NUCLEAR BEHAVIOUR AND DEVELOPMENT OF ASCOCARP IN *SPORORMIA FIMETARIA* DE NOT

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The hyphae of *S. fimetaria* were found to be uninucleate. The development of the ascocarp took place through the formation of the pseudoparenchymatous structure, either derived by the repeated divisions of a single cell or by the fusion of several vegetative hyphae. The vertical orientation of the pseudoparaphyes was independent of the ascogenous hyphae and the asci. Pseudoparaphyses were formed earlier than the ascogenous hyphae. These sterile elements were either attached at both ends or some attached above and free below and some attached below and free above. The ascogenous hyphae originated from the basal cells of the locule. Diplodization process is however, at percent incompletely understood. Development of the asci took place by the formation of croziers in the normal way.

Introduction

Miller [1] considered the origin of the perithecial wall as different from that of stromal tissue. He pointed out that the former originates from the basal cell of the ascogonium as a result of sexual stimulus whereas the latter is formed by the vegetative cells prior to the formation of the sexual organs. Nannfeldt [2] found similar criteria in dividing the Ascomycetes into Ascohymeniales (Hymenial Pyrenomycetes and Discomycetes) and the Ascoloculares in which the asci occur in a stroma. Luttrell [3] divided the Pyrenomycetes into two series, one made up of forms with unitunicate and the other with bitunicate asci. He stated that the presence of the bitunicate ascus is associated with the ascostromatic forms. He further claimed that the division on the basis of unitunicate asci would keep Pyrenomycetes into two distinct series on essentially the same lines as those of the ascostromatic and perithecial forms. Morisset [4] is of opinion that *Sporormia* species are of the ascolocular type, in which ascocarps are developed from a stromatic mass of cells and are accompanied with bitunicate asci, without an apical apparatus. In contrast to *Sporormia* spp., the ascocarps of sordariaceous fungi are formed by the filaments originating from the ascogonium. The asci are unitunicate and possess a more or less complex apical apparatus. These two groups of fungi are considered fundamentally different.

Though there is a limited number of reports on the development of ascocarp in *Sporormia* spp., there is a considerable disagreement at several points. The most important of these are: (i) the nuclear nature of the cells which produce ascocarp; (ii) the origin and attachment of pseudoparaphyses and (iii) the origin of binucleate cells which give rise to the ascogenous hyphae.

The study of the development of *S. fimetaria* was taken up because of two main considerations. (1) In spite of its importance as the type species, *Sporormia fimetaria* De Not was never grown in culture, as a consequence, no developmental studies were carried out with this species. The present workers succeeded in growing this fungus on P.D.A. medium and it was considered worthwhile to study its development so as to strengthen the validity on which the Ascomycetes are divided into Ascohymeniales and Ascloculares. (2) Because of the fact that in spite of a limited number of reports on the development of the ascocarp in *Sporormia* spp., there is a considerable disagreement on several points which have already been mentioned.

Materials and Methods

A single pseudothecium of *S. fimetaria* was placed at a time on an ordinary glass slide with the help of a sterile dissecting needle and the adhering portions of the substrate were carefully removed. Surface sterilization was done in a drop of 10% mercuric chloride and the pseudothecium transferred to the agar surface. In cases of bacterial contamination, streptomycin and terramycin discs were frequently employed. The agar medium used for these studies was P.D.A. (200:20:17).

In addition to the temporary mounts from the tube cultures, microtome sections of the ascocarps made an important part of this work. Tube cultures at an appropriate stage of development were used for microtomy. By tapping the test tube, solid agar medium along with the fungus was obtained on a piece of ordinary filter paper. After removing the superfluous agar by the help of a scalpel, small pieces of the fungus, bearing the developing ascocarps as well as part of the mycelium were obtained and used as sectioning material.

Fixation was carried out in "Bouin's Fluid" for a period of 24 hours. After its completion the material was washed in 50% ethyl alcohol till the picric acid colour disappeared. Dehydration and infiltration were combined by the usual-tertiary

butyl alcohol series. Embedding was done in parafin wax (56°C m.p.). Sections ranging from 5-8μ in thickness were used throughout. After deparafination in xylol the slides were brought to distilled water through 100%, 95%, 80% and 50% ethyl alcohol. For the nuclear staining iron alum haematoxylin (according to Johansen)[5] was employed. Counter staining was carried out by using "Light Green".

Using Canada balsam the slides were made permanent and were used for observations, photomicrographs and Camera Lucida drawings. In addition, temporary mounts made in aceto-carmine were utilized for nuclear staining.

Observations

Cultural Characters.—The growth of the fungus on the P.D.A. medium was slow and the colonies reached a diameter of 1-2 cm. in 10-15 days. In the early stages they were whitish but later on changed to grey. Dark brown vinaceous pigment was produced in the old cultures. This pigment diffused in the medium and was found to be more pronounced on the reverse side of the petri plates.

The production of the pseudothecia started after about ten days and mature pseudothecia were abundantly produced in the cultures ranging from 15-20 days in age.

The hyphae of *S. fimetaria* were septate, branched and more or less of uniform width. There were two types of hyphae which were observed in old cultures. In one type they were branched, septate and the cells were inflated (Fig. $1F_1$). The wall of these hyphae was thin, transparent and lacked the ability to be properly stained by cotton blue in lactophenol. The nuclei of such hyphae were not visible. The other type of hyphae were unbranched, composed of rounded cells in which the nuclei were observed. The cells of these hyphae were also thin-walled, more or less transparent and unable to be stained with cotton blue in lactophenol (Figs. $1F_2$-F_7).

Development.—Microscopic examinations of haematoxylin stained permanent slides as well as Aceto-carmine stained temporary mounts revealed that the vegetative hyphae were uninucleate (Fig. 1A). The development of the ascocarp was found to be associated with a pseudoparenchymatous structure, derived either by repeated divisions of a single cell or by the fusion of several vegetative hyphae. This indicated that in *S. fimetaria* the formation of a pseudoparenchymatous structure is of primary importance rather than the manner in which it has been derived.

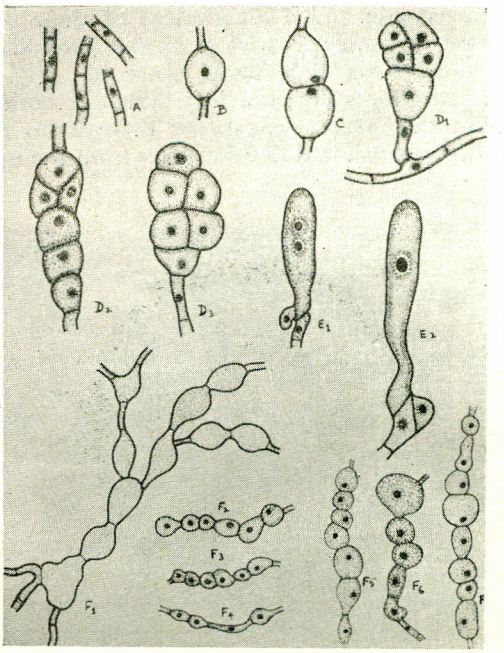

Fig. 1.—Camera lucida drawings: A, Hyphae showing uninucleate cells; B, enlarged and somewhat globose unincleate vegetative cell; C, D_1-D_3- pseudoparenchymatous structures composed of unincleate cells; E_1-E_2, showing the formation of asci by croziers; Fl, thin-walled, branched hyphae consisting of spindle-shaped cells; F_2-F_7, thin-walled, unbranched hyphae consisting of rounded cells.

In cases where a single cell gave rise to a pseudoparenchymatous structure, some of the uninucleate vegtative cells became enlarged and somewhat globose in shape (Fig. 1B). After repeated divisions each of such cells produced a group of uninucleate pseudoparenchymatous cells (Figs. 1C, $1D_1$-$1D_3$). At the initial stages these structures were of different shapes but gradually they attained more or less a globose form. The cells forming these pseudoparenchymatous structures remained uniform in size throughout the developing ascocarp but in the later stages it was found that the cells in the upper part became larger than in the lower. Smaller cells were more pronounced in the region of the ascogenous hyphae. The cells of the outer region became thick-walled and pigmented. These cells finally produced a hard, brittle peridium of the mature ascocarp.

In the early stages of the centrum development, before the ascogenous hyphae were visible, pseudoparaphyses were differentiated and were found to be vertically arranged. This showed that the vertical orientation of the pseudoparaphyses is independent of the asci and the ascogenous hyphae and that the pseudoparaphses are formed earlier than the ascogenous hyphae. The occurrence of

free ends of these elements was not observed on the lower side. The attachment of these structures on the upper as well as on the lower side of the locule was repeatedly observed (Fig. 2). These structures are attached either at both the ends in the intact ascocarp or there is no strict rule that they should develop only from the upper

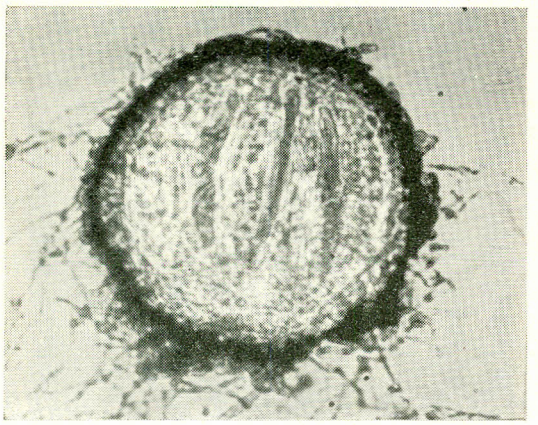

Fig. 2.--Photomicrograph of microtome section of the ascocarp showing pseudoparaphyses attached at the upper part as well as the lower part of the locule.

or only from the lower part of the locule. Their function as sterile elements to guide the asci to the ostiole of the ascocarp seem to be of more importance than the way they developed. The number of pseudoparaphyses seemed to increase with the increase in the size of the ascocarp. There was a limited, well distinct region near the lower part of the locule where the ascogenous hyphae were produced. The cells which gave rise to these hyphae became binucleate. The binucleate condition is brought about either by nuclear migration from one cell to the other or by endomitosis. Some of the cells towards the base of the pseudoparenchymatous structure were observed to consist of darkly stained cytoplasm. The nuclear nature of these cells was not very clear as the cytoplasm possessed a strong affinity for haematoxylin and invariably masked the nuclei. At certain spots these cells were connected to the croziers and possibly the ascogerous hyphae may have originated from them. The formation of asci was noticed through crozier formation in a normal way (Figs. $1E_1$-$1E_2$). In other type of development the formation of pseudoparenchymatous structures took place by the fusion of the vegetative hyphae. In the 9-10 days' old cultures it was observed that the vegetative hyphae intertwined and formed hyphal knots (Fig. 3). These knots gradually became more compact and enlarged. When these structures reached a considerable size the vegetative cells fused with one another producing a pseudoparenchymatous structure (Fig. 4). The cells of these structures were invariably uninucleate. Further development of the ascocarps from such structures was found similar to that in which pseudoparenchymatous structure is derived by the division of a single uninucleate vegetative cell.

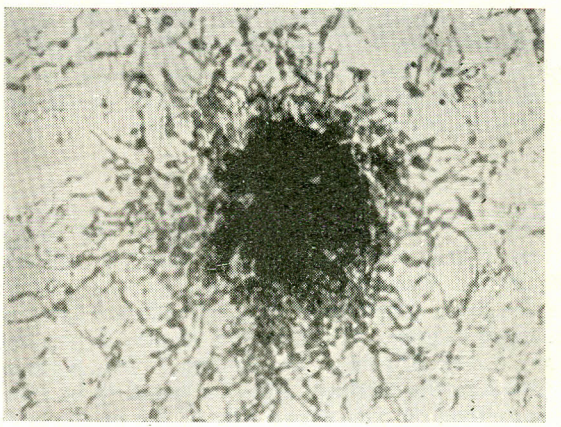

Fig. 3.—Photomicrograph of microtome section showing hyphal knots in the formation of pseudoparenchymatous structures.

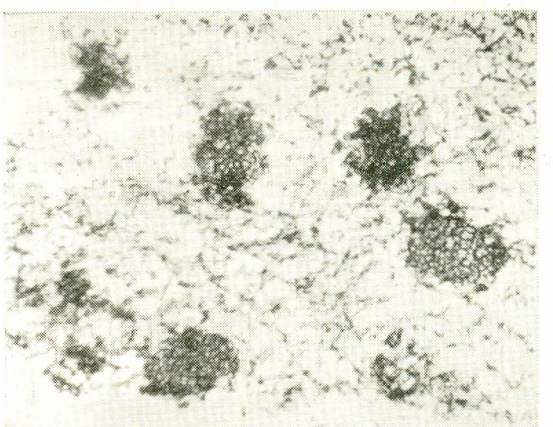

Fig. 4.—Photomicrograph of microtome section showing pseudoparenchymatous structure produced by the fusion of the vegetative hyphae.

In addition to the above observations made on the development of the ascocarp the arrangement of spores in the spore-bundle was detected. In *S. fimetaria*, eight ascospores are arranged in a

parallel fashion, forming a truncate bundle surrounded by a common gelatinous sheath. This arrangement does not permit the observation of the pattern of the orientation of spores in the bundle. In a transverse section of the asci it was possible to detect the arrangement of the spores. The eight spores were oriented in such a way that one was in the centre and seven surrounding it (Fig. 5). This arrangement was found to be consistent for all the spore- bundles.

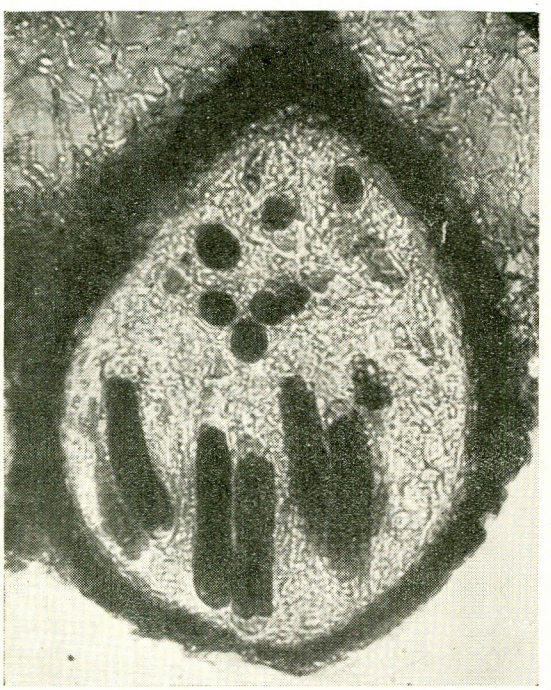

Fig. 5.—Photomicrograph of microtome section of the ascocarp with tranverse sections of the asci, showing one spore in the centre and seven surrounding it.

Discussion

Dangeard[6] claimed that in *S. intermedia* the ascocarp develops from a multinucleate, globose cell while Satin[7] studying the same species did not find such structures. As the observations made by Dangeard[6] and Satin[7] did not agree on this point, it is considered doubtful whether in this species the ascocarp develops from a multinucleate cell. Arnold[8] in his studies on *S. leporina* showed that a single uninucleate cell by its repeated divisions gave rise to an ascocarp. As far as *S. fimetaria* is concerned it agrees with Arnold's studies of *S. leporina* with respect to the uninucleate nature of the vegetative cell which gives rise to the ascocarp. In addition, the cells of the pseudoparenchymatous structure derived by the fusion of vegetative hyphae were also found to be uninucleate.

Arnold[8] described that the pseudoparaphyses originate on the upper part of the locule and the lower ends hang free. These cells elongate by intercalary growth and become enlarged at their tips forming a definite zone at the lower part of the cavity. Through further elongation, the enlarged free tips finally become attached at the bottom of the cavity and become binucleate. It is from these cells that the ascogenous hyphae originate. Such enlarged tips of the pseudoparaphyses forming a zone at the base of the locule were not observed in *S. fimetaria*. According to Luttrell[3] the type of development reported by Arnold is somewhat unusual and the cells of the pseudoparaphyses are uninucleate. It seems probable that presence of binucleate cells at the base of the locule in a close neighbourhood of pseudoparaphyses might have been the reason why Arnold[8] associated the binucleate cells with the pseudoparaphses. On the other hand, according to Dangeard[6] and Satin[7] in *S. intermedia* the binucleate cells giving rise to ascogenous hyphae originate at the base of the locule. Studies on *S. fimetaria* do show that the binucleate cells originate at the base of the locule without involving the end cells of the pseudoparaphyses.

Arnold[8] showed that the pseudoparaphyses are attached above and free below in *S. leporina* whereas Dangeard[6] and Satin[7] reported that these structures are attached below and free above in *S. intermedia*. The studies made by Kerr[9] on *Venturia pirini*, *V. rumicis*, *V. inaequalis* and *Pleospora herbarum* belonging to loculoascomycetes showed that these elements are attached both above and below at all the stages of ascocarp development. As the pseudoparaphyses are not perfectly straight structures, they are liable to be cut in such a way so as to give an impression that they are free at one and attached at another end, whereas in the intact ascocarp they are actually attached at both the ends. On this basis the interpretation of Arnold[8] as well as of Dangeard[6] and Satin[7] becomes extremely doubtful. In *S. fimetaria* the psedoparaphyses are attached either at both ends or some are attached below and free above and some attached above and free below. It is difficult to prove either of the alternatives because the pseudoparaphyses are wavy and liable to be cut at places so as not to give a clear idea about their arrangement. Thus in *S. fimetaria* the pseudoparaphyses could be either attached at both the ends or some attached above and free below while some attached below and free above.

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