in several botanical text books and in certain works of prominent zoölogists. We shall attempt to give a general survey of the subject in its present state with references to the most important papers; but this is not to be an exhaustive account of a literature that is already very large and which can probably be treated far more satisfactorily several years from now when it has passed through the criticism that time will give in a field of very active botanical investigation.

American botanists have reason to be proud of the achievements of their countrymen in research upon the morphology and physiology of the plant cell, for much of the best work of recent years has come from them. This in itself has been a great stimulus to the writer to prepare these brief accounts which he hopes will assist the general botanist to a clearer understanding of the progress in this field. They will also serve to contrast the protoplasmic activities among plants with those of the animal cell which has been so well treated in several foreign works and in English by Wilson's *The Cell in Development and Inheritance*.

The author will feel especially gratified if these papers should help to change an attitude towards investigations on the plant cell that is unfortunately too prevalent among botanists. There is a tendency to regard cell studies as a very special field of botanical research with elaborate technique which the average

botanist cannot be expected to master. Those who work in this field are considered as in a department by themselves and are labeled cytologists which is sometimes given as an excuse for knowing little about their results. Cell studies are nothing more than morphological and physiological investigations which are frequently so broad as to break the mould of the narrower morphology and physiology of former years. Cell studies must be the foundation of all exhaustive work in morphology and physiology. Indeed among the lower plants they constitute almost all there is to morphology and will determine the classification and relationships of great groups. There are no better illustrations of this fact than the effect of Prof. Harper's investigations on the ascus and sporangium upon Brefeld's theory of the origin of the Ascomycetes. And again the results of several investigators upon the multinucleate gametes found among the Phycomycetes and Ascomycetes are of the utmost importance to a correct understanding of the phylogeny of these groups. When students of the plant cell refuse to accept the stamp of cytologist and insist and show that their work is simply fundamental morphology and physiology we shall break away from a past that should be outgrown.

The material of these papers will be treated under the following heads.

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## LITERATURE ON THE PLANT CELL.

Reference to special papers will be given by the authors name and the date of publication through lists presented at the end of every section.

There is no comprehensive treatise devoted to the plant cell but the following general accounts and reviews of the literature are important.

1. Strasburger in the *Lehrbuch der Botanik* and Pfeffer in his *Physiology of Plants* present the best general accounts of the structure and activities of the plant cell.

2. Zimmerman in 1893 and '94 ("Beihefte zum Botanischen Centralblatt" vol. 3 and 4), reviewed the literature on the plant cell under the title "Sammel-Referate aus dem Gesamtgebiete der Zellenlehre" and in 1896 collected the literature dealing with the nuclei of plants in a book entitled *Die Morphologie und Physiologie des pflanzlichen Zellkernes*, Jena, 1896.

3. Dangeard discusses a number of cytological topics in the 6th series of *Le Botaniste* (1898) with especial reference to his studies on the Chlamydomonadeae.

4. Fischer, *Fixirung Färbung und Bau des Protoplasmas* <sup>Jena</sup> ~~Leipzig~~ 1899, presents a critique of the methods of cytological research and the justification of the conclusions based thereon.

5. The most recent analysis of conspicuous activities of the plant cell is that of Strasburger *Ueber Reductionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich, Histologische Beiträge* VI, 1900.<sup>1</sup>

## SECTION I. STRUCTURE OF THE PLANT CELL.

It is customary to apply the term cell in Botany not alone to the protoplasmic units of organization but also to the enclosing wall that generally surrounds the protoplasm. Indeed these walls alone when entirely emptied of protoplasm in specialized

<sup>1</sup> To this list should be added an excellent concise review by Koernicke entitled "Der heutige Stand der pflanzlichen Zellforschung" *Ber. d. deut. bot. Gesell.* 21, (66), 1904. This article appeared too late to be quoted in the earlier papers of this series.

regions of the plant, *e. g.* vascular and certain supporting and tegumentary tissues, are still called cells. When among the lower forms and at certain periods in the life history of many higher plants the protoplasm is naked (*e. g.* zoöspores, sperms, eggs, etc.), these structures are cells in exactly the sense used by zoölogists. We shall consider almost entirely the protoplasmic portion of the plant structure for any extended treatment of the walls would lead us at once into that field of microscopic anatomy termed histology.

### I. Protoplasmic Contents.

The most highly differentiated region of the cell is the nucleus, a structure remarkably uniform in organization among all plants except the lowest Algæ and some very simple Fungi. These more primitive conditions will be considered in Section VI. Besides the nucleus there are present plastids in all groups except the Fungi. Plastids are likewise specialized protoplasmic elements although much simpler in structure than the nucleus. Nuclei and plastids lie in a protoplasmic matrix called the cytoplasm. Cytoplasm is more variable in structure and activity than any other region of the cell. Thus three forms of protoplasm, nucleoplasm, plastidplasm and cytoplasm comprise all the living material of the cell and may be sharply contrasted with the non-protoplasmic contents, mostly food material and waste products, which will be considered under a separate head. Definite masses of nucleate protoplasm, with or without plastids are termed protoplasts and such are either unicellular organisms themselves or units of a multicellular structure.

#### (a) The Nucleus.

The nucleus is bounded by a delicate membrane that is probably largely or wholly a modification of the surrounding cytoplasm. The nucleoplasm very rarely completely fills the nuclear membrane, the remaining space being occupied by a fluid known as the nuclear sap. The elements in the resting nucleus consist chiefly of material that takes the form of a net work so that the

effect is that of a much coiled and twisted thread whose loops are united at intervals to form large and small meshes. The ground substance of this thread is called linin and imbedded in it as in a matrix are deeply staining granules of chromatin. Chromatin is regarded as the most important substance in the nucleus, chiefly because of its behavior during nuclear division, and in critical periods of the life history of organisms as at sporogenesis, gametogenesis and fertilization (to be described in Section V). Just before nuclear division the chromatin becomes organized into bodies named chromosomes which are remarkably uniform in number and definite in shape for each tissue and period of the plant's life. They will be discussed under "The Events of Nuclear Division" (Section II), and in Sections IV and V.

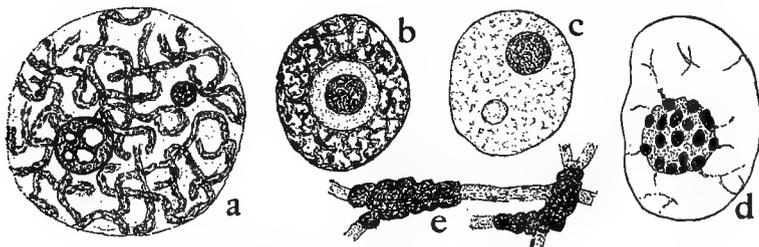


FIG. 1.—The resting nucleus. *a*, Embryo sac of lily with linin thread and two nucleoli. *b*, Root of onion large nucleolus. *c*, Tetraspore of *Corallina* showing large chromatin body and small nucleolus. *d*, *Spirogyra* with central body containing chromatin. *e*, Chromatin on linin net work from egg of pine. After Mitzkewitsch and Chamberlain.

In the meshes of the linin network or lying freely in the nuclear sap may be found one or more bodies, generally globular in form, called nucleoli. (See Fig. 1 *a* and Fig. 1 *b*). The nucleolus is generally regarded as a secretion of the nucleus and it is quite certain that its substance is utilized just previous to and during the period of nuclear division when the spindle is formed. (Strasburger '95 and :00, p. 125, and from the work of others). The structure is not always homogeneous but may show in the interior small vesicles or areas of a different consistency from the periphery. There is often present also a rather thick outer shell or membrane. Sometimes the chromatin in the nucleus may be gathered into a globular body that resembles superficially a nucleolus. Such chromatin bodies are gen-

erally transitory as in *Corallina*, Davis '98, where the structure (Fig. 1 *c*) is only found in the young daughter nucleus and later fragments into many smaller bodies. In *Spirogyra* however (Moll '94, Mitzkewitsch '98, Van Wisselingh :00, '02) the chromatin is supposed to be always in a globular mass mixed with nucleolar substance and recalls the conditions in certain Protozoa. These chromatic structures however should never be confused with nucleoli, whose substance is different and which are not permanent in the cell, since they may disappear before or during nuclear division and be formed *de novo* in each daughter nucleus.

The substance of the nucleolus is not well understood. It is frequently impossible to distinguish it from chromatin except when favorably situated in the cell and there is much evidence that it is closely related to that substance. In large nuclei of higher plants the chromatin is sometimes gathered into globular bodies without apparent relation to a linin thread and these are readily mistaken for nucleoli and have been called such, but this loose usage of the term should be avoided. And true nucleoli may be so closely associated with the linin net work as to have the appearance of chromatin. Some of these conditions have been especially described by Cavara, '98. Chamberlain, '99, has made a study of the egg nucleus of the Pine where masses of chromatin may take very irregular forms on the linin threads (Fig. 1 *e*) and sometimes resemble small nucleoli. But such conditions should always be sharply distinguished from true nucleoli which are often caught in the meshes of the linin net work and appear to be a part of it when in reality there are no organic attachments. It is certain that nucleoli are of secondary importance in the cell and probably by-products of the general constructive activities of the nucleus. In which case they may be secretions, perhaps closely related to chromatin, or even direct transformations of this substance. It is well known that the nucleus has wonderful constructive powers, when the amount of chromatin and other nuclear substances may be immensely increased, facts that are especially well illustrated at reproductive periods of the plant's life as during sporogenesis and gametogenesis.

Chromatin is the only substance in the nucleus that is constant

in its presence throughout all periods in every cell's history. It passes on from cell to cell through the mechanism of nuclear division without interruption. There are periods of cell history when the nucleus consists only of chromosomes as in the stages of nuclear division called metaphase and anaphase. The other structures of the nucleus have their relation to definite conditions that are in part understood. The nuclear membrane probably results from the reaction of the cytoplasm to the secretion of nuclear sap among the chromosomes (Lawson, :03 *a*). It would then be strictly cytoplasmic in character and similar to the plasma membranes around vacuoles. Nucleoli must be regarded as temporary structures since they generally disappear during nuclear division either dissolving or else passing out into the cytoplasm where they may remain for long periods as deeply staining globules (extra nuclear nucleoli). Linin is believed to be derived from chromatin and in its turn may be transformed into the substance of spindle fibers, which are cytoplasmic, so that chemically it holds a position somewhat intermediate between chromatin and cytoplasm. It seems established that the linin net work is a temporary structure related to the activities of chromatin.

#### (b) The Plastids.

These very interesting structures, characteristic of plant cells, have not received the degree of attention that they deserve and much valuable work may be done in the detailed study of their protoplasmic structure and activities at various periods of ontogeny especially through the series of changes that are presented during developmental processes.

The primitive types of plastids are relatively large structures, often solitary in the cells, and generally of complex form. These are called chromatophores and are characteristic of many algæ especially among the lower groups but are not found above the thallophytes (*Anthoceros* and *Selaginella* excepted).

The chromatophores of the simplest algæ are replaced in most of the higher types of these thallophytes and in all groups above by very much smaller structures, generally discoid in

form, which are called chloroplasts when green, chromoplasts when the color is other than green or leucoplasts if colorless. These plastids are without doubt derived from the more primitive chromatophores.

The colors of chromatophores are various. They are believed always to contain some chlorophyll but this green is frequently so completely masked by other pigments that its presence can only be determined when the additional coloring matters have been extracted. Chloroplasts are universally green except when they may be changing into chromoplasts. Chromoplasts generally take their tint from the predominance of other strong pigments in addition to chlorophyll as phycerythrin in the red and phyco-phæin in the brown algæ. But chromoplasts may be derived from chloroplasts whose green has largely or wholly disappeared leaving other pigments present as the yellow, xanthophyll, or the orange red, carotin.

The remaining plastids, leucoplasts, are devoid of color and are found in embryonic regions such as eggs, growing points, and in the various tissues of seeds, underground organs and other structures where the cells are largely or wholly removed from sunlight. The leucoplasts may become green upon exposure to light thus changing into chloroplasts. They are responsible for the secretion of reserve starch in many structures (*e. g.* potato) and in consequence have been called amyloplasts.

Leucoplasts, chloroplasts and chromoplasts are morphologically the same structures. It is well known that they may pass one into the other in the order indicated and that chloroplasts and chromoplasts may lose their color and become leucoplasts. It is generally believed that plastids are not formed *de novo*. They divide by constriction and thus multiplying are passed on from cell to cell and it is believed from generation to generation. They are therefore usually ranked as permanent organs of the cell. However, it is but fair to call attention to the fact that there are some serious difficulties in the way of a complete acceptance of these views.

The protoplasmic structure of the plastids of higher plants is rather simple while that of the chromatophores in algæ is more complex since they contain a special organ termed the

pyrenoid. The detailed structure of chromatophores was first described by Schmitz ('82) and of plastids by Meyer ('83). The most complete study of plastids however is that of Schimper ('85). The body of the plastid is always denser than the surrounding cytoplasm. It has a porous structure that is only visible under high magnification and there are sometimes present very delicate fibrils. The coloring matter, oily in consistency, is held in the pores as minute globules. The plastid may therefore be compared to a very fine-textured sponge saturated with pigment. All of the coloring matter of the plastid may be readily extracted with alcohol leaving the colorless proteid matrix.

The pigments of plastids are then in the nature of secretions held in these specialized regions of protoplasm. Chlorophyll is the principal substance and, as has before been said, is almost always present, but the amount is sometimes so small that its green is completely hidden by the color of other pigments. Chlorophyll itself contains greater or less amounts of two other coloring matters that may be readily separated from the pure green, a yellow xanthophyll and an orange red carotin, both substances closely related to chlorophyll. The other pigments, characteristic of the chromatophores in some groups of algæ, are however quite distinct from chlorophyll. There is phycocyan, found in the blue green algæ (Cyanophyceæ), phycophæin and phycoxanthin, characteristic of the brown (Phæophyceæ) and phycoerythrin of the red (Rhodophyceæ).

Chloroplasts are found almost universally in green plants above the Thallophytes and are also present in the large group of algæ the Siphonales and in the Charales. They are sometimes formed very numerously in the cell, reproducing rapidly by fission (see Fig. 2 *a* 2, 3) and lie in the layer of protoplasm just inside of the plasma membrane. They are sensitive to light and readily shift their position in the cell. Strong illumination results in their retreat from exposed positions to the sidewalls and bottom of the cell where the light is less intense. If the illumination be weak they may all gather on the side most favorable for the reception of light. These facts are well illustrated by the behavior of the plastids in some of the Siphonales (*e. g.*

Botrydium), in the Rhodophyceæ (*e. g.* Polysiphonia) and also in the palisade cells of leaves. Chloroplasts after exposure to light generally contain starch but in some plants this substance is never formed (*e. g.* Vaucheria, Fig. 2 A 1), the first visible products of photosynthesis being other substances more of the nature of oil. It is not known whether the starch grain in the

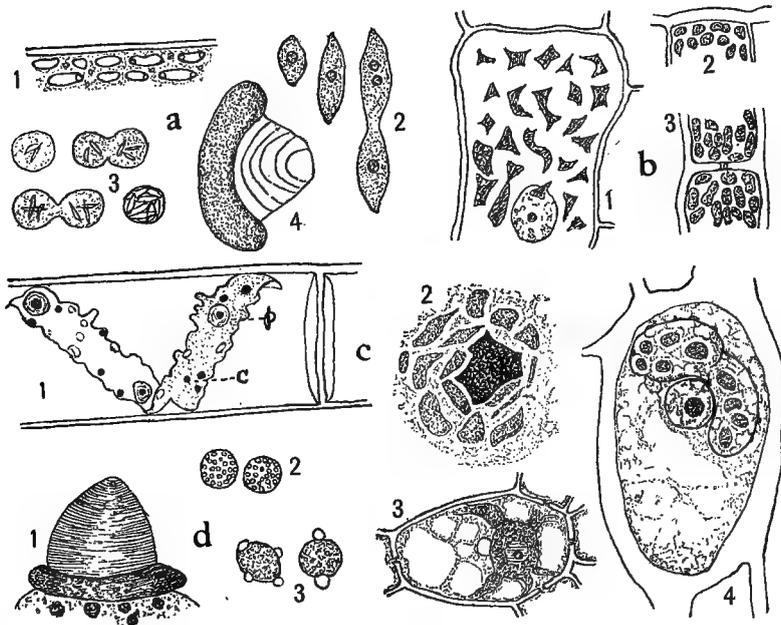


FIG. 2.—Plastids. *a*, Chloroplasts: 1 Vaucheria, with oil globules; 2 Bryopsis; 3 moss (*Funaria*), in division and containing starch grains; 4 *Oxalis*, with a grain of starch. *b*, Chromoplasts: 1 *Tropaeolum*, epidermal cell from calyx; 2 *Fucus*, 3 *Callithamnion*. *c*, Chromatophores: 1 *Spirogyra*, with pyrenoids ( $\beta$ ) and caryoids (*c*); 2 *Hydrodictyon*, pyrenoid forming starch; 3 *Nematium*; 4 *Anthoceros*, in division and containing starch. *d*, Leucoplasts: 1 *Phajus*, plastid and starch grain at the side of the nucleus; 2 *Iris*, from root and containing oil globules; 3 *Iris*, in deeper cells of root, with starch grains. After Meyer, Strasburger, P.lla, Timberlake and Schimper.

chloroplast results from the direct change of some of the proteid substance or whether it is a secretion. The conditions are somewhat different when pyrenoids are present in a chromatophore as will be described presently.

The chloroplasts of higher plants may change color under various conditions and become chromoplasts. Some of the best

examples are found in the colored cells of certain floral parts and fruits (Fig. 2, *b* 1). These pigments are generally either xanthophyll (yellowish) or carotin (orange red). Chloroplasts may also turn brown especially in older cells that are losing their contents. The colors of some leaves and flower parts are due not to the plastids but to substances dissolved or otherwise held in the cell sap of the vacuoles. The brilliant coloration of autumn foliage is of this character as well as some of the tints of petals, hairs and other structures. The chromatophores of the higher brown Algæ (Phæophyceæ) and most of the red (Rhodophyceæ) have the discoid form characteristic of chloroplasts (Fig. 2 *b* 2, 3). They might be called phæoplasts and rhodoplasts if one wished to classify plastids according to their color.

The structure of chromatophores is frequently complicated by the presence of pyrenoids which may be quite numerous in the body. These structures are denser regions of the chromatophore with a definite boundary. They are proteid in character and are known to vary in size with nutritive conditions and may completely disappear if the cell is starved. They have been regarded as masses of reserve proteid material but certain functions of great importance are also associated with them. The arrangement of starch grains in the chromatophores of many algæ is clearly around the pyrenoids as centers. For this reason they have been called amyllum centers. Timberlake (:01) has recently shown in *Hydrodictyon* that segments are split off from the pyrenoids (see Fig. 2, *c* 2) and changed directly into starch grains which naturally lie for a time close to the source of their formation and only later become distributed throughout the chromatophore. It is probable that similar conditions will be found in other algæ (Conjugales, Protococcales, etc.) and we may soon have a much clearer understanding of the pyrenoid. The indications are that the pyrenoid will prove to be a region of the chromatophore differentiated as a metabolic center, more or less prominent according to conditions of nutrition, and that its most conspicuous activity is the formation of starch by the direct transformation of portions of its substance.

Some other structures besides the pyrenoids have been

described by Palla ('94) in the chromatophores of several of the Conjugales and have been named caryoids. Caryoids (Fig. 2, *c* 1) are smaller and more numerous than pyrenoids and are distributed irregularly in the chromatophore but chiefly along the edge. Their function is not known.

The leucoplasts complete the list of plastid structures. They are colorless and may be found in underground or other portions of the plant removed from light or where there is little or no photosynthetic activities as in embryo sacs, seeds, growing points, etc. They become impregnated with chlorophyll under conditions suitable for photosynthesis thus changing into chloroplasts. An important function of the leucoplast is the formation of reserve starch in various parts of the plant. The more recent investigations of this process (Meyer, '95, Salter, '98) claim that it is in the nature of a secretion within the substance of the leucoplast. This view is opposed to the older conceptions (Schimper, '81, Eberdt, '91), which regarded the starch grain as formed by the direct change of proteid material in the plastid. In view of Timberlake's (:01) studies on the pyrenoid of *Hydrodictyon* we may well hesitate to fully accept the views of Meyer and Salter and ask for further investigations of this very difficult subject. In addition to starch leucoplasts may contain proteid crystals and oil globules.

The reproduction of plastids and their evolutionary history in ontogeny and phylogeny offers a very attractive field for research. It is well known that plastids multiply by fission and it is generally believed that they never arise *de novo* but are passed from generation to generation as permanent organs of the cell. The process of division may be very favorably studied in the spore mother-cell of *Anthoceros* (Fig. 2, *c* 4). The fission begins (Davis, '99) by a constriction at the surface as though the bounding membrane of cytoplasm exerted pressure upon an elongating structure. There is no evidence that the interior of the chloroplast undergoes any changes that could assist the process further than a possible tendency of the two separating portions to gather their substance together as division proceeds. The conditions suggest that the division is a mechanical separation of material too bulky for the best advantages of the cell,

for the proper balance of protoplasmic elements in narrow confines, a division prompted by the activities of the cytoplasm rather than emanating from within the plastid.

The view of the permanence of the plastid as a cell organ has received its strongest support from the classical work of Schimper ('85). We are not prepared to deny it and to assert that the plastid may arise *de novo*. Yet those who study the cells of embryonic tissues and reproductive phases know that it is extremely difficult to follow the plastids and that these structures require other than the usual methods of cell research to establish their presence. Several writers (Eberdt, Dangeard, Husek and others) have expressed their belief that plastids may arise *de novo* but no one has thoroughly traced the appearance or disappearance of these structures in any cells.

The plastid in phylogeny has never received the attention that it deserves. Beginning with the conditions among the Cyanophyceæ and the lowest Chlorophyceæ (which will be further discussed in Section VI) we find the pigment distributed so generally throughout the cell that it is doubtful if the term chromatophore should ever be applied to regions so indefinite in outline. Above these groups the pigment is confined to proportionally smaller areas in the cytoplasm and these become chromatophores when their form is clear. The primitive chromatophores were solitary and filled a large part of the cell. The pyrenoids arose in the chromatophores probably as the result of the influence of metabolic centers upon the protoplasm. It is scarcely possible that a large chromatophore should be absolutely homogeneous throughout; there would develop one or more centers of metabolic activity and such would exert some influence on the form of the protoplasm.

But the large single chromatophore does not seem to be the form best adapted to the work of a cell perhaps, if for no other reason, because it requires a mechanical adjustment of other cell organs to itself and would interfere with the quick circulation of material and the general balance of cell activities. It seems possible that mechanical difficulties may have led to the division of large chromatophores and the substitution of numerous small plastids. This change was instituted in the

higher members of the Phæophyceæ and Rhodophyceæ and in the Siphonales, Charales, Cladophoraceæ and some smaller groups of the Chlorophyceæ. The Conjugales whose chromatophores are especially elaborate have cells essentially solitary in their life habits and with a very remarkable adjustment of the cell organs to one another to give almost perfect symmetry. With the splitting up of the chromatophore came the loss of the pyrenoid and the final result was the compact plastid so characteristic of plants above the thallophytes.

(c) Cytoplasm.

There is no region of the plant cell that maintains such varied relations to its environment and performs so many visible activities as the cytoplasm. For this reason the accounts of its structure and behavior have been diverse and there has developed a nomenclature of its parts that is confusing and somewhat difficult to harmonize.

Strasburger has for many years (since 1892) employed the term kinoplasm to distinguish an active portion of the cytoplasm (concerned with the formation of spindle fibers and other fibrillæ, centrospheres, centrosomes, cilia, plasma membranes, etc.) from more passive nutritive regions which he called trophoplasm. Kinoplasm corresponds closely to the archoplasm of the animal cell (Boveri, 1888). This classification has been criticised especially by Pfeffer (:00) on the ground that it employed names signifying physiological differences when the distinctions as far as we know are those of morphology alone. However the physiological behavior of kinoplasm and trophoplasm becomes very real to anyone who studies extensively cell activities and the morphological characters serve to emphasize these peculiarities. The truth seems to be that cell studies cannot be pursued from the standpoint of physiology or morphology alone but must combine these attitudes. And in the union it is hardly possible or perhaps desirable to construct a terminology with strict regard to either field of study. We shall use the terms kinoplasm and trophoplasm grouping the various cytoplasmic structures under these heads.

Cytoplasm has surface contact with three conditions and in each case there is present a delicate plasma membrane, colorless and very finely granular, which is very different in structure from the cytoplasm within. The first of these three membranes is the outer plasma membrane, which bounding the protoplast, is consequently just inside the cell wall. This membrane is called the "hautschicht" by the German botanists, a word for which we have no exact equivalent, the term ectoplast more nearly expressing the meaning than any other but for several reasons not being very satisfactory. Since this outer plasma membrane lies against a moist cell wall it is virtually surrounded by a film of water. The functions of the cell wall in land plants and its developmental history indicate a close relation to the demands of the outer plasma membrane for a fairly uniform environment of moisture, a matter which will be discussed in the last section of these papers.

The second form of plasma membrane surrounds the water vacuoles in the cell. It is very common for the plant cell to have a single large central vacuole containing the cell sap and the membrane around this was named the tonoplast by DeVries in 1885. DeVries believed that this vacuole reproduced itself by fission with each cell division and consequently was a permanent organ of the cell. It is, however, now well known that the large central space containing cell sap is not different from other vacuoles, indeed is frequently formed by the flowing together of several small vacuoles as smaller soap bubbles unite in the froth to form a larger one. A vacuolar plasma membrane is of course bathed by water since it holds the cell sap and its relation to a moist surface is therefore more evident than in the case of the outer plasma membrane.

The third plasma membrane encloses the nuclear sap with the protoplasmic nuclear elements chromatin, linin and the nucleolus. This nuclear membrane was discussed in connection with the nucleus of which it is generally considered a part, but as there stated, the evidence largely indicates that it is cytoplasmic in character, representing a reaction of this protoplasm to the fluid nuclear sap formed around the chromosomes in the daughter nuclei after each division (Lawson :03<sup>a</sup>). The nuclear sap

necessitates the development of a vacuole which becomes bounded by the nuclear membrane. The nuclear membrane in some cases at least differs from a vacuolar membrane in being easily distinguished from the surrounding cytoplasm as a definite film.

The structure of all the plasma membranes is much the same as far as the microscope may determine. The protoplasm is dense, colorless and filled with very minute granules (microsomata). There are no large inclusions such as plastids, particles of food material (starch, proteids, oils, fats, etc.), mineral matter or waste products. These are all held well within the cytoplasm between the outer plasma membrane and the vacuoles. There is good reason to believe that the substance of all plasma membranes is much the same since they perform very similar activities both in relation to the fluids that bathe them and also because their substance in certain cases becomes the protoplasmic basis of cellulose walls. These resemblances are well established for the outer plasma membrane and that which surrounds the vacuoles. Thus, the capillitium of *Myxomycetes* (Strasburger, '84) is formed from the plasma membranes around the vacuoles after the same method as a cell wall from the outer plasma membrane. And again, during cleavage by constriction (see section II) in the plasmodium and sporangium of the molds (Harper, '99 and :00, D. Swingle, :03), vacuoles fuse with cleavage furrows from the outer plasma membrane to form a common membrane which surrounds each spore mass and secretes a wall, thus showing identity of function and structure. The resemblances are less conspicuous for the kinoplasm of the nuclear membrane, only appearing indirectly with certain events of cell division (the formation of the cell plate) which will be discussed in the next section of the paper. The evidence indicates that the three plasma membranes are all kinoplasmic in character, a generalization of some importance since it offers explanations of many peculiar cell activities to be described later.

Since all plasma membranes have these common characters it may well be questioned whether an elaborate terminology is justified for structures so closely related. The terms ectoplast and tonoplast seem undesirable since they were meant to indi-

cate peculiarities of structure and a degree of permanence as cell organs that is not actually present. It seems hardly necessary to define the plasma membranes further than by their position in the cell as the outer, vacuolar and nuclear membranes.

All of the cytoplasm bounded by the plasma membranes with the exception of certain conditions to be described later (centrospheres, centrosomes, asters, filarplasm and blepharoplasts) may be called trophoplasm since it contains structures and substances especially concerned with nutritive functions. Trophoplasm presents an open organization in sharp contrast to the dense kinoplasm. This peculiarity is due in part to numerous small vacuoles which give a spongy appearance to the usual foam like structure and is further complicated by the inclusion of material not strictly a part of the protoplasm in the form of various sized granules. There are sometimes present fibrillæ that impart a somewhat fibrous texture. We cannot discuss here the theories of the structure of protoplasm, which has not been so extensively studied in plants as among animals, further than to point out that it varies considerably in different regions of the cell in relation to peculiarities that will be described later. There is sometimes presented very typically the foam structure of Bütschli but the introduction of small vacuoles generally gives a spongiose appearance. This subject is critically reviewed by Fischer, '99, and has also been treated in several papers of Strasburger especially in '97.

Three well differentiated organs of the cell, probably trophoplasmic in character, require special mention, *viz.*, cœnocentra, nematoplasts and physodes. Cœnocentra are very interesting protoplasmic centers found in the oogonia of certain cœnocytic fungi among the Saprolegniales and Peronosporales during oogenesis. They appear just previous to the differentiation of the eggs as small bodies sometimes with delicate radiations (see Fig. 3, *a* and 8, *f*), and are found one in each egg origin. They are apt to increase in size as the eggs mature and evidently become the centers of the metabolic activities of the cells, drawing the sexual nuclei into their neighborhood where the latter increase in size (Fig. 3, *a 2*). The cœnocentrum disappears in the ripe oöspore and is consequently an evanescent

structure. It is probably the morphological expression of a dynamic center in the egg. Coenocentra have been known for several years and have been given especial attention in the recent investigations of Stevens, '99 and '01, and the author (Davis, :03). They will be further considered in our account of Coenogametes (Section III).

Nematoplasts are exceedingly small rod or thread like

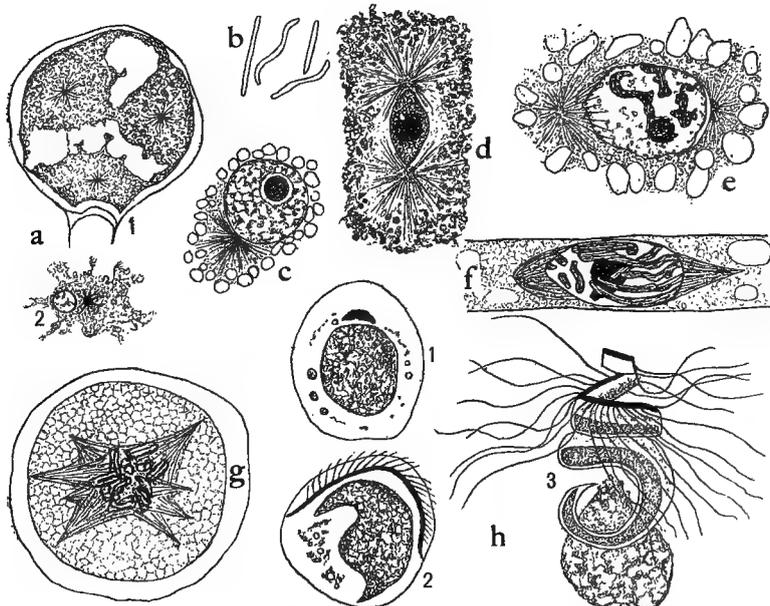


FIG. 3.—Cytoplasmic structures. *a*, Coenocentra of *Saprolegnia*; 1, oögonium, each egg origin with a coenocentrum; 2, coenocentrum and nucleus from mature egg. *b*, Nematoplasts from hair of *Momordica*. *c*, Nucleus from apical cell of *Sphacelaria*, aster with centrosome. *d*, Nucleus from oögonium of *Fucus*, aster with centrosphere. *e*, Nucleus from germinating spore of *Peltia*, centrospheres with short cytoplasmic radiations (aster like). *f*, Nucleus from procambium cell of *Vicia*, kinoplasmic caps. *g*, Pollen mother-cell of *Lilium*, filarplasm in form of multipolar spindle. *h*, Development of sperm of *Gymnogamme*; 1, blepharoplast at side of sperm nucleus; 2, blepharoplast elongating and developing cilia; 3, mature sperm, blepharoplast and nucleus in parallel bands, cytoplasmic vesicle below. After Zimmermann, Hof, and Belajeff.

structures reported by Zimmermann ('93, p. 215) in the cells of hairs of *Momordica* and the root of *Vicia* (see Fig. 3B). It is probable that organs described by Swingle, '98, and Lagerheim, '99, under the names of vibrioides are the same as or closely

related to physodes. Swingle found them in some of the Saprolegniales and certain Rhodophyceæ and Lagerheim in Ascoidea. They are probably not uncommon. Nematoplasts may be proteid crystals but there is evidence that they move, bending slowly back and forth, which suggests a higher degree of organization. They should be further studied.

Physodes are bladder like structures described by Crato, '92, in certain brown Algæ. They contain a highly refractive substance which gives them a very different appearance from vacuoles whose structure they resemble in many respects. Very little is known about the contents of physodes and it may well be questioned whether they are really organs of the cell and not vacuoles set apart to hold some fluids or substances other than cell sap.

There are left for us a group of kinoplasmic structures that are especially prominent and sometimes only present during the events of nuclear division and at the times when cilia are formed. They will be discussed in later sections of these papers (Sections II, III, V and VI) and at this time we shall give but a brief statement of their appearances. They are centrospheres, centrosomes, asters, filarplasm and blepharoplasts.

Centrospheres are rather large areas of kinoplasm that sometimes lie at the poles of nuclear figures and to which are attached the fibrillæ that form the spindle and also those that may radiate into the surrounding cytoplasm. If the centrosphere contains a distinct central body, or if such a small structure be present alone at the poles of the spindle it is called a centrosome. Should either structure be accompanied by definite fibrillar radiations the whole is termed an aster. These latter conditions are sometimes very complex and are the most interesting types of structures. Asters with centrosomes are known for the brown algæ in the growing points of *Sphacelaria* (Fig. 3c), *Stypocaulon* (Swingle, '97) and the spore mother cell of *Dictyota* (Mottier, :00). They are also beautifully shown in certain diatoms (Lauterborn, principal paper '96, Karsten, :00). Asters with centrospheres and occasionally but not constantly containing centrosome-like bodies are found in the oogonium and germinating eggs of *Fucus*, see Fig. 3, *d* (Strasburger, '97<sup>a</sup>,

Farmer and Williams, '98). Especially well differentiated asters with centrospheres are present during the mitoses in the ascus, functioning at the end in the peculiar process of free cell formation (Harper, '97). Large centrospheres accompanied by radiations are present during the germination of the spores in certain Hepaticæ (Farmer and Reeves, '94, Davis, :01, Chamberlain, :03), but are less conspicuously shown in some and are entirely absent in other phases of the life history. Remarkably large centrospheres with inconspicuous radiations are known in the tetraspore mother cell of *Corallina* (Davis, '98). Centrospheres occur in the basidium (Wager, '94, Maire, :02). Centrosomes have been reported during the mitoses in the sporangium of *Hydrodictyon* (Timberlake, :02). Centrosomes have also been described in other types of the thallophytes but we are justified in asking for further work on these bodies since they are generally without radiations and may not have at all the significance indicated. Neither asters, centrospheres or centrosomes seem to be normally present in groups above the bryophytes, nuclear division taking place in these plants by methods, not found in other organisms, which will be described in succeeding sections.

Vegetative and embryonic tissues of plants above the thallophytes present very different conditions from those described in the foregoing paragraph. The centrosphere is replaced by a less definite structure in the form of a kinoplasmic cap which appears at the ends of the dividing nucleus and determines the poles of the spindle (see Fig. 3, *f*). They have been described in the cells of vegetative points of several pteridophytes and spermatophytes by Rosen, '93, Hof, '98, and Nemec, '99 and :01, and in the seta and late divisions in the germinating spore of the liverwort *Pellia* (Davis, :01).

The most highly developed conditions of spindle formation are found in the spore mother cells of the bryophytes, pteridophytes and spermatophytes. Here the nucleus becomes surrounded by a weft of fibrillæ which form a kinoplasmic envelope probably derived in part from the nuclear membrane. The fibrillæ are at first quite independent of one another or of common centers. Most of the fibrillæ enter into the spindle

which may in the beginning have several poles (see Fig. 3, *g*), but these generally swing at last into a common axis so that the spindle finally becomes essentially bipolar. The term filarplasm is applied to this free fibrillar condition of kinoplasm without organized centers. Filarplasm is peculiar to plant cells and its remarkable activities in connection with multipolar spindles have only been found in groups above the thallophytes. Centrospheres, centrosomes and asters among the lower plants resemble in general the same structures in the animal cell. But filarplasm presents a higher form of kinoplasmic structure with perhaps the most complex activities known in the process of spindle formation. We shall consider them especially in Section III when treating the spore mother cell.

The blepharoplasts are in some respects the most complex structures derived from kinoplasm. They are most conspicuous in the sperm cells of higher plants (spermatophytes and pteridophytes) but they are undoubtedly present in lower forms and probably in zoospores. The blepharoplast develops cilia as delicate fibrillæ from its surface. The origin and homologies of the blepharoplast are uncertain. In some forms they resemble centrosomes at the poles of the last nuclear figures in sperm tissue. But in other cases they are entirely independent of such spindles, a character which cannot be brought into harmony with the activities of centrosomes. They finally lie one at the side of each sperm nucleus, see Fig. 3, *h*, and with the development of the sperm they follow the spiral twist, when present, as a parallel band (Fig. 3, *h*, 2 and 3). This structure will receive detailed treatment in our account of the sperm (Section III).

## 2. Non Protoplasmic Contents.

It is not possible to distinguish with certainty all the non-living material of a cell from its protoplasm. We have at one extreme cells from which the protoplasm has almost or wholly disappeared and which are either entirely empty or set apart solely as receptacles for various substances, sometimes waste products and sometimes food materials. In contrast with this

condition are the cells filled with cytoplasm so homogeneous in structure that only the most delicate granules (microsomata) can be distinguished in the clear substance.

Waste products such as mineral matter, resins, certain oils, solutions of tannin and various poisons, such as the alkaloids, may be easily recognized. Most food substances such as starch, proteid grains (aleurone), albumin crystals, oils, fats, etc., are readily separated from the protoplasm in which they lie. But the difficulties are much greater with the smaller particles of proteid material, which are frequently such minute granules as to approach the microsomata in size. These may give to the protoplasm a granular consistency that breaks up the foam or spongy structure characteristic of the pure condition. These granules are undoubtedly in most cases substances intimately concerned with the metabolism of the cell and are members of the chains of constructive and destructive processes that characterize life phenomena.

The other non protoplasmic structures of cells are vacuoles which are essentially bubbles of fluid lying in the denser protoplasmic medium and surrounded by plasma membranes. The watery fluid of vacuoles contains various substances in solution, carbohydrates such as the sugars glucoses and inulin, mineral salts, asparagin, tannin, alkaloids, etc., and occasionally oil and not infrequently crystals. Vacuoles may be formed in large numbers in protoplasm. They tend to run together as do bubbles in a froth and in this way the large central vacuole becomes established in the cell, gathering to itself many smaller vacuoles until the protoplasm is forced to lie as a relatively thin layer next the cell wall. The fluid in the central vacuole (cell sap) is generally thinner and more watery than that in the smaller vacuoles. The latter are apt to be more rich in albumen which may be transformed into proteid grains as is especially well illustrated in the secretion of aleurone. Cell sap may be colored by pigments in solution and the tints of flowers are largely due to this cause alone or to the effects of its color in combination with various plastids in the cell.

It is possible that physodes, described among the cytoplasmic structures, are in reality vacuoles filled with substances other than cell sap, which are not as yet understood.

### 3. The Cell Wall.

Many of the chief peculiarities of plant organization and activities are due to the presence of the cell wall, its influence on structure and mode of life. The cell wall is not an excretion from the cell like a mineral shell but is formed by the direct change of portions of the protoplasm. The regions concerned may be the outer plasma membrane, the vacuolar plasma membrane or the substance that makes up the spindle fibers which form the cell plate. These structures are all kinoplasmic in character and have to do with the formation of cell walls in various ways which will be described in Section II under the topic "The Segmentation of the protoplasm." The transformation of finely granular films of kinoplasm into cellulose is not well understood but there is an evident solution of the granules (microsomata) and the change of the resultant substance into the cell wall. As a chemical process this change means the replacement of molecules of an albuminous nature by those of a carbohydrate substance. The most complete account of the cell wall is that of Strasburger, '98.

Cell walls are chiefly composed of cellulose, but other substances are always present, modifying the structure in various ways to give widely different properties. These modifications are generally due to infiltrations of foreign substances but sometimes cell walls become incrustated with mineral deposits. The group of cellulose compounds is very large and it is extremely difficult to identify the various substances in structures so small as the cell walls. For a detailed treatment of the chemistry of the cellulose group the reader is referred to Cross and Bevan, '95, and for a general account to Pfeffer, :00, p. 480-485. There are microchemical tests for cellulose that give good reactions for most tissues but which cannot be relied upon for some walls (as in fungi and many algæ) yet it is well understood that the cell walls of these organisms are from the biological point of view essentially the same as for other plants. The cell walls of some fungi are very largely composed of chitin.

Several substances known to be present in cell walls give them marked characteristics. Their association with the cellu-

lose is so intimate as to resist very severe treatment and therefore these cell walls are essentially cellulose groups modified chiefly in their physical properties by the presence of foreign substances. The most conspicuous modifications of this character are lignification, suberization and cutinization. Lignified walls are permeable to water and gases. Several substances have been separated from the cellulose of lignified walls, among them lignone, coniferin, vanillin, etc. Suberized and cutinized walls are largely but probably never wholly impervious to water and gases; the one is infiltrated with suberin and the other with cutin, substances that resemble one other very closely. Even walls that appear to be pure cellulose have other substance united with them, the most important being pectose and callose. Cell walls frequently become gelatinous or mucilaginous, when the outer layers swell and lose their form or they may be transformed into gums. These changes are well illustrated in the coats of seeds and fruits and among the algæ and fungi. The cells of algæ frequently secrete gelatinous envelopes or sheaths of substances so closely related to cellulose that were they condensed they would form a firm cell wall.

The cell wall may grow in two directions by methods quite different from one another. There is first surface growth which results in a stretching of the cellulose membrane (growth by intussusception). And second there may be growth in thickness by the formation of successive layers of cellulose inside of one another, giving the wall a striated structure (growth by apposition). The second type of growth is chiefly interesting since it makes possible many peculiarities of structure, because the newly formed layers may not be deposited uniformly inside the primary wall. In some cells the secondary thickenings have the form of rings or spirals or a reticulate structure. The reticulate condition passes insensibly into the pitted cell in which the secondary layers cover the greater part of the surface leaving the primary wall only exposed at the pits. Further discussion of these cells falls more within the range of histology than the purposes of this paper.

The cell wall offers a very interesting field of research among the thallophytes and especially in the lower groups where we

may expect to find these envelopes in a fairly primitive condition and may be able to establish the steps in the origin and differentiation of this very important accessory structure to the plant cell.

(To be continued.)

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## STUDIES ON THE PLANT CELL.— II.

BRADLEY MOORE DAVIS.

### *Section II* THE ACTIVITIES OF THE PLANT CELL.

#### I. Vegetative Activities.

EVERY cell passes through a history whose events repeat in a broad way activities that have become established in the organism by the experience of its ancestors. The most important of these events is nuclear division, which is accompanied in most plants by cell division, the important exceptions being certain groups whose protoplasm is multinucleate throughout all, or almost all, vegetative conditions (*e. g.*, cœnocytic Algæ and Fungi, plasmodia and multinucleate cells in various tissues).

Protoplasm, whose nuclei can no longer divide, becomes incapable of reproducing itself and must take a dependent position in the organism, where the length of its life will be determined by the good fortune of its environment and its vitality. Such protoplasm becomes strictly vegetative in its functions, and while these activities may be very highly specialized and of the utmost importance to the organism as a whole, nevertheless such a cell has lost certain of the constructive, and in consequence reproductive, possibilities characteristic of living matter. The most evident and important of these constructive activities have to do with the increase of nuclear material (chiefly chromatin), which leads to its distribution through nuclear division, and the development of a complicated mechanism (the spindle) to effect this result.

As Weismann first pointed out, from the standpoint of cell studies, there is a stream of germ plasm flowing with every species, protoplasm relatively fixed in its characteristics and potentially immortal. The chief peculiarities of germ plasm are its reproductive powers and the generalized structure that enables it

to turn to any form of activity possible to the species. Portions of the germ plasm are constantly being detached from the main stream and relegated to more or less special duties. Such protoplasm becomes the body plasm, or soma, of the individual. Specialized body plasm generally loses very shortly the reproductive possibilities of germ plasm, and in consequence must finally die, for its nicely adjusted dependence upon surrounding cells cannot last forever. Yet it has been one of the surprises of biological science that specialized tissues may keep for a very long time the reproductive qualities of germ plasm. Investigations on regeneration in particular have brought these facts conspicuously to the front. As an extreme example among plants, it is known that even the epidermal tissues of leaves and scales of certain ferns (Palisa, :00) may sometimes retain the fundamental qualities of germ plasm and reproduce the plant.

There are no visible characters that separate body plasm from germ plasm, excepting, of course, when body plasm begins to show signs of degeneration. Germ plasm may only be distinguished by its potentialities of growth and reproduction, potentialities that cannot be accurately determined because the stimulus to development is, in the last analysis, an external one and the conditions which govern it may be so intricate as to escape close scrutiny.

Germ plasm is found in its most generalized condition in the cells of growing points, in embryonic and meristematic regions, and in the reproductive tissues. These tissues are well recognized as the most favorable for cell studies because they present most clearly the details of protoplasmic activities. Almost all that we know of cell activities have come from investigations of such regions.

One of the first signs of that specialization which transforms germ plasm to body plasm is the slowing up and final end of nuclear and cell division. With this change come a great variety of modifications (peculiarities of cell wall, plastids, cytoplasmic activities, etc.) which may be readily associated with the particular work of that tissue.

The vegetative activities of germ plasm are chiefly those of growth, which in the end mean reproduction, the embryonic cells

drawing upon food that has been prepared for them and is either stored in special structures (as seeds, spores, bulbs, etc.), or manufactured in differentiated organs or tissues (leaves, chlorophyll bearing tissue, phloem, etc.). The vegetative activities of body plasm are far more specific than those of germ plasm. Their tissues have particular and highly developed activities, some dealing chiefly with photosynthetic processes, some (phloem) distributing the organized food over the plant body, some storing the food in large quantities. Besides these there are mechanical functions performed by highly differentiated tissues, even though largely composed of empty cells, as the vascular tissue, supporting tissues, and the external protective integuments.

It is not our purpose to discuss any of these vegetative activities in detail, but only to distinguish as sharply as possible the characteristics of germ plasm with its generalized activities from the specialized body plasm. These generalized characters, as before stated, are constructive activities which mean growth and lead to nuclear and cell division. It is probable that any tissue which presents them has regenerative powers that under the proper environment might be expected to reproduce parts or the entire organism. Germ plasm is distributed more widely throughout the organism than is generally supposed, and many highly specialized tissues still retain the spark of regenerative possibilities. The significance of these conditions is not generally appreciated, perhaps because the environmental conditions of regeneration are little understood and are exceedingly hard to adjust experimentally. There is presented here a very attractive field of botanical investigation, a union of cell studies with the more gross anatomical methods of experimental morphology.

## 2. Cell Division.

Cell division takes place only after periods of growth that have led to a multiplication of nuclei and in the tissues of plants above the thallophytes is very generally a part of the history of each mitosis. This is because of the structure called the cell plate which is essentially an organ of cell division. But the thallophytes present other methods of cell division which bear no especial relation to nuclear activities, and in certain groups of

the thallophytes nuclear division may proceed through the entire vegetative life of the organism without any segmentation of the protoplasm which only takes place during the reproductive phase of spore formation. But fundamentally protoplasmic segmentation depends on increase in the amount of protoplasm which demands the multiplication of nuclei so that nuclear division always precedes cell division, and we shall consider the events in that order.

#### (a) Events of Nuclear Division.

##### 1. Direct Division.

The nucleus divides after one or two methods, either directly by constriction or fragmentation, or indirectly (mitosis) when there is present a fibrillar apparatus called the spindle. Direct division is the only form present in the simplest plants and phylogenetically must have preceded the elaborate mechanism demanded for indirect division. This topic will be given especial attention in Section VI. Direct division is also present in certain specialized cells and tissues of higher plants. These are generally old cells or tissues that are far removed from the generalized structure and potentialities of germ plasm. Yet sometimes direct and indirect division occur in the same cell, *e. g.*, *Valonia* (Fairchild, '94), and such forms might be made the subject of very interesting investigations. In some cases the phenomenon of direct nuclear division accompanies pathological conditions or the degeneration of cells and may take the form of extensive fragmentation. It would be outside of our purpose to discuss such phenomena which is obviously abnormal, and the primitive forms of nuclear division will be taken up later (Section VI). It is possible that direct division in higher plants is in a sense a reversion to early ancestral conditions, a reversion that only comes on when for some reason the normal activities of the germ cell are in abeyance or have ceased.

##### 2. Indirect Division (Mitosis).

Indirect nuclear division, mitosis or karyokinesis, is characterized by a mechanism which varies greatly among plants in its

method of development. The characteristic appearance of this apparatus is a spindle like figure formed of fibrillæ. The poles of the spindle may be occupied by centrosomes or centrospheres or they may be entirely free from such organized kinoplasmic bodies. The essential structures of the spindle are sets of contracting fibers which separate the chromosomes into two groups drawing them to the poles of the spindle where the daughter nuclei are organized. But besides these fibers there are generally present other fibrillæ which complicate the nuclear figure. Some of these extend from pole to pole (spindle fibers) others lie outside of the spindle and end freely in the cytoplasm or attach themselves to chromosomes (mantle fibers), and if centrosomes or centrospheres be present there are likely to be fibers radiating from these centers to form asters.

The events of mitosis are generally grouped into four periods : (a) Prophase, to include the formation of the spindle and preparation of the chromosomes ; (b) Metaphase, the separation of the daughter chromosomes ; (c) Anaphase, the gathering of the daughter chromosomes into two groups which pass to the poles of the spindle ; (d) Telophase, the organization of the daughter nuclei. It is almost needless to say that these periods merge so gradually one into the other that sharp lines cannot be drawn between them. The activities during prophase are especially variable.

*Prophase.* — There are two types of spindles in plants, (1) those that are formed within the nuclear membrane and (2) those whose fibers originate largely or wholly from kinoplasm outside of the nucleus. Intranuclear spindles have been reported in a number of groups of the thallophytes. They seem to be the rule in the mitoses of oögenesis in the Peronosporales. (Wager, '96, :00, Stevens, '99, :01 and :02, Davis, :00, Miyake, :01, Trow, :01, Rosenberg, :03). They are present in Saprolegnia, Fig. 5a (Davis, :03). Fairchild ('94) reports them for Valonia. Farmer and Williams ('98, p. 625) state that the spindle of *Ascophyllum* is largely intranuclear. Harper (:00) has not described them for the Myxomycetes, but very little is known about the prophases of mitosis in that group and their presence is quite probable. Timberlake (:02) is not positive whether the

spindles of *Hydrodictyon* are intranuclear or not; they lie in a clear space which, however, may be a vacuole rather than the outline of a nuclear cavity. It seems probable in such a type that the vacuole is really the nuclear cavity whose plasma membrane (nuclear membrane) becomes less clearly defined. The development of the spindle is very difficult to follow among these lower forms because it is so small. Stevens (:03) found an exceptionally favorable type in *Synchytium* and came to the conclusion that the spindle developed from the threads of the spirem (linin) entirely within and independent of the nuclear membrane.

Very remarkable intranuclear spindles have been described in the central cell of the pollen tube of *Cycas* (Ikeno, '98 b) and *Zamia*, Fig. 5d (Webber, :01). Murrill (:00) found them in the mitosis following the fusion of gamete-nuclei in the egg of *Tsuga*, Ferguson (:01b) at the same period for pine, and Coker (:03) in *Taxodium*. They are also reported by Strasburger (:00) in the cells of young anthers and nucelli of the lily and in growing points (*Viscum*) and possibly may be found quite generally in cells weak in kinoplasmic cytoplasm. The development of the spindles in the above forms has not been studied in detail, but the fibers are probably derived from the linin. We are given a clue to the process by the events of spindle formation in the spore mother cell of *Passiflora* (Williams, '99). In this angiosperm the nuclear cavity becomes filled with a fibrillar network developed from the linin, the nuclear wall becomes transformed into a mesh connecting the intranuclear fibers with a surrounding cytoplasmic reticulum. The fibers in the central region of this net work develop the spindle which is consequently very largely of intranuclear origin.

Among the thallophytes the poles of intranuclear spindles are frequently occupied by deeply staining bodies which have been called centrosomes; but these structures can hardly be homologous with the well-known centrosomes of other thallophytes, *e. g.*, *Stypocaulon* (Swingle, '97) and *Dictyota* (Mottier, :00). They are probably merely temporary accumulations of material with no morphological significance.

Spindles that arise from fibers external to the nucleus (extra

nuclear spindles) are of two main types : (1) those associated with centrosomes, centrospheres or kinoplasmic caps, and (2) those composed of independent fibrillæ developed as a mesh around the nucleus. The latter condition is especially characteristic of the spore mother cell and is perhaps the highest type of spindle formation known for either animals or plants. It is very interesting to trace the relations of this highest condition to the lower types through certain lines of evolution to be discussed in Section VI.

Spindles with centrosomes are known in *Sphacelaria*, *Stypocaulon* (Swingle, '97), *Dictyota*, Fig. 4 *a* (Mottier, :00), the zoö-sporangium of *Hydrodictyon* (Timberlake, :02), in certain diatoms (Lauterborn, principal paper '96, Karsten, :00) and in the basidium (Wager, '94 and Maire, :02). The best accounts of the behavior of the centrosomes are given by Swingle and Mottier. Indeed there is much doubt about the history and significance of the bodies in the other forms, although the constancy of their presence at the poles of the spindles indicates that they are really centrosomes. The conditions in the diatoms are especially complicated; an account of Lauterborn's work has been published in English by Rowley, :03. In *Stypocaulon*, *Sphacelaria* (Fig. 3 *c*, Section I) and *Dictyota* (Fig. 4 *a*) the cells studied have permanent asters which lie at the side of the nucleus and which divide just previous to the mitosis and separate so that they come to lie on opposite sides of the nucleus. Fibers develop from the centrosomes on the sides nearest the nucleus and elongating push against the nuclear membrane and finally enter the nuclear cavity to form the spindle.

Spindles with centrospheres are well known in *Fucus* (Farmer and Williams, '96, '98, Strasburger, '97a), *Corallina*, Fig. 5 *c*, (Davis, '98), in the ascus, Fig. 5 *b* (Harper, '97 and '99), and in the germinating spore of *Pellia*, Fig. 4 *c* (Farmer and Reeves, '94, Davis, :01, Chamberlain, :03). Centrospheres have been reported in other forms but the types mentioned above have received the most careful study. It is probable that the centrosphere is but a larger, more generalized kinoplasmic center than the centrosome, a protoplasmic region whose dynamic activities do not focus so sharply as in the latter structure. There are

bodies, as in the basidium, which stand intermediate in size between centrosomes and centrospheres and are probably only called the former because they are very distinct in outline.

Centrospheres in *Fucus* (Fig. 3 *d*, Section I), *Corallina* (Fig. 4 *b*) and *Pellia* (Fig. 3 *c*, Section I, Fig. 4 *c*) are formed *de novo* for each mitosis by an accumulation of kinoplasm at the poles of the elongating nucleus. The centrospheres in the ascus divide before each of the three successive mitoses and finally remain, one for each nucleus, to instigate the peculiar process of free cell formation characteristic of the ascus. Centrospheres are frequently the centers of asters which, however, are usually not as sharply defined as those with centrosomes, possibly because the fibers are not grouped with the same degree of symmetry as is shown around centrosomes.

Spindle fibers from centrospheres develop in precisely the same manner as from centrosomes, *i. e.* by the growth of the fibrillæ into the nuclear cavity through the dissolving nuclear membrane. The activity is well shown in the oögonium of *Fucus*, and Farmer ('98, p. 638) believes "that the intranuclear part of the spindle is differentiated out of nuclear material that is unused for chromosome formation." The entrance of spindle fibers from centrospheres at the ends of a nucleus has been observed by myself in *Corallina*, Fig. 4 *b* (Davis, '98). The germinating spores of *Pellia*, Fig. 4 *c* (Davis, :01, Chamberlain, :03) furnish especially good illustrations of the entrance of spindle fibers into the nuclear cavity and the development of the spindle in this form is coincident with the dissolution of the nucleus which, according to Strasburger's theory ('95), indicates that the latter structure contributes material for the growth of spindle fibers.

In connection with the centrosphere mention should be made of the blepharoplasts of the cycads and Ginko which are remarkable bodies with radiating fibers. They have been considered by some as asters with centrosomes, but it is known that they take no part in spindle formation or other mitotic phenomena in these forms, and consequently need not be considered at this time. They will be treated in some detail in the account of the sperm (Section III).

Kinoplasmic caps which form spindles are probably an evolution from the type of centrosphere that is developed *de novo* with each mitosis as in *Pellia*. Such centrospheres by becoming less definite in form and lacking radiating fibers would be called kinoplasmic caps. Indeed the centrosphere so evident in the early cell divisions of the germinating spore of *Pellia* becomes a kinoplasmic cap in the later mitoses of the older gametophyte (Davis, :01).

Spindles developed from kinoplasmic caps are characteristic of

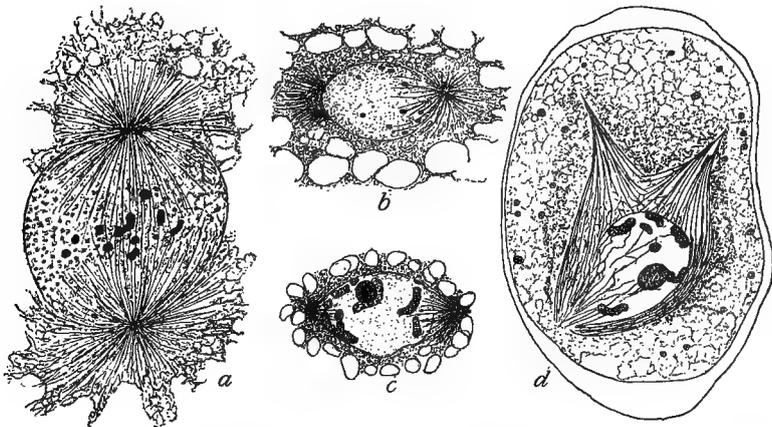


FIG. 4.—Prophases of Mitosis. *a.* *Dictyota*; late prophase in spore mother cell, fibers from the two asters with centrosomes have entered nuclear cavity to organize the spindle, chromosomes gathering to form the nuclear plate. *b.* *Corallina*, early prophase in tetra spore mother cell; two centrospheres, the fibers from one having entered the nuclear cavity, chromosomes shown. *c.* *Pellia*, nucleus in germinating spore; spindle fibers from ill defined centrospheres entering nuclear cavity, chromosomes and a nucleolus present. *d.* *Gladiolus*, first mitosis in pollen mother cell; a multipolar spindle, nuclear wall breaking down at one side and fibrillae entering the nuclear cavity, chromosomes and a nucleolus present. After Mottier and Lawson.

the mitoses in vegetative tissues, meristematic and other embryonic regions. They have been especially studied in higher plants by several investigators and for a large number of forms, those most completely described being *Psilotum* (Rosen, '95), *Equisetum*, *Allium* and *Solanum* (Nemec, '98a and '98b, '99b and '99c), *Pteris*, *Ephedra* and *Vicia*, (Fig. 3 *f*, Section I) (Hof, '98) and *Allium* (McComb, :00). The polar caps first appear as accumulations of kinoplasm on opposite sides of the nucleus which generally elongates. The protoplasm is granular and although

central bodies have been reported most investigators are agreed that they are only granules without regularity or special significance. They are no longer believed to be centrosomes. Fibrillæ are developed from the kinoplasmic caps and grow out against the nuclear membrane and finally enter the nuclear cavity to form the spindle. A large part of the substance of the kinoplasmic cap is transformed into these spindle fibers.

Papers by Schaffner ('98) on *Allium* and Fulmer ('98) on the seedling of the pine are the last attempts to bring the centrosome into the history of spindle formation in vegetative tissues of higher plants. But their results cannot stand against the accumulation of studies which indicate that centrosomes are not present in the cells of any plant above the thallophytes with the possible exception of the mysterious blepharoplast and certain structures appearing in some phases in the life history of Hepaticæ. Centrospheres are unquestionably present in the Hepaticæ and centrosomes have also been reported. The centrospheres are, however, so generalized as to approach the kinoplasmic caps in structure and development and it seems quite possible that they are the forerunners of this manifestation of kinoplasm. The so-called centrosomes of the liverworts do not exhibit the specialized structure or behavior of centrosomes among the thallophytes and it is probable that they are only smaller and somewhat more clearly defined centrospheres. These structures in the Hepaticæ seem to hold an intermediate relation between the definite kinoplasmic bodies (asters, centrosomes and centrospheres) of the thallophytes and the remarkable kinoplasmic activities in higher plants which reach their highest expression in the processes of spindle formation in the spore mother cell. These topics will be treated in Section VI.

Structures resembling kinoplasmic caps have been reported in several other tissues than those noted above. Thus Murrill (:00) finds in the formation of the ventral canal cell of *Tsuga* a dense fibrous accumulation beneath the nucleus which develops one pole of the spindle in essentially the same manner as other polar caps. The other pole of the spindle in this case appears to be formed differently for the fibers seem to be intranuclear.

It would be interesting if two types of spindle formation were present at opposite poles of the same nucleus and further investigation of this subject is much to be desired. The mitoses in the central cell of *Pinus* (Ferguson, :01b, Chamberlain, '99, and Blackman, '98) and *Picea* (Miyake :03a) show spindle formation from accumulations of fibrillæ outside of the nucleus but without conspicuous polar caps. Still more striking than the irregular spindle of Murrill in *Tsuga*, described above, is Miss Ferguson's (:01a) account of the mitosis in the generative cell of the pollen grain of *Pinus*. The spindle here begins to develop as a cap-like accumulation of kinoplasm below the nucleus. The fibers enter the nuclear cavity and in coöperation with a nuclear reticulum form a system of fibers that extend through the nuclear cavity to the inner side of the nuclear membrane beyond. This portion of the nuclear membrane persists until after metaphase so that one pole of the spindle is found wholly within the nucleus while the other is external and of unquestioned cytoplasmic origin. Coker, :03, regards the spindle which differentiates the nucleus of the ventral canal cell in *Taxodium* as almost wholly of nuclear origin and the chromosomes as derived largely from the nucleolus. There are evidently some interesting complications in this form which deserve further study.

It should be noted that whenever spindles are formed in connection with centrosomes, centrospheres or kinoplasmic caps that the fibers have a definite region of attachment from which they extend into the nuclear cavity. Such regions constitute a sort of anchorage for the spindle fibers. In this respect the physiological side of the process of spindle formation in these forms is quite similar to that of the animal kingdom and in sharp contrast to other methods that are found in higher plants, which will now be considered.

When spindles are formed after the second method, *i. e.*, by independent fibrillæ making up a network around the nucleus, there is an abrupt change in the method of development. The kinoplasm becomes distributed around the nucleus as an investing layer and shows no inclination to gather into centers such as kinoplasmic caps or centrospheres. There is developed

from this granular kinoplasm a meshwork of fibrillæ that extends into the cytoplasm more or less radially. When the nuclear membrane becomes disorganized the fibers enter the nuclear cavity and organize the spindle (see Fig. 4 *d*). In some forms, *e. g.*, *Passiflora* (Williams, :00), many or most of the fibers are developed in the interior of the nucleus from the linin and become connected with the extra nuclear reticulum by the dissolution of the nuclear membrane.

The free ends of the fibrillæ that lie in the cytoplasm become gathered into several poles which are distributed variously around the nucleus. This condition constitutes the so-called multipolar spindle (Fig. 3 *g*, Fig. 4 *d*), which in its highest type of development illustrates the most complex method of spindle formation known for animals or plants. During the later periods of prophase the several poles of the multipolar spindle converge and fuse with one another into two poles with a common axis, thus forming the mature bipolar spindle of metaphase (Fig. 5 *f*). The spindle is in a broad sense bipolar, but one may readily see that each pole is made up of several groups of fibrillæ which generally remain quite independent of one another (Fig. 5 *f* 1).

The relation of a multipolar stage to the bipolar spindle of metaphase was first made clear by Belajeff ('94b) for *Larix*, and later was established more widely by the investigations of Osterhout ('97) on *Equisetum*, Mottier ('97) for the lily, and Juel ('97) for *Hemerocallis*. This type of spindle formation is now well known in the spore mother cells of numerous spermatophytes and several pteridophytes. The same conditions in simpler form are found in the spore mother cells of the Hepaticæ, *e. g.*, *Anthoceros* (Davis, '99), *Pellia*, Fig. 5 *e* (Davis, :01), and *Pallavicinia* (Moore, :03). There are a number of very interesting peculiarities in this type of spindle which presents a wide range of variation in the details of its fibrillar organization and development. These will receive special treatment in the account of the spore mother cell (Section III).

The only types of thallophytes known, in which the spindle is partly or wholly of cytoplasmic origin without centrosomes, centrospheres, or kinoplasmic caps, are *Chara* (Debski, '97) and *Spirogyra* (Van Wisselingh, :02). The developmental history is

very difficult to follow in these forms and is not fully known, but multipolar conditions are reported which later change into bipolar spindles.

While the spindle is being organized by kinoplasmic activities outside of the nucleus, some events occur within which form a very important part of the prophases of mitosis. The linin material, which in the resting nucleus generally has the form of a net, becomes organized into a much looped ribbon, called the spirem thread. The chromatin material gathers along the spirem thread as deeply staining globular bodies. These split into halves in the direction parallel with the axis of the spirem thread, and the two sets of chromatic bodies lie in two rows along the edge of the ribbon, which shortens as it grows older. Finally the spirem thread divides transversely into a definite number of segments, and these are the chromosomes. The chromosomes are generally fully formed at the time when the spindle fibers enter the nuclear cavity, and they are readily moved as the fibrillæ develop the spindle. Some of the fibers become attached to the chromosomes, carrying them to the equatorial region of the spindle to form the structure called the nuclear plate, which always indicates the approach of metaphase.

As the spirem thread matures the amount of chromatin is greatly increased, so that the separate globules run together and cannot be distinguished in the chromosome which is homogeneous in structure. Chromatin has its greatest staining power at this period. Whether linin is closely related to chromatin in composition and is actually changed into that substance, or whether it dissolves and contributes its material to the growth of the spindle, is a problem of some importance as yet unsolved. It is possible that the nucleolus may furnish material for the chromosomes, and some nucleolar like bodies are known to be chromatic in character, but it does not seem to be established that any of these are genetically related to an unquestioned nucleolus in any plant form.

Important changes come over the nucleolus coincident with the development of the chromosomes. The structure frequently gives signs of internal modifications early in prophase and before the development of the spindle. It may gradually fade away or

decrease in size, or, if large, it may fragment. Strasburger, in 1895, advanced the view that the spindle actually drew upon the substance of the nucleolus for the material and energy necessary to its development. The evidence in support of this suggestive theory lies chiefly in the development of the spindle coincident with the dissolution of the nucleolus. There is also some evidence that the nucleolus contributes material to the developing chromosomes. Small globules, which stain as the substance of the nucleolus, may sometimes be found adhering to the chromosomes as though becoming incorporated in them. These subjects are naturally very difficult of investigation because stain reactions cannot be depended upon with certainty and are not, of course, chemical tests. Then the behavior of the nucleolus during mitosis is exceedingly variable, since it sometimes disappears quickly and sometimes remains intact, and it becomes a very difficult matter to determine its importance. The nucleolus is probably not absolutely necessary at any stage in mitosis, for both spindle fibers and chromosomes develop apart from this structure; but it does seem to be established that the substance of the nucleolus is generally drawn upon by the cell, especially during prophase, when numerous spindle fibers are organized and the amount of chromatin is being largely increased. Experiments of Hottes, which unfortunately have never been published, have an important bearing on these problems.

If the nucleoli are not entirely dissolved they are frequently thrown out of the spindle into the cytoplasm, where they may lie for long periods as deeply staining globules which are sometimes called extra nuclear nucleoli. It is probable that very many of the bodies that pass under this cumbersome title have no relation whatever to the nucleolus. The cytoplasm frequently contains globules that may be coagulated or precipitated food products, and all of these stain similarly to nucleoli.

*Metaphase.*—The period of mitosis termed metaphase is, to speak precisely, the time when the two halves of the split chromosome separate from one another. However, this is a period of such short duration that for practical purposes nuclei are considered in metaphase when their chromosomes are lined up at the nuclear plate. The metaphase of mitosis is generally the most

conspicuous of the nuclear activities not only on account of the position of the chromosomes (see Fig. 5), but because all kinoplasmic structures (the fibrillæ and centrosomes or centrospheres, if present) are shown to their best advantage.

The best evidence indicates that the chromosomes of plants

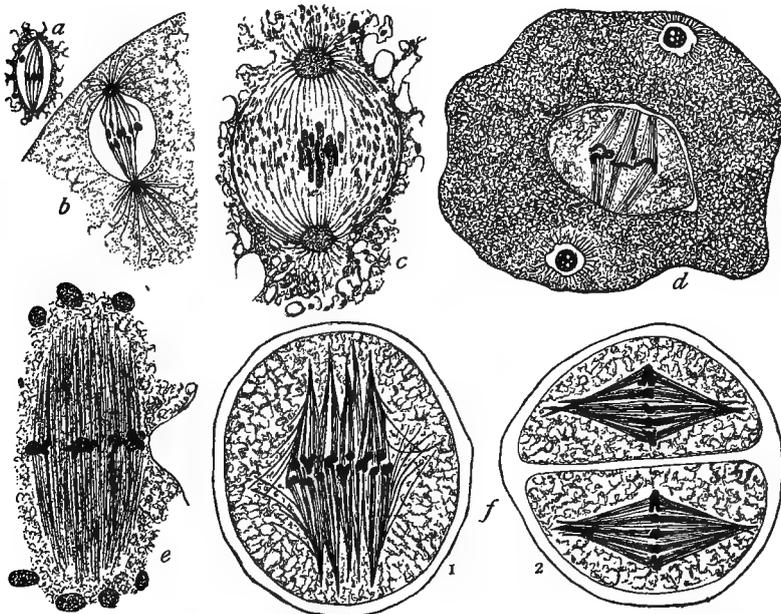


FIG. 5.—Metaphases of Mitosis. *a*, *Saprolegnia*; intranuclear spindle in oögonium, nucleolus outside of spindle. *b*, *Erysiphe*; mitosis in ascus, asters with rather small centrospheres. *c*, *Corallina*; first mitosis in tetraspore mother-cell, very large and well differentiated centrospheres. *d*, *Zamia*; blunt poled intranuclear spindle in central cell of pollen grain; blepharoplasts, their outer membrane about to break up. *e*, *Pellia*; first mitosis in spore mother-cell; broad spindle with rounded poles, the very numerous spindle fibers ending in granular kinoplasm. *f*, *Agave*; first and second mitoses in pollen mother-cells; (1) multipolar spindle just previous to metaphase; the several independent cones of fibrillæ gather more closely together to complete the spindle. (2) metaphase of second mitoses; completed spindles showing however the several independent cones of fibrillæ. (After Harper, Webber and Osterhout.)

only divide longitudinally. This matter has considerable theoretical interest, which will be considered in Section V, and also in connection with the spore mother cell (Section III). The daughter chromosomes are drawn apart by the contraction of the fibrillæ to which they are attached.

Chromosomes may take on various forms during metaphase,

especially while they are being separated. Thus, if the chromosomes are dragged apart from the ends, their form is generally rod shaped; but if the attachment of the fibrillæ is near the middle of the chromosome, the structures are pulled apart as loops or V's, and the pair of chromosomes just previous to their separation may be ring shaped. A further complication is introduced in the spore mother cell by certain premature divisions by which each daughter chromosome becomes a pair of granddaughter chromosomes instead of remaining a single structure. The peculiarities of the heterotypic and homotypic mitoses are due

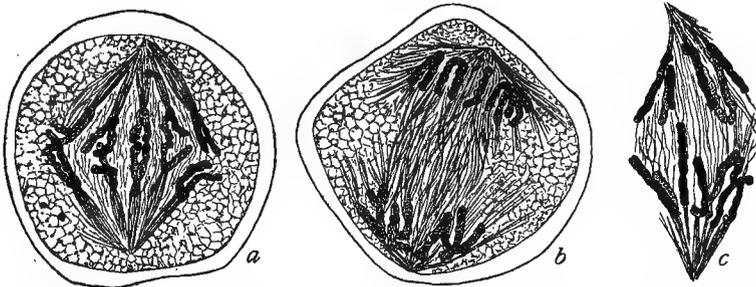


FIG. 6.—Anaphase of Mitosis, spore mother-cell of *Lilium martagon*. *a*, immediately after metaphase of first mitosis; each daughter chromosome consists of two grand daughter segments, adhering at the ends, making the familiar V-shaped figures characteristic of the first mitosis (heterotypic) in the spore mother-cell of higher plants. *b*, late anaphase of the first mitosis; the V shaped chromosomes, each composed of two grand daughter segments adhering at the ends, are very close to the poles of the spindle; the central fibers of the spindle are conspicuous at this stage. *c*, second mitosis; the grand daughter chromosomes, that composed the Vs of the first mitosis, have separated at the nuclear plate of the second mitosis and are being drawn by their ends to the poles of the spindle where they will organize the nuclei of the pollen grain; this mitosis is called homotypic to distinguish it from the usual (typical mitoses in which there are no premature divisions of the chromosomes. All figures after Mottier.

to this phenomenon. (See account of spore mother cell in Section III.

*Anaphase.*—Anaphase begins with the separation of the daughter chromosomes at the nuclear plate (Fig. 6) and ends with the gathering of these structures at the poles of the spindle preparatory to the organization of the daughter nuclei. As the chromosomes move towards the poles the fibers of the central spindle stand out sharply (see Fig. 6 *b*). If a cell wall is to be formed between the daughter nuclei one may expect to find these fibers thickening in the equatorial region of the

spindle where the nuclear plate formerly lay. Such thickenings are granular accumulations formed by the contraction of the central spindle fibers and mark the beginnings of the cell plate (Fig. 8 *d*) that afterwards gives rise to the cell wall. When the daughter chromosomes reach the poles of the spindle they generally lie in a region of granular kinoplasm which results in part from the contraction of spindle fibers and in some cases from the breaking down of organized centrospheres (*e. g.*, *Coralina*, *Pellia*, *Fucus*, etc.). The daughter nucleus at this time

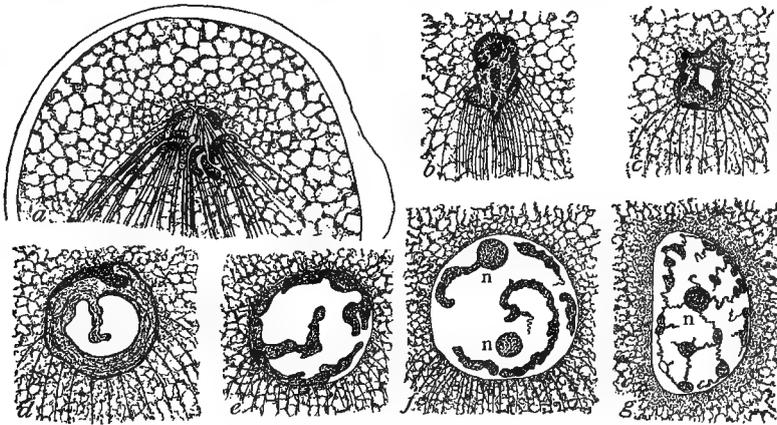


FIG. 7.—Telophase of Mitosis, spore mother-cell of *Passiflora carulea*. *a*, very late anaphase; the daughter chromosomes are collected at the poles of the spindle. *b*, the commencement of telophase; the chromosomes have fused together and the daughter nucleus is represented by an irregularly shaped mass of chromatin. *c*, the presence of small lacunæ within the mass of chromatin indicates the accumulation of nuclear sap in vacuoles. *d*, an increased amount of nuclear sap, still held however within the mass of chromatin, and consequent enlargement of the vacuole destined to become the nuclear cavity. *e*, the chromatin has begun to break up into small masses so that it no longer holds the nuclear sap which has established contact with the cytoplasm and is forming the nuclear plasma membrane. *f*, nuclear sap in contact on all sides with the cytoplasm and a complete nuclear membrane clearly established; chromatin is very much broken up and two nucleoli (*n*) have been formed. *g*, the resting nucleus with chromatin distributed in small masses connected by a network of linin threads; a nucleolus (*n*) is shown; the zone outside the nuclear membrane is kinoplasm and its appearance indicates the approach of the second mitosis in the pollen mother-cell. All figures after Lawson.

(Fig. 7 *a*, *b*) is in its simplest terms, as explained in Section I, a group of chromosomes surrounded by granular kinoplasm and without the nucleolus, linin network or the vacuole which later contains the nuclear sap.

*Telophase*.—Telophase is the closing period of mitosis and completes the organization of the daughter nuclei (see Fig. 7).

The chromosomes come to lie in a vacuole (Fig. 7 *c, d, e*) containing nuclear sap and later the chromatin becomes distributed over a linin network and one or more nucleoli develop (Fig. 7 *f, g*). As was stated in Section I, the nuclear membrane probably represents the reaction of the granular kinoplasm to a fluid secretion around the chromosomes which becomes the nuclear sap (Lawson, :03a). However, the nuclear membrane is generally a definitely organized film, much more sharply defined than vacuolar membranes. The development of the linin network is not well understood. It is readily seen that the chromosomes become joined end to end and sometimes elongate. The amount of chromatin diminishes as the linin substance appears, but it is not certain whether the chromatin is changed directly into linin, or whether the latter substance is a secretion. The best evidence rather favors the former view. Nucleoli are also believed to hold a very close chemical relation to chromatin.

It is uncertain whether or not the chromosomes lose their organic identity in the daughter nuclei. Investigations on this problem are surrounded by many difficulties. It has been claimed by Guignard ('99) for *Naias* and Strasburger (:00) for several forms that the chromosomes may be followed with certainty through the period between the first and second mitosis in the spore mother cell. But other investigators have not been able to trace the chromosomes after telophase and are inclined to believe that the chromosome completely loses its identity in the resting nucleus. One of the last investigations of *Lilium* (Mottier, :03) argues strongly for the latter view, and all who have followed nuclei from one mitosis into another know that the resting nucleus with its linin network and the granular chromatin present conditions that generally make the recognition of chromosomes impossible with the instruments and technique at our command, but this does not prove that they may not be present.

The theory of the permanence of the chromosome has met with much favor because it is argued that otherwise how could the number be maintained so regularly through immense numbers of mitoses. But it can hardly be said that the doctrine is established. It has also found favor because all the events of

mitosis emphasize the importance of the chromosomes which are really the only enduring structures in the nucleus and have led to their being considered as the probable bearers of hereditary qualities.

### 3. The Dynamics of Nuclear Division.

Mitotic phenomena in certain plant cells present evidence that has very direct bearing on some of the theories that deal with mechanical and dynamical explanations of nuclear division. The methods of spindle formation and the various forms of kinoplasmic structures (centrosomes, centrospheres and kinoplasmic caps) which generally in plants seem not to be permanent organs of the cells all tend to support Strasburger's conception of kinoplasm, which is an outgrowth and application to plants of Boveri's well known theory of archoplasm.

The centrosome theory is supported by very few investigations in Botany, the most notable being that of Swingle ('97), for *Stypocaulon*, who believes that the centrosome divides with the aster and is maintained as a permanent organ throughout successive cell divisions. Other examples of similar conditions may be found among the thallophytes which, after all, have received very little attention, and such types as *Dictyota* and the diatoms offer excellent subjects for studies covering a series of cell divisions. But in contrast to *Stypocaulon* it should be noted that the conspicuous centrospheres of *Fucus* and *Corallina* disappear with each mitosis to be formed anew, and the same conditions obtain in the germinating spores of liverworts (*Pellia*). There seems to be no place for the centrosome in spindle formation as presented in the spore mother cells of all groups above the thallophytes (see Sec. III). Neither does mitosis in the vegetative tissues of these groups, characterized as it is by the presence of kinoplasmic caps, conform to the program of the centrosome theory.

∨ The morphological manifestations of kinoplasm are so various that we are driven to a very general conception of its organization. Kinoplasm runs through cycles in which the structure passes from a granular condition to a fibrillar and then back again

to the granular state. By the granular state we mean one in which no fibrillæ seem to be present, but instead the microsomata are densely and homogeneously massed. It is possible that such microsomata form a closely packed network, but no such structure is visible under the microscope. The first appearance of kinoplasm at prophase of mitosis is frequently the granular condition. This state is illustrated by such accumulations as centrospheres and kinoplasmic caps and by the granular zone that has been reported around the nuclei of some spore mother cells.

Granular kinoplasm becomes fibrillar probably by the arrangement of the microsomata into a reticulum from which fibers extend freely into the surrounding cytoplasm. These fibers undoubtedly elongate during prophase, extending in various directions. Some press against the nuclear membrane and when this breaks down grow rapidly into the nuclear cavity. Of these a portion extend from pole to pole and form the central spindle. Others attach themselves to the chromosomes and lie either among the central fibers or somewhat outside of the spindle (mantle fibers). Still others may extend freely into the cytoplasm as astral rays from the pole of the spindle, a very common condition when centrosomes or centrospheres are present. A contraction of the fibrillæ, beginning with metaphase, is just as characteristic of mitosis as their elongation during prophase. The fibers attached to the chromosomes draw the latter to the poles of the spindle. The central fibers in higher plants draw away from the poles and give their substance to the cell plate. The substance of contracted mantle fibers, with other kinoplasm at the poles of the spindle, probably become distributed around the group of daughter chromosomes so that they finally lie surrounded by a sphere of kinoplasm.

It does not seem as if we knew much more about the structure and activities of kinoplasm during mitosis than is indicated in this cycle of change from a granular condition through a fibrillar state back to the granular condition, with a period when the fibers elongate and another when they contract. This with few exceptions is the history for every mitosis. The exceptions deal with peculiar conditions or structures. Thus, for example,

the astral rays of the centrospheres in the ascus instead of contracting to a center or disappearing in the cytoplasm after the last mitosis grow around the nucleus and cut out a portion of the cytoplasm to form the spores, thus contributing their substance to a plasma membrane.

There is little doubt that kinoplasmic fibrillæ actually exist as structural elements in the protoplasm. Their growth and movement in the cytoplasm and nuclear cavity, their multiplication and shifting arrangements as the spindle develops, and their contraction to the poles of the spindle or to a cell plate give these fibers an individuality that cannot be explained on the theory that they merely represent lines of force or paths of dynamic stimuli. They apparently perform all the activities mentioned above by virtue of their own structural organization which is that of rows of microsomata and in this organization resemble and are probably closely related to cilia. There is an excellent discussion of this subject by Allen, :03, p. 302, etc.

Some authors believe that there is a streaming movement in the astral rays (Chamberlain, :03, for *Pellia*) either towards or away from the pole of the spindle. This view is founded on the granular appearance of the radiations which are sometimes very thick in *Pellia* and enlarge at the points where they join the centrospheres or the outer plasma membrane. It is not altogether clear that the larger of these structures are quite the same as spindle fibers since they seem to be actually strands of cytoplasm rather than fibrillæ.

It is probably safe to assume that the forms which kinoplasm takes have relation to dynamic activities, but it is not easy to define these. Thus centrosomes, centrospheres and kinoplasmic caps may well be the centers from which dynamic stimuli extend, and they may be the focal points of other energies. These problems have been very little investigated among plants. It is obvious that differentiated regions of kinoplasm have important physical relations to other portions of the protoplasm, one of the most important being the anchorage which they give to fibrillæ, thereby largely governing the direction of such strains as come about through the contraction of these structures in the later periods of mitosis.

But the essential characteristics of kinoplasm stand out sharply from whatever point the phenomena of mitosis is viewed, and in this protoplasm with its power of forming contractile fibers is vested some of the most conspicuous activities of nuclear division as well as the important powers given plasma membranes in relation to the segmentation of protoplasm to be considered presently.

The dynamic activities concerned with the spindle present only half the story of mitosis. The other important events occur inside of the nucleus. One of these is the dissolution of a portion or the whole of the nucleolus which takes place as the spindle develops and we have already given the views of Strasburger ('95 and :00), supported by the studies of other investigators, that its substance in certain instances furnishes material for the development of the spindle. But the chief events in the interior of the nucleus deal with the accumulation of chromatin on the spirem thread which with the disappearance of the linin indicates that the latter substance may become converted into the former. The splitting of the spirem ribbon longitudinally is of the utmost significance for thereby is made possible an exact and homogeneous distribution of the chromatic material in the nucleus. We do not know how the spirem ribbon splits nor have we as yet any evidence of the origin and evolution of this peculiar activity.

#### (b) Segmentation of the Protoplasm.

Mitosis in the uninucleate cells of plants is generally followed by immediate cell division, which takes place in groups above the thallophytes through the formation and *cleavage of the cell plate* in the equatorial region of the spindle between the daughter nuclei. Among thallophytes, as so far studied, cell division is chiefly through *cleavage by constriction*. There are forms among the thallophytes and also in the spermatophytes whose nuclei gather about themselves a portion of the cytoplasm, wherein they lie, which becomes cut out of the general mass by a cell wall. This is *free cell formation*.

Multinucleate masses of protoplasm, such as plasmodia and

portions of coenocytes, generally divide extensively at reproductive periods and always through *cleavage by constriction* with, however, the frequent coöperation of vacuoles which help to cut the protoplasm in the same manner as the cleavage furrows. *Cleavage by constriction* is undoubtedly the most primitive type; *free cell formation* and *cleavage by cell plates* being special and very highly developed protoplasmic activities.

### I. Cleavage by Constriction.

A simple example of *cleavage by constriction* is presented by such an alga as *Cladophora*. The process consists in the building out of a ring of cellulose from the side wall into the cell cavity. The outer plasma membrane forms a fold, thus placing the two surfaces opposite one another (see Fig. 8 *a*), and the wall is laid down between these. *Spirogyra* forms its wall in precisely the same manner as *Cladophora* with this peculiarity, that the new wall finally cuts through the protoplasmic strands that connect the daughter nuclei. These strands are said to contain spindle fibers (Van Wisselingh, :02) which may contribute to the plasma membranes forming the cell wall, as it is completed. Another illustration of *cleavage by constriction* is presented in the formation of gametes of moulds (*Sporodinia*) and the abstriction of conidia (*Erysiphææ*), both processes having been studied by Harper, '99, p. 506. In these cases a cleavage furrow proceeds from the surface inward and divides the protoplasm. The partition wall of cellulose is formed later between the two free plasma surfaces. The only differences between the processes above described are that in the first forms the cleavage proceeds more slowly and the wall follows the furrow as it progresses in the interior of the cell, while in the latter types cleavage is complete before the plasma membranes develop the wall. Cell division in the red Algæ (*Rhodophyceæ*) is also a process of constriction similar to *Cladophora*, but the wall is not generally formed entirely across the filament so that adjacent cells remain connected by thick strands of protoplasm.

These processes become much more complicated when large masses of multinucleate protoplasm are divided up into many

smaller bodies as during spore formation among the Myxomycetes and Mucorales. Very complete studies have been made of these conditions by Harper, '99 and :00a. In the slime mould (*Fuligo*) cleavage begins by furrows on the external surface which "cut down at all angles into the homogeneous protoplasm." The direction of the cleavage furrows is further complicated by the fact that many of them start from the bottom and sides of deep folds. All of the furrows may bend and secondary cleavage planes strike off from them which in time unite with one another until the protoplasm is divided progressively into very many small masses (see Fig. 8 *b*) that finally round themselves off and secrete walls, becoming spores, sometimes with one nucleus and sometimes with several.

Cleavage in the sporangium of *Synchytrium* and the moulds, as described by Harper, '99, is in general similar to that in the plasmodium with, however, the additional feature that lines or planes of vacuoles are often utilized to assist a cleavage furrow in effecting the segmentation of the protoplasm. The separation of the spore plasm of the sporangium of *Pilobolus* from the filament below begins with a cleavage furrow from the exterior; but this furrow follows and makes use of a curved plane of flattened vacuoles with the result that a dome shaped cleft is developed and two plasma membranes are presented face to face, which form the columella wall between them. The segmentation of the spore plasm in *Pilobolus* is affected somewhat similarly through the coöperation of cleavage furrows from the exterior with vacuoles which cut into the protoplasm at various angles to meet one another and the cleavage furrows. The bodies first formed in the sporangium of *Pilobolus* are not the final spores. Harper suggests that they may correspond to the zoöspores of *Saprolegnia*. They are generally uninucleate and begin immediately a period of growth within the sporangium characterized by extensive nuclear multiplication and several divisions of the protoplasmic body by constriction.

Harper finds that the spore plasm of *Sporodinia* is separated from the filament below by a dome-shaped plane of flattened vacuoles which fuse together and, unlike *Pilobolus*, cut their way to the surface of the sporangium. Thus the cleavage is deter-

mined entirely by the activity of vacuoles. Spore formation, however, is accomplished by cleavage furrows which progress from the exterior inwards and, without the aid of conspicuous vacuoles, cut out multinucleate masses of protoplasm which become the spores.

Dean Swingle (:03) has extended the studies of Harper on spore formation in the molds to *Rhizopus* and *Phycomyces*. He confirms Harper's account of the general processes of cleavage by furrows cooperating with vacuoles, and notes the following characteristics in the types studied. In *Rhizopus* the position of the columella is determined by a dome-shaped series of flattened vacuoles which fuse and meet a cleft that extends upward from the outer plasma membrane at the base of the sporangium. The spores are formed in *Rhizopus* by branching systems of curved furrows that cut the protoplasm into multinucleate masses, and in *Phycomyces* by angular vacuoles that develop into furrows which extend in various directions and unite with one another and with clefts from the region of the columella.

Other excellent illustrations of *cleavage by constriction* are presented in the sporangia of such types as *Hydrodictyon*, *Cladophora* and *Saprolegnia*. Timberlake (:02) has given an account of *Hydrodictyon*, and the events are also fairly well understood for *Saprolegnia*. Segmentation begins in *Hydrodictyon* by the development of cleavage furrows in the outer plasma membrane, which cut into the protoplasmic layer at right angles to the surface and meet similar furrows that make their way from the large central vacuole outward. These cleavage planes spread laterally, uniting with one another, until the protoplasm is all divided into uninucleate masses which become the zoöspores (Fig. 8 c). In *Saprolegnia* (see Davis, :03, for general account) conspicuous cleavage furrows develop from the central vacuole and make their way to the exterior, finally breaking through the outer plasma membrane. When this takes place there is an immediate escape of cell sap, which was under pressure, and a shrinkage of the sporangium so that the zoöspore origins appear to fuse, but this is not really the case, for cleavage is continued and the zoöspores soon separate.

A physiological explanation of *cleavage by constriction* must

consider two sets of factors. There is an evident contraction of the protoplasm in many examples because water is given off. The shrinkage of the surface would undoubtedly form furrows, but, as Harper has pointed out, these furrows do not develop in an accidental manner. Non-nucleated masses of protoplasm are never separated from the nucleated, but the segmentation proceeds after a system by which the final products contain only one nucleus or at most a limited number. So it is probable that the nuclei are the ultimate centers controlling the segmentation which at its commencement may be quite irregular. This explanation of sporogenesis in the plasmodium and the sporangium is not altogether satisfactory for the cell division of *Cladophora*, the abstriction of conidia or the development of the gametes of a mould. In these examples the cleavage begins at definite regions of the plasma membrane, so that the stimulus must be local, and the direction of the plane has a definite relation to the axis of the plant.

It is important to note (see Harper, :00, p. 240-249) how inadequate are some of the well-known theories of the segmentation of protoplasm as explanations of *cleavage by constriction*. Hofmeister's law ('67) that cell division is across the axis of growth obviously cannot be applied to the irregular segmentation in the plasmodium and sporangium, nor is Sachs' well-known law of growth in vegetative points adequate. Sachs, '94, and in the *Lectures on the Physiology of Plants*, chap. XXVII, conceives a growing point of a higher plant or an embryonic structure as a mass of protoplasm whose cell walls are determined by principles of rectangular intersection of perpendicular planes. The outer form of the structure determines the angles of periclinal and anticlinal walls and the transversals conform to these. There is not the slightest hint of such an order in the distribution of cleavage planes in the multinucleate masses of protoplasm just described and Sachs' law in so far fails of general application whether or not it be satisfactory for the conditions with which he especially deals. There are also explanations of cell division, applicable to the tissues of many higher organisms, based on the position of the nuclear figure in the cell, which determines the position of the cell plate but these theories cannot handle the events in the

plasmodium or sporangium where the cleavage planes are formed without regard to the time of nuclear division or the position of mitotic figures.

## 2. Cleavage by Cell Plates.

Cleavage of the protoplasm by means of the cell plate is almost universal in cell division of plants above the thallophytes. It is one of the peculiarities of plant cells, having been found in comparatively few animals and there represented rather imperfectly by the so-called mid-body. The general events of the process have been known since Treubs' studies of 1878, and were clearly described by Strasburger in 1880. Timberlake, :00, in a recent paper gives an historical review of the subject.

When, after the metaphase of mitosis, the two sets of daughter chromosomes separate from one another there is left between them the spindle, made up of the central fibers. The first appearance of the cell plate is a line of granules in the equatorial region of this spindle where the nuclear plate formerly lay. But several important events proceed this condition. The connecting central fibers begin to thicken, first near the daughter nuclei, and then gradually towards the equatorial region of the spindle. The number of fibers may increase greatly, probably by the separation of bundles of fibrillæ composing the spindle into independent elements (Timberlake, :00, p. 94). But there is evidence that new fibrillæ are sometimes formed from the vicinity of the daughter nuclei, some of which may enter the spindle and coöperate with the connecting fibers. In certain forms (*e. g.* Allium) there is an accumulation of a stainable substance between the connecting fibers in the equatorial region of the spindle. The reaction of this substance to stains indicates a carbohydrate composition.

The cell plate really begins with the thickening of the connecting fibers in the equatorial plane of the spindle. In some forms these thickenings are elongated bodies, in others mere granules. The earlier writers (Treub, '78, Zacharias, '88) did not believe that they came from the spindle fibers, but there seems to be now no doubt of their origin from these elements,

which contract and thicken as the plate develops. The bodies composing the cell plate finally lie in a plane extending the entire width of the spindle (Fig. 8 *d*) and they then broaden and come in contact with one another to form a continuous membrane, which, as has been said, may lie in a matrix of carbohydrate material. The cell plate grows rapidly as the central spindle fibers shorten and contribute their substance to the structure. During this contraction the surrounding cytoplasm

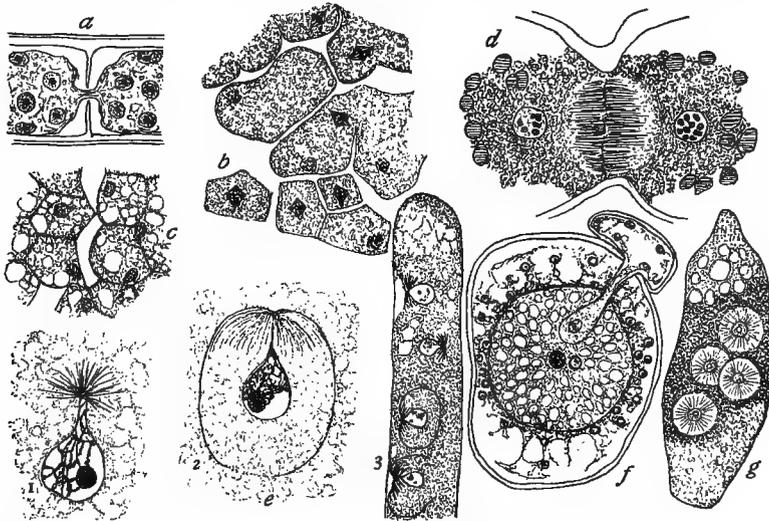


FIG. 8.—Segmentation of the Protoplasm. *a, b, c*, cleavage by constriction. *d*, cleavage by cell plate. *e, f, g*, free Cell Formation. *a*, cell division in *Cladophora*. *b*, cleavage of spore plasm in *Fuligo*. *c*, spore formation in *Hydrodictyon*. *d*, first division of spore mother-cell in *Pellia*. *e*, spore formation in ascus, 1 and 2 (*Erysiphæ*) astral fibers cutting out cytoplasm around nuclei, 3 portion of ascus with developing spores (*Lachnea*). *f*, oögonium of *Albugo*, egg surrounded by membrane pierced by antheridial tube, cœnocentrum and female gamete nucleus within. *g*, egg of *Ephedra* with four embryo cells. After Strasburger Harper and Timberlake.

enters the region between the barrel shaped group of fibers and the daughter nuclei (Fig. 8 *d*). It is probable that the cell plate is composed entirely of the substance of spindle fibers and in consequence is kinoplasmic in character. The cell plate widens with the accretion of material from the central spindle, which in some cases is assisted by the radiating fibers that, lying outside of the spindle, contract and add their material to the edge of the plate. The cell plate thus extends laterally and finally reaches

the neighboring cell walls, fusing with the outer plasma membrane. There are certain mitoses, as in some spore mother cells and in the embryo sac (see Section III) where the cell plates are absorbed into the cytoplasm leaving the original cell with two or more nuclei and without partition walls. It is uncertain whether the edge of the plate is ever extended by the development of additional peripheral fibrillæ (Timberlake, :00, p. 161) from the daughter nuclei.

Cell division is accomplished by the splitting of the cell plate (Strasburger, '98) into two plasma membranes. The division generally begins in the center and the cleft progresses towards the periphery until it reaches the cell wall. During the process the thickened rod shaped portions of the spindle fibers are pulled apart. There are thus left two kinoplasmic membranes opposite one another and continuous with the outer plasma membrane surrounding the daughter cells. The cause of this cleavage is not apparent, but there are reasons for believing that the split is essentially a thin vacuole which, starting near the center, cuts its way through the cell plate to the periphery after a manner very similar to the behavior of vacuoles during the cleavage of the plasmodium and in the sporangia of certain moulds. And there may be shown in this activity a relationship of *cleavage by cell plate* to some of the events of *cleavage by constriction*. After division is complete there follows the formation of a cell wall between the two cell surfaces after the method usual to plasma membranes.

The new cell wall generally begins in the oldest portion of the cell plate where the cleft first appeared and is gradually built out peripherally until it reaches the side walls. The first indication of the wall is the appearance in the cleft of a stainable carbohydrate substance which resembles the material that was primarily present between the fibers of the central spindle and which disappears with the formation of the cell plate. This material is probably the basis of the first deposits on the surface of the two plasma membranes, but the nature of the final substance is exceedingly various. A cell wall may be formed that is homogeneous throughout but often the thickened wall presents three regions, two layers of a cellulose basis formed by the

respective plasma membranes and between them the so-called middle lamella.

The middle lamella has been the subject of much discussion. It is not the remains of the cell plate as was once supposed. Neither is it exactly a cement between two cell walls. Its history is undoubtedly various, for the composition shows much plasticity. The origin of the middle lamella at the surface of a plasma membrane indicates a morphology similar to a cell wall, but the substance, pectic in character, shows transformations far removed from the cellulose compounds that are formed later and which give thickness to the cell wall. Allen (:01) discusses the subject in detail.

The origin of the cell plate is a subject of interest which will be further discussed in Section VI. There are some types, especially among the thallophytes, where a cell plate is present, but apparently in a somewhat undeveloped and rudimentary condition. These forms suggest transitional conditions between *cleavage by constriction with the aid of vacuoles*, so general among the thallophytes, and *cleavage by the cell plate*, characteristic of higher groups. The most interesting examples are *Anthoceros*, *Chara*, *Basidiobolus*, *Pelvetia*, *Fucus*, and *Sphacelaria*.

Cell plates are formed with each of the two successive mitoses in the spore mother cell of *Anthoceros* (Van Hook, :00; Davis, :01, p. 158), but the structure in some species is exceedingly small (*e. g.*, *A. lævis*) and can scarcely extend more than one-tenth of the distance across the cell. It is larger in other forms, as in the one studied by Van Hook; but even there the nuclear figure of the second mitosis is only one-third of the width of the cell. The protoplasm divides simultaneously in the four spores with the characteristic arrangement. If this division were determined entirely by cell plates there would be required an extensive development of fibrillæ, of which there is no evidence in the cell. But their place seems to be taken by numerous delicate strands of cytoplasm which connect the four protoplasmic masses, each of which contains a large chromatophore and an accompanying nucleus. A film is formed in the intermediate region, and this marks the position of the cell wall. It is, of course, quite certain that the two cell plates of the second mitosis are

a part of this membrane and may start its development, but the final structure must contain very much more material than could possibly be contributed by the sparsely developed spindle fibers. Thus, although the splitting of the cell plate may start the process of segmentation, its final course and end is probably determined by cleavage through vacuoles, thus utilizing a method characteristic of the thallophytes.

*Chara* appears to have a fairly well developed cell plate (Deb-ski, '97) which extends almost entirely across the cell, presenting very exceptional conditions among the thallophytes. This peculiarity is in keeping with other characters of the spindle, which begins its development outside of the nuclear membrane and, lacking centrosomes, resembles the nuclear figures of higher plants. It is possible that nuclear studies upon *Chara* throughout ontogeny might show a variation that would be very significant for the evolutionary problems concerned with the structure of protoplasm.

Fairchild ('97) reports a cell plate for *Basidiobolus* when the beak cells are cut off from the gametes. The structure, as figured and described, is not, however, conspicuous. He points out general resemblances between cell division in this form and in the Conjugales, where, as Van Wisselingh (:02) described later for *Spirogyra*, spindle fibers connect the daughter nuclei and may cooperate towards the end of cell division with a cleavage furrow from the side of the cell.

The conditions in the Fucales are not altogether clear. Both Strasburger ('97a) and Farmer and Williams ('98) report that the central spindle disappears in *Fucus* without the formation of the cell plate and that the wall is developed between the daughter nuclei in a region of granular cytoplasm. However, in *Pelvetia* some of the radiating fibrillæ from opposite sides of the daughter nuclei bend around these structures and end in the new wall. It is not plain that they contribute much if anything to its formation in the way of substance, but it would seem probable that they hold a directive relation to the structure (Farmer and Williams, '98).

The Sphacelariaceæ seem to be somewhat similar to the Fucales in their methods of cell division. The beautiful figures of

Swingle ('97) for *Stypocaulon* give details of the region of the cytoplasm that forms the partition wall between the daughter nuclei. There is a zone of fine meshed protoplasm between much larger vacuoles. It is possible that some very long fibrillæ may connect the daughter nuclei with this zone, but they do not form a cell plate. Consequently the wall must be developed in this delicate alveolar layer, which probably splits along some plane of vacuoles. The process of cleavage is then really related to such activities of vacuoles as occur in the sporangium of the Mucorales and in the plasmodium. But the position of the alveolar layer may be determined by the fibrillæ, since it is always situated nearest to the smaller of the two daughter nuclei.

It seems likely that the process of cleavage in the Fucales will be found to be similar to *Stypocaulon* when the details of structure in the internuclear cytoplasm is known. So this group, with others, is likely to furnish conditions in which spindle fibers may determine the position of the cell wall and exert a directive influence upon it without actually laying down a cell plate. As has been pointed out, the splitting of the cell plate is probably a cleavage along a very thin flat vacuole, so that the process in its essential characters is the same as cleavage through a series of vacuoles. Thus *cleavage by the cell plate* is possibly an outgrowth from that phase of *cleavage by constriction* in which the extensive fusion of vacuoles determines the planes of separation. The important advance lies in the new factors, introduced through the activities of fibrillæ, which become very conspicuous as actual contributors of material to the kinoplasmic film which is laid down as the cell plate. This function of the fibrillæ probably developed slowly from conditions such as those in *Stypocaulon* and *Pelvetia*, where their influence upon the position of the cell wall, if any at all, can scarcely be more than directive.

### 3. Free Cell Formation.

Whenever a nucleus becomes the center around which cytoplasm is gathered and separated from the rest of the cell contents, so that the new cell lies freely in the protoplasm of the old, this is *free cell formation*. Illustrations are presented by

the spores of an ascus, the oöspore of the Peronosporales, the embryo cells of Ephedra, and probably other gymnosperms, and in some cases seemed to be exemplified in the conditions presented by the egg and synergids and the antipodals of the embryo sac.

Spore formation in the ascus is known through the studies of Harper ('97 and '99). After the final divisions in the ascus the nuclei lie in the cytoplasm, each with an aster at its side (Fig. 8 *e*, 3). A delicate prolongation carries the aster with its centrosphere away from the main body of the nucleus (*e*, 1). The rays of the aster now bend over and grow around the nucleus, presenting an umbrella-like figure (*e*, 2). They finally meet on the opposite side, and thereby cut out a portion of the cytoplasm which is included in the spore. The substance of the aster fibers forms the basis of a kinoplasmic film which becomes the plasma membrane of the ascospore and develops the spore wall externally after the usual method. This peculiar activity of an aster is unparalleled in plant or animal cells.

Oögenesis in the Peronosporales has been described in some detail by several authors, but the process has not generally been called *free cell formation*. Yet at the end of the process the oöspore, enveloped by periplasm, lies free in the oögonium. In the beginning the oöplasm gathers in the center of the oögonium as a denser alveolar region around that peculiar protoplasmic body (generally present) the cœnocentrum. This accumulation forces the vacuoles, together with most of the nuclei, to the periphery, where they lie in a sort of protoplasmic froth next the cell wall and constitute the periplasm. The spore wall develops at the boundary of the oöplasm, so that it lies close to the large vacuoles (Fig. 8 *f*) in the periplasm. There must be an accumulation of kinoplasm, perhaps from the plasma membranes of numerous vacuoles, to form a delicate layer between the two regions of the oögonium. This layer of kinoplasm probably splits along the line of vacuoles between the oöplasm and periplasm, for the primary walls are certainly established between two plasma membranes, because the secondary layers are added to it from both sides. Nuclei in division frequently lie very close to the boundary of the oöplasm, but there is no evidence

that the kinoplasmic membrane has any relation to these mitotic figures. That is to say, there are no fibrillæ to contribute substance to the membrane, and its development must be concerned with vacuoles alone. In this respect the process recalls the part played by vacuoles in the plasmodium and in certain sporangia during cleavage by constriction.

Free cell formation after the method in the egg of Ephedra (Strasburger, '79), which is also likely to be found among other gymnosperms, takes place during the differentiation of the embryo cells. The cytoplasm collects around each nucleus, forming a sphere (Fig. 8 *g*), and a wall is developed on the outside of this body. Details of the process are not known, and it is not clear whether the position of the membrane is determined by the vacuoles that must border upon this region or whether there are fibers radiating from the nucleus which might lay down a cell plate around the denser protoplasm; but the evidence favors the former possibility.

Somewhat similar conditions are presented in the egg apparatus of many embryo sacs. In certain forms (*e. g.*, the lily so well described by Mottier, '98) the egg nucleus and synergids are thickly invested by radiating fibers, and these, together with the cell plates, may readily determine the position of the plasma membrane that forms the cell wall. But fibers do not seem to be conspicuously present in the egg apparatus of many other embryo sacs (Excellent illustrations can be found among the Ranunculaceæ). In these cases the protoplasm collects around the nuclei as dense areas bordered by vacuolar cytoplasm, and it is possible that the vacuoles by fusing with one another cut out these respective regions and thus determine the plasma membranes of the egg and synergids. Such processes would extend the activities of vacuoles, which accompany cleavage by constriction in the thallophytes, to the highest groups of plants.

It is curious that with all of the work upon the embryo sac we should know less about the segmentation of the protoplasm around the synergid, antipodal, and segmentation nuclei in this structure than in the sporangia of the molds, the ascus, or during spore formation in the Myxomycetes.

(To be continued).

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## STUDIES ON THE PLANT CELL.—III.

BRADLEY MOORE DAVIS.

### SECTION III. HIGHLY SPECIALIZED PLANT CELLS AND THEIR PECULIARITIES.

VERY much of our knowledge of the structure and behavior of protoplasm in plants has been derived from the study of certain cells whose organization has reached an exceptionally advanced degree of differentiation. The peculiarities of these cells are obvious and have proved of great interest but we have as yet scarcely made a beginning in the study which must trace and relate these characteristics of the most complex products of cellular evolution in plants to their more simple progenitors.

This section will describe in some detail the structure and protoplasmic activities of the following six highly specialized cells: 1, The Zoöspore; 2, The Sperm; 3, The Egg; 4, The Spore Mother-Cell; 5, The Cœnocyte; 6, The Cœnogamete.

#### 1. The Zoöspore.

Zoöspores are interesting not only for their own peculiarities but also because they are well known to be the progenitors of the sexual cells or gametes which become later differentiated into the egg and sperm. Comparative studies upon three cells so closely related and yet so diverse in their extremes of structure are sure to yield important results.

The zoöspore is generally an uninucleate cell, colorless in the Fungi, but containing a chromatophore or plastids in all other groups of thallophytes. There are usually two or four cilia attached to the anterior pointed end which is free from coloring matter and at this region one may expect to find a red pigment spot. Some zoöspores are exceptional for special peculiarities, as those of *Vaucheria* which are multinucleate, each nucleus

being accompanied by a pair of cilia, or those of *Cedogonium* whose colorless forward end bears a crown of numerous cilia. The zoospore stands among the higher forms for a type of motile organism that is very close to the bottom of the assemblage of groups and developmental lines which make up the Algæ. The forms most closely related to the zoospore are in the family Chlamydomonadeæ of the Volvocales. But at this general low level of the plant kingdom there are several groups whose members pass most of their lives in motile conditions (Volvocales, Flagellates and Peridinales) and the cells of all of these types resemble zoospores to a greater or less degree in their structure and habits, so that this condition represents a widespread and well defined stage of evolutionary development. Therefore when zoospores are formed in the life history of some higher plant they represent a return on the part of the organism for a short time to the structure and mode of life of an ancestry perhaps related in some way to the groups that still have the motile habits throughout most of their existence.

For these reasons close comparisons in structure between the zoospore and motile Algæ will be interesting and should help to explain the peculiarities of these cells. These peculiarities chiefly concern the organ that forms the cilia (blepharoplast), which becomes very complex in the sperm, and the pigment spot.

Unfortunately studies upon these problems have been few and we are not prepared to make a general statement of the conditions. The most recent investigation on the structure of the zoospore is that of Timberlake (:02), but Strasburger has written extensively on the subject, especially in the *Histologische Beiträge* ('92 and :00). The later paper (:00, p. 177-215) reviews the entire subject of cilia formation. Dangeard has presented an account of the Chlamydomonadeæ, '99, and in :01 described especially *Polytoma*, comparing its structure with that of the animal spermatozoan.

*Polytoma* (see Fig. 9 a) is a colorless organism but its cell structure and life history place it unquestionably among the Chlamydomonadeæ. The two cilia arise from a small body (blepharoplast) situated at the extremity of the cell. A delicate

thread-like structure, which Dangeard calls the rhizoplast, extends from the blepharoplast into the cytoplasm and sometimes ends at the side of the nucleus in a granule (condyle). The cilia grow out from the blepharoplast. This apparatus is not known to bear any relation to centrosomes or to the kinoplasm of nuclear figures present at the time of spore formation. But it should be noted that the blepharoplast is situated directly under if not actually in the outer plasma membrane, which is kinoplasmic. The filamentous connection between blepharoplast and nucleus is probably important, especially since it has also been found in zoöspores (Timberlake, :02, for *Hydrodictyon*) but we do not even know its developmental history much less its function. Further study will be necessary to make clear possible relations to kinoplasm around the nucleus or to centrosomes. Consequently Dangeard's comparison of *Polytoma* to the animal spermatozoön is not convincing for it seems to be established for the spermatozoön that portions of the middle piece at least and the flagellum are derived from a true centrosome. Indeed from the meager evidence now at hand the blepharoplast of *Polytoma* is as likely to be a structure differentiated from the plasma membrane as to have any relation to the nucleus. But detailed studies on sporogenesis may discover a history more in harmony with that of *Hydrodictyon*.

We have summarized a portion of Timberlake's (:02) account of sporogenesis for *Hydrodictyon* in the previous section under the head of "Cleavage by constriction." We shall consider now certain details. Small spherical bodies are found at the poles of the spindles during nuclear division in the mother-cell. They are undoubtedly accumulations of kinoplasm and perhaps stand for centrosomes. However they have no polar radiations nor could they be followed between mitoses when the nuclei were in resting conditions. It is not probable therefore

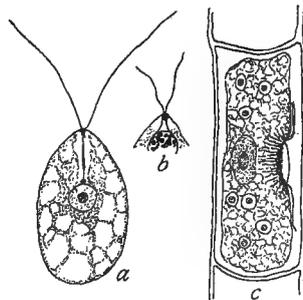


FIG. 9.—The Zoöspore. *a*, *Polytoma*; *b*, *Hydrodictyon*; *c*, Development in *Oedogonium*. (*a*, after Dangeard :01; *b*, Timberlake :02; *c*, Strasburger '92.)

that these structures are permanent in the cell. After nuclear multiplication is ended segmentation proceeds until the nucleate masses of protoplasm separate from one another as zoöspores. Then a body may be found lying in contact with the plasma membrane and bearing a pair of cilia (Fig. 9*b*). This basal body (blepharoplast) by its reaction to stains seems to be entirely distinct from the plasma membrane and is connected with the nucleus by very delicate threads. There is a time just previous to the differentiation of the zoöspores when the nuclei lie very close to the cleavage furrow that finally separates the adjacent zoöspore origins. A granule may sometimes be observed close to these nuclei and it is possible that this is the first appearance of the basal body (blepharoplast). If this should prove correct the structure may have a direct relation to the kinoplasm around the nucleus, a relation that is afterwards maintained through the two or three delicate fibers that connect these structures. Thus the blepharoplast if not directly derived from a centrosome may at least have its origin from the same region of kinoplasm. However these possibilities are mere speculations and the investigation of these points is very much to be desired in a number of algal and fungal types.

We are now brought to the views of Strasburger as expressed in his writings of '92 and :00. His investigations have been chiefly on *Vaucheria*, *Cladophora* and *Ædogonium*. In all of these forms the cilia come from a body (blepharoplast) which he believes to arise from the outer plasma membrane (Hautschicht). The nucleus lies close to the plasma membrane at the time when the blepharoplast is formed and may determine its development there as a dynamic center, but the blepharoplast is not a centrosome according to Strasburger. It is of course kinoplasmic since it develops from the plasma membrane and this would accord with its activities as a cilia forming organ. The blepharoplast is extraordinarily large in *Ædogonium* (see Fig. 9*c*) and develops a ring of numerous cilia on the exterior while at the same time fibrillar rays grow back into the cytoplasm and probably help to give a compact organization to the zoöspore. This structure is very suggestive of the centrosphere and aster that cuts out the ascospore (see Section II, Free Cell

Formation) and in spite of Strasburger's conclusions that it is derived entirely from the plasma membrane we are justified in asking for a fuller description of its development. There is the possibility of a different origin wherein the nucleus may play an important part which, in the light of Timberlake's studies on *Hydrodictyon*, suggests that Strasburger may not have discovered the earliest beginning of the blepharoplast in *Ædogonium*. And the same doubts apply to *Cladophora* and *Vaucheria*.

There is thus considerable divergence in the views of the origin and nature of the blepharoplast in zoöspores, Strasburger believing that they are developed as a specialized region of the plasma membrane with no relation to centrosomes, and Timberlake holding that the structure in *Hydrodictyon* is not a part of the plasma membrane but comes from the interior of the protoplasm. The problem is also involved with conditions in the sperm, where there is likewise a difference of opinion as to the homologies of the blepharoplast but an undoubted origin at least in the pteridophytes and gymnosperms from the interior of the cell. We should naturally expect the blepharoplasts of zoöspores and sperms to be homologous and consequently the problem is of great theoretical interest and will be taken up again in our discussion of the sperm. Its solution demands a most thorough study of the development of some of the larger zoöspores as in *Ædogonium* and certain species of the *Conferales* and *Volvocales*.

The pigment spot is almost universally present in zoöspores and is also characteristic of the cells of many motile organisms as in the *Volvocales* and *Flagellates* while occasionally found in other groups. The structure has been called an eye spot from its fancied resemblance to the simple eyes of certain *Crustacea* (*Cyclops*, etc.) but this term is unsatisfactory since it is not established that the pigment spot is primarily a receptive organ for light or warmth; but even should it prove to be thus sensitive (which is very probable) thereby orienting the cell with respect to the direction of incoming rays, that is not a function comparable to sight.

The coloring matter of the pigment spot is held as a single globule or as a collection of numerous small granules in meshes

of the protoplasm. It is frequently associated with a plastid. The pigment may be readily broken down and dissolved out by such reagents as alcohol and ether. In chemical composition it is very close to hæmatochrome and thus may be related to chlorophyll or a derivative of that substance. The cytoplasm around the pigment spot is undifferentiated and when the coloring matter is removed it is very difficult and sometimes impossible to find the situation of the structure. Consequently the pigment spot can hardly be considered a protoplasmic organ since it is merely an accumulation of coloring matter at some point in the cell. Strasburger (:00, p. 193) states that the pigment spot of certain zoöspores (*Cladophora*, etc.) is formed in the plasma membrane but this is not true of many other motile cells (*Flagellata*) and there is no doubt that in some cells (*e. g.* the gametes of *Cutleria*) the pigment spot is a portion of a plastid. The literature upon the structure and function of pigment spots is reviewed by Zimmermann (*Beiträge z. bot. Centralb.* Bd. 4, p. 159, 1894) and since then Wager ('99) has presented a detailed study of *Euglena*.

## 2. The Sperm.

The sperm is unquestionably derived from the zoöspore through primitive types of gametes which were identical with zoöspores in all essentials of morphology. I have described the origin and evolution of sexual cells of plants in two recent papers (*Popular Science Monthly*, Nov. 1901, p. 66 and Feb. 1902, p. 300). We should expect the simplest forms of sperms to have the characters of zoöspores and this is the fact. The sperms of the *Algæ*, as a rule, have the same number of cilia (usually two) as their ancestral asexual zoöspores. They generally contain a chromatophore, although sometimes much reduced, and there is present the pigment spot. The cilia are attached at the pointed end or at the side, arising from colorless protoplasm that sometimes contains the pigment spot while the chromatophore, when present, and the nucleus lie at some distance from this region of the cell. The sperms of bryophytes and pteridophytes are much attenuated in form and lack the

pigment spot and chromatophore. Those of the bryophytes and the Lycopodineæ are biciliate while other pteridophytes have multiciliate sperms the cilia being distributed on a band (blepharoplast) which lies along one side of the spiral structure. A large portion of the spiral in these sperms is composed of nuclear substance and much of the remaining cytoplasm with granules and vacuolar inclusions may frequently be found in a vesicle attached to the larger end of the spiral.

The only motile sperm among the Fungi is that of *Monoblepharis*. The male cells of other Fungi are non-motile bodies (spermatia) generally formed from the ends of delicate filaments which are found in special organs called spermagonia. Spermagonia have been described in the Uredinales, the lichens and in the Laboulbeniaceæ but their function is only clearly established for the last two groups. They are very highly differentiated in the Laboulbeniaceæ and comprise several types of structure. Another type of male cell, found in certain groups of the Phycomycetes and Ascomycetes, is the cœnogamete (to be described presently) which is however not the homologue of the sperm but of the mother-cell or antheridium that develops such structures. Sperms of the red Algæ (*Rhodophyceæ*) are likewise non-motile and they are invariably formed singly in small cells at the ends of filaments. These non motile sperms of Fungi and red Algæ are exceedingly small uninucleate bodies without further complexity of structure as far as is known.

We shall not attempt to discuss the earlier literature that treats of the structure and development of the plant sperm. In 1894 Belajeff published a German translation of a paper written two years before in Russian which presents the views of previous investigators and to this the reader is referred for such historical references. At that time various opinions were held respecting the organization of the sperm, some writers (Campbell, Guignard and others) believing that it was chiefly or wholly nuclear in origin, while another group (Zacharias, '87, Belajeff, Strasburger, '92, etc.) thought that the cytoplasm shared very largely in its structure. Belajeff ('94a) from studies among the *Characeæ* showed with especial clearness that the cytoplasm was an important constituent of this sperm since the nuclear

material occupied a restricted region in the middle of the spiral structure. This was the first of a series of investigations which have given especial attention to cytoplasmic activities during spermatogenesis and placed the entire subject in a new light.

The year 1897 brought forth almost simultaneously three short papers by Webber ('97a, '97b, '97c) and Belajeff ('97a, '97b, '97c) respectively. Webber had studied the development of the motile sperms of *Zamia* and *Ginko*, Belajeff certain forms of the *Filicineæ* and *Equisetineæ*. These were of the nature of preliminary announcements and both authors published later more detailed descriptions and discussions. The discoveries of motile sperms in *Ginko* by Hirase and of *Cycas* by Ikeno were announced in several short papers during the years 1896 and '97 but without descriptions of their development. This literature together with later papers of Ikeno, Shaw, Belajeff, Hirase, and Fujii is reviewed in Webber's last contribution (:01) and also in Strasburger's discussion of "Cilienbildner" (:00, p. 177) to which the reader is referred for the most complete treatments of spermatogenesis in plants yet published.

The cycads and *Ginko* are the most favorable subjects known for studies in spermatogenesis. Detailed accounts of the cycads are given by Ikeno ('98b) for *Cycas* and by Webber (:01) for *Zamia*, these forms agreeing with one another in all essentials. Two sperms are developed from the daughter cells (spermatids) following the division of the so-called body cell in the pollen tube. The process really begins in the body cell with the appearance of the blepharoplasts. Their development has been followed with especial attention in *Zamia*. They are formed *de novo* in the cytoplasm at some distance from the nucleus and while the latter is in the resting condition. They appear independently of one another, generally on opposite sides of the nucleus but sometimes much nearer together (Fig. 10 a). Each is a large deeply staining body with numerous radiations extending into the cytoplasm. The blepharoplasts then increase in size and, moving farther away from the nucleus, take positions exactly opposite to one another. The nucleus of the body cell now divides, its spindle being clearly intranuclear (Fig. 5 d) and consequently holding no visible relation to the blepharoplasts

which lie at a considerable distance from the structure (Fig. 10 *b*). The latter cannot then be said to occupy the position of centrosomes in relation to this spindle. Meanwhile important changes, which are best known for *Zamia*, take place in the blepharoplast. In this type the structure forms a hollow sphere which breaks up into segments and finally into granules as mitosis proceeds. The radiations disappear without holding any apparent relation to the spindle. During telophase each of the two blepharoplasts

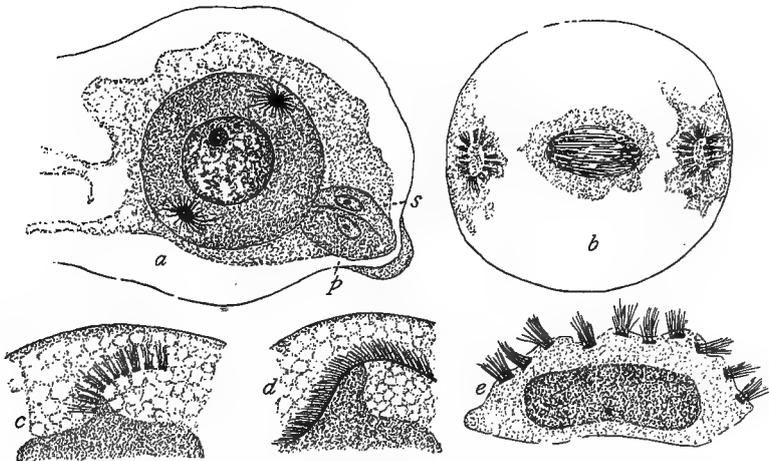


FIG. 10.—Spermatogenesis in *Cycas*. *a*, Body cell in pollen tube with two blepharoplasts; *s*, stalk cell; *p*, prothallial cell; *b*, anaphase of mitosis in the body cell the spindle lying between the two blepharoplasts which have begun to form cilia; *c*, Blepharoplast elongating, in contact with a process from the nucleus; *d*, end of blepharoplast attached to the nucleus at a later stage of development; *e*, sperm showing section of the flattened spiral blepharoplast with cilia projecting beyond the cell. (After Ikeno, '98.)

appears as a mass of granules at some distance from the daughter nuclei which are to become the sperm nuclei. As a result of this division the spermatids (sperm mother-cells) are differentiated. At the close of the mitosis the blepharoplast enters upon its functions of forming in the spermatid a cilia bearing band which is to lie as a spiral around the sperm. The granules first extend as a delicate deeply stained line towards the nucleus and then in the opposite direction. The nucleus in *Cycas* puts forth a papilla (Fig. 10 *c*) which meets this line of granules and remains attached to it for some time. The line thickens into a

band which lengthens and finally takes the form of a spiral of five or six turns which becomes more or less closely applied to the plasma membrane (Fig. 10 *e*, blepharoplast in section). The cilia develop as protuberances from the outer surface of the band (Fig. 10 *c* and *d*) and grow through the plasma membrane to the exterior of the cell. The nucleus in the meantime has increased in size until it occupies the greater part of the top shaped sperm (Fig. 10 *e*).

The history of spermatogenesis in Ginko is strikingly parallel to that of the cycads. The chief features were first described by Webber ('97c) and in greater detail by Hirase ('98). The two blepharoplasts appear *de novo* on opposite sides of the nucleus in the body cell. They show the same high state of differentiation as those of the cycads, being large and the center of a number of prominent radiations. Ginko however presents a peculiarity not reported in the previous group. A large spherical body lies between each blepharoplast and the nucleus in an area of granular cytoplasm. This structure stains deeply like the globules of nucleolar substance which are frequently found in the cytoplasm after nuclear division. They are probably accumulations of a somewhat similar material at these points in the cell to be utilized at later periods of spermatogenesis, since they decrease in size as the sperms mature. The spindle in the body cell is formed between the blepharoplasts but its poles lie at some distance from and are entirely independent of these structures. During this mitosis the spherical bodies pass to one side of the spindle so that the daughter nuclei (sperm nuclei) finally take the position formerly occupied by them. The blepharoplast becomes granular and begins to lengthen into a band, one end of which becomes attached to the nucleus that puts forth a small papilla towards the blepharoplast. The band elongates and takes the form of a spiral which makes several turns around one end of the cell just under the plasma membrane. Cilia then develop along this band as in the cycads. The earlier accounts, describing a short tail on the sperm were founded upon material that was not altogether normal and have been corrected by Webber and Fujii. The mature sperms have essentially the same form as those of *Zamia* and *Cycas*.

There has been some discussion on the morphology of these motile sperms of the gymnosperms. The claim has been made that they are ciliated spermatids (sperm mother-cells) and therefore different from the sperms of pteridophytes which are formed inside of mother-cells that upon their escape are left behind as empty cysts. However a close analysis of their structure will show that the sperms in both groups have an identical protoplasmic organization. There is a nucleus and a greater or less amount of cytoplasm in which the blepharoplast lies and the entire structure is surrounded by a plasma membrane. Any differences in the processes of spermatogenesis can only concern the greater or less development of a cellulose membrane around the spermatids. It may be true that this cellulose membrane is entirely absent in *Cycas* and *Zamia*, but if present it would be merely a shell like envelope around the sperm and cannot affect its morphological unity and agreement with the sperms of pteridophytes. A comparative study of the composition and formation of the walls enclosing sperm nuclei in the spermatophytes is much needed to carefully distinguish between plasma membranes and the cellulose secretions that may be developed by them.

While the cycads and Ginko have very much the largest sperms known and are consequently extremely favorable for an examination of spermatogenesis nevertheless some surprisingly detailed studies have been made among the Filicineæ and Equisetineæ. Following his preliminary announcements ('97a, '97b, '97c), Belajeff published in '98 an account of spermatogenesis in *Gymnogramme* and *Equisetum*. These forms present histories parallel to each other and to the cycads. Two deeply staining bodies (blepharoplasts) appear on opposite sides of each nucleus previous to the final mitosis in the antheridium which differentiates the spermatids. Consequently each spermatid receives a blepharoplast which lies close beside the nucleus. The blepharoplast begins to elongate and is followed by the nucleus so that both structures form two parallel bands which take a spiral form. (Illustrated in Fig. 3½ of Section I.) The rest of the cytoplasm remains as a vesicle which comes to lie at the larger end of the sperm. The cilia of *Equisetum* could be traced to

definite granules in the band as it develops from the compact spherical blepharoplast.

There appeared almost simultaneously with the foregoing contribution of Belajeff a paper by Shaw ('98b) on *Onoclea* and *Marsilia*. Shaw investigated the cell divisions preceding the formation of the spermatids in *Marsilia* and discovered some very interesting conditions. The two blepharoplasts which are found in the mother cell of the spermatid are foreshadowed by smaller bodies which appear at the poles of the spindle in the two previous mitoses. The first of these structures was called a blepharoplastoid. The blepharoplastoid first appears besides the daughter nucleus after the third mitosis previous to the differentiation of the spermatids. There is therefore one for each nucleus of the grandmother cell of the spermatid. This blepharoplastoid divides but the halves remain close together and the pair passes to one side of the cell. With the next mitosis (the second previous to the differentiation of the spermatids) two new structures are formed at the poles of the spindle and from these the blepharoplasts arise. They accompany each daughter nucleus after this mitosis into the mother-cell of the spermatid. Then each divides and the two blepharoplasts pass to opposite sides of the nucleus which prepares for the final mitosis of the series. This division gives a daughter nucleus to each blepharoplast and the spermatid is thus organized. The later history of the spermatid as it changes into the sperm is identical with Belajeff's results.

Belajeff ('99) followed Shaw's account of *Marsilia* with a study of the same form and came to very different conclusions which have to do chiefly with his belief that the blepharoplast is a centrosome, a view that will presently be considered in connection with the opinions of Strasburger and others. Belajeff found centrosome like bodies (blepharoplastoids of Shaw) at the poles of spindles in various mitoses preceding the formation of the spermatids with their unquestioned blepharoplasts. He is not willing to concede that these centrosome like structures pass into the cytoplasm to disappear there as Shaw states for the blepharoplastoids. He also found the blepharoplasts at the poles of the spindles, which was not observed by Shaw, and holds that they have a part in spindle formation.

We are now prepared to take a general survey of the processes of spermatogenesis to harmonize as much as possible the conflicting opinions respecting the homologies of the blepharoplast. Strasburger (:00, pp. 177-215) has critically reviewed the subject and his conclusions are of great interest. He emphasizes the kinoplasmic character of the blepharoplast, whether it be a differentiated region of the plasma membrane (as he believes for the zoöspores of *Cladophora*, *Ædogonium*, etc.) or a special development in the interior of the cytoplasm (pteridophytes and gymnosperms). Strasburger thinks that all kinoplasmic structures, be they centrospheres, centrosomes or blepharoplasts, hold a very close physiological relation to the substance of the nucleolus and that their appearance and size is largely the result of nuclear activities. Accordingly the blepharoplast might occupy the position of a centrosome without being genetically related to that structure, and in fact centrosomes or centrospheres are to be considered more as products of the cells' activities than as self perpetuating permanent organs. There is abundant evidence that the last possibility is the fact in many forms both plants and animals. Since centrosomes are not found at other periods of the life history of gymnosperms and pteridophytes, Strasburger concludes that the blepharoplasts cannot be genetically related (homologous) with such a structure.

Ikeno and Hirase from their earliest writings have considered the blepharoplast to be a centrosome. Ikeno ('98a) held that the blepharoplast corresponded with the middle piece of the animal spermatozoön. Hirase ('94 and '97) although noting for *Ginko* that the blepharoplasts did not divide and took no part in spindle formation nevertheless called them attractive spheres. The conclusions of Shaw ('98) and Belajeff ('99) for the same type (*Marsilia*) have just been summarized and present very different points of view. Belajeff believes that the blepharoplast of *Marsilia* holds the same relation to the poles of the spindles as a centrosome. But Belajeff's conception of the centrosome ('99, p. 204) is that of a morphological and dynamic center which may or may not be easily demonstrated according to the amount of stainable substance present. From these discussions it is

evident that final judgment cannot be passed until certain questions of fact are established by reinvestigations. Shaw and Belajeff cannot both be wholly correct in their observations and interpretations and much depends upon the exactness of future studies upon Marsilia, other pteridophytes, and in the bryophytes. The problems are also related to the processes of zoospore formation among the thallophytes.

With respect to the bryophytes Ikeno (: 03) has recently published an account of spermatogenesis in *Marchantia polymorpha*. He reports for the mitoses in the antheridium, preliminary to the differentiation of the sperm mother-cells, that a centrosome appears at the side of each nucleus and divides, the two daughter bodies passing to opposite sides of the nucleus and becoming the poles of the spindle. He gives evidence that the daughter centrosomes sometimes divide again when at the poles of the spindle in anaphase. The centrosome cannot be found at the side of the daughter nucleus after the mitosis is completed but it appears when the nucleus is ready for the next division. Ikeno's explanation of the reappearance of the centrosome is unusual. He believes that the centrosome is formed within the interior of each nucleus as a deeply staining body among the linin threads. This body moves to the nuclear membrane and is thrust out into the cytoplasm through a protuberance from the nucleus. It then lies outside of the nucleus and becomes the functioning centrosome, dividing to form two centrosomes that separate to preside over the poles of the spindle. After the final mitoses in the spermatogeneous tissue the centrosomes remain to become the blepharoplasts of the sperms. Each blepharoplast passes to the plasma membrane of its sperm cell and develops two cilia. There is formed at this time another deeply staining body in the cytoplasm considered by Ikeno equivalent to a "Nebenkörper." The nucleus begins to elongate and the "Nebenkörper" takes a position between it and the blepharoplast and in this manner the much attenuated sperm is organized from the mother-cell.

Ikeno considers the blepharoplast of *Marchantia* to be actually a centrosome as shown by its behavior during mitosis. His account therefore in the main supports Belajeff's interpretation

of the blepharoplastoids of Shaw which as just described are regarded by the latter author as centrosomes. Both Belajeff and Ikeno are inclined to use the term centrosome with a looseness that is unusual since the first accounts of this structure gave to it a place in the cell which is not strictly followed in these authors' descriptions of spermatogenesis. Ikeno's account of the intranuclear origin of the centrosome is extraordinary. Intranuclear centrosomes have been reported in several animal forms but they do not leave the nucleus in the manner described by Ikeno.

On the whole the writer is more in sympathy with the views of Webber (:01, pp. 70 to 81), Strasburger and Shaw than those of the other authors. Assuming that the observations upon the cycads and Ginko are correct, Webber is certainly justified in emphasizing the striking fact that the blepharoplasts are completely independent of the spindle in the body cell and that they are formed *de novo* at a distance from its nucleus. These are peculiarities which, if established generally throughout spermatogenesis in plants, will remove the processes entirely from the activities of centrosomes in certain thallophytes (*e. g.* *Stypocaulon*, *Dictyota*) and in many animal cells. It is certainly to be expected that a centrosome when present will always hold an intimate relation to spindle formation during mitosis. It need not be a permanent organ in cell genesis and an ever increasing number of investigations indicate that it frequently is not. Therefore many authors hold that the centrosome is rather the morphological expression of a dynamic center than a protoplasmic structure with an individuality comparable to the organs of a cell. But these universal characteristics of centrosomes are apparently not present in the blepharoplasts of the gymnosperms nor, according to Shaw, in the pteridophytes (*Marsilia*). But then the observations of Belajeff and Ikeno are not in accord with those of Shaw and it is possible that studies in zoöspore formation and gametogenesis among the thallophytes may present the subject in new lights.

For as shown in our discussion of the zoöspore it is not clear whether the blepharoplasts in those cells are always derived in the same manner. We have Strasburger's view that the

structures are thickenings of the outer plasma membrane (hautschicht) and opposed to this Timberlake's account for Hydrodictyon in which the blepharoplast is considered as a structure independent of the plasma membrane although lying in contact with it. It must be apparent that the results of Timberlake are in essential agreement with the events of spermatogenesis in the pteridophytes and gymnosperms while those of Strasburger introduce new elements in giving to the plasma membrane the functions of forming a blepharoplast. The process of spore formation in the ascus must also be considered in this connection for in that sporangium a centrosphere associated with each nucleus develops numerous fibrillæ that resemble so much a cluster of cilia as to suggest at once a blepharoplast-like structure, but this centrosphere of course is an important factor in spindle formation during the mitoses in the ascus. Indeed we may well ask for further studies in spermatogenesis and zoöspore formation before we can expect a solution of the problem of the blepharoplast.

Comparisons have been made between the sperms of animals and plants, and some authors (*e. g.* Wilson :00, p. 175, Belajeff '97c) consider the two cells in essential agreement as to structure and development. However these views rest on the assumption that the blepharoplast is truly the homologue of a centrosome. It seems to be established that the locomotor apparatus of the animal spermatozoon is derived chiefly from one or more centrosomes, generally with the co-operation of archoplasm (idiozome, Nebenkern) present in some form near the nucleus. It is true that in plants the locomotor apparatus is derived from kinoplasm which as we pointed out in Sections I and II corresponds closely to the archoplasm of Boveri, but this is very far from implying that structures formed by the archoplasm and kinoplasm respectively need be homologous. Indeed both archoplasm and kinoplasm are distinguished by their physiological activities rather than by their morphological manifestations which are too various to allow of close genetic relationships. Therefore it seems far from established that spermatogenesis in plants is along the same lines as in animals, especially since the weight of evidence at present indicates that the blepharoplast is not a centrosome.

There are numerous problems connected with the physiology of the sperm that bear directly upon its protoplasmic structure. Some of these will be treated in Section IV in connection with processes of fertilization. But at this time it is well to call attention to the intimate association that sometimes exists between the nucleus and blepharoplast. These structures come into actual contact in *Cycas* and *Ginko* through a process put forth from the nucleus. It should also be remembered that Timberlake and Dangeard found the blepharoplasts in the zoöspores of *Hydrodictyon* and in the cells of *Polytoma* connected with the nucleus by one or two fibers. The nuclear beak that bears the aster in the ascus suggests a similar relationship. These conditions indicate that the activities of locomotion may depend vitally upon the nucleus.

### 3. The Egg.

The subject of fertilization is reserved for the next section (Section IV) of this series and the present account will deal only with the structure of the unfertilized egg. As the sperm is derived from a motile gamete identical with the zoöspore, so the egg has had a similar origin. We have traced the steps in this evolutionary process among the algæ in a former paper (*Popular Science Monthly*, Feb. 1903, p. 300). The first indication of a differentiation in the sex of primitive gametes is one of size. The male gametes tend to become smaller while the female contains a greatly increased amount of cytoplasm. One of the important factors determining this differentiation is the number of nuclear divisions which take place in the cells that produce respectively eggs or sperms. There are generally a great many more mitoses in antheridia than in oögonia and consequently a given amount of protoplasm must be very much divided to provide each nucleus with its quota of cytoplasm.

The tendency of oögenesis on the contrary is to conserve the protoplasm for relatively few nuclei, provided for several eggs or for a single nucleus in a solitary egg, with the result that the egg cell is generally richly supplied with protoplasm. Such processes result in large cells with a prominent chromatophore or

numerous plastids and not infrequently a considerable amount of food material. The primitive female gametes were provided with cilia like the male, but with their increase in size came a sluggishness of movement which resulted in much shorter periods of motility on the part of these sexual cells. There are some algæ (*Ectocarpus siliculosus*, Cutleria, Aphanochæte) whose motile female gametes come to rest shortly after their escape from the oögonia and are fertilized as quiescent cells by the active sperms. These female gametes at the time of fertilization behave physiologically like eggs although their development shows a morphology identical with the sperm. When such female gametes dispense with cilia entirely they become eggs.

The absence of cilia does away with very much of the complexity which we have just described for sperms. There is no trace of the blepharoplast in the egg and no indication of the activities associated with this structure, so conspicuous in spermatogenesis. The large motile female gametes of such Algæ as Bryopsis, Cutleria, Aphanochæte and certain species of Chlamydomonas and Ectocarpus will probably show some interesting conditions when the details of their cell structure and development are known, for some of these types are likely to throw light on the relation which the blepharoplast bears to other structures in the cell.

The eggs of all plants (Fungi excepted) are believed to be richly stocked with plastids in sharp contrast to the sperms which are entirely destitute of these structures in all groups above the algæ. The plastids in the eggs of Algæ contain the pigments characteristic of the respective groups giving these cells a very rich coloration and sometimes an elaborate internal structure since these plastids or the single chromatophore generally maintain a symmetrical relation to the nucleus. Leucoplasts (see Fig. 11a) have been found in the eggs of angiosperms (Schimper, '85) but detailed studies on the cytoplasm of such cells in spermatophytes, pteridophytes and bryophytes are greatly to be desired to determine the history of plastids during the development of these germ cells and at later periods after fertilization.

The distribution of the plastids in the eggs of Algæ may be so general that the entire cell is colored as in *Fucus*, *Volvox* and *Sphæroplea*. Or, the plastids may be largely or wholly withdrawn from some portion of the egg. It is usual for eggs retained within the parent cell (oögonium) to present a colorless area of protoplasm that becomes the point at which the sperm fuses with the egg. Such a hyaline region is called the receptive spot and is generally situated (see Fig. 11*b*) at the side of the egg nearest the pore or opening in the oögonium through which the sperms enter. Excellent illustrations are presented among the Algæ in *Vaucheria* (Oltmanns, '95), *Ædogonium* (Pringsheim, '58, Klebahn, '92) and *Coleochæte* (Pringsheim, '60, Oltmanns, '98). It has been suggested that the receptive spot is related to the clear ciliated end of the ancestral motile gamete and zoöspore but the structures have not been critically compared to determine the precise character of their protoplasmic structure and development. The receptive spot in some forms (*Vaucheria*, *Ædogonium*, Fig. 11*b*) lies directly under the opening that is formed in the oögonium and its protoplasm is probably concerned with the fermentative action that destroys the wall at that point.

The red Algæ (*Rhodophyceæ*) do not have eggs although in their sexual evolution they are at the level of heterogamy. The female gamete (carpogonium with its trichogyne) is a cell homologous with an oögonium and its protoplasmic contents correspond to an egg, but the protoplast never withdraws from the cell wall to lie freely as a naked mass of protoplasm within the structure. But the general agreement of the carpogonium and trichogyne with the oögonium and its neck like extension in *Coleochæte* seems to determine without doubt the homologies of the former.

There are very few eggs among the fungi that are strictly comparable to those of the Algæ. *Monoblepharis* (Thaxter '95a) however unquestionably furnishes such an example. But the eggs of the *Saprolegniales* and *Peronosporales* are probably in the author's opinion not directly derived from those of Algæ. They are either a peculiar form of sexual cell called the cœnogamete (Davis :00 and :03) or closely related to this structure

which will be given a separate treatment in this section. The cœnogamete is the homologue of a multinucleate gametangium but its evolutionary tendencies seem to be towards such a reduction in the number of nuclei that in the highest expression of its sexual differentiation the female cell contains a single nucleus and has the general form of an egg. But this process of sexual evolution is entirely independent of the well known lines of development in the Algæ (Davis, *Popular Science Monthly*, Feb. 1903). The female sexual cell of the Ascomycetes (called the ascogonium or archicarp) is probably in most forms the homologue of a gametangium. These subjects will be treated in our account of the cœnogamete.

The egg in the archegonium of bryophytes and pteridophytes is generally reported to have a clearer region on the side nearest the neck and this is called the receptive spot. It is reported by Campbell in his investigations on *Pilularia* ('88), *Iscetes* ('91), *Osmunda* ('92a), *Marsilia* ('92b), and *Marattia* ('94), by Shaw in *Onoclea* ('98) by Thom in *Aspidium* and *Adiantum* ('99) and by Lyon in *Selaginella* (:01). The receptive spot is generally believed to be a portion of the egg differentiated to receive the sperm. It is an open question whether this area is morphologically the homologue of the receptive spot in the eggs of algæ and the clear area at the ciliated end of motile gametes and zoöspores. The problem demands a detailed study of the finer protoplasmic structure to determine whether or not it is ktoplasmic in character. The nucleus is generally situated near the center of the egg and the portions of the cell farthest away from the neck of the archegonium contain coarsely granulate protoplasm which is evidently trophoplasmic, *i. e.*, much of its substance is of the nature of food material and the products of metabolism. The leucoplasts would be supposed to lie in this region of the cell but we know nothing of their presence and behavior in the egg of bryophytes and pteridophytes.

The eggs of gymnosperms generally speaking present sharp contrasts to those of pteridophytes. They are very large, probably the largest uninucleate cells in the plant kingdom, and consequently very attractive for cell studies and some of the best work on the events of the maturation and fertilization of plant

eggs has been done on this group (to be treated in Section IV). Passing over earlier investigations that described accurately the general structure of the egg of gymnosperms we shall consider the results of a number of comparatively recent papers that treat especially the pine, spruce (*Picea*), hemlock (*Tsuga*), fir (*Abies*), cycads, Ginko, Gnetum, Taxodium, etc.

Oögenesis and fertilization in the pine has been the subject of several extensive studies the chief being papers by Dixon ('94), Blackman ('98), Chamberlain ('99) and Ferguson (:01b). The protoplasm of the egg is at first vacuolate but later takes on a denser structure which becomes very puzzling because of numer-

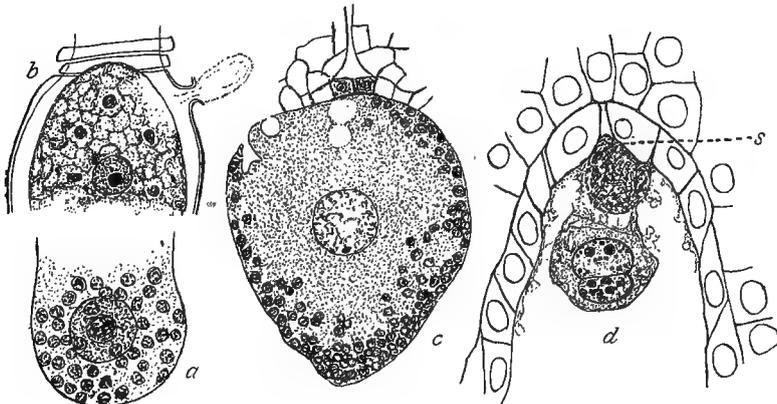


FIG. 11.—The Egg. *a*, *Daphne*, showing leucoplasts; *b*, oedogonium, showing receptive spot; *c*, pine, with numerous proteid vacuoles; *d*, embryo sac of the lily, gamete nuclei fusing, remains of one Synergid (*s*) shown. (*a*, after Schimper, '85; *b*, Klebahn, '92; *c*, Ferguson, :01.)

ous granular inclusions and masses of amorphous material which together with fibers present a very complex texture. The fibers are sometimes collected in fascicles and they may form a sort of weft at the periphery of the egg or radiate out from the nucleus which is generally surrounded by a kinoplasmic sheath. The complexity is greatly increased as the egg grows older by the development of remarkable structures called proteid vacuoles (See Fig. 11*c*) which have been especially described by Blackman and Ferguson. The number of proteid vacuoles is exceedingly variable in the egg but they sometimes fill three fourths of the structure. They are spaces in the cytoplasmic reticulum filled

with granules and irregular masses of a proteid nature, some of which stain like nucleoli. The proteid vacuolee were considered nuclei by earlier writers (Hofmeister and Goroschankin) and recently this view has been revived by Arnold (:oob) who describes the migration of large numbers of nuclei from the cells of the jacket surrounding the egg into that structure. These results have not been confirmed by Ferguson who agrees with the interpretation of other writers that the resemblance of the proteid vacuoles to nuclei is superficial. Miss Ferguson believes that the material of the proteid vacuoles is derived in part from the nucleoli in the cells of the jacket and from those in the egg. A vacuole is reported (Ferguson) at the end of the egg nearest the neck of the archegonium and this is regarded as a sort of receptive spot since the pollen tube discharges its contents into this cavity. The egg nucleus is very large and its contents are not arranged with the regularity generally present in resting nuclei. There are numerous bodies which Chamberlain believes to be chromatic in composition that look very much like nucleoli and have been so designated by that writer. But there is generally one large unquestioned nucleolus and besides this many smaller nucleoli are reported by Ferguson as held in the linin reticulum. Then portions of the linin frequently take irregular forms and stain heavily. There is also present besides the linin, chromatin and nucleoli much granular material (metaplasm), especially in the nuclei of younger eggs, which probably holds some relation to the chromatin although it may readily be distinguished at certain times from that substance.

Recent accounts of the spruce and fir, by Miyake (:o3a and :o3b) describe conditions very much as in the pine. The egg of the spruce (*Picea*) is apparently not so fibrous in structure but proteid vacuoles give it a coarse granular structure. He finds no evidence in support of Arnoldi's (:oob) peculiar views that the proteid vacuoles are derived from nuclei that have passed into the egg from cells of the sheath. They are simply masses of nutritive material. There is some doubt whether the vacuoles present at the end of the egg really represent a differentiated receptive spot. The egg of the fir (*Abies*) conforms in all essentials to the structure in the pine and spruce. There are numerous proteid vacuoles.

It is probable that the eggs of other conifers will be found to present much the same protoplasmic structure and activities as those of the pine. Thus Murrill (:00) describes for the hemlock spruce (*Tsuga*) a vacuolar receptive spot and figures masses of food material very much like the proteid vacuoles. The general features of the egg of *Cephalotaxus* (Arnoldi, :00a), *Thuja* (Land, :02), *Podocarpus* (Coker, :02), *Taxodium* (Coker, '03) have been recently described and those of *Abies*, *Larix* and *Taxus* are familiar from older writers but the pine remains as the type of conifer in which the events of oögenesis are best known as regards the details of protoplasmic activities.

Besides the pine we have had some very complete investigations on cycads and *Ginko* (Hirase, '98, Ikeno, '98b and :01, Webber, :01). In some respects these types and especially the cycads seem to be the most favorable of all the gymnosperms for the study of gametes and the processes of fertilization (to be described in Section IV). The cytoplasm of the egg is comparatively homogeneous in structure so that the cell is relieved from the complicated fibrous structure and proteid vacuoles present in the pine. Ikeno ('98b) finds that the egg of *Cycas* develops a crater like depression just before and at the time of the fusion of the sperm thus presenting a rather highly specialized receptive spot.

We know almost nothing of the detailed structure of the egg in the Gnetales. *Ephedra* (Strasburger, '72) develops archeogonia much like those of other gymnosperms and we should not expect their eggs to be materially different even in details. But the conditions in *Tumboa* (*Welwitschia*) are peculiar and approach more closely those of angiosperms where the egg nucleus is scarcely differentiated from neighboring nuclei lying freely in the protoplasm at one end of the embryo sac. The eggs of *Tumboa* (Strasburger, '72) are merely cells of the prothallus that push out small projections to meet the pollen tubes. *Gnetum* presents a further simplification or reduction since the female nuclei lie freely in the protoplasm at one end of the embryo sac. In *Gnetum gnemon* the lower half of the embryo sac is filled with a tissue (Lotsy '99) but in several other species studied by Karsten ('92, '93) no cell walls are found in the entire sac until after fertilization.

The angiosperms present no especial advance over Gnetum in the organization of the egg except that this structure is generally reduced to a single female nucleus and the cytoplasm immediately around it (see Fig. 11 *d*). This egg nucleus flanked by two companions (synergids) and the accompanying protoplasm compose the egg apparatus whose morphology is still a matter of dispute. It is possible that the synergids may stand for portions of a reduced archegonium, but the two nuclei bear such close relations to the egg and polar nucleus that it seems very probable that they are homologous with these structures which have clearly defined sexual potentialities. In spite of the numerous studies on embryo sacs in various groups of angiosperms we do not yet know precisely how the cytoplasm becomes gathered around the egg nucleus and the synergids. The spindles that are formed between these nuclei in some types (*e. g.*, *Lilium*) have been supposed to lay down walls by means of cell plates. But there are other forms in which the protoplasm seems to separate along planes of vacuoles without relation to spindle fibers.

(*To be continued.*)





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## STUDIES ON THE PLANT CELL.—IV.

BRADLEY MOORE DAVIS.

### SECTION III. HIGHLY SPECIALIZED PLANT CELLS AND THEIR PECULIARITIES (*Continued*).

#### 4. The Spore Mother-cell.

THE spore mother-cell and its homologues the pollen mother-cell and certain embryo-sacs have furnished some of the most interesting subjects for cell studies in the plant kingdom. Sporogenesis in all plants above the thallophytes seems to be a period when nuclear structures are especially clearly differentiated and when the mechanism of mitosis reaches the highest degree of complexity. These intricate conditions are only equalled by processes in the development of the female gametophyte of some angiosperms, and during endosperm formation, also in the events of spermatogenesis and with the segmentation of the egg nucleus of certain gymnosperms.

Sporogenesis is one of the critical periods in the life history of a higher plant since it is the time when the asexual generation (sporophyte) passes over to the sexual (gametophyte). This provides certain important features such as the reduction phenomena concerned with chromosomes and greatly adds to the interest in these cells. These matters will receive special attention in Section V, but they must be borne in mind to appreciate fully the significance of many events of spore formation.

The general history of the spore mother-cell may be described as follows: It is the product of the last mitosis in the reproductive tissue called the archesporium. This mitosis always presents the number of chromosomes characteristic of the sporophyte which is double the number found in the gametophyte. Therefore the nucleus that passes into the spore mother-cell has the sporophyte number of chromosomes. Two

mitoses occur successively in the spore mother-cell in all forms. The first mitosis presents half the number of chromosomes found in the last nuclear division in the archesporium and is consequently the reduced or gametophyte number. The reduction of the chromosomes then takes place during the period of rest between the last mitosis in the archesporium and the first in the spore mother-cell. There are two mitoses in the spore mother-cell. ~~In some forms these are exactly alike and present essentially the same characters as the usual typical mitoses of plants.~~ But among the spermatophytes there are likely to be peculiarities in the arrangement and distribution of the chromosomes. In consequence the first mitosis may be heterotypic and the second homotypic in contrast to the normal typical conditions. The description and explanation of these characters will be reserved for the groups that illustrate them the best. They have nothing to do with qualitative reduction phenomena as was formerly supposed.

There is sometimes a well defined period of rest after the first mitosis with the formation of a wall between the two daughter nuclei, but frequently the second mitosis follows immediately after the first so that the spore mother-cell comes to contain four daughter nuclei. Cell walls may then be formed between these nuclei simultaneously so that the resultant spores are disposed in a radially symmetrical arrangement that is termed tripartite. These cell divisions are almost universally present in the spore mother-cell, the only exceptions being certain spermatophytes whose megaspore mother-cells develop directly into embryo sacs, the two mitoses (heterotypic and homotypic) being included within these structures and forming a part of the gametophyte history. ~~Why the number of spores should generally be four is unexplained. There does not seem to be any physiological significance in the number or other reasons why it should not be more or less.~~ Indeed it is somewhat variable in the spermatophytes for microspore or pollen mother-cells form two and three pollen grains in certain types and five, six and seven have been found in others, while much larger numbers have been occasionally reported. In no case is the microspore mother-cell known to develop directly into a pollen grain, al-

though the megaspore mother-cell regularly becomes an embryo sac in some forms (*e. g.*, *Lilium*). But an increasing number of observations indicate that the megaspore mother-cell generally develops two, three or four potential megaspores although normally only one of these becomes an embryo sac.

The interest in the protoplasmic activities of sporogenesis lie chiefly in the elaborate methods of spindle formation and mechanism of mitosis, in the organization and distribution of the chromosomes, in the functions and activities of the nucleolus, and in the organization of the cell plate and development of the cell wall. There is a very extensive literature on the spore mother-cell some of which, however, merely treats the broad features noted in studies of a general morphological character on the development of sporophylls or floral structures. We shall only attempt to consider the most important contributions, and for convenience will begin our treatment with the Hepaticæ and conclude with the spermatophytes where the conditions are the most complex.

The Hepaticæ or liverworts furnish some remarkable spore mother-cells, and are now the subject of considerable interest and some discussion. They were first brought conspicuously to the attention of botanists by a paper of Farmer ('94) on *Pal-lavicinia decipiens*. Farmer described a remarkable series of events in this type. The nucleus of the spore mother-cell became surrounded before division by dense protoplasm that extended into the four lobes of the cell in the form of a four-rayed star which he called a "quadripolar spindle." After its development four chromatic droplets appeared in the nucleus to indicate its approaching division. These chromatic droplets became four chromosomes which by division were doubled in number. The eight rod shaped chromosomes moved in pairs towards the four lobes of the spore mother-cell. There was a further division of each chromosome, making sixteen in all, and the four groups of four each passed simultaneously to the poles of the "quadripolar spindle" which persisted to the end. It should be noted that the striking peculiarities of Farmer's account lie in the division of the four primary chromosomes into sixteen, and in their simultaneous distribution through a "quadripolar

spindle" to form at once four daughter nuclei. These events are unparalleled, as far as the writer is aware, in the plant or animal kingdom, and consequently the account deserves especial attention. A four-rayed figure around the nucleus is not surprising because the spore mother-cell of the Jungermanniales is four lobed, and its centrally placed nucleus lies in a restricted area. But the simultaneous distribution of quadrupled chromosomes to form four daughter nuclei is a process whose establishment would be of fundamental significance. Farmer also described a centrosome at each pole of the "quadripolar spindle."

Farmer ('95*a*, *b*, and *c*) followed his paper on *Pallavicinia* with studies on other liverworts. He reported the "quadripolar spindle" in the early stages of mitosis in several of the Jungermanniales, but did not find the quadrupling and simultaneous distribution of the chromosomes as in *Pallavicinia*. The "quadripolar spindle" when present was a temporary structure replaced later by the bipolar spindles of two successive mitoses with a longer or shorter interval between. Farmer considers the "quadripolar spindle" of these forms as transitional between that of *Pallavicinia* and the normal bipolar spindle. The Ricciales, Marchantiales and Anthocerotales present two successive mitoses after the usual manner in the spore mother-cell.

The writer has described the events of sporogenesis in *Pellia* (one of the Jungermanniales) in a paper covering the nuclear activities at several periods in its life history (Davis, :01), and confirmed much of Farmer's account of the mitoses in this spore mother-cell. These are two in number and successive, with a very well defined resting period between the first and the second. There is a four-rayed figure present during the prophase of the first mitosis, and this seems to correspond to Farmer's "quadripolar spindle." The nucleus lying in the center of the four lobed spore mother-cell becomes invested by a kinoplasmic sheath which develops a fibrillar structure. Many of these fibrillae extend into the lobes of the spore mother-cell because the nucleus is confined to a narrow space in the constricted central region of the cell and the lobes offer the only possible relief for the crowded conditions. However, the four-rayed structure is not present when the chromosomes are ready for distribution,

but there is found instead one large, broad poled spindle. (See Fig. 5 *e*.) A cell wall is formed between the two daughter nuclei (Fig. 8 *d*) which divide again after a very short period of rest, the two spindles lying at right angles to one another. The poles of the spindles are rather blunt, and there are no centrosomes or centrospheres in either mitosis. The four-rayed structure of prophase must be regarded as preliminary to spindle formation because the chromosomes are not ready for distribution, and when that period arrives the structure has been replaced by the true spindle of the first mitosis. These facts led me to question Farmer's account of mitotic phenomena in *Pallavicinia* and his conception of the "quadripolar spindle," and I suggested that this structure might prove to be a phenomenon of prophase, a view to which Farmer (:01) has taken exception in a criticism of my results.

Recent investigations of Moore (:03) on *Pallavicinia* are flatly contradictory to the conclusions of Farmer for *Pallavicinia decipiens* and support my suggestions. Moore finds that there are two mitoses in the spore mother-cell of *Pallavicinia lyellii*, the second (Fig. 12 *c*, *d*) following immediately upon the first (Fig. 12 *b*), each with bipolar spindles and without centrosomes. The chromosomes, eight in number, appear in the usual way with each mitosis (Fig. 12 *c*, *d*). There is no "quadripolar spindle" in Farmer's sense, no quadrupling and simultaneous distribution of the chromosomes. The prophases preceding the first mitosis present a tetrahedral form as is shown in Fig. 12 *a*. This is accentuated by the fibrillae which gather at the points to make a four-rayed structure extending into the lobes of the spore mother-cell. This condition is identical with similar stages in *Pellia* and in other leafy liverworts, and is a feature to be expected from the fact that the spindle fibers develop chiefly or wholly externally to the nuclear membrane in a rather crowded region of the cell. The nucleus at this time is unquestionably in prophase as shown by the undifferentiated chromosomes and because this stage passes immediately into a bipolar spindle of the normal type (Fig. 12 *b*). It seems very probable that Farmer was mistaken in his conclusions for *Pallavicinia decipiens*, and that the mitoses in the spore mother-cell of this form

are not different in any essentials from those of other plants.

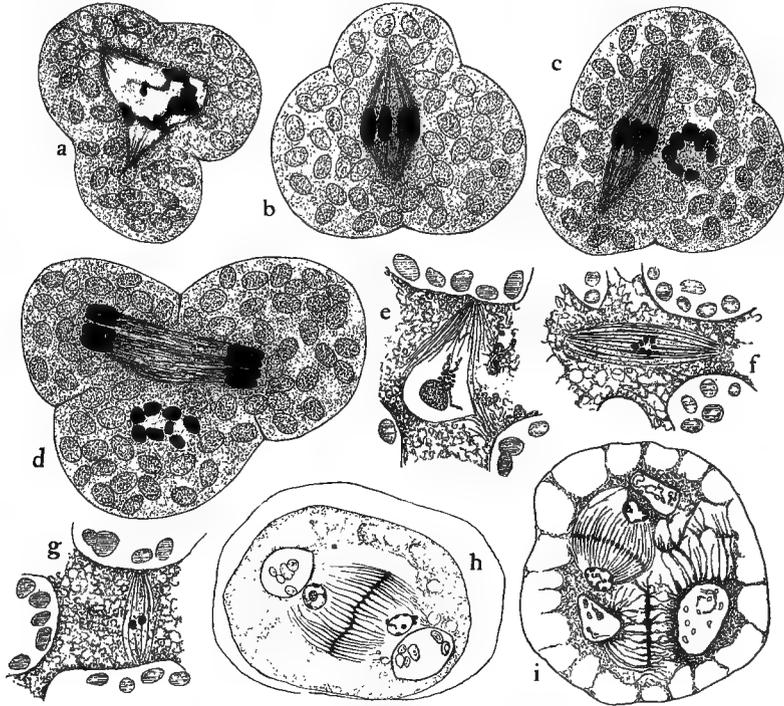


FIG. 12.—Spore mother-cells of Hepaticæ. *a, b, c, d, Pallavicinia lyellii.* *a*, Prophase; the fibrillæ gathered on four sides of the nucleus which has a tetrahedral form pointing into the four lobes of the spore mother-cell; the nuclear membrane has not yet broken down; similar stages of prophase were probably considered by Farmer as quadripolar spindles. *b*, metaphase of the first mitosis; the spindle in all respects a normal bipolar structure without centrospheres. *c*, Metaphase of the second mitosis; one spindle shown in side view, the other, almost perpendicular to the first, presents the eight chromosomes at the nuclear plate. *d*, anaphase of the second mitosis; one spindle viewed from the side, the other from one end shows the group of eight grand-daughter chromosomes. *e, f, g, anthoceros laevis, h, i, a larger species from Italy.* *e*, prophase; one pole of spindle developed. *f*, just after metaphase of the first mitosis; eight chromosomes; blunt poled spindle without centrospheres. *g*, metaphase of second mitosis; very small spindle. *h*, cell plate forming in the spindle between two nuclei. *i*, two nuclei at the side of their respective chromatophores and the cell plate between, after the second mitosis; a third chromatophore shown with strands of protoplasm connecting it with other regions of the cell. (*a, b, c, d*, after Moore, :03; *h, i*, after Van Hook, :00.)

The “quadripolar spindle” proves to be nothing more than a condition of prophase.

Besides Pellia and Pallavicinia, which are the most thoroughly studied of the lower liverworts, we know the processes of sporo-

genesis in the highest type, *Anthoceros* (Davis, '99). This form is exceedingly attractive for such investigations because the spore mother-cells may be found in all conditions upon the same sporophyte. However, the small size of the nuclei and spindles is a disadvantage. Just previous to the first mitosis the nucleus becomes surrounded by a mesh of delicate fibrillæ (kinoplasmic). Later the nucleus takes an angular form, and the fibrillæ are found conspicuously at the prominent poles (Fig. 12 *e*). The nuclear membrane breaks down and the fibers become arranged to form a bipolar spindle (Fig. 12 *f*) without centrosomes or centrospheres. There is a short period of rest after the first mitosis, but no wall is formed between the two daughter nuclei. The small spindles of the second mitosis (Fig. 12 *g*) are likewise bipolar. They lie at right angles to one another and the cell plates that are laid down determine, in part, the position of the walls that are formed between the four granddaughter nuclei and which divide the spore in a tripartite manner. These cell plates are very small (Fig. 12 *h* and *i*), but they have been observed in a favorable species of *Anthoceros* by Van Hook (:00). It is not clear how these plates become extended to the wall of the spore mother-cell unless (as suggested in Sec. II) their edges make use of planes of vacuoles when the protoplasm separates to develop the cleft between the four daughter cells. The poles of the spindles in *Anthoceros* are flattened and entirely free from structures that might be considered centrosomes.

Other interesting events of sporogenesis in *Anthoceros* are the division of the chromatophores and the nuclear condition termed synapsis. The young spore mother-cell contains a single large chromatophore. This increases greatly in size and becomes filled with starch grains. The chromatophore divides successively into two and then four portions which arrange themselves symmetrically in the cell with the nucleus in the center. The mitoses then follow and the four daughter nuclei are distributed, one for each chromatophore in the cell. This provision of four chromatophores long before the mitoses in the cell seems very remarkable (Davis, '99, p. 94 and 95). Synapsis is a condition very common in the nucleus of spore mother-cells before division. The chromatic material becomes gathered into a compact

mass besides the nucleolus. The significance of synapsis is not clear, but the subject will be discussed in Section VI. However, there is good evidence from *Anthoceros* that the phenomenon is a normal event and not an artefact, because synapsis is always found at a certain period of sporogenesis, and nuclei in neighboring spore mother-cells a little older or younger present their chromatic material with the usual arrangement (Davis, '99, p. 96 and 97).

To summarize the conditions in the spore mother-cells of the Hepaticæ, all conclusions, in the author's opinion, indicate: (1) That the spindles develop from a surrounding weft of fibrillæ without the assistance of centrosomes. (2) That the mitoses are always two in number and successive with the same number of chromosomes for each division. (3) That the cell walls may be formed successively as in *Pellia* and some other of the Jungermanniales or simultaneously, to give tetrahedral spores, as in *Anthoceros*, types of the Marchantiales and Ricciales, *Pallavicinia* and some companion forms in the Jungermanniales. It will be interesting to note the essential agreement in these matters between the Hepaticæ and the higher plants.

Nothing is known of the nuclear activities during sporogenesis in the other great division of the bryophytes, the mosses (Musci). The spore mother-cells in this group are always small and unattractive for cell studies but the Sphagnales appear to be rather the most promising for such investigations, which are greatly to be desired.

The pteridophytes have furnished some important contributions to our knowledge of the spore mother-cell. There is first the paper of Osterhout ('97) on spindle formation in *Equisetum*, which was one of a group of three contributions (Mottier, '97, Juel, '97) that did much to dispose of a then prevalent belief that the development of the spindle in higher plants was controlled by centrosomes. This investigation was followed by a study of Smith (:00) on spindle formation in *Osmunda*. Calkins ('97) and W. C. Stevens ('98*a*) considered especially the formation and reduction of chromosomes in several of the ferns, and arrived at contradictory conclusions. Strasburger (:00, p. 76 to 79) has reviewed these results in relation to studies of his own on *Osmunda*.

Osterhout's ('97) account of spindle formation in *Equisetum* is noteworthy. He found that the nucleus of the spore mother-cell became surrounded by a web of delicate fibrillæ, which, extending radially into the surrounding cytoplasm (Fig. 13 *a*), were later (Fig. 13 *b*) gathered into numerous pointed bundles or cones. After the dissolution of the nuclear membrane these

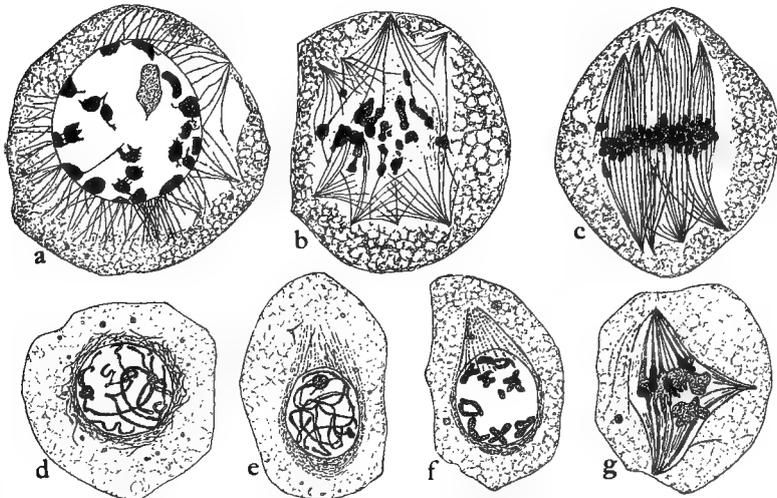


FIG. 13.—Spore mother-cells of Pteridophytes. *a, b, c, Equisetum limosum.* *a*, prophase of first mitosis; the radially disposed fibrillæ are gathering together into cones. *b*, prophase, older than *a*; the nuclear membrane has broken down and the fibrillæ have entered the nuclear cavity; the cones lie in two groups opposite one another. *c*, just before metaphase; the fibrillar cones are nearer together and the chromosomes have gathered to form the nuclear plate. *d, e, f, g, Osmunda regalis.* *d*, very early prophase of the first mitosis; nucleus in the spirem stage surrounded by a granular and fibrillar zone of kinoplasm. *e*, prophase, somewhat older than *d*; fibrillar kinoplasm showing polarity. *f*, still older; chromosomes formed; one pole of spindle developed. *g*, metaphase; a tri-polar spindle. (*a, b, c*, after Osterhout, '97; *d, e, f, g*, Smith, :oo.)

cones arranged themselves side by side in two sets to form the spindle of metaphase (Fig. 13 *c*). The spindle is then from the outset multipolar, and even though some of the cones unite when they become grouped around a common axis, nevertheless the poles of the spindle at metaphase show their composite nature in the absence of a common focal point for the fibrillæ. There are no centrosomes at the poles and no reason for their presence at any stage in the process of spindle formation.

Smith's (:oo) study of *Osmunda* presents an important confirmation of Osterhout's conclusions that the spindle in pterido-

phytes developed without centrosomes, while illustrating a process of spindle formation along somewhat different lines. Smith distinguished a zone of kinoplasm around the nucleus previous to spindle formation. This zone became granular, and then the granules arranged themselves in rows to form fibrillæ (Fig. 13 *d*), which, however, did not extend into the cytoplasm radially, but lay generally parallel to one another, so that the spindle appeared bipolar from the beginning (Fig. 13 *e*). One pole of the spindle was generally formed considerably in advance of the other (Fig. 13 *f*). The fibers did not meet at a common point but over a broad area, and there were no centrosomes. There is, then, normally no multipolar stage in *Osmunda*, although tripolar spindles (Fig. 13 *g*) were occasionally found. During anaphase secondary fibers were put forth from the vicinity of the daughter nuclei and these met in the equatorial region of the cell. The spindle of the second mitosis was formed exactly as in the first. After this division the four granddaughter nuclei lay connected 'with one another by six spindles (two primary and four secondary). Cell plates were laid down in the equatorial regions of these spindles so that the protoplasm became divided simultaneously and symmetrically into tetrahedral spores.

The studies of Calkins ('97) and Stevens ('98*a*) were chiefly upon the division and distribution of the chromosomes in connection with reduction phenomena. Calkins believed that the processes of sporogenesis followed the same course as the maturation of sexual cells in animals, with a transverse division to give a qualitative reduction in Weismann's sense. Stevens disagreed with Calkins in several particulars, holding that the reduction was merely quantitative. Reduction phenomena in plants is now much better understood than at the time of these papers which dealt with plants much more difficult to study than some other forms (*e. g.*, types of the Liliaceæ). We shall consider the subject in Section V, but may state now that Calkins' conclusions have not been sustained.

Strasburger (:00) gives considerable attention to spindle formation in his well known review and critique of cytological literature. He proposes the following classification of spindles in higher plants which lack centrosomes. Those that pass

through multipolar stages and later become bipolar are called multipolar polyarch spindles. When the spindle has a well defined axis from the beginning, as is generally true of the cells in vegetative tissues of higher plants, it is termed multipolar diarch. Strasburger has shown that these types, while easily separated in the extremes, grade into one another so that the classification is not founded on distinctions of a very fundamental character. The spindle of *Osmunda*, for example, resembles a multipolar diarch, but its method of development is more closely related to that of other spindles in spore mother-cells (multipolar polyarchs) than to those of vegetative tissues.

The gymnosperms offer in *Larix* an excellent subject for studies on the formation of pollen, and this type has been treated in several important papers, notably by Belajeff ('94*b*), Strasburger ('95) and Allen (:03). Belajeff's contribution is important as the first investigation that considered the multipolar spindle as a preliminary stage in the development of the bipolar structure. Other authors, at this time and previous to his publication, had noted multipolar and tripolar spindles (Strasburger ('80) and ('88) in several forms), but the lily had received the greatest attention in this connection (Farmer ('93) and ('95*a*), Strasburger ('95), Sargent ('97) and Mottier ('97)). Mottier's investigation presented the first detailed account of spindle formation in this angiosperm and will be discussed presently.

Allen's (:03) paper on *Larix* includes one of the best discussions of the literature bearing on the subject of spindle formation that has yet appeared. He finds that the cytoplasm around the nucleus just previous to mitosis comes to contain a loose network of fibrillæ. Some of the fibers may be followed through the nuclear membrane and may be seen attached to chromatin bodies in the interior (Fig. 14 *a*). Later the cytoplasmic fibrillæ become arranged radially and extend from the nucleus even to the outer plasma membrane at the periphery of the pollen mother-cell. The radiating fibers are connected with one another by branches which indicate that the structure is in part an expanded condition of the original network, but the fibers also grow. The fibers now fold over so that they tend to lie parallel to

the surface of the nucleus and thus form a dense felt around the nuclear membrane. Presently the nuclear membrane which was before a definite film becomes wavy in outline and often granular in appearance. The nucleolus shows signs of dissolution and there is a marked increase in the number of intranuclear fibers, which are chiefly or wholly of nuclear origin. After the

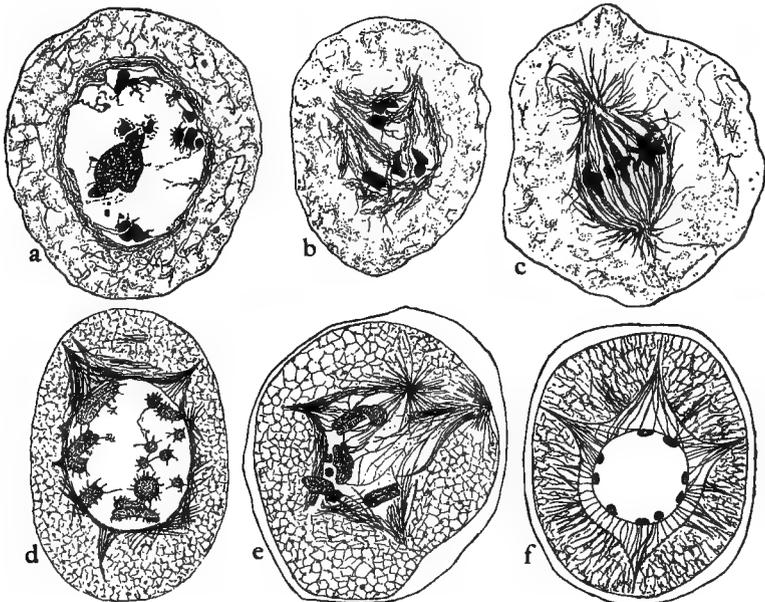


FIG. 14.—Pollen or microspore mother-cells of spermatophytes. *a, b, c, Larix europæa.* *a*, prophase of first mitosis; kinoplasmic fibrillæ forming a felt around the nucleus. *b*, late prophase; the nuclear membrane has broken down and the interior space has become filled with fibrillæ which have gathered to form a multipolar spindle. *c*, metaphase; a completed spindle with polar radiations. *d, e, Lilium candidum.* *d*, prophase of first mitosis; the kinoplasmic fibrillæ have formed a net around the nucleus and are gathered into several cones which would have become poles of the spindle. *e*, late prophase; the nuclear membrane has disappeared and the fibrillæ are entering the nuclear cavity; several cones of the fibrillæ constitute the multipolar spindle. *f, Agave Americana.* Prophase of the first mitosis; the spindle cones on the upper side have pushed through the special membrane around the nucleus. (*a, b, c*, after Allen, '03; *d, e*, Mottier, '97; *f*, Osterhout, '02.)

disappearance of the nuclear membrane some of the peripheral fibers push outward to form several cones of a multipolar figure (Fig. 14 *b*). The fibers attached to the chromosomes gather into bundles that extend towards these poles. Finally the bundles of fibers become more regular and straighten out so that they come to lie approximately parallel to one another, and thus

the multipolar structure, developing a distinct axis (Fig. 14 *c*), becomes bipolar (multipolar polyarch). There is no central body at the poles and no place for a centrosome in this developmental history.

The first detailed study of spindle formation in Angiosperms was, as before stated, that of Mottier ('97) which treated especially of *Lilium*, *Podophyllum* and *Helleborus*. This paper with one by Juel ('97) on *Hemerocallis* and Osterhout ('97) on *Equisetum* effectually disposed of previous views very generally held (Guignard, '91, followed by other authors), that spindle formation and mitotic phenomena in higher plants was involved with the activities of centrosomes or other kinoplasmic centers. Mottier found that the nucleus in the pollen mother-cell of the lily became invested just before mitosis with radiating fibers that shortly after increased in quantity to form a felted web around the structure. Some of the fibers gathered into cones (Fig. 14 *d*) which pointed towards the periphery of the cell so that there resulted, with the disappearance of the nuclear membrane and the entrance of the fibers into the nuclear cavity, a multipolar spindle (Fig. 14 *e*). The poles gradually came to lie parallel to one another in a common axis, some of them disappearing, so that the spindle generally became distinctly bipolar at metaphase (multipolar polyarch). Essentially the same history was repeated during the second mitosis in the lily.

From this time on there have been a succession of papers verifying the general conclusions of Mottier and Juel and extending these results to many other forms until now it seems to be well established that centrosomes are never present in the pollen mother-cell and that multipolar spindles, developed from felted stages and changing to bipolar spindles, may be expected in most if not all forms. Guignard ('97 and '98) described multipolar spindles in several types (*Nymphæa*, *Nuphar*, *Limodendron*, etc.), and while he believed that these poles were occupied by granules that sometimes fused to form typical centrosomes, nevertheless he admitted that the multipolar spindle might be formed independently of centrosomes.

The most important papers on spindle formation in Angiosperms following those of Mottier ('97 and '98) and Juel ('97),

have been Lawson ('98) on *Cobea*, W. C. Stevens ('98*b*) on *Asclepias*, Atkinson ('99) on *Arisæma* and *Trillium*, Duggar ('99) on *Bignonia*, Wiegand ('99) on *Convallaria* and *Potamogeton*, Gregoire ('99) on *Lilium* and *Fritillaria*, Guignard ('99) on *Naias*, Williams ('99) on *Passiflora*, Duggar (:00) on *Symplocarpus* and *Peltandra*, Lawson (:00) on *Gladiolus*, Byxbee (:00) on *Lavatera*, Andrews (:01) on *Magnolia* and *Liriodendron*, Schniewind-Thies (:01) on *Galtonia* and *Osterhout* (:02) on *Agave*.

Of the papers listed above several demand especial attention for the completeness of the studies on the early stages of spindle formation in the pollen mother-cell. Lawson ('98 and :00) found that the nuclei of *Cobea* and *Gladiolus* previous to mitosis were surrounded by a zone of granular kinoplasm which he named perikaryoplasm. This zone developed a felted envelope of fibrillæ from which projections extended to form the cones of a multipolar figure. The cones by fusing in two groups developed the bipolar spindles. The spindle fibers of *Gladiolus* are formed entirely from the perikaryoplasm, the nucleolus and linin apparently taking no part in the development of the spindle. The nucleolus remains intact until after the dissolution of the nuclear membrane when the spindle is practically completely organized. Miss Williams ('99) found for *Passiflora* that the nuclear cavity became filled with a network developed from the linin. The nuclear wall became also transformed into a mesh which connected the network from the linin with the surrounding cytoplasmic reticulum, thus forming a continuous system throughout the cell. The central region of this network, enclosed by a granular zone, developed a multipolar figure whose poles finally fused to form a bipolar spindle. The contrast between this type of spindle in which so much of the fibrous structure is derived from the linin and that of *Gladiolus* just described is very marked. A granular region outside of the fibrous network around the nucleus is much more conspicuous in *Lavatera*, described by Byxbee (:00), than in *Passiflora*. It forms in *Lavatera* a dense zone that suggests a gathering of nutritive material (deutoplasm). The fibrillæ are developed as a felt around the nuclear membrane and enter the nuclear cavity

with the breaking down of this structure. The fibers gather into projecting cones presenting a multipolar structure, and two of these, becoming more prominent, absorb the others and thus form a bipolar spindle.

One of the most recent studies on spindle formation is that of Osterhout (:02) on *Agave*. This investigation is of especial interest for the extensive experimentation in the technique of fixation. The author proposes a new terminology for the stages of mitosis that need not be presented here. *Agave* offers a striking peculiarity in the presence of a special membrane around the early stages of the spindle. The fibrillæ form inside of this membrane and finally push through it radially into the exterior cytoplasm where they gather into cones (Fig. 14 *f*). The cones separate into two opposite groups with a general parallel arrangement of the fibers and in this manner a bipolar spindle is formed.

It is becoming possible to make some general statements respecting the methods of spindle formation in the spore mother-cell. Just previous to prophase it is almost always possible to differentiate a region of kinoplasm around the nucleus. This zone has been found to be either granular, *e. g.*, *Pellia*, *Anthoceros* (Davis, '99 and :01), *Osmunda* (Smith, :00), *Cobea* and *Gladiolus* (Lawson, '98 and :00), or it presents the appearance of a fibrous reticulum, *e. g.*, *Equisetum* (Osterhout, '97), *Larix* (Allen, :03), *Lilium* (Mottier, '97 and '98), etc. The latter condition probably develops from the former by the arrangement of granules into fibers and the gradual expansion of a very close network thus formed into a coarser structure. The fibers in this reticulum sometimes surround the nucleus as with a heavy web. They later extend radially into the cytoplasm, partly by the expansion of the network and partly by their own growth and frequently take a radial arrangement. In some instances the spindle fibers are developed very largely within the nucleus from the linin (*Passiflora*, Williams, '99). They then become gathered into bundles or groups forming the cones which collectively constitute a multipolar figure that is often called a multipolar spindle. By the rearrangement of these cones somewhat parallel to one another, together with more or

less fusion, the multipolar structure becomes a bipolar spindle (multipolar polyarch) generally just previous to the period of metaphase. The formation of cell plates and the disappearance of the spindle fibers have been discussed in Section II under the title "Cleavage by cell plates."

Mention should be made of some irregularities in the division and distribution of the chromosomes that are conspicuous in certain spore mother-cells and which have been the cause of much discussion. The subject has especial reference to certain older views of the reduction phenomena in plants. Chromosomes split once longitudinally in all typical mitoses and the halves are drawn apart in a symmetrical manner which is very easily understood. This division is really determined by the longitudinal fission of the spirem thread. But appearances during the first nuclear division in the spore mother-cell of many forms have puzzled investigators for many years and have given rise to a number of interpretations. It seems to be pretty clearly established now that in these types there is a double longitudinal splitting of the chromosomes at the time of this mitosis. The first division takes place during prophase and the second follows closely after the first and is generally clearly seen at metaphase or during anaphase. Therefore the chromatic bodies which appear at the nuclear plate during the first mitosis are in reality divided or about to be divided into quarters and they separate after this mitosis as pairs of granddaughter chromosomes instead of simple daughter elements. These pairs are either firmly united at one end into a V or irregularly drawn out so that the bodies have very unusual and sometimes bizarre forms. Nuclear figures of this irregular appearance were originally described by Flemming for the first mitosis in the spermatocyte of *Salamandra* and named by him "heterotypic." These in the spore mother-cell of plants are of similar character and the designation "heterotypic" has been adopted by botanists for this condition. The pairs of chromosomes that enter the daughter nuclei after the first mitosis fuse end to end to form a spirem thread which breaks up again during the second mitosis, without longitudinal fission, into pairs of chromosomes which are believed to be identical with those that entered the nucleus after the first mitosis. Since there is

no longitudinal splitting of the spirem thread before the second division this mitosis differs from that of the "typical" mitoses of cells and is called "homotypic" to distinguish it on the one hand from the former and on the other from "heterotypic" divisions. Several illustrations of heterotypic and homotypic mitoses to be described presently are presented in Fig. 15, showing the peculiar V-shaped pairs of granddaughter chromosomes, characteristic of the first group. It is important to note that whatever the significance of this premature fission of the chromosomes before the second mitosis it is not of the nature of a qualitative reduction division in Weisman's sense. The details and significance of reduction phenomena will be considered in other connections (Section V). The topics discussed above have been recently studied and reviewed by Mottier (:03).

We have as yet said nothing of the megaspore mother-cell in Spermatophytes. An increasing number of investigations have clearly established the fact that the embryo-sac in many forms is one of a group of two, three or four cells, each of which is a potential megaspore because its nucleus contains the reduced number of chromosomes. We are accustomed to think of the well known conditions in the lily, where the megaspore mother-cell develops directly into the embryo-sac. But this type with some others (*e. g.*, *Fritillaria*, *Tulipa*, *Erythronium*, etc.) are the exceptions and present a very highly differentiated condition in which the usual developmental history is shortened in a very interesting manner, which will be described presently.

The embryo-sac arose undoubtedly as one of four megaspores developed after essentially the same manner as microspores or pollen grains, excepting that their arrangement was generally in a row, which is even true of some pollen grains (*e. g.*, *Asclepias*, *Zostera*). As stated above, an increasing number of investigations have established the row of four potential megaspores in a large number of forms in various groups. They may not always be distinguished by the form of the group, but their homologies are established by the mitoses that lead to their differentiation. Two mitoses are of course required to establish the group of four cells and both are identified by the reduced number of chromosomes. Some detailed studies on these mitoses have

established the fact for certain forms that the first is heterotypic and the second homotypic, exactly as in divisions of the micro-

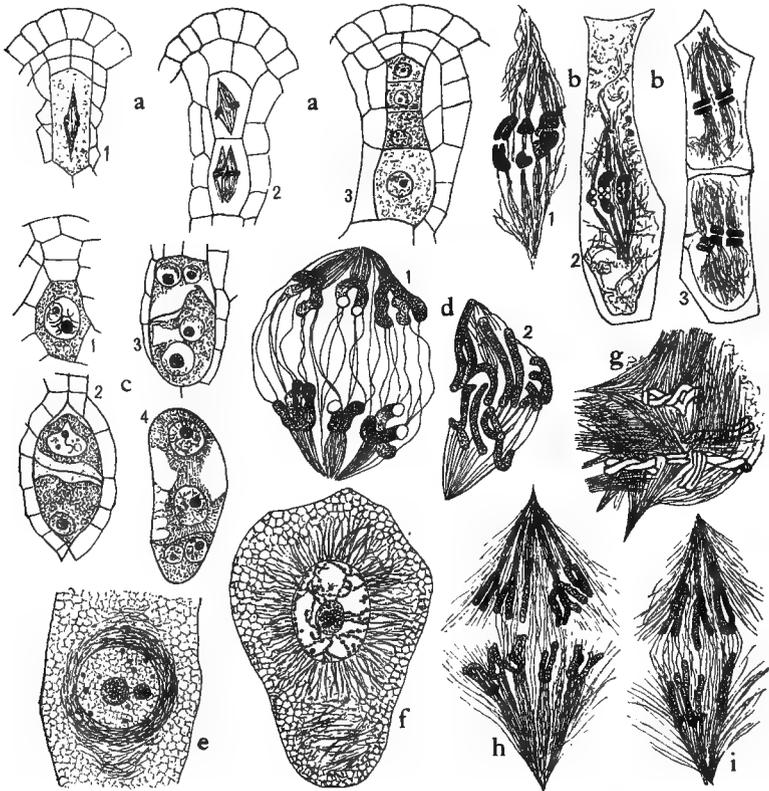


FIG. 15.—Embryo sac or megaspore mother-cell of spermatophytes. *a, b, Galtonia candidans.* *a1*, first mitosis in megaspore mother-cell. *a2*, second mitosis. *a3*, group of four megaspores. *b1* and *b2*, shortly after metaphase of the first mitosis (heterotypic). *b3*, metaphase of the second mitosis (homotypic). *c, d, Scilla Sibirica.* *c1*, megaspore mother-cell. *c2*, after the first mitosis. *c3*, after the second mitosis, the lower cell of the pair to become the embryo sac. *c4*, after the second mitosis, the upper cell of the pair to become the embryo sac. *d1*, anaphase of the first mitosis (heterotypic). *d2*, anaphase of the second mitosis (homotypic). *e, Lilium martagon*: portion of embryo sac mother-cell, nucleus surrounded by a felt of fibrillæ. *f, Lilium candidum*; embryo sac mother-cell, nucleus surrounded by radiating fibrillæ. *g, h, i, Lilium martagon.* *g*, late prophase of first mitosis in embryo sac mother-cell, a multipolar spindle. *h*, anaphase of first mitosis (heterotypic). *i*, anaphase of second mitosis (homotypic). (*a, b, c, d*, after Schniewind-Thies : 01; *e, f, g, h, i*, Mottier '97.)

spore or pollen mother-cells. Schniewind-Thies (: 01) figures very completely the mitoses in *Galtonia*. The first mitosis in the megaspore mother-cell (Fig. 15*a*) is heterotypic because the

chromosomes (Fig. 15*b*, 1, 2) show clearly the V-shaped forms characteristic of this division. The second mitosis (Fig. 15*b*, 3) is homotypic. The lowest cell of the group of four (Fig. 15*a*, 3) becomes the embryo-sac and the mitoses that take place within it as the female gametophyte develops are all typical. This account illustrates a simple history in megaspore mother-cell development and is considered the first of three types in a classification proposed by Schniewind-Thies (:01).

The second type of development is one in which two megaspores are generally developed from a mother-cell and one of these becomes the functional embryo-sac. Schniewind-Thies presents an excellent illustration of this type in *Scilla*. The first mitosis in the megaspore mother-cell (Fig. 15*c*) is heterotypic (Fig. 15*d*, 1) and results in two cells (Fig. 15*c*). The second mitosis in both cells is homotypic (Fig. 15*d*, 2). Either the lower (Fig. 15*c*, 3) or the upper (Fig. 15*c*, 4) of the pair may become the embryo-sac. The embryo-sac then includes the homotypic or second mitosis within its development, making it the first nuclear division of the gametophyte history. The typical mitoses of the gametophyte begin with the second nuclear division in the embryo-sac. Three megaspores may be formed in such a group when the cell of the pair that does not become the embryo-sac divides again.

The third type of development is illustrated by several forms, of which the best known are *Lilium* (Mottier, '98 and :03) and *Tulipa* (Schniewind-Thies :01). The lily has been much studied, but Mottier presents the most detailed account of spindle formation and the behavior of the chromosomes. He supports the observations of Schniewind-Thies, based upon the tulip, and her explanation of this type of development. The megaspore mother-cell of the lily and tulip develops directly into the embryo-sac. The first mitosis in this cell (Fig. 15*h*) is heterotypic and the second (Fig. 15*i*) homotypic. These divisions give the four-nucleate embryo-sac and one more mitosis presents the mature structure. This last is a typical mitosis, the only one found in the embryo-sac before the development of the endosperm and sporophyte embryo. Thus the two mitoses characteristic of the spore mother-cell are here included within the

embryo-sac and appropriated as a part of the gametophyte history.

We can see in these three types of embryo-sac development an evolutionary process of which the third stage is plainly derived from the simpler second and first, and is consequently a highly developed and very complex condition, far removed from primitive gametophyte structures among the angiosperms. The embryo-sacs of these forms (*Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, etc.) are probably the most complex spore mother-cells that we know. The studies of Schniewind-Thies and Mottier have been supported by other investigations, and more especially by the results of Ernst (:02) on *Paris quadrifolia* and *Trillium grandiflorum*, who followed the history of the heterotypic and homotypic mitoses in these forms in detail. They illustrate the second type of embryo-sac development in the classification of Schniewind-Thies.

Spindle formation in the embryo-sac mother-cell has not received as much attention as in the pollen mother-cell, probably because material of the latter structures may be obtained much more readily than the former. There have been numerous descriptions and figures of the spindles but few accounts in full of their development. Of the latter the investigation of Mottier ('98) on *Lilium* is the most complete. This paper was written at the time when the centrosome question was under discussion and served, with other papers on the spore mother-cell (Osterhont, '97, Juel, '97, Mottier, '97) to discredit the presence of these bodies in this structure. Mottier found that the nucleus of the embryo-sac became invested with a close network of fibrillæ (Fig. 15 *c*) from which fibers developed into the cytoplasm radiating from the nucleus in all directions (Fig. 15 *f*). With the dissolution of the nuclear membrane the fibrillæ entered the nuclear cavity, filling it with masses of fibers which gathered into cones to form a complicated multipolar spindle (Fig. 15 *g*). These cones later come together into two poles, but even in the mature spindle the fibrillæ are frequently in several groups at the poles. Essentially the same history is repeated in the second mitosis. A large number of later papers have described and figured multipolar spindles in embryo-sacs, con-

firming the conclusions of Mottier that these structures are developed here after the same methods as in the pollen mother-cell, from surrounding investments of fibrillæ and without centrosomes. Indeed the embryo-sac is remarkable for the quantity of the cytoplasmic fibrillæ present during its mitoses.

In concluding this account attention should be called to some forms whose microspore mother-cells were formerly supposed to omit the mitoses of sporogenesis and develop directly into pollen grains. These conditions were reported in *Zostera*, the Cyperaceæ, and the Asclepiadaceæ. However, Juel (:00) finds the two mitoses present in *Carex acuta*, although three of the nuclei break down and the cytoplasm is appropriated for the fourth to form a single pollen grain whose wall is developed from that of the mother cell. The history is very similar to the development of the megaspore in certain heterosporous pteridophytes (*e.g.*, *Marsilia*, *Selaginella*) and to the embryo-sac, which functions while its companion potential megaspores degenerate. The development of the pollen in the Asclepiadaceæ has been shown to be normal in the nuclear activities by several investigators (Frye, :01, Strasburger, :01, and Gagner, :02), the tetrad consisting of four pollen grains in a row, instead of the usual arrangement. In *Zostera* (Rosenberg, :01) there are longitudinal divisions of the very much elongated pollen mother-cell to give four extraordinary filiform pollen grains.

##### 5. The Cœnocyte.

This remarkable type of cell has reached an extraordinarily high state of development in certain plants, notably among the Siphonales and the filamentous Phycomycetes (Mucorales, Saprolegniales and Peronosporales). Cœnocytes are multinucleate cells. The simplest types are developed by the limited division or fragmentation of a nucleus accompanied by an increase in the size of the cell but without extended growth. Excellent illustrations are found in the older cells of the red algæ, the internodal cells of the Characeæ and in old parenchyma cells of many higher plants.

A higher type of cœnocyte is presented when the multinucle-

ate cells show some definite activity resulting in extensive growth or peculiarity of form. Thus some laticiferous cœnocytes are branching tubes that grow for considerable distances among the cells of the tissues in which they are contained. The embryo-sac and the female gametophytes of *Selaginella* and *Isœtes* in the early stages of their development are interesting cœnocytes. Among the lower algæ there are numbers of cœnocytic forms. (*e. g.*, *Hydrodictyon*, *Cladophora*) whose cells present very little change with age except an increase in size. Yet some of these conditions, especially those illustrated in the *Cladophoraceæ*, are probably related to the higher types of cœnocytes.

The best differentiated cœnocytes are found in the Siphonales, Mucorales, Saprolegniales and to a lesser extent among the Peronosporales and are especially well illustrated in a few aquatic forms, such as *Monoblepharis* and *Myrioblepharis*. The peculiarities of these forms lie in elaborate structures which result from the ability of the cœnocyte to respond to several directive-stimuli in its growth. The most complicated responses and consequently the most highly differentiated morphology is shown among the Siphonales, where some very elaborate forms are found. In many types the plant body is clearly composed of root and shoot regions and in the highest expressions (*e. g.*, some species of *Caulerpa*) there are rhizoids, shoots and leaf-like structures presenting a remarkable degree of specialization. The behavior of the protoplasm in these most highly differentiated types of the Siphonales is known to us chiefly through studies of Noll and Klemm.

There is a very conspicuous layer of clear protoplasm next to the cell wall which constitutes an outer plasma membrane (*hautschicht*). This outer plasma membrane is stationary while the granular protoplasm within changes its position readily and frequently in different portions of the plant streaming in various directions. The nuclei are all situated in the granular cytoplasm so that they must shift their positions with its movements. Noll ('87) by a clever method of coloring the cell wall of living plants of *Caulerpa* was able to prove that the forward growth took place by the protoplasm extending beyond the old wall, thus adding new regions of cellulose to the old. He called this

method of growth a process of eruption in contrast to Nägeli's conception of growth by intussusception. Increase in thickness comes with the laying down of successive lamellæ inside the older wall and is consequently growth by apposition. *Caulerpa* is very favorable for such investigations and Noll's results greatly strengthen the theory that a cellulose wall results from the direct transformation of a plasma membrane in which carbohydrate molecules gradually replace those of albuminous material. Accordingly the cellulose wall is not strictly a secretion and its growth is not by the intercalation of new molecules among the old (intussusception) in a non-living membrane.

The wide space in the interior of the filaments of *Caulerpa* and some other members of the Siphonales is frequently crossed by cellulose bars at various angles. These are at first strands of protoplasm which become gradually filled with a carbohydrate material and finally solidified. Noll ('88*a*), while recognizing that these structures may have value in strengthening the filament, believes that they are also the paths of metabolic exchange between the interior regions of the protoplasm and the water outside the plant. They are surrounded by the plasma membrane which in consequence presents a much greater extent of surface to the water permeating the cell wall.

It is plain that because of the constant movement of the granular cytoplasm carrying with it the nuclei which change their position in the cell, the outer plasma membrane is the only portion of the protoplasm that can receive fixed stimuli for an extended period. Consequently Noll regards this membrane as the responsive or irritable region of the cell that reacts to the stimuli which largely or wholly direct growth. Some of these stimuli are well established. Thus it is light which directs the formation of leaves and shoots. The behavior of *Caulerpa* in relation to prominent stimuli (light, darkness, gravity, etc.) has been studied by Noll ('88*b*) and Klemm ('93). The latter author believes that the response is due to the presence of foods or other substances at certain points which make them especially sensitive to the external stimuli. Injuries to a filament of the Siphonales brings about an immediate flow of protoplasm to the wounded part (Klemm, '94), after which the plasma membrane is quickly repaired and new portions of the wall laid down.

Mitotic phenomena in the Siphonales is known to us only through the investigations of Fairchild ('94) on *Valonia*. He found that nuclei in the same individual may divide directly or indirectly. The first process is one of simple fission, the latter takes place with the formation of an intranuclear spindle. Studies in sporogenesis and gametogenesis are very much to be desired in the Siphonales that we may understand the behavior of the nuclei at these periods. The author's recent studies of oögenesis in *Vaucheria* (Davis, :04*a*) have shown an interesting process of nuclear degeneration similar to that in the Saprolegniales and Peronosporales, and suggests some very interesting lines of investigation.

The protoplasmic structure in the hyphæ of the larger filamentous Phycomycetes, especially the Saprolegniales and Peronosporales, is undoubtedly much the same as in the Siphonales. But the absence of chlorophyll and the greater delicacy of the filaments makes it more difficult to recognize the different regions of the protoplasm. There is an outer plasma membrane inside of which the granular material slowly moves in protoplasmic currents that may sometimes be observed in rapidly growing tips. Delicate strands which are the paths of streaming currents are beautifully shown in developing sporangia of the molds and the oögonia of the Saprolegniales and Peronosporales. The nuclei are undoubtedly carried by the protoplasmic movements, sometimes collecting in considerable numbers in growing regions of the filaments which always contain much dense protoplasm.

Another type of cœnocyte, and in some respects the most remarkable, is the plasmodium of the Myxomycete. These structures are too well known to need description here. We shall only refer to them as they help to break down an old theory that the cœnocyte is a compound structure composed of many energids, represented by the nuclei, which coöperate to make up the whole. The plasmodium and the protoplasmic mass inside the cellulose tubes of the Siphonales and Phycomycetes agree in all essentials of structure and mode of growth. The forward growth of the plasmodium, as is also true of the *Amœba*, begins with the prolongation of the outer plasma mem-

brane (hautschicht, ectoplasm) into a process (pseudopodium) which advances and is followed immediately by an inflow of the granular cytoplasm. And the growth of the filaments of the Siphonales and higher Phycomycetes is a pushing forward of the outer plasma membrane followed by the granular protoplasm, but this growth is slow because the plasma membrane is at all times under the restraint of a cellulose envelope.

Mention should be made of the remarkable cœnocytic zoöspores well known in *Vaucheria* and also described by Thaxter ('95*b*), for the Phycomycete *Myrioblepharis*. In *Vaucheria* the entire contents of the sporangium becomes transformed into an immense multinucleate zoöspore, the cilia being distributed in pairs above the nuclei. In *Myrioblepharis* the contents of a sporangium usually forms four large multiciliate zoöspores.

These zoöspores of *Vaucheria* have often been called compound zoöspores, and the idea has been expressed that they stand for the coöperative union of many hundreds of zoöspores (energids) represented by the nuclei and their respective pairs of cilia. And this explanation of the zoöspore of *Vaucheria* is a part of a broad view, formerly very largely held, that the cœnocyte is an assemblage of energids (uninucleate masses of protoplasm) coöperating in a fused structure.

The theory of the coöperative association of energids in a cœnocyte (Sachs) has been very much modified. While the nucleus and some other organs of the cell, such as groups of cilia, plastids, etc., are homologous with the same structures in uninucleate cells nevertheless the behavior of the cœnocyte is not the same as a group of coöperating protoplasmic units. The cœnocyte reacts to the usual stimuli in precisely the same manner as a uninucleate cell, and must be regarded as physiologically presenting no peculiarities over the latter structure excepting those of an increased bulk of protoplasm demanding a greater number of nuclei for its metabolic processes. The most important contribution presented by the cœnocyte to our knowledge of the physiology of the cell is the establishment of the plasma membrane as the region of the protoplasm responsive to the stimuli that determine the form assumed in growth. The constant shifting of the nuclei and plastids in the movement of the

granular protoplasm eliminates them as structures immediately concerned with the form of a cell or organ thus limiting their functions more especially to metabolism.

#### 6. The Cœnogamete.

The cœnogamete is a multinucleate sexual cell. The name was first applied by the author (Davis, :00, p. 307) to the remarkable multinucleate eggs of *Albugo bliti*, and the conception has been considerably extended since, as explained in his later writings on *Saprolegnia* (Davis, :03, p. 320-331) and on "The relationships of sexual organs in plants" (Davis, :04*b*). Stevens ('99) discovery of the multinucleate eggs of *Albugo bliti* opened a field of research that has been greatly extended in the past four years and which is likely to yield very important conclusions on the relationships and evolution of the Phycomycetes and Ascomycetes. Conditions similar to *Albugo bliti* were reported the following year by Harper, :00*b*, for *Pyronema*, and several later papers have described, with greater or less fullness, the structure and behavior of cœnogametes in some other Ascomycetes, types of the Peronosporales (species of *Albugo*) and in the Mucorales.

We shall not discuss the details of these investigations with their bearings upon the problems of phylogeny as this has become a very complicated subject and is treated elsewhere (Davis, :04 *a-b*), but merely describe the structure and behavior of cœnogametes so far as they are known to us.

Stevens and Harper both found that the multinucleate female cell of *Albugo bliti* and *Pyronema* was fertilized by the introduction of a large number of nuclei from the antheridium. These sexual nuclei paired off and fused, a male with a female, in the common mass of cytoplasm so that the fertilized cell finally contained a large number of fusion nuclei. A similar history was reported later by Stevens (:01), in *Albugo portulacae* and *Albugo tragopogonis*. These events have been so thoroughly studied that we know the processes of fertilization in the above forms as well perhaps as for any plant type.

The structure and especially the nuclear history of other

cœnogametes is less perfectly understood. The multinucleate character of the fusing gametes is well known, but the later distribution and fate of the sexual nuclei has not been followed, and it is by inference that we believe these cœnogametes to behave in essentially the same manner as those of *Albugo* and *Pyronema*.

Cœnogametes fall into two classes according as they involve all of the protoplasm contained within the mother-cell or only a portion of such protoplasm. The first group probably represents the simplest and most primitive conditions.

Cœnogametes of the first class are found in the *Mucorales* (Gruber :01) and in the *Gymnoasceæ* (Dale, :03). In these types the entire contents of the terminally formed sexual cells unite to produce the zygospore in the former group and the fertilized ascogonium in the latter, from which arises the system of ascogenous hyphæ.

Cœnogametes of the second class contain only a portion of the protoplasm in the mother-cell which is usually a terminal structure. The protoplasm that is not involved in the cœnogamete proper generally bears some important relation to the sexual element. Thus the periplasm of the *Peronosporales* assists in the formation of the wall of the oöspore and the conjugation tube of *Pyronema* becomes the path through which the contents of the antheridium enters the ascogonium. But in some forms the superfluous protoplasm is merely cut off from the cœnogamete as a sterile cell (*Monascus*). In *Albugo* and *Pyronema* the sterile and fertile portions of the protoplasm are so closely associated that the mother-cell really acts as a whole, very much as the simplest types of cœnogametes which shows the close relationships between the two. Moreover the antheridia of these forms are types of cœnogametes almost as simple as those of the molds or the *Gymnoasceæ*.

The cœnogamete is a type of sexual cell unknown in the animal kingdom and among plants is probably restricted to the *Phycomycetes* and *Ascomycetes*. The problems of its homologies and origin are very interesting.

The simplest types of cœnogametes (*Mucorales* and *Gymnoasceæ*) are cells situated at the ends of filaments in the same position as the sexual organs of the *Siphonales*. The mother-

cells of the more complicated cœnogametes (oögonia and antheridia) are also terminal cells. All of these sexual organs are multinucleate. In the Siphonales (*Vaucheria* excepted) all of the nuclei are functional gamete nuclei. This is also true of simplest types of cœnogametes, but in the more complicated forms (*Albugo*, *Pyronema*, etc.) large numbers of the nuclei degenerate or fail to function sexually in sterile accessory regions of the protoplasm. The same conditions of sexual degeneration are also found in the oögonia of *Vaucheria* (Davis, :04) and *Saprolegnia* (Davis, :03). The agreement of all of the structures mentioned above in structure and protoplasmic behavior seems to establish beyond question their common homology.

The problems of the origin of the cœnogametes are very difficult with the meager evidence at hand. The author believes that the simplest types have probably been derived from structures like the sexual organs of the isogamous Siphonales, which structures gave up the habits of forming uninucleate gametes and acting as cœnocyctic units became multinucleate sexual elements. A physiological development very similar to such a change must have taken place in *Peronospora* and some species of *Pythium* when their conidia ceased forming zoöspores and took the habit of germinating directly by a tube. This view regards the cœnogamete as a cœnocyte derived from a protoplasmic structure that at one time produced a large number of independent sexual elements, represented in the cœnocyte by the numerous nuclei. Whether the higher types of cœnogametes (*Albugo*, *Pyronema*, etc.) have developed directly from the simpler forms or from levels of the heterogamous algæ, such as are illustrated by *Vaucheria*, are very complicated problems that cannot be treated here. They with other topics, mentioned above, have been considered in recent papers of the author (Davis, :03, ;04a, :04b). Cœnogametes are proving to be among the most interesting types of sexual cells in plants and research in this field is likely to prove very fruitful of results.

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(To be continued.)





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## STUDIES ON THE PLANT CELL.—V.

BRADLEY MOORE DAVIS.

### SECTION IV. CELL UNIONS AND NUCLEAR FUSIONS IN PLANTS.

THE forms of cell unions and nuclear fusions in plants fall into two groups: (1) those which obviously have no sexual significance attached to them, and (2) those which are evidently sexual acts. But apart from these simple divisions there are some very interesting conditions in which it is far from easy to determine whether certain events have a sexual significance either physiologically or phylogenetically. The real test of such problems should lie in the evolutionary history of the processes involved, for every sexual condition in plants has probably developed in obedience to the same physiological demands and in an essentially similar manner. However, we cannot apply the evolutionary test in many cases where we have little evidence of the developmental history of the group and such forms must rest for the present as unsolved problems. We shall treat them in special connections later in the paper.

The material of this section will be presented under the following heads:—

1. Protoplasmic connections between cells (plasmodesmen).
2. Sexual cell unions and nuclear fusions.
3. Asexual cell unions and nuclear fusions.

#### 1. Protoplasmic Connections between Cells (Plasmodesmen).

It has been known for a great many years that the walls between the cells in some plant tissues and more especially between the cells of filaments in certain thallophytes were crossed by delicate strands of protoplasm so that contiguous protoplasts were not entirely separated from one another. This fact

offers at once many interesting possibilities of explaining the close association of many cells and tissues, not alone in delicate dynamic interrelations but even in the exchange and distribution of food material and other products of metabolism. It makes possible the conception of the plant body as a finely adjusted community of protoplasts intimately and sensitively related to a great degree in all parts, a view very different from the old idea of a cell republic. As might be expected, these speculative possibilities were conceived and expressed by such leaders as Hofmeister, Nägeli, Sachs, and Strasburger long before the detailed study of protoplasmic connections gave the mass of evidence upon which have been based the more elaborate conceptions of recent years.

The most obvious protoplasmic connections between cells may be found in the thallophytes where as in the Rhodophyceæ, Volvox, and in certain fungi, the cells in younger structures may be observed under comparatively low magnification to be united by strands of protoplasm so broad as to quite exclude them from the category of fibrillæ. Some of these structures are so conspicuous that it is surprising that more was not made of them by early writers and that they have not been more extensively investigated recently. The greater part of the papers have been on the very difficult phase of the subject, the structure of pores and pits in the tissues of higher plants. The literature treating of protoplasmic connections is too extensive to be given detailed treatment in the compass of this paper. The best review of the subject is that of Strasburger (: 01), supplemented by the more recent paper of Kienitz-Gerloff (: 02).

The earlier papers on the protoplasmic connections in higher plants, following the establishment of perforations of sieve-plates by Sachs and Hanstein, appeared during the years just preceding and following 1880. Thus Tangl ('79-'81) described very clearly the communications between the endosperm cells of *Strychnos nux vomica* and Phoenix (see Fig. 16, a). Tangl noted the resemblance of the complex of connecting threads to the arrangement of spindle fibers associated with the simultaneous division of the protoplasm in the endosperm but was cautious in assuming a relationship, suggesting that the resemblance might be superficial.

Strasburger ('82, p. 246) discussed the permeability of cell walls and Gardiner ('88) gave a general treatment of the subject without, however, any figures to illustrate his conclusions. Gardiner discovered for a large number of forms in a wide variety of families that the pit membranes were frequently pierced by protoplasmic fibrils and that in some cases the fibrils traversed the entire thickness of the cell wall. A more detailed study with better methods, supplementing his former work and accompanied by figures, was published by Gardiner, in 1898, this paper forming an important contribution to the subject. Gardiner (:00) announced himself strongly in favor of the view that the protoplasmic connections between cells were derived from spindle fibers of nuclear figures concerned with each cell division, a possibility which had been suggested by previous writers (Tangl, '79-'81; Russow, '83).

Kienitz-Gerloff ('91) gave an excellent account of the protoplasmic connections in a number of forms, some of them pteridophytes, but especially for *Viscum album*, and followed the history of the wall formation, showing that the spindle fibers disappeared completely before the development of the connecting strands of protoplasm. Kuhla (:00) followed Kienitz-Gerloff with more extended studies on the same form, *Viscum album*, tracing the protoplasmic fibrils between the cells in all the chief tissues and establishing the protoplasmic connections throughout the individual to an extent that was not known before. Hill (:01) described the structure of the sieve-tubes of *Pinus*, dealing especially with the formation of callus and the conversion of the connecting threads of protoplasm into strings of slime. An excellent review is also given of the work of Russow and others, particularly upon sieve-tubes. Kohl ('97) describes clearly protoplasmic connections between the cells of moss leaves.

A classification of protoplasmic connections was suggested by Kohl (:00) who distinguished between the solitary state when each fibril pierces the cell wall independently of its neighbors (Fig. 16, a and b) and a grouped condition when a number of fibers arise close together at the bottom of a pit and pierce the pit-membrane or middle lamella in a spindle-shaped arrangement,

reminding one of the central spindle of a mitotic figure (Fig. 16, c). In general the two types of protoplasmic connections are not found together in the same cell or tissue.

A new point of view was introduced into the discussion by the very important paper of Strasburger, in 1901. He considered the protoplasmic connections as sufficiently clearly differentiated structures to rank as organs of the cell and proposed for them the name plasmodesmen. Strasburger in agreement

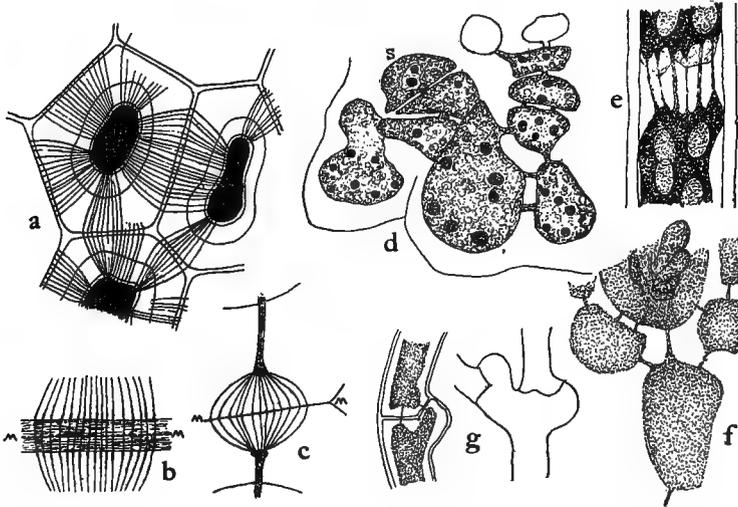


FIG. 16.—Protoplasmic connections between cells of plants. *a*, endosperm cells of *Strychnos nux vomica*; *b*, details of the solitary fibers in the same form, (*m*) middle lamella; *c*, grouped fibers at bottom of pit in endosperm of *Phytelephas* and crossing the pit membrane in a spindle-shaped figure; *d*, cell connections around the sporophytic portion of a developing cystocarp of *Champia*, (*s*) sporophytic elements; *e*, fibers between cells of *Cladophora*; *f*, cell connections around the ascogenous elements in *Laboulbenia*; *g*, clamp connections in *Pleurotus* (*a*, after Tangl, '79-'81; *b* and *c*, Kohl, :00; *d*, Davis, '96 b; *e*, Kohl, :02; *f*, Thaxter, '96; *g*, Meyer, :02).

with Kienitz-Gerloff opposed the view that the plasmodesmen were in any way derived from or related to the spindle fibers associated with the formation of cell plates. He believed them to be developments of the outer plasma membrane as he likewise considers the cilia in certain zoöspores (see account of zoöspore and sperm in Section III, *Amer. Nat.* vol. 38, pp. 571, 576, 1904). Strasburger also holds that pores are formed in the cell walls by the fermentive activities around plasmodesmen. A

recent short paper by Michniewicz (:04) describes clearly the plasmodesmen in *Lupinus*, especially in their relation to masses of intercellular protoplasm which are discussed at the end of this portion of the section.

It is not clear whether all protoplasmic connections may be considered in the same class, as Strasburger would have us believe, or whether there may not be some confusion between the broader cell connections which are especially conspicuous in the thallophytes and certain tissues (sieve-tubes, laticiferous vessels), and the delicate protoplasmic fibrils (plasmodesmen) so general throughout all tissues of higher plants. As is well known, the cells in actively growing regions of the red algæ are connected by broad strands of protoplasm that are obviously left by the cleavage furrow which constricts the protoplasm of daughter cells but does not entirely separate them. These openings may become partially blocked in older portions of the plant by the deposition of material so that the connections are finally fibrillar but they frequently remain open for long periods, particularly in regions where the nutritive processes are active as during the development of cystocarps. At this time new fusions may be developed between neighboring cells (auxiliary cells) so that they become connected in an elaborate network around the cells or filaments (sporophytic) that develop the carpospores (Fig. 16, d). The Phæophyceæ also furnish frequent illustrations of connecting fibrils especially in the Fucales and Laminariales where the cells of internal filaments are sometimes connected by conspicuous strands. Certain elongated filaments which traverse the central region of the larger brown algæ show a complicated group of fibrils that strikingly resembles the protoplasmic connections piercing the sieve-plates of higher plants. Broad protoplasmic connections are conspicuous between the cells of some of the filamentous Cyanophyceæ (*Stigonema*, *Tolypothrix*) and in the Chlorophyceæ have been reported for some species of *Cladophora* (Kohl, :02; Fig. 16, e) and for *Chætopeltis*, one of the Mycoideæ. They do not seem to be present in the Conjugales as was at first reported by Kohl ('91) whose cells show a great degree of physiological independence. In *Volvox*, studied by Meyer ('96), each cell of the

sphere is connected with its neighbors generally by six strands of protoplasm, only a few of which could possibly be left by the successive cell divisions. The majority must have developed as outgrowths from the plasma membrane of the cell.

Numerous instances of cytoplasmic connections among the fungi have been reported by many authors. A general review of the subject is presented by Kienitz-Gerloff (:02) and in a lengthy paper of Meyer (:02). The protoplasmic connections fall into two groups: (1) those that remain in the center of the wall after a cell division, and (2) the lateral unions and clamp connections which are developed entirely independently of cell division. Connections of the first type, *i. e.*, those between daughter cells, appear to be very general in the Ascomycetes and Basidiomycetes and are essentially similar to the strands between cells of the Rhodophyceæ. They are especially well illustrated in members of the Laboulbeniaceæ (Thaxter, '96; see Fig. 16, f). In the second group are the clamp connections (Fig. 16, g), characteristic structures of the tissues of fleshy forms of the Basidiomycetes, and the lateral unions between cells of closely entangled hyphæ which are well known in a number of forms and have been followed in cultures from germinating spores. It is probable that the fusions between sporidia in the smuts are also of this class, although De Bary and others have attached sexual significance to the phenomenon (especially as illustrated by *Tilletia*). Harper ('99a) has studied the fusions of the conidia of *Ustilago* and finds that they concern the cytoplasm alone. However, Federley (:03-:04) has reported a nuclear fusion in one species (*Ustilago tragopogonis pratensis* Pers.) but states that others agree with Harper's account. Extensive experiments of Brefeld have shown that the fusions of sporidia depend largely upon the character of the nutrient media and are less likely to occur when the conditions are favorable. He considers the fusions as purely vegetative processes comparable to the unions of germ tubes of spores (*e. g.*, *Nectria*, *Sclerotinia*, *Rhyparomyces*, etc.) into a common mycelium and to the connections between hyphæ of Basidiomycetes. Recent studies of Blackman (:04 a) indicate also that sexual processes should not be expected at this period in the life his-

tory of smut or rust. One of the best discussions of cell fusions in the fungi is that in Harper's paper ('99a), noted above.

Although most of the protoplasmic connections in higher plants are of the fibrillar character there are some notable illustrations of broad openings between cells, even more conspicuous than those in the red algæ. Such may be found in the pores of sieve-plates traversed in their early stages by strands of protoplasm that later disappear, and even better illustrations are the unions between cells composing laticiferous vessels. But the most interesting conditions are those associated with the nutrition of the eggs of certain cycads. Goroschankin ('83) first noted for the cycads pores or canals in the egg-wall of *Ceratozamia* and described communications between the protoplasm of the enveloping cells of the jacket and the egg. The subject is closely associated with the explanation of the proteid vacuoles in the eggs of gymnosperms which Arnoldi believed to be nuclei that had migrated from the surrounding cells. The conclusions of Arnoldi have not been sustained (see Sec. III, *Amer. Nat.*, vol. 38, pp. 591, 592, 1904) but the presence of pores in the egg-wall of gymnosperms is likely to prove very general with further investigation. A recent paper by Miss Isabel Smith (:04) gives an account of haustoria-like processes from the egg of *Zamia* which pass through the pores of the egg-wall into the cells of the jacket, where they are in direct contact with its protoplasm. These pseudopodia-like processes of the egg apparently absorb material from the cells of the jacket as is indicated by the character of their staining and the streaming movement towards them of the protoplasm in the jacket cells. The relation of the plasma membrane of the processes from the egg to that of the jacket cells is not clear but probably they are merely in contact and not in open communication. The ovules of cycads seem to offer an especially favorable subject for the study of pore formation and the intimacy of protoplasmic connections between cells.

It seems very clear that the cytoplasmic connections in the Rhodophyceæ, Volvox, fungi, and between the egg and jacket cells of cycads involve very much more substance than is generally present in the delicate fibrillæ of higher plants. Meyer

(:02, pp. 167, 168) seems justified in emphasizing their resemblance to pseudopodia rather than to any other structure of the cell. If they should finally be connected by intergradations with the exceedingly fine plasmodesmen of Strasburger, there would stand at one end of the series structures so thick as to be composed of a plasma membrane containing much cytoplasm in the interior and behaving like haustoria or pseudopodia and at the other end delicate fibrillæ. Viewing the problem of their relationships from the lower plants upwards, it is very difficult, if not impossible to follow Strasburger's theory that all cytoplasmic connections (plasmodesmen) are related to developments from the plasma membrane similar to cilia. They seem to be more of the nature of processes put out from the cytoplasm and when necessary penetrating cellulose walls probably in response to chemotactic stimuli since they are most conspicuous when metabolic activities are obviously important (*e. g.*, nourishment of the egg in gymnosperms and sporophytic generation of the red algæ).

In method of development we have seen that protoplasmic connections fall into two classes: (1) those that represent the incomplete separation of daughter cells, and (2) those that result from the coming together or fusion of protoplasmic outgrowths. The types of the first group are always in the beginning open communications which later may become largely or wholly closed; types of the second group may result in broad cytoplasmic fusions (*e. g.*, many fungi) but there is evidence that in many cases, especially among the higher plants, the two processes only come in contact so that the plasma membranes are applied to one another but do not actually unite. It does not seem probable that the two methods of development or the presence or absence of intimate protoplasmic union indicate a different kind of structure. They are more likely to be only varied responses to the demands for a more or less close association of neighboring cells. Broad communications are especially characteristic of regions where there is evidently an extensive demand for the nourishment of a cell or tissue, as in the eggs of the cycads or the cystocarp of the red algæ.

The functions of protoplasmic connections are probably vari-

ous. It is evident that they bind the whole plant body into a cell complex capable of very delicate interrelations. It is natural that physiologists, Pfeffer and others, should associate the structures with the phenomena of irritability as the paths over which stimuli may be transmitted from cell to cell and tissue to tissue. Several writers have reported their presence in unusual numbers in irritable structures of plants. The subject is discussed in great detail by Strasburger (:01, p. 533).

Besides conducting stimuli, there is much evidence that material may be transferred in solid or semifluid form by the protoplasmic connections from cell to cell and that in some instances there is actually a movement or flow of protoplasm. It is even known that nuclei may pass from cell to cell through pores in the wall, especially after some shock, as in the neighborhood of wounds (Miehe, :01), or when temperature is suddenly raised (Schrammen, :02). This literature and other references are discussed by Koenicke (:01 ; :04). A flow of protoplasm between neighboring cells of hyphæ has been reported by Reinhardt ('92) and Charlotte Ternetz (:00). That nuclei may pass through very small space is shown in the development of spores in the Basidiomycetes and in the growth of haustoria from the cells of hyphæ (Smith, :00). There are many forms known, especially among the thallophytes, where the communications between cells are so broad as to admit of a very free circulation of their contents. Such conditions are especially well illustrated in tissues around the developing cystocarps of the Rhodophyceæ and the ascocarp of the Ascomycetes, both structures apparently sporophytic in character and dependent to a great degree upon the gametophyte as a host. It is believed that the vitality of protoplasm in sieve tubes, whose nuclei have degenerated and disappeared, is maintained through protoplasmic connections with neighboring cells and especially the companion cells, when present. Of course where an actual circulation of protoplasm is established between cells or tissues there is made possible a distribution of the products of metabolism in solid form that is very different from the usual diffusion in tissues through cell walls and plasma membranes.

It seems probable that there are really two forms of protoplasmic connections between cells in plants: first, those so intimate that the plasma membranes are pierced and become continuous openings inclosing a strand of granular cytoplasm within; and second, those in which the plasma membranes are merely applied to one another without open communication. The second form comprises the most delicate connecting fibrillæ, structures so fine that their minute structure is not understood and we do not know how intimate may be the application of the fibrillæ to one another or to the surface of the cells. These are the typical plasmodesmen of Strasburger which he considers as organs of the plasma membrane, kinoplasmic in character, and compares to cilia. The broad connections of the first group have exactly the structure that would be expected of fused pseudopodia, as Meyer pointed out. Whether the two types insensibly grade into one another or whether each is a development by itself is a problem of considerable interest, for if the former possibility prove true, Strasburger's conception and classification of plasmodesmen as organs of the cell will hardly seem justified.

When protoplasmic connections become so broad that cytoplasm flows or surges from one cell to another, an actual transfer of nuclei sometimes takes place. Such conditions may illustrate simply one extreme of the series of protoplasmic connections that we have just discussed, but many of them introduce some complexities, mainly through a certain resemblance to sexual processes, so that they should be treated apart from general protoplasmic connections. Some of them will be described later under the head of "Asexual Cell Unions and Nuclear Fusions."

Closely associated with protoplasmic connections is the interesting subject of intercellular protoplasm which is receiving some attention at present. The last papers are by Kny (:04) and Michniewicz (:04) who are studying conditions in the seed, especially of *Lupinus*. By various reactions and physiological studies, Kny has established an apparent identity of nature between an intercellular substance, sometimes with starch inclusions, and the cytoplasm of the neighboring cells. He considers this substance to be intercellular protoplasm, that is,

protoplasm outside of the cell walls, but connected with the cytoplasm within through fibrillæ. The intercellular protoplasm is thus conceived in organic connection with nucleated cells and from the studies of Townsend ('97) we know that non-nucleated protoplasm may live so long as it is united with nucleated, even though it be by very delicate fibrillæ. Michiewicz (:04) confirms Kny's conclusions for *Lupinus* and gives a very clear account of the fibrillæ which connect the masses of intercellular protoplasm with neighboring protoplasts. These studies make clearer a number of observations of several investigators (Sauvageau, Buscalioni, Schenk, Magnin, Strasburger, and others) who have noted similar conditions in the tissues of higher plants which are being investigated in detail by Kny. Some of the lower unicellular forms likewise exhibit an extracellular surrounding film or envelope, which may also be of a protoplasmic nature and consequently in the same position in relation to the protoplast as intercellular protoplasm. Thus it has been known for many years that the cells of the Peridinales, diatoms, and desmids possessed extracellular material, which some authors have considered in the nature of slimy excretions but others — Schutt ('99; :00a; :00b), Hauptfleisch ('88; '95), Müller ('98-'99) — have regarded as protoplasmic in character. Since the cell walls in these forms are known to possess pores, such extracellular substance must be in close association with the cytoplasm of the cell and it is not at all difficult to conceive of it as a part of the protoplasm. Some of the peculiar creeping movements of the diatoms and desmids are perhaps explainable upon these facts.

## 2. Sexual Cell Unions and Nuclear Fusions.

The test of a sexual act must lie with the history of the elements which fuse. If these are shown by their morphology and developmental history to be sexual cells or gametes then their fusion becomes a sexual process. There are cell and even nuclear fusions which have the physiological appearances of sexual acts but cannot be so considered because the elements concerned have plainly no relation to sexual cells, which are

developed at other periods of the life history, or to the primitive conditions always found with the origin of sex. These exceptional processes will be collected and described under the heading "Asexual Cell Unions and Nuclear Fusions," following this portion of the paper.

The union of gametes is generally termed fertilization. The evolution of the sexual process always tends towards a differentiation of the two sexual cells, one becoming more richly stored with food material and containing more protoplasm than the other. This latter gamete is always considered the female and is said to be fertilized when the male gamete, either as a motile sperm or reduced simply to a sperm nucleus generally with some accompanying protoplasm, fuses with it. The most evident morphological feature of fertilization is the close union of the gamete nuclei so that the chromosomes of both enter into the mitotic figure with which the new generation begins.

We shall not discuss the various forms of gametes nor their habits in different types of sexual reproduction. They have been described in two articles by the author on the origin and evolution of sex in plants (Davis, :01 ; :03). A detailed account of the sexual reproduction of well known types throughout the plant kingdom has been recently published by Mottier (:04b) under the title "Fecundation in Plants" a term which he prefers to fertilization. This paper gives in English the most extensive summary of our knowledge of the subject up to the date 1902 and will be read with especial interest as the most available expression in English of Strasburger's general views on the significance of the events connected with sexual reproduction.

A recent paper of Guérin (:04) is confined to an account of fertilization in the phanerogams which are treated in considerable detail. His discussion of double fertilization and parthenogenesis is of especial interest and will be taken up later.

Our purpose is to divest from the events of sexual cell unions and nuclear fusions all secondary and unessential processes and to outline, as are now understood, the fundamental phenomena. And to make the subject more plain we shall try to compare in their essentials the events of fertilization in plants with those

in animals. Probably the most important feature of fertilization is the close union of the gamete nuclei through which the chromosomes of both enter into the first mitotic figure of the new generation. It involves the organization of the first cleavage spindle, which inaugurates the new generation, and the history of the paternal and maternal chromosomes of the gametes at this time when the number becomes doubled.

Several zoölogical papers have developed in the past few years some very important conclusions concerning the individuality of the paternal and maternal chromosomes, as maintained during the fusion of the gamete nuclei and in the formation of the first cleavage spindle. It has been generally believed for some time — see general review in Wilson (:00, p. 204) — that the fusion of gamete nuclei did not involve a coalescence of the chromosomes but that both paternal and maternal chromosomes maintained complete independence of one another and that all entered into the first cleavage spindle as structures quite as distinct as when formed during spermatogenesis and oögenesis. Häcker and Rückert have shown for *Cyclops* that the gamete nuclei divide side by side in the first mitosis following fertilization, and Häcker followed these double nuclei as far as the 16-celled stage when they were still distinct from one another. A few notable investigations of recent years have identified chromosomes accurately as maternal and paternal not only in the first cleavage spindle but through certain succeeding mitoses and finally at the period of gametogenesis when sperm and egg were again formed. The above principles have been established chiefly through a series of papers of Montgomery, the chief being a lengthy investigation of 1901, and contributions of Sutton (:02 ; :03) and Moenkhaus (:04). They have given us clear evidence that the chromosomes not only maintain their complete individuality throughout successive generations but are distributed with gametogenesis and fertilization in various possible combinations that can be expressed by mathematical formulæ furnishing the basis for certain ratios that approximate the teachings of Mendel's law. We shall have occasion to refer to these in Section V when the subjects of gametogenesis, reduction of chromosomes, and hybridization will be discussed.

The same principles have been established in plants by recent investigations, some of which deal with oögenesis and spermatogenesis and will be specially treated in the Section V while others treat of the behavior of the chromosomes when the gamete nuclei fuse and the sporophyte generation begins its development. The latter conditions concern the present discussion.

The history of the chromosomes in plants at the time when the gamete nuclei fuse (fertilization) is most accurately known for the pine. The last paper upon this type (Ferguson, '04) is very complete. Miss Ferguson gives a beautiful series of figures, some of which we have reproduced. The sperm nucleus comes in contact with the egg nucleus and sinks into the latter so that it lies in a depression, but as noted by Blackman ('98), it does not penetrate the membrane of the egg nucleus (Fig. 17, a). Both gamete nuclei thus lie side by side occupying approximately the same space formerly filled by the female. Each shortly gives evidence of preparation for the mitosis following fertilization (first cleavage spindle). The chromatin of the egg nucleus collects in a spirem, very close to the sperm, occupying a relatively small portion of this large female nucleus (Fig. 17, b). The chromatin of the sperm nucleus also takes position as a spirem on the side nearest its companion chromatin of the opposite sex. The remaining space of each nucleus is filled with a granular reticulum of a linin nature. At this time the amount of linin is extraordinarily large in proportion to the chromatin, suggesting that some of the latter substance has become changed to the former. Soon, delicate fibrillæ appear around the two spirems growing outward in various directions and finally crossing from one nucleus to the other. At the same time the two nuclear membranes become less distinct and shortly disappear. Thus the maternal and paternal spirems come to lie in a common area filled with delicate fibrillæ which run out to the granular cytoplasm that lay around the two gamete nuclei (Fig. 17, c). It should be especially noted that at no time in this history has there been a resting nucleus including both maternal and paternal chromosomes within a common nuclear membrane. The fusion of the gamete nuclei has only come with the actual formation of the first cleavage spindle.

The fibrillæ organize a multipolar spindle which is very variable in form, sometimes with broad poles of a multipolar diarch (Fig. 17, d) and at other times almost as pointed as in a typical bipolar spindle (Fig. 17, e). There are, of course, no centrosomes and the entire spindle is essentially of intranuclear origin. The history of its development recalls Miss Williams' account of the spindle in the pollen mother-cell of *Passiflora* (Sec. III, *Amer. Nat.*, vol. 38, p. 738, 1904). During spindle formation the spirems of the sperm and egg nuclei can be readily distinguished as was described by Blackman ('98) and Chamberlain

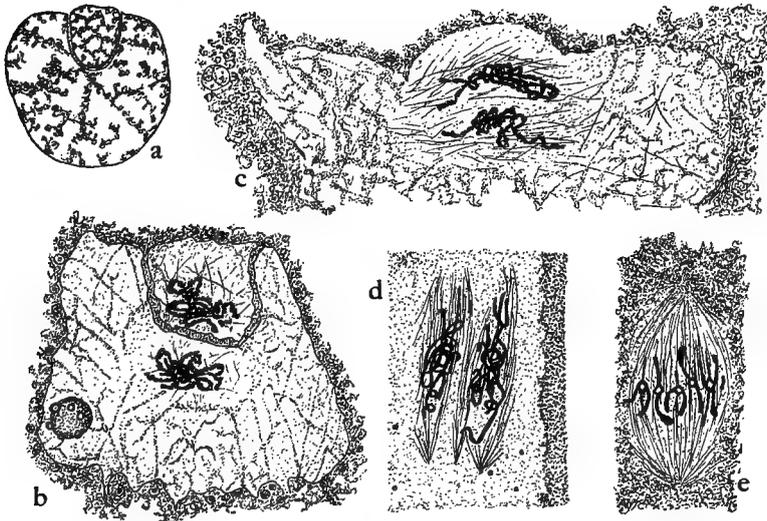


FIG. 17.—Fertilization in *Pinus strobus*. *a*, conjugating gamete nuclei; *b*, the gamete nuclei still separated, with nuclear membranes distinct, the maternal and paternal chromatin in two spirems; *c*, the nuclear membranes have disappeared and the two spirems lie close together surrounded by the fibrillæ which will organize the first segmentation spindle; *d*, prophase of the first segmentation spindle, of the multipolar diarch type, paternal and maternal spirems still distinct; *e*, metaphase of first segmentation mitosis, maternal and paternal chromosomes now indistinguishable, beginning to split in the middle region (after Ferguson, :04).

('99), but after the two sets of chromosomes are formed (twelve of each) the latter are brought so closely together at metaphase of mitosis that the paternal and maternal cannot be separated. All of the chromosomes are exactly alike and there is nothing in the form or size to distinguish one from another as certain

zoölogists have been able to do in some favorable animal types (Montgomery, Sutton, Moenkhaus). The chromosomes divide longitudinally in the usual way, the halves being drawn apart from the points of attachment of the spindle fibers (Fig. 17, e). It is clear that each daughter nucleus receives a full set of 24 daughter chromosomes, 12 of paternal and 12 of maternal origin, and that there is about an equal amount of chromatin from each sex.

It should be especially noted that in the process of fertilization in the pine there is at no time present what is generally called a fusion nucleus, *i. e.*, a single nucleus whose membrane incloses all the material of both male and female gamete nuclei. Such fusion nuclei, as we shall see, have been reported many times in other groups of plants than the gymnosperms where in many cases, however, detailed studies are very difficult and can scarcely be said to have even approached our knowledge of the pine.

Studies of other botanists indicate that the gymnosperms generally will show essentially the same conditions as in the pine. Thus Woycicki ('99) distinguished in *Larix* two groups of chromatin which he regarded as paternal and maternal. And Murrill (:00) states for *Tsuga* that the chromatin of sperm and egg remain separate, forming two spirems, and only after their segmentation into chromosomes are the two sets of structures brought together in the first cleavage spindle. Land (:02) figured the sperm nucleus of *Thuja* imbedded in a depression of the egg nucleus. Miyake (:03a) noted that the sperm nucleus of *Picea* became more or less imbedded in the egg nucleus while the nuclear membrane remained intact, and the same author (Miyake, :03b), reports similar conditions in *Abies*. Robertson (:04) figures the sperm nucleus of *Torreya* lying within a depression in the female and with a large amount of granular cytoplasm (kinoplasm) at the side. Coker (:03) states that the partition between the gamete nuclei of *Taxodium* "does not entirely disappear until immediately before the first division" although the two structures are closely united for some time previously while they pass to the bottom of the egg.

Lawson, studying *Sequoia* (:04a) reports gamete nuclei of

about equal size whose chromatin contents unite in a fusion nucleus to form a common network in which male and female elements cannot be distinguished. A similar condition obtains in *Cryptomeria*, according to Lawson (:04b), where a fusion nucleus is described in which paternal and maternal chromatin are mingled together in a nucleus that passes through a short period of rest before the development of the first cleavage spindle. In view of the work on *Pinus* I think it may safely be questioned whether in *Sequoia* and *Cryptomeria* the maternal and paternal chromatin really does form a common network in the resting fusion nucleus. The subject is one very difficult of study and demands more stages than Lawson seems to have followed.

Fertilization in the cycads is not as completely known as for the conifers. Webber (:01) figures the sperm nucleus of *Zamia* imbedded in the egg nucleus but quite distinct from it as in the pine but the further history leading to the development of the first segmentation spindle was not followed. On the other hand Ikeno ('98b) described in *Cycas* the formation of a cup-like depression in the egg nucleus to receive the sperm nucleus which was said to enter and fuse completely with the female and the same author (Ikeno, :01) reports a complete fusion of the gamete nuclei in *Ginkgo* and did not distinguish the paternal and maternal chromosomes during the formation of the first segmentation spindle. However it is probable that more detailed studies among the cycads and in *Ginkgo* will show a behavior of the sperm nucleus together with the paternal and maternal chromatin essentially similar to that of the conifers. All investigations among the cycads and in *Ginkgo* agree that cytoplasmic structures of the sperm (blepharoplasts, cilia, etc.) are left behind in the cytoplasm of the egg before the gamete nuclei unite.

Our knowledge of the details of fertilization in the angiosperms is surprisingly meager. The only account of the chromatin is that of Mottier ('98 ; :04b, p. 176) for *Lilium*. He describes and figures the two gamete nuclei as uniting with their chromatin in the resting condition. The nuclear membranes disappear at the surface of contact and the two nuclei

fuse into one. The nucleoli unite and so thoroughly does the paternal and maternal chromatin seem to be mixed in the resting condition that the fertilized egg nucleus can scarcely be distinguished from the unfertilized. There would seem to be then a fusion nucleus in the lily with the chromatin in the resting condition. The figures and brief accounts of other botanists indicate that similar conditions may be expected in other angiosperms. But no one has followed the chromatin in the fusion nucleus through its later history, during the organization of the chromosomes preparatory to the first mitosis following fertilization. It would be very surprising if paternal and maternal chromatin did not remain entirely independent of each other as in the pine. The detailed study of fertilization in the angiosperms presents a very attractive subject for investigation.

Some very interesting conditions of fertilization have been

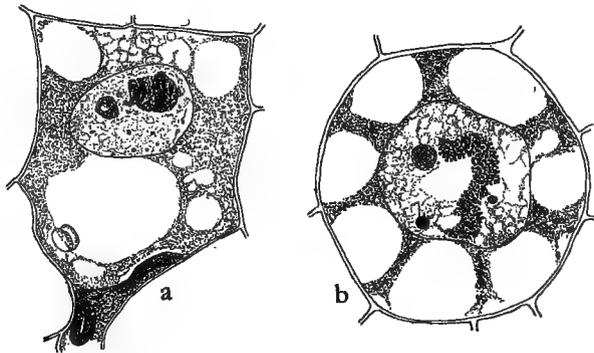


FIG. 18.—Fertilization in *Onoclea sensibilis*. *a*, sperm as a spiral band within the egg nucleus; *b*, later stage, the chromatin of the sperm much less condensed and more widely distributed in the egg nucleus (after Shaw, '98a).

described in the pteridophytes for *Onoclea* by Shaw ('98a), confirmed by Mottier (:04 a; :04 b), and for *Adiantum* and *Aspidium* by Thom ('99). In these forms the male nucleus after leaving in the protoplasm of the egg all of the cytoplasmic structures of the sperm (blepharoplasts, cilia, etc.) enters the egg nucleus as a more or less spiral body which stains deeply and is evidently chiefly or wholly chromatic in composition (Fig. 18). Within the egg the dense structure of the sperm nucleus becomes looser by the separation of the chromatin granules (Fig.

18, b) but the form of the sperm can be recognized for a long time. The chromatin of the egg nucleus is in a resting condition at this period and the densely packed mass of paternal chromatin is very conspicuous in the loose, delicate network of the female chromatin. The mitosis following fertilization does not occur for several days so that it is not easily studied and the organization of the first cleavage spindle with the history of the maternal and paternal chromosomes has never been followed. But it is clear that we have in the pteridophytes a true fusion nucleus containing for several days both maternal and paternal chromatin within the same nuclear membrane.

There is only one paper that gives any details of fertilization in the bryophytes, a contribution of Kruch ('90) on the liverwort, *Riella*, which seems to have been generally overlooked in recent literature. After the sperm enters the egg, a male nucleus is organized which increases in size until it is about equal to the egg nucleus. The chromatin in both gamete nuclei is described and figured as forming 8 chromosomes which are organized before the fusion. The two gamete nuclei were observed, but not figured, in contact and it was not possible to distinguish in size the male from the female. This account is then very different from those of the pteridophytes since the sperm nucleus does not enter the egg nucleus but the two fuse side by side and with their chromosomes fully organized. There are, however, some points in Kruch's paper that require more extended investigation and confirmation in the light of modern research.

There is left only the group of the thallophytes where less is known about the detailed behavior of the chromatin during fertilization than in any region of the plant kingdom. The conjugation of the gamete nuclei has been observed in a number of thallophytes, representing all of the higher groups. All of the authors, with the exception of Chmielewski ('90 b) for *Spirogyra*, describe the product of conjugation as a fusion nucleus, *i. e.*, one in which the nuclear substance of both gametes is contained within a common nuclear membrane. The most detailed accounts of the fusion of gamete nuclei in the thallophytes are those for *Fucus* (Strasburger, '97 a; Farmer and Williams, '98).

The sperm of *Fucus* upon entering the egg loses its cytoplasm and passes rapidly to the egg nucleus as a deeply staining body resembling a plastid in form. This structure is the male nucleus whose chromatin is so densely crowded that it stains too deeply to show much structure. Arriving at the side of the female nucleus, about ten minutes after its entrance into the egg, the male nucleus flattens against the female and increases in size so that the chromatin appears less condensed. The male nucleus is then absorbed so that the paternal chromatin lies within a fusion nucleus but may be distinguished for some time as densely staining material at one side. A second nucleolus often appears in the fusion nucleus in the vicinity of the paternal chromatin and is probably associated with the entrance of the sperm nucleus, although it is not likely to have been brought in as an organized structure but developed later at the expense of material in the sperm nucleus. The fusion nucleus remains quiescent for from 20 to 24 hours during which time the paternal chromatin becomes so distributed that it can no longer be followed. Then two centrospheres with conspicuous radiations appear at opposite poles of the fusion nucleus and the first cleavage spindle is organized. There is no evidence that either of these centrospheres is brought into the egg by the sperm and both appear *de novo* and independently of one another.

The chief accounts of the fusion of gamete nuclei in thallophytes are as follows: *Closterium* and *Cosmarium* (Klebahn, '91); *Rhopalodia* (Klebahn, '96); *Cocconeis* (Karsten, :00); *Sphæroplea* (Klebahn, '99; Golenkin, '99); *Ædogonium* (Klebahn, '92); *Coleochæta* (Oltmanns, '98); *Vaucheria* (Oltmanns, '95; Davis, :04); *Fucus* (Strasburger, '97a; Farmer and Williams, '98); *Batrachospermum* (Schmidle, '99; Osterhout, :00); *Nemalion* (Wolfe, :04); *Basidiobolus* (Fairchild, '97); *Albugo* (Wager, '96; Stevens, '99, :01 b; Davis, :00); *Peronospora* (Wager, :00); *Pythium* (Miyake, :01; Trow, :01); *Achlya* (Trow, :04); *Araiospora* (King, :03); *Sphærotheca* (Harper, '95); *Pyronema* (Harper, :00). Most of these papers with others on fertilization in the thallophytes are summarized by Mottier, (:04 b) in very convenient form for reference.

There is some confusion in the accounts of fertilization in

Spirogyra which should be thoroughly investigated. Chmielewski ('90b) in a paper published in Russian and reviewed in the *Bot. Centralb.*, vol. 50, p. 264, 1892, described a fusion of the gamete nuclei in the zygospore and an immediate mitosis, without a period of rest, followed at once by a second division of the daughter nuclei. These mitoses give the zygospore four nuclei, two of which unite to form a final resting nucleus in the zygospore while the remaining two fragment and their products finally break down. This behavior offers an exception to all sexual processes so far known in the plant kingdom. There are some features which suggest a possible confusion with events as described in the zygospore of the desmid and the auxospores of certain diatoms.

The fusion nucleus in the zygospore of *Closterium* and *Cosmarium* (Klebahn, '91) divides into four at the time of germination and two of these break down while each of the others becomes the nucleus of the two new desmids that are formed. There is then in the desmids the division of the fusion nucleus into four but no secondary nuclear fusions as Chmielewski reports for *Spirogyra*. In certain diatoms, *Rhopalodia* (Klebahn, '96) and *Cocconeis* (Karsten, :00), there is a preliminary division of the nuclei in each of the two cells which form the auxospore. In *Rhopalodia* the mitoses are carried so far that four daughter nuclei are formed in each diatom and the protoplasm divides into two cells each of which fuses with a corresponding cell of the companion pair. In both types the superfluous nuclei break down so that the conjugating cells have each a single functional gamete nucleus. There are then complications in the Conjugales and the diatoms, which make nuclear studies of the sexual processes exceptionally difficult and we seem justified in reserving our judgment of the results of Chmielewski until confirmed. It seems possible that the mitoses following the germination of the zygospore in the Conjugales with the attendant nuclear degeneration are reducing divisions in a simple and primitive type of sporophyte generation but more detailed studies of nuclear behavior during the formation and germination of the zygospore will be necessary to settle the discussion.

We have now finished our account of nuclear fusions in the

sexual act (fertilization) but there remains for consideration the behavior of certain cytoplasmic elements introduced into the sexually formed cell, especially chromatophores and the blepharoplast. Since the blepharoplast bears a very close resemblance to the middle piece of the animal spermatozoon, which sometimes becomes a centrosome in the animal egg, a critical comparison of the behavior of these two structures during fertilization is full of interest.

Except for certain accounts of *Spirogyra*, to be described in the next paragraph, investigators agree that the chromatophores or plastids of gametes never fuse in the sexually formed spore. Plastids have not been found in the sperms of the gymnosperms, pteridophytes, nor bryophytes. The sperms of some algæ also appear quite colorless at maturity but careful examinations have shown in a number of forms a very small chromatophore in the early stages of development. Other less highly differentiated sperms are known to have chromatophores (*e. g.*, *Sphæroplea*, *Cutleria*, *Volvox*). Both gametes in the isogamous types of sexuality among the algæ always have chromatophores or plastids. These have been followed in detail through stages of fertilization in *Ectocarpus* by Berthold ('81) and Oltmanns ('99), and in *Scytosiphon* by Kuckuck ('98) where it is evident that they do not unite and there is no reason for believing that different conditions obtain among any of the lower forms such as *Ulothrix*, *Cladophora*, *Hydrodictyon*, etc., although detailed observations are greatly lacking on this point, chiefly because the conjugating cells are generally very small.

Early accounts of the formation of the zygospore of *Spirogyra* have reported some form of union of the chlorophyll bands of the two gametes. The last work upon the subject, Chmielewski ('90a), reviews the results of previous investigators and gives a detailed account of a species of *Rynchonema* (*Spirogyra*). Chmielewski claims that the chromatophore of the gamete (male) that passes over into the other cell (female) becomes disorganized as the zygospore develops. While the chlorophyll band of the female cell retains much of its color, that from the male becomes yellowish and breaks up into fragments which become scattered in the zygospore and finally break down. This inter-

esting account accompanied by clear figures gives an explanation far more in keeping with what we know and might expect of the behavior of chromatophores in resting spores. That the green chromatophore may temporarily become much modified in color and form is well known in some of the red and orange resting spores of the Volvocaceæ and the zygospores of the desmids. In some of these types the form and color of the chromatophores become quite lost for the time being so that studies on their behavior are very difficult. For these reasons it seems probable that the accounts of the fusion of the chromatophore in the zygospore of *Spirogyra* are incorrect. It is very interesting that the gametes of *Spirogyra* should be so sharply differentiated that the chromatophore of one should be reduced during conjugation in a manner that resembles very closely the behavior of the chromatophore in highly differentiated sperms.

There is no evidence that the pigment spots, so generally present in the motile gametes of lower forms, ever unite. They have been followed into the zygospore and after the germination of this cell and they remain entirely independent of one another as would be expected from their close relationships to chromatophores.

The fate of the blepharoplast will now be considered. This structure is especially interesting because of its close analogy to the locomotor apparatus of the animal spermatozoon, which is formed chiefly from one or more centrosomes generally with the coöperation of archoplasm (idiosome, *Nebenkern*). It is also claimed by a number of zoölogists that in some forms, at least, the centrosomes of the first cleavage spindle are derived from the spermatozoon.

All evidence indicates that the blepharoplast of the plant sperm is left behind in the cytoplasm of the egg when the male nucleus passes into the interior to unite with the female and that centrospheres when present, in the first cleavage spindle, are formed *de novo*. The fate of the blepharoplast is clearly known in *Cycas* (Ikeno, '98b), *Zamia* (Webber, :01) and *Ginkgo* (Ikeno, :01). Soon after the large top-shaped sperm of these forms enters the egg, the male nucleus slips out of the spiral blepharoplast, that partially invests it, and, leaving it with

other cytoplasm of the sperm at the end of the egg, passes quickly to the center to unite with the female nucleus. The blepharoplast remains near the periphery of the egg and may be recognized even after the gamete nuclei have united. It finally breaks down and its substance becomes lost in the cytoplasm of the egg. The most complete account of the history of the blepharoplast in the fertilized egg is that of Webber (:01). We should naturally expect the first cleavage spindle in the cycads and Ginkgo to be developed as in the conifers. Ikeno (:01) described clearly an intranuclear spindle in Ginkgo. In the conifers, as previously described, the first cleavage spindle is intranuclear and the fibers are developed freely from a mesh and form a broad poled spindle without centrospheres. So that not only does the blepharoplast break down at a distance from the egg nucleus but we have no reason to think that there is any place for a centrosome in the history of the first cleavage spindle in the gymnosperms.

We do not know clearly the fate of the blepharoplast in the egg of any pteridophyte or bryophyte, although Shaw's ('98a) studies on *Onoclea* indicate that it breaks down in the cytoplasm. Our knowledge of the thallophytes is equally incomplete as regards the history of the blepharoplast in the egg. But both Strasburger ('97a) and Farmer and Williams ('98) have agreed for *Fucus* that the two centrospheres at the poles of the first cleavage spindle develop *de novo* and independently of one another, and Williams (:04b) holds the same view for the centrosphere which appears at the side of the fertilized egg of *Dictyota*. The sperms of the thallophytes are generally very small cells and it may prove a difficult matter to follow their blepharoplasts so that our opinions of events in these forms are likely to be largely inferential from our knowledge in higher groups.

We can safely say that there is no evidence that the blepharoplast ever enters into the first cleavage spindle which is certainly developed in the spermatophytes and probably in the pteridophytes without centrosomes or centrospheres. Where centrosomes or centrospheres are known for the first cleavage spindle in the thallophytes (*Fucus* and *Dictyota*), the observations indi-

cate that such structures have not come from the blepharoplast. Williams' (:04b) recent work on Dictyota, while incomplete in the series of stages illustrating the fusion of gamete nuclei (fertilization), presents a very interesting comparison of the development of the first cleavage spindle in fertilized eggs with parthenogenetic eggs. In the fertilized egg there is regularly found a centrosphere which apparently divides into two that separate until they lie at opposite poles of the mature spindle. In the parthenogenetic egg, on the contrary, the spindle is multipolar and develops very irregularly from a kinoplasmic mesh which is intranuclear and there is no sign of centrospheres. Williams believes that fertilization enables the fusion nucleus to form *de novo* a centrosphere external to itself which is not possible for the nucleus of a parthenogenetic egg.

It should be noted that these conclusions are all against the view that the centrosome is a permanent organ of the cell and that the blepharoplast holds any direct relation to centrosomes when present in the first cleavage spindle and inferentially rather strengthens the doubt that the blepharoplast is derived from a centrosome, which point was discussed in our account of the sperm in Section III. However, Ikeno (:04) in a paper which arrived too late to be treated in Section III, is very positive that blepharoplasts are centrosomes, presenting his evidence clearly, but his explanation of the conditions under which blepharoplasts are formed from the plasma membrane does not seem to me conclusive, especially in the light of Mottier's (:04a) recent paper on Chara, which also could not be treated in Section III (see *Amer. Nat.*, vol. 38, p. 576, 1904).

### 3. Asexual Cell Unions and Nuclear Fusions.

As stated earlier in the paper, the test of a sexual act must lie with the history of the elements which unite, unless we choose to treat sexuality as a purely physiological process and disregard its relation to morphology in ontogeny and phylogeny. This relation is so precise, *i. e.*, sexuality is so firmly established as a fixed period in the life history of most organisms, that the biologist generally thinks of the sexual process as a part of the

life history, which must take place with as much regularity as the normal development of any organ. As a matter of fact, our knowledge of the structure of sexual elements and the events of sexual phenomena is almost wholly morphological and for the present at least it seems safer to treat and define sexuality from a morphological standpoint.

Under asexual cell unions and nuclear fusions we shall include a number of interesting phenomena which can be arranged in three groups: (1) cell fusions which have apparently no sexual relations; (2) cell fusions which are substitutes for a normal ancestral sexual process now suppressed; and (3) extraordinary modifications of what may have been originally sexual processes but which at present serve some peculiar and special function.

In the first group will be included the extensive union of swarm spores, or the amœboid elements derived from such, best illustrated in the development of plasmodia; also such cell fusions as are clearly for nutritive purposes, as is the union of the sporophytic portion of the cystocarp of the red algæ with auxiliary cells and probably also the fusion of sporidia in the smuts and the conjugation of yeast cells. The second group embraces the interesting fusions of the nuclei in teleutospores of the smuts and rusts and in the basidium with the previous history of the paired (conjugate) nuclei in the mycelium, perhaps also the nuclear fusions in the ascus, and such cell unions as have been reported preliminary to the apogamous development of the fern sporophyte. The third group includes the remarkable phenomenon in the embryo sac, the double fusions of the polar nuclei and the triple fusion of these with the second sperm nucleus, frequently called "double fertilization."

The well known union of the swarm spores of the Myxomycetes as amœboid cells (*myxamœbæ*) to form the plasmodium is one of the best illustrations of a fusion of protoplasm without sexual significance. In this general union of hundreds and perhaps thousands of small cells there are no nuclear fusions so far as is known, but simply the merging of the cytoplasm to form a large multinucleate unit. The whole phenomenon indicates a coöperative process which is probably economical of nutritive functions in the semiterrestrial conditions under which plas-

modia live. It is quite possible that the origin of sex may have been involved with some of the same principles as those which bring about the union of swarmers to form a plasmodium, but the added features of nuclear fusion together with the history of the sexually formed cells which become in higher groups the starting point of a sporophyte generation places the sexual act on a very much higher level of complexity.

There are some records of the union of several zoöspores or gametes to form a zygospore instead of the usual conjugation in pairs. The biciliate gametes of *Acetabularia* (De Bary and Strasburger, '77) sometimes conjugate in threes and large zygotes are figured with five pairs of cilia indicating that as many gametes entered into their formation. The gametes of *Protosiphon*, described by Rostafinski and Woronin ('77) as in the life cycle of *Botrydium*, are reported by them to unite at times several together and four are so figured. Klebs ('96, p. 207) in his account of *Protosiphon* also noted the union of the gametes in threes especially when in organic solutions. The significance of these multiple fusions of swarm spores is not clear for we know nothing of the nuclear history following the union. There is in the habit, however, such a resemblance to the extensive union of swarmers in the *Myxomycetes* as to indicate that primarily sexuality may have been concerned chiefly with cytoplasmic fusions and associated very intimately with nutritive processes. I have recently observed several instances of the conjugation of zoöspores of *Saprolegnia* when the elements united in pairs at the ciliated ends and along the sides exactly as do motile gametes, and the fused cell bore four cilia. The zoöspores of *Saprolegnia* are too far removed morphologically from the highly differentiated sexual organs of the group to justify the explanation of such conjugation as a sexual act and we must think of it as due to some peculiarities of nutritive conditions.

Another class of very interesting cell fusions, associated with nutritive functions, is presented in the union of the sporophytic fertile filaments (oöblastema filaments) in the cystocarp of the *Rhodophyceæ* with auxiliary cells. This phenomenon which was regarded by Schmitz and his followers as sexual in charac-

ter, is considered by Oltmanns ('98b) to have nutritive relations alone. Oltmanns studied the fusion with auxiliary cells in several genera, but especially for *Callithamnion* and *Dudresnaya*, and is satisfied that the cell unions concern only the cytoplasm. Fertilization takes place with the fusion of gamete nuclei in the carpogonia and these cells develop the sporophyte generations. The fusion of fertilized carpogonia or filaments derived from them with auxiliary cells, is a feature of a sort of semiparasitic relation that the sporophyte holds to the gametophyte by which it is nourished in part through organic connections with the gametophyte. The nuclei of the sporophytic structures remain quite apart from those of the auxiliary cells so that the union is purely cytoplasmic. This theory of Oltmanns has received strong support through the detailed nuclear studies of Wolfe (:04) on fertilization and the development of the cystocarp of *Nemalion* who finds cytological evidence of the sporophytic character of the cystocarp. These papers of Oltmanns and Wolfe have been discussed by myself in the *Bot. Gaz.*, vol. 27, p. 314, 1899, and vol. 39, p. 64, 1905.

Writers have at times attached sexual significance to the conspicuous fusions between sporidia of certain of the Ustilaginales (*e. g.*, *Tilletia*). But there seems at present no reason to regard this phenomenon as different from the cytoplasmic connections frequently established between cells of hyphæ which are ultimately associated in a common mycelium where the whole forms a close unit with respect to common nutritive relations. Such protoplasmic connections were treated in the first part of this section. Harper ('99a) studied the union of conidia and cells of the promycelium in *Ustilago* and concluded that the fusions involve the cytoplasm alone, there being no nuclear changes. However, Federley (:03-:04; review in *Bot. Zeit.*, vol. 62, p. 171, 1904) has observed the migration of a nucleus from one conidium to another in *Ustilago tragopogonis pratensis* (Pers.), and a fusion within the latter. This nuclear fusion was not found in some other forms of *Ustilago* which behaved as Harper has described. There is nothing in the morphology of the conidia to indicate that they are sexual cells and from what we know of the life history of Basidiomycetes we should look

for sexual processes at other periods more closely associated with the development of teleutospores or basidia.

The conjugation of yeast cells has many points of similarity to the fusion of conidia in the Ustilaginales. This phenomenon has been discovered in an organism obtained from commercial ginger by Barker (:01), which he calls *Zygosaccharomyces*, and in three species of *Schizosaccharomyces* by Guilliermond (:03). The conjugation in all forms immediately precedes spore formation and involves a nuclear fusion as well as that of the cytoplasm. The conjugation is followed by division of the fusion nucleus and spore formation in the united cells. The conjugating cells are sisters in the species of *Schizosaccharomyces* but apparently may not be closely related in Barker's form, *Zygosaccharomyces*. Both investigators regard the conjugation as a sexual act, and Guilliermond considers the fusion cell to be an ascus with the value of a zygosporangium. These conclusions do not seem to the writer convincing. Spore formation in the yeasts has not been shown to present any of the peculiarities of nuclear division and free cell formation as described by Harper for the ascus, and until such are established it is hardly safe to conclude that the yeasts are Ascomycetes. Whether or not the conjugation is a sexual process becomes a question of phylogeny and we know too little of the history and relationships of the yeasts to assert that the conjugating cells are morphologically gametes. Again, the view that yeasts are derived from conidia or mycelia of higher fungi which have continued a simple growth by budding in peculiar and favorable media is rather against any view that we are dealing here with a simple or primitive sexual act. There are very striking resemblances to the fusions of conidia in the Ustilaginales, which were described in the previous paragraph and do not appear to be sexual processes. It is unsafe to assume sexuality because the conjugation precedes spore formation, because in most yeasts spore formation takes place regularly without conjugation. Is it not rather another illustration of cell and nuclear fusions related to nutritive processes alone?

Some of the most interesting nuclear fusions, apparently associated with the apogamous development of a sporophyte are

the unions of the pairs of nuclei which enter the cells of the developing teleutospores of the Uredinales and Ustilaginales and the basidium of higher Basidiomycetes. It has been established through the studies of a number of investigators (chiefly Rosen, '93; Dangeard and Sapin-Trouffy, '93; Dangeard '93, '94-'95a, c; Poirault and Raciborski, '95; Sapin-Trouffy, '96; Maire, :00 a, b, c, :02; Holden and Harper, :03) that the æcidiospores and the mycelium derived from them and preceding the development of the uredospores and teleutospores contain pairs of nuclei which divide in such a manner (conjugate division) that the nuclei of the pair are derived through two unbroken lines of succession for a long vegetative period and always maintain complete independence of one another. Every young teleutospore and basidium contains such a pair of nuclei which shortly fuse so that the mature structure is uninucleate. Dangeard and Sapin-Trouffy have from the first regarded the nuclear fusion within the teleutospore, whether of rust or smut, as a sexual act and the ripe teleutospore a fertilized egg, regardless of the fact that its morphology was not that of any known sexual organs. Dangeard ('94-'95 c; :00) likewise considered the nuclear fusions in the basidium as sexual. Raciborski ('96) suggested that the series of conjugate mitoses leading to the nuclear fusions in the teleutospore represented a vegetative phase intercalated between the beginning of a sexual act and its finish in the teleutospore. His explanation, in the light of the recent paper of Blackman (:04a), was nearest the truth. Maire (:02) presents the most extensive account of the nuclear structure in the higher Basidiomycetes previous to and during the formation of the basidia. He held that the fusion of the paired nuclei (synkaryon) in the basidium was not the whole act of fertilization which must begin with the formation of the paired nuclei. Maire (:02, p. 189) gave some suggestions as to how and where the paired nuclei arose but neither he nor any of the authors mentioned above knew clearly their origin.

Blackman (:04a) has made the most important contribution to the subject of fertilization and alternation of generation in the Uredinales, showing clearly that the paired nuclei appear in the life history of *Phragmidium violaceum* and *Gymnosporangium*

*clavariæforme* just before the development of the æcidium. They arise in Phragmidium by the migration of a nucleus from an adjacent cell into an element (the fertile cell) which represents a female sexual organ. The morphology of the female organ is not clear but there are suggestions of a structure similar to the procarys of the Rhodophyceæ and Laboulbeniales. The fertile cell, after receiving its second nucleus, develops a chain of æcidiospores, the two nuclei becoming so closely associated in the paired condition that they divide simultaneously (conjugate mitosis) from now on until the teleutospores are formed. Thus the cells of all mycelium beginning with the æcidiospore contain paired nuclei up to the development of the teleutospores, including of course the uredospores when present. This period of the life history may be considered as representing a sporophyte generation, especially since the total of chromatin in the pair of nuclei is double the amount when the nuclei are solitary. The sporophyte phase ends with the fusion of the pair of nuclei in each cell of the teleutospores and in the reduction phenomena that take place with the germination of the teleutospore, including the formation of the promycelium. The sporidia developed by the promycelium are uninucleate and the cells of the mycelium derived from them are uninucleate up to the production of the æcidium. This constitutes the gametophyte phase of the life history. The spermogonia by their morphology seem to be male organs, now functionless.

In such of the Uredinales as have no æcidium, as also in the higher Basidiomycetes and the Ustilaginales, it is probable that both sexual organs are suppressed since no trace of such structures has been found. However, we may expect to discover periods in all of these forms when paired nuclei come into the life history and after a series of conjugate divisions fuse in the teleutospore or basidium. Such pairs of nuclei, as stated before, are known in the Ustilaginales (Dangeard, '93) and in a number of forms of the Uredinales and the nuclear fusions have been followed in the teleutospore. Holden and Harper (:03) have given an especially clear account of the paired nuclei in the mycelium and uredospores of *Coleosporium* together with their fusion in the teleutospore. Maire (:02) describes the paired

nuclei (synkaryons) and their fusion in the basidium in a large number of Hymenomycetes and Gasteromycetes.

Evidence is thus accumulating that the cells in the mycelium of higher Basidiomycetes (Hymenomycetes and Gasteromycetes) are binucleate for extended periods previous to the formation of basidia where nuclear fusions always take place. Binucleate cells in the higher Basidiomycetes were first reported by Maire (:00a; :00b), in the tissue preliminary to spore formation. He also confirmed Dangeard ('94-'95c) in his view that only two nuclei unite in the basidium contrary to accounts of Rosen ('93) and Wager ('99, p. 586) which described a succession of fusions involving sometimes as many as six or eight nuclei. Harper (:02) has given for *Hypochnus* one of the most complete accounts of the behavior of paired nuclei previous to and during the development of the basidium. The cells of the mycelium of this simple Hymenomycete were found to be binucleate as far back as they were studied which included all of the conspicuous vegetative structure. Only a single pair of nuclei enters the basidium and fuses. Harper's results are then in agreement with the extended observations of Maire (:02) as are also the detailed studies of Ruhland (:01) on a number of forms and Bambeke (:03). Taken together they seem to show clearly that the mycelium, for long periods preliminary to the formation of basidia, contains paired nuclei and that the basidia receive each a single pair, which nuclei fuse. There is thus an exact correspondence between the life histories of the Ustilaginales, Uredinales, and higher Basidiomycetes with respect to the period of paired nuclei and their fusion in the teleutospore or basidium. Dangeard called the fusion in the basidium a sexual act and the structure an oöspore regardless of the morphological difficulties of such a conception. Maire (:02, p. 202) states that the origin of the paired nuclei is the only phenomenon strictly comparable to fertilization and Blackman's studies support this view. Ruhland (:01) regards the conditions as a deviation from the normal type of sexuality calling it "intracellular karyogamy." The origin of the paired nuclei is not known for any higher Basidiomycete and the discovery of this period and determination of the events leading to the change from uninucleate mycelium to

binucleate is one of the most interesting problems in this field of botany. This is the point where we should expect to find the remains of sexual organs, if any are present in the higher Basidiomycetes, but it is not likely that they will be found. It seems more probable that the mycelium with the paired nuclei (perhaps sporophytic in character) arises apogamously with a complete suppression of the sexual organs in agreement with such of the Uredinales as have no æcidium and the Ustilaginales.

Blackman's explanation of the history of the paired nuclei in *Phragmidium* is full of interest. As stated before, he regards the fertile cell which develops a chain of æcidiospores, "as a female reproductive cell which undergoes a process of fertilization" by a union with an adjacent cell of the mycelium and its reception therefrom of a nucleus. The mycelium then which arises with the æcidiospore is sporophytic in character and so remains until the fusion of the pairs of nuclei in the teleutospores. The male organs of the rusts are the spermogonia and the male gametes the spermatia which are of course now functionless so that the "process of fertilization" is through the introduction into the female cell of a nucleus which is not phylogenetically a male sexual element. Blackman's (:04 a, pp. 349-353; :04 b) conception of the process as an act of fertilization involves some principles which will be briefly outlined.

Blackman believes for *Phragmidium* "that the primitive normal process of fertilization by means of spermatia has been replaced by fertilization of the female cell through the nucleus of an ordinary vegetative cell" and regards the process as very similar to the phenomenon reported in the apogamous development of ferns by Farmer, Moore, and Digby (:03), which will be considered presently. Blackman points out that normal processes of fertilization such as we have included under the head of "sexual cell unions and nuclear fusions" do not involve in many forms (probably all types with a sporophyte generation) an immediate union of the chromatin of the sexual nuclei which is known to remain distinct during the first cleavage mitosis in a number of types (*e. g.*, *Pinus* and some other gymnosperms). So there is nothing in the delayed fusion of the paired nuclei up to the teleutospore that is seriously against his explanation of the "fer-

tilization" of the female cell of the Uredinales. Indeed, we may expect to find that the actual fusion of paternal and maternal chromatin does not take place in the higher plants until the end of the sporophyte generation in the spore mother cell, as zoölogists have concluded that such union occurs just previous to gametogenesis in animals. But is Blackman justified in regarding the phenomenon substituted for the activities of ancestral sexual organs in *Phragmidium*, now functionless, as a sexual act and is it desirable to apply the term fertilization to the phenomenon?

Blackman (:04 b, p. 153) speaks of the introduction of a nucleus into the fertile cell of the Uredinales and the phenomenon in the apogamous development of the fern after the account of Farmer, Moore, and Digby (:03) as "reduced forms of fertilization." It may be questioned whether the use of the term fertilization is fully justified by the events under discussion. We are all likely to agree with these authors that the physiological aspects of the phenomena in the cases under consideration are similar to sexual acts. But, by the writer, the act of fertilization is always considered in phylogenetic relations and strictly limited to the union of sexually differentiated cells, which are defined by their morphology through principles of homology. Whenever one or both of the gametes are suppressed in a life history and a succeeding generation develops of the sort that normally follows a sexual act, then such a development is apogamous and the phenomena always introduce features which are foreign to the processes of normal fertilization and the fundamental principles of sexuality.

Perhaps the most important characteristic of sexuality from an evolutionary standpoint is the fusion of gametes of unrelated parentage, for in the mingling of diverse protoplasm lie two factors: (1) a physiological stimulus to development, and (2) an increased probability of inherited variation which in new combinations will appear to the advantage of the species. Blackman's "reduced forms of fertilization" which I should prefer to consider apart from normal fertilization as examples of apogamy, and have so classed in this treatment, do satisfy the physiological requirements of a sexual act in that a form of nuclear fusion

is substituted for the union of gamete nuclei but the phylogenetic and evolutionary aspects of sexuality are disregarded. Also, the nuclei that fuse are sometimes very closely related, which is a condition generally avoided in sexual processes except where peculiarities of habit make close inbreeding necessary. It is true that large groups, such as the Basidiomycetes, perhaps certain regions of the Ascomycetes, some Phycomycetes, and some forms of the higher plants and algæ seem to have given up normal sexual processes but there is much evidence that in many cases this loss of sexuality is associated with a certain degree of segregation and with peculiarities of life conditions apart from the normal activities of all organisms or quite different from the ancestral stock. The groups are likely to be distinguished by highly specialized life habits of a sort that make it impossible for inherited sexual organs to function, either through mechanical difficulties or because one or both degenerate. It seems to me much clearer to regard all illustrations of Blackman's "reduced forms of fertilization" under the general term of apogamy even though it may be clear that they are physiological substitutes for sexual acts and to reserve the term fertilization for the union of gametes which can always be clearly identified through morphology in ontogeny and phylogeny. The success of a group even though ancestral sexual processes may be suppressed does not enter into a problem which is at bottom a morphological one. Success is relative and we really have no means of estimating its degree save by actual experiment. It is not likely that any biologist would claim that sexual degeneration is advantageous to any species although the organic world is full of forms which have dispensed with sexuality and still hold their places. These are the reasons why I have grouped cell unions and nuclear fusions as sexual and asexual on a morphological basis founded on phylogenetic principles and why in Section V, we shall devote some attention to the substitutes for sexuality under the head of apogamy.

The Ascomycetes present a phenomenon of nuclear fusion within the ascus which may properly be considered at this time since there is a certain resemblance to the nuclear fusions in the teleutospore and basidium. Dangeard ('94-'95b) gave the

first account of this phenomenon describing it for several forms. The mother cell of an ascus sometimes terminates a hypha but more commonly is situated a little back from the end at a point where the hypha bends abruptly like a knee. The mother cell contains two nuclei, closely related to each other, that unite, after which the fusion nucleus divides to form the ascospores. Dangeard considered this fusion to be a sexual act and the product an oöspore which germinates immediately to form the ascus. He regards the ascus as a sporangium, and equivalent to the promycelium which he calls a conidiophore. Dangeard is not willing to accept any of the evidence that the ascocarp ever results from a sexual act or that sexual organs either functional or abortive are present at any stage in the life history of Ascomycetes. Sexuality, according to him, is reduced to the fusion within the ascus alone. He (Dangeard, '96-'97a, b; :00) discredits the work of Harper on *Sphærotheca*, *Erysiphe*, and *Pyronema* and the older accounts of De Bary and his pupils on sexual organs of the Ascomycetes. A series of short papers in *Le Botaniste* (:03, Fas. 1) presents Dangeard's last attack on the work of Harper and a reaffirmation of his peculiar views.

Harper's description of sexual processes in *Sphærotheca* ('95; '96) *Erysiphe* ('96), and *Pyronema* (:00b) are so convincing that, together with our knowledge of sexual organs in the lichens, Laboulbeniales, and Gymnoascales, we must accept the old view of De Bary that the ascocarp represents a development (probably sporophytic) from a sexual phase even though it may be established that there is much apogamy in the Ascomycetes. Harper gives the clearest account of the nuclear fusion in the ascus of any author without, however, committing himself to speculations on its significance. The subject is well summarized in his paper on *Pyronema* (:00b, pp. 363, 394). He finds in *Erysiphe*, *Pyronema*, and some other forms that the ascus is always developed from a penultimate cell of a hypha which bends sharply so that this cell appears to lie at the tip. There are two nuclei at the end of the ascogenous hypha and these divide simultaneously in a very characteristic manner so that the young ascus receives two of the resultant four nuclei, but each is derived from a different one of the original pair and

consequently they are not sisters. The two nuclei in the ascus then fuse. The origin of the original pair is not known.

No satisfactory explanation of this fusion in the ascus has been advanced. The conditions in the Ascomycetes are not the same as in the Basidiomycetes. There is no series of paired nuclei in the ascogenous hyphæ and no evidence of a delayed fusion of gamete nuclei following a sexual act nor of nuclear fusions associated with the apogamous development of a sporophyte generation. On the contrary, a sexual act with the fusion of gamete nuclei has been clearly established in some forms preliminary to the development of the ascocarp and the nuclear union in the ascus is plainly a supplementary phenomenon. Wager and Harper point out analogies to the account of Chmielewski ('90b) for *Spirogyra*, considered in a previous part of this section, which described a double nuclear fusion in the zygospore. Thus the primary, sexually formed nucleus of the zygospore is reported to divide into four secondary nuclei, two of which break down while the remaining two unite forming the second and final fusion nucleus of the spore. It is hard to see how these second nuclear fusions can be sexual and Groom ('98) is perhaps correct in considering them superimposed on the sexual act, but their physiological significance is not clear.

Some recent papers support in general Harper's investigations on the ascus. Guilliermond (:04a ; :04b) describes the development of the ascus and ascospores in a number of forms. In an unnamed species of *Peziza* he found, however, that the ascus developed from the terminal cell of the ascogenous hypha which received two nuclei (that fuse) of the four that are found at the tip. Maire (:03a ; :03b) has reported a similar history for *Galactinia succosa*. Both Maire and Guilliermond note the resemblance of these conditions to the nuclear associations in the young basidium and Maire does not hesitate to consider the two nuclei in the tip of the ascogenous hypha as much reduced synkaryons, (paired nuclei) appearing for a very short period just previous to the nuclear fusions in the ascus. Maire follows Dangeard in denying the sexual processes described by Harper in the Ascomycetes and would align the events in the ascus with those in the basidium. Guilliermond agrees with

Harper that the number of chromosomes presented in the mitoses within the ascus is large (8, 12, 16, in various species) as against Dangeard and Maire who have claimed that the number is uniformly 4. Guilliermond's account of spore formation in the ascus supports that of Harper (described in Section II) in all essentials and gives especial attention to the structure of the epiplasm and its inclusions.

In summary: the significance of the nuclear fusions in the ascus seems very much of a mystery. If they could be associated with an apogamous development of the ascocarp we should have conditions analogous to those in the Basidiomycetes but following a sexual act as it does in *Sphærotheca*, *Erysiphe*, and *Pyronema* we find a phenomenon whose *raison d'être* is not apparent. However, we do not know the history of the nuclei preceding the group of four at the end of the ascogenous hypha and perhaps it may be discovered that events at this period are concerned with nuclear reduction at the end of a sporophyte generation.

One of the most interesting announcements of recent months is that in a preliminary note of Farmer, Moore, and Digby (:03) on the nuclear history preceding the apogamous development of a species of *Nephrodium*. They found that the cells of the prothallus at the point where the sporophyte arose became binucleate by the migration of nuclei from neighboring cells. The two nuclei might remain separate for some time or fuse at once. The authors speak of the whole process "as a kind of irregular fertilization" and Blackman considers it analogous to the entrance of the nucleus into the fertile cell of *Phragmidium* and the establishment of the paired nuclei in the Uredinales. As we discussed the phenomenon in that connection I considered the use of the term fertilization unfortunate since it included processes which however similar physiologically held no relation morphologically and phylogenetically to normal sexual processes. As stated then, it seems to me much clearer to regard all such apogamous phenomena apart from sexual processes, pointing out as far as possible physiological resemblances but recognizing the wide gap in morphology established by the past evolutionary history of the plant. The interest in the phe-

nomena does not become less by this treatment which certainly avoids much confusion of expression.

There is left for consideration one other group of nuclear fusions which may have sexual significance although such is not obvious, namely the fusions of polar nuclei in the embryo sac of angiosperms and the triple unions of the above with a second sperm nucleus which is often called "double fertilization." Several excellent reviews of this subject have appeared, notably by Strasburger (:00b), Sargant (:00), Coulter and Chamberlain (:03), Mottier (:04a, b), and Guérin (:04). The explanation of this phenomenon is likely to rest finally upon morphological analysis but at present we are uncertain of the homologies of the polar nuclei and the part they play in the evolutionary history of the endosperm. The most striking theory of the endosperm was proposed by LeMonnier ('87) who suggested that the fusion of the polar nuclei gave origin to a second embryo modified to nourish the normal embryo. One of the polar nuclei is always closely related to the egg nucleus so that in the triple fusions (the sperm with two polar nuclei) we have conditions very close to normal fertilization, the discordant element being not the sperm nucleus but the antipodal polar nucleus. The triple fusions would seem at first thought to be rather favorable to LeMonnier's theory although it is plain that with such a diverse mixture of chromatin from three nuclei the resultant structure can scarcely be called a sporophyte embryo from the very grotesqueness of its make-up. Miss Sargant considers the fusion of the second sperm with the micropylar nucleus as sexual in character but so complicated by the introduction of the antipodal polar nucleus that the result is a bizarre structure not strictly comparable to a normal embryo. In the final solution of this problem we must know whether in phylogeny the sperm and micropylar polar nucleus fused first and the antipodal entered into the process later or whether the polar nuclei began the habit and the second sperm nucleus was drawn afterwards into the activities. Should the first possibility be established the sexual nature of the process would seem clear while in the second the events would be of the nature of asexual nuclear fusions. While we know very little

of the origin and evolution of the endosperm in angiosperms there is some evidence in favor of the second possibility.

Strasburger (:oob) holds that the double and triple nuclear fusions in the embryo sac are not true sexual acts even though they may involve an important principle of fertilization, namely, a stimulus to growth. According to him, sexual processes present two distinct features which he designates as "generative fertilization" and "vegetative fertilization." Generative fertilization deals with the mingling of ancestral hereditary substances in the nuclei and establishes the basis for such characters as hold the species true to its past or introduce new qualities as variations into the germ plasm. Vegetative fertilization brings to the fusion nucleus simply a stimulus to growth such as may be given to unfertilized eggs by changes in their physical and chemical environment. We might apply this classification to many of the examples of asexual nuclear fusions which we have discussed, as in the apogamous development of the fern and the origin of the paired nuclei in the rusts, and they have the elements of vegetative fertilization in Strasburger's sense. But such distinctions are very subtle and it seems rather doubtful whether they add much to the clearness of our conceptions. The growth stimulus of "vegetative fertilization" is always an accompaniment of "generative fertilization" and would be expected of any cell unions or nuclear fusions. The peculiarities of sex lie in the phylogenetic features of the phenomena, *i. e.*, in the union of differentiated gametes with their long evolutionary history and not in the mere fusion of any nuclei at any time.

From this point of view the double fusions of polar nuclei or the triple fusions, when a sperm nucleus becomes involved in the phenomenon, are of very doubtful sexual nature since no phylogenetic connections have been established with the normal sexual processes of the spermatophytes. Indeed, there are many irregularities in the process of endosperm formation which complicate the discussion and make it very difficult to trace relationships. Thus nuclear fusions are described in the late stages of endosperm formation when several of the free nuclei become included in the same cell area by the formation of the cell walls

(*Corydalis*, Strasburger, '80; Tischler, :00; *Canna*, Humphrey, '96). Such nuclei are known to unite two or more and sometimes several together within the cells, forming fusion nuclei with a large and variable number of chromosomes. In *Peperomia* and *Gunnera* the endosperm nucleus results from the fusion of several free nuclei and a number of instances are recorded in which no fusion of the polar nuclei takes place, but the endosperm is derived from the division of one or both. Such irregularities, which will probably be greatly increased in number as investigations proceed, indicate that the double and triple fusions preceding the differentiation of the endosperm nucleus are not of phylogenetic importance but are more likely to be special developments in relation to peculiarities of seed formation among the angiosperms rather than of a sexual nature.

However, the triple fusions, when a sperm enters into the composition of the endosperm nucleus, seem to furnish a cytological explanation of the phenomenon of xenia and thus come into very close physiological relations to sexual processes. In xenia we find the effects of hybridization expressed immediately outside of the embryo in the endosperm of the seeds. If paternal chromatin has entered into the composition of the endosperm nucleus or should the sperm nucleus by itself give rise to a series of endosperm nuclei the appearance of paternal characters would be expected. This explanation of xenia was worked out independently by DeVries, Correns, and Webber, the last author having published a particularly clear and full account of the phenomenon (Webber, :00). Even though the relation of xenia to hybridization is apparent, it is nevertheless clear that we are dealing with an exceptional process only possible because of the unusual conditions within the embryo sac which allow a second sperm nucleus to enter into the activities of seed formation and it is certainly not established that these activities have any phylogenetic relations to past sexual processes.

Some interesting studies of Nemec (:02-:03; :04) upon asexual nuclear fusions may open the way for explanations of some of the examples which we have considered as asexual in the latter portion of this paper. Nemec found that mitosis in the root tip of *Pisum sativum* could be checked during anaphase

by treating the material with chloral hydrate so that no walls were formed between the daughter nuclei, which remained in the common mother cell and presently fused with one another. The fusion nucleus presented a double number of chromosomes (twice that of the normal sporophyte) in succeeding mitoses which became reduced in a few hours so that later divisions showed the number characteristic of the sporophyte. Nemec regards nuclear fusions and reduction phenomena as self regulating processes which follow the vital cell fusions characteristic of fertilization. The latter (cell fusions) are then the essential phenomena of sex and nuclear activities follow automatically. Reduction phenomena are atavistic in character. Nemec considers these results in serious conflict with Strasburger's ('94) theory of the periodic reduction of the chromosomes, believing that the number of chromosomes is not so likely to give the characters of the respective sporophyte and gametophyte generations as other factors.

Nemec's contribution is chiefly of interest to us in the present connection as showing that nuclear fusions may result from disturbances of the normal environment very far removed from the conditions that produce sexual cells. And this emphasizes our contention that sexual processes must be judged through phylogenetic analysis and not by physiological resemblances. Thus the nuclear fusions in the ascus, in the basidium, preceding apogamous development of the fern, and perhaps the union of polar nuclei in the embryo sac may be involved with special physiological conditions although they resemble outwardly sexual processes and are sometimes a substitute for these. But nevertheless they are asexual nuclear fusions lacking that fundamental character of sexuality, the result of sexual evolution, namely, a fixed position in a life cycle established by phylogeny and expressed by the classic phrase "ontogeny repeats phylogeny." They are departures from the normal life history either apogamous in character or concerned with some other peculiarity of the plants' existence.

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## STUDIES ON THE PLANT CELL.—VI.

BRADLEY MOORE DAVIS.

### SECTION V. CELL ACTIVITIES AT CRITICAL PERIODS OF ONTOGENY IN PLANTS.

WE shall discuss in this paper the behavior of the protoplasm at a number of critical periods in the life history of plants when the organism passes from one phase to another of a fundamentally different character. At such times great changes take place in the potentialities of the cells which inaugurate the new developments, changes that are generally most conspicuously shown in the structure of the nucleus. Some of the most interesting events of cell and nuclear history take place at these times, as would be expected from the importance of the phenomena. We shall treat the material under the following heads: (1) Gametogenesis, (2) Fertilization, (3) Sporogenesis, (4) Reduction of the Chromosomes, (5) Apogamy, (6) Apospory, (7) Hybridization, (8) Xenia.

#### I. GAMETOGENESIS.

The events of gametogenesis are clearly known for the higher plants but there is some confusion and almost no detailed information in the accounts of the thallophytes where the nuclei are very small and the details of the mitoses preceding the formation of sexual cells exceedingly difficult of study.

There is complete agreement among all investigators that the mitoses which precede the differentiation of gamete nuclei in spermatophytes, pteridophytes, and bryophytes are typical karyokinetic figures not differing essentially in the behavior of the chromosomes from the mitoses generally characteristic of the gametophyte generation. This information is based upon a

large number of studies of nuclear figures in antheridia and archegonia, the generative cell of the pollen tube and micropylar region of the embryo-sac. There are no reduction phenomena in these higher groups at the period of gametogenesis.

The subject is complicated in some types of spermatophytes where the gametophyte phase is so reduced that the mitoses which precede gametogenesis may follow immediately upon the two mitoses characteristic of sporogenesis or be separated from them by only one or two divisions. For example, it is known in several types of the lily family (*Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, etc.) that the two mitoses of sporogenesis (heterotypic and homotypic) are included in the embryo-sac and become a part of that gametophyte history. The third and final mitosis in this history differentiates the egg in the micropylar end of the embryo-sac and is a typical nuclear division. This subject was treated in some detail in Section III of these "Studies" (*Amer. Nat.*, vol. 38, pp. 741-745, 1904). When the mitoses of sporogenesis are not included within the embryo-sac we find almost without exception three typical mitoses preceding the differentiation of the egg in the angiosperms and a very large number in the gymnosperms, and of course in the pteridophytes and bryophytes the whole vegetative period of the gametophyte which is generally green and self-supporting. There are from two to three mitoses in the pollen grain and male gametophyte of the angiosperms before the development of the sperm nuclei and a somewhat larger and more variable number among the gymnosperms. It is necessary at the outset to understand clearly what are the events of gametogenesis in spermatophytes because several authors have carried the phenomena of sporogenesis over into the period of gametogenesis, where it can have no proper place in exact morphology. Such papers will be treated in connection with "Sporogenesis" and "Reduction of the Chromosomes," for they concern primarily these phenomena alone.

Gametogenesis must be considered at present chiefly from our knowledge of the conditions in the higher plants as they furnish almost the only detailed information that we have on the subject. Upon this as a basis we are justified in suggesting

possibilities in the thallophytes which must remain as speculations until investigations have advanced much farther in this difficult field of cell study. The basis of any theories at present must be phylogenetic, a principle that has not been followed in some of the work upon the thallophytes.

Gametogenesis in plants is full of interest because of the sharp differences from the processes of spermatogenesis and oögenesis in animals. In animals the period of gametogenesis is one of unusual activity. After the germ cells are differentiated there follows a period of cell growth, with the peculiar activity termed synapsis, during which the number of chromosomes is reduced to one half the number characteristic of the species. The germ cells emerge from the growth periods as primary spermatocytes or oöcytes which give rise respectively by two successive mitoses to four spermatids or to an egg with its accompanying polar bodies. The gametes have one half the number of chromosomes characteristic of the species, so that the period of gametogenesis is one of chromosome reduction. The character of this process of reduction will be considered when we take up the analogous phenomena in plants after the discussion of sporogenesis. Gametogenesis in plants is in striking contrast to that in animals. In all higher groups (those above the thallophytes) we know that the gametes have the same number of chromosomes as the vegetative cells of the parent plant (gametophyte). There is no reduction of the chromosomes at the time of gametogenesis, that phenomenon taking place at the end of the sporophyte generation with sporogenesis. Also, there are no peculiarities of the mitoses immediately preceding gametogenesis excepting such as concern the development of cilia-bearing organs (blepharoplasts) or slight peculiarities in the form or size of the spindles, for such nuclear figures are frequently different in these particulars from the mitoses in vegetative cells of the gametophyte. The differences concern chiefly the structure of the sperm, and have been described in our account of that structure (*Amer. Nat.*, vol. 38, July and August, p. 576, 1904).

To Strasburger above all others should be given the credit of making clear these important characteristics of gametogene-

sis in plants. Strasburger's paper of 1894 on "The Periodic Reduction of the Number of Chromosomes in the Life History of Living Organisms" (*Annals of Bot.*, vol. 8, p. 281) was the first elaborate presentation of the principles of gametogenesis and reduction phenomena in plants and has become classical as the foundation of the present attitude in botanical science and the basis and stimulus of a large amount of confirmatory research. The matter really crystallized after the discovery that the sporophyte generation of the higher plants possessed nuclei with twice the number of chromosomes characteristic of the gametophyte and that the reduction took place in the spore mother-cell just previous to sporogenesis.

These facts were gradually established by a number of investigations beginning with Strasburger ('84, '88) and Guignard ('84, '85). Guignard ('91) presented the first complete count of the number of chromosomes in the life history of a plant (*Lilium martagon*), determining the reduction period to be in the spore mother-cell, and Overton ('93 a and b) independently reached the same conclusions for the same plant and extended the knowledge of the chromosome count in gametophyte and sporophyte to a number of other types. Overton's paper was important in its suggestiveness for extended research among the higher cryptogams. Other investigations followed shortly in the gymnosperms, pteridophytes, and liverworts, all supporting the view that the nuclei of the sporophyte generation, following the fusion of gamete nuclei, had double the number of chromosomes characteristic of the gametophyte and that the reduction phenomena occurred at the end of the sporophyte generation in the spore mother-cell. The significance of reduction phenomena at sporogenesis must be phylogenetic since it represents a return of the organism at this time to the ancestral gametophyte condition. The details of this literature belong to the account of "Sporogenesis" and "Reduction of the Chromosomes," and will be taken up later. But it is necessary to present the outline at this time to make clear the important fact that no reduction of the chromosomes takes place during gametogenesis in all groups above the thallophytes.

The theories of gametogenesis among the thallophytes rest

upon information which in point of completeness falls very far short of our knowledge of the groups above. Indeed, no forms have been studied with the detail that is known in higher groups chiefly for the reason that the investigator is forced to deal with very small nuclei and mitotic figures whose chromosomes are exceedingly minute and because of various technical difficulties. The theories in general fall into two groups: (1) those which have an obvious basis in attempts to reconcile events with the processes of gametogenesis in animals, and (2) those proceeding from the view that for phylogenetic reasons the periods and phenomena of gametogenesis in the lower plants should correspond with those of the higher.

We may pass over with a few words the early crude attempts to establish structures for plants comparable to the polar bodies of animals. For example at the conclusion of oögenesis in some algæ (*e. g.*, *Vaucheria*, *Ædogonium*) a globule of slime is exuded with the opening of the oögonium. It was suggested that such material is thrown off from the egg but we now know that it is not protoplasmic in character but is apparently derived from a softening of the cell wall. Then the ventral canal cell has been compared to a polar body but it seems clear now that all of the canal cells are homologous and a part of what was formerly an extensive gametogenous tissue within the archegonium. Then the small group of cells cut off below the oögonium of the Charales and the fragmented nuclear material in the trichogyne of the red algæ have been compared to substance thrown off from the egg but without any knowledge of the nuclear structure. Finally the nuclear degeneration which is a very conspicuous feature of oögenesis in certain groups whose oögonia are multinucleate (*Peronosporales*, *Saprolegniales*, *Pelvetia*, etc.) has been considered related to reduction phenomena. But the nuclei in all of these forms bear every evidence of being in each type homologous structures whose large numbers have a phylogenetic *raison d'être* and the extensive degeneration is associated with the principles of sexual evolution which tend to conserve protoplasm for the good of a lesser number of gamete nuclei even to the sacrifice of others that are potentially equivalent.

We will now consider the few instances among the thallo-

phytes in which a reduction of the chromosomes is reported just previous to or during gametogenesis. The best known case is *Fucus* since this type has been studied by three investigators: Farmer and Williams ('98) and Strasburger ('97a). They agree in describing the nuclear figure that differentiates the oögonium from the stalk cell as exhibiting a large number of chromosomes (28 or 30) while the three mitoses within the oögonium, which give rise to the eight eggs, present only one half that number (14 or 15). Apparently there is a reduction by one half just before the mitoses in the oögonium. Since there is no sporophyte generation in *Fucus* it is of course difficult to compare these conditions with those in higher plants, but, as will be explained later, there are some reasons why we should not expect to find reduction phenomena at gametogenesis in any thallophyte.

Reduction phenomena at gametogenesis have also been suggested for various types of the Peronosporales and Saprolegniales but not, however, in exactly the same way as in *Fucus*. There are always, as far as is known, one or two mitoses within the oögonium before the gamete nuclei are organized and it has been held that these are reduction divisions by Rosenberg for the Peronosporales and by Trow for the Saprolegniales. Rosenberg (:03b) described for the oögonium of *Plasmopara* a condition of synapsis in the nuclei preceding the two mitoses and compared this sequence with the events of sporogenesis in higher plants in which the two divisions within the spore mother-cell are preceded by a period of synapsis. Rosenberg did not determine the number of chromosomes in the vegetative nuclei so that he has no positive evidence of reduction in the oögonia. With respect to the two mitoses and the preliminary synapsis I have already pointed out in criticism of Rosenberg's studies (*Bot. Gaz.*, vol. 36, p. 154, 1903) that the number of mitoses is variable in the oögonia of the Peronosporales and Saprolegniales and apparently entirely absent in the species of *Vaucheria* studied by myself (Davis, :04a). Also, the phenomenon of synapsis, which is easily recognized in the large nuclei of the spore mother-cell, would be difficult to establish in the small nuclei within the oögonia of the forms mentioned above. Nuclei can be found

in a number of structures with their contents somewhat massed at one side or in the center but such conditions must not be confused with the remarkable process of synapsis in the spore mother-cell. Among all the excellent studies of gametogenesis in the Peronosporales I cannot find any clear evidence of a reduction of the chromosomes at gametogenesis.

Quite different is the account that Trow (:04) brings forward to support his view of chromosome reduction during gametogenesis in the Saprolegniales. Trow describes two mitoses in the oögonium of *Achlya debaryana*: in the first the number of chromosomes is eight which becomes reduced to four in the second. Trow's account of a second mitosis in *Achlya* is very different in a number of particulars from the results of all investigations on gametogenesis in the Peronosporales and Saprolegniales. Two centrosomes with radiations are said to appear at the poles of the spindle at anaphase, structures which were not present in the first mitosis. Some of these asters become the center of the egg origins and are later accompanied by deeply staining material constituting a body which Trow terms an ovocentrum and which perhaps corresponds to a cœnocentrum. Relatively few of the nuclei in the oögonium are said to pass through this second mitosis and some of their products, with the accompanying asters, break down. The remainder become the functional gamete nuclei of the eggs. There are many complex activities described by Trow in connection with the appearance of the asters during the second mitosis and also at the side of the sperm nuclei which are said to enter the oögonium, events that cannot be correlated with the processes of gametogenesis and fertilization as we understand them for the Peronosporales. They are treated briefly in a review by myself (*Bot. Gaz.*, vol. 39, p. 61, 1905), where, however, I misunderstood a distinction that Trow draws between the aster and the ovocentrum (see an answer by Trow, *Bot. Gaz.*, vol. 39, p. 300, 1905). My impression is that either Trow has been mistaken in his interpretations or that there are present events which must entirely change our conception of gametogenesis in the Saprolegniales and Peronosporales, but which are not fully explained by Trow's paper.

Let us now think of gametogenesis among the thallophytes

with reference to what we know of the process in higher groups and the principles of the origin and evolution of sex and the sporophyte among the lower. It seems clear that the sporophyte generation is characterized by a double number of chromosomes as a result of the fusion of gamete nuclei at fertilization. We must then lay the fundamental inception or origin of the sporophyte to the stimulus of the sexual act. That is, the sexually formed fusion cell must have different potentialities from the germ plasm of the parent gametophyte and it cannot produce a gametophyte again until these potentialities are worked off and the protoplasm returns to the dead level of the ancestral stock (the gametophyte). By the potentialities of the sporophyte plasm we mean primarily a greater energy or growth stimulus which must express itself differently from the gametophyte. Morphologically we can only distinguish sporophyte plasm from gametophyte plasm by the double number of the chromosomes but of course the complexities of the sexual act would make great differences in the chemical structure of the two. The divergences in the history of the gametophyte and sporophyte, as shown throughout ontogeny and phylogeny, are but the final expressions of the different potentialities of the protoplasm in each generation. The morphological forms of expression of the sporophyte are extraordinarily various and in the long evolutionary history of this generation have developed great structural differentiation but with every life history the sporophyte has the same beginning (fertilization, with the doubling of the chromosomes) and the same ending (sporogenesis, with chromosome reduction). Between the beginning and the end is intercalated a vegetative period, short and simple in some forms, and very long and elaborate in others. The history of the development of this vegetative period or the evolution of the sporophyte is a subject far outside of and secondary to the scope of this discussion. We are only concerned with the protoplasmic activities at the beginning (fertilization) and the end (sporogenesis) of the sporophyte generation.

We know nothing of the behavior of the chromosomes in types of the thallophytes which illustrate most closely our conception of the origin of sex and of the sporophyte generation.

I refer to many lower algæ such as *Ulothrix*, forms of the *Volvocaceæ*, *Ædogonium*, *Coleochæte*, and many others. However, the homologies of primitive gametes and their origin from types of asexual zoöspores is very clear in a number of groups. We can see nothing in the morphology and mode of development of these reproductive cells to suggest reduction phenomena when gametes are produced. The primitive gamete is generally somewhat smaller than its homologue the zoöspore, often because the protoplasm of the gamete mother-cell becomes distributed in a greater number of daughter elements. It is well known that the conditions that lead to conjugation are exceedingly variable, depending upon environmental factors and one often cannot tell at the time whether a swarm spore will show sexual habits or germinate without conjugation. The most satisfactory theory of the origin of sex in plants regards primitive gametes as weaker or lacking in certain potentialities of vegetative growth and the conjugation as a mutually coöperative process resulting in a rejuvenescence of the protoplasm. The fact that many simple types of gametes will germinate without fertilization and produce small and weak sporelings shows that vegetative possibilities are not entirely lost. Investigations on the chromosome history among these forms, difficult though they be, are some of the most interesting subjects of botanical research. We know some general principles of the origin and evolution of sex in plants (Davis, :01b, :03a) but of the chromosome history in the simplest types of gametogenesis nothing is known.

With respect to the history of the chromosomes in the simplest sporophytes we are also as ignorant as in the simplest types of gametogenesis. We have excellent reasons for believing that the sporophyte generation is represented among the thallophytes in a number of very simple conditions. Numbers of zygospores and oöspores (*e. g.*, *Ulothrix*, *Ædogonium*, forms of the *Conjugales* and *Volvocaceæ*, etc.) give rise on germination to several daughter cells. In higher forms this growth period is lengthened to the formation of a reproductive tissue (*Coleochæte*) and in the great groups of the *Rhodophyceæ*, *Ascomycetes*, and *Basidiomycetes* there is present an extensive development from the fertilized female cell (or its equivalent when apogamy obtains)

involving the development of a vegetative structure before the period of sporogenesis. From the studies of Wolfe (:04) we know that the sporophyte portion of *Nemalion* (the cystocarp) contains nuclei with double the number of chromosomes (about 16) present in the gametophyte (about 8) and that the period of chromosome reduction is apparently just previous to the development of the carpospores (sporogenesis). Williams (:04a and b) has recently determined that the asexual plant of *Dictyota* is a sporophyte generation with double the number of chromosomes (32) found in the sexual plant (16). The reduction occurs here during a rather long period of preparation on the part of the nucleus in the tetraspore mother-cell and the reduced number appears in the two mitoses that form the tetraspores. These events closely parallel those in the spore mother-cell of higher plants and will be discussed further under "Sporogenesis."

William's (:04b) account of gametogenesis in *Dictyota* is the most complete that we have for any thallophyte. The oögonia and antheridia are cut off from a stalk cell by a mitosis which presents 16 chromosomes, the number characteristic of the gametophyte. The contents of the oögonium forms a single egg and consequently presents no mitotic phenomena. The antheridium develops over 1500 sperms thus exhibiting a large number of successive divisions. These all show 16 chromosomes and the mitoses are typical, not differing in any essential from the division in the stalk cell. The entire absence of mitoses in the oögonium and the great number in the antheridium are striking facts which show that no especial significance can be attached to nuclear divisions within sexual organs of this type. There is no place for reduction phenomena within these sexual organs and none precede their development.

These studies of Williams and Wolfe justify us in expecting that other thallophytes will support their discoveries that the product of the sexual act will have a fusion nucleus with double the number of chromosomes present in the sexual plant (gametophyte) and that reduction phenomena may be expected to follow the sexual act and not precede it as in animals. In such thallophytes as have no sporophyte generation we may suppose, as Strasburger ('94a) suggested, that the number of chromo-

somes is reduced with the germination of the sexually formed cell so that the protoplasm returns at once to the potentialities of the gametophyte. It is quite possible that the four zoöspores produced from the oöspore of *Ædogonium* and the four nuclei found in the germinating zygospores of the desmids and *Spirogyra* may indicate divisions concerned with reduction phenomena similar to those in the tetraspore mother-cells of *Dictyota* (which may also be expected in the tetraspore mother-cell of the red algæ) and in the spore mother-cell of the higher plants.

For these reasons we seem to be justified in taking a critical attitude towards the accounts of chromosome reduction at gametogenesis among the thallophytes. The logic of the situation would lead us to expect that every sexual act gives a doubling of the chromosomes and an impulse towards the development of a sporophyte phase in plants which must be worked off before the protoplasm is in condition to reproduce the parent gametophyte. Reduction phenomena should follow then every sexual act. If it takes place immediately with the germination of the sexually formed cell there is of course no sporophyte generation. Because the conception of the sporophyte generation with reduction of the chromosomes at sporogenesis is so clearly established in higher groups, those investigators who claim reduction phenomena at gametogenesis must expect their views to be severely scrutinized and accept the responsibility of presenting very clear and convincing proof of their conclusions. The author does not think that this evidence is supplied in satisfactory form by any investigation so far.

## 2. FERTILIZATION.

In Section IV of these "Studies" we described the most important phenomena of fertilization under the caption "Sexual Cell Unions and Nuclear Fusions." It will not be necessary to discuss the facts of the phenomena in detail again. This account will take up the more theoretical aspects of the events of fertilization and their relation to other critical periods of ontogeny.

Plants are in complete agreement with animals in the follow-

ing chief events and principles of fertilization. Thus Van Beneden's conclusion of 1883 that sexual nuclei are equivalent in their chromatin content at the time of fusion irrespective of differences in size is admirably borne out by Miss Ferguson's (:04) studies on the pine. In this form as in the gymnosperms generally the male nucleus is much smaller than the female and comes to lie in a depression in the latter before the actual fusion takes place. After the fusion the paternal and maternal chromosomes are found in two groups side by side preparatory to the first cleavage mitosis and are indistinguishable except for their position; the chromatin of the two sexes is equal in amount as far as can be seen. Then the observations of the Hertwig brothers, in 1887, and Boveri, in 1889 and 1895, that the sperm nucleus could enter and cause the development of denuded eggs or their fragments thus taking the part of a female nucleus in parthenogenesis, were established for plants by Winkler's (:01) experiments on *Cystoseira*. Winkler was able to divide the egg of this brown alga into a nucleated and a non-nucleated portion and he found that sperms entered the non-nucleated parts and caused them to develop sporelings side by side with the fertilized nucleated portions. The sporelings from the non-nucleated fragments, controlled by the sperm nuclei alone, developed about half as rapidly as those from the originally nucleated portions which of course were dominated by sexually formed fusion nuclei, but the two sets of sporelings were alike in form as far as they were grown. Only with respect to Boveri's celebrated theory that the sperm brings to the egg in the centrosome the mechanism of cell division, do plants fail to support the conclusions of certain zoologists with respect to the most important events of fertilization. This point upon which zoologists are not in full accord will be discussed later. There is general agreement in the view that the male nucleus of plants supplies chromosomes equal in number and equivalent quantitatively to the female, and general accord in the conclusions that the chromosomes by their individuality, apparent permanence of structure, and fixed behavior must be bearers of hereditary characters.

Evidence from the most recent investigations upon favorable forms of both animals and plants indicates that the chromosomes

from both gametes maintain their independence and never fuse at the immediate time of fertilization. We have reason to assume, chiefly from zoölogical studies, that the paternal and maternal chromosomes of plants remain independent throughout the entire sporophyte generation and that no fusion takes place until the period of chromosome reduction at sporogenesis. If no sporophyte generation is present we should expect the fusion and reduction of the chromosomes to occur after the sexually formed cell had passed through a period of rest (for all reduction phenomena seem to require considerable time) unless there be actually such reduction during gametogenesis in the thallophytes as reported for *Fucus* and *Saprolegnia*. The morphology of the chromosomes is probably unchanged by the immediate act of fertilization. The fusion nucleus simply contains double the number of chromosomes present in each gamete nucleus which increases by so much the metabolic possibilities which lie in these structures.

Besides chromatin the sperm brings into the egg a certain amount of cytoplasm. Some of this may be the substance of the blepharoplast or other kinoplasm associated with the nucleus but there is often besides considerable granular trophoplasm, sometimes with inclusions of starch and other food substances, and the male gamete of certain thallophytes contains a chromatophore. There is no reason to suppose that development especially characteristic of fertilization, the sporophyte generation, has any relation to this trophoplasm with its food inclusions, excepting as it may stimulate growth which is to be expected whenever organic food material is introduced into protoplasm. But we can hardly believe that the formative elements or the rudiments of further development especially those of a sporophytic character lie in this region of the protoplasm. They must be sought in the nuclei and in the only stable elements of the nuclei, the chromosomes.

It has been held at times by botanists, following the lead of certain zoölogists, that the sperm or sperm nucleus introduced a centrosome into the egg which organized the first cleavage-spindle and thereby played a necessary part in starting cell division. Such a centrosome would naturally be sought in the

blepharoplast which is clearly analogous to the middle piece of the animal spermatozoön. We have no evidence that such events ever take place in the eggs of plants. On the contrary we know that the first cleavage-spindle in the eggs of spermatophytes develops without centrosomes from a mesh of fibrillæ. Also the blepharoplasts of the gymnosperms *Cycas*, *Zamia*, and *Ginkgo* remain in the cytoplasm at a distance from the fusion nucleus and Shaw's account of the fern, *Onoclea*, indicates that similar conditions obtain there. We know less about the history of the blepharoplasts within the egg of thallophytes where the first cleavage-spindle frequently has very handsome centrospheres and asters (*e. g.*, *Fucus* and *Dictyota*). Strasburger ('97a) pointed out that one of the asters of the first cleavage-spindle in *Fucus* arose near the point where the male nucleus united with the female. However, Farmer and Williams ('98) believe that centrospheres of the first cleavage-spindle in *Fucus* are formed *de novo* and Williams (:04b) came to the same conclusion for *Dictyota*. There are some very interesting features in the comparative study that Williams (:04b) has made on the development of the first segmentation spindle in the fertilized and parthenogenetic eggs of *Dictyota*. The spindle in the parthenogenetic egg is multipolar and develops from an intranuclear kinoplasmic mesh and there are no centrospheres. But in the fertilized egg a centrosphere always appears at the side of the nucleus and apparently divides into two which separate until they lie at opposite poles of the mature spindle. Yet Williams after a very careful study concludes that this centrosphere arises *de novo* and believes that the stimulus of fertilization enables the fusion nucleus to form a centrosphere external to itself, a thing which is not possible for the nucleus of the parthenogenetic egg.

It seems then probable that the only structures of the sperm that preserve their morphological entity in the fertilized eggs of plants are the chromosomes. Whatever may be the relation of the blepharoplast and other cytoplasmic structures as stimuli to the development of the egg they cannot be regarded as fixed factors in the problem of heredity. It is very probable that they introduce valuable food material, perhaps important fer-

ments, substances of great service, although possibly not absolutely necessary to the successive metabolic processes which characterize growth and development. But the fact remains that we have in the chromosomes the only new morphological elements. And the progress of research seems ever to strengthen the general view that in the chromosomes are contained the directive rudiments of development and that they are the bearers of hereditary principles. Nuclear studies on apogamous forms will certainly prove of great interest in this connection. We have reason to expect some very important results from thorough cell studies on apogamy and apospory.

The best developed theory of fertilization in plants is that of Strasburger and a statement of his views should precede any comments of other authors. Strasburger has written much on the phenomena of fertilization; important considerations may be found in his papers of '94a, b, '97c, :00a, b, :01, and :04a. Strasburger points out that the protoplasm of the egg is predominately trophoplasmic in character because of the proportionately very large amount of cytoplasm with granular inclusions that are evidently food material or the products of metabolism. On the other hand the cytoplasm of the sperm contains relatively little trophoplasm and much kinoplasm, especially when the sperm is a ciliated cell with a large blepharoplast. As Strasburger conceives kinoplasm to be the active substance of spindle formation, he concludes that the sperm might bring to the well nourished egg, rich in trophoplasm, the substance necessary to start the mechanism of mitosis. In its broad aspects this view is very similar to the celebrated theory of Boveri, 1887, that the spermatozoon supplied the animal egg with the centrosome which is conceived as necessary to start mitotic processes and that the egg is powerless to divide before fertilization because it lacks such a structure.

Another feature of Strasburger's views (advanced in his paper of :00b) appears to have grown out of the discovery of the so called "double fertilization" in the embryo-sac and other nuclear fusions whose sexual significance is not clear, together with the phenomena of parthenogenesis as produced experimentally in many studies of recent years. Strasburger considers that two

processes are involved in the sexual act. The first, termed "vegetative fertilization," is simply the stimulus to growth which results from the fusion of two nuclei or other masses of protoplasm. The second, called "generative fertilization," involves deeper factors than those of mere growth stimulus. These lie in the union of germ plasm of diverse parentage with the mingling of hereditary racial characters and individual variations and the establishment of a new organism which may have possibilities of development quite different from the parent form. The effects of "vegetative fertilization" may be imparted to protoplasm artificially by chemical and physical stimuli as has been done in the numerous experiments of Klebs and Loeb on the conditions which induce parthenogenetic development. "Generative fertilization" has a phylogenetic significance and a background which is entirely apart from the mere vegetative processes of cell growth and division.

It is apparent that Strasburger's theory is open to the same line of criticism that has been brought against the universal application of Boveri's hypothesis that the spermatozoön brings to the egg the agent of cell division as a centrosome. The investigations of several zoölogists indicate that one or both of the centrosomes in the first cleavage-spindle may be derived from the egg or may be formed *de novo* (see Wilson, :00, pp. 196, 208). The kinoplasm of the plant sperm, whether in the form of a blepharoplast or as an ill defined accompaniment of the sperm nucleus has not been shown to take part in the formation of the first cleavage spindle. There is no evidence that the blepharoplast retains its organic entity in the egg to pass over into a centrosome or centrosphere. Of course the kinoplasm which lies immediately without the nuclear membrane of the sperm, and there is sometimes a conspicuous amount of this densely granular protoplasm, must merge with similar kinoplasm associated with the egg nucleus at the time of fusion. For example Miss Robertson (:04) and Coulter and Land (:05) note in *Torreyia* that the sperm nucleus brings to that of the egg a large amount of accompanying kinoplasm which forms an investing layer around the fusion nucleus. It is reasonable to suppose that the mixing of these masses of kinoplasm with the

fusion of the gamete nuclei would give material for a larger and more highly differentiated nuclear figure in the first cleavage of the egg.

Williams' (:04b) observations and conclusions on Dictyota are especially interesting in this connection for he shows that the first cleavage-spindles in the parthenogenetic eggs are intranuclear and multipolar, showing no dominant kinoplasmic centers while the fertilized eggs form each a well differentiated centrosphere with radiations, exterior to the nuclear membrane, which clearly guides the whole process of spindle formation. Williams does not hold that this centrosphere comes as an organized structure from either sperm or egg but is developed *de novo* by the fusion nucleus as the result of the general stimulus of fertilization. The evidence, then, furnished by studies on fertilization in plants, indicates that the chromosomes alone maintain morphological independence throughout the process of fertilization and that the kinoplasmic (archoplasmic) elements play no part in the phenomena as fixed morphological structures but simply contribute their substance to the general union of cytoplasm with cytoplasm, and that any specialized kinoplasmic structures of the first cleavage spindle are formed *de novo*. While it is true that the sperm brings to the egg much kinoplasm it may well be questioned whether such kinoplasm is a necessary factor in the formation of the first cleavage-spindle. It seems more probable that the development of achromatic structures in the first mitosis following fertilization is due rather to the general stimulus of cell and nuclear fusion than to particular structures supplied by either sperm or egg.

The second phase of Strasburger's theory of fertilization concerns a separation of the two processes in the sexual act: (1) the mere growth stimulus, "vegetative fertilization," that may be expected with the union of any two masses of protoplasm, and (2) the clearly defined sexual phenomena, "generative fertilization," which lies in the union of germ plasm of different parentage and diverse potentialities and which leads to the inheritance of these characteristics. It seems clear that the two processes are really present and can be clearly distinguished. But it may be strongly questioned whether the factors charac-

terizing the first (vegetative fertilization) should really be considered a part of the sexual act. Strasburger regards the processes of "generative fertilization" as essential to the sexual act. The growth stimulus "vegetative fertilization" is always to be expected as an accompaniment of fertilization. It may be given to cells in other ways than by the sexual act and is found in cell and nuclear fusions which for phylogenetic reasons are plainly not sexual.

The experimental work of recent years on the conditions determining artificial parthenogenesis have done much to define the sorts of factors which stimulate growth and division of sexual cells when the process of fertilization is suppressed. Klebs for plants and Loeb for animals have been foremost in these studies and they have shown that what seem to be very minor changes in the environment of the sexual cell may suffice to give a gamete the power of immediate development without fertilization. Thus the egg of the sea urchin will develop parthenogenetically to an advanced stage when placed for a short time in sea water containing magnesium chloride and then brought back to normal sea water. Nathansohn (:00) found that a small proportion (about 7%) of the eggs of *Marsilia vestita* would germinate parthenogenetically when the megaspores were cultivated for 24 hours at the rather high temperature of 35° C. and then left to continue their development at 27° C. There are then a number of factors such as varying osmotic pressure, temperature, and in some cases chemical reagents which may induce gametes to further development without the usual sexual processes. These reactions seem to be of a similar character to the processes in that phase of sexual reproduction termed "vegetative fertilization" by Strasburger. They give the stimulus to growth but without that essential feature of sexuality, the mingling of germ plasm of different parentage which distinguishes the processes of "generative fertilization."

It seems to the author, for the sake of clearness, that we are trying to include too much under the term fertilization. If the features of "vegetative fertilization," *i. e.*, the growth stimulus, can be introduced experimentally as in artificial parthenogenesis then they cease to be fundamental qualities of the sexual act.

They are accompaniments of sexual processes which may always be expected but nevertheless are not the essential characteristics. The essence of the sexual act (fertilization) is the union of germ plasm with such possibilities of new developments as come from the inheritance of mixed characters from two lines of ancestry. And the more diverse and complex are the characters of the parents the more conspicuous are the essential features of the sexual act. Among lowly organisms and in simpler types of sexual processes the growth stimulus becomes exaggerated in our attention because the features of heredity are not so prominent as in the higher forms. But in the higher groups the varied characters of offspring express clearly the subtle factors concerned with the mingling of diverse germ plasm in the process of fertilization and the growth stimulus recedes into the background.

For these reasons it seems to me that the term fertilization should only be used for the mingling of germ plasm with the possibilities of new combinations in the potentialities of the resulting sexually formed cell and that the growth stimulus should be treated as an accompaniment but quite apart from the essentials of the sexual act. And for these reasons I was careful to include in Section IV under the caption "Sexual Cell Unions and Nuclear Fusions" only illustrations in which the sexual nature of the phenomena was clearly shown by applying a morphological or phylogenetic test to the elements concerned in the process of cell fusion. The phylogenetic test seems to me the only sure way of determining the sexual nature of the members of a cell fusion and there are very few cases in which there can be any hesitation in deciding whether or not such elements are morphologically gametes.

I included under "Asexual Cell Unions and Nuclear Fusions" in Section IV a number of cases in which the sexual nature of the act is under dispute for the reason that none of these satisfy the phylogenetic test. It is perfectly clear that the growth stimulus is a conspicuous feature of these cell and nuclear fusions and that in this feature they resemble sexual processes but this does not, to my mind, make them acts of fertilization or the equivalent of sexual processes. The union of sporidia in the

smuts and of yeast cells, the fusion of nuclei in the teleutospore and basidium and in the apogamous development of ferns, the double fusion of polar nuclei and multiple nuclear fusions in the embryo-sac (*Corydalis*) illustrate phenomena which I cannot regard as sexual even though they have in them elements associated with sexual processes and in certain cases may be substitutes for a former sexual act. In none of these instances can we be positive that the nuclei concerned are morphologically and phylogenetically gamete nuclei. This point was discussed in some detail in Section IV. It seems to me that Blackman's (:04 a, p. 353) conception of the cell fusions preceding the æcidium in *Phragmidium* as "reduced forms of ordinary fertilization" or Farmer's (:03) explanation of apogamy in the fern "as a kind of irregular fertilization" leads to a confusion of a substitute process with a true sexual act. The substitute processes have their true place as phenomena of apogamy. They can, however, only have a sexual significance if they represent the origin of a new set of gametes in the organism, a proposition which is not likely to be maintained by anyone.

### 3. SPOROGENESIS.

We are employing the term sporogenesis, as must have been apparent in preceding divisions of this paper, to designate a characteristic and highly specialized type of spore formation that is universal in all plants above the thallophytes. The process always terminates the sporophyte phase in ontogeny of these higher plants, and is especially distinguished as the period of chromosome reduction in the life history. The cell activities of sporogenesis are therefore of particular interest, and, since spore mother-cells are generally large and their nuclear and cytoplasmic structure especially clearly differentiated, we have perhaps obtained more knowledge of mitotic phenomena from the study of these elements than of any other tissues of the plant body.

The reduction phenomena of sporogenesis have been established in some forms of the thallophytes, certainly in the tetraspore mother-cell of *Dictyota* (Williams, :04a). There are also reasons for suspecting that the oöspore of *Ædogonium* and the

zygospores of Conjugales on germinating present similar events. The teleutospore and basidium are probably also the seat of chromatin reduction (Blackman, :04b) in the formation of spores either directly or through the promycelium. The ascus holds a position at the end of a sporophyte phase which suggests a similar relation in this group of fungi. Chromosome reduction may also be expected in the tetraspore mother-cell of the Rhodophyceæ, as in Dictyota, but this subject has never been investigated. There are occasional red algæ in which the tetraspores are sometimes borne on the same plant with the sexual organs, conditions which may be difficult to explain on the theory that the tetrasporic plant is a sporophyte. Thus *Spermothamnion turneri* on the American coast frequently bears both procarps and tetraspores on the same plant, and I have also seen cystocarpic plants of *Ceramium rubrum* some of whose branches contained tetraspores. Lotsy (:04a) also reports similar conditions in *Chylocladia kaliformis*. The other extremely varied methods of spore formation (zoöspores, conidia, etc.) in the thallophytes do not concern the present discussion. They seem to have no fixed place in the life history and there is nothing to indicate any relation to reduction phenomena, although we actually know nothing about the chromosome history among these lowly forms.

The importance of sporogenesis as a critical period in the life history of higher plants became at once apparent with the discovery that fertilization doubled the number of chromosomes in the nuclei of the sporophyte phase and that the double number was reduced during sporogenesis. As stated in our account of gametogenesis, these facts were first established for a number of spermatophytes by the work of Strasburger ('84, '88, and '94), Guignard ('84, '85, and '91), and Overton ('93 a and b). Guignard ('91) presented for *Lilium martagon* the first complete account of the number of chromosomes in the life history of a plant, and his results were also established independently by Overton ('93 a and b). Then followed confirmatory investigations among the bryophytes in the work of Farmer ('94, '95 a, b, c) and in the pteridophytes by Strasburger ('94, p. 294) for *Osmunda*. Since 1895 the investigations among the spermatophytes have so multiplied that we know the number of chromo-

somes in sporophyte and gametophyte for more than fifty forms. This list may be found in Coulter and Chamberlain's recent text-book, *The Morphology of the Angiosperms*, 1903, p. 81. Farmer's accounts of the number of chromosomes in the Hepaticæ have been confirmed and extended by myself (Davis, '99, :01a) and by Moore (:03). The more recent literature, especially as it concerns the events of spindle formation in the mitoses characteristic of sporogenesis has been treated in our account of the spore mother-cell (*Amer. Nat.*, vol. 38, p. 725, Oct., 1904).

There are two chief periods in the processes of sporogenesis as illustrated in all groups above the thallophytes: (1) a growth period and (2) a period of cell division. In the growth period the spore mother-cells become differentiated from the general sporogenous tissues through a great increase in the amount of protoplasmic material. At some time in this growth period the nucleus of the spore mother-cell exhibits the phenomenon of synapsis, a very characteristic event, recognized by the very much contracted condition of the chromatin network in the interior of the nucleus. Synapsis is believed to hold fundamental relations to reduction phenomena as the time when chromosomes unite with one another in pairs. The period of cell division follows synapsis and is characterized by two mitoses in the spore mother-cell, the second following immediately upon the first, and a segmentation of the protoplasm, sometimes by two successive divisions, and sometimes by a simultaneous cleavage, into four spores. The two mitoses present certain peculiarities in the structure and behavior of their chromosomes which are unlike the events of typical mitoses. The first is known as the heterotypic and the second as the homotypic mitosis. These peculiarities have been recognized for a long time and have furnished the subject of much investigation and contradictory explanations. They were briefly described in Section III (*Amer. Nat.*, vol. 38, p. 740, Oct., 1904) but recent studies of Farmer and Moore (:03, :05) have opened again a discussion which seemed closed at that time. The details of synapsis and the heterotypic and homotypic mitoses will be taken up under the caption, "Reduction of the Chromosomes."

Contrary to a statement in Section III of these studies (*Amer.*

*Nat.*, vol. 38, p. 726, Oct., 1904) there is probably a deep significance in the fact that two mitoses are almost universally present in the spore mother-cell so that four spores are formed. It is probable that these mitoses are always heterotypic and homotypic, although this fact has only been clearly established in comparatively few favorable forms, and that they are indispensable to the mechanism of reduction phenomena. The latest accounts describe the first mitosis as the separation of the two portions of a bivalent chromosome, that is of two chromosomes joined either side by side or end to end, giving it a unique position among the mitoses of the life history. According to these theories the two mitoses of sporogenesis are features of a remarkable mechanism by which the paternal and maternal chromatin after its union in synapsis may become distributed in proportions that can be expressed by mathematical ratios. The peculiarities of the homotypic mitosis depend on a premature fission of the daughter chromosomes of the heterotypic division as will be explained in the next portion of this section. Thus the four spores are the result of these peculiar mitoses and have morphological significance. We are even justified in suspecting that the groups of four spores when found in the thallophytes, as the tetraspores of Dictyota and the red algæ, the four spores formed on the basidium and promycelium and the four spores of nuclei present in the germinating oöspore and zygospore of *Cædogonium* and the Conjugales indicate the presence of reduction phenomena simply because the number four is so constant. Williams (: 04a) for Dictyota and Blackman (: 04b) for types of the Uredinales have discovered clear cytological evidence of this reduction phenomenon but we know nothing of the chromosome history in other types of thallophytes.

We have already referred to the fact (Section III, *Amer. Nat.*, vol. 38, p. 743, Oct., 1904), that in the spermatophytes the two mitoses characteristic of sporogenesis are very close to the mitoses which differentiate the gamete nuclei. In the male gametophyte of the Angiosperms there are generally only two mitoses between the events of sporogenesis and gametogenesis and in gymnosperms there is a somewhat larger and variable number. The female gametophyte of the angiosperms usually

presents three mitoses after those of sporogenesis before the egg nucleus is formed. But in a number of types in the lily family (*e. g.*, *Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, etc.), the mitoses of sporogenesis are actually included in the embryo-sac and the very next mitosis, which is typical, differentiates the egg (see Section III, *Amer. Nat.*, vol. 38, pp. 741-744, Oct., 1904). This is the furthest point attained in the reduction of the gametophyte which in such forms actually includes but a single nuclear division in its history. But however close the mitoses of sporogenesis come to those of gametogenesis it is perfectly clear through the long phylogenetic history in the lower spermatophytes, pteridophytes, and bryophytes that the two are morphologically distinct processes and are always separate. It is unfortunate that the terms spermatogenesis and oögenesis should be applied to processes of sporogenesis as has been done by several authors, for such usage involves a confusion of two events which phylogeny clearly shows to be different in origin and to have back of them the diverging history of sporophyte and gametophyte from the times of thallophyte ancestry, the most remarkable evolutionary history in the plant kingdom.

It is conceivable that some plants may finally reach a stage in their evolutionary history when all the gametophytic mitoses in the pollen grain and embryo-sac will be suppressed and the nuclei resulting from sporogenesis become gamete nuclei. But it is clear that in such an event the gametophyte phase would be obliterated and we should have an entirely new type of life history. There would then be only one organism (derived from the sporophyte) whose gametes would be formed immediately with the differentiation of the pollen grain and embryo-sac. Such an organism would present reduction phenomena with the differentiation of the gametes and its type of life history would be identical with that of animals. We should look for such a reduced life history in groups related to forms in which the mitoses of sporogenesis are included in the embryo-sac and the gametophyte phase is represented by a single nuclear division (*e. g.*, *Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, etc.). Search among some of the most highly specialized Monocotyledonæ may actually reveal examples of the complete suppression of the female gametophyte.

The speculative possibilities of a suppression of a sexual generation and the assumption of sexuality by an asexual phase were clearly in the mind of Strasburger when he suggested ('94b, p. 852) the possibility that the two mitoses characteristic of oögenesis and spermatogenesis in animals might signify the remains of a former sexual generation now entirely suppressed in the Metazoa. This suggestion was based on the striking similarity of the events of sporogenesis in plants to those of gametogenesis in animals and on the history of sporogenesis as shown in plant phylogeny. This history is remarkably clear and there can be no question but that the phenomena of sporogenesis have developed as the result of sexual processes and are always associated with an asexual generation (sporophyte). It is also clear that the ancestral primitive sexual generation (gametophyte) has steadily degenerated until now it is almost lost in such embryo-sacs as include the two mitoses of sporogenesis within their history. If the sexual generation should become entirely lost the life history of a higher plant would present the same features with respect to the period of chromosome reduction as that of an animal: there would be but one organism, the homologue of the sporophyte which would produce gamete nuclei with reduction phenomena previous to gametogenesis just as in animals. Several authors have expressed views similar to Strasburger's suggestion ('94b, p. 852) or carried the speculation even farther than he. Beard ('95a, p. 444) along these lines of argument combined with conclusions from Bower's ('87) studies on apospory, announced a belief that "Metazoan development was really bound up with an *antithetic* alternation of generations." Lotsy (:05, p. 117) expresses unequivocally the view that the animal body represents an asexual phase (2x generation) and that the sexual phase (x generation) is confined to the sexual cells. Chamberlain (:05) simultaneously with Lotsy and in much greater detail presents a comparison of the phenomena of sporogenesis in plants with gametogenesis in animals tracing the resemblance in the events of chromosome reduction step by step and states his belief that "animals exhibit an alternation of generation comparable with the alternation so well known in plants."

This is not the place to consider this theory in detail from a

zoölogical standpoint since it bears only indirectly upon the material of these papers. Zoölogists have, however, discussed critically Strasburger's suggestions (see Wilson, :00, p. 275, and Häcker, '98, p. 101). The difficulties of accepting this view of a possible *antithetic* alternation of generations in animals seem insurmountable. In the first place there is not the slightest evidence of *antithetic* alternation of generations in the Metazoa or for that matter anywhere in the animal kingdom. The examples of alternation of generations which the zoölogists present among the Cœlenterates are all illustrations of *homologous* generations derived from buds. There is no indication of spore formation comparable to the process in the higher plants, so far as I am able to judge, in any group of animals. And also there seems to be accumulating evidence of reduction phenomena previous to the development of sexual cells in the Protozoa which is essentially of the same character as in the Metazoa (see Wilson, :00, pp. 227, 277, and Calkins, :01, p. 233). It is very interesting and remarkable that reduction phenomena should show the same order of events in animals and plants and the facts should be clearly recognized. But I cannot follow those botanists who carry over to the animal kingdom the phylogenetic conclusions which are so clear in plants. The remarkable agreement of the events of sporogenesis in plants with gametogenesis in animals appears to me likely to prove only another illustration of similar biological phenomena which have evolved independently of one another, an illustration comparable with the independent origin of sex, of heterospory, and probably even of the sporophyte generation itself (involving the processes of sporogenesis) in various groups of the plant kingdom.

We have considered this comparison of reduction phenomena in plants with animals chiefly to emphasize the clear cut morphology of the process as understood by the botanist. It does not matter how close the events of sporogenesis may come to those of gametogenesis in the higher angiosperms, the whole background of plant phylogeny, which is wonderfully clear as a whole, shows that reduction phenomena are the product of the asexual generation. It represents, as Strasburger has so well

expressed it ('94a, p. 288), a return on the part of the plant organism in each life history to the condition of an ancestral sexual generation (gametophyte). Reduction phenomena in themselves are not the result of a gradual evolution, whatever may be the complicated history of the sporophyte generation, for they consist always in the sudden reappearance of the primitive number of chromosomes, characteristic of the generation in which sex arose (gametophyte). The cause of reduction phenomena is phylogenetic. The interval that may separate this phenomenon from the responsible sexual act varies immensely in the plant kingdom according to the evolution of the groups concerned. But the suddenness of the appearance of sporogenesis tells in every case the same story of an immediate and total change in the potentialities of the protoplasm in the spore mother-cell, a change which can only be understood as a phylogenetic process deeply seated in the race.

When the events of sporogenesis in plants are considered as processes of spermatogenesis or oögenesis we disregard the most remarkable historic outlines that plant phylogeny can present, to the confusion of clear thought. Botanical science may well be proud of its achievement in outlining with such exactness the relations that the critical periods of gametogenesis, fertilization, and sporogenesis bear to reduction phenomena and too great stress can hardly be laid upon the importance of the results.

#### 4. REDUCTION OF THE CHROMOSOMES.

There are perhaps no activities of the cell which have been the subject of more investigation and discussion than those of chromosome reduction in animals and plants. The reasons are clear. The events of gametogenesis in animals and of sporogenesis in plants have the deepest significance for an understanding of the organization of protoplasm because these are periods when great changes are made evident in the structure of the cells concerned and at the same time in their potentialities. We are forced to conclude that some of the structural changes at least are the cause of the new potentialities and the attempt to establish the cause and effect has been one of the most fruitful and

interesting subjects of cell research. Reduction phenomena also have a deep phylogenetic significance whose history in plants at least can be traced with a remarkable degree of exactness.

We are confident that sporogenesis in plants signifies the sudden return of the organism to the condition of an ancestral sexual generation with the reappearance of a primitive number of chromosomes. The short time consumed in the process and the details and precision of the cell activities show that we are dealing with phenomena whose complicated mechanism can only find explanation in a long phylogenetic history. In the study of reduction phenomena and fertilization we have reached the conclusion that the chromosomes are intimately concerned with the transfer of hereditary qualities and are probably the chief or even the sole bearers of these characters. And thus we enter upon some of the most far reaching problems of biology, those of heredity, hybridization, and the basis for the remarkable ratios of inherited characters which Mendel first clearly set forth.

It seems quite certain for both animals and plants that numerical reduction of the chromosomes takes place through an association of the paternal and maternal chromosomes in pairs to form the reduced number of bivalent chromosomes (dyads). We have presented in Section IV ("Sexual Cell Unions and Nuclear Fusions") the evidence which indicates that paternal and maternal chromosomes do not unite at the immediate time of nuclear fusion in fertilization. On the contrary, in all higher animals and plants the paternal and maternal chromosomes are believed to remain separate throughout the long series of cell divisions in the new generation up to the time of sporogenesis in plants and gametogenesis in animals, both events being characterized by reduction phenomena. The fusion of the chromosomes takes place in the growth period which differentiates the spore mother-cell in plants from the archesporium or the primary gametocyte in animals from the preceding gametogenous tissue. The growth period is one of general protoplasmic accumulation and increase in the chromatin content of the nucleus, and is especially characterized by that peculiar activity in the nucleus termed synapsis. Evidence is accumulating that synapsis is the characteristic

feature of that period when the number of chromosomes is reduced by half.

Synapsis is followed very shortly by the two mitoses characteristic of sporogenesis. These nuclear divisions have given rise to a lengthy literature in which well known investigators have shifted their positions more than once. The discussions have centered on the methods of fission and distribution of the reduced number of bivalent chromosomes which appear in the first mitosis following synapsis. Assuming that the chromatin is organized into smaller units, represented by the chromatin granules (chromomeres, Fol, 1891), which compose the chromosomes, it is at once apparent that these finer elements may become variously distributed according to the structure of the bivalent chromosomes and the character of the mitoses of sporogenesis. Each fusion bivalent chromosome is composed of two chromosomes joined (1) end to end or (2) side by side or (3) it is possible that the chromatin is intricately mixed in the structure. With respect to the mitoses a transverse division of the fusion chromosomes might be expected to give a very different proportionate arrangement of the maternal and paternal chromatin from longitudinal divisions. Should the chromatin granules differ qualitatively from one another then different parts of a chromosome might be expected to have different characteristics which would be distributed by the mitoses of sporogenesis in various proportions or ratios.

It has long been known that the mitoses of sporogenesis present peculiarities in the mode of division and arrangement of the chromosomes at the nuclear plate which make them unlike the typical mitoses of cell division. These peculiarities have led to the designation of the first mitosis as heterotypic and the second as homotypic, terms which are now applied by both botanists and zoölogists although we have now a much more extended knowledge of each type than when Flemming first proposed the classification in 1887. We described the characters of the heterotypic and homotypic mitoses in Section III, "The Spore Mother-cell" (*Amer. Nat.*, vol. 38, p. 740, Oct., 1904), and will presently treat them further since some papers of the past year have opened again a discussion which seemed

closed a few months ago. The chief points of issue in discussions of reduction phenomena have centered around the significance of the heterotypic and homotypic mitoses. A typical mitosis is believed to present merely a quantitative division of each chromosome into two halves equivalent in their potentialities. The evidence for this view lies in the longitudinal fission by which each chromatin granule on the spirem is supposed to divide and contribute half of its substance to each daughter chromosome. Can there be a qualitative division of a chromosome by which one of the parts differs in character from the other, and are there such divisions at the time of sporogenesis in plants and gametogenesis in animals when reduction phenomena take place? These have been the chief topics of dispute in studies of this character for two decades.

The problem then ultimately concerns the structure of the chromosome and the reason for the constant reappearance of the number characteristic of the species at the beginning of each new gametophyte generation. All the prominent theories of heredity assume that the chromosomes are made up of simpler elements which stand for characteristics of the race. These may form various combinations of higher orders and collectively give the qualities of germ plasm. The simplest members that can be observed in such a series of structures are the chromatin granules (chromomeres) which may be found at almost all times in the nucleus and are especially conspicuous when arranged in a row on the linin thread of the spirem. Weismann has developed the most complex conception founded on the above principles and with the most elaborate terminology. Starting with the chromatin granule, which he named an *id*, Weismann assumed that this element is composed of still smaller structures called determinants and biophores, the last being the ultimate living units. Groups of *ids* make up *idants* or chromosomes. The *id* was conceived to possess all the essential characters of the specific germ plasm concerned but *ids* vary somewhat among themselves, determining thus the individual variations of the species. Therefore a chromosome or *idant* will have a varying structure according to the character and distribution of the *ids* which compose it.

When a chromosome divides longitudinally so that each id splits in half, the daughter chromosomes are exactly equivalent and the division of the chromatin is merely quantitative. But should a chromosome divide transversely then two sets of entire ids would be separated from one another and the two daughter chromosomes would differ in proportion as their component ids varied, *i. e.*, the division of the chromatin would be qualitative. These conceptions of the possible structure and mode of division of chromosomes outline the basis of Weismann's theory of heredity and will serve to illustrate the general attitude of those biologists who approach the subject from the standpoint of preformation, although none have cared to formulate such elaborate assumptions as Weismann. However, there is a general agreement among biologists of this school that elements are present in the chromatin which do carry hereditary characters and that the chromatin granule and chromosome have a definite architecture and organic value because of these elements.

Weismann's theory of heredity rests on an interpretation of the complexities of mitosis presented by Roux in 1883. Roux assumed that chromatin was not homogeneous in structure throughout the nucleus, but differed qualitatively in various regions. The elaborate history of mitosis with the formation and division of the chromosomes and their distribution through the mechanism of the spindle seemed inexplicable to Roux except on the theory that portions of the chromatin represented specific characteristics which were sorted and distributed accurately according to some system. There could be no need of such a complicated mechanism as mitosis if the distribution of the chromatin was to be merely quantitative for simple direct nuclear division could perform that operation as effectively as mitosis. Mitosis then became a device for the qualitative distribution of chromatin as well as quantitative and the characters of the daughter cells were determined chiefly by the specific elements which were given to one or the other.

Weismann siezed upon Roux's suggestion of a possible qualitative distribution of chromatin in mitosis and this assumption became a very important feature of his theory of heredity. Weismann postulated two methods of mitosis. By the first the

chromosomes are assumed to split longitudinally into equivalent halves, which are the facts in all vegetative or somatic mitoses so far as is known, and the chromatin is distributed quantitatively. By the second method chromosomes were conceived to split transversely so that one half is carried to each daughter nucleus, and if the two ends of a chromosome differed in the character of their fundamental elements (ids and determinants) the chromatin would be distributed qualitatively. Weismann prophesied in 1887 that this second type of nuclear division (qualitative mitosis) would be found and ever since investigators have steadily searched for a transverse division of the chromosomes. They have been reported in connection with the mitoses of chromosome reduction both for animals and plants and the history of these investigations forms an important part of the subject of reduction phenomena. But the present interpretation of these transverse divisions involves the consideration of factors that were unknown to Weismann and are very different from the significance assigned by him. The effect of Weismann's speculations, as a stimulus to investigations in these lines can, however, hardly be overestimated.

Botanical literature dealing with the two mitoses of sporogenesis presents a confusion of statements respecting the presence or absence of a transverse division of the chromosomes. Strasburger has changed his opinion three times. In his early studies Strasburger ('95) believed that the chromosomes divided longitudinally in both mitoses of sporogenesis. Then, led by studies of Mottier ('97) he concluded ('97b) that the fission of the chromosomes in the second mitosis was transverse. Almost immediately, however, Strasburger and Mottier reverted to the former opinion that the chromosomes divided longitudinally, a view which Strasburger maintained in his lengthy considerations of reduction phenomena in 1900a. Finally in a recent paper (:04b) Strasburger gives a very different interpretation of the events of the first mitosis (heterotypic), based on the study of *Galtonia*, and in general agreement with the most recent conclusions of Farmer and Moore (:03). Farmer ('95b), Farmer and Moore ('95), Miss Sargant ('96, '97), Guignard ('99a), Grégoire ('99), Lloyd (:02), and Mottier have also held that the divisions

of the chromosomes in the mitoses of sporogenesis were longitudinal with somewhat varying views, however, as to the exact time when the two divisions take place. On the other hand Ishikawa ('97), Calkins ('97), Belajeff ('98), and Atkinson ('99, for *Trillium*) have claimed that the second mitosis presented a transverse division. Dixon ('95, '96, :00) and Schaffner ('97) held a position apart from all these investigators, believing, that the chromosomes of the first mitosis of *Lilium* resulted from loops whose free ends became appressed or twisted together finally separating at the angle of the loop and thus constituting a transverse division in this first mitosis. These latter observations accord with the latest conclusions of Farmer and Moore (:03) and Strasburger (:04b). Most of this literature is reviewed in detail in Strasburger's paper of 1900a. We shall omit an historical discussion of this early work for the entire subject is approached from quite a different standpoint in the series of papers which have appeared in the past three years (1903-05) and which give hope of much clearer information on the mitoses of the spore mother-cell.

The remainder of this treatment of "Reduction of the Chromosomes" will take up the recent papers and try to show the drift of the present investigations. These papers had not appeared when the author described the behavior of chromosomes during mitosis in Section II (*Amer. Nat.*, vol. 38, p. 445, June, 1904) and presented the account of the spore mother-cell in Section III (*Amer. Nat.*, vol. 38, pp. 726, 740, Oct., 1904). At that time it seemed probable that Strasburger's conclusions of 1900 held true for all plants, namely, that the chromosomes split longitudinally in both mitoses of sporogenesis as well as in all other mitoses of the life history. Whether these views may have to be materially changed in the light of the most recent work is now a matter of dispute. Yet the ground has shifted so frequently in these perplexing problems that it is hard to feel sanguine of final conclusions even in the hopeful situation of the present. I shall take up the events of sporogenesis in order, beginning with the growth period and synapsis and ending with the two mitoses of the spore mother-cell.

The growth period always extends over a considerable length

of time and may occupy even weeks or months. During this interval the spore mother-cells increase to many times the size of the archesporial cells from which they were derived. There is an immense accumulation of protoplasmic material and a corresponding increase in the size of the nucleus and its chromatin content. The growth may be continued in the spores after the mitoses of sporogenesis, as is characteristically illustrated in the great increase in the size of the megaspores in the pteridophytes and certain embryo-sacs. The most striking nuclear activity of the growth period preceding the mitoses is synapsis. This term is applied to a very characteristic gathering of the chromatin and linin material in a compact tangle or ball at one side of the nucleus and usually near the nucleolus. Nuclei are sometimes in a state of synapsis for several days or perhaps weeks as is shown by the frequency of the stage in sporogenesis. Thus during the entire period of sporogenesis in *Anthoceros* from the inception of the spore mother-cell to the final differentiation of the spores (which must take many days) the period of synapsis occupies from one eighth to one sixth of the entire time (Davis, '99, p. 104). Synapsis has proved to be a very difficult subject for study and few investigators have made detailed observations upon its events. Some have claimed that synapsis is an artifact due either to poor fixation or to a particularly sensitive condition of the cell nucleus by which the chromatin was especially susceptible to shrinkage but it seems certain now that the phenomenon is entirely normal. Miss Sargent ('97, p. 195) has observed synapsis in the living pollen mother-cell of *Lilium martagon*. *Anthoceros* presents a particularly favorable subject for the study of the effects of fixing fluids on spore mother-cells because one may present all stages in the same sporophyte to identical conditions. In a series of experiments on this form (Davis, '99, p. 97) with a number of standard fixing fluids I have always found synapsis at exactly the same period in sporogenesis and at no other time in the process. True synapsis, characteristic of reduction phenomena must be carefully distinguished from other somewhat contracted conditions of the chromatin which are occasionally found in cells. Thus Miyake (*Annals of Bot.*, vol. 17, p. 358, 1903) noted the resemblance to synapsis

of an accumulation of granular material in the nucleus of the central cell of *Picea* and other cases might be cited which superficially resemble synapsis but have no fundamental relation to this peculiar nuclear activity.

Evidence is steadily accumulating that synapsis is a very important period of sporogenesis. Some authors hold, as will be described presently, that it is the time when paternal and maternal chromosomes, which have remained separate throughout the sporophyte generation, become associated in pairs to give the reduced number of the gametophyte. This conclusion makes synapsis the actual period of chromosome reduction and the two succeeding mitoses become merely distributing divisions of the newly formed chromosomes. Montgomery (:01) first suggested for animals that synapsis involved a union of maternal and paternal chromosomes in pairs. Other views, however, regard the reduction of the chromosomes as merely the temporary union of paternal and maternal elements, end to end, to form a bivalent chromosome characteristic of the first or heterotypic mitosis. According to this view the bivalent chromosomes divide transversely so that the halves are distributed as whole chromosomes in the first mitosis.

Two very important papers on reduction phenomena have appeared this year (1905) both of which were preceded by preliminary publications, that of Farmer and Moore (:03) and Allen (:04). These two accounts best represent the attitude of the opposing schools and will be made the chief texts of our treatment. The fundamental points of difference concern the events of synapsis and the heterotypic mitosis while there is complete agreement in the general interpretation of the homotypic mitosis. All authors have reached essentially the same conclusions as regards the purpose and final results of the reduction divisions but the details of the processes of synapsis and the prophase of the heterotypic mitosis are described in radically different ways by various investigators. However, as has been stated, the views fall into two groups or schools, one led by Farmer and Moore with whom Strasburger's recent paper, "Ueber Reduktionsteilung" (:04) expresses essential agreement. The other school includes Allen, Rosenberg, and the

botanists of the Carnoy Institute, Grégoire and Berghs. To the writer the conclusions of the second school seem better founded and we shall present them first. Allen's last paper (:05) gives the most complete statement of their interpretations.

Allen's conclusions (:03, :05) are based on the study of the pollen mother-cell of *Lilium canadense* and his account of synapsis in this form is of great interest for the simplicity of his explanation of the events of this phenomenon and their significance. The nucleus of the young pollen mother-cell following the last mitosis in the archesporium and previous to synapsis contains a network of large irregular masses connected by fibers of varying thickness. The irregular masses, which probably contain both chromatin and linin, are derived from the chromosomes of the previous mitosis but these structures cannot be recognized in the resting nucleus. Nucleoli are present among the irregular masses or chromatin knots but are readily distinguished from them. As the nucleus grows larger the chromatin knots become more widely separated, but synapsis does not occur until it has reached its full size.

During synapsis the reticulum becomes transformed into a definite spirem. The fibers connecting the chromatin knots increase in length and become more uniform in thickness while the knots become less conspicuous as though their material were drawn out along the fibers. The fibers of the reticulum are now seen to arrange themselves in pairs and a general contraction of the network takes place which is the beginning of synapsis. Allen believes that this contraction is associated with the approximation of the fibers. The contracting network occupies at first the center of the nucleus but later moves to the periphery where the nucleoli may be found flattened against the membrane. There is now a continuous spirem in the nucleus, plainly composed of two slender threads lying side by side and probably with no free ends. These two threads often run closely parallel, sometimes loosely twisted about one another, sometimes in contact and apparently fused and sometimes rather widely separated. It is clear that the double nature of the thread is not due to a fission but that two independent threads are developed indepen-

dently out of the reticulum. The two threads gradually fuse so that in older stages of synapsis the nucleus appears to contain a single relatively thick spirem which is shorter and more loosely coiled than in the earlier stages. The minute structure of the threads of the spirem can be determined by careful staining. They consist of a series of chromatin granules (chromomeres) imbedded in the ground substance, linin. As the two threads fuse the chromomeres generally come together in pairs and unite to form a single row of large chromomeres which project from the side of the larger single (fusion) spirem.

The single (fusion) spirem on emerging from synapsis becomes uniformly distributed throughout the nucleus. There appear to be no free ends in the much convoluted and looped thread. Some of the loops become fastened to the periphery of the nucleus but there is no regularity in the number of loops and no relation to the number of chromatic segments that are formed later. While thus evenly distributed the single spirem undergoes a longitudinal fission which is preceded by the division of each chromomere. This is the first longitudinal fission of the spirem which is well known through the descriptions of Guignard, Grégoire, Strasburger, Mottier, and others. The fission is not simultaneous throughout the length of the spirem, for some portions remain undivided for some time when contiguous parts are plainly split. The result is a condition very similar to that presented just before the fusion of the two systems of threads during synapsis which produced the single (fusion) spirem. It seems probable that the threads which become separated are morphologically the same as those which fused during synapsis although the union at that period seems complete. The split spirem remains uniformly distributed throughout the nucleus exhibiting, however, a tendency to become somewhat massed in the center of the nuclear cavity leaving fewer loops attached to the nuclear membrane.

The split spirem now segments throughout its length into the reduced number of chromosomes (12) characteristic of the heterotypic mitosis. The segmentation is not simultaneous, but the first free ends appear near or at the periphery of the nucleus where the split spirem breaks apart at the loops. As segmen-

tation proceeds the number of loops becomes fewer and the free ends more numerous. Allen finds the breaking apart of the arms of the loops, whose heads are at the periphery of the nucleus, as described by Schaffner ('97) and Farmer and Moore (:05), but cannot accept the interpretation of these latter authors (to be described presently). Allen's studies show that the loops are the points of separation of adjacent split chromosomes and not a point where the spirem bends on itself to form a pair of chromosomes. The ends of the split chromosomes when properly stained are seen to be distinct even though they may be in contact or apparently fused. At the time of the segmentation of the split spirem the two threads are generally twisted about one another.

The split chromosomes now shorten and thicken, the number of twists is reduced and the pairs of elements take on the many forms characteristic of the heterotypic mitosis and described as I's, J's, X's, Y's, V's, and O's. These chromosomes of the heterotypic mitosis are of course pairs of chromosomes, *i. e.*, bivalent chromosomes or dyads. They are believed to represent morphologically the full number of sporophytic chromosomes (24) now associated in pairs forming the reduced number (12) of bivalent chromosomes. The two threads which fuse are believed to represent two spirems of maternal and paternal origin and the chromosomes in the pairs are derived from different parents.

Shortly after the segmentation of the spirem the sporophytic chromosomes of each bivalent element or dyad may show evidence of a second longitudinal fission, first recognized by Grégoire ('99), Guignard ('99), and Strasburger (:00) which is completed during the metaphase of the heterotypic mitosis. The evidence consists in the appearance of a double row of granules in each sporophytic chromosome, the result of the division of the chromomeres. However, these chromomeres soon become indistinguishable from the linin and the chromosomes appear homogeneous from now on.

While the spindle of the heterotypic mitosis is being organized the position of the sporophytic chromosomes shifts with the development of the spindle fibers until they are brought to the

nuclear plate still grouped in pairs as dyads (bivalent chromosomes). The details of spindle formation and the heterotypic mitosis do not concern the present discussion of reduction phenomena. The reduction has occurred with the formation of the dyads and the mitosis simply distributes the 24 chromosomes (generally called daughter chromosomes) which are believed to be the morphological equivalents of the sporophytic chromosomes that entered the spore mother-cell from the archesporium.

Just before the separation of the sporophytic chromosomes during metaphase of the heterotypic mitosis a longitudinal fission appears suddenly in each element extending almost the whole length. This is the second longitudinal fission as interpreted by Grégoire ('99), Guignard ('99), Strasburger (:00), Mottier (:03), and others, with whom Allen is in full agreement. It is of course a premature division of the chromosomes preliminary to the homotypic mitosis. The second fission is probably completed at this time but the elements of each pair (formerly called granddaughter chromosomes) remain clinging together at one end by a peculiar overlapping of the hooked tips forming thus a V-shaped pair whose apex is drawn to the poles of the heterotypic spindle. The daughter nuclei following the heterotypic mitosis are not in a true resting condition and the chromosomes while forming a spirem show abundant evidence of independent structure. They emerge from the spirem at the prophase of the homotypic mitosis as the same morphological entities (*i. e.*, as V-shaped pairs) and are thus brought to the nuclear plate from which they are distributed generally as fairly straight rods to form the nuclei of the pollen grains.

Rosenberg's (:03a, :04a, :04b) studies on the hybrids of *Drosera* furnish further evidence that the chromosomes from different parents fuse in pairs during the prophase of the heterotypic mitosis. The gametophyte number of chromosomes in *Drosera rotundifolia* is ten and in *D. longifolia* twenty and those of the former species are larger than those of the latter. The sporophyte number in the hybrid is thirty as would be expected. At the heterotypic mitosis of sporogenesis, however, twenty chromosomes appear in the hybrid, half of which are plainly double structures and consist each of a larger and a

smaller element. During this mitosis the ten double chromosomes divide but the single chromosomes remain entire and either pass to one pole or the other or are left out in the formation of the daughter nuclei. The explanation of these conditions must be that ten chromosomes of *D. rotundifolia* fuse with ten from *D. longifolia* leaving ten of the latter without mates. Rosenberg's last paper (:04b) on *Drosera* describes in considerable detail the union of chromosomes in pairs in both species of *Drosera* during sporogenesis. The sporophytic chromosomes which at first are scattered throughout the nucleus in the early prophase of the first mitosis come together in pairs and unite so closely that there is hardly a trace of their dual nature in the resultant larger bivalent chromosomes, which are of course the gametophyte number. Rosenberg is very positive that the pairs of chromosomes are preliminary to a fusion and not the result of a fission of already reduced segments of a spirem thread. Rosenberg believes that the two halves of the bivalent chromosomes are separated in the first (heterotypic) mitosis and that each splits lengthwise prematurely during the first mitosis in preparation for the second. The fused bivalent chromosomes then appear to divide twice longitudinally but the first division may be only a separation of the two sporophytic chromosomes that entered into the fused pair.

We shall consider now the conclusions of Berghs and Grégoire of the Carnoy Institute, Louvain, whose publications have appeared practically simultaneously with some of those which we have just discussed. Berghs has published three papers (:04a, :04b, :05) treating of the early history of sporogenesis in *Allium*, *Lilium*, and *Convallaria*, and concludes from a study of synapsis that the spirem immediately preceding the heterotypic mitosis arises from the close association, side by side, of two delicate threads. These threads are organized previous to and during synapsis and their coming together brings about that contraction of the chromatic material characteristic of synapsis. The threads contain sporophytic chromosomes of the last mitosis in the archesporium. The apparent longitudinal fission of the spirem which precedes the heterotypic mitosis in the spore mother-cell is interpreted as being these two threads

which are believed to have never actually fused during synapsis. The reduced number of segments derived from the spirem preceding the heterotypic mitosis are then bivalent chromosomes composed of pairs of sporophytic chromosomes lying side by side. The heterotypic mitosis distributes the sporophytic chromosomes in two sets resulting in a numerical reduction of their numbers by one half. It will at once be noted that while Berghs and Allen have independently arrived at similar conclusions respecting the structure of the chromosomes of the heterotypic mitosis there are some important differences in the mode of origin. Allen reports an actual fusion of the two threads (paternal and maternal) during synapsis and a later fission of the spirem previous to the heterotypic mitosis. But the accounts of both authors have much in common in their interpretation of the structure of the spirem and chromosomes of the heterotypic mitosis which is fundamentally different from the accounts of Farmer and Moore, and Strasburger to be described later.

Grégoire (:04) in a general discussion of reduction phenomena confirms the observations of Berghs and takes a very positive position against the interpretations of Farmer and Moore and Strasburger. The chief features of his conclusions are in harmony with the results of Allen. The sporophytic (somatic) chromosomes are believed to become associated in pairs by the application of two delicate threads throughout their length during synapsis. These threads are believed to retain their autonomy and never actually to fuse although they may come in close contact. Consequently the reduced number of chromosomes are pairs of sporophytic chromosomes which have retained complete independence. Allen, on the contrary, reports a complete union of the two threads involving the fusion of chromomeres in pairs and a later longitudinal division throughout its length of the single (fusion) spirem. Grégoire does not regard the heterotypic mitosis as a true nuclear division but as a special process designed to effect this numerical separation of the sporophytic chromosomes and intercalated between typical mitoses, while Allen would apparently treat it as a true mitosis and regard the chromosome reduction as effected by the fusion of two sporophytic spirems during synapsis.

Rosenberg (:05) has recently published a general review of reduction phenomena based on studies upon *Listera*, *Tanacetum*, *Drosera*, and *Arum*, taking a position in essential agreement with Allen and the investigators of the Carnoy Institute and in opposition to the theory of Farmer and Moore and Strasburger. Rosenberg does not quote Allen's preliminary paper (:04) which anticipates his conclusions. He finds that the spirem which emerges from synapsis is preceded by a condition when the structure is clearly made up of two threads (spirems) which lie parallel to one another. These two threads are frequently joined together, and in places spirally twisted but here and there they may be seen to be entirely separated from one another. They finally form the single spirem which follows synapsis and which divides into the reduced number of chromatic segments. But the chromatic segments throughout the entire processes are shown to be double in structure (bivalent chromosomes), *i. e.*, composed of two chromosomes lying very close together side by side or even united. What appears to be a longitudinal fission of the chromatic segments of the spirem immediately preceding the first mitosis is really then a line of union along which the two independent threads have come together. The phenomenon of synapsis consists of this close association of two threads which are themselves simple spirems into a double spirem which segments into pairs of sporophytic chromosomes each of which may be regarded as a bivalent chromosome.

Farmer and Moore published a preliminary communication in 1903 which aroused much interest in their theory of chromosome reduction. The full account (:05) has recently appeared. Their studies are upon *Lilium*, *Osmunda*, *Psilotum*, *Aneura*, and the cockroach, *Periplaneta*. *Lilium* and *Osmunda* among the plants were given chief attention and since the lily was the type studied by Allen it will serve best to contrast the conclusions of these two investigators. The accounts of Allen and Farmer are so fundamentally different as regards the events of synapsis and the prophase of the heterotypic mitosis that it seems scarcely possible that both can be right in their respective material, *Lilium candidum*, Farmer's type, and *L. canadense* of Allen's description. Farmer and Moore intro-

duce the terms "maiosis" and the "maiotic phase" to cover the whole series of nuclear changes included in the heterotypic and homotypic mitoses. The maiotic phase is regarded as similar in its essential details in both animals and plants but the fact of its appearance at different points in the life histories precludes any probability of relationship in such widely divergent lines. The events of synapsis and the consequent peculiarities of the heterotypic and homotypic mitoses are considered as intercalated between the series of typical mitoses in the life history.

Farmer and Moore's conclusions for *Lilium candidum* may be briefly summarized as follows. A definite spirem with the chromatin distributed as granules appears in the young spore mother-cell before its separation from neighboring elements. A "first contraction figure" now appears and the spirem thread becomes densely coiled in the vicinity of the nucleolus, this condition persisting for some time. Then the coils of the spirem loosen and become distributed about the periphery of the nuclear cavity, from the point of contraction as a center. A longitudinal fission of the spirem thread then appears, the chromatin granules dividing so that they come to lie in two parallel rows on the edge of the split ribbon. The fission is irregular and open loops appear at places. The spirem then shortens and the split gradually closes up and becomes very difficult to recognize. Many of the convolutions of the thread are attached to the nuclear membrane while the remainder form a tangle in the interior around the nucleolus which is believed to give up much of its substance to the chromatic portion of the spirem. Farmer and Moore then fail to find the double thread and its union during synapsis to form a single (fusion) spirem which is a fundamental feature of Allen's account.

There follows then a stage which has been the subject of much discussion. According to Farmer and Moore the spirem thread becomes pulled out into V- and U-shaped loops, shown with especial clearness where the bend of the loop is attached to the periphery of the nuclear membrane. The arms of the V's then come to lie parallel and so close together as to give the appearance of a fission in a structure which is really the result of an approximation of the two free ends of what was a loop.

The spirem thread thus breaks up into segments which, however, lie in pairs represented by the V's in the reduced (gametophyte) number. The pairs are bivalent chromosomes, each composed of two sporophytic chromosomes which were arranged serially on a single spirem thread. The pairs are not always organized through the approximation of the arms of V-shaped loops but this is a very characteristic type of structure. The V's have been interpreted by other authors as the approximation of portions of the spirem thread (Dixon, '95, '96, : 00) or the separation of their free ends at the bend of the loop as a transverse division of a reduced number of looped chromosomes in the heterotypic mitosis (Schaffner, '97). The two parts of the bivalent chromosomes (which are pairs of somatic chromosomes) now become shorter and thicker and all trace of the original fission of the spirem thread is lost.

The essential features of Farmer and Moore's interpretation of the prophase of the heterotypic mitosis are, then: (1) a single spirem with the sporophytic chromosomes arranged serially, which splits only once longitudinally, the fission afterward becoming obliterated when the chromosomes are organized, and (2) the organization of bivalent chromosomes in the reduced number largely by the approximation of the free ends of loops which entails a separation at the bend of the loops of the two sporophytic chromosomes, giving the appearance of a transverse division.

The heterotypic mitosis, then, according to Farmer and Moore involves merely the distribution of the sporophytic chromosomes arranged in pairs (bivalent chromosomes) as univalent elements to each daughter nucleus. This is of course the general conclusion of all recent investigators, the different views being the result of varying accounts of the method of organization of the bivalent chromosomes. During this distribution in the heterotypic mitosis the split of the original spirem appears in each univalent element (sporophytic chromosome) and the halves open throughout the greater part of their length giving the peculiar V-shaped daughter chromosomes so characteristic of this mitosis in the lily. The arms of these V's become the daughter chromosomes of the homotypic mitosis which are thus

formed prematurely during the heterotypic as was first described by Grégoire ('99). However, Grégoire and most botanists have considered the split between the V's as a second longitudinal fission of the original spirem in the spore mother-cell while Farmer and Moore regard it as the reappearance of an original single fission. This view of Grégoire, which has had the support of Guignard ('99), Strasburger (:00), and Mottier (:03), is the theory of a double longitudinal splitting of the chromosomes previous to the heterotypic mitosis and is also maintained in Allen's (:05) recent paper.

The homotypic mitosis brings about the final separation of the arms of the V-shaped longitudinally split univalent (sporophytic) chromosomes of the heterotypic division. The fact that the arms of these V's finally break apart at the ends does not constitute a transverse division as has been claimed by some earlier writers (Ishikawa, '97; Calkins, '97; Belajeff, '98; Atkinson, '99, for *Trillium*). The peculiarities of the homotypic mitosis are then due to the premature fission of the univalent chromosomes during the heterotypic. As a type of nuclear division the homotypic mitosis is not fundamentally different from the typical divisions of other periods of the life history. All recent authors are in agreement on this interpretation of the events of the homotypic mitosis.

Gregory (:04) gives an account of sporogenesis for several leptosporangiate ferns and accepts Farmer and Moore's explanation of reduction phenomena. He finds the same sort of U-shaped segments in the reduced number at the heterotypic division and considers them bivalent chromosomes which divide transversely so that the original sporophyte chromosomes are distributed in two sets during this mitosis. The various positions assumed by the limbs of the U-shaped segments give appearances very similar to the tetrads described in the heterotypic mitosis of animals and which Calkins ('97) reported for *Pteris* and *Adiantum* and regarded as resulting from the transverse division of the halves of a longitudinally split chromosome. Gregory of course cannot accept the conclusions of Calkins.

Williams (:04a) applies the theory of Farmer and Moore respecting the bivalent character of the chromosomes in the

heterotypic mitosis to his studies on the first division in the tetraspore mother-cell of Dictyota. But it can hardly be said that his account offers any material support to the theory. There is a clear synapsis stage preceding the mitosis in this form from which a spirem emerges as a beaded thread. This spirem then becomes split longitudinally and later the chromosomes are organized and show a longitudinal fission. The form of the chromosomes at metaphase of the first mitosis is heterotypic, a ring form being prevalent, and Williams concludes that it is developed by the bending and closing of the free ends of a loop. The events of synapsis are not clearly enough known to make possible a comparison with the accounts of Allen and Berghs.

We are now ready to take up the latest conclusions of Strasburger (:04b) which are closely associated with views expressed in a recent paper of Lotsy (:04). Lotsy gives a clear statement, illustrated with many diagrams of the various ways in which sporophytic chromosomes may be conceived to unite in pairs previous to the first mitosis in the spore mother-cell and the manner in which the resultant bivalent chromosomes may be divided and distributed by the two mitoses of sporogenesis. Lotsy makes parallel comparisons between sporogenesis in plants and gametogenesis in animals and proposes the term "Gonotokonten" ("Nachkommenbildner") for the mother-cells which inaugurate reduction phenomena. The paper presents no new observations but discusses the problems of reduction in their broad aspects. An excellent summary is given by Kœnicke (:04).

Strasburger's (:04b) most recent paper, "Ueber Reduktionsteilung," is based chiefly on studies of Galtonia and Tradescantia and presents an entire change of view from his conclusions of 1900. Galtonia seems to be a very favorable form for study since the gametophyte number of chromosomes is only six and the structures are exceptionally clearly differentiated in the spore mother-cells, which Strasburger calls "Gonotokonten" after Lotsy. A single spirem is reported to split longitudinally but the two daughter threads remain close together. The spirem then shortens and thickens and becomes distributed in heavy

loops. It finally divides into six segments which are interpreted to be six pairs of chromosomes joined end to end. These six segments are then bivalent chromosomes. The two chromosomes of each pair (segment) finally come to lie side by side in various positions by the bending of the original looped segments and the separation of their two ends in the middle. The halves of the six bivalent chromosomes (segments) are distributed by the first mitosis so that there is the effect of a transverse division of six chromosomes at this time, but really the process is one of the distribution of twelve chromosomes in two sets of six each. The longitudinal fission of the spirem thread becomes more conspicuous towards the end of the first mitosis so that the twelve chromosomes become partially split and pass as V's to the poles of the first spindle during telophase. This premature division is preparatory for the second mitosis (homotypic) when the separation is finally effected. There is then only one longitudinal fission of the original spirem in the spore mother-cell and this prepares the chromosomes for the second mitosis, which differs only from the typical mitoses in the premature splitting of its chromosomes. The first mitosis is merely the separation of pairs of chromosomes joined end to end. Strasburger interprets the conditions in *Tradescantia* and *Lilium* in a similar way believing that the complications there simply arise from a more involved looping of the spirem thread. Strasburger's account of *Galtonia* then supports in all essentials the theory of Farmer and Moore.

Strasburger in the same paper (:04b) gives an account of synapsis which cannot be brought into harmony with that of Allen. The chromatin granules are reported to gather during synapsis into as many centers, which he names "Gamozentren," as will finally form the reduced number of bivalent chromosomes (six in *Galtonia*). The "Gamozentren" then become arranged and drawn out into the spirem which emerges from synapsis. The chromatin granules are named "Gamosomen" and the bodies formed in the "Gamozentren" which afterwards become the bivalent chromosomes of the first mitosis are called "Zygosomen." There are then no organized chromosomes during synapsis and no place in Strasburger's account for the fusion of

a fully organized paternal and maternal spirem as described by Allen. The identity of the sporophytic chromosomes becomes entirely lost, according to Strasburger's explanation of synapsis, and the chromatin granules ("Gamosomen") may be variously distributed in the new set of bivalent chromosomes ("Zygosomen"). These "Zygosomen" are a new creation in the cell. All of the other theories, on the other hand, preserve the morphological entity of the sporophyte chromosomes which are of course of maternal and paternal origin but allows their distribution in various ratios to one another during the first mitosis of sporogenesis. The chromosome, however, remains a fixed morphological structure from one generation to another. These are fundamental differences which have a vital bearing on the discussion of hybridization, which will follow shortly, since one of the most important features of the problems concerns the preservation of the relative purity of the germ plasm.

The chief characteristics of the two theories of reduction may be summarized as follows:—

(1) According to Allen, Rosenberg, Berghs, and Grégoire, the phenomenon of synapsis presents a close association of two parallel chromatic threads (probably of maternal and paternal origin) which finally unite to form the spirem that precedes the heterotypic mitosis. This single (fusion) spirem is then double in nature and the longitudinal fission which follows, is the separation of the two threads that entered into its composition. The reduced number of chromatic segments of the heterotypic mitosis are bivalent chromosomes or more precisely pairs of sporophytic chromosomes derived from the two (maternal and paternal) threads of the synapsis stage. The heterotypic mitosis distributes the sporophytic chromosomes in two sets thus effecting a numerical reduction by one half. The sporophytic chromosomes divide prematurely during the heterotypic mitosis in preparation for the homotypic thus presenting a second longitudinal fission of the segments derived from the single (fusion) spirem. A special feature of Allen's studies is the fusion of chromomeres in pairs during the organization of the single (fusion) spirem and a subsequent splitting of each larger chromomere with the longitudinal fission of this structure.

(2) Farmer and Moore, Gregory, Williams, and Strasburger hold that there is primarily only a single chromatic thread in the nucleus of the spore mother-cell which is the spirem of synapsis and the heterotypic mitosis and which most of these authors believe to be composed of the full number of chromosomes (sporophytic) joined end to end. This spirem splits longitudinally but the fission is a premature division which prepares the chromosomes for the homotypic mitosis. The chromosomes of the heterotypic mitosis are formed from loops of the spirem which include a pair of sporophytic chromosomes joined end to end. The members of this pair come to lie side by side by an approximation of the arms of the loops and a breaking apart at the head of the structure. This transverse fission of the spirem is not of course a transverse division of a chromosome but merely the separation of a pair of chromosomes joined end to end. The line between the two arms of the loop marks a region of contact due to approximation and not a line of fission. The heterotypic mitosis effects a numerical reduction of the chromosomes as in the first view but these chromosomes are formed on entirely different principles. A single premature fission of the spirem or its segments prepares the chromosomes for the homotypic mitosis.

Comparing the two schools, it may be noted that they both explain reduction phenomena as a numerical reduction of the double set of sporophytic chromosomes by a distribution in two sets. The fission of the chromosomes is always quantitative and there is no hint in any of the views of a qualitative division in Weismann's sense. Furthermore, most of the investigators are firmly convinced of the individuality of the chromosomes which means that they are <sup>view</sup>convinc~~ed~~ as morphological entities persisting from one generation to the next. This is an important agreement in relation to theories of heredity and hybridization which we shall discuss at another time (see treatment of "Hybridization"). The differences lie in questions of fact regarding the organization of these chromosomes in the spore mother-cell and their behavior during synapsis and at other periods of prophase in the heterotypic mitosis. There is entire accord in that the chromosomes of the homotypic mitosis appear

during the metaphase of the heterotypic but a fundamental difference in the accounts of the manner in which these structures are formed.

In conclusion, we may very briefly note the fact that the zoölogists are divided into two schools in their accounts of reduction phenomena, apparently along similar lines to those of the botanists. Some recent papers (Winiwarter, :00; Schoenfeld, :01; and the Schreiners, :04) have described the union of parallel threads (maternal and paternal) during synapsis to form a single spirem in the rabbit, man, bull, hag-fish, and shark. Winiwarter and the Schreiners regard a later longitudinal fission of the spirem as a separation of the two threads which originally entered into the structure. The chromosomes in the hag-fish (*Myxine*, the Schreiners, :04) are organized in pairs side by side and a second longitudinal split appears in each. The heterotypic mitosis separates the groups in the plane of the first fission and the two parted chromosomes are divided by the homotypic. This history is essentially similar to Allen's account of the lily. On the other hand there is a large body of observations founded on the investigations of Häcker, vom Rath, Rückert, Montgomery, and others, indicating that bivalent chromosomes are formed consisting of somatic chromosomes joined end to end and that these elements or their derivatives are distributed either with the heterotypic or homotypic mitosis. This of course involves a transverse division which is, however, interpreted as the separation of adjacent chromosomes and not as a qualitative division in Weismann's sense. The attitude of the first group is clearly similar to that of Allen, Rosenberg, Berghs, and Grégoire among the botanists, while that of the second shows many points of similarity to the theory of Farmer and Moore and to Strasburger's last view (:04). There are a number of accounts of a double longitudinal fission of chromosomes especially among the vertebrates, which have not been harmonized with the last view but may find explanation along the lines of the more recent investigations.

It is of course conceivable that there are two distinct types of arrangement of sporophytic and somatic chromosomes in animals and plants at synapsis during gametogenesis and sporogenesis.

It is possible that they may be grouped in pairs (bivalent chromosomes) either side by side through two parallel threads (paternal and maternal spirems) or end to end in a single chromatic thread. But it will certainly be interesting if animals and plants both show variations in these respects and very remarkable if the same genus, as *Lilium*, should present contrasting types of reduction phenomena. And on these points must be concentrated the future investigations in this field.

While we are making progress in our understanding of the behavior of the chromosomes it must never be forgotten that in them we are dealing only with the most conspicuous form of germ plasm and that there are much finer elements which in their turn will demand attention. We may hold to the view of the individuality of the chromosomes as morphological entities but nevertheless we must recognize the fact that the substance of these bodies which stand for parental characters, the idioplasm of Nägeli, may pass through remarkable changes which are far from understood. There is much evidence that the parental idioplasm may mix or combine during synapsis in the organization of the spirem from which are developed the reduced number of bivalent chromosomes. Allen has described the actual fusion of sets of chromomeres believed to be of maternal and paternal origin and there are many possibilities of the two idio-plasms reacting upon one another to bring about intimate and fundamental interrelations. These become important principles in discussions of heredity and hybridization and will be considered later. Allen (:05, pp. 246-252) presents an admirable analysis of these problems.







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## STUDIES ON THE PLANT CELL.—VII.

BRADLEY MOORE DAVIS.

### SECTION V. CELL ACTIVITIES AT CRITICAL PERIODS OF ONTOGENY IN PLANTS (*Continued*).

#### 5. APOGAMY.

APOGAMY is the suppression of the sexual act and the development of a succeeding generation asexually. The term was first proposed by De Bary in 1878, following Farlow's ('74) discovery of the phenomenon in *Pteris cretica*. The succeeding generation may arise in one of two ways: (1) by the development of an unfertilized egg or gamete which is termed parthenogenesis, or (2) by some form of vegetative outgrowth from the sexual plant, a process which has been called vegetative apogamy. We shall not attempt to give a detailed account of apogamy in the plant kingdom but will confine ourselves chiefly to the consideration of a few detailed studies of recent months which have taken up the cell problems concerned. The cell problems naturally treat of the processes which may be substituted for the sexual act in ontogeny and the fundamental problems of the behavior of the chromosomes under these conditions.

Parthenogenesis has been known for many years among the thallophytes which furnish illustrations in a variety of groups. In the algæ we have the well known examples of *Chara crinita*, *Cutlaria*, *Dictyota*, some species of *Spirogyra* and *Zygnema*, and a number of types in the lower Chlorophyceæ and Phæophyceæ whose motile gametes will germinate like zoospores should they fail to conjugate with one another. The recent studies of Williams (:04b) on *Dictyota* give the only observations which have been made on nuclear activities during the parthenogenetic development of eggs in any algal form and will be considered presently. The fungi furnish beautiful illustrations of partheno-

genesis in the Saprolegniales. Trow (:04) believes that some of these forms are sexual but there can be little doubt that the group as a whole is generally apogamous. There is probably much apogamy in the Ascomycetes and an almost entire suppression of sexual organs in the Basidiomycetes but no clear instance of parthenogenesis (*i. e.*, a development from a cell whose morphology is unquestionably that of an egg) is known in either of these groups.

Parthenogenesis is not known in the bryophytes and pteridophytes excepting for Marsilia (Shaw, '97; Nathansohn, :00). Although there is much apogamy in the pteridophytes, especially in the leptosporangiate Filicales, <sup>where</sup> the new generation generally develops as a bud-like outgrowth on the prothallus (vegetative apogamy). There have been no nuclear studies on the parthenogenetic Marsilia but an interesting preliminary account has appeared announcing nuclear fusions in the apogamous development of Nephrodium (Farmer, Moore, and Digby, :03).

Parthenogenesis is now known in the spermatophytes for *Antennaria alpina* (Juel, '98, :00), several species of *Alchemilla* (Murbeck, :01a, :01b, :02; Strasburger, :04c), *Thalictrum purpurascens* (Overton, :02, :04), *Gnetum* (Lotsy, :03), a number of forms of *Taraxacum* (Raunkiaer, :03; Murbeck, :04), several species of *Hieracium* (Ostenfeld, :04a, :04b; Murbeck, :04), *Wikstrœmia indica* (Winkler, :05), and is suspected for *Ficus Treub*, :02) and *Bryonia dioica* (Bitter, :04). A number of cases of polyembryony were formerly considered examples of apogamy but are now known to be developments from the nucellus and consequently vegetative buds of sporophytic origin and entirely independent of gametophytic activities. The best known of these forms are *Funkia*, *Cœlebogyne*, *Citrus*, *Opuntia*, and *Alchemilla pastoralis*. Vegetative apogamy is illustrated in the development of embryos from antipodal cells as in *Allium odorum* (Tretjakow, '95; Hegelmaier, '97) or from the cells of the endosperm as in *Belanophora* (Treub, '98; Lotsy, '99). Synergids have been reported to form embryos in a number of forms but many of these have proved to be cases in which the synergid is fertilized by a sperm nucleus and not examples of apogamy. However, synergids are known to develop embryos apogamously

(or parthenogenetically if the antipodal be considered the homologue of an egg) in *Alchemilla sericata* (Murbeck, :02). A summary of the various types of vegetative apogamy, parthenogenesis, and sporophytic (nucellar) budding, supplementing a list of Ernst (:01) is given by Coulter and Chamberlain (:03, p. 221).

We will now take up the few investigations which consider the cytological details of parthenogenesis. That of Williams (:04b) on *Dictyota* is the only one treating of a lower type. It seems probable that parthenogenesis in *Dictyota* is in no sense normal and would not lead to mature plants, since the germination of unfertilized eggs in the cultures of Williams presented many irregularities. The spindles instead of being formed from asters with centrosomes are intranuclear in origin, multipolar, and very irregular in their form. As a result the 16 chromosomes become scattered and a cluster of daughter nuclei is formed containing varying numbers of chromosomes, sometimes one and sometimes several. It is clear in *Dictyota* that the fertilization of the egg results in the development of an aster with a centrosome which exerts a directive influence in mitosis preventing a scattering of the 32 chromosomes and conducting the mitosis in a normal fashion. Williams does not believe that the centrosome is introduced as an organized structure into the egg by the sperm but that it is formed *de novo* as a result of the increased metabolic activities present in the fusion nucleus as compared with that of the unfertilized egg.

There have been several important studies on parthenogenesis in the spermatophytes. Some of these papers while establishing the facts of parthenogenesis in various forms, give no details of nuclear history or behavior of the chromosomes. But the studies of Juel (:00), Overton (:04), and Strasburger (:04), present some very interesting data on the cytological features of parthenogenesis in *Antennaria alpina*, *Thalictrum purpurascens*, and several species of *Alchemilla*.

Several recent papers indicate that parthenogenesis may prove to be general in certain genera or even characteristic of large groups and therefore a far more widespread phenomenon than has been supposed. Raunkiaer (:03) (abstract in English in *Bot. Centralb.*, vol. 93, p. 81, 1903) proved by cutting off the

tops of young flowers that several species of *Taraxacum* produced normal seeds apogamously and concluded that the embryo must develop parthenogenetically since Schwere, in 1896, traced its origin from the egg. Ostenfeld (:04a, :04b) from failure to find pollen on the stigma of *Hieracium* and failure to make it germinate in a number of solutions, was led to try similar experiments to those of Raunkiaer in cutting off the anthers and stigmas of flowers. He found that a large number of species of *Hieracium* were able to set seed apogamously and he believed parthenogenetically but histological investigations were not made to establish the last point. The experiments of Raunkiaer and Ostenfeld are interesting as showing how a form by virtue of its parthenogenetic habits might become segregated and quite removed from the probability of hybridization. Murbeck (:04) in a short paper announced that the embryos in *Taraxacum* and *Hieracium*, developing from flowers whose stamens were cut out (as in the experiments of Raunkiaer and Ostenfeld) actually do develop from the egg cell and are therefore parthenogenetic. Murbeck also failed to find pollen tubes in the ovules where pollen had been applied to the stigma. Winkler (:04) reports that *Wikstrœmia indica* matures very little perfect pollen and produces its seeds apogamously, as proved by experiment. The embryos are stated to develop parthenogenetically from the egg but no details are given in this preliminary paper of the chromosome history. This group of contributions while very interesting, presents no data on the fundamental problems in a cytological explanation of parthenogenesis.

Murbeck (:01a) concluded for *Alchemilla* that true tetrads were formed previous to the differentiation of the embryo-sac but nevertheless found evidence that there were no reduction phenomena so that the nuclei within the embryo-sac contain the sporophytic number of chromosomes. Murbeck's evidence of tetrad formation was not satisfactory and in the light of recent studies of Strasburger (:04c) cannot be accepted. His view was, however, correct that there is no reduction of the chromosomes in the formation of such embryo-sacs as produced parthenogenetic embryos.

Juel (:00) gives a critical comparison of the development of

the embryo-sac in the parthenogenetic *Antennaria alpina* with *A. dioica* whose ovules are normally fertilized. In *A. dioica* the embryo-sac is one of a group of four cells (tetrad) which are formed through two successive mitoses (heterotypic and homotypic) showing the characteristic features of sporogenesis. A clear stage of synapsis precedes the first mitosis. The type of embryo-sac development in this form is then entirely normal. Not only are tetrads suppressed in the parthenogenetic *Antennaria alpina* but there is no trace of the heterotypic and homotypic mitoses in the embryo-sac. The number of chromosomes is very large (about fifty) and evidently the same as is found in other periods of the life history. There is then no reduction of the chromosomes during the formation of the embryo-sac in the parthenogenetic species and the egg and other nuclei in this structure have consequently the sporophytic number. There is no need of fertilization to bring the egg to a condition when with respect to chromosomes it is prepared to develop a sporophyte embryo. Juel (:04) notes certain peculiarities in the development of the embryo-sac of *Taraxacum officinale*. Tetrad formation is reduced to a single mitosis and this is not heterotypic, since there seems to be no reduction of the chromosomes. Details are not given.

Overton (:04) finds normal reduction phenomena in the pollen mother-cell of *Thalictrum purpurascens* which establishes the number of chromosomes to be 24 for the sporophyte and 12 for the gametophyte generations. These mitoses are thoroughly typical of sporogenesis being preceded by a synapsis stage. The development of the embryo-sac is of two types. In some cases a tetrad of four megaspores is formed from a megaspore mother-cell. The nucleus of this cell passes through a synapsis and the first mitosis is heterotypic showing the reduced number of chromosomes. The lower cell of the tetrad becomes the embryo-sac. But many embryo-sacs pass through a different history. There is no heterotypic mitosis and no reduction of the chromosomes which remain 24 in number. Thus in some ovules the mitoses of sporogenesis are omitted and true tetrads are not formed, with the result that the embryo-sac contains nuclei with the sporophyte number of chromosomes (24) in

place of the gametophyte (12). The details of the nuclear history in these embryo-sacs have not been followed but it is plain that their eggs have the requisite number of chromosomes to develop sporophyte embryos parthenogenetically. The varying proportions of parthenogenetically developed seeds which may be found on plants of *Thalictrum purpurascens* indicate that the suppression of normally developed embryo-sacs is not very firmly established in this form.

We now come to a recent paper of Strasburger (: 04c) which is the most important contribution to the subject of parthenogenesis that has yet appeared. Strasburger studied a number of species of *Alchemilla* from the section *Eualchemilla*, the group which formed the subject of Murbeck's important discoveries. Most of the forms develop pollen in a normal manner and Strasburger was able to follow reduction phenomena in this process without difficulty. The nucleus of the pollen mother-cell passes through a synapsis followed by a heterotypic mitosis in which the structure of the chromosomes as bivalent elements is apparent. The bivalent chromosomes are in the reduced (gametophytic) number. Similarly Strasburger found that some species (*e. g.*, *Alchemilla pentaphylla*, *gelida*, and *grossidens*) formed embryo-sacs in a normal manner with the presence of a tetrad and a characteristic reduction division (heterotypic). But the development of the embryo-sac in apogamous species (*e. g.*, *Alchemilla speciosa*, *splendens*, and *fallax*) cuts out the two mitoses of sporogenesis and no tetrads are formed. The nucleus of the megaspore mother-cell emerges from synapsis with the sporophyte number of chromosomes and the first division which follows is a typical mitosis and not heterotypic. The embryo-sac then comes to contain a group of nuclei with the sporophytic number of chromosomes in place of the gametophytic and a parthenogenetic development of the egg takes place. Strasburger regards the parthenogenetic tendencies of *Eualchemilla* as associated with excessive mutations among these forms through which sexual processes are becoming displaced by apogamous methods of reproduction.

This clear evidence that the cause of parthenogenesis in *Antennaria*, *Thalictrum*, and *Alchemilla* lies in the suppression

of chromosome reduction during the formation of the embryo-sac seems to offer an explanation of other examples of apogamy presented by the embryo-sac. Thus apogamous developments of embryos from synergids as in *Alchemilla sericata* (Murbeck, :02) or from antipodals as in *Allium odorum* will not seem strange if reduction processes are suppressed in the production of an embryo-sac and its nuclei retain the sporophyte number of chromosomes. Such nuclei have in them the same potentialities of development as do those of the nucellus whose cells form embryos vegetatively and entirely independent of gametophytic activities in a number of forms (*e. g.*, *Funkia*, *Cœlebogyne*, *Citrus*, *Opuntia*, *Alchemilla pastoralis*, etc.). This type of apogamy from a gametophyte which retains the sporophyte number of chromosomes may be found to hold a very close relation to apospory for there is the same reduction or omission of the processes of sporogenesis as is found in that phenomenon. However, since we know nothing of the cytological events of apospory it is unwise at present to follow the speculation further.

The peculiarities of parthenogenesis in the spermatophytes do not seem so remarkable since the discoveries recorded above. It is not strange that an egg should form an embryo without fertilization when its nucleus contains the sporophyte number of chromosomes. The most remarkable feature in this suppression of reduction phenomena in *Antennaria*, *Thalictrum*, and *Alchemilla* is the possibility of developing an embryo-sac with nuclei in the number and arrangement typical of the female gametophyte and yet with the sporophyte count of chromosomes. The embryo-sacs with their contents have clearly the morphology of female gametophytes and must be so considered in spite of the fact that their nuclei contain twice as many chromosomes as usual. It is clear that the potentialities of sporophyte and gametophyte involve other factors besides those of the chromosome count. This is a very important conclusion because we have been accustomed to lay great weight on the number of chromosomes as the cause of sporophytic and gametophytic developments respectively. We must recognize the presence of other factors determining alternation of generations besides the chromosomes.

There are two types of parthenogenesis in plants : (1) that in the thallophytes where there is no sporophytic generation, and (2) that in higher forms when the life history is complicated by an alternation of generation. We know nothing of the cytological conditions in the first group including such types as *Chara crinita*, *Cutlaria*, some species of *Spirogyra* and *Zygnema* and numbers of the lower Chlorophyceæ and Phæophyceæ whose motile gametes will germinate like zoöspores should they fail to conjugate with one another. But since there is no reason to suppose that there are reduction phenomena at gametogenesis, the unfertilized gamete is fully prepared with respect to the number of chromosomes to continue the parent stock. Dictyota must be excluded from this list since the parthenogenetic developments here are abortive. In the second group parthenogenesis is likely to prove to be the result of a suppression of reduction processes during sporogenesis by which a gametophyte generation retains the sporophyte number of chromosomes and in consequence is prepared to dispense with sexual processes in the development of a new sporophyte. Parthenogenetic development in animals seems to be similar in its essential cytological features to parthenogenesis and apogamy in plants. There may be a suppression of reduction processes somewhat comparable to that discussed above, which takes place, however, at the time of gametogenesis, whereby the egg nucleus retains the number of chromosomes characteristic of the parent. Or, through a fusion with the nucleus of the second polar body the egg nucleus is brought back to the normal condition with respect to the number of chromosomes of the parent stock. We cannot, however, consider in detail the forms of parthenogenesis in animals. They have been recently treated by Blackman (:04b) in comparison with conditions in plants.

Apogamous developments which involve wholly or in part other elements than gamete cells and nuclei are likely to be established in a number of groups of the thallophytes. The author has long believed that the cystocarps of some of the Rhodophyceæ develop apogamously, basing his conclusions on certain general peculiarities of the group and more particularly on a study of *Ptilota* (Davis, '96). Three species of this genus

were investigated and no developments from the carpogonia were found, but the cystocarp in all cases arose from a cell near the base of the group of procarps. These conditions together with the rarity of male plants on the American coasts (none have ever been reported) give strong evidence for apogamy in *Ptilota*. There are a number of genera of the Rhodophyceæ where similar conditions seem to obtain and which lead one to suspect that apogamy may not be very exceptional. However, the subject has been very little studied.

As is well known, the Ascomycetes furnish numbers of illustrations where ascogonia have not been found or appear in what seem to be reduced conditions and even when accompanied by so called antheridial filaments these latter have not been established as functional. De Bary recognized the possibility of apogamy in the development of the ascocarps of these forms and very little critical study has been given to them since his time. The trend of investigations in this group has been towards the more interesting problems of the establishment of sexuality in a few well known forms (*e. g.*, *Gymnoascus*, *Sphærotheca*, *Pyronema*, *Monoascus*, and among the lichens and *Laboulbeniaceæ*.)

It is generally believed that no sexual organs are present in the higher Basidiomycetes (*Autobasidiomycetes*). But the recent studies of Blackman (:04a) in the Uredinales, taken in relation to the well known nuclear fusions in the basidium, preceded by a mycelium containing paired (conjugate) nuclei, make it seem very probable that former sexual processes in the Basidiomycetes have been replaced by a remarkable type of apogamous development of a sporophyte generation. Blackman has traced the origin of the paired nuclei in the Uredinales (*Phragmidium*) to a structure preceding the æcidium, a structure which seems to be the remains of a female sexual organ. We will take up this investigation presently. There is then much reason for believing that a sporophyte generation in the Basidiomycetes arises apogamously in the creation of the paired nuclei and terminates with their fusion within the teleutospore or basidium.

The leptosporangiate ferns have furnished some of the best

illustrations of apogamy. Since Farlow's discovery in 1874 of an asexual sporophytic growth from the prothallus of *Pteris cretica* the list of apogamous pteridophytes has steadily increased until now the phenomenon is known in perhaps 25 forms. Farlow's investigation was followed by an extended study of De Bary ('78) on a large number of forms in the Polypodiaceæ and resulted in the establishment of similar sporophytic outgrowths in *Aspidium falcatum* and *Aspidium filix-mas cristatum*. De Bary proposed the term *apogamy* ('78, p. 479) for the general phenomenon and distinguished two forms, *apandry* the suppression of the male sexual organs which results in a parthenogenetic development of the egg, and *apogyny* for the suppression of the female. Sadebeck in the following year reported apogamy in *Todea* one of the Osmundaceæ (Schenk's *Handbuch der Botanik*, vol. 1, p. 231, 1879) thus extending the phenomenon to another family. And later apogamy was found in *Trichomanes alatum* one of the Hymenophyllaceæ (Bower, '88) and in *Selaginella rupestris* (Lyon, :04, p. 287).

The most important recent contribution on apogamy in ferns is by Lang ('98, abstract in *Annals of Bot.*, vol. 12, p. 251). This paper presents an able discussion of the phenomenon in its relation to alternation of generations and adds the very interesting discovery of sporangia borne directly on prothalli that were grown from spores. These sporangia were found in clusters on a thickened lobe or process from the prothalli of *Scolopendrium vulgare ramulosissimum* and *Nephrodium dilatatum cristatum gracile*. The sporangia were perfectly normal in structure and they matured spores. It is probable that the process is itself sporophytic in character, *i. e.*, made up of cells with double the number of chromosomes of the true gametophytic portion of the prothallus, but cytological details are not known. Lang's study of the apogamous development of sporophytic buds on several forms of the Polypodiaceæ is the most detailed work on apogamy in the pteridophytes yet published. The apogamous growths appeared as the result of cultures which were watered entirely from below and exposed to direct sunlight, important departures from normal conditions surrounding fern prothalli. In all cases the prothalli developed normal

embryos when the conditions permitted of fertilization. We shall refer to some general considerations of Lang in our summary and conclusions on apogamy.

The spermatophytes present some exceedingly interesting examples of apogamous developments of embryos from nuclei within the embryo-sac other than the egg, as from antipodals (*Allium odorum*, Tretjakow, '95; Hegelmaier, '97) or synergids (*Alchemilla sericata*, Murbeck, :02) or nuclei in the endosperm (*Belanophora*, Treub, '98; Lotsy, '99) but in these cases the sporophyte number of chromosomes is apparently present through a suppression of the reduction phenomenon of sporogenesis in the development of the embryo-sac.

We will now consider two studies which describe nuclear fusions preliminary to the appearance of apogamy (Blackman, :04a; Farmer, Moore, and Digby, :03).

Blackman's (:04a) observations on *Phragmidium* have cleared up to a great degree our understanding of the life history of the Uredinales. The chains of æcidiospores have been found to arise serially from "fertile cells" which form a group at the spot where an æcidium is to be developed. Each fertile cell has above it a sterile cell which, however, breaks down. The sterile and the "fertile cell" together may represent a female sexual organ, the sterile cell perhaps standing for the remains of a receptive structure similar to a trichogyne. The spermogonium consists of a large mass of antheridial filaments that abjoin sperms which are no longer functional. It is of course uncertain whether the "fertile cells" are morphologically the original female gametes since they may readily be other cells drawn into the process of apogamy. The "fertile cell" is stimulated to activity by the entrance of a second nucleus either from an adjacent hypha or from the cell below. The second nucleus does not fuse with the original nucleus in the "fertile cell" but the two come to lie close together as a paired or conjugate nucleus. The two nuclei of the pair divide simultaneously (conjugate mitosis) throughout a long series of nuclear divisions, beginning with the formation of æcidiospores and through the vegetative history which follows up to the production of the teleutospores where the members of the last pairs unite to form

the single fusion nuclei within these reproductive cells. There is much evidence that the period in the life history characterized by the presence of paired nuclei represents a sporophyte phase.

Blackman (:04a, p. 353) regards the process by which the second nucleus enters the "fertile cell," resulting in the conjugate nuclei, as a reduced form of ordinary fertilization. I have already pointed out in Section IV, "Asexual Cell Unions and Nuclear Fusions," what seem to me to be serious objections to the use of the term fertilization when it is clear that the second nucleus in the pair is morphologically not a gamete nucleus, and the subject was also taken up in the account of fertilization in the present section. Whatever may be the physiological interpretation of this remarkable phenomenon it seems to me clearly a substitute process for a former sexual condition and involves other elements than the original gametes and as such is a typical illustration of apogamy.

It seems probable that further studies in the Basidiomycetes will determine a similar origin for the paired nuclei preceding the basidium to that of Phragmidium but without any trace of former sexual organs at least in the higher groups. And these conditions must signify the complete disappearance of structures representing sexual organs and the substitution of an apogamous development of the sporophyte generation for the sexual act. In this connection the interesting nuclear fusions in the ascus are of great interest for they may hold relations to degenerate sexual conditions in the Ascomycetes.

Farmer, Moore, and Digby (:03) have reported some remarkable nuclear fusions preceding the apogamous development of the sporophytes of *Nephrodium*, which have many points of resemblance to the apogamous phenomena in the Uredinales just described. These authors find that cells of the prothallus from which the sporophytic outgrowths arise, become binucleate through the migration of nuclei from neighboring cells. The two nuclei may remain separate for some time or they may fuse at once. They regard the whole process "as a kind of irregular fertilization" by which the outgrowth destined to form the sporophyte becomes supplied with nuclei containing the double number of chromosomes. It seems to me unfortunate to asso-

ciate the term fertilization with this phenomenon, whatever may be the physiological significance of the nuclear fusions, because we are not dealing with gametes and there cannot be involved in the process anything of the long phylogenetic history of sexual differentiation in the group. We considered these matters in some detail in that portion of this section entitled "Fertilization."

With respect to the factors which determine apogamy it must be confessed that we are still in the dark. Lang's ('98) studies on fern prothalli, however, throw some light on the problem. In some twenty forms of the Polypodiaceæ apogamy resulted when the prothalli were kept from direct contact with the water (*i. e.*, were watered from below) and exposed to direct sunlight. When watered from above these same forms developed normal embryos from eggs. It is clear that the suppression of conditions which make fertilization possible (*i. e.*, water over the surface of the prothallus), possibly aided by sunlight which may cause irregularities of growth, induced the development of cylindrical processes from which the apogamous sporophytes arose and which bore sporangia in two forms. It seems hard to draw more precise conclusions from these experiments other than that the normal life history is checked at a critical period (fertilization) and the plant is forced into expressions of vegetative activity. The conclusions of Farmer, Moore, and Digby (:03) offer an explanation of how the developments may take on sporophytic characters through the fusion of nuclei in the tissues and the establishment of a sporophyte number of chromosomes.

Strasburger suggests that apogamy in *Alchemilla* may be the result of a weakening of sexual power associated with excessive mutative tendencies. This would seem to imply that exceptional vegetative activity, with the appearance of much variation under favoring conditions, may be combined with apogamy. It is of course a well known fact that a high degree of cultivation tends to lessen the fertility of a form unless guarded by careful selection. A weakened sexual fertility due to excessive vegetative activity is likely to be replaced by forms of vegetative reproduction. When the process of sporogenesis becomes so

reduced or modified that the female gametophyte retains the sporophyte number of chromosomes as in the embryo-sac of *Alchemilla* and *Thalictrum* the apogamous development of embryos is to be expected.

The discovery of apospory in such variable and perhaps mutating genera as *Alchemilla*, *Taraxacum*, and *Hieracium* suggests quite a new line of research with possibilities of a clearer understanding of the origin of mutations. It is very interesting that these widespread and successful genera should give evidence of such strong apogamous habits for it seems to indicate an evolutionary tendency in the higher plants of great significance. These forms with *Thalictrum* are representatives of three large, divergent and very successful orders (Ranales, Rosales, and Compositales) and it suggests the probability that apogamy will be found to be widespread in the spermatophytes. Its bearing on the establishment of extreme variations and mutations may be of the utmost significance for it is clear that the suppression of sexuality would remove sports and mutants at once from the swamping effects of cross-fertilization. The sudden appearance of mutants in some groups and their ability to hold true may indeed be found to rest on the establishment of apogamy in the form. This is at least a possibility which must be considered in cytological investigations on mutants and has not yet received attention.

The subject of apogamy touches another topic of importance, namely, the theory of *homologous* generations as contrasted with *antithetic* generations in comparisons of sporophyte with gametophyte. We shall not take up this discussion in detail here. It must have been apparent to the reader that the present treatment of the critical periods in the life history of plants is based on the conviction of the correctness of the latter view which has had the support of Celakovsky, Strasburger, Bower, Vaisey, and Klebs. The theory of *homologous* generations as held by Pringsheim and Scott is admirably discussed by Lang ('98) in connection with his studies on apogamy and also in a briefer note (*Annals of Bot.*, vol. 12, p. 583). Lang seemed inclined to the opinion that the facts of apogamy and apospory in ferns lent support to the theory of homologous generations since the

prothallus can so readily take on sporophytic potentialities and the sporophyte develop gametophytes vegetatively. But Lang recognized that the importance of this evidence would be minimized should it be found to depend on changes of nuclear structure. These nuclear changes have been established at least for apogamy, either in the suppression of the reduction phenomena of sporogenesis or by the substitution of asexual nuclear fusions for the sexual act, and the argument for antithetic alternation of generations seems to the writer stronger to-day than ever before.

#### 6. APOSPORY.

Apospory is the suppression of all processes of sporogenesis and the development of a gametophyte generation directly from the sporophyte. The term was first proposed by Vines (*Jour. of Bot.*, 1878, p. 355) in a discussion of the life history of *Chara* and adopted by Bower ('86, '87) in a general treatment of the subject based on Druery's ('86a, '86b) discoveries of prothalli developed in place of sporangia directly upon the leaves of *Athyrium filix-femina* and its variety *clarissima*. The phenomenon of apospory is best known among the ferns where it has been most extensively studied but so far no cytological investigations have been published. Since apospory results in the development of a gametophyte generation (presumably with the gametophyte number of chromosomes) without the preliminary process of sporogenesis it becomes a very interesting problem to know just how this reduction of the chromosomes is effected.

Apospory is probably not uncommon in the mosses and has also been reported for the liverwort *Anthoceros*. The independent studies of Pringsheim ('76) and Stahl ('76) established the facts that pieces of the sporophyte stalk (seta) of *Hypnum*, *Amblystegium*, *Bryum*, and *Ceratodon* when placed on damp soil developed a protonema which in its turn produced leafy moss gametophytes. Stahl also found in *Ceratodon* that protonemata may arise from the capsule wall and Brizi ('92) discovered a similar development from the atrophied capsule of *Funaria hygrometrica*. Correns ('99a, p. 421) has confirmed the conclu-

sions of Pringsheim and Stahl in species of *Funaria*, *Hypnum*, and *Amblystegium* and obtained negative results in a number of other forms, and presents an excellent review of the subject. Lang (:01) discovered that small pieces of the sporophyte of *Anthoceros lævis* when laid on damp sand produced green outgrowths which took on the structure of young gametophytes and developed rhizoids. These aposporous gametophytes most commonly arose from subepidermal cells, but they may come from any layer of the cortex down to the archesporial cylinder. It seems probable that the mosses at least among the bryophytes are able to reproduce themselves apogamously without difficulty, when normal processes of sporogenesis are interfered with and if the sporophytic tissue is in contact with moisture.

The leptosporangiate ferns, however, furnish the most conspicuous illustrations of apospory as they do of apogamy. Indeed, the two phenomena are known to occur in the same form in a number of instances (*e. g.*, *Athyrium filix-fœmina*, *Nephrodium filix-mas*, *Scolopendrium vulgare*, *Trichomanes alatum*, etc.). Beginning with the discovery by Druery ('86a, '86b) of apospory in *Athyrium filix-fœmina* and its variety *clarissima* the list has steadily grown until now apospory is recorded for about ten forms. In Druery's forms the prothalli developed from arrested sporangia and the spore alone is left out of the life cycle. But Bower ('86) very shortly brought forward in *Polystichum angulare pulcherrimum* a form in which prothalli are developed as simple vegetative outgrowths from the tips of the leaves and the life history is thus shortened by the omission of both spores and sporangia. This condition is exactly analogous to the development of protonemata from vegetative cells of the sporophytes of mosses and *Anthoceros*. The following year Bower ('87) presented a very full account of the forms of *Athyrium* and *Polystichum* just described, and a general discussion of the phenomenon of apospory. Bower ('88) then extended the illustrations of apospory to two species of *Trichomanes*, of the Hymenophyllaceæ; Farlow ('89) reported it for *Pteris aquilina*, and Druery ('93) in *Lastrea pseudo-mas cristata* and ('95) for *Scolopendrium vulgare crispum*. The exceptional amount of fern variation both in nature and under cultivation has not been

generally appreciated and the studies on apospory and apogamy indicate that much of it is associated with these fundamental modifications of the life history (Druery, :01).

As to the cause of apospory we are as much in the dark as in the case of apogamy. The phenomenon is clearly associated in some forms with disturbances in the normal vegetative life of the sporophytes. This is particularly true in the cases of mosses and *Anthoceros* and has been suggested for the ferns. Thus aposporous developments in *Pteris aquilina* are from leaves which are generally smaller than the normal and whose margins are curled so that the leaf often appears somewhat withered and is easily recognized at a distance. Bower ('87, p. 322) is inclined to regard the phenomenon in the ferns as a sport and does not consider that it has deep morphological significance or that it offers serious difficulty to the acceptance of the theory of an antithetic alternation of generations.

As we have stated there have been no cytological studies upon apospory but there seem to be two possible explanations. That which is likely to suggest itself first calls for reduction phenomena at the time of the aposporous development by which the nuclei of the sporophytic tissues may come to contain the gametophyte number of chromosomes and are therefore capable of developing the sexual generation. But there is another possibility which has not yet been considered. We know for several of the spermatophytes (*Antennaria*, Juel, :00; *Thalictrum*, Overton, :04; *Alchemilla*, Strasburger, :04c) that the processes of sporogenesis may be suppressed and yet a structure be developed with the morphology of the gametophyte generation. Thus the embryo-sac will contain the usual number of nuclei grouped in the typical manner but these nuclei still have the sporophyte count of chromosomes. It seems probable then that the development of a gametophyte may result through an interference with the normal life history and under conditions favorable to the gametophyte even though the nuclei retain the sporophyte number of chromosomes. And it is possible that some of the aposporous developments in bryophytes and pteridophytes may be of this character. It is quite futile at present to carry this speculation further. What is desired is some cytological facts.

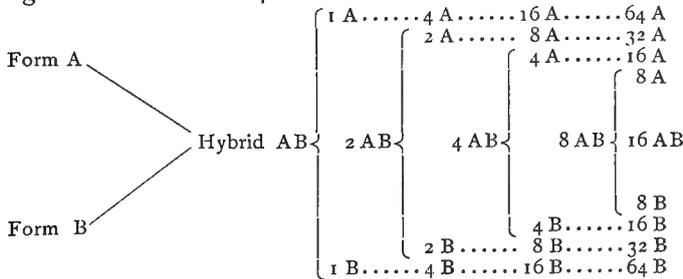
## 7. HYBRIDIZATION.

This is not to be a detailed discussion of the facts and theories of hybridization, a subject far too extensive for the purposes of our treatment. We shall only consider some of the bearings of the recent studies on fertilization and reduction phenomena upon the problems of hybridization treating it as a critical phase in the life history of the organisms concerned. Until recently the attempts to formulate definite laws for the formation of hybrids and their progeny upon a physical basis have not been satisfactory. But the work of a number of breeders all of whom owe their results in large part to a quick appreciation of Mendel's epoch-making contributions have brought much order out of what was a very confused subject. And accompanying the work of this group must be added the equally important conclusions of a number of cytologists whose investigations on the structure and behavior of nuclei in the critical periods of fertilization and chromosome reduction have done much to place Mendelian principles upon a cytological basis. We shall deal with the work of the latter group, for their contributions concern intimately the subject matter of these papers.

We shall not review the conclusions of Mendel except to point out the relations of some of his principles to cytological phenomena. The two papers of Mendel appeared in the proceedings of a natural history society of Brünn, Austria, under the dates 1865 and 1869. They lay buried until 1900 when De Vries, Correns, and Tschermak independently rediscovered them and called the attention of the scientific world to their worth. Soon after, Bateson published a translation of the two papers (*Mendel's Principles of Heredity*, Cambridge, 1902) with an introduction and a defense against the criticisms of Professor Wheldon. There have naturally been many reviews and short discussions of Mendelian theories and among them that of Castle entitled "Mendel's Laws of Heredity" (*Science*, vol. 18, p. 396, 1903) and Professor Bailey's "Lecture IV" in *Plant Breeding*, 1904, will perhaps give the reader the clearest and most concise statements.

The most striking feature of Mendel's investigations and those

of others, who have confirmed his conclusions, is the discovery in a number of animals and plants that the germ cells of the hybrid may be pure with respect to certain characters of the parents which are crossed. This principle is not without exceptions where the conditions are apparently complicated by unusual factors but the phenomenon when present is so striking as to command immediate attention and call for an explanation on a cytological basis. The purity of the germ cells of hybrids means in the words of Castle that "the hybrid, whatever its own character, produces ripe germ cells which bear only the pure characters of one parent or the other." Thus if two forms A and B are crossed the hybrid will have embodied in itself the characters AB, one of which however may lie latent, *i. e.*, may not be visible in the hybrid. Such a latent character when present is termed recessive while the prominent character is termed dominant. In a simple case some of the offspring of the hybrid AB will be found to have the character of A alone, some of them of B alone, and some of them will again have the mixed characters AB. If experiments are carried out on an extensive scale the proportions of these offspring from the hybrid may exhibit the remarkable fact that there are about twice as many forms of AB as either A or B, *i. e.*, the proportions of A's, AB's, and B's are in the ratio of 1 : 2 : 1. Furthermore the offspring of A when bred among themselves remain absolutely true producing a succession of pure forms all A's and the same results follow when the offspring of B are closely bred. But when forms with the mixed characters AB are bred with one another their offspring break up as before into three types A, AB, and B in numerical proportions expressed by the same ratio 1 : 2 : 1. The history is simply told in the following diagram where the number of offspring is assumed to be 4.



This remarkable proportion of forms derived from the hybrids AB, *i. e.*, A, AB, and B in the ratio 1 : 2 : 1 can only be explained on the assumption that the germ cells of the hybrid are pure with respect to the characters of either one or the other of the parents. The gametes from the hybrid, with the pure characters of either A or B and approximately equal in number, may unite with one another in three possible combinations AA, AB, or BB forming three types of offspring, one pure A, another mixed AB, and the last pure B. By the law of chance the proportions of these combinations (AA, AB, and BB) in a simple case will be in the ratio 1 : 2 : 1. This assumption of the purity of the germ cells of hybrids has been found to conform with the facts in a number of simple experiments where two characters such as A and B were sharply contrasted. When one of the characters in the hybrid is dominant and the other recessive the ratio can be expressed as D : DR : R as 1 : 2 : 1 which is merely a substitution of D and R for the characters A and B.

There are of course many factors which tend to modify the ratios as stated above and complicate the results. Thus the normal number of gametes may be of varying vigor and mortality so that there will be proportionately more or less of one type of fusion than is called for by the law of chance. Sometimes the characters of the parents remain evenly balanced in the hybrid and refuse to split up in the succeeding generations, remaining in a stable union in the germ cells produced by the hybrid. Such conditions prove exceptions both to the law of dominance and to that of purity of the germ cells. From these exceptions and particularly the last it is difficult to believe that any large proportion of the germ cells is *absolutely* pure, *i. e.*, bearing only the pure characters of one parent or the other. However, there is much evidence from our knowledge of the distribution of the chromosomes from one generation to the next, that certain relations are possible in the separation of germ plasm which approximate the ratios of Mendel's law and while rarely giving *absolutely* pure germ cells nevertheless do make possible a large proportion of *relatively* pure cells.

Let us examine now the chromosome history as a possible physical basis for the Mendelian principles. Such considerations

must rest on the assumption of what is termed the individuality of the chromosome. This means that the chromosome is believed to be a permanent organ of the cell which never loses its organic entity although the form may be frequently obscured, as in the resting nucleus, and which reproduces by fission during mitosis. We have given in other connections the evidence upon which the above view rests, evidence accumulated from the studies of the critical periods of gametogenesis, fertilization, and sporogenesis (with its reduction phenomena) in plants and of gametogenesis and fertilization in animals. All investigations indicate that paternal and maternal chromosomes maintain complete independence in the sexually formed cell or fertilized egg and in the mitoses of cleavage so far as these have been followed. Also, descendants of the chromosomes which became associated with fertilization have been recognized by their form at the end of the life history during the reduction phenomena of gametogenesis in certain animals (Sutton, :02, :03; Montgomery, :04) and of sporogenesis in the hybrids of *Drosera* (Rosenberg, :04a, :04b). Furthermore, the entire history of chromosome reduction in both animals and plants finds a satisfactory explanation only in the belief that descendants of maternal and paternal chromosomes are distributed as organic entities by the peculiar mitoses of this period.

There is a general agreement that the somatic chromosomes of animals and the sporophytic of plants become grouped in pairs to form bivalent structures before the heterotypic mitosis of the reduction division whether this be present in the primary gametocyte (animals) or the spore mother-cell (plants). The bivalent chromosomes (pairs of chromosomes, dyads) may become transformed into tetrads before the heterotypic mitosis by a division of each chromosome in the pair, as is characteristic of animals, or this division may be delayed until a somewhat later period during the heterotypic mitosis, as in plants. We are not concerned now with the dispute as to how the pairs of chromosomes come to lie side by side to form the bivalent structure or how tetrads are developed, activities which may indeed be various in different types and which will only be understood by a greater body of observations than we have at present (see dis-

cussion of "Reduction of Chromosomes"). The important point for us is the belief that the appearance of the bivalent chromosomes during reduction is due to the temporary union of somatic or sporophytic chromosomes in pairs and further that the reducing divisions distribute the members of the pair, which are believed to be descendants of the maternal and paternal chromosomes of the previous generation, as organic entities to the generation which is to follow.

It is difficult to overestimate the importance of this generalization. If the program prove to be correct as stated above and if the chromosome is established beyond doubt as a self-perpetuating organ of the cell and a bearer of hereditary characters we have then the possibility of studying the actual manner in which these structures are passed on from one generation to the next and perhaps determine the ratios or combinations through which the distribution is effected. The difficulty of making an exact determination of ratios in any form so far studied lies in our inability to distinguish the chromosomes of maternal and paternal origin. There is much evidence that the pairs of somatic and sporophytic elements, which form the bivalent chromosomes of the reduction mitoses of animals and plants respectively, are of different parentage but we do not know whether or not there is any rule in the arrangement of the pairs on the spindles of these mitoses although this is hardly to be expected. Cannon (:02, :03a) and others have held that the mitoses of reduction brought about the complete separation of the maternal and paternal chromosomes so that two of the resultant four nuclei contain chromosomes from one parent and two from the other, and the germ cells are in consequence *absolutely* pure in character. But this view was soon shown by Sutton (:03, p. 233; accepted by Cannon, :03b) to be at variance with the facts of breeding for if germ cells of hybrids are *absolutely* pure there could be no further change by cross-breeding and the first cross would be repeated over and over again without any divergence from the type, which is contrary to experience and fact. The pairs of chromosomes are probably arranged in every possible order and the maternal and paternal elements are distributed in every possible combination by the reducing

divisions. If this is true then by the law of chance the proportions of germ cells of the hybrid which are *absolutely* pure (containing chromosomes entirely from one parent) would be small. Likewise there would be a small proportion of germ cells in which the paternal and maternal chromosomes are equally distributed. And in contrast to these two groups the great majority of germ cells would have a marked preponderance of chromosomes derived from one parent or the other and this condition may be termed one of *relative* purity.

We shall now summarize the cytological evidence for the conclusions of the paragraph above, first with respect to the actual distribution of the somatic and sporophytic chromosomes as entities during the mitoses of reduction, and second as to the probability of the bivalent chromosomes consisting of a pair of maternal and paternal elements. The evidence on the first point has been treated as regards plants in our own account of "Reduction of the Chromosomes" and need not be repeated. With respect to the possibilities of distinguishing maternal and paternal chromosomes throughout a life history and especially at the period of chromosome reduction we must consider briefly the remarkably favorable studies of Sutton, Montgomery, Moenkhaus, Baumgartner, and Rosenberg.

Sutton (:02, :03) discovered in the "lubber grasshopper" (*Brachystola magna*) a form in which the somatic chromosomes, 23 in number, are markedly different in size, presenting a graded series with respect to pairs in which the two elements are approximately equal. There are then 11 types of chromosomes in two groups, a pair of each type, and in addition an accessory chromosome which remains apart from the rest in a special vesicle of its own. These two sets of 11 chromosomes appear with regularity throughout the mitoses leading up to the reduction divisions of spermatogenesis. Previous to the reducing divisions the chromosomes of each pair become closely associated end to end so that 11 threads appear which form 11 bivalent chromosomes (dyads) that later become tetrads through the division of each chromosome in the pair. Sutton concludes that the somatic chromosomes which make up each bivalent structure conjugate during synapsis and that the transverse fission which

appears during the formation of the tetrad simply separates the two somatic chromosomes of the pair, while the longitudinal fission is the usual division of chromosomes, appearing prematurely at this time. The conclusion is natural that the two series of the 11 pairs consist of maternal and paternal chromosomes which are distributed as organic entities by the reducing divisions. But there are no reasons for supposing that all of the paternal chromosomes will pass into one set of germ cells and all of the maternal into another but rather that the ratios of distribution will be by the law of chance according to which the great majority of germ cells will have a marked preponderance of chromosomes from one parent or the other, and will therefore be *relatively* pure. An exceedingly small proportion of germ cells may, by the law of chance, contain chromosomes entirely of maternal or paternal extraction, and an equally small proportion may contain 6 chromosomes of one parent and 5 of the other. The accessory chromosome divides but once during the mitoses of spermatogenesis so that two of the spermatozooids have 11 chromosomes and two 12. No accessory chromosome appears in the mitoses of oögenesis indicating that the female insect lacks this structure which confirms the belief of McClung (:02) and others that the accessory chromosome is a determinant of the male sex.

Montgomery in a series of studies upon insects and Amphibians, which are summarized in a recent paper (:04), reached conclusions in striking support of the theories of the individuality of the chromosomes, the association of pairs of chromosomes during synapsis to form bivalent structures and the probability of the elements of each pair (bivalent chromosomes) being of maternal and paternal origin respectively. His results on the last point are of especial interest in relation to hybridization. In a large number of insects, chiefly Hemiptera, Montgomery has found pairs of chromosomes, which he terms heterochromosomes, much smaller or much larger than the others and these may be followed through mitosis from one nucleus to another. The heterochromosomes of each pair are known to unite with one another during synapsis to form the bivalent chromosomes of the reduction mitoses and they then become separated, each

dividing once, so that every germ cell receives a single heterochromosome of whatever sort. Fertilization then brings the heterochromosomes together again in pairs until the next period of chromosome reduction. This history is then parallel to Sutton's account of the lubber grasshopper (*Brachystola*), the difference being that the latter form presents a remarkably graded set of paired chromosomes. Montgomery regards the small chromosomes and the accessory chromosome as structures tending to disappear in a process of evolution from a higher chromosomal number to a lower.

Moenkhaus (:04) crossed reciprocally two species of fishes (*Fundulus heteroclitus* and *Menidia notata*) and obtained hybrid embryos which reached an advanced stage of development. The chromosomes of the parents are readily distinguished by size and form. These chromosomes were followed throughout the development of the hybrid embryo and were found to retain their peculiarities so that the two sets may be easily separated in favorable tissues. This investigation furnishes some of the strongest evidence of the individuality of the chromosome and the complete independence throughout the life history of the two sets derived from each parent. Could these hybrid embryos be raised to maturity we should expect to find during spermatogenesis and oögenesis an association of the chromosomes in pairs, those of paternal extraction with those of maternal to form the bivalent chromosomes preliminary to the reducing divisions, and a distribution to the sexual cells in varying proportions which would, however, give a very large ratio of *relatively* pure germ cells.

Baumgartner (:04) in studies upon spermatogenesis in the cricket (*Gryllus*) was able to distinguish the chromosomes by their form, following them through the mitoses of reduction. Most of the chromosomes have the form of straight or bent rods but there are apparently two rings in each set in *G. domesticus*. The variation in the form of chromosomes in the nucleus is well known but it has not been supposed that a definite form might be characteristic of an element and be maintained throughout the successive mitoses of a life history as seems probable from Baumgartner's results.

Rosenberg's (:04a, :04b) studies on hybrids of *Drosera rotundifolia* (with ten chromosomes in the gametophyte) and *D. longifolia* (with twenty chromosomes) offer clear evidence that the chromosomes which unite in pairs to form bivalent structures preliminary to the reduction phenomena of sporogenesis are of different parentage. The sporophyte number of chromosomes in the hybrid is thirty, as would be expected. The reduced number appearing at the first mitosis of sporogenesis is, however, not fifteen but twenty chromosomes, ten of which are plainly double the size of the other ten. The explanation of this interesting condition is that the ten chromosomes of *D. rotundifolia* unite with one half of the twenty chromosomes of *D. longifolia* giving ten large bivalent structures accompanied by the ten chromosomes of *D. longifolia* which are without mates. This explanation finds clear support in the facts that the chromosomes of *D. rotundifolia* are larger than those of *D. longifolia* and that the bivalent structure consists of a larger and a smaller element thus giving clear evidence that the pairs of chromosomes which unite in *Drosera* are of different parentage. The single chromosomes which are without mates may pass to one or the other of the poles of the spindle or may be left behind when the daughter nuclei are formed.

This group of investigations illustrates very clearly the character of the evidence that is leading many biologists to assign to the chromosomes the functions of bearing and distributing hereditary characters. The question at once comes up as to whether or not the chromosomes may differ among themselves to a greater or less extent even in the same species or individual. Montgomery, Sutton, with others, have established a difference in the *size* of chromosomes. Baumgartner distinguishes differences in *form* in the same species and the studies of Moenkhaus and Rosenberg have shown that the chromosomes of different parents may retain their peculiarities of *form* in hybrids and be really separated. To these investigations should be added the recent conclusions of Boveri (:02, :04), that chromosomes actually differ in *function*. Boveri found that the chromosomes of eggs of echinoderms that were fertilized by two or more sperms are distributed by multipolar spindles to a varying number of

blastomeres which in consequence received a varying number and assortment of chromosomes. Boveri then separated these blastomeres and followed their independent development into larval stages which exhibited marked differences in form that could be correlated with the irregularities in the number of chromosomes contained in each, thus suggesting that specific chromosomes have specific functions. With this sort of evidence accumulating from both the morphological and physiological side it is not surprising that many biologists believe that specific characters are actually held or are controlled by chromosomes or groups of chromosomes.

Such views of course presuppose that the chromosomes retain a high degree of independence of one another and that variation is expressed chiefly through different combinations of chromosomes and not by modifications of the chromosomes themselves. Yet there is strong evidence of an actual mixing or interchange of the idioplasm among the chromosomes. This possibility which is of course contradictory to the view of the *complete* independence of the chromosomes finds its chief support in the close association of the pairs of chromosomes with the organization of the reduced number of bivalent structures during synapsis. These pairs have been reported so intimately united as to be actually fused. Allen (:05) has described for *Lilium* the union of two sets of chromomeres, one believed to be derived from a paternal spirem and the other from a maternal, which come to lie side by side during synapsis and unite to form a spirem with a single series of fusion chromomeres. This single (fusion) spirem later splits longitudinally and the two halves are regarded as again representing maternal and paternal spirems but there are evidently opportunities during the period of fusion for significant reciprocal interaction between the two idioplasms. This conception of the fusion of idioplasm from the two parents is an old view which has been held by such well known biologists as Hertwig and Strasburger.

De Vries (:03) has recently discussed the significance of the pairing of chromosomes before the heterotypic mitosis in relation to the theory of pangenesis. He conceives the paternal and maternal chromosomes as coming together during synapsis in

homologous pairs so that corresponding pangenes or groups of pangenes are brought together and that there may be a mutual interchange or transfer of idioplasm with the result that the chromosomes after separating may contain a mixed set of pangenes although each is supposed to have a complete assortment. The interchange makes possible all forms of combinations of the pangenes in the two sets, according to the laws of chance, which might be expressed in proportions that would approximate in some cases the ratios of Mendel. If the parents are widely different from one another their idioplasm may not correspond sufficiently to make possible this union and interchange of pangenes so that the process is suppressed and the hybrid is sterile.

Allen (:05, p. 247) points out that the union of two spirems during synapsis with the fusion of two sets of chromomeres, according to his account of the lily, offers a number of possibilities with respect to the constitution of idioplasm following the reduction mitosis. (1) There may be such a fusion of elementary units that a single idioplasm is formed different from either parent which would of course be distributed equally to the reproductive cells by the subsequent double longitudinal fission of the single (fusion) spirem. This would be expected to give hybrids of much the same form in every instance and these would remain stable (constant). (2) There may be a greater or less mixing or modification of units but without the actual union and formation of a new idioplasm in the hybrid. Then by the splitting of the single (fusion) spirem there might result a distribution of the mixed idioplasm following ratios or proportions approximating Mendel's law. (3) There may be in part a fusion and in part a mixing of idioplasm which would be expected to result in a varied combination of parental characters in the offspring. (4) While the chromosomes may be distributed according to ratios similar to Mendel's principles their respective characters may be greatly modified by their temporary union during synapsis. (5) Portions of the idioplasm may interact upon one another so that when they are separated by the reduction mitoses their character has become variously modified. (6) Finally, Allen, of course, recognizes the possibility that parental

idioplasm may be separated so purely by the longitudinal splitting of the single (fusion) spirem or through the distribution of unmodified sporophytic or somatic chromosomes as to give *absolutely* and *relatively* pure germ cells through Mendelian laws.

Allen's discussion, very briefly summarized above, is important for the emphasis which is laid upon the significance of a possible mixing of the parental idioplasms in the more or less complete union of chromatic material, which is generally recognized as characteristic of synapsis. There is a general tendency to rest content when the chromosomes are accounted for as units while they are merely the grosser form of expression of the idioplasm whose final architecture is intricate far beyond our present powers of analysis. Allen's own studies upon the events of synapsis in the lily with the regular fusion in pairs of chromomeres of different parentage may well cause one to hesitate in a full acceptance of the chromosome as fixed and unchanged in its organic constitution throughout the life history. The phenomenon of hybridization is far too complex to be explained in terms of simple ratios and while some characters may be paired or correlated in proportions that can be expressed by mathematical formulæ there is little probability that the assemblage of characters which make the species can be so definitely grouped as the strongest disciples of Mendel may hope. However, a great forward step has been taken and we may expect important results from the empirical methods so clearly defined by Mendel and by the close investigation that cytologists are making of the history of idioplasmic structures (chromosomes) during ontogeny.

#### 8. XENIA.

Xenia is the "immediate or direct effect of pollen on the character of seeds and fruits." The term was first proposed by Focke, in 1881, and is now well established. Xenia has long been known to the plant breeder as one of the most interesting and puzzling problems of hybridization. The botanist has naturally looked for the results of hybridization in the development of the embryo from the seed since this structure has received

the substance of the sperm nucleus of the male parent. But facts have clearly shown that the pollen may also affect the structure of the endosperm in the seed as well as cause the development of the embryo. Since the endosperm holds no genetic relation to the embryo it has seemed very remarkable that it should take on hybrid qualities. It has also been claimed that other regions of the seed or fruit, such as portions of the pericarp were also affected, but it is doubtful whether this is really so or at least whether such changes are truly a feature of the protoplasmic structure and thus deeply seated in the organism as a feature of hybridization.

It is only within recent years that a satisfactory theory has been suggested for the influence of pollen outside of the embryo. And this explanation rests on the discovery of the activities of the second sperm nucleus which enters the embryo-sac and which is known in some cases to unite with the polar nuclei constituting a triple nuclear fusion within the sac that is generally known as "double fertilization." We have briefly referred to the phenomenon in the latter part of the account of "Asexual Cell Unions and Nuclear Fusions" in Section IV and shall take it up now in greater detail. The best account of xenia is a very clear treatment by Webber, in 1900.

The explanation of xenia upon the facts of "double fertilization" was proposed almost simultaneously by De Vries ('99, :00), Correns ('99b), and Webber (:00). Double fertilization was first observed by Nawaschin ('98) in *Lilium* and *Fritillaria* and shortly after was described in greater detail by Guignard ('99b) in other species of the same genera and in *Endymion*. Since these discoveries the phenomenon has been reported by a number of investigators in many other forms representing widely divergent groups in the Monocotyledonæ and Dicotyledonæ and there is every reason to believe that it is widespread in the angiosperms. A review of the recent literature is given by Coulter and Chamberlain (*Morphology of the Angiosperms*, 1903, p. 156). There is no fixed order in the events of the triple nuclear fusion of "double fertilization." The polar nuclei may have united at the time when the pollen tube enters the embryo-sac, in which case the second sperm nucleus coalesces with an organized fusion

endosperm nucleus. Or, the two polar nuclei and the sperm nucleus may all three fuse together practically simultaneously. And again the sperm nucleus may unite first with one of the polar nuclei and the second be drawn later into the triple fusion. But no cases seem to have been reported in which but one polar nucleus unites with the sperm leaving the other free although such a combination may be expected. Also, no one has observed an independent division of the sperm nucleus within the endosperm, although as we shall see, there are reasons for believing that such a development may sometimes take place.

We have already given in Section IV the reason why these triple nuclear fusions may be kept apart from sexual phenomena since we have no knowledge of the phylogenetic history of the processes involved. It seems best at least for the present to regard the phenomenon as a special development associated with the peculiar and highly specialized conditions within the embryo-sac. This detailed and highly difficult problem of phylogeny has no especial bearing on the physiological features of xenia with which we are at present concerned.

The best understood examples of xenia are found in the hybrids of maize and are clearly described in the very interesting paper of Webber (:00). As is well known, some of the varieties of corn are distinguished among other characters by the color of the kernels, which are blue, red, yellow, and white, and also by the surface, which is smooth in the starchy corns (flint or dent) and wrinkled in the sugary sweet corns. When well marked pure races are grown out of reach of chance cross-pollination, the offspring remain true to their seed characters but it has long been known that the varieties of corn hybridize very readily so that when grown close together the ears will very frequently present seeds mixed as to color and texture. Thus when exposed to cross-pollination a corn which is characteristically yellow or white may bear blue or red kernels or a form with wrinkled and starchy kernels may develop smooth starchy corn if varieties with these characters are in the vicinity. The color character is known to lie in these examples in the outer layer of the endosperm (aleurone layer) and of course the food material whether prevailing starch or sugar, which gives the

surface a texture smooth or wrinkled, is stored within the endosperm.

The clearness of xenia in the maize has led to a number of careful studies on cross-pollination beginning with the work of Vilmorin (1866), Hildebrand (1867), and Friedrich Körnicke (1872). The possible explanation of xenia in maize through "double fertilization" which introduces qualities of the male parent from the pollen into the endosperm was suggested by experiments of De Vries on hybridizing maize in the summers of 1898-99 and Correns and Webber in 1899. De Vries ('99, :00) pollinated a wrinkled-seeded sugar corn from a variety of smooth starchy corn and obtained smooth starchy kernels which when cultivated in the succeeding summer were found to be true hybrids. He concluded that this furnished experimental proof that the endosperm of the sugar corn was affected by the entrance of a sperm nucleus from the starchy variety according to the theory of "double fertilization" proposed by Nawaschin ('98).

Correns ('99b) in the same year expressed similar conclusions in a clear statement of the theoretical aspects of the problem of xenia as found in *Zea mays*. Correns advanced a number of propositions some of which should be noted for their speculative interest. Thus he states (proposition 7) that the influence of the new pollen (*i. e.*, from the male parent of the hybrid) is expressed as xenia only in the endosperm and (proposition 8) only in the pigment present or the chemical nature of the reserve material whether starchy or sugary. If the two races differ only in the presence of one character, as in the color of the aleurone layer, that character is only found in xenia when brought by the pollen (proposition 10). Xenia is then only expressed in a hybrid (proposition 14) by the formation of a pigment which the race of the female parent does not possess or of a more complicated chemical compound (such as starch) in place of a simpler (as dextrin). Correns (:01) later presented in a lengthy paper, beautifully illustrated, the full results of his studies on xenia in maize with a discussion of the hybrids.

Webber (:00) also simultaneously with De Vries and Correns conducted extensive experiments in hybridizing a number of

varieties of corn distinguished by the color of the kernels, which were white, yellow, red, or blue and by the texture whether smooth, hard, and starchy (dent or flint corn) or wrinkled and sugary (sweet corn). The results of his investigation are admirably presented with excellent illustrations. He found that the smooth kernel and starchy endosperm of the dent and flint corn were transmitted very conspicuously as *xenia* when these forms were employed as the male in crossing with the sweet corns whose kernels are wrinkled and sugary. The characters of the sweet corns do not seem to be expressed as *xenia* when smooth, starchy, dent corn is used as the female member of the hybrid. This experiment would seem to support Correns' proposition number 14 that a more complicated compound is always formed in *xenia* in place of a less complex. But Webber found that flint corn, which is smooth and starchy, when pollinated with a form of sweet corn developed the wrinkled kernel and sugary type of endosperm of the male member indicating that this rule of Correns is not universal. And McClure ('92) obtained similar results in crossing a white dent race with pollen of Black Mexican which is a sugar corn with black kernels. The product in this case showed *xenia* clearly in having the wrinkled blue-black kernels of the male sugar corn.

Some of Webber's most striking results were obtained in pollinating yellow and white corns with blue-black and red races. The color was transmitted as *xenia* in a most striking manner. Webber agrees with other authors that the color is only present in the endosperm of the kernels. Thus the red of certain dent corn, which lies in the pericarp, is not passed on as *xenia* and McClure observed the same facts in experiments with cranberry corn whose color lies in the seed coat and is not transmitted when employed as the male member in crossing with white corns. Webber's experiments show, as do those of other investigators, that the absence of color in the kernels of the male parent does not seem to affect the tint of the kernels when the female is markedly colored, in agreement with Correns' proposition number 10. But Webber is not convinced that some influence might not be exerted on colored corn when pollinated from races with colorless endosperm, because of certain experiments on variegated *xenia* which will be described presently.

These experiments of De Vries, Correns, Webber, and others have established experimentally the facts of xenia and Nawaschin's theory of double fertilization seems to offer the only explanation of the phenomenon. But it was left to Guignard (:01) to make the concluding observation that a second sperm nucleus does actually enter into the composition of the endosperm of maize, and this fact clinched the argument which up to this time had been a speculation.

Webber has made a very important addition to the theory of "double fertilization" as an explanation of xenia in some observations and speculations on a mottled condition which is sometimes present when white corns are pollinated by colored. He found that the color was sometimes only transmitted in spots as when Hickory King was pollinated by Cuzco, or perhaps only half a kernel may be colored. Webber offers the hypothesis that the second sperm nucleus may enter the embryo-sac but instead of uniting with the two polar nuclei to form a triple fusion may itself divide separately and thus gives rise to a progeny very different from the other endosperm nuclei. There might then be two sets of nuclei in the endosperm one of which is composed of nuclei which would come directly from the male parent. These latter then might become distributed throughout the embryo-sac but would tend to remain in groups as multiplication progressed and would certainly be expected to influence the character of the tissue which is formed later when the walls are developed around the free nuclei. As Webber expresses it, there might be formed islands of tissue in the endosperm whose cells contain nuclei derived directly from the second sperm and such tissue would be expected to show characters of the male parent in spots as xenia. Again, if the sperm nucleus should unite with only one of the polar nuclei and the other should give rise to an independent progeny we should expect similar mixed conditions in the endosperm, with xenia only expressed in the areas dominated by nuclei containing material derived from the sperm.

There have been reported illustrations of xenia in tissues outside of the endosperm but we are fully justified in awaiting their confirmation before accepting them, especially since some

have failed to stand the test of critical investigation, in the light of the present theory. Thus certain investigators have reported xenia in the color of the seed coats of certain peas. But Giltay ('93) in a series of experiments found no instance where color was transmitted to these tissues. The pigments in these plants lie in the cotyledons of the embryo which of course are readily visible through the thin coats of the seed. While the present theory of xenia is very recent and has been critically applied in few forms, it seems thoroughly satisfactory in every particular with no clearly established evidence against it.

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# STUDIES ON THE PLANT CELL.—VIII.<sup>1</sup>

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## SECTION VI. COMPARATIVE MORPHOLOGY AND PHYSIOLOGY OF THE PLANT CELL.

WE shall devote this section to the discussion of a number of topics some of which have received brief mention in the preceding papers of the series but with other subjects will now be considered in some detail. The material will be treated under the following five headings:—

1. The simplest types of plant cells.
2. Comparisons of the structures of some higher types of plant cell with simpler conditions.
3. Some apparent tendencies in the evolution of mitotic phenomena.
4. The essential structures of the plant cell and their behavior in ontogeny.
5. The balance of nuclear and cytoplasmic activities in the plant cell.

### I. THE SIMPLEST TYPES OF PLANT CELLS.

There are three groups of plants which are conspicuous for the simplicity of their cell structure. They are: the Cyanophyceæ (blue-green algæ), Schizomycetes (bacteria), and the Saccharomycetes (yeasts). All three groups have received much attention and there has accumulated an extensive literature which we shall not attempt to treat in detail, since it has been handled very fully by the specialists in these subjects. We shall, however, present the most important conclusions and

<sup>1</sup> This paper concludes the series of studies on the plant cell. The author has a number of complete sets of reprints of this and the earlier sections. Enquiries may be addressed to Professor Bradley M. Davis, University of Chicago.

try to give the present status of investigations in these most difficult subjects.

*Cyanophyceæ (Blue-green. Algæ).*—The most recent and comprehensive papers on the cell structure of the Cyanophyceæ are by Fischer ('97), Macallum ('99), Hegler (:01), Bütschli (:02), Kohl (:03), Zacharias (:00, :03), and Olive (:04). Olive gives an especially clear analysis of the situation in this field of investigation at the present time and an excellent historical review of earlier literature may be found in Hegler (:01). The discussions center chiefly around (1) the presence or absence of a nuclear structure and its behavior in cell division, (2) the distribution of the blue-green pigment (phycocyan) and the structure of a possible chromatophore, and (3) the nature of certain conspicuous inclusions within the cell, called cyanophycin granules and slime globules. An outline in tabular form of the views of some thirty investigators on these subjects is given by Olive (:04, p. 10).

Writers from the earliest periods of cell studies on the Cyanophyceæ have recognized the presence of a central body in the interior of the cell more or less sharply differentiated from the peripheral region, which holds the coloring matter and certain inclusions. The central body contains granular material which stains and behaves in other particulars like chromatin. But as a rule this granular material is not confined within a membrane or vacuolar cavity which has proved the most serious difficulty to its acceptance as chromatin and the central body as a nucleus. Then many investigators have not been able to satisfy themselves that the central body exhibits the phenomena characteristic of nuclear division even in a simple form. Consequently much doubt has been expressed as to its morphology and possible relation to a nucleus.

The most recent and detailed investigations have, however, brought forward much evidence to the effect that the granular material in the central body is chromatin which becomes organized into chromosomes that are distributed by a form of mitotic division. In the vegetative cells, which generally divide rapidly, the chromatin is never held within a nuclear membrane but in young heterocysts and spores such inclosing membranes have been found (Olive, :04).

Olive (:04) has given especial attention to methods of sectioning and staining on the slide and presents the most detailed account of the structure and behavior of the chromatin and the simple apparatus which brings about the division of the central body. The central body is made up chiefly of dense kinoplasm with a fibrillar structure in which lie chromosomes that may be counted and whose number is found to be constant in several species. Thus there are eight chromosomes in a species of *Glœocapsa* and *Nostoc* and sixteen in certain forms of *Oscillatoria*, *Phormidium*, and *Calothrix*. The chromatin in some cases was observed to be organized into what seemed to be a simple type of spirem (especially clear in *Glœocapsa*) within the central body, and the chromosomes are formed by a concentration of material at certain points which are constant in the cells of the same plant.

Olive found evidence that the chromosomes split during the process of division of the central body and are gathered in two groups at the ends of the achromatic structure which is generally flattened at the poles and conforms in other particulars to the shape of the cells. The two sets of chromosomes are finally separated by the cell wall which develops from the periphery during cell division and cuts the achromatic structure in the middle region. That portion of the central body which remains between the two sets of daughter chromosomes is regarded by Olive as equivalent to the central spindle so well defined in stages of anaphase and telophase in mitoses of higher plants. The central body during this process of division has certainly very much the appearance of a simple type of spindle although there are not present the large fibers so characteristic of nuclear figures in higher plants. Moreover it can scarcely be held that the division is one of simple fusion when chromosomes are present in constant numbers and split into two groups with each division of the cell. Olive believes that the achromatic structure, present during cell division, is a disc-shaped, generally flat-poled spindle, densely fibrous in structure and that the fission of the chromosomes and their separation into two sets constitutes a true mitotic division of the central body, which is a nucleus.

Other authors as Scott ('88), Hegler (:01), Bütschli (:02), and Kohl (:03), also believe that the central body is a nucleus which divides mitotically but none has described the process as so closely similar to nuclear division in higher plants as in the account of Olive. Some of their results are criticized by Olive as based on preparations in which the stain was not properly differentiated or the sections were too thick. Among the recent writers Wager (:03) stands alone as holding that the nucleus divides directly (amitotically) by a process of simple fission. Both Kohl and Wager conceive the chromatin as in a network or convolute spirem which breaks up into segments which are drawn apart, thread by thread, quite a different process from the splitting of organized chromosomes. Other authors have held that the granules in the central body were chromatin although they were not willing to admit the structure as a nucleus. Thus Macallum ('99) found that the central body contained phosphorous and "masked iron" to a conspicuous degree and he, with other investigators, has shown that this structure resists the action of artificial gastric juice, solutions of pepsin, etc. These chemical reactions are considered confirmatory of the theory that the granular material is a proteid of a high order of organization such as would be expected of chromatin. However, such chemical tests are very difficult to apply and do not seem to the writer so important in establishing the nature of the central body as does the careful study of its structure and activity during cell division. The objection that the central body lacks a membrane, universally present around resting nuclei of higher plants, is not regarded as vital by Olive. In the first place such a membrane may be found around the resting nuclei in young heterocysts and spores and its absence in vegetative cells is probably explained by the rapidity of the successive cell divisions. There are some recent writers, as Massart (:02) and Zacharias (:00, :03) who are still unconvinced that the granules in the central body are chromatin and that the structure is the equivalent of a nucleus. Their papers and figures, however, clearly show that they have failed to find the detailed structures of other investigators.

Fischer ('97) has been the most conspicuous opponent of the

view that the cells of the Cyanophyceæ and also of the Schizomycetes contain nuclei, taking a position in sharp opposition to that of Bütschli ('96). Fischer's conclusions were based on his failure to find that differentiation of the protoplasm within the cell demanded by the conception of the central body and the activities of this structure during cell division as described by other authors. He presented a sharp criticism of the conclusions based on the reaction of stains in determining the nature of protoplasmic structures, criticisms largely directed against the investigations of Bütschli. He showed by some ingenious experiments upon emulsions of albumen fixed on a slide that stain reactions were a purely physical phenomenon. Thus the same combinations of stains, such as saffranin and gentian violet, may be made to give exactly opposite results in differentiating a mixture of large and small globules of albumen when used in reverse order. He attached no importance to the so called affinity of a protoplasmic structure for a particular stain and would not accept such apparent affinity as evidence of its chemical nature. The fact that the central body takes chromatic stains did not seem to him important evidence of its nuclear character and he was very positive in his belief that the cells of the Cyanophyceæ do not contain nuclei and that the central bodies should not be considered the phylogenetic forerunners of such structures.

This attitude of Fischer towards conclusions based on stain reactions was later presented in more elaborate form in his critique ('99) on methods of fixing and staining protoplasm and has had an important influence on methods of cytological investigation and interpretation. The stain reaction is now regarded as probably merely a physical phenomenon but an effective means of differentiating protoplasmic structures. The determination of their morphology rests with an understanding of their history and behavior in the activities of the cell. Although Fischer's general criticism of methods of cell research was timely and in some instances richly deserved, nevertheless his particular conclusions respecting the cell structure of the Cyanophyceæ and the Schizomycetes have not been sustained by investigators who have followed the history of the protoplasmic structures in the cells of these organisms.

We may pass now to the peripheral region of the cell which holds the blue-green coloring matter of the Cyanophyceæ. A number of investigators, as Wager (:03), Kohl (:03), Hegler (:01), and Hieronymus ('92), have held that this pigment was contained in minute granules distributed throughout the cytoplasm under the cell wall. These granules have at times been termed chromatophores or plastids and Hegler has proposed for them the name cyanoplastids. Other authors, especially Fischer ('97), Nadson ('95), Palla ('93), and Zukal ('92) have been unable to find these color-bearing granules and have believed the coloring matter to be uniformly diffused throughout the peripheral region of the cell. Fischer has made a particularly thorough study of the reactions of the pigmented region to various acids in comparison with the chromatophores of higher algæ and concludes that no plastids are present but that the color is held in a hollow cylindrical or spherical outer layer of protoplasm which may be termed a chromatophore. Olive supports Fischer, approaching the subject from a very different point of view. If minute plastids are present they should be visible in fixed and stained material and Olive is unable to find any trace of Hegler's cyanoplasts. The granules of the outer region of the protoplast seem to be colorless inclusions.

Perhaps the most confused part of the discussion on the structure of the cell of the blue-green algæ is that which deals with certain inclusions. There are apparently two sorts which are very common in the cells: (1) the cyanophycin granules (Borzi) and (2) the slime globules. The cyanophycin granules are very apt to lie along the cross walls in filamentous forms or in other peripheral regions of the cell. They are generally believed to be a form of food material and it has been suggested that they are the first visible product of photosynthetic processes, but their chemical nature is under dispute. The slime globules lie more frequently in the interior region of the cell close to the nucleus and frequently within this structure. They have been termed nucleoli by some authors and also confused with chromatin. Besides these two bodies, other minute globules have been described as oil or fat and some remarkable crystalloid structures have been figured, especially by Hieronymus ('92).

Indeed the entire subject is so confused that it does not seem desirable for us to take it up in detail at this time, especially since these inclusions are apparently all secretions or excretions and not morphological features of the cell. The most comprehensive discussions of the subject will be found in the papers of Hegler (:01), Kohl (:03), and Zacharias (:03).<sup>1</sup>

*Schizomycetes (Bacteria).*—The history of research upon the cell structure of the Schizomycetes has run in large part parallel with that on the Cyanophyceæ. The clearest results have come from studies upon the larger forms of the sulphur bacteria, especially certain species of *Beggiatoa*, and on certain forms of *Spirillum*. The more minute types and pathogenic forms in particular have proved very baffling because of their small size and it can scarcely be said that we fully understand their cell structure. As in the Cyanophyceæ, investigators of the bacteria fall into two groups: one holding that the Schizomycetes entirely lack a nucleus and the other that there is present a structure, often termed a central body, which is the equivalent of a nucleus.

Bütschli ('96, :02) has been the most conspicuous advocate of the latter view. He described and figured clearly a central body in the cells of *Beggiatoa*, *Chromatium*, and *Spirillum* with the same organization as given in his account of that body in the Cyanophyceæ. The central body contains granular material which Bütschli regards as chromatin and the structure is shown in stages of division. Bütschli has no hesitation in giving the central body the value of a nucleus. It lies within a peripheral

<sup>1</sup>Since the above was written a lengthy paper by Fischer, "Die Zelle der Cyanophycean" has appeared (*Bot. Zeit.*, vol. 63, p. 51, 1905), too late to be included in these reviews. Fischer has not changed his conclusions on the chief points as discussed in his earlier papers. The chromatophore is a closed cylindrical structure; the cyanophycin granules are proteid in character; glycogen and another carbohydrate, anabænin, are conspicuous substances in the cell; the central body is not a nucleus but the seat of important metabolic processes concerned with these carbohydrates, and its contents and behavior in cell division have only a superficial resemblance to nuclear structure and mitosis; the chromatin granules of Bütschli, Olive, and others are masses of anabænin (a carbohydrate). Fischer's criticisms are fundamental and it is evident that the morphologists must clearly establish the proteid nature of the central body and its contents (especially the "so called" chromatin granules) before they can expect the acceptance of their conclusions as to its nuclear character.

region of protoplasm as in the Cyanophyceæ. There is of course no blue-green pigment (phycocyan) in the cells of bacteria and consequently no chromatophore but several sorts of inclusions may be present in the protoplasm. The nature of some of the inclusions is not clear and this subject has not been given as much attention as in the Cyanophyceæ. It is significant that this cell structure should be found so clearly in the *Beggiatoa* since this organism seems very close to *Oscillatoria* in its morphology. Some of the larger species of *Beggiatoa* may be expected to yield conclusions similar to those of Olive's investigation on *Oscillatoria* if sectioned and critically stained, especially as the cells are very large in some forms and there is probably less extraneous matter to complicate the interpretation of the preparations.

As has been stated, investigations upon the smaller species of bacteria and especially upon pathogenic forms have met with great difficulties. These led at one time to the ingenious theory of Bütschli ('90), followed by Zettnow ('97) that possibly the entire protoplast had the value of a nucleus. That is to say, an outer peripheral region of cytoplasm had either never been developed in these organisms or, if present, had become so closely associated with the chromatin that it could not be distinguished as a special region of the cell. A peripheral region of cytoplasm is represented in some of the larger forms by the cilia and by accumulations of protoplasm at the ends of the cells, especially clearly shown in *Spirillum* (Bütschli, '96; Zettnow, '97). Later Zettnow ('99) and Feinberg (:00) applying the staining method of Romanowski, followed by several later investigators with improved technique (Nakanishi, :01, and others), succeeded in differentiating a minute body in the cells of smaller bacteria and pathogenic forms, which is regarded now as similar to the central body of the sulphur bacteria and a true nucleus. This structure is very minute since it occupies a portion of these exceedingly small cells. Naturally it will be very difficult to obtain any detailed knowledge of its structure and behavior during cell division. But enough seems to be known to justify the belief that differentiated nuclear structures are probably present even in the smallest types of bacteria. A recent paper

of Vejdovsky (: 04) describes and figures a simple type of spindle in *Bacterium gammeri* and *Bryodrilus ehlersi* with a separation of two groups of chromatin granules during mitosis.

The chief critics of the conclusions that the cells of Schizomycetes are nucleated have been Migula ('95) and Fischer. The latter author in particular has devoted considerable attention to the group especially in his paper of 1897 which is largely a discussion of Bütschli's ('96) results on studies of the blue-green algæ and bacteria. Fischer considers the central body described by Bütschli in the sulphur bacteria as merely a vacuolate region of the cell made conspicuous by the arrangement of the sulphur grains and that the structure does not appear in cells which are free from sulphur. The granular material, considered as chromatin by others, is regarded by Fischer as reserve material. The central body described by Bütschli in the cells of *Spirillum* is stated to be a product of contraction. In general the same criticism which Fischer applied to the methods of staining and interpretation of structures in the Cyanophyceæ is presented for the Schizomycetes. Fischer cannot justify Bütschli's ('90) view that the smaller bacteria are chiefly composed of nuclear substance, a view which probably has few if any followers to-day and could scarcely claim to be more than a passing suggestion. In short, Fischer finds no evidence of a nuclear structure in the Schizomycetes but curiously ends by declaring that the group has no affinities with the Cyanophyceæ but that its forms are closely associated with the Flagellata.

*Saccharomycetes (Yeasts).*—The structure of the yeast cell has been perhaps the subject of as long a series of investigations as the cells of the Cyanophyceæ and Schizomycetes, and the problems in both cases have many similar features. The chief problem in the yeasts has concerned the presence or absence of an organized nucleus or its equivalent in the form of some simpler structure. The accounts range from a complete denial of its presence to descriptions of a nuclear apparatus of considerable complexity which passes through some rather involved activities during cell division. It is impossible for us to treat the subject historically. We shall only consider the accounts of the most recent investigators and try to determine the probable

bearing of these studies. An admirable review of the early literature is presented in Wager's paper of 1898.

Wager ('98) himself has made one of the most detailed studies of the yeast cell and his conclusions on the presence of a "nuclear apparatus" will be made the starting point of our discussion. The yeast cell contains a structure, termed by Wager a "nuclear body," generally situated at one side, close to the cell wall. This body resembles the nucleolus of higher plants in its homogeneous structure and reaction to stains. Besides the "nuclear body" Wager finds a vacuole always present which contains granular material and is an important part of the nuclear apparatus. This "nuclear vacuole" must be carefully distinguished from other vacuoles of the usual type which merely contain glycogen. There are besides some globular bodies in the protoplasm whose nature may be oil in some cases and proteid in others. The "nuclear body" is always in close contact with the "nuclear vacuole" but is never within it. The amount of granular material in the nuclear vacuole is variable but it sometimes contains a dense mass. This content is believed to be chromatin from the behavior to stains and insolubility in digestive fluids. Sometimes the nuclear vacuole disappears but in such cases the granular network is found in contact with the nuclear body and sometimes distributed about it in a very regular manner. The chromatic granular material appears then to be a permanent substance in the cell and always closely associated with the nuclear body, sometimes distributed about it and sometimes included within a special vacuole.

Wager concludes that the nuclear apparatus consists of (1) a nucleolus (nuclear body) and (2) a store of chromatin in a network, either enclosed in a vacuole in close contact with the nucleolus or lying freely about the nucleolus or sometimes disseminated in granules generally throughout the cytoplasm. Wager believes that the nuclear vacuole arises from the fusion of numerous small vacuoles which lie around the chromatin granules which thus come to lie within a common vesicle. This mode of origin seems reasonable from what we know of the history of the nuclear vacuole which arises around the chromosomes that gather at anaphase of mitosis to form daughter

nuclei in higher plants. The earlier investigators for the most part failed to recognize the chromatic granules and network and considered the nucleolar body (nucleolus) to be the nucleus of the cell. Janssens and Leblanc ('98), however, described a nucleus with a membrane containing caryoplasm and a nucleolus, and other authors noted the vacuole and believed that it held some relation to the nucleus.

Both the nuclear vacuole and the nuclear body (nucleolus) take part in the process of bud formation. The bud appears on the opposite side of the cell from the nuclear body and the nuclear vacuole lies between. The bud contains at first cytoplasm alone; then the nuclear vacuole begins to pass into it and the nuclear body takes a position in the vicinity, between the mother-cell and the bud. The nuclear body now divides by simple fission and one half enters the bud. The nuclear vacuole gradually constricts and is drawn apart in the canal between the two cells. The two daughter nuclear vacuoles and nuclear bodies then pass to opposite ends of the mother- and daughter-cells respectively. If the nuclear vacuole is absent the chromatin network is drawn apart so that a division is effected in a similar manner.

At the time of spore formation, the chromatin is reported by Wager to become so closely associated with the nuclear body that the two substances cannot be easily separated and behave as one. The resultant structure elongates and divides by constriction and the subsequent divisions are of the same character. Strands of deeply staining protoplasm between the daughter nuclei are of interest as suggesting the possibility of a simple type of spindle. Wager describes the formation of spore walls around the nuclei enclosing a portion of the protoplasm and thus cutting the spores out from the remaining non-nucleate cell contents. The details of this process are not known and might prove very interesting since the process, from Wager's account, would seem to be one of free cell formation without, however, the characteristics described by Harper in spore formation within the ascus. It should be more thoroughly studied for it is possible that the division will be found to involve cleavage furrows and really prove to be a type of segmentation by constriction (Section II, *Amer. Nat.*, vol. 38, p. 453, June, 1904).

Several papers have appeared on the structure of the yeast cell since Wager's account of 1898. Marpmann (:02) and Feinberg (:02) described much simpler conditions than are reported by Wager, and recognize scarcely more than a deeply staining body which they term a nucleus. Hirschbruch (:02) gives an extraordinary description, accompanied by diagrammatic figures, of a nuclear structure and a body, staining red and blue respectively, which are supposed to fuse previous to the development of a bud, but the account is so unsatisfactory as to merit little attention. Janssens (:03) reviews the work of these investigators and others in comparison with his earlier results (Janssens and Leblanc, '98). Guilliermond (:04) has published the most recent paper presenting more completely his conclusions of an earlier investigation in 1902.

Guilliermond's conclusions have some points of resemblance to those of Wager. He finds a nuclear vacuole containing a granular network believed to be chromatin and a nucleolar structure. The entire body seems to be a true nucleus, not differing in its essentials from the nuclei of other fungi. Sometimes all the material in the nucleus seems to be condensed into a central body, a sort of chromatin nucleolus (chromoblast) somewhat resembling a similar structure in Spirogyra. Guilliermond figures the nucleus as constricting during the process of budding, one part passing into the daughter cell. His figures show clearly deeply stained material outside of the nuclear membrane in a position similar to that of Wager's nucleolar body (nucleolus).

These points of agreement seem to justify at least in part Wager's account, but of course the peculiarities of both lead one to suspect that there are important features in the structure of the nucleus and in the events of nuclear division which have not been determined. It certainly seems probable that chromatin is present in definitely organized bodies (chromosomes) sometimes within a vacuole and sometimes lying around a nucleolar structure. The latter also holds an intimate relation to the chromatin, which is frequently true in higher plants: There are indications that a simple type of spindle is present at least in the nuclear divisions during spore formation. In view of

Olive's results in studies on the Cyanophyceæ it does not seem unreasonable to hope that more accurate staining of very thin sections will bring the peculiarities of these accounts into harmony with mitotic phenomena of higher forms.

The accounts of conjugation in yeasts (Barker, :01 and Guilliermond, :03) which were discussed under "Asexual Cell Unions and Nuclear Fusions" in Section IV give no additional information on the essential structure of the yeast cell.

## 2. COMPARISONS OF THE STRUCTURE OF SOME HIGHER TYPES OF PLANT CELL WITH SIMPLER CONDITIONS.

Some of the most fruitful and interesting fields of investigation in cell structure are likely to be in those border groups between the very simplest conditions of the lower algæ and fungi and the higher regions where the nucleus and processes of mitosis have clearly the essential features which are generally ascribed to this structure and its activities. At present the gap seems very great between the simple conditions of the Schizophyta and the groups of algæ and fungi on the next higher general level. But as a matter of fact we know almost nothing of the nuclear structure in the lowest groups of the Chlorophyceæ, *i. e.*, among the simplest of the unicellular green algæ. It is rather remarkable that this region should have been so neglected.

*The Nucleus.*—Comparative studies on the nucleus naturally treat chiefly of the chromosomes and nucleolus. One of the most interesting features of more recent research on the nucleus has been the steady accumulation of evidence indicating that the nucleolus holds a very important relation to the chromatin content. There are types among the lower algæ in which the whole or a greater part of the chromatin is gathered into a dense nucleolar body in the resting nucleus. Spirogyra is the best known illustration of this condition and has been studied by several investigators. Similar phenomena have been reported by myself in *Corallina* (Davis, '98), by Golenkin ('99) for *Sphæroplea*, and by Wolfe (:04) for *Nemalion*. Some nuclei, however, particularly in the higher plants have nucleoli whose

substance does not seem to contribute directly to the chromosomes and these have been regarded as secretions within the nucleus. Strasburger believed that such were masses of reserve material drawn upon by the kinoplasm during the process of spindle formation. The term plastin has been applied to such substance in the nucleolus and also in the linin as cannot be directly connected with chromatin. A nucleolus may consist of plastin alone, or have with this substance varying quantities of chromatin. Nucleoli consisting of chromatin alone may be expected among the lower plants from the studies on *Spirogyra*, *Corallina*, *Sphæroplea*, and *Nemalion*. Plastin and chromatin are probably closely related substances.

A recent paper of Wager (:04) indicates that the nucleolus of some higher plants holds a far closer relation to the chromosomes than has been supposed and rather weakens Strasburger's theory of the structure as a reserve mass drawn upon during mitotic activities. This study and recent papers by Miss Merriman (:04) and Mano (:04) have all been upon the cells of root tips while the conceptions of Strasburger and others have been founded largely on the structure and behavior of the nucleolus in the spore mother-cell during the mitoses of sporogenesis. Wager treats of the root tip of *Phaseolus*, Miss Merriman of *Allium*, and Mano of *Solanum* and *Phaseolus*. They are important contributions to the subject of the nucleolus and should be considered in any treatment of this structure. The papers appeared too recently to be noted in our brief account of the nucleolus in Section I which is consequently incomplete. Wager's paper especially presents an excellent review of the literature on the nucleolus in the plant cell.

Wager concludes that the nucleolus is really a portion of the nuclear network and that the spirem is derived in part at least from this structure. Material from the nucleolus then passes into the chromosomes. Also, in the reconstruction of the daughter nuclei the chromosomes are massed together at a certain stage and from this mass the nucleolus emerges, taking out with it the greater part of the chromatin. Wager then considers the nucleolus as a store of chromatin which must be taken into account in theories of heredity based on the morpho-

logical independence of the chromosomes. Miss Merriman reports the origin of the nucleoli as masses among the meshes of chromatin from which they draw their substance. Mano, in contrast to Wager, holds that the nucleoli appear as globules independent of the chromatin network and later flow together into a single body. The chromosomes are also believed by Mano to be morphologically independent of the nucleolus and if the latter furnishes material to the former it is not by the emergence of strands as described by Wager. Mano then holds the nucleolus to be an accessory structure without morphological relation to the chromosomes.

The theory of the individuality of the chromosomes is of course vitally concerned with the problem of the morphology of the nucleolus but this topic we have reserved for later treatment under the caption: "The Essential Structures of the Plant Cell and their Behavior in Ontogeny." The chromatin and nucleoli within the nucleus of a higher plant lie in a vacuole whose fluid content is bounded by a plasma membrane similar to that around any vacuole in the cell. Lawson (:03) and Grégoire and Wygaerts (:03) have emphasized this structural condition in recent papers but the central idea seems to be an old one running through the writings of Strasburger from an early period.

We bring up these striking conceptions of nuclear structure in the higher plants because it seems very probable that a much clearer understanding of the problems will come through investigations upon the simpler conditions in the lower plants. There, we may hope to find evidence of the primitive forms of nucleolar and chromatic associations with perhaps some clues as to the manner of the development of the higher types of structure. Thus the yeast cell, as reported by Wager ('98) with its chromatin sometimes collected within a vacuole and sometimes distributed in the cytoplasm and a nuclear body (nucleolus) in close association with the nuclear vacuole, but not within, is of the greatest interest as presenting intermediate stages in the complexity of nuclear structure and illustrates what may be hoped from further research among the lower forms.

*The Chromatophore and Plastid.*—In considering the great variety of chromatophores and plastids exhibited among the

thallophytes one notices at once certain features of their distribution in various groups. The large chromatophores are characteristic of the cells of simpler and more primitive groups and the small plastids, numerous in the cells, are generally present in types which are at a fairly high evolutionary level. There are exceptions of course to this general statement but some of these are probably significant of phylogenetic relations.

The evidence all indicates that the primitive type of chromatophore was a large structure in the peripheral region of the protoplast and with an ill defined boundary or occupying the entire surface of the cell. This type of structure is at present characteristic of chromatophores of the Cyanophyceæ and is also present in numbers of the lower groups of green algæ. Thus we may find many types in the Pleurococcaceæ whose cells contain a pigment so diffused that it is impossible to establish definite limits and similar conditions often appear in the cells of some of the higher algæ as in Hydrodictyon and certain simple forms of the Ulothricaceæ.

The simple diffused types of chromatophores of the lower algæ become replaced in higher groups either by sharply differentiated structures of definite form and often showing internal organization in the form of pyrenoids or by numerous plastids. There is considerable evidence that the plastids have arisen by the successive splitting or division of large organized chromatophores. The most highly differentiated chromatophores are found in the Conjugales and the remarkable size and symmetry of these cells is emphasized by the same peculiarities of the chromatophores. They are generally so placed in the cells as to give an almost perfect balance of protoplasmic structure. This principle is especially clearly illustrated among the desmids and in such forms as *Zygnema* and *Mougeotia* while even *Spirogyra* illustrates the principle strikingly in the distribution of its spirally wound chromatophores.

Plastids are characteristic of the Siphonales, Charales, most of the Rhodophyceæ, the higher Phæophyceæ, and all groups generally above the thallophytes. It seems to be the type of structure best suited to cell activities since with few exceptions it is found in groups in the highest lines of plant evolution in

various directions. The only striking exceptions to this broad principle are *Anthoceros*, whose cells contain each a single large chromatophore, and *Selaginella*. *Selaginella* is especially interesting for, while the cells of the meristematic region and young organs contain but a single chromatophore, this structure may divide later in some types to form a chain of discoid plastids in older cells connected with one another by delicate strands of protoplasm. Thus in the life history of certain species of *Selaginella* we have plainly shown the change from a single chromatophore to a number of plastids. It seems probable that this history repeats in general outline the evolutionary history of the condition characterized by numerous plastids within a cell from a primitive type of cell structure with but a single chromatophore. *Anthoceros* and *Selaginella* may be regarded as forms whose cells still retain the primitive conditions with respect to the single large chromatophore. There are somewhat similar illustrations in the Rhodophyceæ as in *Nemalion* and *Batrachospermum* whose cells hold a single large chromatophore while most of the more highly organized red algæ have numerous plastids. A beautiful series of stages illustrating the evolutionary principles outlined above might be worked out in the Phæophyceæ.

What is the fundamental principle underlying the substitution of numerous plastids in a cell in place of a single chromatophore? The author believes that it must have relation to the preservation within large cells of a certain balance of the metabolic centers. The fission of a plastid is a process of constriction and studies on *Anthoceros* (Davis, '99, p. 94) indicate that the bounding cytoplasmic membrane exerts pressure upon the elongating structure. It seems probable that the division is due to the mechanical separation of material that is too bulky for the most effective results of photosynthesis which in the case of a single chromatophore are centered in a particular region of the cell. By the division of a chromatophore into numerous plastids the photosynthetic activities are distributed among several centers and a much better balance results within the cell. It is very interesting that the large elaborate chromatophores with their peculiar internal differentiations, the pyre-

noids and caryoids, should have been displaced by the much simpler and apparently homogeneous plastids.

A comparative study of chromatophores and plastids from the point of view of their evolutionary history is much to be desired and such research would necessitate extensive studies among the lower groups of algæ and especially in the Proto-coccales. Such studies would involve far more than the general morphology of the chromatophore and plastid. The structure and activities of the pyrenoid are a very important subject as shown by the investigations of Timberlake on *Hydrodictyon* and nothing is known of the function of the caryoid. A detailed investigation of the chromatophore or plastid throughout ontogeny is yet to be made.

*The Cytoplasm.*—There is no region of the plant cell whose structure is more varied and as little understood as that presented by the cytoplasm with its diverse conditions. We have throughout these papers held to the classification of Strasburger that the cytoplasm may be separated into two forms: kinoplasm and trophoplasm, which show certain structural peculiarities and are characterized by very different forms of activity. While it must be acknowledged that kinoplasm and trophoplasm are very similar in certain regions of the cell and at certain periods of the cell history, still the distinctions are in general clearly marked.

Kinoplasm is homogeneous in structure, either minutely granular or consisting of delicate fibrillæ composed of very small granules placed end to end. The homogeneous condition is characteristically shown in the three forms of plasma membranes which cytoplasm places between itself and external or internal surface contacts. The three membranes are: the outer plasma membrane, the nuclear membrane, and the vacuolar membranes. They are certainly closely related and probably identical in structure and appear to be the natural expression of protoplasm to contact with a fluid (water) medium. The fibrillar condition appears during mitosis and serves important functions in the mechanism (spindle) through which the chromosomes are distributed and in most of the higher plants determines the position of the cell wall that is generally formed with each nuclear division.

But the manifestations of kinoplasm during nuclear division and also in relation to cilia-bearing surfaces are exceedingly various and it is among these structures that our ignorance of relationships and modes of origin is deepest. These kinoplasmic structures have been described in various connections throughout this series of papers and especially in Sections I, II, and III, and need not be treated here. But the point which should be emphasized in this connection is the necessity of the close study of their simplest expressions in the lower regions of the thallophytes. The most varied forms of kinoplasm are in the thallophytes where asters, centrospheres, and centrosomes obtain and where ciliated cells, presumably with blepharoplasts, may occupy long periods of the life history. It is here that we must search for information that will bring order out of the confusion of our present accounts and insufficiency of knowledge. The most vital problems relating to kinoplasm concern the origin and the events of the simplest types of mitotic phenomena and the formation of cilia. We have a fairly clear understanding of the general features of mitosis in the groups above the thallophytes and their relation to the lower types and these will be briefly treated in the following portion of this section under the head: "Some Apparent Tendencies in the Evolution of Mitotic Phenomena." But the events of mitosis among the thallophytes are exceedingly various and difficult to understand and nothing is known of their origin or relation to the simpler conditions which must be present in the lowest regions of the Chlorophyceæ and in the Cyanophyceæ.

Trophoplasm comprises all of the cytoplasm included within the plasma membranes. While this region does not give rise to such highly differentiated cell organs as the kinoplasm, nevertheless some remarkably interesting structures are developed. Cœnocentra and Physodes are specialized structures of exceeding interest and our ignorance of the latter is truly remarkable. Nematocysts if trophoplasmic offer another attractive subject for investigation. In a sense, chromatophores and plastids may be considered trophoplasmic but their high grade of specialization and fixity as cell organs gives them a certain independence of other structures in the cell. Respecting the structure of the

groundwork of trophoplasm, whether fibrillar, granular, or presenting the structure of foam, botanical science has as yet furnished very little systematic study and this field of research is one of exceptional opportunity for the student of the plant cell.

*The Cell Wall.*—The cell wall may be treated from two points of view: either with respect to the strict chemistry of its organization and development or more largely for the biological and morphological features involved. The chemistry of the cell wall is an exceedingly complex subject which has developed a special literature of its own. In the substance termed cellulose we are not dealing with a single body but rather with a large group of closely related bodies. And besides the members of the cellulose group there may be present foreign substances so intimately associated with the carbohydrates as to resist very severe treatment. We cannot even touch this phase of the subject; a brief review of its complexities and problems is presented by Beer (:04) and there are further references in Section I of these "Studies."

There are, however, some biological features of the process of wall formation, the morphological and physiological aspects of the phenomena as they are related to protoplasm, which offer some exceedingly interesting problems especially among the thallophytes. It has long been a matter of dispute whether the cell wall is a secretion from the surface of a plasma membrane or is formed wholly or in part by the transformation of such a membrane.

It seems to be established now that substances of the cellulose groups are only formed in contact with plasma membranes, that is, they are not formed actually in the interior of protoplasm although they may appear to lie in such situations. Thus the material of the capillitium of the Myxomycetes which is of the same character as the chief substance in the exterior covering of the fructification, is laid down within vacuoles in the protoplasm, and is therefore in contact with the surface of vacuolar plasma membranes precisely as the outer covering lies in contact with the surface of the outer plasma membrane. The morphological relation of capillitium and outer covering to the surface of plasma membranes is therefore precisely the same.

And similarly the cross wall which takes the position of the cell plate at the end of mitosis is not developed from the transformation of a film of protoplasm but is laid down between two surfaces that separate to form a thin vacuole which later spreads to the edge of the cell and the wall is deposited between these two membranes which are almost in contact. There are a number of cases in which large strands or masses of protoplasm have been described as changing directly into cellulose but it is probable that these examples upon further study will exhibit the same relation of the cellulose substances to plasma membranes as in the typical cases of wall formation. There are many interesting examples of cellulose formation whose precise relation to the protoplasm has not yet been determined.

Respecting the exact method by which a cellulose wall is laid down by a plasma membrane there is very little real information. It is clear now that the cellulose is not a secretion from the plasma membrane comparable to a mineral shell. There is much evidence that protoplasm is actually sacrificed in the development of cellulose. There are numerous illustrations, as in the tracheids and other cells empty of protoplasm, where the final secondary thickenings are deposited as the protoplast grows smaller and eventually disappears, a large part of its substance evidently contributing to the deposits which are members of the cellulose group. But of course it cannot be supposed that the molecules of the proteids are changed directly into those of the carbohydrates. Nevertheless it does seem clear that the carbohydrates appear simultaneously with the disappearance of the proteids and occupy the position formerly held by the latter. It is probable that with the splitting up of the proteid molecule, carbohydrate material is formed which displaces the proteid substances. So in a broad sense the cellulose deposit actually does represent a transformation of a plasma membrane.

The evidence in general favors the view that the wall, lamellæ, and other deposits of cellulose only increase in amount when in actual contact with a plasma membrane. Some apparent exceptions to this principle are easily understood. Thus cell walls or portions of such may swell greatly and become much softer in consistency and perhaps even mucilaginous. There are no

reasons for regarding such transformations as an actual increase in the carbohydrate material for it is clear that the substance is a body with a greater amount of water in its organization than is present in the more usual forms of cellulose compounds. But there are some cases which are not so easily understood and perhaps the most widely known are the megaspore walls of certain species of *Selaginella*. These spores are remarkable for a differentiation of the spore wall in which the outer layer seems to be entirely separated from the inner by a space and yet is able to increase enormously in size and take on marked peculiarities of structure, but apparently without any relation to the protoplast. It may, however, be justly questioned whether the apparent space between the inner and outer wall is really a cavity and may not be filled with plastic material which holds the two walls in intimate organic relation to one another and to the protoplast. Miss Lyon has recently given this subject attention and announced her belief that the latter condition obtains. Her conclusions will be awaited with interest.

As regards the way in which a cell wall increases in size we are still limited to the two conceptions termed (1) growth by apposition and (2) growth by intussusception. The first method consists in the laying down of successive layers by the plasma membrane and results in a thickening of the cell wall. It is of course a comparatively simple process. Growth by intussusception is a stretching or expansion of the substance which seems to be greatly increased in quantity although the morphology of the structure remains the same. The current explanation outlined by Nägeli assumes that new molecules of carbohydrates are intercalated among the old. It seems more probable that the increase in bulk is due to some modification or rearrangement of existing molecules, involved, perhaps with an increase of material but not through the actual intercalation of new molecules of the same or original carbohydrates. The chemistry of the carbohydrates is so complex that great changes of form, bulk, or optical properties may be readily assumed which would quite change the appearance of a structure without, however, necessitating the transportation of new carbohydrate substance to it directly.

There are many forms, particularly among the lower plants, where studies on the processes of wall formation are sure to throw much light on the fundamental problems which we have discussed. And a particularly interesting study might be made of the evolutionary history of the cell wall among the thallophytes and in the modifications introduced when plants pass from aquatic habits to aërial or terrestrial conditions. Our attention has been chiefly centered on the structure of the protoplast and the morphology and behavior of its parts. We are likely soon to give more study to the carbohydrate membranes and walls and this subject is likely to be very fruitful for investigation.

### 3. SOME APPARENT TENDENCIES IN THE EVOLUTION OF MITOTIC PHENOMENA.

Our brief descriptions in Section II (*Amer. Nat.*, vol. 38, p. 431, June, 1904) of the various kinoplasmic structures developed during mitosis in different groups of plants brings up the problem in their relationships to one another, *i. e.*, the evolutionary tendencies in the differentiation of mitotic phenomena. We have seen that the thallophytes present an especially diverse assortment of kinoplasmic structures associated with the spindle and its method of development. The spindle fibers, whether formed within the nuclear membrane (intranuclear) or arising from without (extranuclear), are associated with centrosomes or centrospheres to form asters in a number of well known types as *Stypocaulon*, *Dictyota*, *Fucus*, *Corallina*, certain diatoms, the ascus, and the basidium. Centrospheres are found in certain phases of the life history of liverworts as in the germinating spore of *Pellia*. A second type of kinoplasmic structure resembling in certain features the aster but with some fundamental differences has been termed the polar cap. The polar cap is an ill defined region of kinoplasm, generally larger than a centrosphere and without clear boundaries, which forms a region for the insertion of spindle fibers. Polar caps are well illustrated in the mitoses of vegetative tissues and meristematic regions, especially among the higher plants (pteridophytes and sperma-

tophytes). They sometimes approach the centrosphere very closely in their morphology.

The third and highest type of spindle formation in plants is that illustrated in the mitoses within the spore mother-cell which were given special treatment in Section III (*Amer. Nat.*, vol. 38, p. 725, October, 1904). In this remarkable cell the spindles develop from a mesh of independent fibrillæ which at prophase more or less completely surround the nucleus. The poles of the spindle arise by the grouping of cones of fibrillæ so that a single axis is finally established but without any kinoplasmic centers at the poles. This type of spindle formation which may be termed the free fibrillar type is one of the most interesting cytological peculiarities of plants. It has been found in all types whose sporophytic phase terminates its history with a spore mother-cell, although the accounts in the Hepaticæ are not in full accord.

Is it possible to connect the various types of spindle formation with one another and to establish any evolutionary tendencies in the processes involved; and have the different manifestations of kinoplasm such as centrosomes, centrospheres, polar caps, free fibrillar condition, and the mysterious structure called the blepharoplast any genetic relation to one another? The confusion is so great among the thallophytes that the author sees little hope at present of establishing clearly any relationships between the types of centrospheres and centrosomes with their systems of radiations (asters) and we must patiently wait for more information. And respecting the origin of these structures from the simpler types of mitosis we are absolutely in the dark. But the relation which polar caps and the free fibrillar type of spindle formation bear to centrospheres is less perplexing and it seems possible to define certain common features among these structures which hold them together with a degree of unity in their relations to mitosis. That phase of the subject will be considered in this treatment. The Hepaticæ as a group occupy an interesting position with respect to the character of mitotic phenomena at various periods of ontogeny, between conditions in the pteridophytes, which are obviously similar to the spermatophytes, and conditions in the thallophytes. This was brought

out by the work of Farmer whose accounts of centrosomes and centrospheres in the germinating spores of *Pellia* and within the spore mother-cell of various liverworts, together with his account of a "quadripolar spindle" made it evident that the group offered some very interesting cytological problems. They led the author to the study *Anthoceros* (Davis, '99) and *Pellia* (Davis, :01), investigations which have been followed by Van Hook (:00) on *Marchantia* and *Anthoceros*, Moore (:03) on *Pallavicinia*, Chamberlain (:03) and Grégoire and Berghe (:04) on *Pellia*, while Ikeno (:03) has studied the processes of spermatogenesis in *Marchantia*.

My studies on sporogenesis in *Anthoceros* and *Pellia* led me to conclude that the processes of spindle formation did not differ in any essentials from those in the pteridophytes and spermatophytes. There are present two successive mitoses and the spindles are formed from a surrounding mesh of fibrillæ developed from the kinoplasm associated with the nuclear membrane and without achromatic centers (centrospheres or centrosomes). They exhibit clearly the free fibrillar type of spindle formation although in somewhat simpler form than in the pteridophytes and spermatophytes. The poles of the spindles generally end bluntly in areas of granular kinoplasm but these seem to me too indefinite in form to deserve the designation of centrospheres and such granular inclusions as may be present are too variable in number and position to be termed centrosomes. There is clearly present in *Pellia* during the prophase of the first mitosis a four-rayed achromatic structure which is later replaced by a typical bipolar spindle. This four-rayed kinoplasmic structure is evidently the same as Farmer's "quadripolar spindle" which he described as associated with a simultaneous distribution of the chromatin in *Pallavicinia* to form at once four daughter nuclei. I was led to doubt this account and to suggest that the "quadripolar spindle" might prove to be simply a phenomenon of prophase associated with the peculiar four-lobed structure of the spore mother-cell in the *Jungermanniales*. I stated my belief that the distribution of the chromosomes during sporogenesis in all liverworts would be found to take place through two successive mitoses after the usual manner. Moore (:03)

has recently studied an American species of *Pallavicinia* and has failed to confirm Farmer's conclusions. He found the four-rayed figure, which Farmer terms a "quadripolar spindle," a conspicuous feature of the first mitosis here as in *Pellia* but there was no indication of a simultaneous distribution of quadrupled chromosomes to form four daughter nuclei as reported by Farmer. The four-rayed figure was merely preliminary to the first mitosis whose spindle at metaphase was bipolar and the first mitosis was followed shortly by a second, so that *Pallavicinia* offers no exception to the essential features of sporogenesis as known in all groups above the thallophytes.

Farmer (*Bot. Gaz.*, vol. 37, p. 63, 1904) has taken exception to the restriction of the term spindle by Moore and myself to the structure found at metaphase and holds that the four-rayed structure is a part of the spindle apparatus. In this discussion he appears to avoid the issue, which is not the broader or narrower application of the term spindle, a mere matter of usage, but concerns the fundamental character of the mitoses during sporogenesis whether they are two in number and successive in all forms or whether *Pallavicinia* presents an extraordinary exception in a distribution of the chromatin to form four daughter nuclei simultaneously in the spore mother-cell. Farmer (:05) has recently reaffirmed his view that the poles of the four-rayed figure in *Aneura* and presumably in other *Jungermanniales* are occupied by centrospheres and that sometimes a central body (centrosomes) may be distinguished in each. This statement involves again a matter of usage in which I should differ from Farmer for my studies and those of Moore do not seem to me to justify the application of these terms to regions of kinoplasm whose form is so ill defined and history so transient within the cell.

These disputed points which were also discussed in Section III (*Amer. Nat.*, vol. 38, pp. 727-732, October, 1904) are of importance in relation to the mitotic phenomena in other periods of the life history of liverworts which will now be considered. It may be stated, however, that other investigators who have studied the processes of sporogenesis in the liverworts (Van Hook, :00; Chamberlain, :03; Grégoire and Berghs, :04) sup-

port my general program of sporogenesis with the free fibrillar type of spindle formation. There seems to be little question but that centrospheres are present and conspicuous in the early mitoses within the spore of *Pellia*. They have been especially studied by Farmer and Reeves ('94), Davis (:01), Chamberlain (:03), and Grégoire and Berghs (:04). All of these authors have agreed that asters are clearly defined in the early mitoses within the spore and most of them have termed the region of kinoplasm in the center of the aster a centrosphere. The structures are less prominent in the third mitosis and are perhaps replaced in later periods of the gametophyte history by kinoplasmic polar caps. Polar caps are characteristic of the mitoses in the seta of *Pellia* (Davis, :01). However, Van Hook has described centrospheres with radiations at the poles of the spindles of the archegoniophores of *Marchantia*, whose centers sometimes contained centrosomes, and it is possible that the centrosphere runs through a considerable period in the life history of liverworts. There is complete agreement that the centrospheres when present arise *de novo* and independently of one another during the prophase of mitosis and that they disappear at telophase. Ikeno has, however, described centrosomes during the mitoses within the antheridium which are said to divide and pass to opposite sides of the nucleus where they become the poles of the spindles. They cannot be found after the mitosis is completed, but are described as formed *de novo* in the interior of the nucleus and thrust through the nuclear membrane into the cytoplasm previous to each mitosis. After the final division in the antheridium, the centrosome remains to function as a blepharoplast.

Thus we see that the liverworts present during their life history an almost complete range of kinoplasmic structures associated with the nuclear divisions from centrosomes and centrospheres to polar caps and that type of spindle formation characterized by free fibrillæ gathered into cones but entirely independent of definitely organized centers. There is also present the blepharoplast. I emphasized this range of kinoplasmic structure in my paper on *Pellia* and it seemed to me one of the most interesting features of the liverworts. In this paper

(Davis, :01, p. 171) are outlined the changes in form which kinoplasm may assume in the mitoses of the liverworts upon which is based a theory of a cycle through which kinoplasm may run in the history of a cell. On this theory, centrosphere, polar cap, and the free fibrillar condition are all secondary developments from a primal finely granular kinoplasm which is the only form of kinoplasm that is in any sense permanent in the cell. This finely granular kinoplasm is always present in characteristic form in the plasma membranes of the cell. The substance of centrospheres, polar caps, and fibrillæ arises from accumulations of granular kinoplasm during prophase and these structures return to the same undifferentiated granular kinoplasm at the end of mitosis or become lost in the general cytoplasm of the cell.

The cycle is from an undifferentiated finely granular kinoplasm through certain specialized conditions either wholly or in part fibrillar in structure back to the granular state. The centrosphere and polar cap are regions from which fibrillæ develop at least in part and to which they may remain attached as to an anchorage. The polar cap is a less clearly differentiated kinoplasmic center than the centrosphere but does not differ from it in the essentials of its organization. It seems to me that the two structures are very closely related in the liverworts and that in this group we may readily conceive the polar cap as derived from the centrosphere. The free fibrillar type of spindle formation is a step farther in the direction of such a distribution of the kinoplasm that no very positive centers for the development of the spindles may be distinguished. The four-rayed structure (quadripolar spindle) so characteristic of the spore mother-cell in the Jungermanniales represents a group of four temporary centers for the formation of fibrillæ and there is clearly a gathering of kinoplasm at these points but the regions are so vague in outline as hardly to justify the designation of centrospheres. From the fibrillar state, kinoplasm returns to the finely granular condition by the contraction of the fibers which thus contribute their substance to some common area. The area may lie around the chromosomes of the daughter nuclei where it becomes later in part at least a nuclear mem-

brane. Or the area may be a cell plate whose halves on division finally merge with outer plasma membranes of the cells. The spindle fibers which cut out the spore areas in the ascus form the basis of a plasma membrane. Thus the fate of all kinoplasmic fibrillæ seems to be a final return to the undifferentiated, finely granular condition so characteristic of plasma membranes which according to this theory is the condition from which they arose.

Thus I believe the liverworts present rather striking evidence of a relationship between the centrosphere, polar cap, and the free fibrillar condition of spindle formation and establish an evolutionary tendency from the first two types of kinoplasmic differentiation towards the latter. The free fibrillar type of spindle formation is found in a very simple form in this group, sometimes with temporary centers, as in the four-rayed figure (quadripolar spindle) of prophase, whose poles have accumulations of kinoplasm in the position of centrospheres. The polar caps are likely to prove a much simplified type of centrosphere whose kinoplasm is no longer gathered to form conspicuous spherical centers. With respect to the problem of the homologies and nature of the blepharoplast, the liverworts furnish as yet no material assistance and this structure stands at present as one of the most interesting puzzles of plant cytology. As stated in the beginning, the variety of centrosomes and centrospheres with and without radiations in various types of the thallophytes seems to me too confusing to promise an understanding of their relationships at present.

Grégoire and Berghs (:04) have interpreted the structure of the mitotic figure in the germinating spore of *Pellia* in a very different manner from the accounts of Farmer, Chamberlain, and myself. They consider the asters to arise through a rearrangement of the cytoplasmic network around the nucleus. They affirm that there are no true centrospheres nor any accumulations of granular kinoplasm to constitute the centers of origin for the spindle fibers or the radiations around the poles of the spindle. The centers of the asters ("vésicules polaires") are said to have a vesicular structure and neither they nor the nucleus contributes to the building up of the spindle which is

developed entirely out of the cytoplasmic network. The authors are unable to distinguish a kinoplasm distinct from the general network of the cell. These are vital points of difference which are fundamental to the understanding of mitotic phenomena and rest of course on matters of fact. The chief points at issue concern the structure and development of the asters and the nature of the material at their centers. My own studies and those of Farmer and Chamberlain have convinced me that there is an accumulation of substance (kinoplasm) in the centers of the asters and polar caps to such an amount that it must be regarded as a definite structure in the cell and its morphology and relations to the spindle have certainly justified us in considering it as similar to the centrosphere of the thallophytes.

#### 4. THE ESSENTIAL STRUCTURES IN THE PLANT CELL AND THEIR BEHAVIOR IN ONTOGENY.

The cell is composed of a series of osmotic membranes between which are included a number of protoplasmic structures whose morphology and minute organization is various. They are: the outer plasma, the vacuolar, and the nuclear membranes. Each of these sustains a relation to some fluid which bathes its surface. The fluid nature of the nuclear sap and cell sap is obvious but the outer plasma membrane is also against a moist surface since the cell walls of tissues are normally saturated with water. The structure of the plasma membranes is apparently the same. They consist of the homogeneous finely granular protoplasm that is designated kinoplasm. The protoplasmic structures included within the plasma membranes may be grouped as cytoplasmic and nuclear. The greater part of the cytoplasm, including that which is termed trophoplasm, has an organization peculiar to itself and very different from that of the plasma membranes. This structure has been described as alveolar or of the nature of foam and sometimes fibrillar and with various large granular inclusions. The cytoplasm also contains the characteristic organs termed plastids. The conspicuous structures of the nucleus are: the chromatic elements

appearing as chromosomes during mitosis and the nucleoli. These structures are so easily recognized and play such important parts in the events of nuclear division that they command attention at once as the essential elements in the nucleus. The nucleus may also contain other material such as linin which, however, does not seem to have a fixed form or behavior in the cell. Finally there are certain kinoplasmic structures, as centrosomes, centrospheres, and blepharoplasts, whose behavior throughout cell history has been much discussed. We shall now consider the most important of these structures, those which seem essential to the cell in ontogeny.

The outer plasma membrane naturally retains its morphological entity throughout all cell divisions with such slight changes as when new parts are intercalated into its area through the vacuoles that are utilized in the segmentation of protoplasm. Vacuolar membranes are constantly shifting and cannot be followed during cell division excepting in such cells as have one large central vacuole (the tonoplast of De Vries). Such a central vacuole is much more characteristic of old cells and tissues than of young or embryonic regions. There is certainly no reason to suppose that it has organic existence through any very extended period of the life history. The nuclear membrane becomes lost during the prophase of mitosis and there is much evidence that its kinoplasm contributes in some cases to the formation of spindle fibers. Thus the nuclear membrane disappears as a structure in the cell during mitosis and new vacuoles are formed around the assemblages of daughter chromosomes during telophase, leading of course to the formation of fresh nuclear membranes at their surface of contact with the surrounding cytoplasm.

There is perhaps no region of the cell protoplast that presents such different appearances through long periods of the cell history as the trophoplasm. This is largely due to the varying character of the inclusions which are not in themselves protoplasmic but which give a mixed structure to the trophoplasmic regions of the cell. The inclusions may be carbohydrate or proteid bodies held within spaces in the trophoplasmic groundwork or they may be globules of oil or fatty substances. These

inclusions occupy small spaces in the trophoplasm which are essentially vacuoles. There is also a class of granular inclusions of a proteid nature which probably represent material in very close organic relation to the substance of protoplasm. Trophoplasm does not then have so clearly defined a type of structure as do the other regions of the protoplast, but it is hardly probable that its essential nature changes very materially throughout the life history. The organization of trophoplasm is itself a matter of dispute but the prevailing views favor an alveolar or foam structure with a fibrous character at times somewhat resembling the texture of sponge.

Ever since the classical investigations of Schimper upon the plastid it has generally been held that these structures are permanent organs of the cell, reproducing by fission, and carried along from one cell generation to the next with as much permanence as the nucleus. Schimper discovered plastids in the oospheres of certain spermatophytes and in a variety of embryonic tissues and concluded that the structures passed from parents to offspring as leucoplasts when no trace of color could be found in the reproductive cells or embryonic tissues. There has been, however, no systematic study of the plastid throughout the life history of higher plants and in most of the green thallophytes there are reproductive phases, such as resting spores, where we have no knowledge of the structure or distribution of the chromatophores in the cell. It is very important that the plastid be investigated with the same degree of attention which has been given to the nucleus, and that it be followed through all periods of the life history in forms where the color becomes greatly modified or is absent in the reproductive cells and embryonic (meristematic) regions of the plant. Anyone who has studied the embryonic tissues of plants will realize the difficulties of the investigation which will probably involve the development of methods of technique, especially of staining, somewhat different from those generally employed in cell studies.

We may now consider the elements in the nucleus and their behavior during ontogeny. This is one of the most interesting subjects in cell studies, for the importance of the chromosomes

and chromosome history in relation to problems of development, heredity, hybridization, and variation is clearly understood, and these subjects have already been treated in Section V, "Cell Activities at Critical Periods of Ontogeny in Plants." Also some recent papers on the nucleolus of which Wager's (:04) is the most comprehensive, have brought this structure into very close relation with the chromatin content of the nucleus, and the nucleolus must now be considered in any treatment of the chromosomes. The problems hinge on what is termed the individuality of the chromosome, which is the question whether or not the chromosome is a structural entity maintaining its independence completely through each and all of the cell divisions in a life history. There is also involved the view that the chromosomes have come down from a line of ancestral structures, reproducing by fission in every mitosis throughout the history of the race.

There are two extremes in the views on this exceedingly interesting conception and also an intermediate position. The one extreme has recently been set forth by Boveri (:04) in a very clear statement. This view regards the chromosomes as structural entities, possibly elementary organisms, which maintain an organic individuality and independent existence in the cell. They are further regarded as in their typical form when present as rods or short filaments during mitosis. Their behavior in the resting nucleus is one of great metabolic activity which affects their morphology for the time being.

Those who are inclined to doubt the individuality of the chromosomes and to hold off from a full acceptance of the theory, base their attitude on the extreme difficulty or perhaps impossibility of following the chromosomes as entities through the resting nucleus from one mitosis to another. These difficulties are well known to those who have studied chromosomes even in nuclei which are most favorable for the investigation of their morphology. The chromosomes which enter the daughter nuclei from a mitosis generally lose their form and the chromatin becomes so distributed on a linin network or in a nucleolar structure that the outlines of the original structures become quite lost. Mottier (:03) in his recent studies on the spore

mother-cell of certain angiosperms has emphasized these points and Grégoire and Wygaerts (:03) have also shown the difficulties of following the chromosomes in the resting nuclei of the root tip and spore mother-cell of *Trillium*, stating that the structures become resolved into an alveolar network.

On the other hand Rosenberg (:04) claims that the chromosomes may be clearly recognized in the resting nuclei of some forms and cites *Capsella bursa-pastoris* as a particularly good illustration. In this plant the chromosomes are described as small granular bodies scattered throughout the nucleus in fixed number at various stages of ontogeny. Thus there are 16 in cells of the gametophyte and 32 in those of the sporophyte while 48 of these bodies were counted in the nuclei of the endosperm as would be expected if these nuclei are descendants of a triple fusion in the embryo-sac. Similar conditions are reported in other forms and there is considerable evidence giving weight to the view that chromosomes may be actually followed through all periods of the nuclear history in some favorable types.

Apart from the actual demonstration of the chromosomes in the resting nuclei and their recognition as structural entities through successive cell divisions there is much general evidence in support of the theory of the individuality of the chromosomes. This evidence lies in the nuclear fusions of fertilization and the mitoses of processes of segmentation that follow where the chromosomes are known to remain separate and have been distinguished as maternal and paternal. Also, as we have seen from the discussions of reduction phenomena at sporogenesis and the behavior of the chromosomes in hybridization, there are good reasons for believing that maternal and paternal chromosomes remain separate all through the sporophyte generation and are distributed to the offspring during sporogenesis. The importance of these events in the minds of all investigators has rested very largely on the behavior of the chromosomes and has led to the very general assumption that they must stand for units of organization and may be counted as constant factors in the problems of heredity. It is not necessary to adopt Boveri's extreme views to hold still the theory of the individuality of the chromosomes. Nor is it necessary to assume that the structures

have a distinct organization which holds throughout the life history. The form of the chromosomes certainly does change with different periods of the cell's history especially within the resting nucleus and yet the centers of chromosome activity may always be present to organize the chromatin into a new set of elements for the next mitosis. It is perhaps difficult to believe that the chromatin granules (chromomeres) find their way back to the same chromosome with the prophase of each mitosis but the existence of chromosome centers may be readily conceived within the resting nucleus which would hold the number of chromosomes true to the cell's history.

With respect to the nucleolus there is abundant evidence that the structure is not a permanent organ of the cell. When containing chromatin, the nucleolus is found in its characteristic globular state only during the resting condition of the nucleus. Its chromatic substance passes into the chromosomes at prophase of mitosis and the nucleolus generally disappears before metaphase. Or if any substance is left after the chromosomes are formed the remaining structure either gradually dissolves or is thrust forth bodily into the cytoplasm surrounding the mitotic figure where it disappears sooner or later. The nucleolus in higher types of mitosis never divides to pass on with the chromosomes to the daughter nuclei, but such a history is reported in the yeast cell. If the nucleolus has any function in heredity, as has been claimed (Dixon, '99), such function must relate to the chromosomes which contribute to its substance or derive material from it. Besides the nucleoli which are composed wholly or largely of chromatin, there are also those which seem to have little if any relation to the chromosomes. Such are well known in the spore mother-cells of higher plants and no investigator has been able to connect these with the formation of chromosomes as Wager (:04) has been able to do in the root tip. It was upon nucleoli of this class that Strasburger founded his theory that the structure was a mass of reserve material utilized by the kinoplasm during mitosis in the process of spindle formation. Such nucleoli generally fade away during the prophase of mitosis and either entirely disappear or the remaining substance is thrust out into the cytoplasm where it may some-

times be recognized as deeply staining globules (the so called extranuclear nucleoli).

There is left for our consideration that group of kinoplasmic structures termed centrosomes, centrospheres, and blepharoplasts which, when accompanied by radiations, are called asters. Some authors regard these structures as homologous and believe them to be present in one form or another as permanent organs of the cell in certain types (see discussion of Ikeno, :04). Against this view stand the well established facts of an increasing list of forms, both animals and plants, in which these structures unquestionably arise *de novo* at certain periods in the cell's history. To the author this evidence seems insurmountable and he cannot believe that the aster is in itself a permanent organ of the cell. We shall not take up the subjects of relationships here for such discussions have proved of little profit except in special cases where the various types of structure are found in closely related forms or in the same life history, and these have scarcely been studied at all. We know so little about the relationships in the thallophytes, where relationships must be sought if present at all, that a satisfactory treatment of the subject is hardly possible at present. One point seems to have escaped attention in the writings of those who have discussed the centrosome problem. The active elements of the asters are not the central structures (centrosomes, centrospheres, or blepharoplasts) but the fibrillæ which play such important parts as spindle fibers or cilia. This fibrillar condition of kinoplasm has a fixed place in the cycle of cell division appearing with each mitosis and at the time of cilia formation, but the fibrillæ are not permanent structures of the cell. There is some evidence that the centrosomes, centrospheres, and blepharoplasts are merely regions for the development and attachment of these fibrillæ and as such may stand as the morphological expression of fibrillæ-forming dynamic centers rather than as organs which actually induce the development of fibrillæ.

## 5. THE BALANCE OF NUCLEAR AND CYTOPLASMIC ACTIVITIES IN THE PLANT CELL.

Two regions of the cell are sharply distinguished from one another with respect to both morphology and physiology. They are the nucleus and the cytoplasm. The nucleus soon dies if isolated from cytoplasm and the latter, lacking a nucleus, cannot be kept alive indefinitely unless it be in organic connection with a nucleated mass of protoplasm. The necessary connection may be only through delicate strands, as was established by Townsend ('97), and also seems to be illustrated in the instances of intercellular protoplasm which Michniewicz (:04) reports are connected by delicate fibrillæ (plasmodesmen) with neighboring cells. Some very interesting adjustments of the nucleus and cytoplasm to one another have been reported in a series of investigations of Gerassimow beginning in 1890. His most recent papers of the past year (Gerassimow, :04a, :04b) present a general summary of his studies and constitute a very important contribution to the subject. They will furnish much of the material for this discussion.

Gerassimow has found that the cells of *Spirogyra* and other members of the Conjugales offer admirable material for the study of the relations between the nucleus and cytoplasm, and throw important light on the functions, physiological activities, and interdependence of both structures. By subjecting filaments of *Spirogyra* during cell division to a temperature of 0° C. or treating them for a short time to the anæsthetic influence of ether, chloroform, or chloral hydrate it is possible to arrest the processes of mitosis at different stages with the result that the protoplasm may become variously distributed in the daughter cells. (1) A daughter cell may be formed lacking a nucleus but containing a portion of the divided chromatophore in a peripheral layer of cytoplasm. (2) A single cell may contain the two daughter nuclei either separated from one another or more or less intimately associated and perhaps wholly fused depending upon how far the processes of mitosis have progressed before the cells have been subjected to the shock of the

experiment. (3) Binucleate cells may continue their growth with subsequent mitoses which when treated as before may give daughter cells with three nuclei and one nucleus respectively or with two each or indeed a cell containing four nuclei. Furthermore these nuclei may fuse with one another to give structures with a greatly increased chromatin content. (4) In place of the non-nucleated cells there may be formed chambers containing cytoplasm and chromatophores, but without nuclei, which remain in open communication with the nucleated companion protoplast because the cell wall is not formed entirely across the mother-cell.

Gerassimow has made some extended observations on these various types of cells, and presents his results in many elaborate tables and diagrams. We can only give an outline of his conclusions. (1) Cells which come to contain unusually large nuclei through the suppression of mitosis or by the reuniting of partially divided daughter nuclei increase proportionally in size and their further cell division is postponed. The nuclei of such cells have of course the peculiarity of an increased amount of chromatin content. The large nuclei may later fragment into two or more structures which separate and generally come to lie at a distance from one another in the cytoplasm. The fragments finally lose their powers of reproduction and exhibit marked evidence of degeneration. (2) Cells which lack nuclei may form starch in the usual manner in the presence of light and exhibit for a short time a weaker general growth than normal nucleated cells. The power to develop a gelatinous sheath also becomes markedly weakened. Finally there result a decrease in the volume of the cell, a fading of the chromatophore, and conditions which lead to eventual death. (3) Chambers which lack nuclei but are in protoplasmic union with nucleated cells may be contrasted sharply with the non-nucleated cells. They exhibit a much stronger growth for a longer time and with a greater power to form starch, although not so marked as in the nucleated cells, and the chromatophores retain their color. There is also a conspicuous development of the gelatinous sheath.

Haberlandt, Klebs, Pfeffer, Strasburger, and others have dis-

cussed the relations of the nucleus to the surrounding protoplasm with respect to both dynamics and morphology. Klebs ('88) indeed anticipated some of the work of Gerassimow, studying the non-nucleated cells of *Zygnema* and *Spirogyra* and noting the ability of their chromatophores to form starch in considerable quantities but the inability of the protoplast to add to the cell wall. Klebs was able to keep these non-nucleated cells alive in a sugar solution for from four to six weeks. But for the most part the discussions of the balance of nuclear and cytoplasmic activities in the plant cell have been very general in character.

Some principles have been, however, widely held for several years and may be summarized. The necessity of the nucleus to the life of the cytoplasm has been clearly understood but the studies of Klebs and Gerassimow indicate that the nucleus is not directly concerned with the process of photosynthesis which apparently may go on in non-nucleated cells as long as the cytoplasm retains a certain degree of vitality. A non-nucleated cell may enlarge slightly but it is not probable that the amount of protoplasm is increased. An especially interesting feature of non-nucleated cells is the inability of the outer plasma membrane to form cellulose walls or outer membranes. But the very interesting studies of Townsend ('97) have shown that this power may be retained provided the non-nucleated mass of protoplasm is connected by delicate cytoplasmic fibrils with a nucleated mass. It thus seems clear that the membrane-forming possibilities of the outer plasma membrane are absolutely dependent upon dynamic relations with the nucleus. While the chromatophore may carry on the processes of photosynthesis independently of the nucleus, nevertheless the general health of the cell requires the activities of the latter so that the nucleus becomes necessary to any extended photosynthetic work.

It has frequently been stated that the size of the nucleus is directly proportionate to the amount of cytoplasm in the cell. There are many favorable illustrations of this statement, as the extraordinarily large eggs of the gymnosperms, especially the cycads, whose nuclei are by far the largest in the plant kingdom. And in general an increase in the amount of cytoplasm is accom-

panied either by a marked enlargement of the nucleus with a corresponding increase in the chromatin content or by mitoses which distribute to the cytoplasm a greater number of nuclei whose sum total of material is very much greater than before. Conversely a sudden increase in nuclear material through nuclear fusions either sexual or asexual is followed almost immediately by general cell growth and increase in the amount of cytoplasm. However, such fixed growth relations between nucleus and cytoplasm can hardly be an established physiological law for certain highly specialized sperms have an insignificant amount of cytoplasm proportionately to the chromatin that is contained within the gamete nucleus. It is evident that the interrelations of the nucleus and the cytoplasm are so intimate that the growth activities of the one must benefit the other, but that this principle can be formulated in definite mathematical ratios seems improbable.

The dependence of the nuclei upon favorable situations in the cytoplasm is clearly shown in cells when a partial or general nuclear degeneration takes place. Thus during the processes of oögenesis in the Peronosporales, Saprolegniales, and in Vaucheria there is present a period when the most of the numerous nuclei within the oögonia begin to break down and finally become disorganized. The causes of the nuclear degeneration are not entirely clear but apparently the organ is unable to supply all of the nuclei in their respective situations in the cytoplasm with the conditions necessary for their life. There is consequently a sort of struggle for existence among these numerous nuclei and only those that are favorably placed in the cell are able to survive. In all forms the surviving nuclei occupy a situation in the center of the masses of protoplasm which are to become the eggs and those that break down are at or near the periphery of the cell. In several genera (*e. g.*, *Albugo*, *Peronospora*, *Pythium*, *Sclerospora*, *Saprolegnia*, and *Achlya*) the surviving nuclei seem to owe their good fortune to a very close association with the cytoplasmic structure termed the cœnocentrum. The cœnocentrum is a clearly differentiated region of the cytoplasm and is probably the morphological expression of a dynamic center in the eggs of these fungi. Stevens' ('99, :01)

studies on *Albugo* showed that the *cœnocentra* exert a chemotactic influence upon the nuclei in their vicinity, drawing them towards the mass of granular material in this favored region of the cell, and it is clear that they are greatly benefited in this situation since they increase in size while the nuclei at the periphery break done. This subject is discussed in detail in my paper on *Saprolegnia* (Davis, :03, pp. 240–243) a form which also illustrates exceptionally well the same principles of a survival of certain nuclei among many which degenerate, because of their favorable position in the central region of the eggs in close proximity to *cœnocentra*. There are then undoubtedly regions of the cell more favorable for the nutrition of nuclei than others and the positions of these may be marked by morphological characters as illustrated in the *cœnocentra*. That similar dynamic centers may also be present when there is little morphological evidence of their existence is indicated in the processes of oögenesis in *Vaucheria* (Davis, :04) which exhibits the same principles of extensive nuclear degeneration as are found in the *Peronosporales* and *Saprolegniales* and the survival of a single nucleus in the oögonium, apparently because it comes to lie in a mass of granular cytoplasm near the center of the oögonium.

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