Some recent work on the cytology of fungus reproduction, II.

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In the first article of this series[1]) the results of cytological investigations published during the year 1911 were considered. Certain papers were unintentionally omitted and accounts of them are here incorporated with the idea of making the series as complete as possible for the use of those interested in this branch of the subject. A section of the fungi which abound in interest are the *Archimycetes*, a group of microscopic fungi which usually infect water plants, and which are remarkable for the little vegetative structure they possess. As in the case of many simple organisms a controversial point is whether they are primitively simple or simple by reduction. Barrett (1912) has worked at three species of *Olpidiopsis*, *O. vexans* (= *O. Saprolegniae* A. Fisch.), and *O. luxurians* Cornu. The zoospores are biciliate having two equal cilia arising from the same point. One cilium, in motion, trails behind, and, crossing the upper end of the zoospore at an angle, and leaving it usually at the side, gives it the appearance of the short lateral cilium that has been described in this and in some other closely related genera. There is present a type of diplanetism, a phenomenon common in the zoospores of the *Saprolegniineae*. The zoospore penetrates into the host and is, after a brief time, lost to view. Its individuality is maintained, however, and it gives rise to a single sporangium, there being no plasmodium formed. The young parasite is uninucleate, but soon the nucleus divides rapidly and the organism becomes multinucleate. Fragmentation of the protoplasm is believed to be simultaneous throughout the sporangium. The resting-spores arise sexually. The larger oogonium and the smaller adjacent antheridium are at first naked but soon surround themselves with cell walls which become fused at the point of contact. Both cells contain a number of nuclei, twenty-five to thirty in the youngest oogonia seen, though the number in the later stages is not stated. The contents of the antheridium pass through a small fusion pore into the oogonium, which soon closes up by the growth of the surrounding wall. "There is no contraction of the oogonial protoplasm to form an oogonium and no apparent changes comparable to those taking place in oogenesis in the higher *Oomycetes*. . . . . There is no apparent difference in the male and female nuclei, and this fact, coupled with their extremely small size, makes it impossible to definitely follow their subsequent relation to each other. However, there are indications which strongly suggest a fusion of nuclei." After fertilization the oogonium becomes the oospore directly. The resting nucleus possesses a deeply-staining, somewhat prominent nucleolus, a slightly granular nucleoplasm, and a rather indistinct network with frequent

1) See Mycol. Centralbl. 1912, 1, 202—207, 259—267.

deeply-staining thickenings. The first stages of division resemble somewhat the synaptic knot of higher plants but Barrett does "not believe that to be their nature". The chromosomes become massed together into a large, deeply staining body from which the spindle appears to emerge. There is no indication of centrosomes or of any structure which would suggest nuclear polarity.

This account recalls what Löwenthal recorded in *Zygorhizidium Willei* (1905) where however the sexual organs are uninucleate and a copulating tube is put out from the smaller antheridium.

One of the *Archimycetes* in which a sexual process has long been known is *Polyphagus Euglenae.* Wager (1898) gave a brief account of the structure of the nuclei and of their behaviour during the formation of the zygote. Dangeard (1900) confirmed in general these results. Wager (1913) has again studied the cytology of the fungus. Reproduction takes place by the production of zoospores in sporangia, which may be formed on the ordinary vegetative cells, on cysts, or on the sexually produced zygotes. The thallus is unicellular and uninucleate, and is provided with delicate pseudopodia which penetrate the *Euglena* cells. The structure of the resting nucleus differs somewhat from the normal structure in the higher plants and recalls what was described by Percival in *Synchytrium endobioticum.* There is an arc-shaped nucleolus and a central, more or less spherical, lightly staining hyaloplasm connected to the nuclear membrane by delicate radiating threads. The nucleus is surrounded by a mass of deeply staining chromidia. In the nuclear divisions in the zoosporangium a large portion of the nucleolus is extruded into the cytoplasm, only a small part being retained for the formation of chromosomes. The zoospore possesses a single cilium at the base of which is an oil drop in close connection with the nucleus. A chromidial mass surrounds both the latter and appears to be continuous with the cilium. The zoospore germinates immediately. Sporangia are formed as outgrowths of vegetative cells, cysts or zygotes according to nutrimental conditions. Cysts resemble smooth-walled zygotes but contain a single nucleus. The zygote is formed by the fusion of two ordinary vegetative cells which, instead of producing zoosporangia, become transformed into gametes. The gametes are usually different in size, the smaller one functioning as the male, the larger one as the female. The zygote wall may be either spiny or smooth, the former apparently being the normal form. No difference was noted in the formation of these. This is in agreement with Dangeard's observations though opposed to those of Nowakowski (1876). The male gamete puts out a delicate copulating pseudopodium-like process which, in contact with the female cell, swells and produces the zygote. "Immediately following the appearance of the zygote, the protoplasmic contents of the male cell pass through the delicate pseudopodium into the zygote. A perforation appears in the wall between the young zygote and the female cell and the contents of the latter pass through it into the zygote[1])."

The nuclei of the fusing gametes have exactly the same structure as those of the ordinary vegetative cells. The male nucleus is usually smaller

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1) Dangeard, in describing the formation of smooth-walled zygotes states that the female nucleus ordinarily passes in first, then the male.

than the female nucleus and contains less chromatin. The two nuclei come together in the zygote and the male nucleus grows until it is almost exactly similar in size to the female nucleus. The two nuclei then move apart to opposite sides of the cell and extrude chromidia, a more or less distinct group being formed by each. Shortly after their appearance these chromidial masses fuse together and form a "chromidiosphere" or "chromidiocentrum" in the centre of the cell. Fusion of the generative nuclei does not occur until the contents of the zygote pass into the sporangium. It is only in the sporangium that nuclear division occurs. The divisions have been studied in the asexual sporangia though the first division was not observed. The spindle is internal. The chromosomes number about ten or twelve and are very minute only a small amount of the chromatin mass being used up. The nuclear wall breaks down at the poles and centrosome-like structures become visible. The prophases and anaphases appear to be those of normal mitosis. WAGER thinks that *Polyphagus* shows relationships with the *Mucoraceae* and the other *Oomycetes* and with the *Protozoa*, and that the chromidial fusion and the later nuclear fusion "may afford some clue to the explanation of the delayed nuclear fusions and double nuclear fusions observed in the higher Fungi".

GRIGGS (1912) has worked at *Rhodochytrium Spilanthidis* which "seems to occupy a transitional position between the protococcoid algae [such as *Phyllobium*] and some of the chytridiaceous fungi. . . . It has no chlorophyll and is strictly parasitic in its mode of life. . . . Although entirely incapable of photosynthesis, it develops abundant starch. But the starch grains are apparently built up directly in the cytoplasm, for neither plastids nor pyrenoids have been found" — a "paradoxical combination of characters". The plant has more or less of red pigment in all its stages. In both resting cysts and zoosporangia there is an extensive system of haustorial rhizoids proceeding from the basal portion. The two are distinct from the very beginning but their nuclei undergo the same development which, in the case of the resting spore leads to a remarkable, but seemingly universal and perfectly normal shriveling preparatory to the long dormant period, and in the zoosporangium to mitosis. At the end of the vegetative activity of the resting spore the rhizoids are ultimately cut off by cell walls and the mature spore has a two layered cellulose exospore and a thick non-cellulose endospore. On the beginning of germination in the spring, the nuclei again become turgid though they are apparently smaller than before shriveling up. In the later developmental stages of the zoosporangia there is always one large vacuole which occupies the upper half of the cyst. The latter reaches its full size before there is any indication of nuclear division, but when this commences the stages follow each other in rapid succession until a large but variable number of nuclei have been formed. Then segmentation occurs which appears to be brought about by the precipitation of membranes around the protospores which quickly round off and ultimately give rise to zoospores. These frequently contain starch grains, but are colourless except for the red anterior end. They possess two anterior cilia which are of a more highly type than those of the *Chytridiales*. The zoospores correspond in every important particular save in the absence of chlorophyll with algal zoospores in general. They are uninucleate and there is a deeply staining body at the base of the cilia which is connected with the nucleus. When there is an insufficiency of fluid present, the zoospores fuse in pairs. "The process of conjugation is not different from that common in various algae." In development the nucleus grows from 4 or 5 μ until it may reach the enormous size of 50 to 60 μ in the largest zoosporangia (cf. *Synchytrium*). The primary nuclei have enormous nucleoli and peculiar masses of chromatin as in the case of *S. decipiens*. "In the first type of mitosis the spindle, which is usually unipolar at first, is formed from coarse acicular fibers that appear within the nuclear cavity; it has no connection with the nuclear membrane. The spirem is formed from that part of the chromatin which lies in the equatorial region, the rest being cast out; it is frequently entirely within the spindle. The second type of mitosis presents no unusual features. No centrosomes or true asters were seen.... The cytology of *Rhodochytrium* bears a strong resemblance to that of *Synchytrium*. These resemblances suggest that *Synchytrium* was derived from protococcoid anestors."

Kusano (1912) has studied the life history and cytology of *Olpidium Viciae*. "In the fungi, the copulation of motile isogametes has hitherto received no special attention." Sorokin (1874) described the mycelial vegetative body as derived from the zygospore resulting from the zoospores in *Tetrachytrium*. Fisch (1884) observed the formation of the zygote by copulation of zoospores in *Reesia* and *Chytridium Mesocarpi*. Griggs (1910) working with *Monochytrium* states that the swarm spores perforate the cell wall and enter the cells where they present amoeboid forms within the protoplasm of the host. Some of these fuse in pairs, the nuclei remain distinct and the zygote forms a binucleate resting spore. Other swarm spores grow to from zoosporangia.

In *O. Viciae* the zoospores are colourless and possess a long posterior cilium. The swarming period depends largely upon the temperature. The zoospores undergo an "amoeboid deformation" several times before coming to final rest, when they soon begin to encyst. Copulation of zoospores is quite usual. A motile zygote is formed which behaves differently according to conditions. No external difference can be observed between the fusing zoospores, "but from the fact that any two spores coming into contact are not always induced to fusion . . . it appears highly probable that a certain internal differentiation is called forth in becoming the gamete". Kusano thinks there is "no attracting action between the zoospores" but that there is a certain period or age at which copulation is possible. "It will be seen that the sexual action . . . is at an exceedingly primitive state and in reality a sexual distinction between the gametes is still obscure." After encysting, the zoospore and the zygote present a quite similar phase of development up to a certain stage: the former later develops into the zoosporangium, the latter into the resting sporangium. The mode of infection of the host plant is of the usual type. When the zoosporangium is approaching its maximum growth a membrane appears on its surface. At first it is uninucleate but with growth the nuclei increase in number. While the nuclei are few the cytoplasm is compact, then it becomes vacuolate and later reticulate. At this stage the nuclei become larger presenting a distinct structureslet, a nucleolus, chromatin and a few linin threads being recognisable within a well defined wall. In certain *Phycomycetes* this period is considered to be the stage just before zoospore formation but Kusano records here as in *Synchytrium Puerariae* (1909) that further growth takes place increasing the amount of cytoplasm and the number and size of the nuclei, the mature condition being representend by a dense consistency of the cytoplasm. In no case was a typical mitotic division observed during the growth stage; the divisions resemble amitosis but there is a disappearance of the nuclear membrane and the chromatin as well as linin. "It is very likely the "Promitose" proposed by Naegler in the nuclear division of *Amoeba*." During the reproductive stage mitotic division takes place. The nucleolus disappears but the exact processes were not made out. This difference in the two divisions is already known in other *Chytridiales* and in the *Plasmodiophoraceae*. Zoospore formation was not fully investigated "as the minuteness precludes an exact observation".

Among the spherical naked bodies in the host cells are found certain larger ones which are binucleate and have arisen from the encysted zygote. In the earlier stages only a small nucleolus is prominent in the nucleus. Later the nuclear membrane becomes clearer, the enlarging nucleolus becomes vaculate and disc-shaped, and on maximum size being attained, the linin threads and chromatin granules become conspicous, assuming a reticulated structure. When fully grown, a membrane appears on the surface of the resting sporangium, its centre is occupied by a large vacuole and the two nuclei occupy peripheral positions usually opposite to oneanother (cf. zygote of *Polyphagus*). The nucleoli now appear as if displaced outside the nuclear membranes, and a nuclear budding takes place at the tip of the nucleolus. "This bud gradually increases in size and can attain nearly the form and size of the mother-body, resulting in the formation of a dumb-bell-shaped nucleus with the nucleolus in the median position. Later the budded portion disintegrates, its wall is broken and the contents are thrown into a central vacuole." Afterwards these dissolution products begin to be distributed from the vacuole into the surrounding cytoplasm. The vacuole becomes replaced by highly stainable cytoplasm, and the resting period is entered upon. Just before germination the cytoplasm appears homogeneously granular and hardly stainable. "Among such sporangia we find several stages of the nuclear feature indicative of karyogamy." A few sporangia were found in which only a single larger nucleus occurred centrally situated. Stages of fusion were difficult to observe. The author thinks that the first divisions of the fusion nucleus are karyokinetic and represent reduction divisions. The stages are difficult to make out as the nuclei exhibit only an obscure outline and give no definite figure.

Němec (1911) working with *Olpidium Salicorniae* has obtained results which are very interesting in the light of the researches of Griggs and Kusano. The first stages observed were naked uninucleate cells with at first dense, granular cytoplasm which later becomes vacuolate. The nucleus is large and contains an ellipsoidal, strongly staining nucleolus. The naked cells become either zoosporangia or resting sporangia. In the first case the nuclei increase in number, the parasite surrounds itself with a rather thin membrane and its contents break up into a large number of uniciliate swarmspores. These appear to be all of the same size and posses a nucleus which frequently sends off a short process to the base of the cilium pointing to a connection between the two. In other cases resting cysts arise from the naked parasites. They possess a very thick double membrane, a dense, vacuolate cytoplasm with coarse granular contents and fat bodies, and a rather large, centrally placed nucleus, which appears nipped in the centre in most cases as if it arose from the fusion of two nuclei. Sometimes the cyst contains two nuclei lying close together. Since the naked parasites are uninucleate, the author searched for fusing naked cells but did not find them. He records the fact that he has met with zoosporangia and resting sporangia in close proximity: they can even develop in the same cell. This he holds could not be brought about by different external conditions but could be explained by a fusion in the one case and its absence in the other. "Parasiten, die nicht Gelegenheit fanden zur Copulation, bilden Zoosporangien, jene, welche copuliert hatten, bilden Dauercysten." The author holds that direct nuclear division may possibly take place in the resting spores similar to the amitosis in *Synchytrium*.

Němec (1912) has also studied the development of *Olpidium Brassicae*. Faworsky (1910) had already worked at this fungus and Němec confirms, and adds to, his results. The youngest stages observed were naked spherical bodies with granular protoplasm. As growth proceeds the protoplasm becomes vacuolate and the nucleus shows a finely granular or fibrous structure and a large spindle-shaped nucleolus lying against the nuclear membrane. The nuclear contents next become thread-like and a paired arrangement of the threads is often seen. The parasite grows considerably before nuclear division occurs. The first and second divisions were not observed, though cases were seen suggesting that the nucleoli persist during the division. After further divisions (the number probably depending upon nourishment), the vacuoles disappear, the cytoplasm becomes finely granular, the nuclei reduced in size almost to a point, and the nuclear contents coarsely granular. The parasite now surrounds itself with a membrane and forms an exit tube. Nuclei enter this and probably divide once or twice. All nuclear figures found occur at the same stage; the spindle is very weakly developed and the chromosomes could not be counted. During division the nucleolus disappears. The cytoplasm breaks up into uninucleate zoospores by the segmentation of the protoplasm from the exterior and by the formation of interior vacuoles. Most of the cysts observed were uninucleate but in a few cases multinucleate cysts were found which were obviously preparing for zoospore formation.

Entophlyctis Brassicae and *E. Salicorneae* were also studied but chiefly from a morphological standpoint. The young stages of the former have a single nucleolated nucleus and dense cytoplasm which sometimes contains deeply staining masses. When the parasite reaches a certain definite size the nucleus begins to divide and zoospores are formed. The characteristic resting spores are also formed. These, at maturity, are uninucleate and have a dense cytoplasm containing deeply staining masses. The vegetative stage of *E. Salicorniae* is also uninucleate and later becomes transformed into a zoosporangium or a resting spore.

Němec (1911) has investigated an organism which he names *Sorolpidium* and places among the *Myxochytridiineae* [1]). The first stages seen were naked uninucleate cells with clear vacuolate cytoplasm. The

1) For a criticism of this view see Bally, Mycol. Centralbl. 1913, 2, 292—293.

nucleus has a rather large nucleolus and an indefinite structure. The nucleus later becomes oblong and thickened at the ends. Preparatory to division the nucleus elongates a little, stainable material appears at the periphery and "centrosomes" at the poles. The nucleolus divides equally and the halves place themselves more or less in the longitudinal axis of division. A nuclear plate, the structure of which could not be properly made out, arises in the equatorial plane. In full grown parasites another type of nuclear division occurs. The nucleus appears poor in content, the chromosomes can later be relatively clearly made out but the centrosomes are scarcely visible. The nucleolus quite disappears. The nuclear membrane is now dissolved, the chromosomes arrange themselves on the nuclear plate and the division proceeds quite normally. Besides this first division where the chromosomes are rather small there is a second division where the chromosomes are large and rod-shaped. Němec considers these divisions the first and second "Fortpflanzungsteilungen", the second division sometimes occurring after the cytoplasmic contents are segmented. This segmentation was not closely followed. The first formed portions apparently divide to give rise to the zoospores. The segments contain no typical resting nucleus, but a heap of more or less distinct chromosomes. The author thinks the heaps divide. The zoospores are uniciliate and possess a small nucleus which has no apparent nucleolus.

Bodies are also found which surround themselves with a membrane and divide into uninucleate portions, whose nucleus at first shows chromosomes. The cells secrete a membrane and later round themselves off remaining together though the original membrane surrounding the group can no longer be seen. Němec considers each cell a sporangium and the group a sporangial sorus. The sporangia give rise to zoospores. In many cases it could be seen that the nucleus as well as the protoplasm had divided. In the same cells as the sporangial sori were frequently found small uninucleate vegetative parasites. These were a little larger than the zoospores and probably arose from them. „Copulation von Schwärmsporen wurde nicht beobachtet."

The genus which has received most attention in the *Chytridiaceae* is *Synchytrium*. Dangeard (1890) recorded that in *S. Taraxaci* the large primary nucleus divides by ordinary amitosis. The nuclei sometimes divide mitotically and both methods may occur side by side in the same cytoplasm. Rosen (1893) described a direct division of a different type. The chromatin loops into a spireme, the nucleolus divides and the halves migrate to the forming daughter nuclei. The nucleus then constricts in the middle thus completing a division of the nucleus in its spireme condition without the aid of the usual achromatic structures. The following divisions take on more and more the character of mitosis. F. L. and A. C. Stevens (1903) first stated that the primary nucleus in *S. decipiens* divided mitotically and most observers have confirmed this statement. Striking points about the mitosis are the vacuolisation of the nucleolus and the shortness and thickness of the few spindle fibres. According to Griggs (1909) the numerous recorded peculiarities occur mostly in a somewhat definite "period of irregularities" immediately following the division of the primary nucleus. In this stage direct division is more frequent than mitosis and takes place by at least two processes: 1. "Nuclear gemmation" where the karyosome of the parent nucleus gives off a small karyosome which migrates through the nuclear membrane, forms a vacuole and a membrane about itself, and becomes an independent small nucleus, the process being repeated until the parent nucleus is converted into small nuclei often forming a definite group and 2. "Heteroschizis" where the membrane of the parent nucleus dissolves and the karyosome fragments into a number of pieces each of which becomes a new nucleus.

Bally (1911) has published his results in *Synchytrium Taraxaci*. The primary nucleus in the young stages possesses a large nucleolus rich in chromatin. Later, secondary nucleoli arise from this and can pass out of the nucleus to become the bases of secondary nuclei. The process recalls that of nuclear gemmation. Indirect division of the primary nucleus was not observed. The secondary nuclei divide indirectly. They differ from the primary nucleus principally in possessing a greater number of secondary nucleoli and better developed linin threads. The division stages seem to agree with those found by previous observers. The nucleolus remains outside the nuclear membrane. The telophase does not show the karyodermatoblast present in *S. decipiens* and *S. Puerariae*. Also the spindle instead of being drawn into the daughter nuclei persists for a time in the cytoplasm and then dissolves. After the segmentation of the cyst there is a different type of division. Here the nucleolus, instead of persisting, is used up in the formation of chromosomes and the intra-nuclear fibres. In both types of division four chromosomes are present a low number being apparently the rule in the genus. In the sorus before segmentation, all the nuclear divisions are simultaneous: after cleavage, they are simultaneous in each sporangium but without any reference to neighbouring sporangia.

Chrysophlyctis endobiotica was also studied and most of Percival's (1910) results confirmed. Unlike the latter he does not regard the fungus as a species of *Synchytrium*. The uniciliate swarm spores possess a pair of small chromatin particles. The youngest parasitic stages, however, show a pronounced nucleus with a very large nucleolus but a very small quantity of dense cytoplasm. A nuclear membrane, not observed by Peecival, is present. From these arise either sporangial sori or resting sporangia. In the resting sporangia no mitotic divisions of the primary nucleus were seen. Percival's account of the origin of the zoospores is confirmed. The nucleus by a method of nuclear gemmation sheds chromitin as chromidia into the surrounding cytoplasm which pass unchanged into the zoospores. The primary nucleus remains undivided during this process. Its nuclear membrane dissolves finally and the remains of the nucleolus etc. are found amongst the swarmers. According to Percival the nuclei in the sporangial sori divide by mitosis, the spindle being intranuclear and the nucleolus disappearing. Bally did not see this division: neither could the differences in the structure of the nuclei in the two types of spores, recorded by Percival, be substantiated. The transitions to sporangial sori are represented by multinuclear stages with nuclei of different sizes. *Chrysophlyctis* thus differs from *Synchytrium* in the manner of zoospore formation, in the resting sporangia, and, further, in the manner of penetration into deeper lying tissues.

A third species investigated was *Urophlyctis Rubsaameni*. Schröter and Magnus held that in this genus there is sexual reproduction; two vesicles copulate one of which empties its contents into the other.

Vuillemin regarded the process as simply a phenomenon of budding and Fischer considered the attached cells as „Sammelzellen". The non-sexuality of the organs has been shown by Maire and Tison (see below) and by the present study. „Wenn hier Sexualität vorhanden ist, so muß sie an einem ganz anderen Ort gesucht werden, nämlich entweder bei den Schwärmsporen, deren Copulation mir nicht unmöglich erscheint". The young stages of the parasite completely fill the host cell with a tangled mass of extremely thin hyphae. In the capitate swellings at the ends of the hyphae arise the young spores which have at first a single large nucleus possessing a prominent nucleolus and some linin threads. As growth proceeds nuclear division occurs. The nuclei in the following stage are smaller but very numerous. In the further developed cells the nuclei are few and large. When a certain size is reached zoospore formation commences. True mitosis was never observed but nuclear gemmation and heteroschizis were not at all infrequent. Budding was found chiefly in the stages preceding zoospore formation. The zoospores are formed independently of the nuclei. Bally divides the *Chytridiales* into two groups one where the uninucleate stage continues until the nuclear divisions previous to zoospore formation *(Synchytrium, Chrysophlyctis)*, and the other where nuclear division goes on together with the growth of the cell *(Olpidium, Cladochytrium)*. He follows Pavillard in believing that the *Synchytriaceae* show great cytological similarities with the *Sporozoa* and were probably derived from them.

Previous to the appearance of Bally's paper, Maire and Tison (1911) published a short account of the cytology of the *Cladochydriaceae (Cladochytrium, Physoderma* and *Urophlyctis)*. The "vésicule collective" of Vuillemin is found at the interior of the host cell near the point of infection. This gives rise to a certain number of new pedicellate vesicles by budding and the secondary vesicles bud in their turn in the same way. At each budding the lower vesicle empties itself into the upper one. Finally the terminal swelling produces only a single bud with a short pedicel. The bud swells and forms the "chronisporocyste" which, like the vesicles, is plurinucleate. There is no trace of karyogamy at any stage seen. The nuclei vary much in form and dimension. Typically formed of a nuclear membrane, a lateral karyosome and a loose achromatic network they appear to multiply solely by amitosis. In the developing chronisporocyst certain nuclei swell and their karyosome vacuolates giving rise to large masses of a substance which accumulates in the centre of the cell. Around this substance which appears to play the rôle of reserve material, some nuclei are found which remain intact.

In *Physoderma Urgineae*, the fungus presents itself in the form of small uninucleate amoeboid masses. These naked cells multiply by simple divsion but do not remain joined. They then become multinucleate, surround themselves by a membrane, and become chronisporocysts. "La formation des chronisporocystes représente ici un simple enkystement se produisant à la suite d'une schizogonie. . . . Le développement endophytique est la même dans le *Physoderma Gehrhartii*." From the researches of Lüdi (1901) it is known that *Cladochytrium Menyanthes* forms its chronisporocysts in a manner analogous to *Urophlyctis*. The *Cladochytriaceae* "sont donc asexués au moins pendant leur vie endophytique". The authors consider the group to be fairly homogeneous though at first sight *Physoderma* seems distant from the other two genera because of the total absence of connection between the schizonts.

It would seem from these several accounts that the longstanding problems of cytology and reproduction which the *Archimycetes* present will be cleared up in the immediate future. At any rate it is clear that in some cases there is a copulation of zoospores, a phenomenon, the presence or absence of which has long been debated.

In the *Mucorineae*, Gruber has taken up the study of *Zygorhynchus Moelleri*, but his results do not agree with those of Moreau[1]). Zygospore formation usually begins, as Blakeslee and others have pointed out, by the union of the end portion of an upright aërial hypha with a side branch of the same hypha. The end portion, which becomes cut off by means of a transverse septum, has usually been interpreted as the male branch. Gruber, however regards it as the female, and the larger side branch which curves and swells up into a club-shaped structure in applying itself to this as the male branch. At the place of contact a pear-shaped elevation (the progamete) arises perpendicular to the long axis of the female hypha, while the club-shaped end of the male branch builds the other progamete. Contrary to Blakeslee's account, a septum was not formed in the male progamete but only in the female. A cross septum is now partially laid down about the middle of the female gametangium: it arises from the periphery and is rarely completely formed usually being soon dissolved. Each gametangium contains numerous very small nuclei regularly distributed in a dense cytoplasm. The author thinks that in the gametangia nuclear divisions take place during which reduction is brought about but this he was unable to demonstrate. In the male progamete a portion of the protoplasmic contents containing from twenty to thirty nuclei separates off from the remainder. It differentiates clearly in staining and is regarded as being the male gamete. It applies itself to the membrane separating it from the female gametangium. This it dissolves in one place and passes over, amoeba-like, through the small opening into the female gametangium, whereupon the opening in the membrane soon closes up again. The further behaviour of the nuclei could not be followed because of their small size but the author holds it probable that the male nuclei fuse with a corresponding number of female nuclei. He also suggests that the nuclei in the female gametangium might undergo some differentiation into vegetative and generative nuclei when that structure becomes partially segmented. Since the zygospore (or oospore) contains a very large number of nuclei it appears that the fusion nuclei must divide repeatedly. Gruber considers that the processes recall what occurs in the *Peronosporiineae* and the *Saprolegniineae*, especially *Achlya racemosa* where Pringsheim saw amoeba-like bodies wander over from the antheridium to the oospheres and bring about fertilization. He considers that *Zygorhynchus* can be regarded as occupying a position between the *Zygomycetes* and the *Oomycetes*, the general habit and method of sporangium formation showing characters of the *Mucorineae*. The many small variations observed during the study, the vanishing cross wall in the female gametangium and the lack of a cross wall at the base of the male gamete leave the suspicion that the form dealt with is in a state of variation.

1) See Mycol. Centralbl. 1912, 1, 204—205.

Moreau (1912) immediately criticised Gruber's results. "Nous n'hésitons pas à dire que Grüber a été la victime d'une erreur." He considers that the large suspensor of *Z. Moelleri* has been mistaken for a reproductive structure: that the general method of zygospore formation in the *Mucorineae* has been disregarded: that it has been forgotten that the fusion in all cases studied (in particular three species of *Zygorhynchus* near *Z. Moelleri)* presents all the characters of a gametangium, and that, lastly, all the homogeneity which the *Mucorineae* owe to the characters of their reproductive structure is taken away. Moreau's work was known to Gruber when he published his results and must have been considered as it is mentioned by him[1]).

Mc Cormick (1912) has published a preliminary account of her work on *Rhizopus nigricans* (isogamous and heterothallic). Namylowski (1906) worked at a species which he considered the true *R. nigricans* but found it indifferently isogamous or heterogamous, and homothallic. Each gametangium contained large numbers of nuclei but neither division, copulation, nor disintegration of nuclei were seen. Mc Cormick found that the young gametangia each contained numerous nuclei. The walls cutting of the gametangia from the suspensors may not be formed simultaneouly and in each wall there is left a central pore. The wall which separates the gametangia from each other often thickens considerably before disintegration, and fragments of the thickened wall may be found in quite old zygospores. The many nuclei from each gametangium increase in size after the disintegration of the wall. All the nuclei except two disintegrate and these two nuclei are embedded in a coenocentrum. There are indications that the coenocentrum has its origin at the point of contact of the two suspensors before the gametangia are cut off. Neither fusion nor division of nuclei have yet been observed. Mc Cormick believes that the two nuclei left in the coenocentrum fuse. "From this stage to maturity many changes appear in the appearance of the zygospore, but their interpretation is not yet quite clear. The coenocentrum persists until quite late, and in the mature zygospore there are many nuclei of the same size as those in the mycelium." This account of the fusion of two nuclei seems to agree with that of Lendner (1908) for *Sporodinia grandis.* Here, however, is the first record of a coenocentrum occurring in the *Mucorineae* though its presence is so very common in the *Oomycetes.*

Moreau (1913) has published a full account of his studies on the *Mucorineae.* First considering the case of asexual reproduction, he divides the group into a) those with sporangia, b) those with conidiophores and c) those in which the endogenous or exogenous origin of the spores is disputed. Many observers have studied the cytology of the sporangia. Harper (1899) laid the foundations of our present knowledge when he showed that although the ripe spores of both *Pilobolus* and *Sporodinia* were plurinucleate, in the former case the protoplasm fragmented into uninucleate segments (protospores) which later became plurinucleate, in the latter the segments were plurinucleate from the beginning

Moreau finds that the spores in *Circinella conica, Mucor spinescens, Absidia glauca, A. septata* and *Zygorhynchus Moelleri,* are at first

1) For a critism of Gruber's work from a morphological standpoint see Blakeslee, Mycol. Centralbl. 1913, 2, 241—244.

normally uninucleate those of *Rhizopus nigricans* and *Phycomyces nitens* plurinucleate. Studying the segmentation of the protoplasm in the sporangium Moreau found that in *C. conica*, *R. nigricans* and *P. nitens*, species with plurinucleate nature spores, irregular vacuoles separate fragments of protoplasm which, after the formation of a membrane, become spores. Their separation gives the impression of a retraction of the protoplasm. They form masses of amoeboid appearance joined by trabeculae which become thinner and break. In *Mucor spinescens* (and possibly *A. glauca*) the protoplasm becomes vacuolate and takes the form of elongated threads enclosing the nuclei. The thread nips off into the usually uninucleate spores.

In *Cunninghamella echinata* and *C. Bertholletiae* the swelling which supports the conidia arises as in the above cases. The protoplasm separates into two layers the exterior portion of which contains denser protoplasm and more numerous nuclei. Small swellings with narrow sterigmata arise and the nuclei from the peripheral layer pass into these conidia which enclose from three to eight at maturity. "Au bien de constituer des spores à l'interieur de la tête renflée, le protoplasma et les noyaux forment des conidies à la périphérie."

Much discussion has taken place as to whether *Piptocephalis*, *Syncephalis* and *Syncephalastrum (Cephalideae)* should be considered as having conidia or sporangia. Moreau studied *Syncephalastrium cinereum* and *S. racemosum*. The sporiferous head arises in the manner of a sporangium. Tubes bud out around the swelling. Into these the nuclei pass sometimes to the number of twenty. Often the protoplasm of the tube differentiates into two layers. The spores arise by the condensation of the protoplasm around the nuclei. Each spore contains usually one nucleus but may contain several, and surrounds itself with a cell wall. The formation of spores "doit être décrite comme une production de spores internes à l'intérieur d'une sorte de sporange allongé; . . . le sac qui les produit n'est pas non plus un véritable sporange homologue de celui des *Mucor*".

The results of the study of zygospore formation in twelve species is given. *Mucor genevensis* (heterogamous, heterothallic), *Absidia spinosa* (heterogamous, homothallic), *Rhizopus nigricans* and *Phycomyces nitens* (isogamous, heterothallic) are the species not considered in the previous résumé[1]).

The two first agree with what was described by the author in other species of the same genera. *Phycomyces nitens* is, like *Sporodinia grandis*, unfavourable for study. Multiple nuclear fusions were, however, clearly observed in the zygote. The account of *R. nigricans* differs greatly from that given by Mc Cormick. There is no coenocentrum present. Most of the nuclei arrange themselves in pairs and fuse, a few only degenerate. Moreau's account is consistent. The zygospore in all cases results from the fusion of two multinucleate gametangia. Mitotic divisions of the same type as those in the mycelium occur in the young zygospore. The nuclei then fuse in pairs or degenerate. In all cases studied except *Zygorhynchus Dangeardi* the fusions preponderate and

1) The *Mucor* sp. (Mycol. Centralbl. 1912, 1, p. 204) is now identified as *M. silvaticus*. The two unnamed species of *Zygorhynchus* are regarded as new species, the one with only four functional nuclei being called *Z. Dangeardi*, the other *Z. Bernardi*.

disintegration is rare. The author assumes that the reduction division takes place on the germination of the zygote.

A confirmation of MOREAU's work will be eagerly awaited. Modern investigation shows clearly that the gametangia are multinucleate. Division and degeneration of nuclei have been recorded in many cases. Larger bodies have also been often reported, but it hardly seems credible that what have been variously interpreted as mucorine crystals, large nuclei and coenocentra could be the same structures.

BUCHOLTZ (1912) published his investigations on the genus *Endogone.* This subterranean genus was previously little known and has been placed in various systematic positions. BREFELD put it in his *Hemiasci.*

In *E. lactiflua* the author discovered a sexual process resembling that of the *Phycomycetes.* The hyphae are multinucleate but non-septate. The male and female progametes arise as sac-like outgrowths of the hyphae, the male being usually smaller than the female. The nuclei in the progametes undergo a simultaneous division such as has been often described in the *Phycomycetes.* There is no differentiation into periplasm and ooplasm in the female. At the end of the simultaneous division a nucleus appears in the centre of each gamete. It is not clear what the origin of these nuclei, but it is thought that they have wandered from the periphery. The peripheral nuclei now wander down into the suspensors; if any remain in the gametangia they degenerate. Septa cutting off the uninucleate gametangia or gametes from their suspensors are formed practically simultaneously. Almost at the same time copulation begins between the two gametangia. No receptive papilla nor fertilizing tube is formed. Meanwhile the two nuclei have grown considerably. There is an increase of chromatin and an excentric nucleolus is visible in each nucleus. The male nucleus passes over into the female gametangium but the nuclei do not fuse. At the summit of this organ a portion of the wall is gelatinised and a papillate outgrowth appears which gradually enlarges. As a rule the whole of the contents of the gametangium pass into this outgrowth which is the zygote or zygospore. It enlarges further, rounds itself off and thickens its membrane. The nuclei were not observed to fuse, but it is thought that fusion occurs at germination. (In an unnamed variety of *E. lactiflua,* the nuclei appear to fuse during the ripening of the zygote.)

The only other species in which sexual reproduction was found was *E. Ludwigii.* In the youngest stages obtained the zygote was already formed. In the zygote were seen either two nuclei, or one larger nucleus which presumably was formed by their fusion. In *E. macrocarpa* and *E. microcarpa* only asexually formed chlamydospores were present in the fruit bodies. These contain a large number of nuclei. No fusion of nuclei was seen. The author holds that because of its resemblance to a zygote, the chlamydospore might be considered as an azygote.

In *E. pisiformis* the ends of the hyphae swell and become multinucleate sporangia. The nuclei apparently undergo division. The process of spore formation resembles that observed in many *Mucorineae.*

In *E. lignicola* and *E. fulva* the method of reproduction was not made out. The author proposes forming a sub-group *(Endogoneae)* in the *Phycomycetes* for the reception of this genus. He points out the close resemblance between the sexual process in this genus and that in the

Mucorineae, and also the resemblances in the method of spore formation. He regards the swelling which afterwards becomes the zygote, into which the fused male and female nuclei pass, as being possibly analogous to an ascogenous hypha. The development of vegetative hyphae round the zygote also recalls the *Ascomycetes*.

It is interesting to find in this genus such differences from the ordinary *Phycomycete* type of sexual process. Whether they are to be regarded as being anything more than adaptations to a subterranean mode of existence seems doubtful. In any case, the *Phycomycetes* apparently have their hypogeal section as well as the *Ascomycetes* and *Basidiomycetes*.

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Edouard Griffon war am 24. Juli 1869 in Vault-de-Lugny (Yonne) geboren. Seine Eltern waren Bauern. Er hatte anfangs die Lehrerlaufbahn eingeschlagen, studierte dann aber Landwirtschaft, wurde Ingénieur-agronome und Licencié-ès-sciences. Unter Bonnier arbeitete