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**Study of the Nuclear Behavior in the Basidia and Basidiospores of
Schizophyllum commune Fries**

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STUDY OF THE NUCLEAR BEHAVIOR
IN THE BASIDIA AND BASIDIOSPORES
OF SCHIZOPHYLLUM COMMUNE FRIES

By

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A Thesis submitted to the Faculty
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in
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INTRODUCTION

Schizophyllum commune Fries is a member of the Hymenomyces. This genus has long intrigued mycologists because of the peculiar "split gills" it characteristically possesses. For a long time, however, not much importance was attached to the fungus until it was reported from many parts of the world and its pathogenicity as a wood-destroying agaric established. Despite these facts relatively little is known about the fungus. Perhaps the phase of development most completely described was the development of the "gills", Essig (1922). Because of the location of the hymenium and stipe attachment, Essig (1922) suggested that the fungus more properly belongs in the family Thelphoraceae rather than the Agaricaceae; however, further work in the development of the fungus, especially the cytology, must properly be accomplished before such a change can be made. The cytological work on Schizophyllum is very incomplete, especially the cytology of the basidia; and many stages of development involving work in this field have not been demonstrated. Problems are presented by the exceedingly small nuclei, the small basidia, the hardness of the sporophore, difficulty in staining, and the difficulty of sectioning the sporophore thin enough for cytological investigation.

STATEMENT OF PROBLEM

The purpose of this investigation is to demonstrate

the cytology of the nuclear behavior in the basidia and the development of the basidiospores with special reference to the nuclei. This work is only, however, a preliminary for further work on the cytology and other phases of investigation.

HISTORICAL

The nuclear behavior in the basidium of the four-spored members of the Hymenomycetes as described by Wagner (1893), Marie (1900), Harper (1902), and Sass (1929) can be generally outlined as follows: The fertile hyphae of a sporophore form, at their ends, single cells - the basidia. The young basidium contains two nuclei, each with a single nucleolus; and the union of the two nuclei occurs, with some variance, when the basidium is one half to two thirds its mature size. The nuclei migrate close to each other and become flattened at the points of contact. The nuclear membranes disappear at the area of contact and the nuclei become continuous. The constriction in the fusion nucleus gradually disappears and the nucleus becomes spherical. It has not been stated how the chromatin masses are combined, but the two nucleoli are considered to unite. As the basidium increases in size, the fusion nucleus migrates to the apex of the basidium and divides. A second division immediately follows. As the basidium elongates further, the four nuclei migrate to the middle of the basidium. Four long, slender sterigmata bud out from the apex of the basidium;

and the nuclei migrate to the base of the sterigmata. Here the nuclei become slightly smaller (Wagner, 1893), narrowed, and elongate. One nucleus then passes through each sterigma into each basidiospore, one basidiospore being formed at the end of each sterigma by this time. The spores elongate and remain uninucleate; or the nucleus in each basidiospore divides once resulting in binucleate basidiospores, Marie (1900).

Since 1719 when Schizophyllum was mentioned in Dellenius' "Catalogus plantarum sponte circa Gissam nascentium", some of the most important work done on the fungus has been in the field of genetics. Kniep (1920, 1923) established that Schizophyllum was heterothallic and quadrisexual. Sass (1929) demonstrated the cytological basis for homothallism and heterothallism in some other members of the Hymenomycetes. Buller (1941) explained the "diploidization" process with some reference to Schizophyllum commune.

So far as the author is aware, the most extensive work on the cytological aspect of meiosis in Schizophyllum was performed by Essig (1922) on S. commune Fries. He reported that the basidia are born at the ends of thin-walled hyphae, and are only slightly larger in diameter than the hyphae which bear them. After maturing in succession, the mature basidia project beyond the hymenial surface, each having four long, slender sterigmata and bearing four basidiospores. The basidia measured $5\mu \times 20\mu$ and the granular nuclei, in

the vegetative mycelium, ranged from 0.3 μ to 0.5 μ in diameter. He reported having seen two nuclei in some basidia, four nuclei in others, and two nuclei in the basidiospores. Since two nuclei are characteristically found in the young basidium, he presumed that the usual fusion of the two nuclei occurred; and meiosis followed, resulting in four nuclei in the basidium as described by Wagner (1893), Maire (1900), Harper (1902), and Sass (1929) for some of the other Hymenomycetes.

MATERIALS AND METHODS

Small portions of a number of pilei of Schizophyllum commune Fries were removed from sporophores and killed and fixed. The killing and fixing solutions used were Flemming's Medium Solution; Nawaschin's type formula I, consisting of 20 parts 1% chromic acid, 75 parts 1% acetic acid and five parts 40% aqueous formaldehyde (Sass 1940); and one of Carnoy's formulas consisting of three parts absolute ethyl alcohol and one part glacial acetic acid. After twenty-four hours fixation, the Flemming's and Nawaschin's solutions were removed by washing in running tap water for twenty-four hours. Specimens killed and fixed in the three parts absolute ethyl alcohol and one part glacial acetic acid solution were not washed but placed directly into 70% ethyl alcohol.

Dehydration was accomplished with the ethyl alcohol method to 70%, and further dehydration and infiltration

with paraffin was accomplished by the iso-amyl technique (Steil, unpublished) according to the following schedule:

Dehydration with Ethyl Alcohol

15%	-----	15 minutes
30%	-----	30 minutes
50%	-----	50 minutes
70%	-----	24 hours (indefinitely)

Dehydration with Iso-Amyl Alcohol

70%	-----	4 hours
80%	-----	4 hours
90%	-----	4 hours
Absolute	-----	overnite

Infiltration with Iso-Amyl Alcohol and Paraffin

- 1) One fresh change of absolute iso-amyl alcohol was made and paraffin chips were added immediately.
- 2) The vial was placed in an oven at 52°C. for twenty-four hours with the stopper removed.
- 3) Three changes of melted paraffin were made twenty-four hours apart.

The specimens were embedded in paraffin, trimmed into blocks, and mounted and sectioned with a microtome.

Schick injector and Enders razor blades were used in sectioning the material. Sectioning at various room temperatures was attempted. The temperature levels used were 60°F., 55°F., 50°F., 45°F., 40°F., and 26°F. Ice was used for cooling the microtome blade and paraffin blocks at all

temperatures except that of the 26°F. level which was done in the refrigerator of the Milwaukee Public Museum. Sections were obtained at thicknesses of 5 μ , 4 μ , 3 μ , and 2 μ .

The sections were mounted on glass slides using Mayer's albumin affixative, and dried on a warm plate for twenty - four to forty-eight hours. The paraffin was removed from the sections by placing the slides for two minutes in each of two changes of xylol, and the xylol removed by placing the slides for two minutes in each of two changes of 95% ethyl alcohol. The alcohol was removed by washing in tap water. Those sections killed and fixed with Flemming's were oxidized in a 3% solution of peroxide for one to two and one-half hours in order to remove the osmic acid. The following stains and techniques were used:

Iron haematoxylin

Iron haematoxylin and aniline blue

Iron haematoxylin and eosin

Iron haematoxylin and orange green

Iron haematoxylin and fast green

Iron haematoxylin and picric acid

Harris' haematoxylin

Harris' haematoxylin and fast green

Harris' haematoxylin and picric acid

Harris' haematoxylin, aniline blue, and fast green

Harris' haematoxylin, Lugol's solution, and picric acid

Newton's modification of Gram's stain

Flemming triple stain

Safranin and fast green

Safranin, Harris' haematoxylin, and fast green

Safranin, Harris' haematoxylin, and orange green

The Feulgen technique (see outline below)

The Feulgen technique and fast green

The Feulgen technique as used in this investigation has been so completely revised (McDonough, unpublished) that it is really a new technique. The schedule is as follows:

- 1) Process the slides in the usual way until ready for staining.
- 2) Hydrolyze in N/HCl at 60°C. for 5 to 7 minutes.
- 3) Stain in decolorized basic Fuchsin for 1 to 24 hours.
- 4) Wash in tap water
- 5) Rinse in a solution of 50% glacial acetic acid.
- 6) Rinse in a solution of 3 parts absolute ethyl alcohol and 1 part glacial acetic acid.
- 7) Rinse in a solution of 9 parts absolute ethyl alcohol and 1 part glacial acetic acid.
- 8) Rinse in 95% ethyl alcohol.[†]
- 9) Place slides in 70% ethyl alcohol for 12 hours.
- 10) Rinse in 95% ethyl alcohol.*
- 11) Dehydrate with absolute ethyl alcohol.

- 12) Clear with clove oil.
- 13) Add a drop of Canada balsam.
- 14) Add a cover glass.

OBSERVATION AND DISCUSSION

Essig (1922) reported that the alcohol dehydration and paraffin embedding method could not be used because the alcohol hardened the material and made sectioning impossible. He found it necessary to freeze the material and section it with a rotary microtome. He could obtain only fragments of the hymenium when the lamellae were sectioned at 5μ , and he did not report having made sections any thinner. With the use of the iso-amyl technique in this investigation, no hardening of the material was encountered; and it was possible to embed in paraffin and section with the ordinary microtome successfully.

In sectioning, the temperature was found to be a crucial factor. It was found that better and thinner sections were obtained not only by cooling the microtome blade and paraffin block with ice but by sectioning at low room temperatures. Sectioning at room temperatures of 60°F . and 55°F . was fairly successful at 5μ ; but compression was too great in thinner sections. Sectioning at 50°F . and 45°F . was excellent for thickness of 5μ , good for 4μ , and fair for 3μ ;

* At these points one can proceed by adding a drop of euparal and a cover glass, omitting the steps in between.

compression was too great at 2 μ . Sectioning at 40° F. was excellent for thickness of 5 μ , 4 μ , and 3 μ ; sections 2 μ in thickness were good. At the 26° F. temperature level it was found that a little too much compression was present in attempts made to date. From the above data, it appears that the optimum temperature for sectioning material involved in this investigation is 40° F.

All killing and fixing agents used yielded good results.

Of the staining techniques attempted thus far, those involving the use of iron haematoxylin were, with the exception of iron haematoxylin and orange green, the most successful. Generally, iron haematoxylin and fast green or eosin were the best for showing the structure of and defining all parts of the basidium.

Harris' haematoxylin and picric acid was rather good for differentiation of the nuclei, but was only fair for differentiation of the remainder of the basidium. No better results were obtained by using fast green as a counter stain.

The Feulgen technique proved successful in that the nuclei were stained well; however, nuclear definition and differentiation of the remainder of the basidium was not good, even with the aid of a fast green counter stain. The technique was useful, however, in confirming the nuclear behavior described in this investigation.

Other staining techniques attempted were not satisfactory.

In studies made thus far, the actual union of the two nuclei characteristically found in the young basidium could not be demonstrated. Since the union nucleus is present in the basidium when the basidium is approximately one-half its mature size (Pl. 1, Fig. 1), it must be assumed that the union of the two nuclei occurs at the time the basidium is approximately one-half its mature size as reported by Harper (1902) in *Hypochnus*. The basidium at this stage is only very slightly larger in diameter, if at all, nearer the apex than the base of the basidium. The cytoplasm in the basidium stains heavily at this stage since it is very finely granular and dense, with no vacuoles or just a few small ones. Therefore, it is difficult to determine the exact outline of the nucleus. The nucleus is more or less oval and lies about two-thirds of the length of the basidium from the base.

As it elongates, the whole basidium increases only slightly in diameter, the area near the apex increasing only slightly more in diameter than the base; and the union nucleus migrates to the apex of the basidium (Harper, 1902, with *Hypochnus*) and becomes more or less spherical (Pl. 1, Fig. 2). If present at all, the vacuoles are few and small; and the cytoplasm does not stain as heavily as that of the young basidium.

Here the first meiotic division occurs, the spindle being formed transversely across the apex of the basidium (Harper 1902 with *Hypochnus*), (Pl. 1, Fig. 3). The cytoplasm

may be slightly more vacuolated at this stage; however, the staining is not deep, and the apex of the basidium enlarges slightly more. The two resulting nuclei are oblong and lie near the apex against the wall of the basidium, their long axes being more or less parallel to the long axis of the basidium (Pl. 1, Fig. 4).

It cannot be stated definitely at this time whether the second meiotic spindles are formed perpendicular to the first, but there are indications that this is the case.

After the second meiotic division, the four nuclei arrange themselves at the same level near the apex of the basidium. They are equidistant from one another, oblong with their long axes more or less parallel to the long axis of the basidium, and are located against the wall (Pl. 1, Fig. 5). The cytoplasm is not deep staining at this stage, and a few small vacuoles may be present near the base of the basidium.

The four nuclei then migrate to the middle of the basidium, and the sterigmata begin to form at the apex. (Harper 1902 with *Hypochnus*). The nuclei lie close to each other and the cytoplasm around the nuclei stains so deeply that it is difficult to see the nuclei at this stage. The remainder of the cytoplasm of the basidium stains lightly, and small vacuoles are present especially at the base of the basidium (Pl. 1, Fig. 6).

The elongation of the four sterigmata continues until they become long and slender, and the formation of one

basidiospore is initiated at the tip of each sterigma. When the basidiospores are about one-third their mature size, they are oval or elliptical in shape. Either by migration or the passage of the cytoplasm of the basidium into the basidiospores, the four elongate nuclei begin to move from the middle of the basidium toward the apex. The nuclei become further apart and seem to move toward the apex along the sides of the basidium. Some of the cytoplasm around the nuclei still stains darkly, and small vacuoles may be present in the lower portion of the cytoplasm (Pl. 2, Fig. 7). The amount of the cytoplasm in the basidium decreases as the spores increase in size.

One of the four nuclei is then located at the base of each sterigma (Wagner 1893 with *Agaricus*). Here they become more or less tear shaped as they begin to narrow and elongate once again for passage through the slender sterigmata into the basidiospores. Only a single nucleus enters each basidiospore. Immediately after entry the nuclei are again more or less tear shaped. (Pl. 2, Fig. 8). The spores at this time may be one-half to two-thirds their mature size, and the cytoplasm of the basidium continues to enter the spores. While the base of the basidium seems largely void of cytoplasm small vacuoles may be present in the cytoplasm remaining in the basidium at this stage.

Very soon after a nucleus enters each spore the nucleus divides once (Maire 1900). The division figure may be three-fourths as long as the basidiospore. The cytoplasm of

the basidium which may have one or more large vacuoles at this stage continues to enter the basidiospores.

(Pl. 2, Fig. 9).

After division is complete, the spherical or elongate nuclei lie far apart from one another; sometimes at almost opposite ends of the basidiospore. The spores continue to enlarge as the cytoplasm of the basidium continues to enter them (Pl. 2, Fig. 10). At this stage one or more large and numerous small vacuoles may be present in the remaining cytoplasm of the basidium, and one or more very small vacuoles may be present in the cytoplasm of the basidiospores.

After the entrance of all or practically all of the cytoplasm of the basidium into the basidiospores, the basidium sometimes appears to have started collapsing. There may be one or more small vacuoles in the basidiospores and the nuclei begin to migrate closer together toward the apex of the basidiospores (Pl. 2, Fig. 11).

When the basidiospores are mature two spherical or elongate nuclei lie very close together at the apex of the spore. It seems characteristic of the spores that they remain in a very tight tetrad until the time of discharge (Pl. 2, Fig. 12).

As Essig (1922) reported, the walls of the basidiospores and basidia are very thin; however, the basidia are not always slightly larger than the hyphae which bear them. In this investigation it has been found that the basidia

frequently seem to originate from large parenchyma-like sub-hymenial cells. The nuclei do not always appear granular, but the dark staining chromatin is frequently found in aggregations of various sizes, especially around the periphery of the nucleus (Eg. Pl. 1, Fig. 2). No nucleoli have been seen in the nuclei during this investigation. The mature basidiospores, in addition to possessing just two nuclei, may have one or more large and many small vacuoles.

SUMMARY

- 1) Small portions of a number of pilei of Schizophyllum commune Fries were killed and fixed using three solutions; Flemming's Medium Solution; Nawaschin's type formula I consisting of 20 parts 1% chromic acid, 75 parts 1% acetic acid, and five parts 40% aqueous formaldehyde; and three parts absolute ethyl alcohol and one part glacial acetic acid. All yielded good killing and fixation.
- 2) The material was run up with the iso-amyl technique (Steil, unpublished) and paraffin, and yielded excellent results.
- 3) Sectioning was most successful at 40° F., and sections were cut at 5 μ , 4 μ , and 2 μ .
- 4) The iron haematoxylin and fast green technique was found to be the best for staining. The Feulgen technique was used for confirmation of the nuclear behavior.
- 5) The union nucleus is present when the young basidium is about one-half its mature size.
- 6) The basidium elongates and the union nucleus migrates to the apex.
- 7) The first meiotic division is transverse to the long axis of the basidium.
- 8) Four nuclei result after the second meiotic division and are arranged equidistantly in a plane which is perpendicular to the long axis of the basidium.
- 9) The four nuclei migrate to the middle of the basidium,

and four sterigmata are formed at the apex. Basidiospore development is initiated as the nuclei move toward the apex of the basidium. The nuclei arrange themselves at the base of the sterigmata.

10) One nucleus enters each basidiospore and divides once resulting in binucleated basidiospores.

11) During the various stages of the nuclear activity the shape of the nuclei varies being elongate, amoeboid or spherical.

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EXPLANATION OF FIGURES

Plate 1

- Fig. 1 Young basidium about 1/2 mature size with union nucleus 2/3 of the length of the basidium from its base. Iron haematoxylin and fast green. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 2 Union nucleus at apex of basidium (note dark staining chromatin aggregations at periphery of nucleus). Harris' haematoxylin and picric acid. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 3 Basidium showing first meiotic division of the nucleus, near the apex, transverse to the long axis of the basidium. Harris' haematoxylin and picric acid. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x2080.
- Fig. 4 Basidium with two nuclei resulting from first meiotic division arranged at periphery toward the apex. Harris' haematoxylin and picric acid. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 5 Four nuclei resulting from meiosis arranged equidistantly at the same level near the wall of the basidium (drawn at an angle). Harris' haematoxylin and picric acid. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 6 Four nuclei in the center of the basidium surrounded by dense cytoplasm. The beginning of the formation of sterigmata at the apex of the basidium. Iron haematoxylin and fast green. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.

PLATE 1



Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6

EXPLANATION OF FIGURES

Plate 2

- Fig. 7 Four nuclei approaching the base of the sterigmata, elongation of the sterigmata, beginning of the formation of basidiospores, and entrance of cytoplasm into the basidiospores. Iron haematoxylin and fast green. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 8 One nucleus entering one sterigma, one nucleus entering one basidiospore, one nucleus immediately after entering one basidiospore, and continued entrance of the cytoplasm. Harris' haematoxylin and picric acid. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 9 Two basidiospores each showing division of the single nucleus in the basidiospore and continued entrance of the cytoplasm. Iron haematoxylin and fast green. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 10 Two nuclei in each of two basidiospores resulting from division. Continued entrance of the cytoplasm. Iron haematoxylin and fast green. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.
- Fig. 11 Two basidiospores each showing the migration of nuclei toward the apex. No cytoplasm remains in the basidium, and the basidium is beginning to collapse. Iron haematoxylin and fast green. 2 μ . Fixing medium, Flemming's. Camera lucida, x 2080.
- Fig. 12 Mature basidiospores with nuclei at the apex of the spores. Only fragments of cytoplasm remain in the basidium. Iron haematoxylin and fast green. 4 μ . Fixing medium, 3 alcohol and 1 acetic. Camera lucida, x 2080.

PLATE 2



Fig. 7



Fig. 8



Fig. 9



Fig. 10



Fig. 11



Fig. 12

Approved

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