THE STRUCTURE AND BEHAVIOR OF THE NUCLEUS IN
THE LIFE HISTORY OF PHYCOMYCIES NITENS
(AGARDH) KUNZE AND RHIZOPUS
NIGRICANS EHRLBG

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Among the contributions to various phases of the life history
of the Mucoraceae, a number of the writers do not include any
clear statement of the structure of the nucleus or how it behaves
in division. Most of the workers, who have attempted to follow
closely the behavior and structure of the nucleus in zygospore
formation, have described the resting nucleus in the forms studied
as consisting of a nucleole, a nucleoplasm containing little or no
stainable substance, and a nuclear membrane. In the consideration
of nuclear division they have generally described a type of mitosis
with a spindle formation, but in the details of this process the
descriptions are incomplete. Many of these have described numer-
ous nuclear fusions during the maturation of the zygospore together
with a disorganization of other nuclei that do not fuse.

Dangeard and Léger (1894a) in a cytological study of zygospore
formation in Sporodinia grandis and two species of Mucor have
described the structure of the nucleus in the vegetative hyphae and
the sexual branches of these forms as vesicular, consisting of a
surrounding membrane and a centrally placed nucleole, which
are separated by a non-staining "cytoplasm" containing a little
chromatin. In the young zygospores of these forms they find two
sizes of nuclei.

In another report, Dangeard and Léger (1894b) describe without
figure the structure of the nuclei in Mucor nucedo and M.
racemosus as similar to that described above. In the ripe zygo-
spore of Sporodinia the small and, later, the large nuclei disappear.
After their disappearance the zygospore contains a varying num-
ber of deep-staining bodies.

Léger (1895a) figures the nucleus of the vegetative mycelium
as a vesicular body with a centrally placed nucleole. He describes
karyokinetic figures as occurring in the vegetative hyphae but does not figure nor describe the process of nuclear division. He finds that the nuclei, evident up to this time, begin to disintegrate as the zygospore matures and its protoplasm becomes spongy and filled with oil. This disintegration is a process in which the nucleole becomes smaller and finally disappears. The nucleus then becomes vacuole-like. A few nuclei, which are two or three times larger than those entering the zygospore at the time the gametes fuse, remain in the zygospores longer than do the other nuclei, but ultimately disappear. In another paper, without figures, Léger (1895b) has reported on the conditions he has found in species of Pilobolus, Rhizopus, Chaetocladium and Mortierella, together with those forms previously studied with Dangeard. He describes the structure of the nucleus in the vegetative hyphae in all the forms as similar to that described by Dangeard and himself. He indicates a point of interest as to the fate of the nucleus in the mycelium and the columnella in that the nuclei become reduced to nucleoles which persist after all other traces of the protoplasm have disappeared.

Istvanffy (1895) describes the nucleus in Mucor sp. He finds that the nuclei are scattered throughout the entire protoplasm; that they are elliptical or spherical in shape and usually provided with a nucleole. In spore formation only one nucleus enters each spore. During preparation for germination, the spore is observed to have eight to ten nuclei. In the tips of hyphae where he considers that the nuclei are youngest, he states that they may consist of only a small homogeneously-stained body, not exceeding 1 μ in diameter.

Harper (1899) in his description of spore formation of Pilobolus states that the vegetative nuclei of this form divide in the basal bulb, thus giving rise to the nuclei that enter the sporangia during its development. He also finds that the nuclei divide in the protosporangium and in the swelling spore in preparation for germination. In none of these references to nuclear division does he describe the structure of the nucleus or indicate how it divides. However, he figures several nuclei, each consisting of a dense central body enclosed by a granular nucleoplasm and a nuclear membrane. He also illustrates several division figures, each consisting of an elongated body, at the two poles of which are several dense granules; the two groups of granules are connected by one or more fibrous strands.
Swingle (1903) in a report of spore formation of Phycomyces and Rhizopus describes the nuclei in the sporange of either form as in a resting condition. In either form each nucleus is approximately spherical in shape, and consists of one or two nucleoli, a finely granular chromatin, and a surrounding membrane. In the columella of Rhizopus he describes the nuclei as disintegrating. According to him the process of disintegration consists of the appearance of a red-stained mass on one side of the nucleus, followed by the nucleus taking on the appearance of a shrunken homogeneous mass often irregular in shape and staining much as do the crystalloids of the protoplasm. In Phycomyces the structure of the nucleus is similar except that it may have as many as three nucleoli, and in this form he has described the disintegration of the nuclei in the mycelium as in the columella of Rhizopus.

Gruber (1901) in a description of nuclear behavior in zygospore production of Sporodinia grandis agrees with Léger up to the point at which the protoplasm of the two gametes become mixed. From this point on he does not find nuclei of two sizes, nor fusions taking place between paired nuclei, nor evidence that nuclei are disintegrating, but rather that the nuclei are clearly in evidence after fourteen days of development of the zygospore. After five or six weeks and again at the end of six months he still finds nuclei in the same condition as when the zygospore was formed.

In a later paper, reporting on the sexual process of Zygorhynchus Moelleri, Gruber (1912) describes nuclear fusions as occurring between the nuclei of the male gamete and an equal number of those of the female gamete.

Dangeard (1903) describes the nuclear structure and behavior in a species of Mucor and in Sporodinia. In either of these forms a nucleus of the gametes consists of a small nucleole and a homogenous achromatic nucleoplasm. After the fusion of the gametes the nuclei divide one or more times, then nuclear fusions occur. A daughter nucleus, thus arising in the zygospore, consists of a nucleole, a network of granular chromatin, and a nuclear membrane. In the process of nuclear fusion, the membranes fuse at the point of contact. At first the two nucleoles rest within the membrane thus formed and then fuse. Some of the nuclei do not fuse but disintegrate. In rather mature zygospores, this author finds large deep-staining bodies throughout the protoplasm, which he suggests have arisen from mucorine crystals.
Moreau (1911a) and 1911b) in describing cytological studies, especially in zygospore formation, of species of Mucor, Zygorhynchus, Circinella, Rhizopus and Sporodinia states that a nucleus of these mucors consists of a chromatin nucleole, a nucleoplasm, and a nuclear membrane. The nucleole, is either centrally, eccentrically, or laterally placed. In some cases in Mucor a centrosome, chromatic in nature, is observed on the external surface of the nuclear membrane. In the columella of Rhizopus he finds a modification of the nucleus in that there is no nuclear membrane and the nucleus consists simply of a homogeneous body. According to Moreau, the nucleus divides mitotically in the vegetative hyphae and in the zygospores of the forms studied. The process of mitosis is inaugurated by the disappearance of the nuclear membrane and of the nucleole. No other stages characteristic of prophases are described. He describes an equatorial plate stage in which double chromosomes are borne on a straight spindle, terminated at each pole by a centrosome. He describes and figures a later stage in which two daughter chromosomes are in process of moving toward each pole. Stages in the reorganization of the daughter nuclei are not described. In the zygospore, the mitotic nuclear divisions take place, according to Moreau, as if activated by the mixing of the protoplasm from the two fusing gametes. He also describes a form of amitotic nuclear division of the homogeneous nuclei in the columella of Rhizopus. In Mucor, following the nuclear divisions in the young zygospore, the nuclei fuse in pairs, giving rise to a large number of fusion nuclei. A number of nuclei fail to fuse and later disintegrate. In Zygorhynchus the nuclear disintegration takes place before fusion, and in the zygospores of this form, only two fusion nuclei are formed. The fusion nuclei of all the forms studied by Moreau are similar in structure. Each contains a single large nucleole. He states that the fusion nuclei persist in the mature zygospore and form the basis of the nuclei of the thallus arising from the germination of the zygospore.

Moreau (1913) has published the results of very extensive cytological research of a large number of the Mucoraceae. These later results are in accord with his previous work. In the case of Phycomyces he states that he saw clearly stages in the fusion of the nuclei in the young zygospore at the time the spiny exospore was being formed. The fusion of the nuclei in the zygospore, of those forms for which he describes the process, consists in the fusion of the two membranes at the point of contact, thus forming one
nuclear cavity. At first the two nucleoles lie separate, but later fuse. In his description of nuclear division in this paper, he conforms to his previous report.

Miss Keene (1914) in her cytological studies of Sporodinia grandis finds that the nucleus in this form is granular in structure and contains a centrally placed nucleole which she considers chromatin in nature. She believes that nuclear divisions occur in the tips of the two sexual branches. As the mixing of the two fusing gametes takes place, nuclear fusions occur. Miss Keene describes no divisions in the zygospore preceding nuclear fusions, as was described by Dangeard and Moreau. She describes nuclear degeneration of unfused nuclei in the zygospore, but her description of the process is somewhat different from that given by other workers. According to her, the process is first accompanied by an enlargement of the nucleole which does not stain as deeply as in preceding conditions. The nucleus eventually becomes a homogeneous-staining mass. According to her the fusion nuclei are in evidence in zygospores two and three months old.

Later, Miss Keene (1915) has contributed results of cytological studies of Phycomyces nitens. She has figured the nuclei in germinating asexual spores as containing one or two deep-staining bodies. The resting nucleus is described by her as bounded by a membrane and containing a deep-staining body, probably the nucleole, and chromatin granules throughout the nuclear cavity. She states that in the germinating spores, the young sporangium, suspensors, and progametes, the nuclei show conditions that are very suggestive of division figures. She suggests that in nuclear division figures containing three bodies, two may be chromosomes and the third the nucleole. In the young zygospore she finds that many of the nuclei are arranged in groups of twelve to sixteen. Some of these nuclei fuse in pairs; others do not fuse. Later the unfused nuclei of the zygospore coalesce to form one or two large amorphous masses that persist in the zygospore several months old. Similar masses are formed within the suspensors. The fused nuclei persist in the zygospore and are confined to a thin peripheral zone of the cytoplasm.

Burgeff (1915) has contributed a very interesting description, without figures, of his studies of the cytology of Phycomyces nitens and mutants of this species. His observations as to the structure of the nucleus or as to the facts of nuclear fusion are not fully in accord with those of either Keene or Moreau. He describes the
nucleus of the vegetative mycelium as consisting of a very small homogeneous body, and suggests that it is identical with a chromosome. Division of a nucleus in the vegetative hyphae consists simply in the separation of the chromatin body into two daughter nuclei. He finds that in spore formation in the sporangium from five to twelve of these chromatin bodies are enclosed within each spore. He describes no change in the structure of the nucleus during zygospore formation, except that after the fifth day of development the nucleus is spongy and contains a single chromatin body. He reports no nuclear divisions or fusion during the formation and maturation of the zygospore.

The nuclei in the zygospore preparing to germinate are surrounded by a membrane but have only one chromatin body and by the time the germ tube pushes out, eleven to twelve days after sowing, the nuclei are more or less irregular in outline and provided with several chromatin bodies and a nucleole. At this stage they are several times larger than the nuclei of the sexual generation. In the germ tube and sporangium, nuclear divisions occur among the large nuclei. Burgeff states that all stages in mitosis are difficult to make out. Clear prophases occur in which the nucleole disappears. The chromatin is separated into twenty-four (estimated) chromosomes. Twelve chromosomes move to each pole within the membrane; no equatorial plate stage is observed. Burgeff characterizes this as a heterotypic division, following which, he states, a homotypic division occurs in which distinct chromosomes are present. Following the homotypic division successive divisions take place, giving rise to a large number of membraneless nuclei. These nuclei, with a number of nuclei surrounded by membranes, become the nuclei of the spores formed within the germ-sporangium. He suggests that the nuclei with membranes are either unfused nuclei of the gametes or else nuclei that have not passed through reduction division. In spore formation only one nucleus enters into the formation of a spore. After spore formation, as they mature, the nucleus of each spore divides successively so that each mature spore contains a number of nuclei. In his summary, however, Burgeff states that he has not observed the division of nuclei with membranes within the spores of the germ-sporangium.

Burgeff (1920) describes the nuclei of the parasitic mould, Chaetocladium, and of its host, Mucor, as being very similar. The nucleus of either form consists of a deep-staining body with or without a clear zone surrounding it. The clear zone is more fre-
quently observed in poorly nourished hyphae. Burgeff calls the deep-staining body chromatin, states that it is nucleole like, and that it is frequently located eccentrically in the nucleus. In well-nourished hyphae the chromatin body is much larger than in poorly nourished hyphae. His special study of the nuclei has been in the galls formed at the point of attack of the parasite. He points out that the nuclei in the sporangiophores contain a much larger chromatin body than do those in the vegetative branches; he attributes this to the presence of reserve food in the sporangiophores. He states that the Mucor nuclei in the gall divide mitotically, but gives no figures nor details of the process. He also describes crystalloid bodies as arising from degenerating nuclei.

**Materials and Methods**

The material from which the preparations were made was grown in petri dishes on potato-glucose agar prepared with distilled water. Flemming’s medium fixing reagent was used almost exclusively, after a wide variety of reagents was tried out. The imbedded material was cut in sections 4μ–12μ thick and the preparations stained with Flemming’s triple stain. Heidenhain’s iron-alum-haematoxylin method of staining was also used, but in no case gave the minute differentiation obtained by the use of the triple-stain.

For the germination of the zygospores of Phycomyces, zygospores that had remained on the original substratum, upon which they grew, in a dark room at an average temperature of 20 degrees C., germinated readily when transferred to a non-nutrient 2% agar medium. The zygospores germinated, forming a single germ-sporangium within four or five days after transferring. The cultures of germinating zygospores were grown in a light room, at room temperature. On account of interruption in the work, the study of nuclear behavior in the germ sporangie and of the sex of the germ-sporangiospores has not been completed.

**Phycomyces Nitens**

In the swollen spores of Phycomyces about to germinate there are one or more vacuoles with several (4–13) nuclei imbedded within the cytoplasm. After the germ tube has been formed (fig. 1) the nuclei are distributed throughout the enlarged cell by the increase of vacuolar volume. The nuclei show no change in structure up to this period. So far as I have observed the
nuclei show no change in structure until some time after the germination of the spore. As soon as the first hypha has several branches, one observes the first changes in the nuclei as described below.

A nucleus in the resting spore and during early stages after germination consists only of a homogeneous, deep-staining, approximately spherical body which I consider to be chromatin in nature. Its surface is undoubtedly membranaceous, although no membrane is differentiated. With the triple stain this body stains deeply with the safranin while the surrounding cytoplasm stains orange. The first change that occurs in such a nucleus is the swelling of the chromatin body accompanied by the formation of a vacuole within it. In some instances the chromatin becomes distributed for a time in the peripheral zone. With further growth of the vacuole the chromatin becomes separated at one or more points. If separated at one point, the chromatin may present a somewhat crescent-shaped optical section (fig. 2f). On the other hand, it may be separated so as to present a somewhat horseshoe-shaped section with a chromatin body in the opening of the horseshoe (fig. 2b). Usually the chromatin substance is separated into from three to six bodies, as the growth of the vacuole progresses (figs. 2a, 2c, and 2d). Figure 2 illustrates the distribution of such nuclei associated with vacuoles from a hyphae in the substratum of a three-day-old culture.

The question naturally arises in this connection concerning the nature of the membrane surrounding the nuclear vacuole. The vacuole evidently originates below the surface of the chromatin. But as soon as the chromatin is separated sufficiently at any point a thin membrane remains surrounding the vacuole; the membrane does not stain with the safranin as does the chromatin but stains more as does the surrounding cytoplasm. This may be due to the fact that it is so thin as not to appear differentiated from the surrounding cytoplasm, but I am inclined to believe that, while the membrane originates within the chromatin, its composition becomes changed and so differentiated from the chromatin.

The above-described vesicular body has undoubtedly been interpreted by most workers as the nucleus. Since, as I shall point out later, such a vesicular body functions as a single structure of the protoplasm at certain stages in the life history of the plant, perhaps it would not be incorrect to consider it a nucleus. However, on account of its behavior in connection with division and distribu-
tion of the chromatin in rapidly growing mycelium, I consider the vesicle a vacuole, and each portion of chromatin at its periphery a nucleus.

The nuclei that have been thus formed are destined soon to become disassociated from one another and in subsequent nuclear division give rise to daughter nuclei by a similar process.

There appear to be two different forces that bring about the ultimate separation of the daughter nuclei. One is the growth of the vacuole from within as just described. The other force is the streaming of the protoplasm within the hyphae, giving rise to elongated division figures and most frequently observed in the hyphae of the substratum where their tubular form is not uniform in outline and very angular in contour. In such hyphae of living cultures I have frequently observed that the rate of streaming is not uniform throughout a given diameter. This character of the streaming evidently accounts for the fact that the vesicular division figures are sometimes elongated and often curved in the hyphae of the substratum (figs. 2c, 2d).

Where the enlargement of the vacuole alone is operating, the daughter chromatin masses and the vacuole with which they are associated present a spherical figure (figs. 2a, 2e). While the outline of the vacuole is still unobliterated, one often finds the daughter nuclei beginning a subsequent division (fig. 2e). In this figure the daughter nuclei have lagged behind the progress of the vacuolar membrane and appear within the space bounded by the membrane that was carrying them apart. Here, too, the original vacuolar membrane is losing its identity and becoming thickened and evidently is about to be incorporated into the slimy portion of the cytoplasm. It is also observed that slimy cytoplasm is being formed within the cavity of the vacuole between the daughter nuclei.

In the sporangiophore and sporangium one does not usually find division figures of the nucleus elongated or otherwise distorted, for evidently the protoplasm in which the nuclei are imbedded is moving uniformly or is at rest, as is the condition of the protoplasm of the sporangium and sporangiophore as the sporangium reaches maturity. In these structures, therefore, one usually finds the nuclei associated with approximately spherical vacuoles (figs. 3a and 3b). Figures of similar form are also found in the gametes, suspensors, and immature zygospores.
In some instances a vacuole associated with a nucleus may arise without bringing about a division of the nucleus. In such a case the vacuole originated below the surface of the chromatin mass and gradually enlarges eccentrically, giving rise to a vesicular structure consisting of a vacuole with a single chromatin body at its periphery (fig. 3a). In this case the vacuole is not destined to play a part in nuclear division. In some cases the vacuole enlarges uniformly around the chromatin body, leaving the chromatin suspended within the vacuole (figs. 4f, 4g and 5d). Thus it appears that numerous vacuoles of the cytoplasm arise from the chromatin bodies; they may or may not have divided nuclei as they were formed.

In figures 4a-4k are represented a number of nuclei taken from the two gametes of a sexual apparatus. All show stages in nuclear division except figure 4d where the vacuole was formed at one side as described above. Figures 4e, 4f and 4g represent the type of structure in which the vacuole was formed by progressing on all radii of the sphere, leaving the chromatin body within the vacuolar sap. A nucleus thus suspended within the vacuolar sap forms a second vacuole within itself to bring about division (figs. 4f and 4g).

During the process of nuclear division strands of protoplasm are frequently observed, extending across the vacuole from one chromatin body to another (fig. 4a). They vary considerably in different figures and do not always appear. Such strands are evidently formed by the chromatin. In some cases the strands connecting the daughter chromatin bodies appear to be so closely associated with the vacuolar membrane as to cause the membrane to be somewhat flattened, to conform to the straight lines of the connecting strands, and thus present a vacuole with a broken surface of flat faces rather than the curved surfaces of other vacuoles (figs. 4f, 4h and 4i).

In many cases the ultimate destiny of a vacuole of a division figure is that it becomes obliterated by an incorporation of the membrane with the slimy cytoplasm, and the space within becomes gradually filled with slimy cytoplasm, either reticulate or homogeneous. Figures 4j and 4k represent such groups of nuclei after the vacuole has thus become obliterated. The group of seven nuclei represented in figure 4k evidently originated from two sister nuclei. The three in the lower portion of the group and to the left are
evidently from one of the nuclei, and in this group each has begun
to form a vacuole.

After the fusion of the gametes and during the growth of the re-
sulting zygospore, I find no evidence of a pairing or fusing of the
nuclei. During the growth of the zygospore, nuclear division
occurs continuously until the protoplasm finally takes on its resting
condition as described below. All of the nuclear division figures
of the zygospore are of the spherical type. The only observed
change in the protoplasm after the fusion of the gametes is the
increased affinity of the cytoplasm for the safranin and gentian
stains. As a result of this change in staining reaction, the differ-
entiation of the nuclei is not as definite in the zygote as in the sus-
penors or the vegetative hyphae. The nuclear division figures of
the zygospore are of the same form as has been described for them
in the sporangae (figs. 5a–5e). Figure 5 represents a zygospore
during the growing period while the exospore is being formed and
shows the fragments of the walls of the gametes which fused to
form the first wall of the zygospore.

During the growing period of the zygospore many of the divid-
ing nuclei are found in groups of four to eight. In figure 5a six
dividing nuclei of such a group, at the same optical level, are
shown in their relative positions. Evidently these have originated
from the same nucleus through two successive divisions, but, since
the vacuoles bringing about the divisions have enlarged but little,
the daughter nuclei of each division remain in close proximity to
each other. On the other hand, the isolation of other daughter
nuclei in the zygospore is accounted for by the greater expansion
of the vacuole separating the nuclei. Figure 5b contains both
isolated and grouped nuclei.

The nuclei of a zygospore which has attained nearly its full size
are found associated with smaller vacuoles than are the nuclei of
a zygospore during the rapidly growing period, or of the vegetative
hyphae. Compare figures 5c and 5e of a full-grown zygospore with
figures 3a and 3b from a sporangae, and with figures 6a and 6b from
a growing zygospore.

In the stained preparation of the protoplasm I have found no
evidence of fat, nor structures identified with its formation. How-
ever, a test with Sudan III of protoplasm crushed out of zygospores
in all stages of development shows very clearly the presence of fat.
Undoubtedly the fat in the zygospores occupies many of the larger
spaces that appear as vacuoles in the preparations. The fat is
evidently dissolved by xylol or chloroform during the clearing and imbedding processes.

A mature zygospore two weeks old shows very little of the slimy portion of the cytoplasm. Figure 7 shows the condition of the protoplasm in a zygospore from an eleven-day-old culture, in which the zygospores would vary in age from three to seven days. At this stage one finds that the protoplasm consists of three distinct portions, vacuoles, the slimy portion of the cytoplasm, and nuclei. The vacuoles evidently represent spaces filled with oil, cell sap or gas. Some of the vacuoles contain a slightly-staining granular substance, as has often been described by other workers, but this is not so specially characteristic of the vacuoles of the zygospore as it is of the vacuoles of the vegetative hyphae. The slimy portion of the cytoplasm is still in evidence but in a much reduced proportion. Most of the nuclei together with the vacuoles associated with them present a different aspect than has been described for them heretofore in the life history of the plant. Some of the vacuoles associated with the nuclei appear as has been previously described; the others now contain a substance that stains with the same reaction as do the chromatin bodies at the periphery of the vacuoles. It appears that the chromatin substance has increased in volume accompanied by a reduction in the proportion of the cytoplasm.

In figure 7a a distinct vacuole is present, but the surrounding membrane has become thickened and stains deeper. Figure 7b illustrates a nuclear vacuole that has become filled with a stainable substance. Figure 7d represents the final stage in the development of such a nuclear structure in the mature zygospore, the form in which most of the protoplasm, except the fat, is found. These structures are often so closely crowded together that their surfaces are compressed flat against each other (fig. 7d). During this change the same stain-absorbing elements as are contained in the chromatin characterize all the material contained in the nuclear vacuoles. I call these structures "reserve food bodies." Each consists of a variable amount of food reserve, which occupies the space of the nuclear vacuoles, and one or more chromatin masses which may usually, though not always, be observed as deeper-staining bodies at the periphery (fig. 7b).

My interpretation is that during the process of maturation of the zygospore there are no nuclear fusions. After the fusion of the two gametes the nuclei continue to divide rapidly, as they do in
the actively growing vegetative mycelium, until the zygospore is mature. Then the nuclei, acting as metabolic centers, proceed to transform available food into a reserve form of protoplasm which is stored in the space previously occupied by the vacuolar sap of the associated vacuole. As for the fat, I am unable to conclude whether it is a metabolic product of the cytoplasm or of the nuclei.

In preparations of zygospores in which the protoplasm is in a resting condition, the proportion of stainable substances and of the spaces appearing as vacuoles varies considerably. This variation is undoubtedly due to a difference in the amount of nutrition available for the given zygospores. In some zygospores a comparatively large proportion of the space may be unoccupied by the deeply-stainable substances (fig. 7). In others the same substances occupy a much greater proportion of the space within the zygospores. In some cases this material is located largely in the peripheral zone, in others distributed more or less irregularly throughout.

In crushing zygospores under water, bubbles always appear from within the rupture of the inner, leathery spore wall. Therefore, I conclude that the clear spaces appearing in preparations are in some instances occupied by gas resulting from metabolism.

The protoplasts of zygospores five months old are unchanged in appearance from those that have matured up to the resting stage. After the zygospores are placed on moist agar for germination, one finds that many of the dense nuclear bodies become separated from each other by vacuolated cytoplasm. This change is most evident in the peripheral zone. In the more central region the nuclear structures stick together in larger masses. In the peripheral zone there are many cases of a single nucleus with a vacuole or a group of several such nuclei surrounded by a very dense mass of slimy cytoplasm. The vacuoles associated with some of these nuclei have lost a large portion of their deep-staining substance. Figure 8 illustrates a section of a germinated zygospore with a portion of the germ tube. It contains many of the nuclear structures filled with food reserve as has been described for the zygospores before germination; many of them persist during zygospore germination and the formation of the sporangium. In the peripheral zone are many of the dense cytoplasmic masses surrounding one or more nuclei. Figure 8a shows such a cytoplasmic mass, wherein one nuclear structure is still unchanged, and from at least two of the nuclear vacuoles the reserve food has been dissolved. The later condition of the nuclear vacuoles is clearly illustrated in figures
8b, 8c and 8d. Such figures correspond very closely to those of nuclei observed in the zygospore during maturation when the vacuoles were beginning to receive the reserve substance.

An insufficient number of stages were fixed to warrant reporting on the process of spore formation in the germ sporangium. However, it has been determined that nuclei of the type shown in figures 8a, 8b, 8c, and 8d pass into the sporangiophore and are found there before and after spore formation in the sporangia. There appear to be no nuclear divisions in the germinating zygospore or in the germ sporangiophores either before or after spore formation.

**Rhizopus Nigricans**

A complete study of the nucleus of *Rhizopus nigricans* has been made in the vegetative mycelium, in the sporangia, in the mature and germinating spores, in the gametes, and in the zygospores during maturation. Since no germination of zygospores has been obtained, it has been impossible to follow the nuclear behavior through that process.

It is very evident that nuclear behavior is, in general, the same in Rhizopus as has been described for Phycomyces in the processes of nuclear division and in the formation of reserve protoplasm stored in the vacuoles associated with the nuclei of the zygospores.

The nuclear vacuoles do not become quite as large in Rhizopus as they do in Phycomyces. Compare figures 9a, 9b, and 9c taken from the sporangium of Rhizopus with figures 3a and 3b taken from the sporangium of Phycomyces; both sporangia were at about the same stage of development.

In Rhizopus the obliteration of the vacuole, causing the separation of the chromatin masses, takes place sooner after the separation of the daughter nuclei, and, therefore, in the case of Rhizopus one finds more frequently the nuclei disassociated from a vacuole.

Figure 9c illustrates a case in which two sister nuclei evidently were at first associated with the same vacuole and later one of them formed a second vacuole, which separated the two chromatin masses still further. A third small vacuole also has formed between them. A somewhat similar case is illustrated in figure 11b in which a nucleus from a young zygospore has divided and one of the daughter chromatin masses is forming a vacuole within another vacuole. Figures 10a, 10b, and 10c are dividing nuclei taken from a vegetative hypha.
Another minor variation in the process of nuclear division, occurring in Rhizopus as compared with Phycomyces, is that in Rhizopus the chromatin mass is separated more frequently into two portions rather than into three, four, or more as in Phycomyces (figs. 10a, 10b, 10c). In a few cases observed, however, in Rhizopus the chromatin may be separated into several portions (figs. 13a and 13b).

Figures 11a and 11c illustrate cases in which the vacuolar membrane has progressed further than the two daughter chromatin bodies, thus leaving them within the vacuole, and figure 13b shows one such chromatin body forming a second vacuole within itself. Figures 13a, 13b, and 13c represent nuclei from a zygospore at a time when the associated vacuoles are becoming filled with homogeneous reserve substance, as was described for the nuclear vacuoles of Phycomyces.

Figure 12 represents a spore from the sporangium of Rhizopus before the spores have shrunk and their walls have thickened. The nuclear structures observed here apparently shrink as the spore matures and shrinks. Each structure thus becomes one of the dense nuclei observed in the mature spore and that appear as soon as the spore germinates, as was figured for Phycomyces (fig. 1).

**Nuclei of Aged Mycelium**

The nuclei of the vegetative, aged mycelium behave very similarly to those in the maturing zygospores. Nuclear division continues in the vegetative hyphae up to a time which I conclude is determined by a change in water balance. For, at a time when no further growth of mycelium occurs, its nuclear vacuoles take on the appearance of those which contain reserve food in the zygospores (figs. 14a and 14b). In figure 14a, taken from a 36-day-old culture of Phycomyces, the vacuole has become filled with a homogeneously-staining substance, and some of the nuclei associated with the vacuole are still partially differentiated. Figure 14b represents a similar condition of a nuclear structure taken from a vegetative hypha of a 14-day-old culture of Rhizopus.

As the mycelium becomes dried out in aging cultures, the slimy cytoplasm does not disappear from the mycelium on a large scale as it does in the zygospores. However, in the aged mycelium partially dried out, some of the hyphae appear empty, except for a few of the deep-staining nuclear structures and a very slight
amount of slimy cytoplasm; other hyphae contain a large amount of slimy cytoplasm and nuclear structures filled with reserve food (fig. 16).

Mycelium of Phycomyces forty days old was placed on moist nutrient agar and after sixteen hours many hyphae had grown out from the old mycelium. Preparations made from sections of this material showed that nuclear structures of the type described above for aged mycelium were abundant in the new hyphae in the substratum. Some had lost completely the reserve food of the vacuoles and the nuclei were in a state of active division. Most of the nuclear structures were in a transitory condition in which the vacuolar portions had swollen considerably; the reserve food was partially dissolved and some of the nuclei were beginning division. Figure 15a shows several nuclei in a very dense cytoplasm which apparently has been formed from the reserve substance of the vacuolar portion. Figure 15b shows a nuclear structure in which most of the reserve food has been dissolved and the vacuole is quite clear; one of the nuclei is in progress of division and a second nucleus is apparently unchanged.

The foregoing nuclear behavior fits in well with the growth habits of these plants. The plant grows rapidly with a favorable water supply and during the growing period the nuclei divide rapidly and are distributed into the newly formed portions of the plant. As soon as a decrease in available water takes place or, at least, after a certain minimum is reached, growth stops and at the same time nuclear division ceases. As this change occurs most of the nuclei are at a certain stage in division. The nuclear vacuoles cease to expand but apparently proceed to absorb food reserve from the surrounding cytoplasm, giving rise to nuclear structures, filled with reserve food, as has been described above in old dried-out mycelium. When such mycelium is again supplied with water, new hyphae are observed growing out from the aged hyphae. The nuclei and associated structures resume activity, giving up the food reserve and dividing as previously described.

**DISCUSSION**

In the above report it has been difficult to use the term "nucleus" and convey the full idea that the writer holds as to its form and structure. It is evident that the fundamental unit of structure composing the nuclei is a small, homogeneous mass that stains
readily with safranin. This is undoubtedly the body seen and
described by many writers as the nucleole. The vesicle that I have
called a vacuole associated with one or more chromatin bodies
seems to have been considered by others the nucleoplasm.

As I interpret the structure of the protoplasm of Phycomyces
and Rhizopus, the homogeneous body is nucleus; it has the general
nature of chromatin in that it stains readily and is present in all
stages in the life history of these fungi.

Single masses of this chromatin may exist unassociated with
others, as in the spores, where the single body appears to have origi-
nated from the reassembling of two or more small bodies that had
previously been formed by the division of a single chromatin body,
and in the vegetative hyphae, where individual bodies have be-
come disassociated from others by the obliteration of a vacuole that
previously had been active in bringing about division. Most fre-
cently several nuclei are associated with a vacuole and these
nuclei may or may not be connected by slender strands of proto-
plasm other than the vacuolar membrane. A vacuole thus asso-
ciated with one or several nuclei seems to have been formed within
the chromatin mass. Usually, the vacuole grows in such a way as
to rearrange the chromatin substance into several bodies around
its periphery. At other times, as it grows, the vacuole forms eccen-
trically in the chromatin body, leaving most of the chromatin un-
disturbed.

Although it has been observed that slimy cytoplasm seems to be
formed in the immediate vicinity of the nuclei, as in the germinat-
ing zygospores and in the propagation of the mycelium from aged,
dried plants, and that at the time this cytoplasm is being formed
the nuclear vacuoles apparently lose their reserve, one can hardly
conclude whether the nuclei are active metabolic centers contribut-
ing to this metabolism by forming cytoplasm from the reserve of
fat outside of the nuclear structures, or whether the food has been
stored in the nuclear vacuoles, and, later, cytoplasm formed di-
rectly from it. Possibly both the reserve in the nuclear vacuoles
and the fat outside are used. But it appears evident that the nuclei
are very intimately associated with the processes that bring about
the renewed vegetative condition of the protoplasm.

The nuclei in the mature spores of the sporangia are dense, ho-
ogeneous bodies. When the spore is first formed in the sporangia it
contains several nuclear vacuoles with two or more chromatin
bodies associated with each. The condition of the nuclei in the
mature spore appears to come about through a reverse in the process of nuclear division brought about by the expansion of the nuclear vacuoles. At about the time the spores are formed there seems to be a change from expansion of their protoplasm to a contraction, due to a loss of water. At the same time the nuclear vacuoles shrink in size and the chromatin bodies of each nuclear structure reassemble to form a single chromatin mass. They remain in this condition until after spore germination, at which time they resume division.

Although it is impossible to state definitely which structures described by other workers correspond to structures that I have described, it is quite plain that most workers have found many of the same structures in different mucors.

The non-staining “cytoplasm” of Dangeard and Léger (1894) is undoubtedly the vacuole that I have described as associated with most nuclei. Léger (1895 a) believes that in aged hyphae the nuclei becomes reduced to nucleoli and persist after the rest of the protoplasm disappears. I find a similar condition in many of the aged hyphae, considering that the nucleole described by Léger is identical with what I have described as a nuclear structure, consisting of one or more nucleoli associated with a vacuole which has become filled with a reserve protoplasm.

Istvanfi (1895) describes the nuclei in the growing tips of the mycelium as small homogeneous bodies. This conception of a nucleus is what I hold for the nucleus throughout the life history of the two forms I have studied.

I find no formations in the two forms I have studied that correspond to the dividing nuclei in the protosporae of Pilobolus as figured by Harper (1899), in which he interprets fibrous strands between two nuclei as the remains of spindle fibers.

Swingle (1903) has described the nuclei of Rhizopus and Phycomyces as having two or three nucleoli. These are undoubtedly what I have described as the daughter chromatin bodies of a single nucleus. His description of disintegrating nuclei in the columella of Rhizopus and aged mycelium of Phycomyces corresponds very closely to my description of the nuclear structures with reserve material in aged mycelium, which later may become active upon resumption of growth on the part of such mycelium. Other workers have frequently referred to similar nuclear disintegration. It seems probable that the disintegrating nuclei in zygospores men-
tioned by Miss Keene (1915) are the same as the reserve food bodies of zygospores described by me.

As to the question of nuclear fusions in the zygospores of the various forms of the Mucoraceae, a review of the various reports shows that even in the same species there is little agreement as to when fusion takes place or how many nuclei take part. Several, as my review of the literature shows, do not find nuclear fusion at all in the early formation of the zygospore. Burgeff (1915) describes nuclear fusions as taking place at or shortly after zygospore germination. Many of the workers have described nuclear fusions as taking place soon after fusion of the gametes. For this period I have described rapid nuclear division.

Burgeff postulated a theory as a result of his work to account for the effects brought about by some nuclei fusing and others not fusing. If the nuclei do not fuse anywhere in the life history of the plants, as my results seem to indicate, the behavior of the nuclei in the germ sporangium remains to be demonstrated before any theory to explain the significance of sexual process in the Mucoraceae can be advanced.

**Summary**

1. The nucleus in *Phycomyces nitens* and in *Rhizopus nigricans* is a dense, homogeneous, chromatin body.

2. The nucleus divides by a method of fragmentation into several portions; the complete separation of these fragments is brought about by growth of a vacuole formed within the body of the mother nucleus. In some instances the separation of the daughter nuclei is aided by the streaming of the protoplasm.

3. Vacuoles frequently form eccentrically in a nucleus in such a way as not to bring about nuclear division.

4. Nuclear division continues during the growth of the fungus in the sexual generation. In the vegetative hyphae nuclear division is arrested by the discontinuance of water absorption by the mycelium; in the spores of the sporangium, by a reverse of water absorption to water excretion; and in the zygospores by maturation, which is undoubtedly accompanied by cessation of water absorption.

5. In zygospore formation no nuclear fusions seem to occur.

6. When nuclear divisions stops in the zygospore and in the vegetative hyphae, reserve material is stored in the vacuoles associated with the nuclei.
7. The nuclei in process of division in the embryonic sporangiospores reverse their progress and the portions reassemble to form the nuclei of the mature spores.

8. Upon germination of the zygospores of *Phycomyces nitens* the reserve material in the nuclear vacuoles contributes to the formation of slimy cytoplasm. There is no evidence of nuclear division in the germ tube or in the zygospore.

9. Upon the resumption of growth of mycelium, the reserve of the nuclear vacuoles contributes to the formation of new cytoplasm, as do the similar structures of the germinating zygospores.

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**BIBLIOGRAPHY**


**EXPLANATION OF FIGURES**

All figures drawn with the aid of the camera lucida.
Figs. 1—10, 14a, 15a, 15b and 16 are from *Phycomyces nitens*.
Figs. 11—13 and 14b are from *Rhizopus nigricans*.
Magnification of figures 7 and 8, 90 diameters; figure 5, 186 diameters; figures 2 and 16, 1050 diameters; figure 5b, 2100 diameters; and all other figures 3250 diameters.

**PLATES XIV AND XV**

Fig. 1. Germinated spore.
Fig. 2. Hypha from substratum, from culture 3 days old, grown from spore.
Figs. 2a—2f. Nuclei from hyphae similar to that in fig. 2.
Figs. 3a and 3b. Nuclei from sporangia before spore formation.
Figs. 4a—4k. Nuclei from gametes showing various stages in nuclear division.
Fig. 5. Young zygosporée during growing period, showing portion of a suspensor to the right.
Fig. 5a. A group of nuclei from zygosporée shown in fig. 5.
Fig. 5b. Detail of protoplasm from zygosporée in fig. 5.
Figs. 5c and 5e. Dividing nuclei from zygosporée in fig. 5.
Fig. 5d. Nucleus of same zygosporée around which a vacuole has formed.
Figs. 6a and 6b. Nuclear division figures from a maturing zygosporée and with comparatively large nuclear vacuoles.
Fig. 7. Mature zygosporée, about 5 days old. The numerous dense bodies are the nuclear structures, referred to in text, in which the nuclear vacuoles are filled with reserve material.
Figs. 7a-7d. Nuclear structures with reserve material.
Fig. 8. Germinated zygosporée with dense cytoplasm around nuclear structures in peripheral region at the right; nuclear structures with reserve still unchanged in the central region. A small portion of germ tube appears.
Fig. 8a. Cytoplasmic mass formed around nuclear bodies.
Figs. 8b-8d. Nuclear structures from which reserve material has been dissolved.
Figs. 9a-9c. Nuclear division figures from sporangia before spore formation.
Figs. 10a-10c. Nuclear division figures from vegetative hyphae.
Figs. 11a-11c. Nuclear division figures from young zygosporée.
Fig. 12. Embryonic spore. Nuclear division arrested in progress at about this stage.
  Figs. 12a and 12b. Stages in the maturation of spores, and shrinking of nuclear vacuoles.
  Figs. 13a and 13b. Showing nuclear vacuoles becoming filled with reserve material in a zygospore.
  Fig. 13c. Four sister nuclei from zygospore; one is enlarged somewhat by a vacuole within, and is becoming filled with reserve material.
  Figs. 14a and 14b. Nuclear vacuoles filled with reserve material, from aged hyphae.
  Figs. 15a and 15b. Nuclei from hypha propagated from aged mycelium. In 15a slimy cytoplasm is forming around the nuclear structure; in 15b the reserve material has been dissolved.
  Fig. 16. Aged hypha after 16 hours on culture medium, after its protoplasm has renewed activity.