I. Introduction and History

The desirability of a study of fertilization in the Mosses was suggested by the fact that this is the one great group of plants in which no thorough investigation had been undertaken concerning nuclear fusion. The question of fertilization in the Liverworts, which are similar to the Mosses in their structural aspects, has received considerable attention in recent years. In the Liverworts three distinct types of nuclear fusion are encountered. Inasmuch as the observations regarding fertilization in the Mosses are so meagre and since the processes in the Liverworts are so diverse, the present investigation was undertaken.

The literature contains fragmentary observations on the subject, the earliest of which is that of Hofmeister ('62), who observed in Funaria an antherozoid moving down the neck of an archegonium which was ready for insemination. In the case of dioecious species no fruit or sporophyte was formed unless male and female plants were growing in the same locality. He observed that the young sporophyte when consisting of from one to four cells remained free in the ventral cavity, but, after further division, grew down into the tissue of the archegonium.

Roze ('72), studying the development of the archegonium in Sphagnum, depicted an archegonium with several antherozoids in the neck canal and one antherozoid in contact with the egg. The antherozoids entered with the ciliated portion foremost and remained in the ventral cavity. The thickness of the archegonial wall prevented him from determining the progress of penetration of the antherozoid into the egg.

An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.
Arnell ('75) observed, during an examination of archegonial material of *Discellium nudum* (Dicks.) Brid., one plant of which the archegonium had a visible canal. During his observations the top cells separated and the archegonium opened. The antherozoids were drawn to the mouth of the archegonium as if attracted by a magnetic power. In addition he noticed a rocking motion of the egg due to the movement of the antherozoids which were surrounding it.

Gayet ('96), in connection with his study of archegonial development in *Bryum capillare* L., observed antherozoids swimming down the neck canal and one of them penetrating the egg. After penetration the antherozoid assumed a crescent shape and became located above the egg nucleus.

In *Fissidens incurvus* Schwaegr., Gayet perceived that in fertilization a large number of antherozoids penetrated the egg, but that only one united with the egg nucleus. The antherozoid, in the cytoplasm of the egg, became crescent-shaped at first and then spherical. The female nucleus possessed four chromosomes which were attracted to the male nucleus. After fusion only four chromosomes were discernible in the fertilized egg, and Gayet inferred that each male chromosome fused with a female.

W. and J. Van Leeuwen-Reijnvaan ('08a) reported a very bizarre process in the fertilization of *Polytrichum*. From their observations they inferred that reduction divisions occurred both in the archegonium and in the antheridium. The ventral canal cell and the egg then fused, and the product of this fusion was fertilized by two antherozoids. In this way the sporophyte had double the original number of chromosomes. The same authors reported a similar situation in *Mnium*.

Wilson ('09) found in *Mnium*, however, that reduction division took place in the division of the spore-mother-cells to form spores. In studying spermatogenesis in other Bryophytes he found no evidence of a reduction division in the formation of the antherozoids.

Vandendries ('12) studied spermatogenesis in *Polytrichum* with reference to the chromosome number. He showed quite conclusively that there was no double reduction occurring in the formation of the antherozoids.
Walker ('13) found no fusion of the egg with the large ventral canal cell of *Polytrichum*. He contended that the bizarre process described by J. and W. Van Leeuwen-Reijnvaan was doubtful since his studies showed no such process occurring.

Bryan ('20) described in complete detail the fusion of the ventral canal cell and the egg of *Sphagnum subsecundum* (Nees) Limpr. His results indicated that the number of cases in which this abnormal fusion occurred about equalled those in which the ventral canal cell had disintegrated. He reported two cases in which the egg nucleus disintegrated and in which the nucleus of the ventral canal cell remained distinct and sharply defined.

Harvey-Gibson and Miller-Brown ('27) published a preliminary note on the fertilization of Bryophytes. Their work consisted of observations made on mites which visited both male and female heads of *Polytrichum commune* L. The mites carried sperms on their bodies from antheridial heads to archegonial heads and in this way brought about insemination of distant archegonial heads.

The Liverworts, on the other hand, have received considerable attention within recent years. Rickett ('23) has summarized the earlier literature on the subject, and it need not be repeated here.

In *Sphaerocarpos*, according to Rickett, the male nucleus after penetration into the cytoplasm of the egg swells markedly, becoming spherical and reticulate but remaining smaller than the female nucleus. The two gametic nuclei come into contact but remain distinct until each has organized its chromosomes preparatory to mitosis. The two nuclear membranes then disappear and a spindle figure is formed, thus initiating the metaphase of the first embryonic division.

Showalter ('26, 27a, 27b, '28) has made careful studies of the fertilization processes in three of the Anacrogynae. In *Riccardia* ('26) the male nucleus remains, with scarcely any perceptible change of form, in the cytoplasm of the egg for from twenty-nine to thirty-six hours. Then it penetrates endwise and passes slowly into the female nucleus, where it forms first a vesicle of deeply staining chromatic material, and later a compact reticulum that loosens up more and more until after three to four days it
is no longer distinguishable from the reticulum of maternal chromatin.

In *Pellia* ('27b) the male nucleus, after penetration into the egg, moves slowly toward the female nucleus and gradually assumes a reticulate form. The cytoplasm between these two nuclei recedes, leaving the mass of paternal chromatin almost in contact with the membrane of the female nucleus. The membrane of the female nucleus seems to dissolve in the region of contact with the paternal chromatin, and a common membrane encloses the paternal chromatin and the female nucleus. The union of the two nuclei occurs usually the second day after insemination. The paternal chromatin quickly assumes the condition of the maternal chromatin, and except for the presence of two nucleoli the dual nature of the fusion nucleus is distinctly evident for a short time only.

In *Fossombronia* ('27a) actual penetration of the male nucleus into the female nucleus was not observed, although Showalter found that after forty-eight hours the chromatic mass about the nucleolus had become a reticulum which occupied approximately one hemisphere of the nuclear cavity. In the other hemisphere was a dense mass of chromatic substance which was more intensely stained than the chromatic reticulum, and it seemed probable that this dense mass was the substance of the male nucleus.

Showalter ('28) has studied hybrid fertilization in four varieties of *Riccardia pinguis* (L.) S. F. Gray. In this study he found that nuclear fusion between the four types was in accord with that described in his earlier paper on *Riccardia* ('26).

The salient points in these investigations are shown in table II, where they are compared with the results obtained in *Funaria*.

**II. Materials and Methods**

*Funaria flavicans* Michx. is similar in its morphological features to *Funaria hygrometrica* (L.) Sibth., with which it is often associated in nature. It may be distinguished from *Funaria hygrometrica* by its smaller size, its erect pedicel, and its more pointed leaves. The capsule, which is furrowed less deeply than that of *Funaria hygrometrica* and which has a non-apiculate lid,
matures a week or two earlier. *Funaria hygrometrica* is almost cosmopolitan in its distribution, whereas *Funaria flavicans* has been reported only from the mid-central and southern portions of the United States. Both species are monoecious. *Funaria flavicans* is abundant around St. Louis, Missouri, and was found to grow well in cultures. Hence, it was selected for the experimental work described in this thesis.

Mature sporophytes were obtained in the spring of 1929, near Festus, Missouri, where a great number of plants were growing on an outcrop of St. Peter sandstone. Specimens of the material were sent to Dr. A. J. Grout, who kindly verified the identification. The spores were sown on a sterile mixture of sand and soil in six-inch pots which were then set in granite pans containing tap-water. This procedure permitted the soil to remain moist and at the same time prevented spontaneous insemination. The cultures were kept in a north greenhouse in which the temperature was relatively cool. When both archegonia and antheridia were mature insemination was brought about by flooding the cultures. The pots were tightly corked from below, placed in a container of water, and then covered with water. These precautions were taken in order that the water would not seep down into the soil, carrying the antherozoids with it. At the end of half an hour, that being the time determined necessary for the antherozoid to escape from the gelatinous envelope surrounding it, the pots were removed from the container, uncorked, and the excess water permitted to drain out at the bottom of the pots. Fixations were made at intervals after flooding. The killing fluids employed were chromo-acetic, Flemming's medium, Showalter's modification of Flemming's medium ('26), Navaschin's chromo-acetic formalin as described by Babcock and Clausen ('29), and Benda's fluid. Although Flemming's medium and Benda's fluid produced perceptible plasmolysis, Showalter's modification and Navaschin's fixative gave excellent results.

After washing, dehydration, infiltration and imbedding in paraffin, the material was sectioned and stained. Sections cut 12 μ in thickness often included the entire egg in one section. The material was stained, some with Flemming's triple stain,
some with Haidenhain's iron-alum haematoxylin, and some with the gentian violet-iodine combination. The iron-alum haematoxylin did not give as clear nuclear differentiation as was obtained with the triple or with the gentian violet-iodine combination.

In studying the slides, recourse was made to the standardized scheme of systematic and objective observation developed by Fry ('30) in connection with his studies of fertilized echinoderm eggs and recently outlined by him.

III. Observations

1. SPORE GERMINATION AND DEVELOPMENT OF THE LEAFY SHOOT

The first visible indication of germination of spores sown on soil is the modification in spore color. The spores when sown have a definite orange color, but with the development of more chlorophyll in the protoplasts the orange color is gradually transformed first to a brown, then to a deeper brown, until the distinction between the brown color of the spore and the green color of the developing protonemata is scarcely perceptible. These faint patches of green color are discernible three days after the spores are sown. The development of the protonemata continues rather rapidly, and under favorable temperature conditions, around 20° C., the entire pot is covered with protonemata within four weeks. After six to seven weeks the first leaves of the gametophores appear. Their development continues, and within three months the antheridia are discernible at the tips of leafy branches as small green knob-like structures. As the antheridia mature they change in color from green to bright orange, and with the discharge of the antherozoids they become very dark brown.

Since Funaria flavicans Michx. is monoecious the archegonia are developed on the same plant as are the antheridia. However, the archegonial heads arise as lateral branches of the male gametophore. Their development takes place at a later date, and the relationship of the archegonial branch to the antheridial when the antheridia are maturing is indicated in fig. 1. Following insemination and fertilization the archegonial branch develops rapidly and quickly surpasses the antheridial branch in size, until the latter is quite insignificant in comparison with the former.
The young sporophyte is visible to the unaided eye within ten days after fertilization. At this time its structure is exceedingly long and narrow, indicating a very small mass of potential sporogenous tissue, but this tissue increases in amount with continued growth of the sporophyte. The sporophyte with fully developed spores reaches maturity five weeks after insemination.

2. DEVELOPMENT OF THE ANThERIDiUM

The antheridium develops from a superficial cell (pl. 43, fig. 1). Nuclear division takes place and a cross wall is laid down between the two daughter nuclei (pl. 43, fig. 2). The division of these cells continues, with walls being laid down at various angles, until a many-celled structure is developed (pl. 43, fig. 3). The outer layer of cells develops into the wall of the antheridium, and the two cells at the apex are characterized by their unusual size (pl. 43, fig. 4). These two cells function in the discharge of
the antherozoids. The antherozoid when discharged is surrounded by a gelatinous envelope which is readily dissolved, after which the antherozoid may be observed swimming about (pl. 43, figs. 5–6). In material which has been fixed and stained the two cilia are very distinct, but in living material these structures are not readily observed.

In the antheridial head are found sterile hairs or paraphyses which are multicellular and contain numerous chloroplasts. In very young heads it is observed that these paraphyses are filiform and very similar to those found in the archegonial heads (pl. 43, fig. 7). The changes undergone in the transition from the filiform to the clavate condition are unusually interesting and apparently have never been described (pl. 43, figs. 8–9). The nucleus in the one-celled filament possesses a very large nucleolus which in a later stage is apparently in the process of division and represents what might be interpreted as an intra-nuclear division (pl. 43, fig. 10), such as is found in certain of the lower fungi. The cytoplasm of the young paraphysis contains very definite rod-shaped structures that increase in thickness and later are distinguished as early stages in plastid development. In comparing living material with fixed material the stages in plastid development are readily observed. In the unicellular paraphysis the chloroplasts are elongated structures which appear as rods in fixed material. These chloroplasts change from the elongated rods into small spherical bodies. In the mature clavate paraphysis the chloroplasts have increased in size and their structure is characteristic of the mature plastid.

There is a considerable number of mature antheridia in one head with the majority in the same stage of development, that is, nearly all the antheridia develop concurrently and mature almost simultaneously. Occasionally an antheridial head is found in which a number of developmental stages was found, but this was somewhat rare.

3. DEVELOPMENT OF THE ARCHEGONIUM

The archegonium, like the antheridium, develops from a superficial cell, and in the very early stages it is impossible to distinguish a young archegonium from a young antheridium
(pl. 43, fig. 11). In somewhat later stages the paraphyses may be used as a criterion, since they are characteristically different in the two kinds of heads. In the archegonial head they are always unicellular and filiform and may be distinguished from the young filiform paraphyses of the antheridial head by the less dense cytoplasm and by the fact that they are long and slender, whereas those of the antheridial head are shorter and more nearly uniform in transverse diameter (pl. 43, fig. 14).

The cell which gives rise to the egg and the ventral canal cell is located in the basal portion of the archegonium and completely fills the ventral cavity (pl. 43, fig. 15). The division of this cell is somewhat unequal, the egg receiving approximately three-fourths of the cytoplasm of the original cell, the ventral canal cell the remaining fourth. The fact that there is an invagination of cytoplasmic material at the point where the two cells divide gives one the impression that division is brought about by furrowing rather than by formation of a cell plate, but this invagination may be due to the rounding up of the newly formed cells (pl. 43, fig. 16). An insufficient number of observations prevents the writer from making any precise deductions.

The ventral canal cell, after formation, has a clearly defined nucleus. This disintegrates eventually and in later stages is recognized merely as a dense mass within the cytoplasm (pl. 44, fig. 17). When this nucleus ceases to be recognizable as such the beginning of disorganization of the neck canal cells may be observed, the ventral canal cell disintegrates along with the other canal cells, and the egg is ready for fertilization.

The number of archegonia in a head varies from one to many. These are generally at various stages of development, although there may be two mature archegonia in one head at the same time. Both of these archegonia may contain eggs in the same stage of fertilization. This is encountered in about 17 per cent of the material examined, and in one plant three mature archegonia were found with their eggs in precisely the same stage of fertilization. The archegonia apparently exercise no ill effects on each other, for several instances were noted in which two young sporophytes in a multicellular condition were found in the same head. These sporophytes showed no indication of being in a dwarfed condition.
4. FERTILIZATION AND NUCLEAR FUSION

In material fixed immediately after flooding, the spherical egg with densely granular cytoplasm is found either in the central portion of the ventral cavity or toward the bottom. The unfertilized eggs range in diameter from 7.0 to 8.6 μ, whereas the ventral cavities range from 8.0 to 12.6 μ. The nucleus, which occupies the central portion of the egg, is from 2.7 to 3.3 μ in diameter and possesses a very large nucleolus. Very little chromatin material, other than the nucleolus, could be definitely recognized. However, a distinct granular zone was differentiated about the nucleolus (pl. 43, fig. 18). At this particular stage only one archegonium was found in which the antherozoids had passed down the canal and were near the egg. The gelatinous envelope which surrounds the antherozoid when discharged from the antheridium requires from twenty to thirty minutes for dissolution. Inasmuch as this period of time is required and since the neck canal is exceedingly long, it is probable that in material fixed immediately after flooding the antherozoids have not had the opportunity to reach the egg in the archegonium.

With complete dissolution of the gelatinous envelope the antherozoids swim down the neck canal and into the venter. A large number of them approach the egg so that it has the appearance of being covered with very fine threads (pl. 43, fig. 19). Immediately following the entrance of the antherozoids into the venter a mucilaginous plug appears in the canal (pl. 43, fig. 20). This plug seems to be a secretion of the first two tiers of cells above the venter, inasmuch as it is always found associated with these two tiers of cells and never with any others. The plug, furthermore, is connected in some way with the process of fertilization. In all archegonia in which antherozoids have entered, the plug is present and remains not only throughout the fertilization process but also in early stages of sporophytic development. A few archegonia are found in which the ventral canal cell has not completely disintegrated and into the venter of which antherozoids have entered. In these no indication of the mucilaginous plug is discernible. The plug, which stains less deeply than the egg or any of its components, appears to have the ability to prevent the entrance of any more antherozoids, because
in the majority of cases there is present above the plug a large number of antherozoids tangled together. In some instances one to several antherozoids have been caught in this mucilaginous secretion.

The antherozoids approach the egg from all angles and tend to become closely adpressed to its surface which is slightly indented along the lines of contact (pl. 44, figs. 21–22). Those antherozoids, which become adherent to the egg, take a deeper stain than do those which are present in the ventral cavity and in the canal, since whenever the triple stain has been used the former are violet in color whereas the latter are red—depending upon the intensity of the stain. Notwithstanding the fact that the antherozoids lose their cilia on becoming adpressed to the egg, those present both in the canal and in the cavity are still equipped with cilia.

The antherozoid is a long slender structure with the anterior region somewhat enlarged and spherical. The two cilia which are present are attached to the antherozoid at this anterior region.

The antherozoid after becoming attached to the surface of the egg begins to pierce the membrane in its spherical portion (pl. 44, fig. 23). The substance of the antherozoid gradually passes into the cytoplasm of the egg, after which the original form is resumed. The antherozoid, in 63 per cent of the cases observed, penetrates the surface of the egg in the side toward the base of the archegonium. If penetration has not occurred in this region it takes place at one side (30 per cent of all cases observed) of the nucleus and only rarely above (7 per cent) the level of the nucleus. The time required for penetration is rather short, material fixed two hours and twenty minutes after insemination showing antherozoids in the cytoplasm of all the eggs in condition to be fertilized.

The entrance of an antherozoid into the cytoplasm of the egg seems to stimulate it to increase gradually in size. With this gradual increase in size there is a concurrent increase in the size of the ventral cavity as well as in the width of the archegonium. The size of the egg nucleus, however, is affected only slightly by this penetration of the antherozoid. Table 1 gives the average
measurements of these structures and indicates the gradual increases in size.

**TABLE I**

<table>
<thead>
<tr>
<th>Fixation number</th>
<th>Interval after flooding</th>
<th>Egg diameter in microns</th>
<th>Ventral cavity diameter in microns</th>
<th>Archegonium diameter in microns</th>
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<tbody>
<tr>
<td>1</td>
<td>5 minutes</td>
<td>7.7</td>
<td>10.1</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>35 minutes</td>
<td>7.1</td>
<td>10.9</td>
<td>—</td>
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<td>12.3</td>
<td>35.6</td>
</tr>
<tr>
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<td>8.5</td>
<td>12.8</td>
<td>40.1</td>
</tr>
<tr>
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<td>13.8</td>
<td>37.0</td>
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<tr>
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<td>40.0</td>
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<td>38.6</td>
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<td>14.0</td>
<td>36.4</td>
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<td>12.6</td>
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<td>12.5</td>
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<td>13.9</td>
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<td>16 hr.</td>
<td>8.8</td>
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The variations in the widths of the ventral cavity and of the archegonium are explained by the fact that the region containing the egg is not always the median portion of the archegonium. If the egg is situated toward the outer area of the ventral cavity the archegonium will be narrower in width at this point than in the region directly at the center of the ventral cavity.

Penetration, moreover, is not restricted to a single antherozoid. Instances of polyspermy, however, are relatively few, only a small number being noted and these in the very early stages soon after
insemination. It is quite likely that the supernumerary antherozoids disintegrate, inasmuch as no case was found at a later stage in which they were present in the cytoplasm of the egg. Plate 44, fig. 26, shows an egg in which the spherical portions of several antherozoids have penetrated, the elongated portions not being readily distinguishable in the dense cytoplasm.

In two instances where the ventral canal cell had not completely disintegrated both the aforementioned cell and the egg were surrounded by antherozoids. There is no indication, however, that the antherozoids ever penetrate the ventral canal cell.

The antherozoid does not remain in the cytoplasm in a quiescent condition. It becomes shorter and thicker and passes to a position near the female nucleus immediately after penetration is completed (pl. 44, fig. 27). In material fixed three hours and thirty minutes after insemination the male nucleus has come in contact with the egg nucleus (pl. 44, fig. 28).

There was but one case observed in which the supernumerary antherozoids were still surrounding the fertilized egg. Those which do not penetrate the membrane apparently disintegrate, for with this one exception no instances have been found in which the supernumerary antherozoids remain adjacent to the egg longer than three hours and thirty minutes after insemination. On the other hand, those antherozoids which are present in the cavity are still recognizable as such in material fixed ten hours and thirty minutes after insemination.

The cytoplasm becomes less dense with the entrance of the antherozoid, and material fixed in Flemming's medium and stained with the iron-alum haematoxylin shows the presence of very definite rod-like and spherical bodies (pl. 44, fig. 35). The dense mass about the nucleolus becomes less granular and moves toward the periphery of the nuclear cavity, leaving a clear zone about the latter structure. The cells of the archegonium become vacuolated, and in the basal portions mitotic figures occasionally are found.

The male nucleus as it comes in contact with the female nucleus causes a depression in the surface of the latter. The antherozoid or sperm nucleus becomes shorter and thicker and is distinguished at one side of the female nucleus or below it as
a slightly elongated ovoid structure (pl. 44, figs. 29, 30). The male nucleus penetrates the female nucleus, and its chromatin substance passes into the female nuclear cavity where it assumes a more or less definitely ovoid form (pl. 44, figs. 31, 33, 34). The exact method of penetration could not be determined because of the minute size of the nucleus. Whether the membrane disappears at the point of contact has not been definitely determined. The membrane is not visible at the point where the male nucleus is in contact and later enters, but immediately after complete entrance a membrane is again very distinct and definite.

The male nucleus, moreover, does not remain in a resting state. The nucleolus of the egg nucleus, which is in reality the condensed reticulum enclosing the true nucleolus, tends to become vacuolate after entrance of the male nucleus into the female nucleus, and the mass of paternal chromatin is attracted to it (pl. 44, fig. 32). The dense mass which previously surrounded the condensed reticulum has practically disappeared, and about the two masses present in the nuclear cavity there is distinguished a zone which is quite clear. During the entrance of the paternal chromatin a definite staining area is evidenced, indicating the penetrating substance. The two distinct masses of chromatin tend to come together in the center of the cavity. The nuclear membrane becomes irregular in outline and eventually disappears completely (pl. 44, figs. 36–39). The two chromatin masses come into contact and gradually fuse, the two bodies being vaguely distinct and discernible only as darker-staining regions (pl. 44, fig. 40; pl. 45, fig. 41). The complete intermingling of the two masses of chromatin is more or less gradual.

The fused mass of chromatin is easily recognized by the fact that it is a somewhat spherical body with specific regions that are much darker than others. It remains in the central portion of the egg and without any perceptible membrane for several hours after fusion. During this time there is no apparent change in the structure of the fusion product. The cytoplasm is less granular at this stage and tends to become somewhat vacuolated toward the periphery of the egg. After ten to twelve hours the granular portion of the cytoplasm tends to become aggregated about this fusion nucleus. The aggregated cytoplasm
shows a tendency to become dense and gives the appearance of a definite granular zone similar to the one observed in the earlier stages. As this zone increases in density the outlines of a membrane being laid down become visible, at first considerably irregular, but gradually more regular and definite (pl. 45, figs. 44-48).

The fusion nucleus, as it is now recognized, remains in a resting stage for some time. A fertilized egg in this stage is distinguished from an egg just prior to insemination by its larger size, as well as by the appearance of the condensed reticulum.

In some of the material fixed forty-five hours and twenty-five minutes after insemination, the mucilaginous plug indicating that insemination has occurred, very definite plastid-like bodies are observed in the egg. These bodies are much too large and regular in appearance to be considered as chondriosomes. The condition of the nucleus is somewhat masked from view by the presence of these plastid-like bodies. In later stages, however, no such bodies are discernible, and it is a matter of speculation whether their preservation in this case is attributable to the particular fixative used, Benda's fluid, or whether the egg was not fertilized, as a result of which the plastid-like bodies developed.

The nucleus remains in the resting condition for a considerable period, after which it undergoes the changes for the prophase of the first division. The condensed reticulum, or the body which represents the fusion of the maternal and paternal chromatin, presents an appearance similar to that found in other nuclei at an early prophase stage in the division process. The chromatin becomes transformed into a spireme which is located not in the peripheral portion of the nuclear cavity as is customary, but within the region previously occupied by the condensed chromatin (pl. 45, fig. 50).

The first division of the nucleus and cell is transverse to the long axis of the egg and the archegonium. The two daughter nuclei which are formed tend to pass through a short resting stage before going through the second division which is at right angles to the first. There is an enormous increase in the size of the cell with the formation of the daughter nuclei. An embryo
in the bi-nucleate condition has a diameter of 25 μ, whereas the fertilized egg in the later stages before division has a diameter of 11 μ. The nuclei are quite large, possessing large nucleoli, and present an appearance similar to that found in the resting nuclei of the mature egg (pl. 45, figs. 51, 52). Further divisions occur rapidly until a multicellular sporophyte is formed. This sporophyte remains free in the ventral cavity and does not grow downward into the tissue of the archegonium until some time later. The archegonium increases considerably in size, and this increase is correlated with the increase in size of the young sporophyte.

5. SPORE FORMATION

The sporogenous tissue originates as a single row of cells toward the outer periphery of the columella. The cells are at first rectangular and are in an active stage of division. They increase in size and become rounded off so that at the time they are matured into spore-mother-cells they are quite spherical in shape.

The nucleus of the spore-mother-cell divides, and the resulting daughter nuclei go to opposite ends of the cell. These daughter nuclei do not appear to undergo a resting stage but pass from a very late telophase into the early prophase of the second division. In the second division the plane of division of one daughter nucleus is at right angles to that of the other daughter nucleus. This conclusion is reached from the fact that the majority of spore-mother-cells shows only three nuclei in focus, the fourth nucleus being seen when the focus is changed (pl. 45, figs. 54–56).

Cytokinesis of the spore-mother-cells is by cell-plate formation, the cytoplasm displaying no indication of furrowing either after the first division or after the second division. No walls are laid down after the first division, but those formed after the homeotypic division are laid down before the daughter nuclei are completely reconstructed.

6. CHROMOSOMES

An attempt was made to determine not only the structure of the chromosomes but also the specific number. Mitotic figures are frequently found in various tissues of the plant. The lower
portion of the archegonium shows a large number of mitotic figures, one of which gives an excellent polar view (pl. 45, fig. 57). By careful focusing, ten chromosomes can be brought into view. It is very likely that these represent both poles, inasmuch as the sections were cut rather thick. With the highest magnification available, it has been impossible to determine the exact number of chromosomes. Mitotic figures in the antheridium are less helpful than those of the archegonium. Certain of the spore-mother-cells, after the first division, show the chromosomes being transformed into the spireme of the daughter nuclei. Such figures display approximately ten short rods becoming more or less entangled with one another. Previous to the homeotypic division very definite chromosomes, unusually small and irregular in shape, were observed, but the exact number could not be determined.

IV. Discussion

The fertilization process in Funaria flavicans varies considerably from the processes described in the Bryophytes which have been investigated by other authors.

The mature egg of Funaria, at the time of insemination, is much smaller than that of any Liverwort studied. In Sphaerocarpos the egg is 40 x 20 μ, in Fossombronia it is about 25 μ in diameter, in Riccardia about 20 μ, whereas in Pellia no actual measurements are given although it is stated to be larger than that of Riccardia. In Funaria the average size of the egg at the time of insemination is 7.7 μ in diameter, a third the size of any other egg studied. The nucleus is correspondingly small. In Sphaerocarpos the nucleus of the mature egg is 13 x 10 μ, in Fossombronia, as well as Riccardia, it is about 10 μ, and in Funaria 2.9 μ, a third the size of the other nuclei. The volumes, determined from these measurements, give a much better indication of the comparative sizes of the structures studied in these different forms. These volumes are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Egg</th>
<th>Nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphaerocarpos</td>
<td>14,137.9 cu. μ</td>
<td>796.3 cu. μ</td>
</tr>
<tr>
<td>Fossombronia</td>
<td>8,181.9 cu. μ</td>
<td>523.6 cu. μ</td>
</tr>
<tr>
<td>Riccardia</td>
<td>4,189.0 cu. μ</td>
<td>523.6 cu. μ</td>
</tr>
<tr>
<td>Funaria</td>
<td>239.1 cu. μ</td>
<td>12.8 cu. μ</td>
</tr>
</tbody>
</table>
The relatively small size, not only of the egg but of the nucleus as well, increases the technical difficulties and may be one of the reasons that no previous cytological work has been done on the fertilization of the Mosses.

Rickett ('23) observed a quantity of mucilaginous material resulting, supposedly, from the disintegration of the ventral canal cells and the neck canal cells in *Sphaero carpos*. This material not only filled the neck canal but also a part of the venter and was seen extruding from the neck. No such mucilaginous material was observed in the other Liverworts that have been studied, nor was it seen in *Funaria*, although an apparently similar phenomenon was observed. This will be discussed later.

The manner of the penetration of the antherozoid into the cytoplasm of the egg is dissimilar in the Bryophytes that have been studied. In *Sphaero carpos* the membrane of the egg is very delicate and thin, and actual penetration of the antherozoid into the cytoplasm of the egg was not observed. The process is presumably instantaneous, for in eggs fixed fifteen, twenty, or forty-five minutes after insemination the antherozoid was observed as a slender curved body in the cytoplasm. The entrance of the antherozoid was not restricted to any one portion of surface of the egg. Some entered at the distal end, others at the basal end, whereas still others entered at one side.

In *Riccardia* penetration of the antherozoid is a gradual process. The antherozoid becomes applied to the surface of the egg which becomes depressed along the line of contact, and the antherozoid or its nucleus passes laterally into the cytoplasm. Material fixed twenty to thirty minutes after insemination showed the antherozoid in the surface membrane of the egg. In *Pellia* penetration is similar to that in *Riccardia*. In *Fossom bronnia* penetration seems to be nearly instantaneous, accompanied by a swelling of the antherozoid. In plants fixed six minutes after insemination a number of eggs was found which had been penetrated by antherozoids.

In *Funaria* the time required for penetration is somewhat longer. In material fixed one hour and thirty-five minutes after flooding only three out of seventeen eggs examined showed
partial penetration of antherozoids. However, in material killed two hours and twenty minutes after flooding all eggs showed complete penetration of the antherozoid. In *Funaria*, moreover, there is a tendency for the antherozoid to enter at the basal end of the egg, although some showed penetration at one side and a very few at the distal end of the egg. It is doubtful whether this basal end functions as a "receptive spot" as stated by Shaw ('98) in *Onoclea*. In *Onoclea* there is a definite concavity of the egg, which is not present in *Funaria*.

The length of time which the antherozoid remains in the cytoplasm is not the same for all Bryophytes that have been studied. In *Sphaerocarpos* the male nucleus remains in the cytoplasm approximately forty-six hours; in *Pellia* for about twenty-four to thirty-six hours, during which it undergoes a change in form, preparatory to nuclear fusion; in *Riccardia* it remains almost without change of form in the cytoplasm of the egg for the same length of time, after which it begins an endwise penetration of the female nucleus. In *Fossombronia* actual penetration of the male nucleus into the female nucleus was not observed. In *Funaria* the male nucleus does not remain in the cytoplasm for a long period of time, but almost immediately undergoes a change in form and position. In eggs fixed three hours and thirty minutes after flooding the male nucleus, in the majority of cases, was found to be in direct contact with the nucleus of the egg.

The greatest variation between these species exists in the methods of nuclear fusion. *Sphaerocarpos* displays the type in which the nuclei come in contact with each other, the chromatin material undergoes the formation of chromosomes, the nuclear membranes disappear, and the first division of the zygote occurs.

The penetration of the male nucleus into the female nucleus of *Fossombronia* has not been observed. The fusion nucleus shows the two masses quite distinct in the nuclear cavity.

In *Pellia* the male nucleus comes in contact with the female nucleus, whereupon the membrane of the latter disappears at the point of contact. A new membrane which is formed about the male nucleus is continuous with the female nucleus, and as a result the two nuclei are surrounded by a common membrane.
Fusion occurs, and the fusion nucleus is distinguished by the presence of two nucleoli.

In *Riccardia* the male nucleus penetrates endwise by piercing the membrane. The passage of material into the female nucleus is very slow. The two masses of chromatin are quite distinct, each occupying separate regions of the nuclear cavity, but these become optically indistinguishable before division is initiated.

The situation as described in *Funaria* offers a good many points of contrast. Penetration of the male nucleus into the female nucleus occurs very shortly after insemination. The process of penetration is similar to that in *Riccardia*, but the behavior of the two masses of chromatin is distinctly different in the two cases. In *Funaria* there is a very definite fusion of the two masses with the disappearance of the nuclear membrane. The fusion nuclear body, resulting from the coalescence of the male nucleus and the condensed reticulum of the egg, remains very distinct and definite in the central region of the egg, but is for a time not delimited by any perceptible membrane. The reappearance of the membrane some time after fusion is another point of contrast, and the fact that the chromatin material, at the inception of the prophase of the first mitosis, is distinguished at the periphery of the fusion nuclear cavity places *Funaria* in a category by itself, as far as fertilization is concerned. This particular method, distinct and unique, has not been described for any species of plant.

The fact that in all Bryophytes previously studied there is a prolonged period of time before the fusion of the two nuclei is interesting. There seems to be a very definite period in the Liverworts during which the male and female nuclei retain their identity. In *Funaria* penetration of the male nucleus into the female nucleus is followed shortly by their fusion. However, the fusion body remains in the cytoplasm of the egg for some time before mitosis occurs. Hence, the two-celled embryo is encountered in *Funaria* about the same number of hours after flooding as it is in *Sphaerocarpos*.

Rickett (’23) finds that the length of time required in *Sphaerocarpos* for visible sporophytic development is from two to eight weeks. However, in *Funaria* the length of time required for mature sporophytic development is five weeks.
The ferns, which also belong to the group of plants known as Archegoniates, have distinct methods of nuclear fusion. The processes of nuclear fusion which have been described in the ferns do not present any points of similarity to the process found in Funaria.

Table II represents in a condensed form the more essential points of contrast between the fertilization processes of Funaria and the other members of the Bryophytes which have been studied.

The presence of a mucilaginous plug in the neck of the archegonium of Funaria is another characteristic restricted apparently to this particular group of plants, although no other moss has been studied adequately. No record has been found of any previous mention of this plug. Rickett ('23) considers the mucilaginous material present in the cavity of the venter and the neck in Sphaerocarpos as analogous to the "fertilization membrane" described by other workers. The mucilaginous plug observed in Funaria is not comparable in structure and location with the mucilaginous material of Sphaerocarpos, although the latter may be similar in origin and function. In the first place, there is no indication of any such substance being present in the canal of any archegonium in which insemination has not occurred. In the second place, this mucilaginous plug is always associated with the two tiers of cells of the neck adjoining the venter, never with any other cells. In 334 archegonia examined with reference to this particular point, 219, or 65½ per cent, showed this mucilaginous plug to be present. Some of the archegonia studied were sectioned transversely; hence, if the plug were present it would not be visible in the same sections as the eggs (but the sections through that part of the neck might be expected to show it). It is entirely possible that the percentage would be much higher if all the material had been sectioned longitudinally. In all of these archegonia in which the mucilaginous plug is present it is associated with the two tiers of cells of the neck adjoining the venter. Never do any other than these two tiers of cells secrete the mucilaginous material, and hence it is inferred that this plug is a secretion of these cells but its function is as yet undetermined. If the plug were analogous to a fertili-
<table>
<thead>
<tr>
<th></th>
<th>Fasoria</th>
<th>Riccardia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of egg</td>
<td>40 x 20 µ</td>
<td>25 µ</td>
</tr>
<tr>
<td>Size of nucleus</td>
<td>13 x 10 µ</td>
<td>10 µ</td>
</tr>
<tr>
<td>Penetration of male nucleus into egg</td>
<td>Fierces membrane</td>
<td>Fierces membrane</td>
</tr>
<tr>
<td>Time required for penetration</td>
<td>15 minutes</td>
<td>0-6 minutes</td>
</tr>
<tr>
<td>Penetration of male nucleus into female nucleus</td>
<td>No penetration, two nuclei come in contact</td>
<td>No penetration, fusion in reticulate condition</td>
</tr>
<tr>
<td>Role of nuclear membrane</td>
<td>Disappears</td>
<td>Remains</td>
</tr>
<tr>
<td>Behavior of chromosome</td>
<td>Organized into chromosomes</td>
<td>Not known</td>
</tr>
<tr>
<td>Time elapsed before fusion</td>
<td>44 hours</td>
<td>40-60 hours</td>
</tr>
<tr>
<td>First division of nucleus (two-embryo)</td>
<td>73 hours, 45 minutes</td>
<td>168 hours</td>
</tr>
</tbody>
</table>
zation membrane its function would be to prevent the penetration of any supernumerary antherozoids into the egg. The presence of a relatively large number of antherozoids within the ventral cavity, after penetration of one antherozoid, makes this assumption doubtful. The fact that an unusually large number of antherozoids appears to have accumulated above the plug gives one the impression that the plug prevents further entrance of antherozoids into the cavity. It may also be conceived to afford protection against injury to the zygote, preventing evaporation of water or entrance of bacteria.

There is no indication of any fusion between the ventral canal cell and the egg cell, such as has been described by Bryan ('20) in the case of Sphagnum. The egg cell in Funaria is approximately three times the size of the ventral canal cell. The ventral canal cell, as it disintegrates, decreases in size until it is scarcely perceptible above the egg cell and then disappears completely. The rounding up of the egg cell and decrease in size of the two cells leaves a space between the two cells. This space makes it evident that the cells do not come in contact with each other, thus eliminating the possibility of fusion.

The structures observed in the cytoplasm of the egg, in the fixations which were made approximately forty-five hours after flooding, have been definitely determined to be plastids. Throughout the development of the fertilized egg, at various periods after flooding, rod-like structures and granules have been observed in the cytoplasm of the egg, but this particular lot is the only material in which definite plastids have been identified. The plastids present the same vacuolated appearance and are similar in structure to those found in other tissues of the plant except that they are smaller. Sapehin ('13) finds plastids in the egg of Bryum. From his drawing, however, it is very evident that the egg has just been separated from the ventral canal cell, since it does not display the definitely rounded appearance associated with mature eggs. The plastid-like bodies appear to be small, spherical, and relatively few in number. In addition to these small spherical structures Sapehin represents definite rods and minute granules. These latter structures in Bryum are very similar to those which are found in eggs of Funaria three hours and thirty minutes after insemination.
Showalter ('27b) includes a drawing of *Pellia* showing very definite starch granules. These appear in a mature egg which contains two male nuclei, but in which no nuclear fusion has occurred. Showalter ('28) has observed in eggs of *Riccardia pinguis*, type C, inseminated with antherozoids of *Riccardia pinguis*, type B, that plastids with starch grains were sometimes quite conspicuous. Motte ('28) figures very definite rods, together with some irregularly shaped bodies in the cytoplasm of the egg of *Hylocomium*. He has used the particular technique which has been developed for the study of plastids. He assumes that these irregularly shaped bodies are plastids, and considers them, inasmuch as the archegonium is advanced in age, to be plastids which develop as a result of non-fertilization of the egg and to be forerunners of cellular death. In *Funaria hygrometrica* he finds no indication of plastids in the cytoplasm of an egg that is somewhat past the mature stage.

The fixations of *Funaria flavicans* were made for other purposes than the studying of plastids, but it is interesting to note that these rod-like structures and spherical bodies are present in the cytoplasm of the egg regardless of the killing fluid or the stain used. The fluid which brought out the definite plastid bodies was that of Benda, and in other lots of material fixed in Benda's fluid no plastids, rods, or granules were observed in the cytoplasm. The rod-like structures and granules, which have been observed, are present in those eggs which show very clearly the male nucleus in contact with the female nucleus. In the later stage in which the definite plastids are observed, the presence of the mucilaginous plug would indicate that insemination had taken place. It is questionable whether nuclear fusion has occurred, since the presence of these bodies makes it somewhat difficult to determine the nuclear structure. Not all of the eggs of this particular lot show these plastids, and it is possible that they have developed because of non-fertilization. The very scanty amount of evidence prevents one from making any definite inferences regarding these plastids.

The condition of the nucleus with reference to the condensed chromatin shows some definite points of similarity to those described by Showalter ('28) for *Riccardia*. He observes that,
after penetration of the male nucleus into the cytoplasm of the egg, the chromatin of the female nucleus condenses into a compact mass about the nucleolus, leaving a region in which there is no staining substance present. In early stages the chromatin is readily distinguished about the nucleolar body. In Funaria, however, the region about the nucleolus is very dense in appearance and does not seem to display any of the details characteristic of true chromatin. This dense mass gradually disappears with the entrance of the male nucleus into the female nucleus, leaving a region in which there is no staining substance present. No measurements were made of the nucleolar body to determine if there was an increase in its size, which at all times is extremely minute, making detailed observations difficult. It seems logical, however, to assume that the nucleolus of the egg nucleus in Funaria is in reality a condensed mass of chromatin enclosing the true nucleolus and is imbedded in some cytoplasmic material which undergoes structural changes with the occurrence of fertilization.

Cytokinesis has not been thoroughly investigated in the Mosses. Wilson ('09) depicted very definitely division by cell-plate formation in Mnium. Allen ('16) found that the spore-mother-cell of Catharinea presented a lobed appearance and stated that this was the first observed occurrence of lobing in the Bryales. The lobing may be interpreted as furrows which grow in dividing the spore-mother-cell into tetrads. The method of division in Funaria is doubtless that of cell-plate formation. The definite thickenings which appear at the equator between the poles and which grow out toward the periphery of the cell are regarded as incipient cell-plates.

**Summary**

1. Sporelings, obtained from spores of *Funaria flavicans* Michx. sown on sterile soil, were grown under controlled conditions. At the time when the archegonia and antheridia were mature, insemination was brought about by flooding the cultures. Fixations were made at intervals after flooding and the material studied microscopically.

2. The volume of the egg of *Funaria* was found to be approximately one-eighteenth that of the egg of *Riccardia*. The
volume of the nucleus of *Funaria* was found to be one-fortieth that of *Riccardia*.

3. The antherozoid penetrates the cytoplasm of the egg by a gradual process, and takes place, for the most part, at the basal end.

4. The male nucleus, having assumed a spherical form, comes in contact with the female nucleus and then passes into the nuclear cavity.

5. The region about the condensed chromatin of the female nucleus is very clear, whereas the region about the male nucleus is chromophytic.

6. After penetration of the male nucleus the nuclear membrane about the female nucleus becomes irregular in outline and disappears, leaving the condensed chromatin and the male nucleus in the center of the nuclear cavity.

7. The condensed chromatin of the female nucleus gradually fuses with the male nucleus. After this fusion has occurred, a nuclear membrane reappears around the fusion nucleus.

8. In connection with fertilization, a mucilaginous plug is developed in the neck of the archegonium. It is thought to be a secretion of the first two tiers of neck cells above the venter, since it is always found associated with these cells.

9. No fusion of the egg cell with the ventral canal cell, such as that reported in *Sphagnum* by Bryan, was observed.

10. Cytokinesis of the spore-mother-cell is by cell-plate formation.

11. These results, the only ones so far obtained in connection with fertilization in Mosses, are compared with those in other Bryophytes.

**Acknowledgments**

The writer wishes to express her extreme gratitude to Dr. George T. Moore for the use of the facilities of the Missouri Botanical Garden Library, to Dr. A. M. Showalter, under whose direction and guidance this problem was undertaken, to Dr. Edgar Anderson for his helpful criticisms and suggestions, and to Dr. A. J. Grout for his kindness in identifying the material used.
Bibliography


EXPLANATION OF PLATE
PLATE 43
All figures were drawn with the aid of the camera lucida at the magnification indicated.
Figs. 4, 5, 6, 9, 10 were drawn from living material. Figs. 1, 2, 3, 7, 8, 11–57 were drawn from stained preparations.
Fig. 1. Single-celled antheridium. × 1500.
Fig. 2. Two-celled antheridium. × 1500.
Fig. 3. Multicellular antheridium. × 1500.
Fig. 4. Mature antheridium. × 380.
Fig. 5. Single-celled paraphysis. × 380.
Fig. 6. Several-celled paraphysis. × 380.
Fig. 7. Mature paraphysis. × 380.
Fig. 8. Intranuclear division. × 1500.
Fig. 9. Mature antherozoids in gelatinous envelope. × 750.
Fig. 10. Sperm—cilia not visible. × 750.
Fig. 11. Single-celled archegonium. × 1500.
Fig. 12. Several-celled archegonium. × 1500.
Fig. 13. Mature archegonium. × 150.
Fig. 14. Paraphysis of archegonial head. × 380.
Fig. 15. Cell before egg and ventral canal cell have been cut off. × 1500.
Fig. 16. Cell showing invagination of cytoplasm. × 750.
Fig. 17. Venter with degenerating ventral canal cell. × 750.
Fig. 18. Mature egg immediately after flooding. × 1500.
Fig. 19. Egg being surrounded by antherozoids, 35 min. after flooding. Diagrammatic × 1500.
Fig. 20. Mucilaginous plug. × 1500.
BEARDSLEY—FUNARIA FLAVICANS
EXPLANATION OF PLATE

PLATE 44

Fig. 21. Antherozoids adpressed to egg, 1 hr. 35 min. after flooding. × 1500.
Fig. 22. Antherozoids adpressed to egg, 2 hr. 20 min. after flooding. × 1500.
Fig. 23. Antherozoid in process of penetration, 1 hr. 35 min. after flooding. × 1500.
Fig. 24. Antherozoid in cytoplasm of egg, 2 hr. 20 min. after flooding. × 1500.
Fig. 25. Antherozoid in cytoplasm of egg, 2 hr. 20 min. after flooding. × 1500.
Fig. 26. Egg showing poly sperm, 1 hr. 35 min. after flooding. × 1500.
Fig. 27. Antherozoid assuming a spherical form, 3 hr. 30 min. after flooding. × 1500.
Fig. 28. Antherozoid coming in contact with egg nucleus, 4½ hr. after flooding. × 1500.
Fig. 29. Male nucleus in contact with female nucleus, 3½ hr. after flooding. × 1500.
Fig. 30. Male nucleus in contact with female nucleus, 3½ hr. after flooding. × 1500.
Fig. 31. Male nucleus penetrating female nucleus, 4½ hr. after flooding. × 1500.
Fig. 32. Egg nucleus showing vacuolation of condensed chromatin, 4½ hr. after flooding. × 1500.
Fig. 33. Male nucleus within female nuclear membrane, 4½ hr. after flooding. × 1500.
Fig. 34. Male nucleus within female nuclear membrane, 4½ hr. after flooding. × 1500.
Fig. 35. Egg showing rod-like bodies in cytoplasm, 2 hr. 20 min. after flooding. × 1500.
Fig. 36. Membrane becoming irregular, 5½ hr. after flooding. × 1500.
Fig. 37. Membrane becoming irregular, 5½ hr. after flooding. × 1500.
Fig. 38. Membrane becoming irregular, 6½ hr. after flooding. × 1500.
Fig. 39. Disappearance of membrane, 6½ hr. after flooding. × 1500.
Fig. 40. Maternal and paternal chromatin in process of fusion, 6½ hr. after flooding. × 1500.
BEARDSLEY—FUNARIA FLAVICANS
EXPLANATION OF PLATE

PLATE 45

Fig. 41. Maternal and paternal chromatin in process of fusion, 7½ hr. after flooding. × 1500.
Fig. 42. Fusion body, 14 hr. after flooding. × 1500.
Fig. 43. Fusion body, 14 hr. after flooding. × 1500.
Fig. 44. Cytoplasm becoming dense around fusion body, 14 hr. after flooding. × 1500.
Fig. 45. Cytoplasm denser, 16 hr. after flooding. × 1500.
Fig. 46. Cytoplasm denser, 18 hr. after flooding. × 1500.
Fig. 47. Irregular membrane, 22 hr. 20 min. after flooding. × 1500.
Fig. 48. Definite membrane, 48 hr. 45 min. after flooding. × 1500.
Fig. 49. Egg showing presence of plastids, 45 hr. 20 min. after flooding. × 1500.
Fig. 50. Egg showing dispersal of chromatin about periphery of fusion body, 93 hr. after flooding. × 1500.
Fig. 51. Binucleate embryo, 93 hr. after flooding. × 1500.
Fig. 52. Embryo showing nuclei in resting stage, 93 hr. after flooding. × 1500.
Fig. 53. Three-celled embryo, 93 hr. after flooding. × 750.
Fig. 54. Spore-mother-cell. × 1500.
Fig. 55. Spore-mother-cell during first division. × 1500.
Fig. 56. Spore-mother-cell during second division. × 1500.
Fig. 57. Chromosomes in archegonium. × 1500.