Some recent work on the cytology of fungus reproduction. I.

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Since the introduction of the compound microscope the reproduction of fungi has always interested, and at the same time puzzled, investigators. One of the most interesting points is the great variation in the reproductive structures — structures which are more or less constant throughout most, if not all, of the other great plant groups. Many controversies have waged regarding the sexual processes in fungi, and when there has been agreement as to the phenomena, there have still been great differences with regard to the interpretation to be given to them. As a group, the *Phycomycetes* have offered the least difficulty, the sexual organs being fairly easy to observe. The majority of the old investigators held that in most of the genera, a normal process of fertilisation obtains, and during the last twenty years this view has been confirmed by various workers using modern cytological methods.

A section of this group, the *Saprolegniineae*, however, gave great difficulty. Certain members of the family possess both antheridia and oogonia; others possess only oogonia; whereas still others, which have oogonia, may or may not have antheridia the presence or absence of these depending upon external conditions. Pringsheim held that normal fertilisation occurred in certain cases, but de Bary (and after him Humphrey, Ward, Hartog, and others) considered that the family as a whole was apogamous, i. e. that the antheridium, even where present was never functional. Trow opposed this view and held that in certain species of *Achlya* he had proved normal fertilisation, but his work was not quite free from doubt until 1904, when he clearly showed that a male nucleus fused with a female nucleus in the oosphere of *Achlya de Baryanum* and *A. polyandra*. Normal fertilisation has since been shown to take place in *Saprolegnia monoica* (Claussen 1908). Mücke (1908) saw the male and female nuclei close together in the oosphere of *Achlya polyandra*, but he did not see actual fusion.

Kasanowsky (1911) has published an account of his researches on *Aphanomyces laevis*. In this monoecions species both oogonium and antheridium are multinucleate. In the oogonium a large central vacuole develops which, as it enlarges, forces the protoplasm to the periphery, and many of the nuclei degenerate. Those that remain undergo a mitosis as also do the nuclei in the antheridium. In each organ all the nuclei degenerate except one. The oophere is formed in the middle of the oogonium by the gathering of the protoplasm towards the centre round a coenocentrum which acts as a centre of nutrition. The female nucleus

1) A paper similiar in part to the above was published in the Trans. Brit. Mycol. Soc. 1911.

lies near the coenocentrum and increases in size. The single male nucleus passes over together with some protoplasm and fuses with the female nucleus. The oospore is uninucleate.

This account is quite in accord with what has been described in the other *Saprolegniineae*. In all recent work the sexual organs are described as being multinucleate at first: all the nuclei degenerate except one male nucleus and one female nucleus. These fuse in the oosphere. No series has yet been found which corresponds with that discovered in the *Peronosporineae*, e. g. in *Albugo*, where a regular transition can be traced from multinucleate antheridium and oogonium, the nuclei of which fuse together in pairs, to a case where all the nuclei degenerate with the exception of one male and one female nucleus, which then fuse. Also in the *Saprolegniineae* (with the exception of the anomalous *Pythium*) there is no periplasm formed as in the *Peronosporineae*. The only questions which seem to be debatable are (1) whether the body in the oosphere is a coenocentrum and equivalent to the similar structure found in the *Peronosporineae*, at least physiologically (Davis and Kasanowsky), or whether it is a centrosome with, or without, additional structures (Trow, Claussen and Mücke); (2) whether two mitotic divisions take place in the sexual organs, as Trow states. Trow thinks that in *Achlya de Baryanum* he has seen two divisions which constitute a true reduction division, the number of chromosomes being halved during the process. Two divisions have been observed in the sexual organs of several of the *Peronosporineae* e. g. *Albugo Bliti*, but it is not known whether this is usual: indeed various observers have differed in their accounts of the same species. It seems extremely doubtful that the two divisions observed constitute a reduction division, as Davis (1903), working on a form of *Saprolegnia monoica*, which was without antheridia, observed a division in the oogonium.

Another section of the group, the *Mucorineae* have been rather neglected from a cytological standpoint although their morphology and physiology is probably better known than that of any other fungi. The meagre cytological results are all at variance. The isogamous species (which are at the same time homothallic) were the first investigated. *Sporodinia grandis*, because of the ease by which its zygospores can be obtained, is the species which has been most studied. It is hardly a suitable species however, because of the large number of small nuclei in the gametes and the presence of oil globules, and mucorine crystals. Dangeard and Léger (1894) first showed that the young zygospore was multinucleate. Léger (1895/96) continued the work and extended his researches to many other genera. The zygospores of *Sporodinia grandis* and *Mucor mucedo* were particularly studied. Léger stated that in the zygospores the nuclei gradually disappear. At the precise moment of disappearance two groups of small spheres („sphères embryogènes“) probably arising from the union of a certain number of nuclei appear. At a later stage all the spheres of each group fuse forming the notorious „sphères embryonnaires“. At germination these spheres fuse.

Dangeard immediately dissociated himself from Légers work. Gruber (1901) examined *Sporodinia*. He found numerous nuclei which were at first more numerous in the parietal layer but afterwards were evenly dispersed. He found neither degeneration of nuclei nor fusion of nuclei although he assumed that the latter probably occurred.

Dangeard (1906) worked at *Mucor fragilis* and interpreted the phenomena seen in *Sporodinia grandis* in light of the obtained results. In the former species a division of nuclei takes place in the young zygospores. The nuclei at a later stage fuse in pairs. Nuclei are afterwards seen of three kinds which Dangeard interprets as being nuclei before, immediately after, and some time after fusion. The nuclei which fail to copulate disappear.

Very similar results were obtained in *Sporodinia* but the number of nuclei in each gamete is more than one thousand. In the old zygospores from ten to twenty rather large deeply staining bodies are seen. They resemble coenocentra but are masses of mucorine.

Lendner (1908) then took up the study of *Sporodinia*. His account of the early stages agrees with that of the other authors. One of the progametes penetrates more or less into the other which is perhaps a sign of sexuality. Two large nuclei, one from each gamete, are present as well as numerous small nuclei. The latter which are dispersed everywhere but are more numerous near the walls, divide. This division Lendner thinks Dangeard misinterpreted as nuclear fusion. These small nuclei do not degenerate but seem to preside at the formation of the zygospore membrane. The two large nuclei fuse and occupy the middle of the zygospore.

Moreau (1911) has published three notes on the *Mucorineae*. He has studied *Sporodinia*. „Le similitude des observations de Dangeard et des nôtres sur ce point nous dispensera, dans cette note préliminaire, d'en donner le détail." In a species of *Mucor*, he finds that the protoplasm in the progametes presents a vacuolate appearance and is multinucleate. Shortly after the mixing of the protoplasm the nuclei show karyokinetic figures. This mitosis has the same characteristics as that in the mycelium: two chromosomes, two centrosomes, no nuclear membrane nor nucleolus. In the zygospore however the spindles are much shorter. It is interesting to note that the nuclei of the *Ancylistes* and *Basidiomycetes* have nuclear membranes, where as these are absent from the nuclei of the *Ascomyeetes* and most *Siphomycetes*. The protoplasm finally becomes reticulate alveolar and the zygospore surrounds itself with a spiny endospore. „A ce stade, la plupart des noyaux presentent des aspects qui ne laissent aucun doute sur l'existence de fusions multiples." All the nuclei do not fuse. A few degenerate but it is not a case of those degenerating which fail to fuse as fusion and degeneracy are two concomitant phenomena.

Moreau has extended his researches to heterogamous and heterothallic species. The heterogamic species have a peculiar interest. Their morphology has been studied and species which showed the faintest trace of heterogamy have been regarded as showing the beginings of sex differentiation. Blakeslees work (1906) however showed that often where the heterogamy was most marked e. g. in *Zygorhynchus* there was no physiological differentiation, the fungus being homothallic. Vuillemin considers that far from indicating a well-marked sexuality, heterogamy indicates a tendency to form azygospores.

Moreau has worked at four species of *Zygorhynchus*, *Z. Mölleri*, *Z. Vuillemini* and two unnamed species. In the two former the phenomena are difficult to setablish because of the smallness of the nuclei but

they appear to agree with those in one of the unnamed species; the nuclei in the young zygospore, after dividing, fuse in pairs with the exception of a few which degenerate. In the remaining species the nuclei are small and numerous and, as is usual, there is nothing to distinguish the nuclei coming from either gamete. All the nuclei except four degenerate and these fuse two by two but not until much later than is usual in the other cases studied.

Absidia Orchidis is a species which is indifferently isogamous or heterogamous. It is however heterothallic. The nuclei are very numerous in the young zygospore. They fuse in pairs with the exception of a few which degenerate. Similarly with *Mucor hiemalis* another heterothallic and heterogamic species, but here the nuclei are large in size and few in number and the fungus is therefore particularly suitable for study. The results obtained by MOREAU seem fairly consistent. The facts brought out by his researches seem to be that in the *Mucorineae* there is a fusion of nuclei and a degeneration of nuclei. The variation depends upon whether the first or the second phenomenon is dominant.

The deferred nuclear fusion in one of the *Zygorhynchus* sp. may be present only in heterogamous species but we have not yet sufficient facts upon which to base theories. It is interesting to note that the phenomena seem practically identical no matter whether the species is homothallic or heterothallic, isogamous or heterogamous. There is greater variation in the nuclear phenomena in *Sporodinia grandis* according to the various published accounts than is here recorded for eight different species.

In the *Ascomycetes* the controversies have been much more severe. Apart from the older observers one has only to consider the work of HARPER, DANGEARD and CLAUSSEN on *Pyronema confluens* to realise that we are at present far from having agreement as to the facts.

In the *Helvellineae*, CARRUTHERS (1911) has published a paper on *Helvella crispa*. There is no ascogonium present in this species and in this respect it agrees with *H. elastica* (MC CUBBIN 1910). BROWN (1910) found an ascogonium in *Leotia lubrica*, although his specimens were too for advanced to work out its structure and development. This difference between the two genera is interesting as BOUDIER in his classification of the *Discomycetes* widely separates the *Helvellaceae* from the *Leotiaceae* on account of the method of opening of the ascus, the former being placed in the *Operculés* the latter in the *Inoperculés*. The data however is not yet sufficient to base any conclusions on, as we know that in the same genus even, an ascogonium may be present or absent e. g *Humaria* where there is a well marked ascogonium in *H. granulata* whereas such a structure is entirely absent from *H. rutilans*. In *Helvella crispa* the hypothecium is a loose tangle of hyphae with a variable number of nuclei in the cells. Certain of these nuclei where observed to fuse in pairs as in *Humaria rutilans* (FRASER 1908), but there was no evidence of nuclear migration such as occurs in that species. In *H. elastica* MC CUBBIN found a very marked difference between fertile and vegetative hyphae, the former containing several nuclei in each cell, the latter only two. This exact differentiation between the two kinds of hyphae could not be traced in *H. crispa* although in most cases the cells of the paraphyses are binucleate and the cells of the fertile hyphae multinucleate.

The latter are generally larger than the former and their nuclei resemble in size and appearance the fusion nuclei of the hypothecium. A certain amount of evidence was obtained as to mitosis in both the vegetative and the fertile hyphae; the number of chromosomes in the first appears to be two, and in the latter four, and then eight. "The nuclei are, however, so minute that it would be unwise to attach any great importance to these phenomena." In the divisions in the ascus brachymeiosis (a second reduction as first recorded by Fraser in *Humaria rutilans*) occurs. The first division is heterotype with four bivalent chromosomes, the second homotype with four monovalent chromosomes, and the third brachymeiotic with two chromosomes. This latter number is confirmed by the fact that in mitosis in the spore two chromosomes go to each pole. Neither Mc Cubbin nor Brown found a second reduction in the ascus.

In the *Pezizineae* Guilliermond (1911) has criticised the work of Fraser and her pupils on the divisions in the ascus. He has again worked at *Humaria rutilans*, the species in which Fraser first recorded brachymeiosis. He had previously (1904—1905) published an account of the nuclear divisions in the ascus of this species, and unfortunately did not cut fresh material for his present study, but used his old slides. The nuclei of *H. rutilans* are very suitable for the study of these phenomena. Guilliermond now considers that Fraser's account of the first two divisions, a heterotype followed by a homotype, according to the scheme formulated by Farmer and Moore for the reduction divisions in both animals and plants, is probably correct. He admits that he missed "plusiers stades" recorded by Fraser. However, he considers there is no second reduction. He finds there are sixteen chromosomes present in the first and second divisions and from a study of all his figures he thinks that in the third division the number of chromosomes is certainly greater than eight, and approaches sixteen although he could not count the number exactly because of their length and twisting. "Comme, d'autre part, les figures de Fraser ne sont pas plus démonstratives que nos préparations (et ne peuvent l'être), nous nous permettrons donc d'émettre des doutes très sérieux sur l'exactitude de l'interpretation de cet auteur et de considérer son opinion comme une simple théorie qui aurait besoin de trouver sa demonstration."

Guilliermond also re-examined his old slides of *Peziza catinus* and *Pustularia vesiculosa*, the latter being one of the species in which Fraser and Welsford state that brachymeiosis occurs. In *P. catinus* he finds the first two divisions favourable to Fraser's view, but the number of chromosomes remains constant throughout the three divisions. In *P. vesiculosa* the chromosomes are less in number and smaller in size than in the other species investigated and their enumeration is much easier and allows of remarkable precision. He insists that there is no numerical reduction in the chromosomes their number being eight throughout the three mitoses.

The author also again studied *Galactinia succosa* but did not rely on his old preparations as the series was not complete. In his previous investigations he had thought that the divisions took place according to Maire's scheme, which seemed to agreesome what with Fraser's ideas concerning the significance of the third division in the ascus, but he holds that his latest study shows conclusively that the number of chromosomes

remains constantly eight, and that the processes of division agree absolutely with those in all the other *Ascomycetes* he has investigated.

Brown (1911) has studied the development of the ascocarp of *Lachnea scutellata*. The archicarp, when mature, consists of a row of about nine cells which reminds one of the scolecite recorded in some of the *Ascobolaceae*, though there seem to be no pores in the septa of the archicarp. In this connection one may notice that the speciecs in which Woronin first described the scolecite, namely *Ascobolus pulcherrimus*, has been placed in the genus *Lachnea* by certain authors e. g. Cooke, Gillet, Saccardo.

The nuclei in the ascogonium divide karyokinetically, centrosomes being present. "Five daughter chromosomes proceed to each of the opposite poles . . . . The two groups of chromosomes are usually separated far enough so that when they reorganise the daughter nuclei are separated by an appreciable distance. Frequently, however, the daughter nuclei reorganise so close together that after a slight growth they are pressed against each other and resemble fusing nuclei." The nuclei do not divide simultaneously and all stages can be found in a single ascogonium. No fusion of uuclei was observed in the ascogonium. In a number of cases nuclei were seen pressed against each other but in every case the nuclear membranes between the nuclei were intact and every appearance suggested that the two nuclei were daughter nuclei of the same nucleus which had reorganised close together. Also fusion nuclei are often simulated by the fact that during prophase, when the nuclei are large, the chromosomes sometimes mass into a nucleolus-like group. "It may be said that a fusion of the nuclei would be hard to find, but they have been looked for very carefully in a large number of well fixed and stained preparations. The slight decrease in the size of the nuclei during the development of the ascocarp and the persistence of the same number of chromosomes throughout the ascogonium and ascogenous hyphae, moreover, indicate very strongly that a fusion of nuclei during this stage is not to be expected." The first division in the ascus is heterotypic, the second and third divisions are of the same type as those in the ascogonium. The number of chromosomes is five in all the nuclear divisions throughout the life history of the fungus and there is thus no second reduction. The author's figures of the nuclear divisions are all text figures and it is very unfortunate that all those which deal with the division in the ascogonium, the critical portion of the paper, should be labelled "× 11,200".

(Fortsetzung folgt.)

Referate.

Knoll, F., Untersuchungen über den Bau und die Function der Cystiden und verwandter Organe. (Jahrb. f. Wiss. Botanik, 1912, **50**, 453—501, Tafel VI.)

Die Fruchtkörper vieler *Hymenomyceten* besitzen eigene Organe für die Absonderung von Wasser in tropfbarflüssiger Form (Hydathoden); dieselben können an der sterilen Oberfläche des Fruchtkörpers, aber auch