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BY

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SUSIE PERCIVAL NICHOLS.

INTRODUCTION.

Rees (20) was among the first to attempt a careful study of the mycelium of the *Basidiomycetes* with reference to the question of the origin of the carpophore. By making artificial cultures of *Coprinus stercorarius* in dung decoction on slides he was able to observe the formation of erect short hyphae on which he believed sexual cells, spermatia and carpogonia were borne. He also believed that he found a spermatium fused with a carpogonium. After fertilization, branches arise from the base of the carpogonium which developed into the carpophore.

Van Tieghem (25) also germinated the spores of *Coprinus stercorarius* and *radiatus* in dung decoction and studied the development of the carpophore. In his first paper he agreed with Rees. He found the swollen end cells on the lateral branches of the mycelium. These "carpogones" usually terminated in a papilla with which the spermatia fused. The carpogone then divided into three cells, the two lower developing a system of lateral branches which curve around and enclose the terminal cell. Their further development in slide cultures was prevented by lack of nutriment. But by observations made on larger cultures they were seen to be the beginnings of carpophores. Later Van Tieghem reversed this opinion.

Brefeld (3, 4) grew mycelium of *Coprinus stercorarius* from single spores in dung decoction and figures a series of stages in

the development of the young carpophore. From the older portion of the mycelium a perpendicular hypha very rich in protoplasm arises. This hypha branches profusely forming a dense snarl from the center of which a bundle of parallel hyphae develop forming the first indication of the stipe. Lateral branches are formed increasing the size of the mass and at the same time the stipe grows rapidly in length. The pileus and gills are differentiated very early in the development of the fruit body. Brefeld found no evidence of the existence of sexual organs at the formation of the carpophore.

With the study of the nuclear phenomena new stand-points arose. The work of Rosen (21), Rosenvinge (22), Wager (26, 27, 28), and Dangeard (5) has established the fact that the cells of the carpophore are frequently multinucleated while the basidia are at first binucleated. In typical basidia the two nuclei fuse and the fusion nucleus divides into the four spore nuclei.

Maire (15) found that the cells of the young carpophore are binucleated and that the cells of the hymenial layer never have more than two nuclei, but that the cells of the stipe and pileus may become multinucleated through the amitotic fragmentation of the two nuclei originally present. The young basidium when it is formed from the hymenial cells receives two and only two nuclei which unite to form the large fusion nucleus of the basidium. He further states that the nuclei in the series of binucleated cells in the young carpophore divide by conjugate divisions so that the two nuclei which fuse in the basidium are of widely different origin. But his evidence is not conclusive on this point.

Maire describes the division of the nucleus in the basidium in detail. The nuclear membrane disappears and the spindle appears at about the same time. The chromatin filaments break up into irregular granules or protochromosomes which are placed on the spindle without any definite order. At the end of the prophase these protochromosomes unite into two definite chromosomes. That the formation of only two chromosomes is not universal among the Basidiomycetes as Maire assumes has been shown by Wager, Ruhland and others. Maire states further

that the chromosomes after a longitudinal splitting are pulled apart at the center and move to the poles. The second division is similar to the first. The four centrosomes remain at the summit of the basidium while the nuclei move to the center or base of the basidium. Soon a sterigma is formed above each centrosome, and fibres appear extending from the centrosome to the nuclei which now move to the summit, probably through the influence of these fibres.

Two notes of Maire's (16 & 17) in the Comptes Rendus report that the last two or three cells of the ascogenous hyphae of *Pustularia vesiculosa, Galactinia succosa* and *Acetabula acetabulum* are binucleated. The ascus like the basidium is the last of a series of binucleated cells. In order that such a comparison should have any value we must know how the ascogenous hyphae originate.

In *Hypochnus* Harper (12) was able to trace a series of binucleated cells from the hymenium to the mycelium in the substratum. The mycelium did not form dense wefts or strands but spread through the decaying wood where it could be readily studied. The cells were regularly binucleated. The stages of nuclear fusion and division were similar to those described by Wager. At the equatorial plate stage the chromosomes were distinct and at least six or eight in number.

The origin of the binucleated cells was not determined by these observers. Maire states that the two nuclei in the spore of *Coprinus radiatus* pass into the germ tube and a cross wall may or may not be formed between them. The mycelium is then of two types, the one apocytic, the other cellular. The cells of the latter are uninucleated. He did not observe the transition from these stages to the binucleated condition found in the young carpophore.

In a preliminary notice Blackman (2) has given a brief account of the life history of two of the *Uredineae*. He finds that the spermatia do not have the structure of conidia but of male cells; a thin wall, no reserve material, a very large nucleus with no nucleolus and cytoplasm greatly reduced in amount. He also studied in detail the development of the aecidium of *Phragmidium violaceum*. The aecidium arises as a layer of uninu-

cleated cells just beneath the epidermis of the leaf. Each of these cells divides into a sterile cell above and a fertile cell below. The fertile cell becomes binucleated not by the division of its original single nucleus but by the migration through the wall of the nucleus of a neighboring vegetative cell of the mycelium. He says, "In the presence of the spermatia with their special cytological characters, etc., the only view that seems capable of explaining the facts is that the fertile cell was formerly fertilized by the spermatia, but that now the process has become reduced, fertilization by means of spermatia having been replaced by the more certain method of fertilization by the nucleus of a neighboring vegetative cell." In view of these facts he holds it to be evident "that the Uredineae present an alternation of generations which is as sharply marked as that of the higher plants."

Since the young carpophores invariably have binucleated cells these must originate either at the first formation of the carpophore or sometime during the growth of the mycelium. The latter hypothesis is suggested by Harper's observations on *Hypochnus*. In order to obtain some evidence on this point the study of the nuclei in the mycelium of some of the *Agarics* was begun at the suggestion of Professor R. A. Harper under whose direction the work was carried on.

METHODS.

Spores were collected from a large number of *Agarics* in the following manner. Zinc racks were washed in alcohol and passed through a flame and then placed in plates which had been washed in 95 per cent. alcohol. The racks were covered with bell jars which were also washed in 95 per cent alcohol. Two racks were placed side by side under each jar. Slides were washed in alcohol and passed through a flame and then placed on the lower bars of the racks, each rack holding four. The pileus from a mature fruit body was carefully removed from the stipe and placed on the upper bars of the rack. When the basidia discharge their spores they fall on the slides below thus lessening

the danger of infection from the gills. After the spores were discharged the pileus was carefully removed and the bell jar replaced the slides remaining on the racks until needed. Spores preserved in this manner remained pure for a year.

A decoction of string beans, (about 392 grams to a liter of water) proved to be the best nutrient although a decoction of *Coprinus* and of dung was used for some forms. For the early stages in the development of the mycelium small cultures were made in dishes holding 10 c. c. of the nutrient solutions. Large quantities of spores were sown and at the end of 12, 24, and 48 hours, the nutrient solution was removed by a pipette until only 2 c. c. remained, the dish was then filled with fixing solution which would thus be reduced about one-fifth in strength. After fixing 24 hours the spores were stippled on the slide by the method described by Harper (11) in his paper on the nuclear phenomena in the smuts.

Spores were also sown in thin films of agar-agar on sterilized slides. When the mycelium had attained the desired growth the entire slide was immersed in fixing solution. If the film loosened from the slide it was easily fastened again by a film of albumen.

To obtain mycelia, cultures were made similar to those described by Falk (8). Rye bread cut in slices two or three inches thick were moistened in bean decoction and fitted into battery jars five inches deep by four wide. For covers petri dishes four and a half inches in diameter were used. A thin layer of cotton was placed between the cover and the dish to allow free circulation of air. Agar-agar plates were also used.

The spores germinate in from six to eight hours and at the end of two or three days form a growth of mycelium which appears as a white mat about a quarter of inch in diameter on the surface of the bread. The mat increases in size rapidly until it is two or three inches in diameter. At the same time there appear all over the culture small white dot-like masses of mycelium. These are new growths from oidia scattered from the first mycelium. Falk has also described and illustrated such oidial colonies. These small secondary growths were removed

whole with about a quarter of an inch of the substratum. Larger mycelia were cut into small pieces for fixation. In order to force the fluid through the thick felt which the mycelium forms the material was placed in a small bottle and well covered with the fixing fluid. The bottle was then fitted into the end of a rubber tube which was connected with the air pump. The air was pumped out from the closely matted hyphae, after which the fixing fluid was renewed.

The material was fixed in Flemming's solutions both the stronger and the weaker. Merkel's and Herman's solutions were also used. The best results were obtained from Flemming's weaker solution. Both Flemming's triple stain and Heidenhain's iron haematoxylin gave satisfactory results.

Hypholoma perplexum, Pk.

The spores of *Hypholoma perplexum* were collected in great abundance. The carpophores appeared in great profusion on decaying stumps and logs of oak throughout September and October. The spores were collected and stored after the manner already described. A large per cent of the spores obtained from mature pilei germinated in a shorter time than those obtained from younger ones. The spores were sown in the hanging drop agar cultures made with bean decoction. The cultures were kept in a dark box at a temperature of 20° c. As all the spores do not germinate at the same time but vary from four to forty-eight hours in the time of the appearance of the germ tube a large number of stages are obtained from the same slide.

The mature spores were studied in the drop cultures and also in the cross sections of the gills where they were still in connection with the sterigmata.

The mature spore of *Hypholoma perplexum* has a dark brown opaque wall which before germinating swells to two or three times its original thickness becoming much lighter in color and transparent. At this time it is easy to distinguish two nuclei lying close together near the center of the spore. They show the usual structure of the resting nucleus and are small spherical bodies with a well defined membrane, a large distinct nucleole