THE CYTOLOGY AND MORPHOLOGY OF SORDARIA FIMICOLA CES. AND DE NOT

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INTRODUCTION

Recent cultural experiments dealing with the homothallism and heterothallism of fungi relating to the Sordarias has made it worth while to study the origin and nature of the structures initiating the formation of fruiting bodies in these forms.

Special attention has therefore been given to the growing of Sordarias in pure cultures so that the presence or absence of sex organs could be noted, as well as the nature and function of such organs during their development. Gross morphology was noted, but the nuclear phenomena, especially as to the behavior of the nuclei during the early stages in formation of the perithecium, has been given special consideration. As a preliminary to this study, a careful review has been made of the accumulated data, both cytological and morphological, of previous workers on the Ascomycetes.

HISTORICAL REVIEW

Pyronema confluens

The development and nature of the sex organs in Pyronema confluens have interested several investigators. The first descriptions were supplied by De Bary (1863), by the Tulasne brothers (1865, 1866), by Van Tieghem (1884), and by Kihlman (1885). These early investigators observed the elongated antheridium, the globular oogonium, the trichogyne, and the ascogenous hyphae characteristic of Pyronema. More detailed work of a cytological nature was necessary, however, before the history of Pyronema confluens could be considered complete. This led to the investigations of Harper (1900), followed by Dangeard (1903, 1907), and by Claussen (1912).
As a result of Harper’s observations on *Pyronema confluens*, there has been obtained a detailed life history, a review of which follows. The vegetative mycelium is multinuclear, from six to twelve nuclei appearing in each cell. Although the mycelium is sparse and loose, the reproductive organs are abundant and produce ascocarps which, when mature, are seen crowded together.

The first appearance of sex organs may be noted when certain thick hyphae tend to stand at right angles to the substratum. These hyphae, from the same or different mycelia, may be seen grouped in pairs. One of the two branches becomes swollen and differentiates into an upper spherical cell, the oogonium, and a lower portion, the stalk, composed of a few cells; the other branch has an upper cell cut off which remains slender and is called the antheridium, with a lower portion forming the stalk cells. Very early in the formation of these sex organs, the trichogyne appears as a slight elevation at the “apex” of the oogonium. This trichogyne elongates, becomes multinucleate, and is separated from the oogonium by a wall.

The mature oogonium is a spherical or flask-shaped cell filled with dense cytoplasm and containing many nuclei larger than those of the vegetative cells. The nuclei of the antheridium are almost as large as those of the oogonium but the cytoplasm is less dense, due perhaps to the lack of reserve materials.

Before fertilization, hyphae from the ascogonial and stalk cells and from surrounding cells begin to grow up and later envelop the sex organs.

The trichogyne and the antheridium grow towards each other, the tip of the trichogyne finally coming in contact with the apex or side of the male organ. When fertilization is about to take place, there is a single receptive spot at the end of the trichogyne free from nuclei and made up of dense, finely granular cytoplasm. At the point of contact of the antheridium and trichogyne, a pore is formed by dissolution of the cell walls. During this process, the nuclei of the trichogyne degenerate and when they are completely disorganized, the nuclei of the antheridium migrate through the pore and into the trichogyne, in which the contents have degenerated still further so that the cytoplasm
and nuclei together form a densely staining mass. The male nuclei continue to pass into the trichogyne which often appears filled and slightly swollen as a result. The wall at the base of the trichogyne now breaks down. This allows an opening for the male nuclei to enter the oogonium.

The greater part of the cytoplasm remains in the antheridium and trichogyne and does not enter the oogonium. After migration of the male nuclei through the trichogyne and into the oogonium, a new wall appears at the base of the trichogyne so that the oogonium is again a single cell. The male nuclei pair with the female nuclei and these paired nuclei then fuse within the oogonium. During this time slight protuberances make their appearance in the walls of the oogonium which is called an ascogonium after fertilization takes place. These projections elongate and are young ascogenous hyphae into which the fusion nuclei soon pass. These hyphae lengthen in a crooked fashion, become septate, and grow among the vegetative branches which have arisen from the stalk cells of the oogonium. The vegetative branches grow faster than the ascogenous hyphae and continue to grow upward, their extremities becoming paraphyses. The ascogenous hyphae also grow in a vertical direction and appear among the paraphyses, but their upward growth soon stops and they then grow horizontally, branching repeatedly, forming a network at the base of the hymenium.

The tips of the ascogenous hyphae which extend upward among the paraphyses, become curved. They contain, at first, two nuclei which divide simultaneously so that four nuclei are found in the curved portion, and these nuclei are arranged in such a way that the two nuclei from separate spindles lie together in the "crook" of the branch, one nucleus in the tip and one below the curve. Cell walls then appear, cutting off the tip with one nucleus, the bent upper part of the branch with two nuclei. It is from this binucleate cell that the young ascus arises as an apical projection into which the two nuclei pass and then fuse. This fusion-nucleus or primary nucleus, as it is usually called, divides to produce two, then four, and finally eight nuclei
arranged throughout the entire length of the now fully elongated ascus.

Each nucleus has a beak and to this are attached the aster rays which still remain. These fibers grow back around the nucleus until they meet and a membrane is formed around each nucleus. Thus the ascospores are cut out by a process called a "free cell-formation." Later a wall forms around each uninucleate spore.

Harper, therefore, finds that the nuclei in the antheridium pass through the trichogyne and into the oogonium where they pair with the female nuclei and fuse. These fusion-nuclei then pass into the ascogenous hyphae. Harper thus finds two fusions in Pyronema confluens, one in the oogonium and one in the ascus when the two nuclei from the penultimate cell unite to form the primary nucleus of the young ascus. Then chromosomes were counted by Harper in the divisions of the nuclei in the ascogenous hyphae and in the divisions of the primary nucleus in the ascus but whether this number represents a double number resulting from the fusion in the oogonium or whether reduction division has already taken place, Harper was unable to decide.

Dangeard (1903–1904) disagrees with these conclusions given by Harper. The sex organs, he believes, are not functional. The wall between the oogonium and the trichogyne does not disappear so that the migration of male nuclei beyond the trichogyne can not take place. Ascogenous hyphae arise from the functionless sex organs and the asci possess characteristics of a sex organ in which the nuclei fuse as gametes. This fusion-nucleus then divides by heterotypic division followed by two homoeotypic divisions. There is, therefore, according to Dangeard, only one nuclear fusion and that in the young ascus.

The theory of a single nuclear fusion is also advanced by Claussen (1912). His conclusions, concerning the development and function of sex organs and the migration of male nuclei through the trichogyne and into the oogonium, agree with those of Harper. But, according to Claussen's investigations, the male nuclei pair with the female nuclei but there is no fusion in the oogonium. These paired nuclei pass into the ascogenous hyphae and then fuse in the young
ascus as described by Harper and Dangeard. A single division then follows with two homoeotypic divisions in the ascus. Claussen has counted twelve chromosomes in these divisions.

Brown (1915) finds no union of antheridium with trichogyne, no fusion of nuclei in the ascogonium and none in the ascogenous hyphae. He agrees with Claussen and Dangeard in the single fusion theory, that fusion taking place in the young ascus.

**Phyllactinia corylea**

In *Phyllactinia corylea*, Harper (1905) finds a small coil composed of the functional oogonium and antheridium which appear at about the same time as lateral branches from separate hyphae but from the same mycelium. The oogonium is cut off from its hypha by a cross wall so that a large upper cell and a small lower cell results. The upper cell enlarges to form the oogonium and contains a single nucleus, the lower cell divides to form the stalk cells of the oogonium. The antheridium is formed in a similar manner but remains slender and erect and contains one nucleus. The oogonium grows more rapidly and twists around the short, erect antheridium. The walls separating the two sex organs, at the point of contact, break down and a pore is thereby produced for the passage of the male nucleus into the oogonium. A part or all of the cytoplasm remains in the antheridium. Fusion of the two nuclei then occurs in the oogonium. The pore between the two sex organs closes and the antheridial cell degenerates. While fertilization is going on, protective branches grow up from the stalk cells of both sexual branches and begin the formation of the perithecial envelope.

The oogonium, after fertilization, is called the ascogonium. It enlarges and a division of the fusion-nucleus takes place. This binucleate cell remains in this condition until the ascogonium is completely enclosed by the enveloping hyphae. Then the two nuclei divide and just how many divisions occur before cell walls appear, Harper was unable to observe. However, from three to five cells in a row appear in the ascogonium, the end cell with one nucleus, the others
with one or two nuclei. Ascogenous hyphae arise as lateral branches from the ascogonium cells and whether they come from one cell or from several of the upper cells is unknown. It is certain that some arise from the penultimate cell. The ascogenous hyphae develop considerably before they become septate; then cells with two nuclei and with one nucleus result. From the binucleate cells, the asci are formed as lateral outgrowths probably in the same manner as in Pyronema.

Each young ascus has two nuclei. The ascogenous cells which formed the asci contained two nuclei, but these nuclei are not necessarily daughter nuclei of the same nucleus. As the asci elongate, the two nuclei in each ascus fuse, forming the primary ascus-nucleus which moves to the lower end of the ascus. By this time the asci have become long, narrow structures with short stalks at the base. The primary nucleus undergoes three nuclear divisions so that eight nuclei are formed. By free cell-formation, eight spores are cut out.

The perithecium at this time has a three-layered wall; the innermost layer several cells in thickness, thin-walled, and probably supplying food for the developing asci. Outside of this layer is a strengthening and protecting zone of cells with walls which appear lignified. The outermost layer of the perithecium consists of thin-walled cells from which characteristic appendages arise as stiff, pointed hairs with swollen bases.

*Ascobolus magnificus*

Dodge (1920) found that cultures containing two strains of mycelia were necessary for the production of ascocarps. In such cultures four to six days old, he found paired branches. These club-shaped branches elongate. One of them usually grows faster than the other, coils about this shorter branch, and is called the ascogonium; the shorter structure remains a two or three-celled antheridium. Very often both branches arise a short distance apart. In this case, one remains an erect, short antheridium, the other elongates into an ascogonium and forms a trichogyne which grows to and becomes coiled about the antheridium.
Dodge was able to prove the presence of an antheridium in any normal case by separating this same structure from the trichogyne coil before fusion had occurred and if hyphal branches had not grown out from the stalk of the oogonium to envelop the male organ.

In normal development, the ascogonium enlarges and often ascogenous hyphae grow out before sterile hyphae begin to form the fruiting body.

Since two strains of mycelia are necessary for the production of fruiting bodies, there is good indication that sex has not been lost or reduced in at least this species of Ascobolus.

By germinating ascospores of *Ascobolus magnificus*, Dodge obtained papulospores—defined by him as a spore "in which one or two large storage cells are surrounded by a covering of hyphae which develop from blister-like outgrowths of the storage cell." He transferred mycelia of the single papulospore strains to different kinds of media but obtained no ascospores of Ascobolus. Hotson, at Dodge's request, studied papulospores from cultures sent by the latter and concluded that they were not the asexual stage of *Ascobolus magnificus*. He was unable to obtain ascospores from the cultures.

E. S. Schultz also made single spore cultures of this papulasporea for Dodge, but was unable to obtain ascocarps.

Recently Dodge proved that papulospora is not a case of parasitism on Ascobolus as he once thought, but is an example of what has been described as self-penetration or self-parasitism. Internal hyphae running in and out of larger hyphae were found with papulospores arising from branches growing out of these internal hyphae.

As already noted, ascocarps could not be obtained from single spore cultures of papulospores and there is no record of producing ascocarps from single ascospore cultures of *A. magnificus*. Two strains are thus necessary for sexual reproduction and the formation of ascocarps. Dodge planted single ascospore cultures in separate petri dishes and obtained mycelium and papulospores but no sex organ branches or ascocarps. Of the seven strains obtained he planted five of them as follows:
Strains

<table>
<thead>
<tr>
<th>Results after 10 days</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papulospores but no fruiting bodies</td>
<td>2 alone</td>
</tr>
<tr>
<td>Ascocarps</td>
<td>2 and 1</td>
</tr>
<tr>
<td>Papulospores but no fruiting bodies</td>
<td>2 and 3</td>
</tr>
<tr>
<td>Ascocarps</td>
<td>2 and 4</td>
</tr>
<tr>
<td>Papulospores only</td>
<td>2 and 5</td>
</tr>
<tr>
<td>6 lost</td>
<td></td>
</tr>
<tr>
<td>7 not used</td>
<td></td>
</tr>
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</table>

The results prove that strains 2, 3, and 5 when grown in the combinations given in the table are sterile; the same is true for strains 1, 4, and 7. Fertile cultures, however, are produced when any one of the first group is grown with the different strains in the second group.

Strains 2 and 4 were further tested. It was found that each is sterile when grown alone, but fertile when combined with the other. Strains 2 and 1, although sterile when grown alone, produced ascocarps when grown together. But strains 4 and 4 grown together in one petri dish will show a zone between them free from hyphae. When opposite strains 2 and 4 are planted, there is no such zone between them and sex organs appear throughout the culture. Therefore, according to Dodge, each strain is self-sterile and in a single strain culture produces no sex organs. Sexual reproduction occurs only in cultures containing two strains properly chosen.

**Venturia inaequalis**

Frey (1924) has reported the presence of an ascosgonium, a trichogyne, and antheridal branches which indicate sexual conditions in *Venturia inaequalis*. The coil usually consists of two branches which arise from the same filament. One branch functions as an ascosgonium, the other is not an antheridium but may function as a nutritive organ. Sometimes a single branch may be found producing a coil. The coil increases in size, stains deeply, and sends out an elongated projection called the trichogyne. Early stages of the trichogyne and ascosgonium are non-septate—the former seldom has a nucleus, while the latter is multinucleate. From the filaments near the ascosgonium, the antheridal hyphae arise. Their apical cells are enlarged and some appear multinucleate, probably functioning as
antherids because they come in contact with the trichogyne and seem to fuse with it. Just when, in the development of the coil, fertilization occurs, is not known, but since later stages show paired nuclei in cells of the ascogonium, it probably takes place during the non-septate stage.

After fertilization, the ascogonium becomes septate and contains seven or eight cells with paired nuclei, as already mentioned. These nuclei do not fuse in the ascogonium. Later there is an increase in number of cells in the ascogonium, some of which may contain as many as four nuclei. These cells branch extensively, are septate, and certain of these cells may become asci directly, other cells may again branch, and these branches then become septate and form asci. Frey does not consider these branches equivalent to the ascogenous hyphae described by Harper and Claussen.

The ascogenous cells contain two or four nuclei which enlarge and pair and these paired nuclei then fuse in the young ascus. The nuclei resulting from the division of this primary nucleus lie near the center of the ascus, but after the second division when the ascus has elongated, the nuclei pass to the periphery. After the third division the spores are cut out by the astral fibers as described by Harper. These spores are two-celled and uninucleate.

Killian (1915) reported a large trichogyne, a coiled ascogonium, and also a branched antheridium in *Venturia inaequalis*. Frey found a few lobes of the apical cells of antheridial branches and perhaps these lobes correspond to the branched antherids found by Killian. Killian suggests the possibility of a septate archicarp at the time of fusion of antherid and trichogyne because of pores found in cells of the ascogonium.

*Polystigma rubrum*

In *Polystigma rubrum*, Nienburg (1914) found a coil composed of an ascogonium, a trichogyne, and an antheridium. The ascogonium is an elongated cell with one nucleus; the trichogyne is multinucleate, does not function as a sex organ but perhaps has a nutritive value; the antheridium is an elongated cell with many nuclei. The antheridium applies itself to the ascogonium and the walls at the point of contact between the two cells break down. The
male nuclei then pass from the antheridium into the ascogonium where one male nucleus enlarges to become the functional male nucleus—the remaining nuclei degenerate. No fusion of nuclei in the ascogonium was observed by Nienburg. From the ascogonium, ascogenous hyphae, the cells of which contain two nuclei, arise and whether this binucleate condition is due to a “constriction” division is an unsettled question. Development of the ascus could not be followed.

Nienburg did not observe a fusion of nuclei in the ascogonium or in the ascogenous hyphae and believes there is but a single fusion and that in the ascus.

Blackman and Welsford (1912) disagree with Nienburg. They claim that the ascogenous hyphae develop from vegetative cells, and that two nuclear fusions occur, one in the ascogenous hyphae and one in the ascus.

*Podospora anserina*

An organism more closely related to Sordaria than the forms described above, is *Podospora anserina*, a saprophyte commonly found on waste material. It was investigated for the purpose of determining spore formation because the number of spores produced in a few species of Podospora varies from four to sixteen or more.

Wolf (1912) made a study of *Podospora anserina* and found coils made up of two hyphae, but with no differentiation of sex organs. The nuclei are very small and for this reason, fusion of nuclei was not observed. Vegetative hyphae arise from the coil, envelop the coiled hyphae and develop into the pear-shaped perithecium.

Near the base of the perithecium, the asci develop in groups, several asci branching from a single hypha. The young ascus contains one large primary nucleus, much larger than those found in the vegetative mycelium where each cell is multinucleate. This primary nucleus undergoes divisions; only a few stages, however, were observed by Wolf. After heterotypic and homoeotypic divisions there is a short period of rest followed by an enlargement of the nuclei containing knots of chromatin which may represent chromosomes. After the third division a region of
less dense protoplasm appears between pairs of the resulting eight nuclei. It was found that four spores are cut out of the ascus in *Podospora anserina*, but whether only one nucleus of each pair takes part in the process of spore delimitation or astral rays from the two concerned are effective was not determined. Since two nuclei are normally included within each of the four spores, no disintegration of the eight nuclei in the epiplasm takes place.

As the spores mature they increase in size, the nuclei migrate to the ends, the upper part enlarges and the lower half becomes the hyaline appendage. The nuclei increase in size and the colorless, young spores gradually change to green, then to dark brown. The appendage remains hyaline, becomes equal in length to the body of the spores, and often disappears in mature spores.

*Sordaria fimbicola* Ces. and De Not

*Sordaria fimbicola* Ces. and De Not. was first described by Roberge in 1849 and given the name of *Sphaeria fimbicola* later in 1865. Cesalpini and De Notaris described the fungus more fully and gave it the name *Sordaria fimbicola*, which name has been generally accepted. More recently, Griffiths and Seaver, in listing the Sordariaceae for the North American Flora, dropped the genus *Sordaria* as such and substituted *Fimetaria* and gave the new combination *Fimetaria fimbicola* (Roberge) Griffiths and Seaver. As *Sordaria fimbicola* has been so long accepted and is found as such in most literature, that name will be used in this paper.

**Materials and Methods**

Pure cultures of *Sordaria fimbicola* Ces and De not. available for study, were easily isolated from refuse material and from impure cultures of Sordaria, and grown at room temperature on artificial media. It develops rapidly on potato agar, forming a great number of black, flask-shaped perithecia which stand upright above the medium. Spores from the most characteristic perithecia were then chosen and transferred to agar plates. Single spore cultures were then made from these cultures and pure strains of *Sordaria*
**Observations and Discussion**

The cytoplasm in the mycelium of *Sordaria fimicola* is highly vacuolate with strands of varying thickness extending throughout the cells. Close to the walls can be seen a thin, densely granular layer of cytoplasm. Imbedded in the cytoplasm are numerous mitochondria, either scattered singly, in aggregations, or often in pairs, probably as the result of recent division. The cells are multinucleate. With the exception of the nucleole which stains heavily, the content of the nucleus is not readily distinguishable. Cross walls with special thickenings as shown in figures 1, 4 and 5, are frequently present. A similar condition has been reported in *Pyronema* by Harper, and in various Basidio-mycetes by several workers. These thickenings appear as darkly stained granules or as a mass of material centrally located on either side of the cross wall.

In several instances, branches arising at right angles to the mycelium and apparently from separate hyphae, grow toward each other and come in contact (figures 1–6). This is not a condition of ordinary anastomosing, but rather one of actual fusing of cell contents from the two closely applied hyphae as the result of a dissolution of the separating walls. This may occur at the tips or at various places in the lateral walls of the two branches (figures 4, 5, and 6). There is no differentiation of cytoplasmic content in the two branches and the nuclei in each cell are variable, but in several cases a difference in size can be noted in the two hyphae and in the mycelium from which the hyphae arise.
(figures 2, 3, 6). It is probable that the fusion of cell contents observed may be an initial step in coil formation, but intermediate stages, which might give conclusive results, are lacking. Miss Miller (1927), a research student in botany, made many single spore cultures of Sordaria and in every instance secured normal perithecia containing normal spores.

Repeated experiments with single spore cultures and with mycelium from single spore cultures gave the same results. She then made plantings of two spores and with portions of mycelium from different cultures to see what the effect would be, and in more than one hundred cultures of this type the perithecia appeared throughout the cultures indicating that all the strains with which she worked were homothallic.

In the majority of cases a typical coil arises where two hyphae come in contact (figures 7–20). In plate cultures four or five days old, upright branches which arise at right angles to the mycelium make their appearance. These branches originate from separate hyphae, grow toward each other and come in contact. One of them usually swells considerably, grows faster than the other, and initiates the formation of the coil. The two branches elongate and continue to coil about each other until a large coil is produced. Dodge (1920) finds that the coil in Ascobolus magnificus is differentiated into a definite male organ, the antheridium, which does not elongate but remains a short erect branch, and into a female organ, the oogonium which elongates, the end cell functioning as a trichogyne and coiling about the antheridium. Harper, in Pyronema, finds an elongated antheridium, a large, spherical oogonium with a trichogyne produced at the “apex”; the trichogyne and antheridium bring about fertilization. But in Sordaria fimicola no true oogonium, trichogyne or antheridium are distinguishable. Sordaria resembles Podospora anserina in this respect because both have little differentiation of sex organs. Due to the swelling of one branch in the initiation of the coil, there is a difference in size of the two sex organs as seen in figures 16–20, suggesting the presence of male and female elements.

The sex organs composing the coil are, therefore, elong-
ated coiled branches with cross walls cutting off cells containing from one to seven nuclei. After fusion of certain of these cells an increased number of nuclei can be found in each cell of the coil proper. These fusion cells, probably representing simple or reduced sex organs, give rise to a variable number of hyphae which seemingly function as ascogenous hyphae in that the ultimate branches will give rise to ascii. Whether they are true ascogenous hyphae such as are found in Pyronema and other forms or whether they more nearly resemble the ascogenous structures found in Venturia, it is impossible to state. The nature and arrangement of nuclei in these structures suggest that fusion has taken place, but the actual process of fusion has not been noted.

These hyphae branch repeatedly and as they develop they become more and more like the ascogenous hyphae described by others. The curved end of such a branch often contains four nuclei arranged in a row. Cross walls are then laid down in such a manner that the end cell contains a single nucleus, the second cell two nuclei, and the third cell again contains only a single nucleus (figures 22–25). The growth of the second (penultimate) cell causes the tip cell to bend backwards. In many instances it was found that all the cells except that at the tip contained two nuclei. In figures 23 and 24, the terminal cell can be seen to curve downward and the whole to give the appearance of the typical “shepherd’s crook” commonly found at the ends of ascogenous hyphae in many Ascomycetes.

Most of these hyphae remain in large part within the coil, but occasionally (figures 20–25) the hyphae project beyond the coil and are then more characteristic of true “ascogenous hyphae.”

Since the above was written, a paper by Arnold has appeared in which he gives the development of the peritheciun and ascogenous hyphae in *Sporormia leporina* Niessl. The perithecium, which originates as a single enlarged cell of the vegetative mycelium, becomes a large rounded structure composed of several layers of cells, the innermost of which break down so that a cavity remains in the center. Extending into this cavity are the long hyphae which arise from the apex of the perithecium. Their enlarged tips,
found near the base of the perithecium, give rise to ascogenous hyphae which bend upwards, become hooked, and finally cells are formed in the usual way as described in other forms.

Faull (1905) finds asci in *Sordaria fimicola* arising from the terminal cells as well as from penultimate cells of the ascogenous hyphae. In my material, the asci usually arise as lateral outgrowths from any of the cells which contain two nuclei. The young asci are short, swollen structures (figures 24 and 25) with dense cytoplasm and a large primary nucleus, the result of a fusion of the two nuclei which were present in the ascogenous cells which develop into young asci. The asci elongate and extend in a vertical direction beyond the coil which is located in the basal region of the now enlarging perithecium (figures 26, 27).

The primary nucleus lies at the center or above the center of the ascus. It is a well defined and easily distinguishable structure in contrast with the small indistinct nuclei of the vegetative cells. Within the nuclear membrane is one large nucleole and a network of chromatin material. This chromatin is not in strands as Harper (1905) finds in *Phyllactinia*, where the resting nucleus contains chromatin threads attached to a central body located against the nuclear membrane. These strands of *Phyllactinia* extend into the nucleus and correspond in number to the number of chromosomes counted later. In *Sordaria*, the primary nucleus in the resting condition has the chromatin in the form of a network characteristic of nuclei in higher plants (figures 28–30). The division of the primary nucleus has not been observed in any of the material studied, but results of division of the primary nucleus have been found. As division goes on, the asci elongate and finally eight nuclei are formed. Here again only the nucleole is visible in each nucleus (figures 31–34). Further elongation takes place as the asci mature. They become long, slender structures with the eight nuclei in a single row, extending throughout the entire length of the ascus. Eight spores are then cut out of the cytoplasm surrounding the nuclei, probably by “free cell-formation” as Harper observed in several Ascomycetes, although no intervening stages were observed from the time eight nuclei were visible until eight uninucle-
ate spores were produced. No more than eight nuclei have been found in a single ascus but spores have been observed in which there are two nuclei, a condition which probably results from division of the nucleus within the spore. The spores are round when first formed, but become ellipsoid, acutely rounded at one end with a vacuolated cytoplasm. When mature, they have a thick wall surrounded by a gelatinous sheath (figure 35).

In addition to these main studies, attention has been given to the initiation of the peritheium. Vegetative hyphae arise in the neighborhood of the coil and envelop this structure. They begin to enclose the sex organs when the coil is still quite immature and by the time the "ascogenous hyphae" are formed, the vegetative hyphae have formed several layers of the peritheium. Sections of perithecia were observed in which a coil had been retarded in its development although the perithecial walls were fully formed and young asci were present. In such cases, the initiation of the peritheium probably takes place with the development of another coil from which the young asci originate. The inner layers of the fruiting body remain thin-walled, but towards the exterior the walls become impregnated with waxy substances, are thicker, and take the stain very readily.

At the time of ascus formation, hyphae resembling paraphyses can be seen in the interior of the peritheium and among the young asci. Later these hyphae disintegrate and when the asci are mature, no paraphyses or hyphae resembling them are present.

I wish to express my sincere appreciation of the helpful advice and criticism given by Dr. E. M. Gilbert during the progress of this work.

**SUMMARY**

*Sordaria fimicola* has recently been proved a homothallic species. In single spore cultures, perithecia appear in large numbers and in plate cultures inoculated with two strains, perithecia are abundant throughout the material, not limited to the region where the two different mycelia meet.
The sex organs are elongated, multicellular branches which coil about each other, forming large coils. They are undifferentiated except for a slight difference in size; no true antheridium, oogonium, or trichogyne are distinguishable.

"Ascogenous hyphae" arise from fusion-cells within the coil. Each hypha curves at the tip, forming a "crook" in which are found a uninucleate cell at the end, a binucleate penultimate cell and others with one or two nuclei.

Asci arise as lateral outgrowths from the binucleate cells, usually the penultimate, and the two nuclei fuse to form a primary nucleus. This nucleus undergoes three divisions and eight nuclei result which finally are cut out with a portion of the surrounding cytoplasm to form spores.

The uninucleate spores become binucleate as a result of the division of the nucleus present at the time the spores were formed.

As soon as the coil is well organized, hyphae cells increase in number and very soon completely enclose the coil and by a continued growth give rise to the various types of cells which characterize the perithecium of a Sordaria.

EXPLANATION OF PLATES

PLATE 6

All figures were drawn with an Abbe camera lucida, using a Leitz 4-ocular and a 1/16 oil immersion objective; magnification of 1850.

Photomicrographs were made with a Leitz 4-ocular, 1/16 oil immersion, magnification of 1800, with the exception of fig. 29, which was made with a 6 mm. objective, magnification of 500.

Figs. 1, 2. Two hyphae in contact. Cross walls with special thickenings are present, cells are multinucleate with only nucleoles visible. Fig. 2 shows hyphae of different size.

Figs. 3–6. As in Figs. 1 and 2. Dissolution of walls at place of contact and fusion of cell contents.

Figs. 7–29. Coil formation. No differentiation of sex organs, merely a difference in size of the two hyphae can be noted.

PLATE 7

Fig. 21. Tips of ascogenous hyphae showing multinucleate condition.

Figs. 22–25. Septate ascogenous hyphae. Fig. 23 shows the binucleate penultimate cell which usually gives rise to asci. Figs. 24, 25 show young asci developing as lateral outgrowths.

Figs. 26, 27. Elongating asci with a primary nucleus visible in some.
FIGS. 28–30. Portions of asci showing large primary nucleus with distinct nucleole and a network of chromatin material.

FIG. 31. Portions of ascus after first division of primary nucleus.

FIG. 32. Ascus with two nuclei.

FIGS. 33, 34. Asci with 8 nuclei, only 6 visible.

FIG. 35. At left, a spore from an ascus containing 8 spores. At right, a spore from an ascus with 4 spores.

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