THE MORPHOLOGY, CYTOLOGY AND SEXUALITY OF THE HOMOTHALLIC RHIZOPUS SEXUALIS (SMITH) CALLEN.

by

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THESIS

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CONTENTS.

Introduction.

The fungus forming the subject of this study was originally isolated in 1922 by Mrs. M. N. Kidd in the Botany School, Cambridge, England, from a rotting strawberry. Through the courtesy of Prof. Brooks, a culture of this fungus was received by the Dept. of Mycology, University of Edinburgh, a few years later, where it has been maintained ever since.

When received, it was said to be a homothallic Rhizopus, greatly resembling Rh. nigricans. It was never identified until Smith (1939) described it as a new species, Mucor sexualis. He used a culture also obtained through Prof. Brooks, but gives practically no details, apart from the technical descriptions.

Preliminary observations had shown that this fungus belongs to the genus Rhizopus. It is therefore proposed to give a detailed account of the morphology and behaviour of this unusual and interesting fungus.

Microtechnique and Cultural Methods.

It was essential for a close study of the morphology of the fungus, and particularly for the examination of the nuclei of the mycelium and developing reproductive organs, to be able to examine the fungus repeatedly under high magnification without contamination. The agar film technique of Kniep, as modified by Sass (1929) and Noble (1937) was found to be ideal. The slide, with the agar film on the underside, acted as the lid of a cell, thus enabling the mycelium to be examined without fear of contamination. Pure cultures could be maintained for three weeks in such cells.

The most satisfactory medium was found to be that used by Noble (1937), as it gives a clear evenly staining preparation, even when a thicker film is used. The agar film technique is particularly suited to this homothallic Rhizopus, owing to the scanty production of aerial mycelium and its method of zygospore and sporangiospore formation. The technique is not suited to most other fungi however, as they completely fill the cell with mycelium, and the zygospores are buried in it.

The fixing fluid as recommended by Sass (1929), and used by Noble, though satisfactory for the film, was quite unsuitable for the mycelium, as the tips

of the hypae burst immediately the film was immersed in the fluid. In most cases the suspensors, the progametangia, or even the zygospores burst, causing extrusion of the protoplasm. Numerous attempts were made to find a suitable fixative which did not cause bursting of the tips of the hyphae, which did not cause plasmolysis and which would harden the film sufficiently. Karpechenko's modification of Navashin's fluid (Rawlins, 1923) was ultimately used, as it caused practically no bursting of the hyphae, fixed the film well, and caused no apparent plasmolysis. Fixing for 18 hours, and washing for 18 - 24 hours was found to give satisfactory results.

With this fixative, iron alum haematoxylin was most unsatisfactory, and Breinl's triple stain did not give sufficient contrast. A combination of aqueous Gentian Violet (with a few drops of commercial anilin oil added), iodine and erythrosine was found to be ideal, as it gave the whole preparation a rose pink colour, except the nuclei, which stained a deep purple. The successful staining of the Gentian Violet appears to rest largely on the presence of an impurity in the commercial anilin oil used. Great care had to be excersised in using the iodine, as, if it was allowed to act too long, spherical deposits were found on the inside of the wall, making the preparations useless for /

for further study.

For the other cultural work, a 2½% malt extract agar was used, occasionally with the addition of 2% sucrose. When using Sporodinia grandis, some rice starch was introduced into one half of the Petrie dish befor the medium solidified. The value of this measure is rather doubtful.

Material for microtome sectioning was usually fixed in Fleming's weaker solution, though Navashin's solution as modified by Karpechenko was also used. The former was more successful, though no amount of treatment with hydrogen peroxide would remove the excessive blackening of the maturing zygospores. Colson (1938) met the same difficulty, though in the present case it appears to be due to the retention of the osmic acid by the oil reserves, as the suspensors stained normally.

The cultures have all been kept in the dark at a temperature of from 18 - 20 °C, except during the height of the summer in Würzburg, Bavaria, when the temperature rose to nearly 30°C, though it did not appear to affect them adversely.

Sources of Material.

The fungi used in the hybridisation experiments were obtained from the following sources :

Rhizopus nigricans Ehrenb. (+) and (-)ve.

Mucor hiemalis Wehm. (+) and (-)ve.

M. Ramanianus Möl.

Absidia glauca Hag. (+) and (-)ve.

Zygorhyncus Moelleri Vuill.

from the collection maintained in the Dept. of Mycology, University of Edinburgh.

Mucor hiemalis Wehm. (+) and (-)ve.

M. mucedo Fres. (+) and (-)ve.

Absidia glauca Hag. (+) and (-)ve.

A. cylindrospora Hag. (+) and (-)ve.

Phycomyces nitens (Kz) v.Tiegh. et Le Mon. (+) and (-)ve.

from the collection maintained in the Botanisches Institut der Universität, Würzburg, through the courtesy of Prof. Hans Burgeff.

Rhizopus Oryzae Went et Pr. Geerl.

Rh. tonkinensis Vuill.

Rh. japonicus Vuill.

Absidia caerulea Bain.

A. Regnieri (Luc. et Cost.) Lend.

A. capillata v.Tiegh.

Circinella /

Circinella minor Lend.

C. spinosa v.Tiegh. et Le Mon.

Sporodinia grandis Link.

from the National Type Collection, Lister Institute, London, through the courtesy of Dr. St. John Brooks.

The previous history of these fungi need not be considered here, except to record that a few are recent isolations, but the majority have been maintained in culture for considerable periods, in most cases a number of years.

The Mycelium.

In all cultures, the mycelium that first appears is of the submerged type, and is closely adpressed to the medium, or actually embedded in it. It is hyaline in appearance, and grows out rapidly in a radial direction around the inoculum, till the mycelium is some 48 hours old, when the superficial type makes its appearance. This second type appears in the centre of the inoculum, and gradually spreads out radially.

With increasing age, long stolons are soon developed, which give the culture a characteristic appearance. These stolons, typical of the genus Rhizopus, are strong thick hyphae which develop rapidly and soon grow beyond the edge of the submerged mycelium, giving forth numerous "rhizoids" where they come into contact with the medium. These "rhizoids" remain hyaline, and develop into the submerged type of mycelium. For some reason not yet understood, the stolon may branch in mid-air, producing from four to six branches, which then grow forward and take root in the medium.

As the mycelium develops further, the long stolons become fewer in number, and their development appears to be arrested at an early stage, and by the end of the fourth day, the submerged mycelium is only giving rise to these small stolons, which now assume a sexual function /

function, in other words they become zygophores. The stolons are therefore homologous with the zygophores. This aspect will be dealt with more fully in a subsequent chapter.

The superficial mycelium is light silvery grey in colour, but with age it loses its lustre, and ultimately assumes a light buff colour. It is never very profuse and does not rise much from the medium. As has already been noted, this fungus is ideal for study with the agar film technique, on account of the scanty aerial mycelium and the fact that fewer stolons are produced. The smaller number of stolons produced in slide culture is due to a nutritional limiting factor.

According to Burgeff (1924), Rhizopus nigricans produces what he terms "Fanghyphen", which might be translated as "trapping hyphae". They are produced in the normal course of growth before copulation takes place, and are thin much branched hyphae arising from the ageing mycelium. Rh. sexualis does not normally produce these trapping hyphae until the culture is several days old, and the aerial mycelium has developed, and then generally only if the culture is in a sufficiently saturated atmosphere. As the cultures become older, the new zygophores are very much thinner, and the zygospores correspondingly very much smaller too. /

too. The zygophores branch excessively, and show a great similarity to the trapping hyphae. It appears highly probable that in this homothallic species, the trapping hyphae and the zygophores are homologous, which suggests that trapping hyphae in Rh. nigricans are really only zygophores. In Absidia, the trapping hyphae are suppressed when sexually similar mycelia are plated together, which shows them to be sexually specific organs, according to Burgeff (1924), and comparable to the facultatively sexual stolons. Köhler (1935 a) was able to point to certain structures in the heterothallic Mucor mucedo, which he considered homologous with the zygophores. This suggests that structures comparable to zygophores might be found in other heterothallic species.

In Rhizopus sexualis very fine hyphae may also be produced by the older stolons, zygophores and suspensors, if the atmosphere is sufficiently saturated. Their production may however be stimulated in young cultures by the presence of other fungi, but generally only in those parts of the homothallic mycelium in contact with the other fungus. These very fine hyphae are produced by the stolons only when they have started to collapse, and this is true also of the zygophores. In the case of the suspensors however, the fine hyphae are generally produced before they collapse. Further,

hyphae /

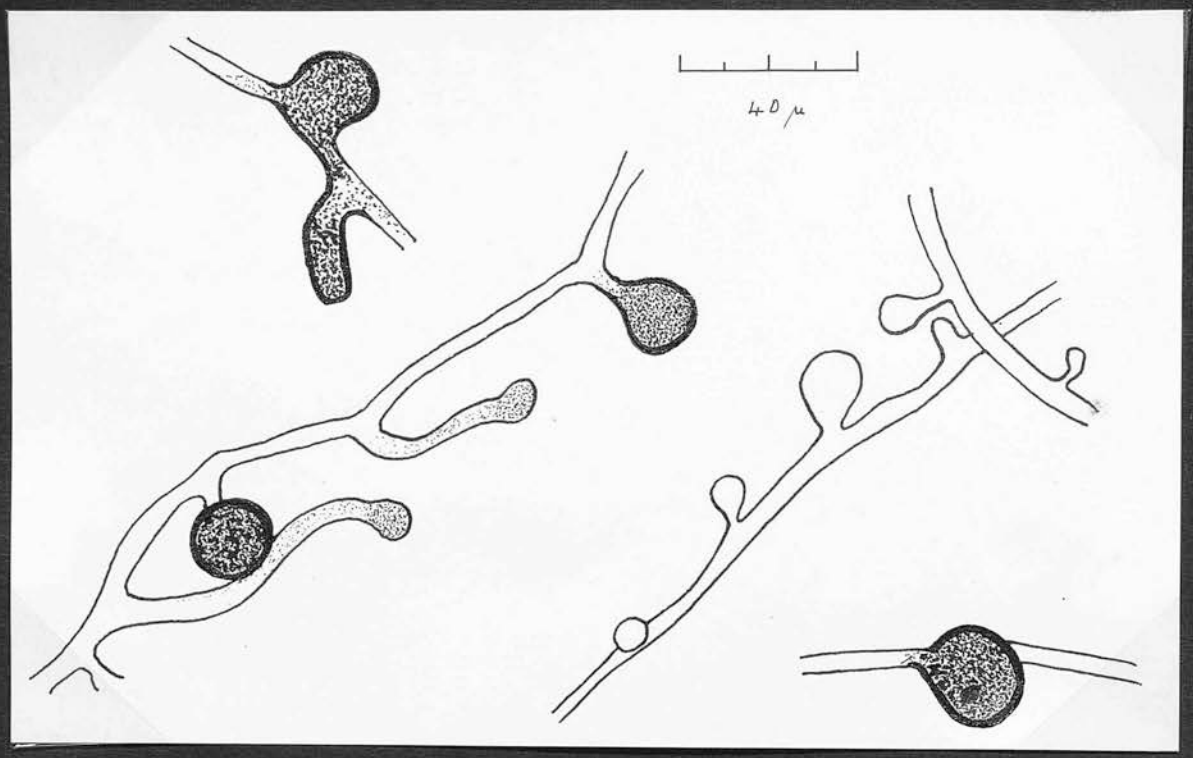

Fig. 1. Reservestoffblasen.

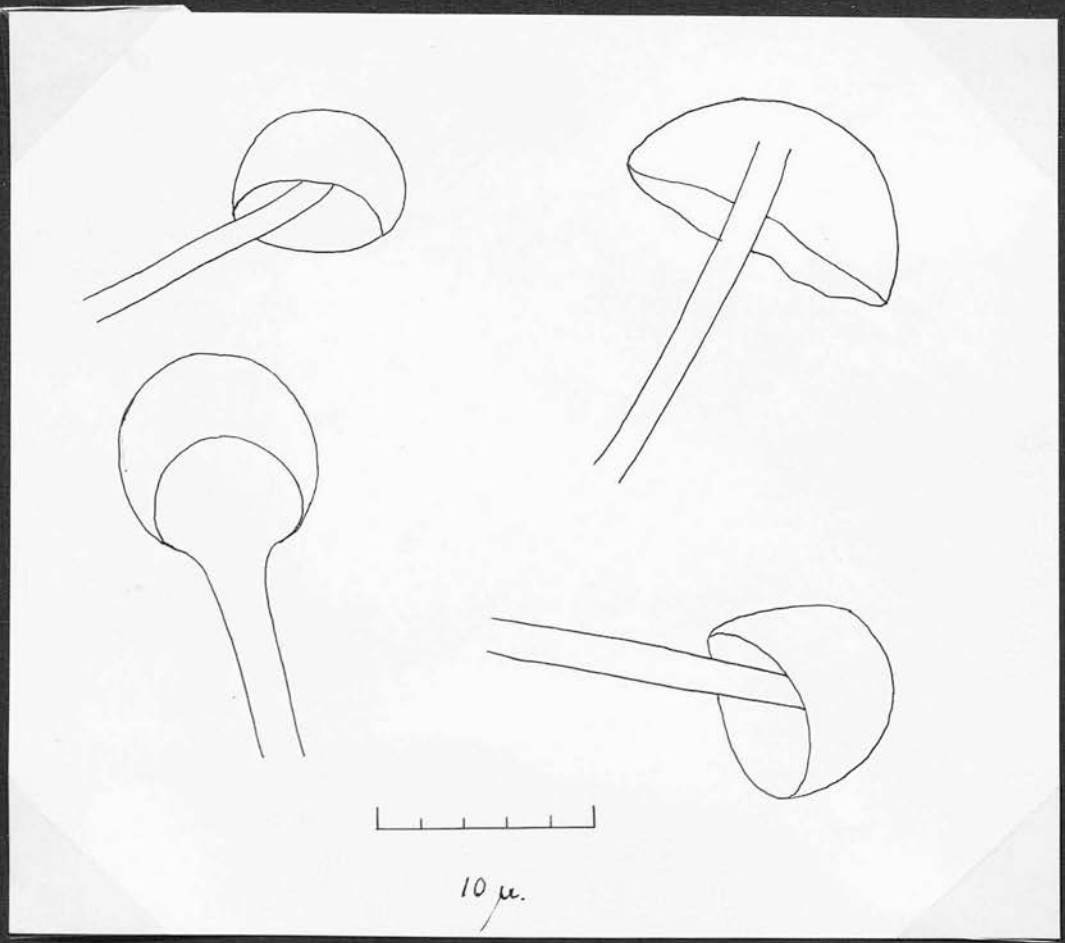

Fig. 2. Sporangia

hyphae in the air near the zygospore are induced to produce these fine hyphae as well, and with those of the suspensors, help to envelope the zygospore and impart a fur-like appearance to it.

Chlamydospores, as defined by Zycha (1935) are not produced by Rhizopus sexualis, though present in other Rhizopus species. Giant cells are however produced, when the edge of the mycelium reaches the limits of the nutrient medium. The tips of the hyphae grow forward over the glass surface for only a short distance, when they start to swell and to branch. The tips of these hyphae become very much swollen and distorted, ans are filled with a dense mass of protoplasm and nuclei.

In addition to the giant cells, another type of gemma is produced, which might be called "Reservestoffblasen" but which Zycha (1935) classed under the term "Dauergemmen" . When nutriment becomes scarce, small spherical swellings are developed at short intervals along the main growing or trunk hyphae. They are outgrowths of the wall, and are either terminal on short branches, or sessile. They are completely filled with protoplasm, and ultimately assume a dark greenish colour, though the wall remains hyaline very much longer. When fully mature, a cross wall is laid down, cutting /

cutting off the spherical body from the rest of the mycelium. Although the term "Reservestoffblasen" has been used, it does not mean that they are identical with the swellings found in Phycomyces (Burgeff 1924). In the latter they are swollen or distorted portions of the hyphae, whereas in Rhizopus sexualis we are dealing with an actual outgrowth of the hyphal walls.

There is a very marked protoplasmic flow in this species, either to the growing tips of the mycelium, or to a developing reproductive organ. Reversal of the flow has also been observed, even away from the reproductive organs.

Zygospore formation.

When the mycelium is some 18 hours old, strong rapidly growing hyphae rise up into the air from any branch of the mycelium, except trunk hyphae. These aerial hyphae generally originate near the tips of the branches; the latter are then cut off by a cross wall from the rest of the mycelium and shrivel up. The aerial hyphae take on the function of the branch, developing rapidly, and as there is a constant streaming of protoplasm to them, no vacuoles are formed. Up to this point the mycelium has developed no sporangia.

The first of these aerial hyphae to be produced continue their rapid growth, and become stolons, producing copious guttation water as drops at short regular intervals along their whole length. The aerial hyphae formed further from the centre of the culture slow down their growth, and produce a lateral branch, generally at least one third of the way up. Burgeff stated verbally, and also in his paper (1924), that the terminal portion of the aerial hyphae cease their growth in all homothallic species known to him, and that cessation of growth is a necessary factor for the production of the lateral branch. It has become increasingly evident in Rhizopus sexualis however that this is not necessarily the case. The lateral grows rapidly, and in a short time /

time curves towards the less rapidly growing tip of the terminal. These two constitute the zygophores. With the realisation that these aerial hyphae are really the beginnings of the zygophores, comes confirmation of Burgeff's statement (1924, p.79), that the sexual phase is initiated before the asexual.

Measurements of the zygophores show that the lateral is always thicker, but that formed from the terminal portion of the aerial hypha does not decrease in size when growth continues more rapidly after the lateral has been produced. The measurements for the base of the aerial hypha, and the terminal portion are identical, and are 11- 18 μ in diameter, whereas for the lateral they are 14 - 22 μ. If however the aerial hypha produces the lateral close to its own base, where it arises from the medium, then they both have the same diameter, namely that of the base and the terminal, 11 - 18 μ.

In no case have swellings been observed on the zygophores until they have come into contact, which leads to the conclusion that the progametangia are formed through contact stimulus. In the few cases where swellings have been observed without apparent contact (always in fixed preparations) the zygophores have been pulled apart during the fixing, The view that /

that contact stimulus causes the formation of the progametangia is strengthened by the fact that the apparently compatible zygophores, that is, a terminal and its lateral curve towards each other, but do not develop gametangia unless they come into contact.

The progametangia are essentially equal in size, but if one happens to be larger at the beginning, as often is the case, the other develops more rapidly later, so that they are ultimately of equal size. Development continues in the normal way, and though the progametangia are equal in size, the actual gametangia cut off, are in almost all cases unequal. The slightly larger gametangium is cut off from the progametangium on the lateral, i.e. thicker zygophore. Guttation water is formed on the aerial hyphae before the lateral is produced, and is found on the zygophores soon after progametangium formation. One single drop appears on the zygospore when the two gametangia have completely fused.

The zygospore takes some twelve hours to develop fully from the time of contact of the zygophores. More or less barrel-shaped at first, the zygospores tends in time to assume a more spherical shape, so that after three or four months they are more oval than barrel-shaped. They vary greatly in size, the first ones produced may be small, but soon the larger ones

are /

are produced. As the culture ages, the zygospores formed become increasingly smaller, as do the zygophores and progametangia, and eventually when fully mature, the smaller ones are less than half the size of the larger ones. In size, the zygospores are 54-172.5 X 70.5-220 μ (100 measurements) (Smith 60-180 μ in diam.), the first measurement being taken across the zygospore between the two suspensors, and the second at right angles to that. The suspensors measure 40-110 X 48-128 μ taken across the suspensor form zygophore to zygospore, and at right angles to that respectively.

During development the suspensors are hyaline, and it is the young zygospore that first shows pigment development. As it reaches maturity however, the suspensors gradually assume a light brown colour, when seen by transmitted light, caused by carotin formation. The carotin is formed in the suspensors first, and then spreads down the zygophores, but is much less intense there. It is not formed in the mycelium buried in the medium, as Köhler (1935 a) found was the case in Mucor mucedo. Through the courtesy of Prof. Burgeff, a comparison between Köhler's Mucor mucedo and Rhizopus sexualis was possible, and showed that the two fungi differed entirely in the place of their carotin formation. Crystals were also formed in the suspensors,

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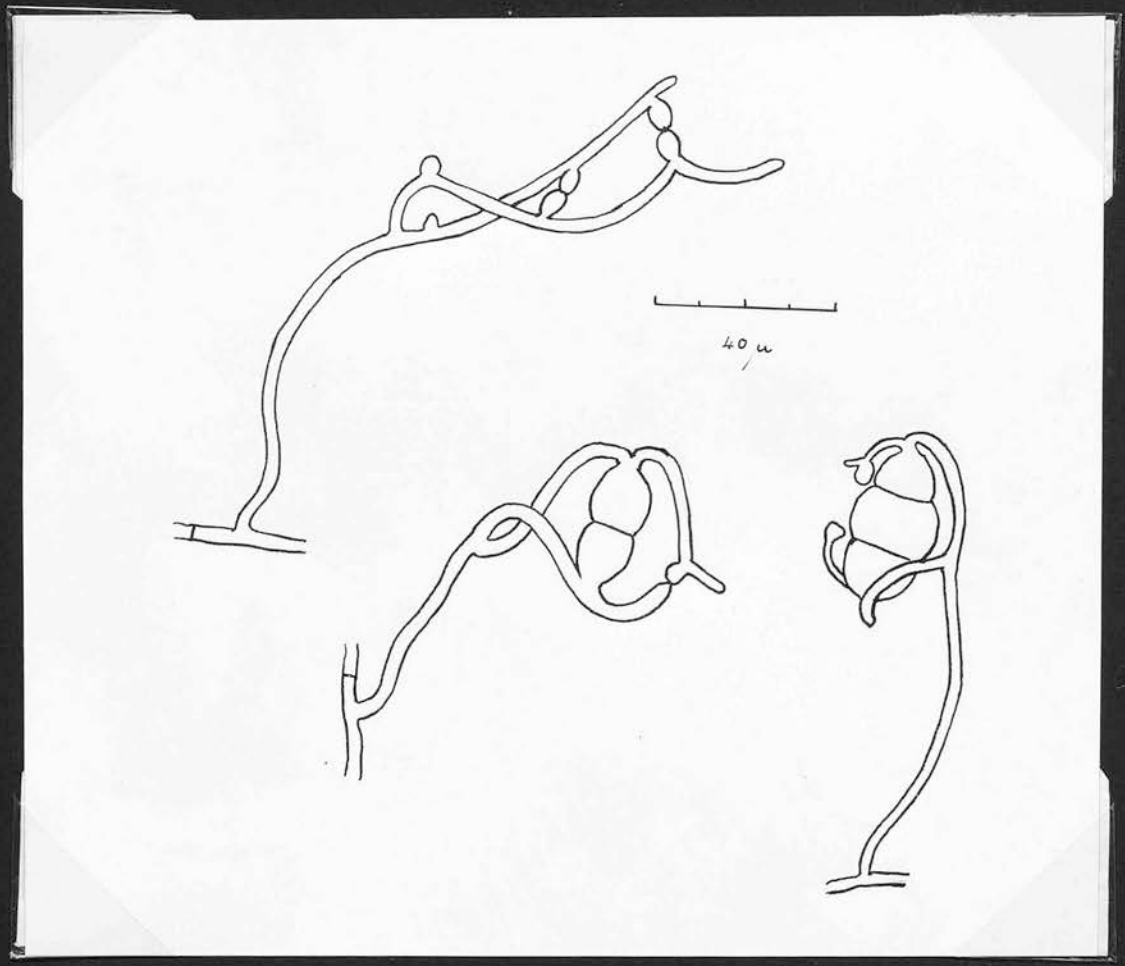

Fig. 3.

a character common to the genus Rhizopus.

Although it is usual for only one zygospore to be produced by the pair of zygophores, the ends of the zygophores may continue their rapid growth and form yet another zygospore, ladderwise, which may or may not mature. The mycelium is well able to nourish two zygospores from one pair of zygophores, but the second one is often unable to develop fully, as the rapid growth of the first may cause the pulling apart of the progametangia of the second one (fig. 3). This production of zygospores ladderwise in homothallic species, is proof that the terminal hypha does not completely stop its growth on production of the lateral, but merely slows down, and that it can take up its rapid growth again. The nearest approach to this type of zygospore formation has been observed in Absidia spinosa by Burgeff (1924) and Nielsen (1927), where one zygophore was found to be capable of producing two zygospores by contact with two different laterals, but that one of them is nearly always abortive, due to insufficient nutriment as both authors suggest.

The zygophores may not come to zygospore formation at first however, in which case they produce laterals and these again laterals, which in various combinations may give rise to as many as three or four zygospores

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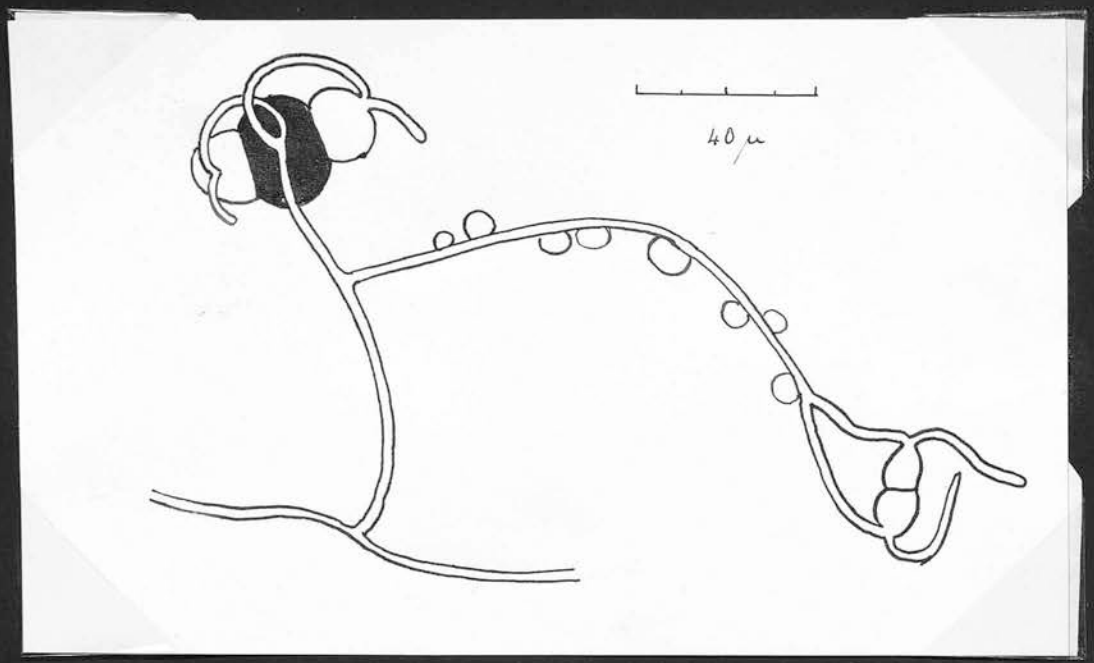


Fig. 4.

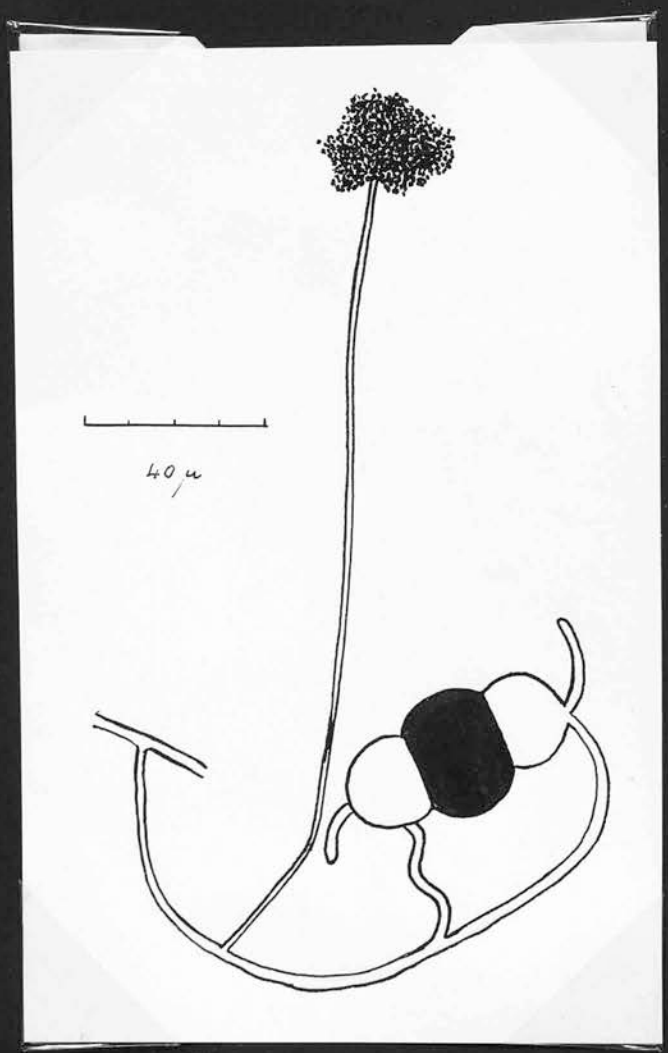


Fig. 5.

on the original pair of zygophores. If there is no zygospore production, the zygophores either wither, or or continue to grow rapidly and become long stolons.

After several days growth a number of variations can be observed in the cultures. One of the most common is that the base of the aerial hypha gives rise to a lateral which continues the reproductive phase, and does not develop into a stolon. This lateral in its turn gives rise to a lateral branch, and we have a new pair of zygophores which produce a mature zygospore (fig. 4), or, apparently if insufficient nutriment is present, the second lateral does not develop, and the first lateral produces a sporangium at its apex (fig. 5). The sporangiophore may be either long or short, but is of the auxilliary sporangial type (see later section).

Other variations that have been observed include, that two unbranched aerial hyphae (zygophores) may react together, and produce a mature zygospore (fig. 7). They are apparently of different lengths and ages, and sometimes one of them has a minute withered lateral. It may also happen that the terminals of two different sets of zygophores copulate. In other words there does not appear to be a hard and fast rule inorder to obtain a sexual reaction.

On a highly unfavourable medium, the stolons are capable /

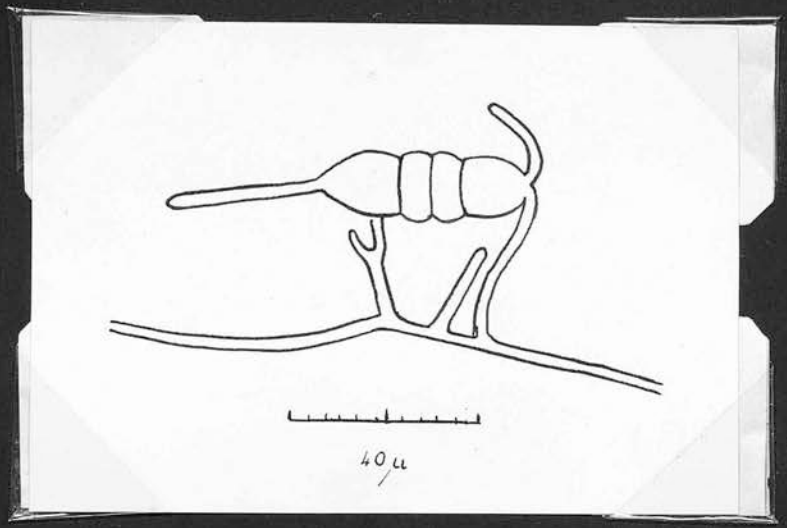


Fig. 6.

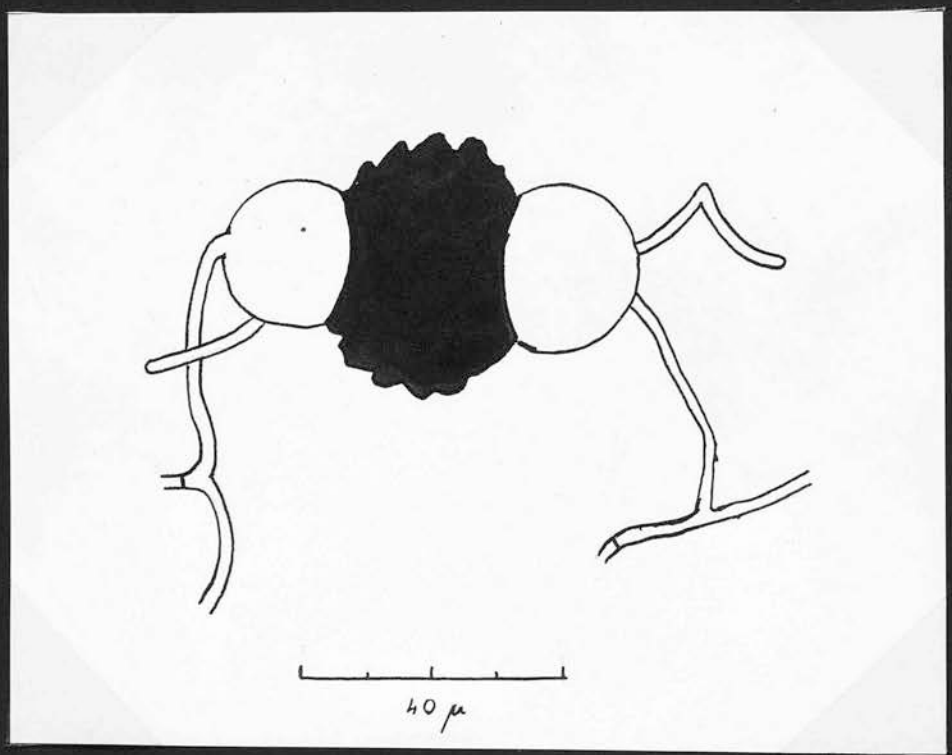


Fig. 7.

capable of taking up the sexual phase, for at a later stage they are capable of giving rise to a lateral near the tip, which reacts with the terminal portion to give a mature zygospore. The stolons are incapable of reacting sexually except at the tip, but are capable of producing laterals in the sexually inactive region, which can react together and form mature zygospores (fig. 6). Curiously enough these laterals always seem to be produced on the same side of the stolon.

Summarising therefore, it would appear that any young hypha is potentially capable of reacting sexually, if sufficient nutriment is present. This follows as a natural corollary to the statement already made (p.13), that the sexual phase precedes the asexual. That the sexual phase does precede the asexual even in the heterothallic fungi, has been demonstrated for Phycomyces nitens by Burgeff (1924), who stated that the change from vegetative to sexual reproduction takes place much nearer to the growing tips of the mycelium, than the change form sexual to asexual reproduction.

A medium of acid reaction (pH 5.0) produces as many zygospores as the medium of pH 6.4 normally used, but there is a far more abundant production of zygophores, a great number of which do not copulate. They branch excessively and produce sporangia and azygospores, or both. /

both. These cultures on the acid medium do not show any sign of producing the trapping hyphae, so characteristic of the normal growth already described, as well as of other Rhizopus species. As has been pointed out however, these branched zygophores, which ultimately wither in a ten day culture, are homologous withthe trapping hyphae.

Zygophore formation, and particularly the branching can be much stimulated by the presence of other fungi, as for example when hybridising with other members of the genus Rhizopus or other genera of the Mucorineae. This point will be further dealt with in a later section however.

Suspensor regeneration.

At first numerous attempts to get the suspensors of this homothallic species to germinate were completely unsuccessful. Although one month old zygospores were repeatedly sown on 2½% malt extract agar, and left there for a fortnight, no regeneration took place. When however one week old zygospores were used, it was possible in four cases out of sixty-four to isolate the developing mycelium from a single suspensor, and in all four cases the typical homothallic mycelium was obtained.

Later, when four month old zygospores were sown on damp sterile earthenware plates in Petrié dishes in an atmosphere saturated with water vapour, many of the suspensors germinated, and it was possible to isolate a number of them. To ensure that the mycelium really was due to regenerated suspensors, and not to germinating sporangiospores, which might still have been adhering to the exosporium inspite of repeated washings, each suspensor was carefully scrutinised with a binocular microscope. Fifteen isolations were made, but eleven were rejected as not showing the origin of the mycelium to be definitely from the suspensor. Each of the four remaining regenerations developed into the homothallic mycelium. According to a verbal communication from Burgeff, he himself has been able to isolate only homothallic /

homothallic mycelia from the suspensors of true homothallic species. Satina and Blakeslee (1930) mention regeneration of isolated progametangia in Dicranophora, " - which may be published later -", which supports the facts above, that the plasma of the progametangia are undifferentiated sexually.

Owing to unforseen circumstances, only six out of some dozen isolations survived, being temporarily abandoned on the Continent during an international crisis. These six show a rather remarkable variation amongst themselves. Two of them have the typical scanty aerial mycelium with stolons, but the others develop a varying quantity of aerial mycelium, which is at first sight white in colour, but later turns a light buff, and in the extreme case almost obscures the zygospores produced beneath it. When subcultured, each isolation retains its own special characteristics.

This difference in the aerial mycelium suggests that there has been a genetical change, and the possibility that other characters have changed. With a view to testing this supposition, each isolation was plated in triplicate against the Würzburg strain of Mucor hiemalis (-)ve, with the original Rhizopus sexualis as control. Rh. sexualis gives perfect hybrid zygospores with M. hiemalis (-)ve as will be shown later (p.56).

The /

The plates were carefully examined and marked according to the degree of response. The two isolations which had shown the scanty aerial mycelium, gave a response equal to that of the control. Two others showing a medium development of aerial mycelium, gave a distinctly poor response to Mucor hiemalis, whilst the last two isolations, in which there was so much aerial mycelium that the zygospores could scarcely be seen with the naked eye, gave no response at all. In other words it appears that the amount of aerial mycelium present, is in inverse proportion to the response to hybridisation in this species, and would therefore appear to be an indication of the virility of the strains.

Another variation observed was that one of the cultures developed guttation water of a clear deep crimson hue, when seen by transmitted light, or black by reflected light. In subcultures the mycelium retained this character, and it was observed in four successive generations. It cannot have been due to a difference in the medium, as it was always subcultured onto the same batch of medium as the others.

No further tests have been carried out on these isolations.

Sporangial production.

As has already been seen, the zygophores are produced within twenty-four hours of inocculation, but it is only after some forty-eight hours, when the centre of the culture has developed a relatively dense weft of aerial mycelium, that the sporangia appear. They are at first exclusively formed in those portions of the mycelium where there is a "Hemmung", or slowing down of the growth, and only later are they formed in the sparsely covered areas. It can clearly be seen therefore, that the sexual phase is initiated before the asexual, as has already been pointed out (p.13).

The first sporangia to appear are of the type called "Zwergsporangien" by Burgeff (1924), or as he now prefers to call them, "Nebensporangien" (in Köhler, 1935 a), in English "auxiliary sporangia". They have b been observed by Burgeff in Phycomyces nitens, and by Köhler in Mucor mucedo, and their observation here in Rhizopus sexualis suggests interesting comparisons. In appearance these auxiliary sporangia are merely miniatures of the normal ones, except that they have no rhizoids, and the sporangial wall is very much thinner and diffluent. They are produced from the lateral branches of the mycelium, generally the aerial mycelium, and occur singly, though on three occasions branched ones have /

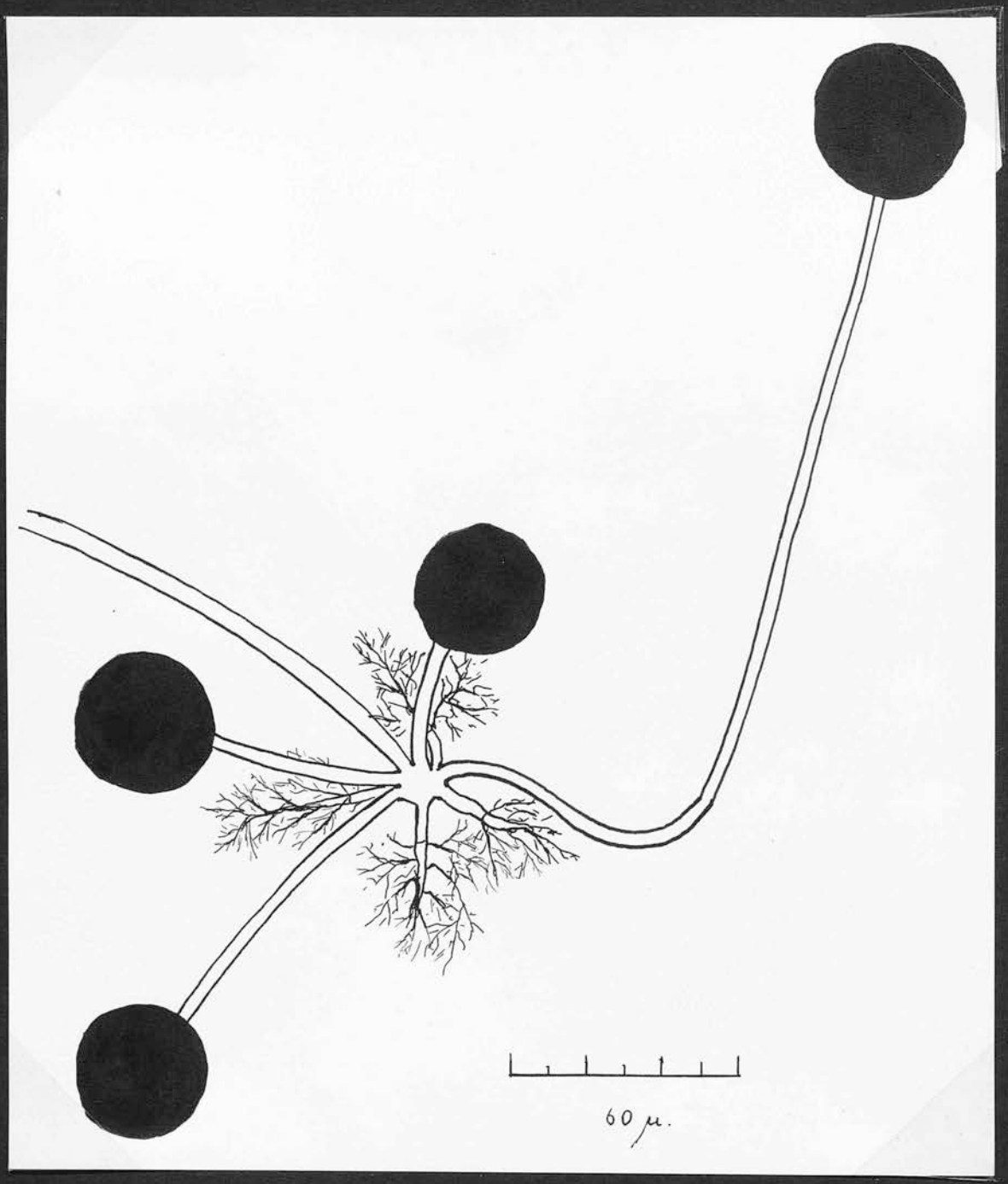


FIG. 8.

have been observed. Cross walls are sometimes laid down, either in the parent hypha or in the sporangiophore itself, though as a general rule none is present. It would appear that the presence or absence of cross walls is governed by the amount of protoplasm available in the parent hypha; if well filled, a septum is laid down. The sporangium figured by Smith (1939) is of the auxiliary type, and shows the diffluence of the sporangial wall very well. As the aerial mycelium spreads, so does the production of these auxiliary sporangia.

The normal sporangia are produced by the stolons, but only under certain conditions. If a stolon touches the medium where it is covered with the submerged type of mycelium, then one or more sporangia are formed at the point of contact, and rhizoids are produced, which soon develop a deep brown colour. It is therefore only where there is a "Hemmung" or check to the growth, that they are produced, and their absence in Smith's cultures must have been due to the absence of stolons. In young cultures the stolons often branch in the air, rooting beyond the edge of the submerged mycelium, and consequently no sporangia are formed. If the branch does not manage to reach the edge of the mycelium however, it produces a single sporangium, or occasionally up to /

up to five, and ramifies itself in the substrate amongst the submerged type of mycelium. On the other hand, if the stolon touches the glass, rhizoids are produced, generally without sporangia. This is in direct contrast to Rhizopus nigricans, where bunches of sporangia are usually formed at all points where the stolons touch the glass.

In the most recent monographic treatment of the genus, Zycha (1936) states that in the genus Rhizopus as a whole, in any species, the only fairly constant character, morphologically, appeared to be the height of the sporangiophores, and the size of the sporangia and the spores. In Rhizopus sexualis however, the sporangiophore length varies considerably, for although to all appearances fairly uniform when grown in Petrié dish or test tube culture, when examined more carefully, as is possible with the agar film technique, they are seen to vary considerably in older cultures, namely anything from 30-192 µ in length. This is due to the fact that there is no sharp demarkation between the normal and auxiliary sporangia. With little difficulty a complete set of transition forms can be selected.

The sporangia also show a wide range in size, 24 -253 µ (Smith 40-85 µ) in diameter, varying more or less with the length of the sporangiophores. The spores themselves /

themselves are more constant in size however, and even those produced from the auxiliary sporangia, practically fall within the limits of the normal spore range, being if anything a trifle larger. The normal spore measure 6-17 μ (average of 100, 10 μ) (Smith 8-18 μ in long axis) and those of the auxiliary sporangia 9-18.5 μ (average of 50, 12 μ). As the latter had been lying on the agar film for several hours, the spores of the normal sporangia were allowed to lie an an agar film till they were about to germinate.

Attempts were made to influence the production of sporangia and zygospores by growing the fungus on acid media, but little difference could be observed. By accident some malt extract agar was allowed to become contaminated, which, though it had a pH of 6.4 (due no doubt to the buffer effect of the agar), had a sickly smell, owing to the decomposition of the malt extract. This medium caused a retardation in the growth of the mycelium, but plentiful sporangia were produced after three days in culture, but only of the auxiliary type. The sexual phase remained in abeyance till the cultures were much older, a reversal of the normal procedure. Rhizopus nigricans behaved differently on this medium. No sporangia were produced in three days, but very obvious zygophores appeared, which curled and twisted into /

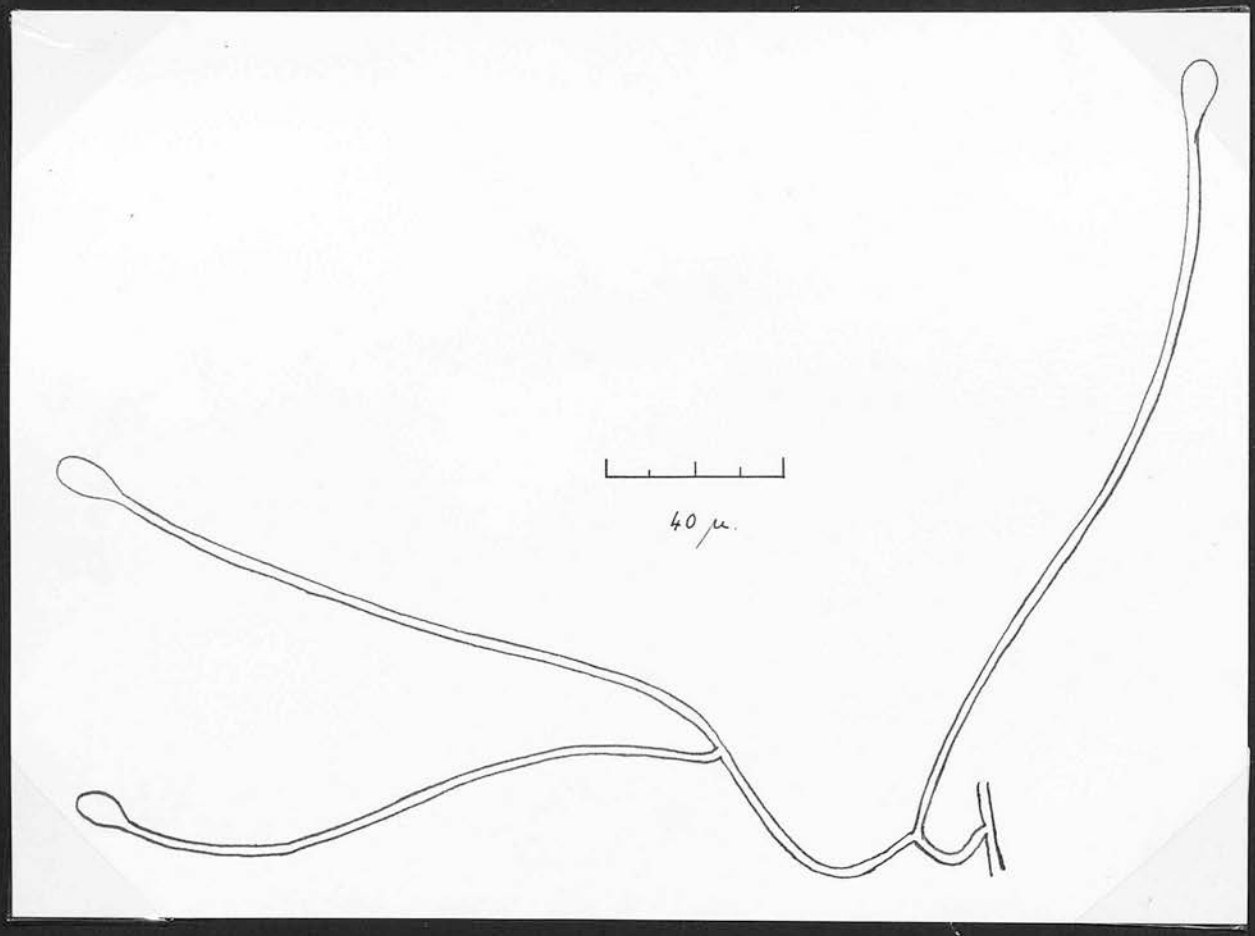


Fig. 9.

into knots instead of growing straight out.

A number of abnormalities have been observed, some of which have already been recorded for Rh. nigricans by Vuillemin (1902) and Lendner (1908). The latter depicts sporangia giving rise to smaller ones from the sporangia themselves, or from the sporangiophores. Both these types have been observed in Rh. sexualis. On three occasions it has also been observed that the sporangiophore can give rise to a second one , and the second one in its turn to yet another (fig. 9). They are always separated from the parent sporangium by a septum in the parent sporangiophore, just above their point of origin.

Auxiliary sporangia are also produced by the zygophores under certain circumstances, as has already been mentioned (p.17). This is actually a common occurrence amongst the Mucorineae.

Azygospore formation.

Azygospore formation is a fairly frequent occurrence in the Mucorineae, in some of them even, such as Mucor tenuis, M. vicinus and M. neglectus, no normal zygospores are produced at all. Others such as Mucor racemosus form normal zygospores and azygospores in almost equal numbers (Bainier, 1883). Kniep (1928) gives a list of over twenty-five species known to produce azygospores. He places Rhizopus nigricans amongst them on the evidence of Zopf (1890) and Namyslowski (1907), with the note, "azygospores rare". Zopf does not mention azygospore formation however, and only mentions the species in a list of fungi whose zygospore formation was already known. Namyslowski describes two types however, thigmospores and ordinary azygospores, and it is the latter which are described as rare.

In the present homothallic species, azygospore production is actually an uncommon occurrence, as only about a dozen can be found in a Petri dish culture (3½ inch diameter) containing some 27.000 zygospores. They may be of two types as described by Namyslowski for Rh. nigricans, but the double or thigmospore type is much less frequent. The percentage of azygospores produced can be considerably increased when Rh. sexualis is plated against certain other species. Absidia cylindrospora /

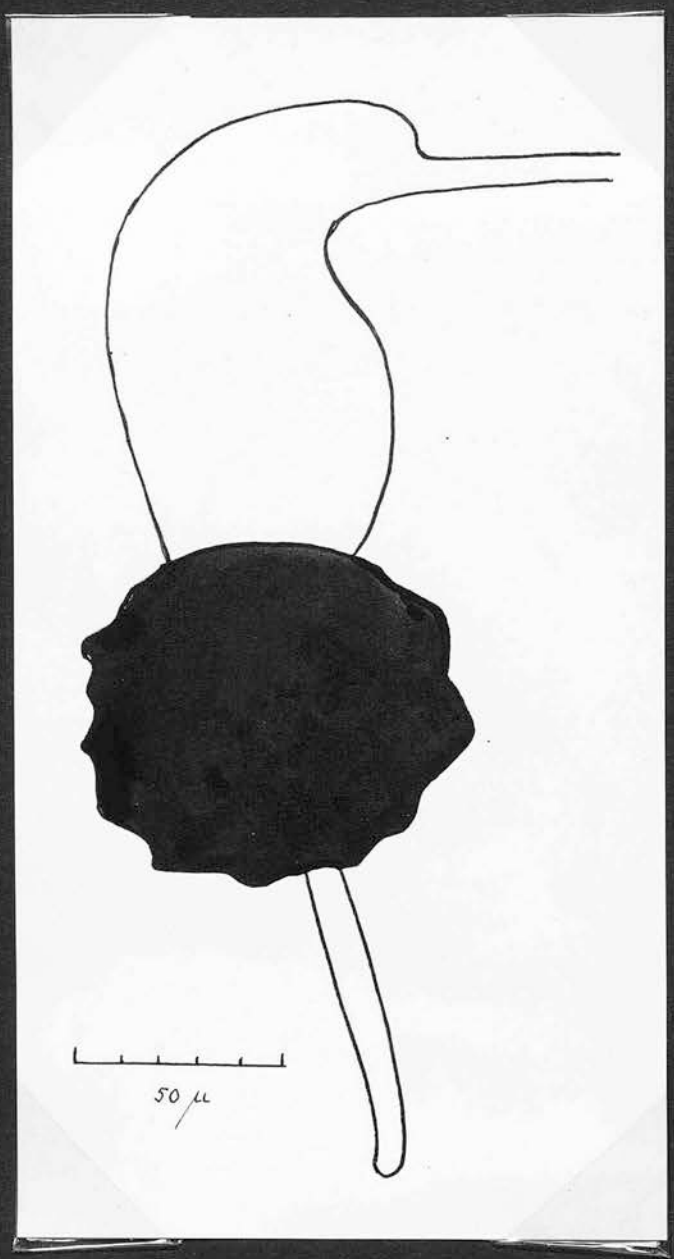


Fig. 10.

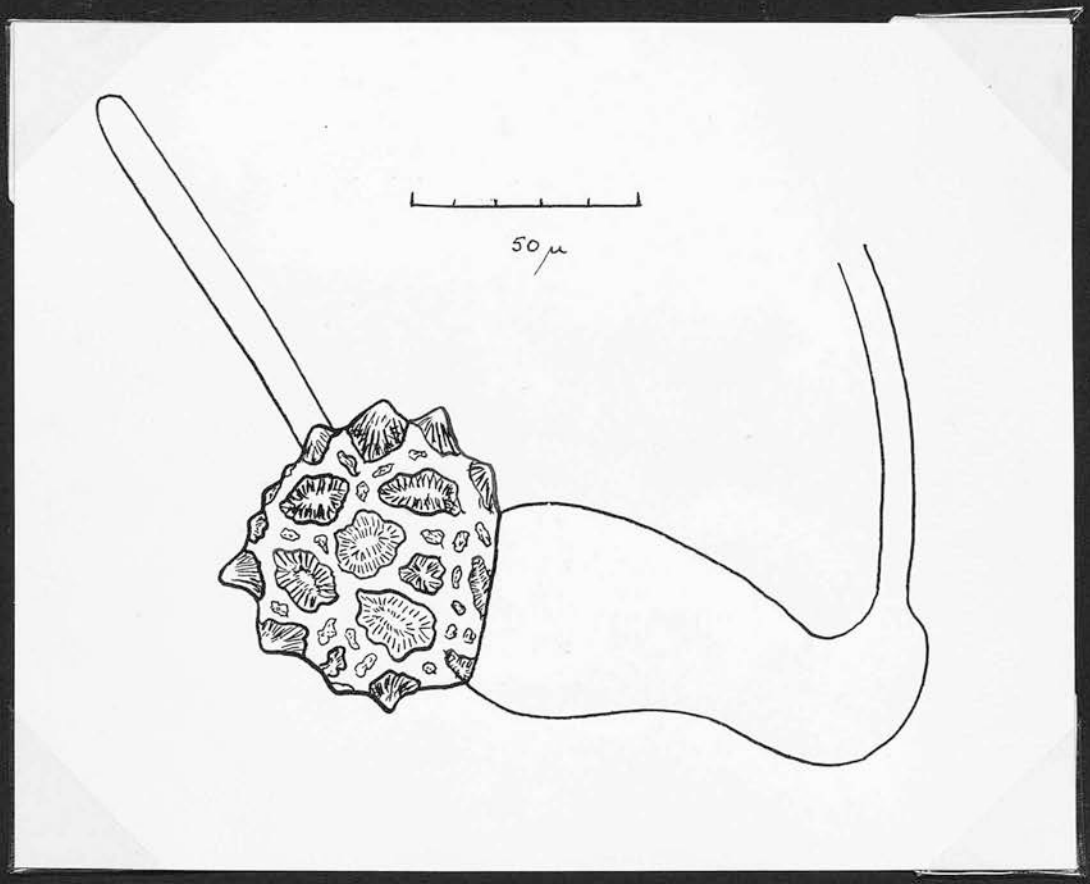


Fig. 11.

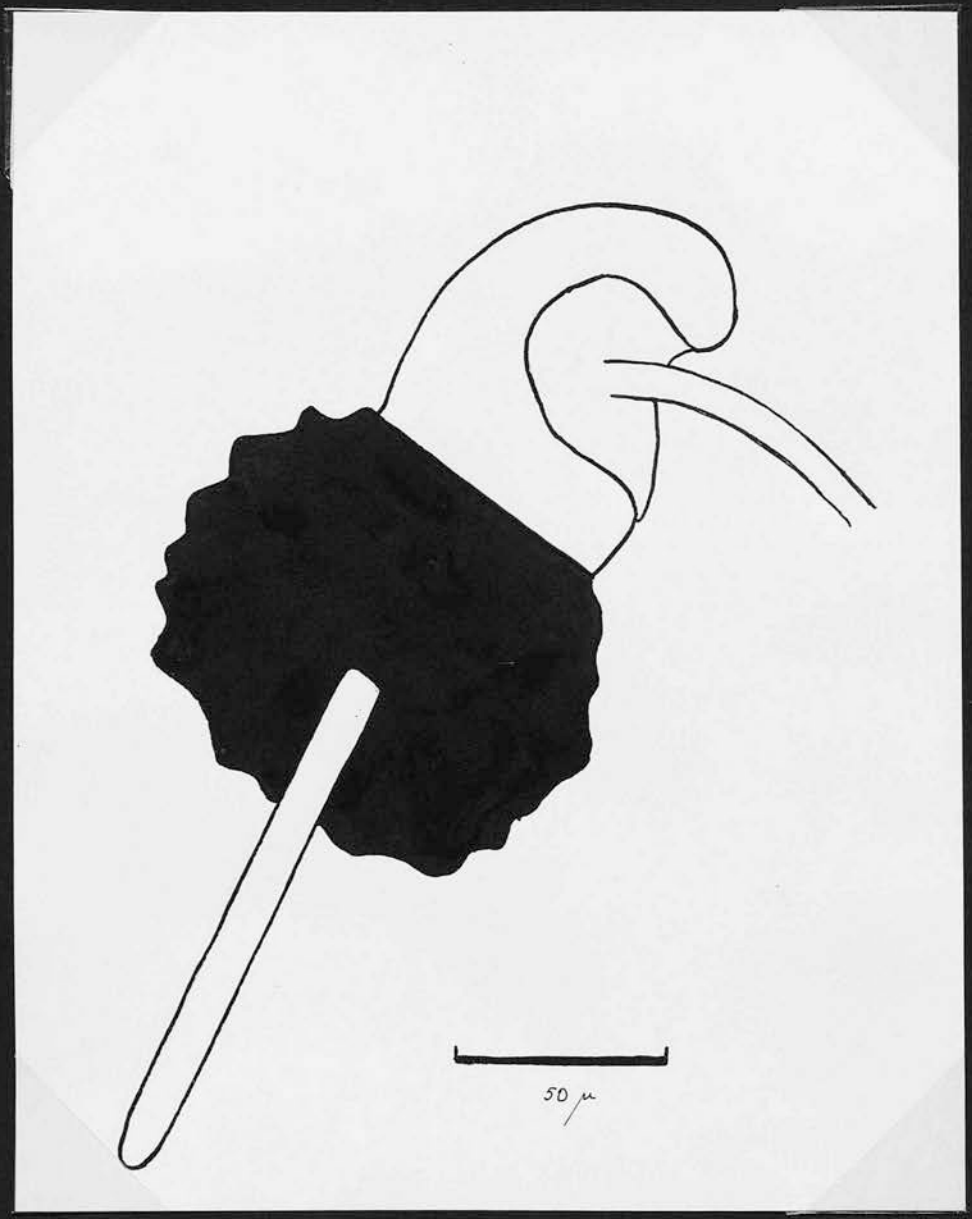


Fig. 12.

cylindrospora causes a large increase in the number of azygospores, though many never reach maturity, that is to say, do not develop the thich brown exosporium. A curious structure observed on most azygospores is what looks like the continuation of the zygophore projecting from one side of the azygospore (fig. 12). No mention of such a structure can be found in the literature, though Ling-Young (1930) figures a great number of abnormalities in azygospore production in the Mucorineae. This projection may develop an exosporium at the same time as the azygospore, though it may only be for part of its length.

On a medium of acid reaction (pH 5.0) there was no apparent increase in azygospore production, contrary to what Kanouse (1923) found in the homothallic Mucor parvisporus.

Though the actual number of mature azygospores produced is very small, the number of pseudophores is very much larger. In hybridisation experiments, where the azygospore production is greatly stimulated, the pseudophore production may be equally stimulated, as for exanple when Rh. sexualis is crossed with Absidia cylindrospora. This does not always appear to be the case however, for in crosses with A. glauca, though the azygospore production is greatly increased, the number of /

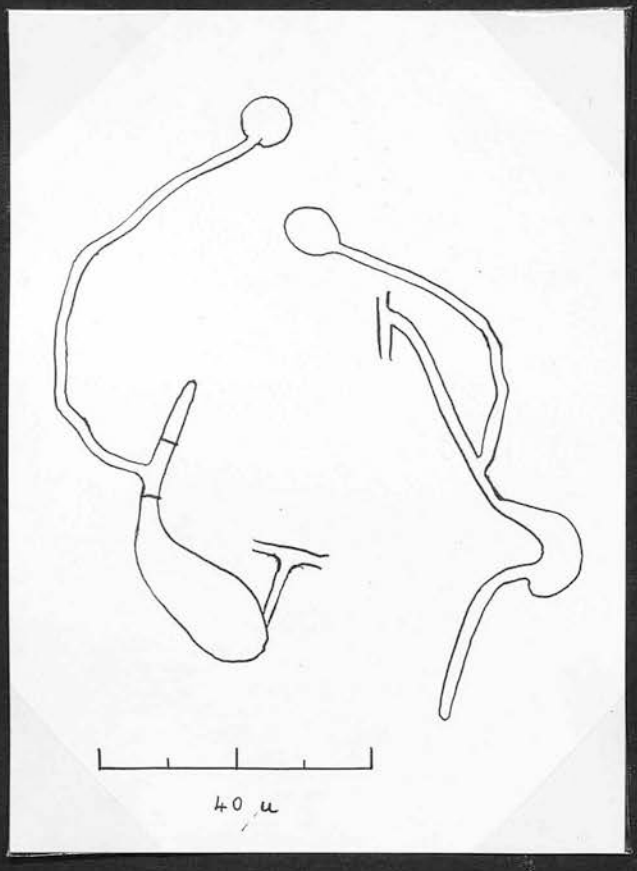


Fig. 12 a.

Fig. 12 b.

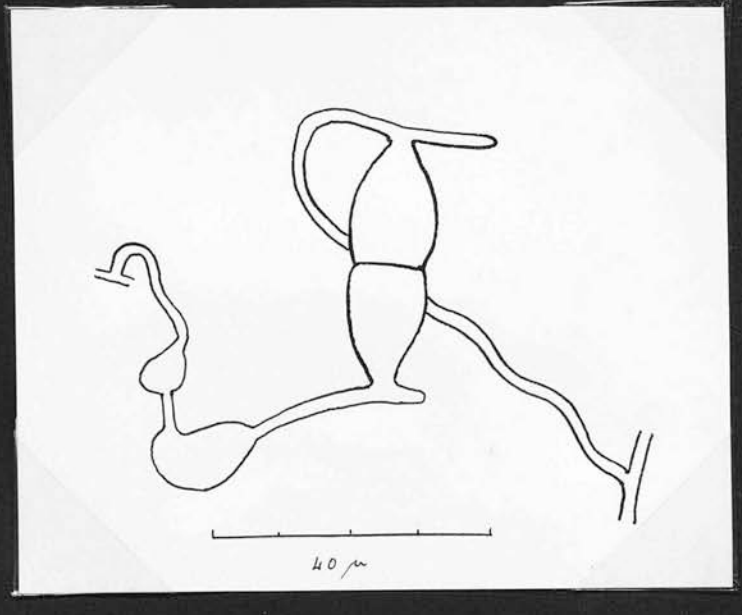


Fig. 12 c.

of pseudophores remains relatively small. The explanation of this would appear to be that some fungi can stimulate the homothallic to bring a far greater number of pseudophores to azygospore production than others, From an examination of several hundred cultures, one is forced to the conclusion that if the mycelium is sufficiently stimulated, pseudophores can develop without the contact stimulus of another hypha. One branched zygophore can in extreme cases produce two or three pseudophores (fig. 13). They may be terminal or more usually subterminal. In the latter case, the terminal portion of the zygophore may continue its growth and give rise to an auxiliary sporangium (fig. 12a), or even take part in a sexual reaction, either by producing a lateral itself (fig. 12b), or with another zygophore (fig. 12c).

Plasma excretion from the pseudophores has been observed on several occasions. This curious phenomenon has been recorded for Phycomyces nitens by Orban (1918) and Burgeff (1924), but is quite unknown in any other fungus. Orban suggests that it is due to the bursting of the pseudophore, but as Burgeff points out, the pseudophore remains turgescent after this excretion. He finds that the plasma is apparently excreted along with water, as it is always to be found in a large drop of water on top of the pseudophore. He treated these pseudophores /

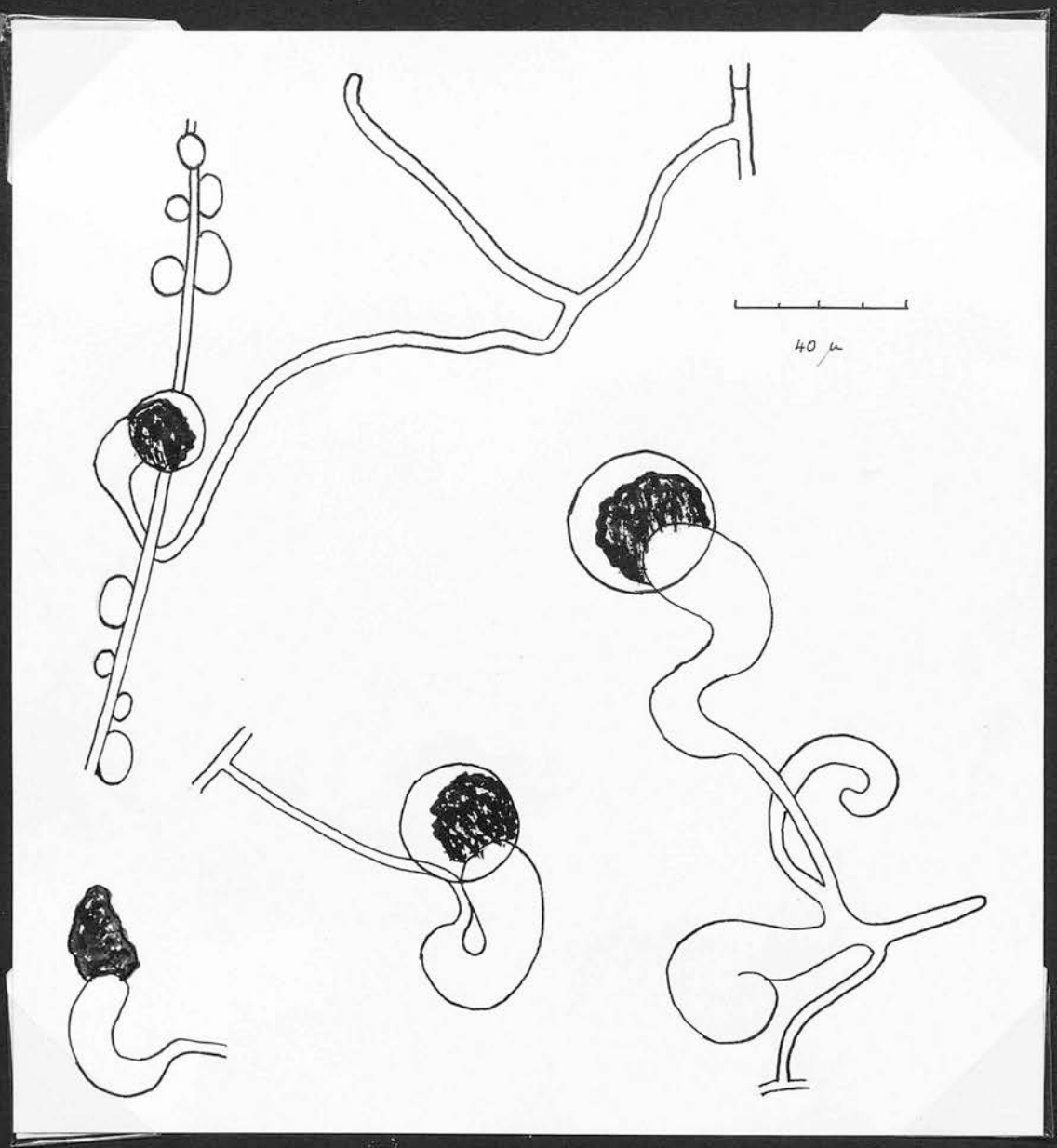


FIG. 13.

Plasma excretion in Rh. sexualis.

pseudophores with weak alcahol, and found that a small cap separated off from the tip, leaving the pseudophore intact underneath.

In Rhizopus sexualis this excretion of plasma has been observed on at least seven occasions, though actually the phenomenon is not of such a rare occurrence as that would lead one to believe. On four of these occasions the plasma was lying in a large drop of water, as both Burgeff and Orban had also seen it, but on three other occasions the plasma has been found on top of the pseudophore, but there was no drop of water present. On closer examination of some of the hybridisation experiments, this latter "dry" excretion can be observed relatively frequently in a Petri dish culture. This phenomenon would appear to be much more common than has been suggested. The explanation of the rather puzzling appearance of the tops of the pseudophores only occurred to the writer after much of the hybridisation work had been carried out. It would therefore appear that the presence of the drop of water on the pseudophore is only accidental, and that it is not excreted with the plasma as Burgeff suggests. In two of the observed cases, where there was a drop of water present, it was obviously guttation water from a neighbouring stolon (fig.13). Actually only a very small percentage of the pseudophores /

pseudophores produced reach the excretion stage, as most of them are subterminal, and have the continuation of the zygophore projecting from the tip. With this plasma excretion the pseudophore reaches its highest development.

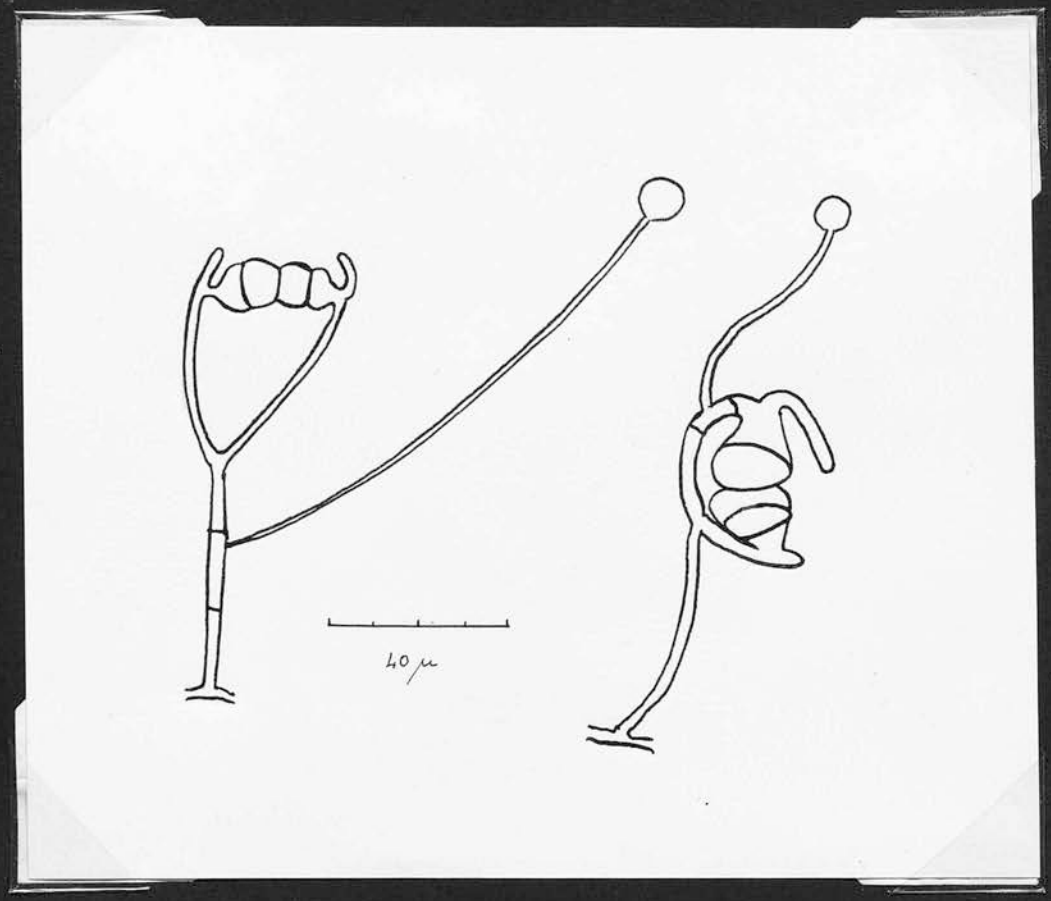


Fig. 14.

Changes from sexual to asexual reproduction.

As has already been noted (p.13), the sexual phase precedes the asexual, under normal circumstances, and it is therefore not surprising to find the zygophores giving rise to sporangia. This is a frequent occurrence amongst the homothallic Mucorineae, and Rhizopus sexualis is in no way an exception. Sporangia may occur on a number of different places on the zygophores, the commonest of which is depicted in fig. 3, where the zygophores have already given rise to a mature zygospore. The production of another lateral is normally the first step towards the production of a second zygospore, as shown in fig. 4. Instead of this, the lateral grows on and develops into a sporangium as shown in fig. 5. There is always a cross wall laid down in the zygophore to separate the sporangium from the zygospore, though it is not formed if the lateral develops a branch and a zygospore instead.

It may happen that in the normal sexual reaction the gametes are formed, but that they do not fuse, in other words that a thigmospore is formed. In this case one of the zygophores generally gives rise to a sporangium (fig. 14), but completely isolated by two cross walls in the parent hypha. This auxiliary sporamgium is very small in size, and produces only a very /

very few spores, and it is doubtful even if they develop fully.

A further instance of the change from the sexual to the asexual can be seen in the production of sporangia on zygophores bearing pseudophores. Blakeslee (1906), Burgeff (1914) and Orban (1918) have all recorded this type of change. Burgeff (1924) expresses the opinion that the production of these auxiliary sporangia is a sign of age, just as is the production of cross walls. He admits that his earlier statement (1914), that the sporangia are developed from the pseudophores themselves, is erroneus. In the present fungus, the sporangia were observed to originate from the zygophore, either below or infront of the pseudophore, but never from the pseudophore itself.

There appears to be no regular rule about the laying down of cross walls; in some cases two are present, one on each side of the origin of the sporangiophore, in others one wall is present only, while in still others no walls are produced. The intermediate condition, where one wall is present, is by far the commonest.

From a consideration of the foregoing, it is rather suggested (der Gedanke liegt nahe) that the cause of this change from sexual to asexual is nutritional.

After /

After one zygospore has been formed by the zygophores, there is still sufficient nutriment accumulated there to form a second one. Before that can take place however, some of the protoplasm and food material is withdrawn to some other part of the mycelium. This reversal of the protoplasmic flow, away from the zygophores has been observed on two occasions. Perhaps the actual gametangia of this potential zygospore have been formed, though they have not actually fused. There is still the sexual stimulus present, but insufficient food material for the completion of the zygospore, although still sufficient for a sporangium to be formed. If there is not even sufficient material for that, the whole zygophore shrivels up rapidly.

If we assume this nutritional basis inorder to explain the change, we can also explain the reversal of the two phases on the decomposing medium (p.26), where as has already been mentioned, the asexual proceeds the sexual phase. On this medium the mycelium was very slow growing for the first three days, producing sporangia only, and the zygospores appearing later. Thus it was only when the mycelium had been able to accumulate sufficient nutriment that the sexual phase was initiated.

Apart from the foregoing case, the change from asexual to sexual has never been observed, though Burgeff /

Burgeff (1924) records the production of a zygophore from a sporangium in Mucor mucedo and in Zygorhyncus exponens. A constant close watch was kept on several occasions on zygophores and stolons which had come into contact with sporangiophores, but there was not even the suggestion of a swelling on either hypha.

Cytology.

Examiation of the sporangiospores by means of the agar film technique shows that there are no fixed number of nuclei present, but that the number varies more or less directly with the size of the spores. There is no difference to be observed in the number of nuclei contained in the spores from the auxiliary and normal sporangia.

There are from 4-20 nuclei in each spore, with either 10 or 12 as the most frequent number. An uneven number of nuclei may ~~be~~ sometimes be observed, but in that case two nuclei are lying side by side, as if they are the daughter nuclei of a recently divided nucleus. This suggests that it is possible, that the number of nuclei originally in the spore has been smaller, and that it increased during the period of swelling, as the counts were made just prior to germination. It is also possible on occasion to find two nuclei present, which are very much smaller than the others; always two, and never one alone. Further, in those spores with only four nuclei, the nuclei are of an unusually large size. This suggestion that a nuclear division takes place in the spore can only be confirmed by an examination of the sporangium, and will be referred to again later.

On germination the nuclei enter the germtube singly, and /

and may undergo division almost before they are in the germtube, or after they have progressed some distance down it. Later the spore is emptied of all nuclei and protoplasm. The well known fact that the sister nuclei do not divide simultaneously in the thallus is illustrated in this fungus.

The zygophores when first formed, are like any other hyphae, but additional nuclei are soon brought along by the flow of protoplasm. When the aerial hypha branches to give the lateral zygophore, no nuclear divisions have been observed, as a result of which one daughter nucleus proceeds up each zygophore. There is no evidence to suggest that there is a separation of sexes, or anything other than the ordinary mitotic division occurring in the zygophores. The mere fact that a single regenerated suspensor gives the homothallic mycelium, is sufficient proof of this, even though the nuclear divisions cannot be followed.

The following is the record of the development of a single zygospore, as observed on the 15th January 1938. It cannot ofcourse be taken as a record of the exact length of time taken for the development or dissolution of any one structure, as it is impossible to follow these processes in the living unstained material.

1. Young /

1. Young progametangia in contact.	11.09 a.m.
2. Walls separating two gametangia have fused.	12.14 p.m.
3. Walls cutting off gametangia have been formed.	12.55 p.m.
4. Wall between gametangia in course of dissolution.	1.20 p.m.
5. Large vacuole has appeared in centre of zygote.	2.25 p.m.
6. Guttation water has appeared on zygote.	3.15 p.m.
7. Disappearance of all external traces of junction of gametangia.	4.13 p.m.
8. Exosporium formed, about to break through external layer of zygote.	5.20 p.m.
9. Zygospore quite opaque, larger than suspensors.	7.45 p.m.
10. Zygospore still growing in breadth, i.e. between suspensors.	9.19 p.m.
11. Zygospore still increasing in breadth, though not in hight.	9.35 p.m.

When the progametangia are formed, the number of nuclei found in them increases rapidly, both by multiplication and migration, though chiefly by the latter. Soon after their formation, a thin deeply staining layer is seen on the adjoining walls of the progametangia. It appears that there is no special aggregation of nuclei in these layers. These layers may be found in both progametangia, or at first in one only, though not necessarily in the one on the lateral zygophore. Some

fifteen /

fifteen to twenty minutes later however, there is a definitely deeply staining mass of protoplasm on each side of the common wall. By this time the two walls have become fused together to form a single one, and the two progametangia can no longer be pulled apart without damaging them. Half an hour later, that is when approximately 2½ hours old, the progametangia have elongated. The darkly staining masses of protoplasm are still present, and have grown in amount, but are still crescent-shaped. Behind them however is a zone, which is darker staining than the normal protoplasm, but not as dark as the first masses. These lighter staining zones contain a greater number of nuclei, and the mucorine crystals have already made their appearance. These crystals stained deeply with the Gentian Violet. At a slightly later stage, the lighter staining zones have assumed the same dark colour as the original dark small ones, sothat the two are indistinguishable, and the nuclei are becoming evenly distributed throughout. There is no suggestion that they are lying close to the dividing wall, and there is also no suggestion of the bulging of either progametangium into the other, either at this stage or at a later one. When the two zones have become indistinguishable, a wall is laid down separating the gametangia from the suspensor. /

suspensor. The gametangia are formed approximately three hours after contact of the zygophores, and they contain a large number of deeply staining nuclei, which are more clearly defined than those of the mycelium and suspensors. They consist of a deeply staining spherical central mass of chromatin, surrounded by a clear non-staining area. The nuclei are all of then same size, and stain equally densely.

Soon after the gametangia have been formed, the common wall between them breaks down, starting in the central region, and working outwards. There is no sign of the protoplasm of one gametangium being extruded into the other, as has been observed by Keen (1914) in Sporodinia, and which suggest that one gametangium is male, and the other female. When the wall between them has broken down, the pressure in the suspensors causes the suspensor walls to bulge into the zygote, squeezing its contents into smaller bulk. This is made possible through the great number of loosely reticulated vacuoles contained in the zygote, which had already been present in the gametangia. An association of nuclei in pairs can now be observed, but it is doubtful whether any of them actually fuse at this stage. A number however appear to be somewhat larger than their fellows. Some do not take the stain so readily, the central mass of chromatin /

chromatin being ill defined, and the clear non-staining area surrounding it is less evident. These are presumably nuclei in the process of degeneration.

The maturing zygote soon grows sufficiently to press back the suspensors. When this has been achieved, all traces of the common wall have disappeared inside the zygote, except that there is sometimes still a mass of protoplasm in the midline to indicate where the wall previously existed. Externally the line of junction is still visible as a furrow that rings the zygote, but that too soon disappears, heralding the appearance of the exosporium under the external wall of the zygote.

Some four hours have passed since the formation of the progametangia, and the contents of the zygospore have become remarkably uniform in texture. The nuclei are still as clearly defined as before, with some quite definitely larger than their fellows. These number upwards of twenty, and appear to be increasing in number, though no actual fusions have been observed. Two nuclei may come to be associated, the two deeply staining chromatin masses lying side by side within one clear oval area. They tend rather to lengthen than to retain their spherical shape. This association of nuclei can be first observed when the gametangia are fusing, but it does not appear to be necessary for the two /

two nuclei to originate from the different gametangia, as they have been observed associated at points as far removed as is possible from the line of junction, whilst the common wall was only partially dissolved.

The zygospore continues its rapid development, and when it has reached a stage seven hours from progametangium formation, the suspensors stain a purple shade, in contrast to the zygospore which still stains a rose pink. This is a sign that the suspensors have reached and are past their maximum development, and the nuclei are all in the course of disorganisation. It is also the stage when the mucorine crystals have reached their highest development, and anything up to ten or more can be counted in a single zygospore, as well as several in each suspensor, generally close to the wall joining it to the zygospore.

When the zygospore is examined at later stages, as for example when four days old, the contents are still homogeneous and contained in a thick, but transparent membrane which fits closely to the exosporium, even penetrating up the spines. On bursting this envelope and staining, the first point to notice is that the contents stain a deep purple, showing that the zygospore is in a resting condition. The contents are uniform, there being no differentiation into zones, and no oil globules /

globules are visible. The nuclei seem to be fewer in number, but that would appear to be due to the fact that they are lying in pairs. They are large, but not so clearly defined as before, and though in pairs, there is no suggestion of nuclear fusion. There is no sign of the degenerate nuclei. With increasing age there appears to be no further change in the nuclei, and even at four months old, the condition is the same as that described above.

It has so far unfortunately not been possible to bring the zygospores to germination, in spite of repeated attempts. Some 25.000 four month old zygospores were sown on moist sterile circular earthenware plates, and though kept in a saturated atmosphere, and in a diffuse light for fourteen days, there was no sign of germination. Zygospores up to the age of nineteen months have been sown on malt agar, after having been allowed to dry up and been kept at room temperature, but they also refused to germinate.

The development of the sporangia is the same as that for any other species, so that a detailed description is superfluous, as it is so well known. When the mass of protoplasm in the sporangium is being divided into little islands, the future spores, from one to four nuclei can be counted in each. It appears that /

that at this stage the nuclei are actively undergoing division, so that it is possible that four is not the original number, and that originally only one or two nuclei were contained in each spore mass.

Owing to the very small size of the nuclei (they are 0.42 - 0.57 μ in diameter in the actively growing regions) it is extremely difficult to follow the ordinary mitotic division. Moreau (1911) described an amitosis in Rhizopus nigricans, which appeared to be the usual method of division in the older hyphae, particularly in the columella. This does not appear to take place in Rh. sexualis however, as divisions can be observed in old vacuolated hyphae and in the columella after the spores have been formed.

The divisions are intranuclear, and a clear non-staining area surrounds the dividing chromatin mass, as has been observed in the Mycetozoa as well (Wilson & Cadman, 1928) (Cadman, 1931). The first sign of approaching division is that the chromatic mass stains more deeply. The prophase is indicated by an increase in size of this mass, which assumes an irregular oval shape, and stains unevenly. It has unfortunately not been possible to follow the development of the spindle in detail during the prophase, A lighter staining cone-shaped mass has sometimes been observed on one side /

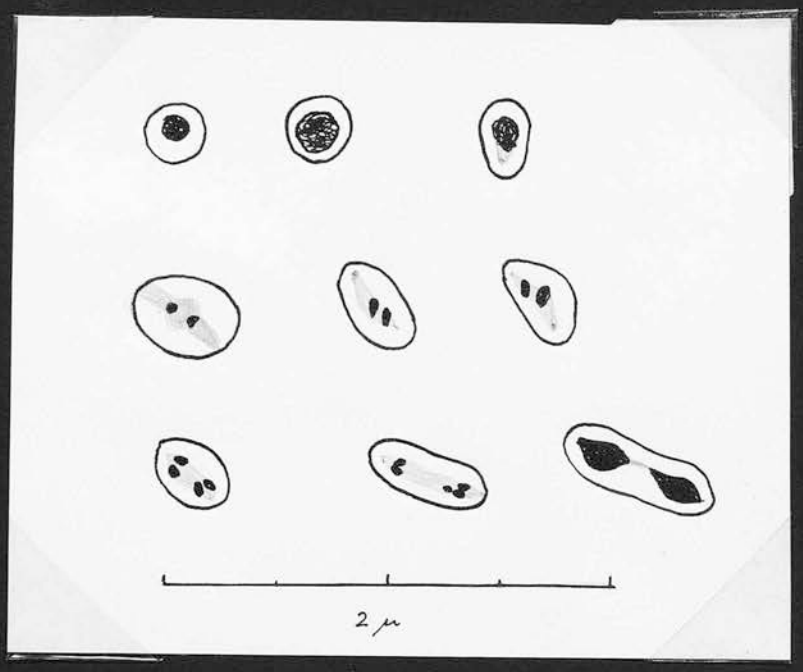


Fig. 15.

1. Resting nucleus.
2.-3. Prophase.
4.-6. Metaphase.
7.-8. Anaphase.
9. Telophase.

side of the chromatin during the prophase. This might be regarded as a portion of the spindle, but no definite statement can be made owing to the very small size of the nuclei. With the development of the metaphase, the spindle assumes a more or less central position over the mass of chromatin. Centrosomes appear to be absent, though a small deeply staining body can sometimes be observed at one of the apices of the spindle. It is pointed at both ends, and possesses a uniform structure, in which no trace of fibrils can be discerned. The mass of chromatin now divides into two oval masses, each of which can sometimes be seen to be divided into two portions of chromatin. With the beginning of the anaphase, these two masses move to the poles, and in the telophase they are still seen to be connected by the now thread-like spindle. The nuclear membrane elongates as they move apart, and begins to constrict in the mid-line, and ultimately the two daughter nuclei separate completely. A curious structure may often be seen, in what would be the late anaphase. There are two chromatin masses joined together by the spindle, the whole thing being crescent-shaped, with the chromatin masses at the apices of the horns. It has further several times been observed that the whole chromatin mass appears to move to the one pole, the meaning of which is not understood.

Hybrid experiments.

Quite a number of investigators have succeeded in crossing the (+)ve and (-)ve races of different species, and even of different genera. Of particular interest however are the studies of Blakeslee (1915), which showed that the contrast between homothallic and heterothallic species can give imperfect sexual reactions. Different homothallic species were found to respond in different ways when contrasted with heterothallic species. Some of them reacted with both (+)ve and (-)ve races (Mucor genevensis), some (Sporodinia grandis) did not show any reaction at all, whilst others showed either a (-)ve tendency (Absidia spinosa, Zygorhyncus Moelleri, Z. Vuillemini) and reacted predominantly with the (+)ve races, or had a (+)ve tendency (Z. heterogamus) and showed only reactions with (-)ve races.

Burgeff (1924) was the next to investigate the hetero-homothallic crosses on a large scale, and to use the imperfect sexual reaction as an aid in determining the sexuality of the zygophores. He used three different homothallic species and the (+)ve and (-)ve races of two heterothallic species, but in each case only one heterothallic with one homothallic. From his experiments, he drew the conclusion, that the predominant (-)ve tendency of the homothallic species used by Blakeslee /

Blakeslee, seemed to rest on the fact that he used a particularly active (+)ve race of Mucor hiemalis, known as Mucor V , as a tester, rather than on a specific (-)ve tendency in the homothallic species themselves.

Nielsen (1927) repeated a number of Burgeff's crosses, with a view to studying the sexuality of the zygophores.

Satina and Blakeslee (1929, 1930) carried out an extensive programme of crosses, in which they crossed homothallic species with heterothallic and with homothallic species. They reached the same conclusion as Blakeslee had reached before, that heterogamous homothallic species may have (1) a (-)ve sexual tendency (5 species), or (2) a (+)ve sexual tendency (1 species).

Of great interest are the crosses between homothallic species. Burgeff (1924) crossed Zygorhyncus exponens (according to Blakeslee of (+)ve tendency) and Absidia spinosa (Blakeslee: (±)ve tendency) and obtained a number of imperfect sexual reactions in some cases, but did not pursue this line of investigation further.

Satina and Blakeslee (1930) carried out a number of crosses between various homothallic Mucorineae, chiefly Zygorhyncus spp., and found that an imperfect sexual reaction took place between them, when one was of a /

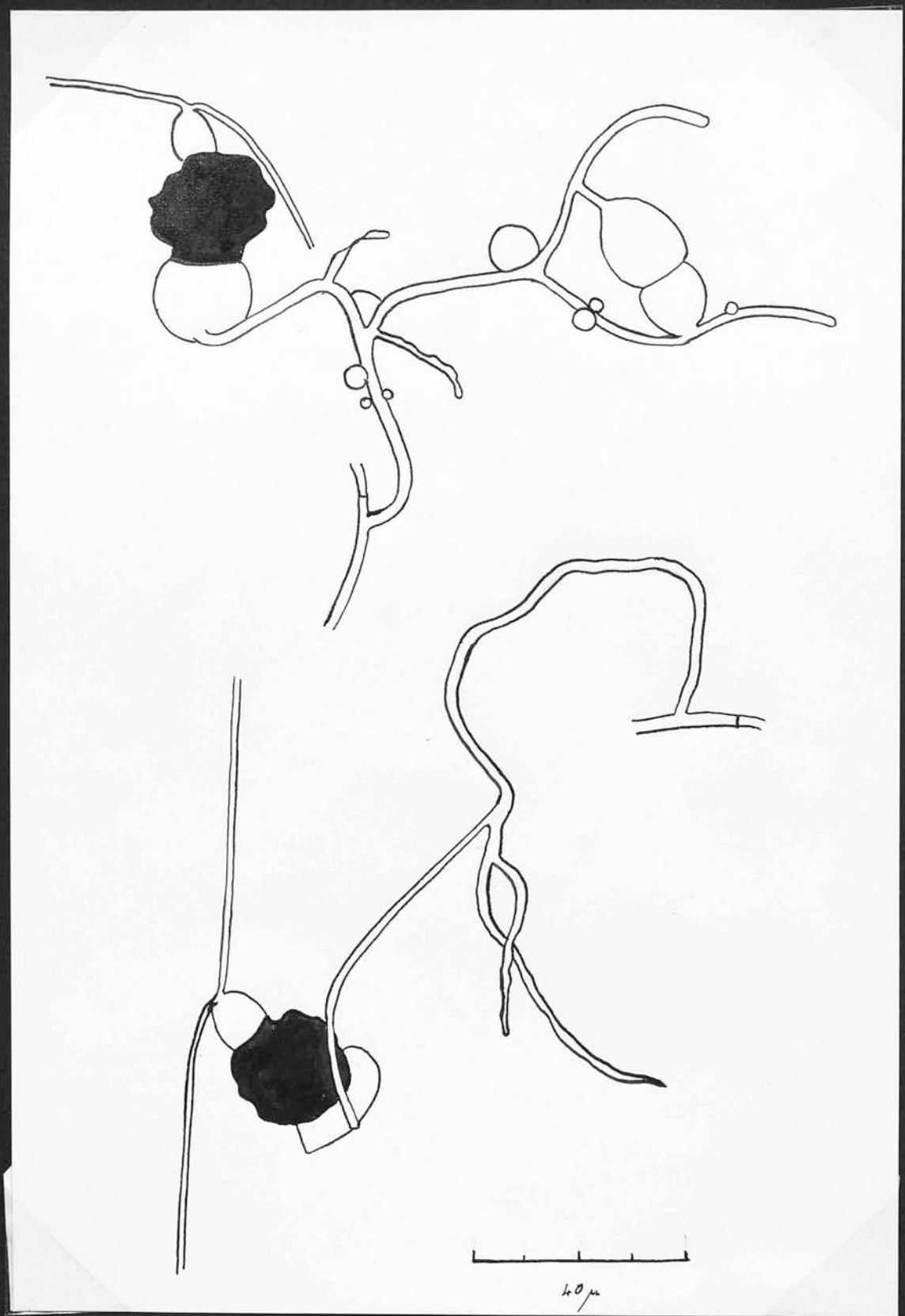


Fig. 16.

Rh. sexualis x Rh. nigricans. (−) ve.

of a (+)ve tendency and the other of a (-)ve tendency, as they had expected.

The hybridisation of species is of sufficient importance, not only to an understanding of the sexuality in the Mucorineae, but also for the theories of sex in general, to warrant a more intensive investigation. For these reasons a study of the imperfect sexual reactions of Rhizopus sexualis was undertaken, using both heterothallic and homothallic species.

In the first instance, Rh. sexualis was contrasted with other species of the same genus. Perfect hybrid zygospores were obtained with Rh. nigricans both (+)ve and (-)ve, though a much larger number was obtained when contrasted with the (+)ve race than with the (-)ve race. There is not so much variation in size amongst these hybrid zygospores, as there is in the homothallic species itself, and as a general rule they are smaller than the homothallic zygospores, and of the same size as the heterothallic ones, or a trifle smaller. There is a definite difference in exosporium pattern, that of the cross Rh. sexualis X Rh. nigricans (+)ve being smoother and resembling the heterothallic zygospore, whilst the cross Rh. sexualis X Rh. nigricans (-)ve resembles the homothallic zygospore.

The zygospores are not found at the point where the /

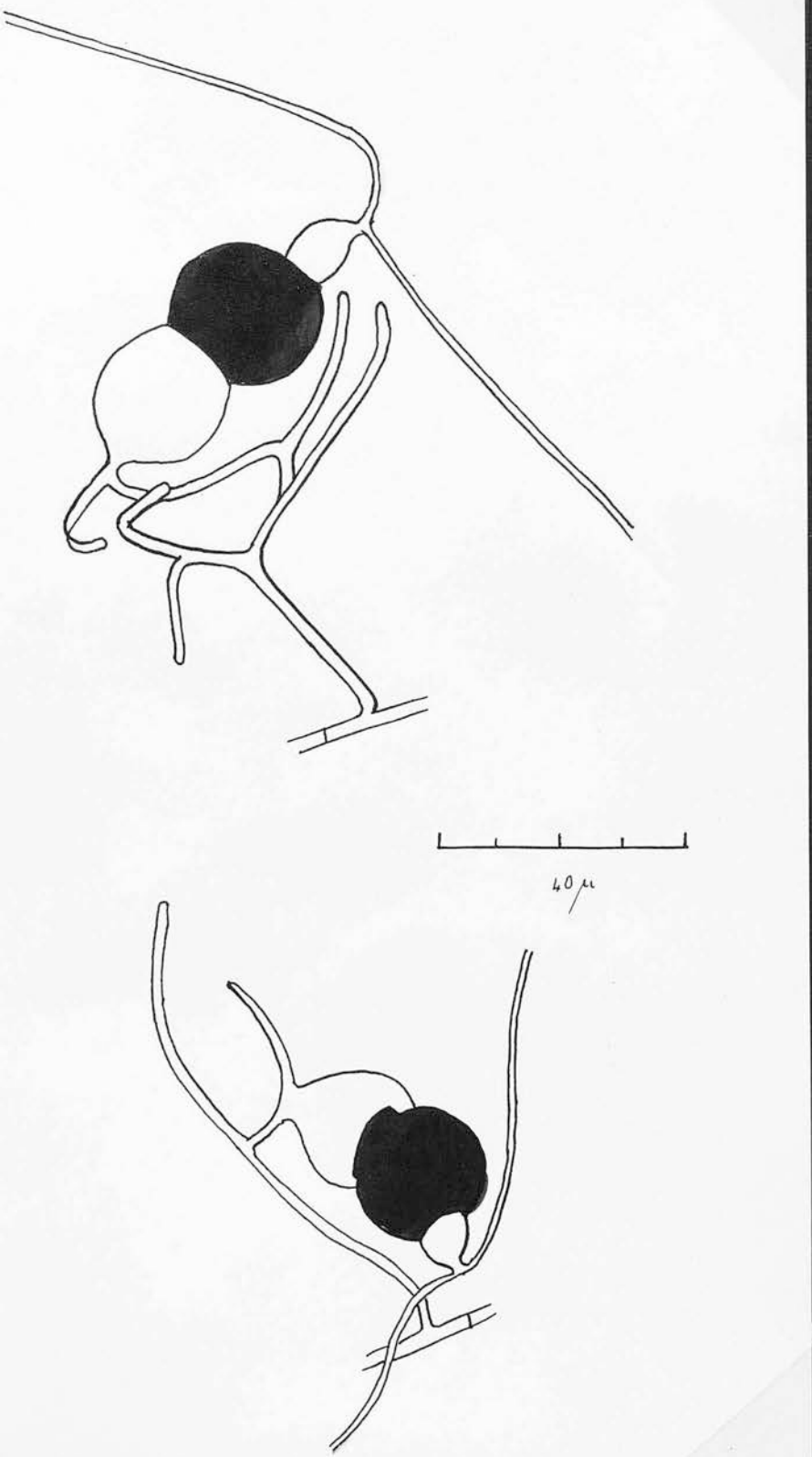


FIG. 17.

Rh. sexualis × Rh. nigricans (+) ve.

the two mycelia first meet, but a little way into the homothallic mycelium. The hybrid zygospores have therefor to be distinguished from the normal homothallic ones. This is a fairly simple matter, as the homothallic species has large hemispherical suspensors of equal size that vary little, whereas the two races of Rh.nigricans used had thin conical suspensors. In the very youngest stages of development of the zygospore, it is impossible to identify the hybrids by means of their suspensors, as they are more or less equal at first, but by observing their point of origin, i.e. from a branched zygophore or a main hypha, it is possible to distinguish them clearly from the homothallic ones.

In the hybrid, the one suspensor is of the large homothallic type, whilst the other is not of a thin conical shape, as is to be expected, but small and semi-ellipsoidal. This is surprising, as the two heterothallic races used have never shown anything other than the long conical shape. On the whole, the suspensors of the (+)ve race were larger in the hybrid than the (-)ve, as was also the case when the (+)ve and (-)ve were plated alone together. Another feature that is often emphasised in the hybrid, is the angle at which the suspensors are placed in relation to each other. In the homothallic race, they are at an angle of 180° i.e.

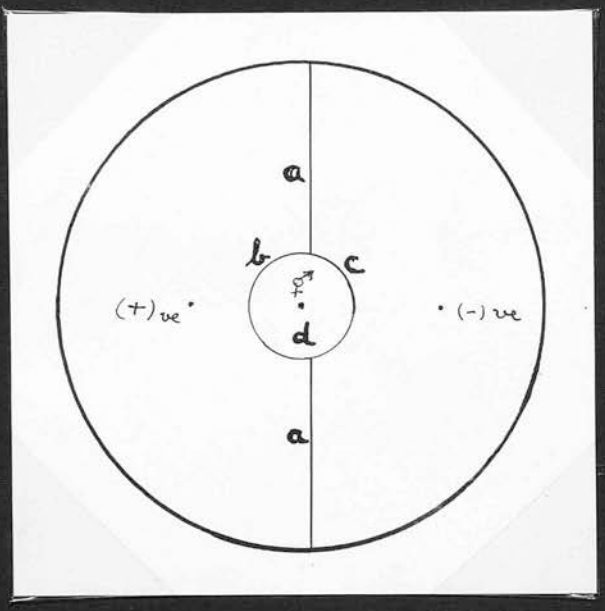


FIG. 18.

Types of zygospores.

a. Rh. nigricans (+) X (-) ve

b. Rh. nigricans (+) ve X Rh. sexualis.

c. Rh. nigricans (-) ve X Rh. sexualis.

d. Rh. sexualis.

i.e. opposite, but in the heterothallic races they are generally at an obtuse angle. This is often emphasised in the hybrid, to such an extent sometimes, that they are at right angles to each other. In this case, if the smaller suspensor happens to be hidden by the zygospore, it appears as if one is dealing with an azygospore.

It has several times been shown by Blakeslee (1906) that a homothallic species plated between two races of a heterothallic, reacts with both these races. As was to be expected therefore, when Rh. sexualis was plated between the (+)ve and (-)ve races of Rh. nigricans in the same Petri dish, zygospores were formed with both races, and as the heterothallic races grew much faster than the homothallic one, four types of zygospores were formed, as shown in the accompagning diagram.

Azygospores and thigmospores were produced in large numbers by the homothallic species in this cross with Rh. nigricans. Actually, thigmospores were observed six times, but this is more frequent than in any other cross, or than can be observed in the homothallic species itself.

There are certain characters which have been observed to differ in the crosses. The first of these is the production of trapping hyphae, and of fine hyphae by the /

by the suspensors and zygophores. Rh. sexualis produces these normally in older cultures, with a sturated atmosphere. In the cross with Rh. nigricans (-)ve, trapping hyphae were absent, but fine hyphae were produced on the suspensors and zygophores of a four day culture, in the areas nearest to the heterothallic race. It was noticeable too that the zygophore branching was greatly stimulated, and zygospore production increased. Curiously enough, pseudophore production appeared to be normal.

The next species contrasted with Rh. sexualis was Rh. Oryzae, which when received, bore no indication of its sexuality. It was contrasted with Rh. nigricans both (+)ve and (-)ve races, but proved to be neutral. When contrasted with Rh. sexualis, imperfect sexual reaction took place, but this reaction did not proceed beyond progametangium formation in its earliest stages. There was a very greatly stimulated azygospore formation, and thigmospores were also present, six having been counted. Pseudophore production was also accentuated, but most important of all, there was a secretion of protoplasm not always accompagnied by a drop of water. There was an extensive production of trapping hyphae, and fine hyphae from the suspensors, zygophores and stolons.

The /

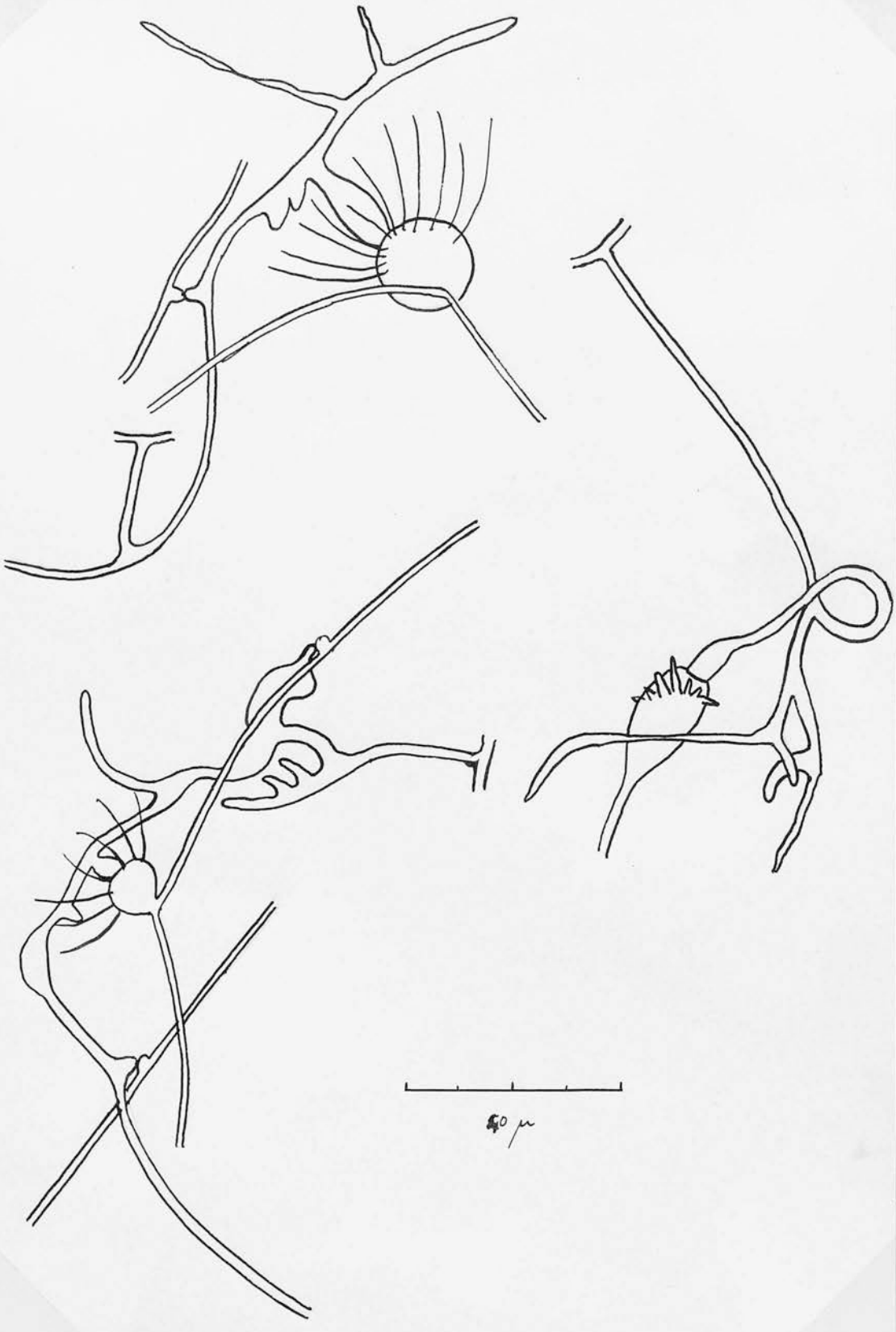


Fig 19.

The other species of the genus Rhizopus contrasted with Rh. sexualis were Rh. japonicus and Rh. tonkinensis, which Zycha (1935) classed as varieties of Rh. Oryzae. In neither case was an imperfect sexual reaction observed. In four day culture with Rh. japonicus, the homothallic produced no azygospores, very few pseudospores, and no trapping hyphae at all. With Rh. tonkinensis, the homothallic showed a certain stimulation of zygophore branching. A few azygospores and pseudophores were produced, and a moderate number of fine hyphae were produced by the suspensors, zygophores, and to a lesser extent by the stolons, and a few trapping hyphae were also developed.

Rhizopus sexualis was then contrasted with a number of members of the genus Absidia, first of all with A. glauca. With the (-)ve race there was a definite and marked imperfect sexual reaction, often reaching gametangium formation. These gametangia seemed to be unable to fuse, and give perfect hybrid zygospores. In the (+)ve there was only a slight reaction, the imperfect sexual reaction never passing the early progametangium stage. In response to both races, very few azygospores and pseudophores were formed. When contrasted with the (+)ve there was a copious production of fine hyphae by the suspensors and stolons in a four day /

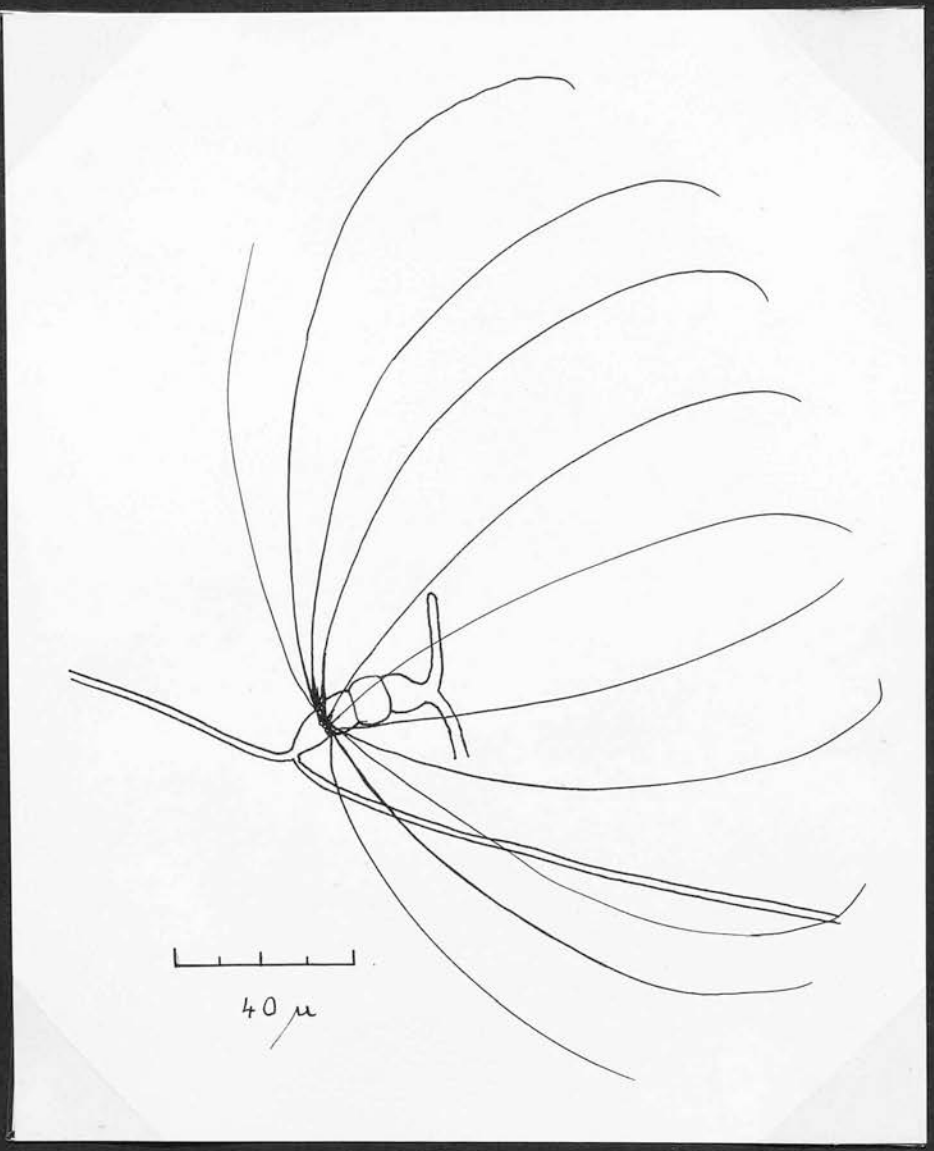


FIG. 20.

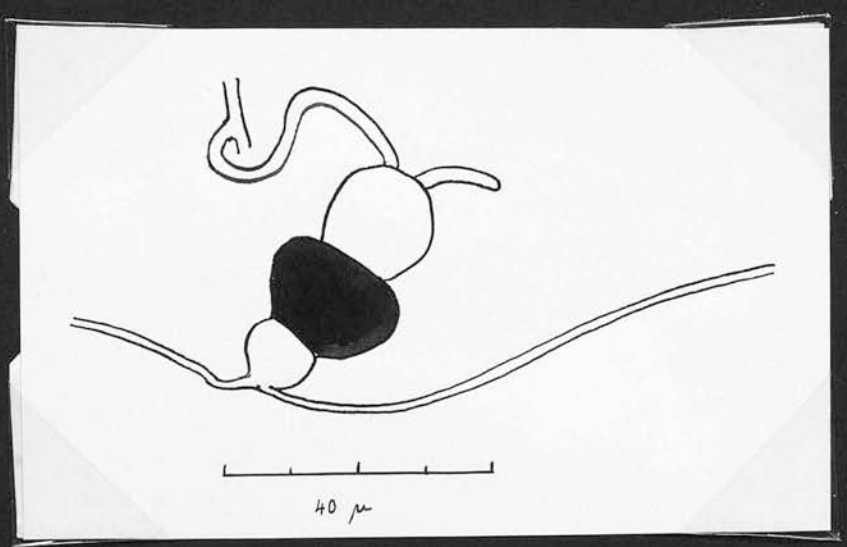


FIG. 21

day culture, and an equally copious production of trapping hyphae. With the (-)ve the fine hyphae were only starting to be produced in an eight day culture. The (-)ve race stimulated zygophore branching in the homothallic, which the (+)ve did not.

Absidia cylindrospora was the next to be contrasted with Rh. sexualis, and with both races there was an imperfect sexual reaction, very marked with the (-)ve race, but less so with the (+)ve. Azygospore formation and pseudophore production was more marked with the (-)ve race, and there was a greater stimulation to zygophore branching given by the (-)ve than by the (+)ve one. In a four day culture, the fine hyphae were absent, and there was only a slight production of trapping hyphae with the (-)ve race, and none at all to be seen with the (+)ve race.

The next of the Absidia species to be contrasted with the homothallic was A. caerulea, which when received, bore no indication of sexuality. It was therefore plated against (+)ve and (-)ve races of Absidia glauca, but appeared to give no reaction. When contrasted with Rhizopus sexualis, Absidia caerulea gave a slight imperfect sexual reaction. The number of azygospores produced was small, and even fewer pseudophores were formed. In a four day culture there were no trapping

or /

or fine hyphae to be seen. On the eighth day the latter were produced by the suspensors. In this eight day culture there was a good deal of zygophore branching. It is of interest to note that Satina and Blakeslee (1930) recorded that they had failed to obtain a reaction between Mucor genevesis (Blakeslee: (+)ve and (-)ve tendencies) and Absidia caerulea (-)ve, though there was an imperfect sexual reaction with the (+)ve race.

Next in this series was Absidia capillata, which also bore no indication of its sexuality. It too was contrated with Absidia glauca (+)ve and (-)ve, and proved to be neutral. There was no imperfect sexual reaction, and in a four day culture azygospores and pseudophores were conspicuous by their absence. Trapping and fine hyphae were also absent, though they were developed in eight day culture. Zygophore branching was much stimulated however in the eight day culture, though evident in the four day. A most surprising fact was that the production of auxiliary sporangia was stimulated, and apparently not at the expense of the zygospores.

Last of its genus to be used was Absidia Regnieri, which also bore no indication of its sexuality when received. It too was contrasted with the (+)ve and (-)ve races of A. glauca, but gave no reaction. This strain /

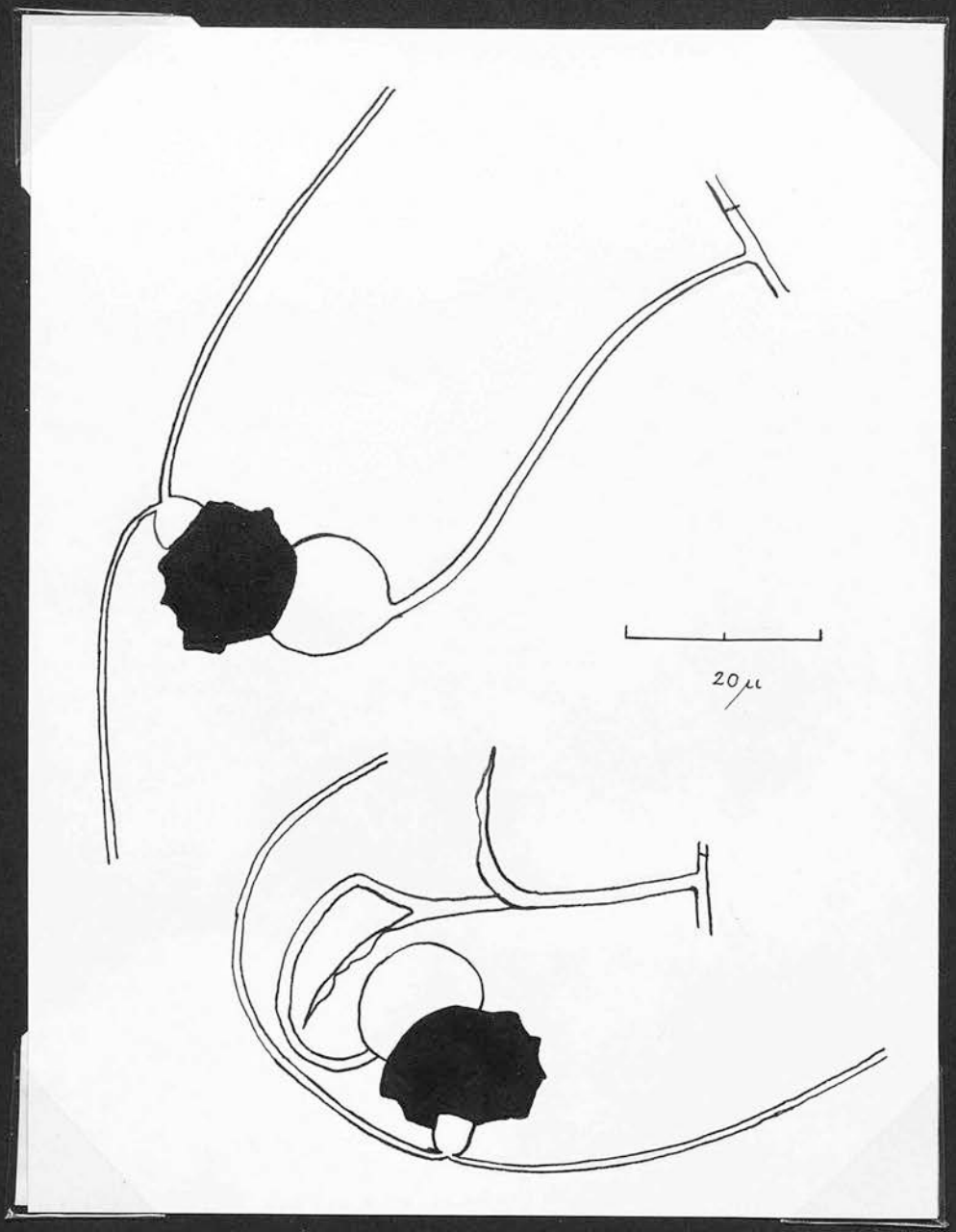


Fig. 22

strain was extremely slow growing, and as might be surmised, showed no effect on Rh. sexualis in a four day culture. In an eight day culture it could be seen that the zygophore branching had been stimulated, but there was no sign of trapping hyphae or fine hyphae. Azygospore production was very slight, but there was moderate pseudophore formation.

Several Mucor species were next crossed with Rh. sexualis, the first being M. hiemalis. With the (-)ve race perfect hybrid zygospores were formed, though there was only an imperfect sexual reaction with the (+)ve race. The hybrid zygospores were smaller than either of the parent species. The exosporium resembled that of the homothallic parent, but was more irregular. Both races of M. hiemalis caused stimulation of zygophore formation in the homothallic, but more particularly the (-)ve. As a result of the extensively branched zygophores, there was an abnormal number of small zygospores formed, and many progametangia which never developed fully. Azygospore and pseudophore formation remained almost entirely in abeyance. Trapping hyphae were being produced in a four day culture, and the suspensors and zygophores of the homothallic were producing fine hyphae in the same culture. This applies to both the (+)ve and (-)ve races of M. hiemalis.

With /

With Mucor mucedo there was no imperfect sexual reaction, either with the (+)ve or (-)ve races. There was a moderate stimulation to zygophore branching, but the zygophores shrivelled up much sooner than is normally the case. There was a slight azygospore and pseudophore production. Trapping hyphae were just being formed in a four day culture, and the suspensors and zygophores were giving fine hyphae. The amount of stimulation caused by the (+)ve and (-)ve races was approximately equal, though sometimes one and sometimes the other caused a greater stimulation of one of the features, the reason for which is not clear.

Mucor Ramanianus was the last of its genus to be used in these series of experiments. Here there appeared to be no reaction at all, except that the zygophores were slightly more stimulated than normally. Trapping hyphae remained in abeyance, even in a nineteen day culture, and fine hyphae were also not produced. As the M. Ramanianus colony is extremely slow growing, and closely adpressed to the medium, Rh. sexualis soon grows over it and produces bunches of sporangia over it. Bunches of three sporangia of the Rhizopus originating from a single stolon could often be observed against the pink background of the Mucor colony.

Two species of Circinella, C. minor and C. spinosa were /

were contrasted with Rhizopus sexualis. No imperfect sexual reactions took place however. With C. minor in an eight day culture, fine hyphae were just being produced, and there appeared to be a certain amount of extra zygophore branching. Azygospores and pseudophores were scanty, and trapping hyphae absent. With C. spinosa the zygophoric branching is much stimulated in an eight day culture, but the azygospore and pseudophore production is meagre. No trapping or fine hyphae were produced.

The last of the heterothallic species to be contrasted with Rh. sexualis in this series was Phycomyces nitens. There was no response apart from a slight stimulation of zygophore branching and azygospore and pseudophore production.

The only two homothallic species contrasted with Rhizopus sexualis were Zygorhyncus Moelleri and Sporodinia grandis. In neither case was a sexual reaction of any description encountered. With Z. Moelleri the zygophore branching appeared to be stimulated, and a few azygospores and pseudophores were produced, otherwise Rhizopus sexualis remained quite passive. With Sporodinia grandis it responded even less, and apart from a moderate production of pseudophores, no other response could be observed.

From /

Table I.

Heterothallic and neutral species.	Rhizopus sexualis	
	perfect hybrid zygospores	imperfect sexual react.
Rhizopus nigricans (+)ve	XXX	0
" " (-)ve	XX	0
Rh. Oryzae	0	X
Rh. japonicus	0	0
Rh. tonkinensis	0	0
Absidia glauca (+)ve	0	X
" " (-)ve	0	XXX
A. cylindrospora (+)ve	0	X
" " (-)ve	X	X
A. caerulea	0	X
A. capillata	0	0
A. Regnieri	0	0
Mucor mucedo (+)ve	0	0
" " (-)ve	0	0
Mucor hiemalis (+)ve	0	X
" " (-)ve	X	X
M. Ramanianus	0	0
Circinella minor	0	0
C. spinosa	0	0
Phycomyces nitens (+)ve	0	0
" " (-)ve	0	0
Homothallic species		
Zygorhyncus Moelleri	0	0
Sporodinia grandis	0	0

X slight response.
XX moderate response.
XXX good response.
0 no reaction.

From this series of contrasts, one point emerges. Though there is a certain order of response called forth in Rhizopus sexualis by the other fungus, this order is not always retained. In the usual order, stimulation of zygophore branching is the first response, then azygospore and pseudophore production, followed by the production of fine hyphae by the suspensors, then by the zygophores, and finally the production of trapping hyphae, and of fine hyphae on the suspensors.

The sexual reactions of all the above fungi with Rhizopus sexualis are summarised in Table I. The results obtained with the limited number of fungi employed, are very encouraging. In those species in which both the (+)ve and the (-)ve races were employed, the (-)ve race gave a more vigorous response than the (+)ve, with the exception of Rhizopus nigricans where this was reversed, and Phycomyces nitens where there was no response at all. The exceptional behaviour of Rh. nigricans may be accounted for by the assumption that we are dealing with an unusually active (+)ve race. This rather suggests that in Rhizopus sexualis we are dealing with a homothallic that is predominantly (+)ve. This is of great interest, as only one other such species is known, namely Zygorhyncus heterogamus, which reacts in the same way. An extensive series of hybridisation experiments would have /

have to be carried out however to prove this conclusively.

Sexuality.

When first received, Rhizopus sexualis was producing copious zygospores, and was believed to be definitely homothallic. Monospore isolations soon confirmed this. Several investigators have attempted to determine the sexuality of the zygophores in the homothallic species of the Mucorineae.

Blakeslee (1904) came to the conclusion that what had been designated (+)ve in one species, was not necessarily the same sexually, as what had been designated (+)ve in another species. He therefore embarked on a series of hybridisation experiments, and was able to determine the sex of a given race in relation to the others, and which could otherwise not be determined. He applied this (1913) to homothallic species, where he had found that the lateral zygophore gave rise to the larger gametangium, and had therefore been designated female.

Burgeff (1924) observed that the terminal and lateral zygophores often did not come to copulation. They continued their growth instead, and ultimately the lateral gave rise to a lateral of its own. The question which he tried to solve was, how does the second lateral stand in relation to the others, sexually. He stated that a sexual substance is manufactured in the tips of the growing hyphae, whether the other partner is present or /

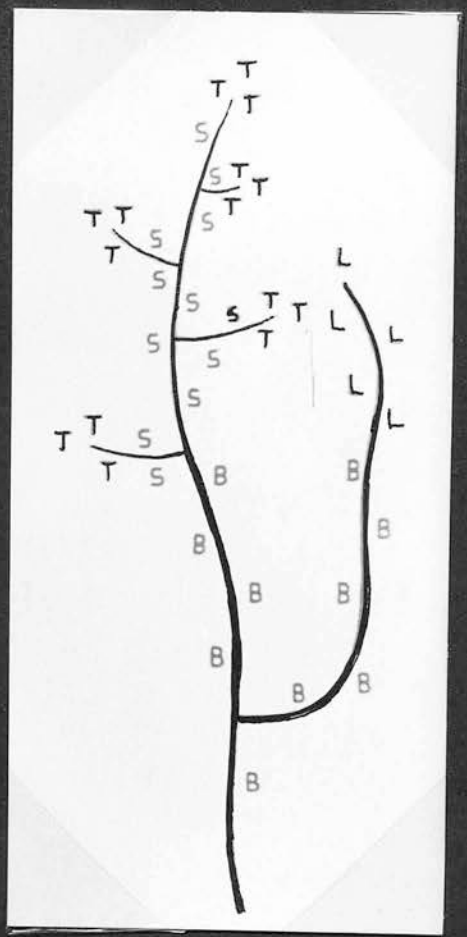


Fig. 23.

(after Burgeff)

T = terminal
S = subterminal } react (−) vely.

L = lateral } reacts (+) vely.

B = basal } non-reacting.

or not, and which can be transmitted through the substrate or the air.

Bearing this in mind, Burgeff turned to the zygophores of the homothallic species Absidia spinosa. He found that the second lateral zygophore could copulate either with the first lateral, or with the terminal. He therefore postulated that the terminal zygophore reacts (-)vely, and that the first lateral reacts (+)vely, but if they do not come to copulation, the first lateral changes to the (-)ve (i.e. becomes terminal) and produces a lateral ("second lateral") in its turn, which is then (+)ve. He postulated that the lateral branches can change only once, from the (+)ve into the (-)ve, if they do not come into contact with another zygophore, and that they remain (-)ve.

From a study of a second homothallic species, Zygorhyncus exponens, and its sexual reactions with heterothallic species, he was able to draw up a scheme of the sexual zones of a zygophore.

The essential point, according to Burgeff (verbally) is that the two reacting hyphae must be of different ages, which means that they will therefore be of different sexes.

Nielsen (1927) carried out observations on Absidia spinosa, and considered the fact that a single zygophore /

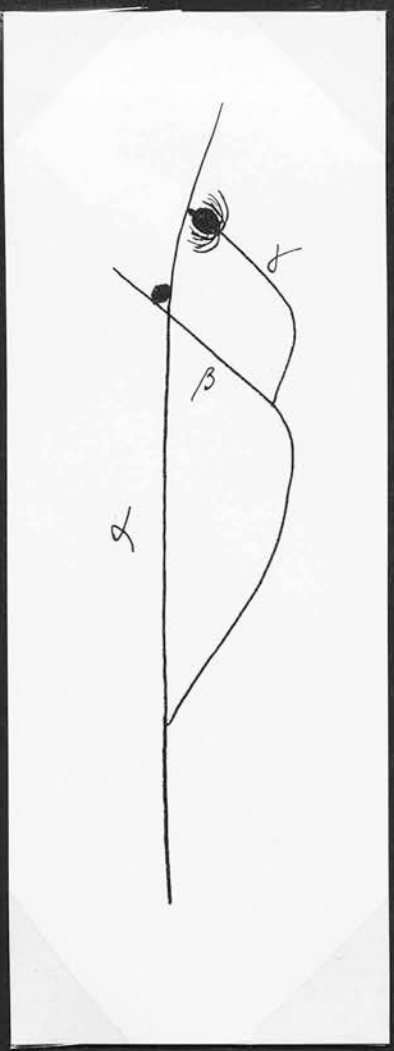


Fig. 24.

(after Nielsen.)

zygophore can form more than one zygospore is not consistent with Burgeff's theory. He found that the second lateral hypha (γ, (+)ve according to Burgeff), which originated from the first lateral hypha (β, (-)ve according to Burgeff), having first formed a zygospore with the terminal one (α, (-)ve according to Burgeff), the first lateral hypha had then formed an abortive zygospore with the terminal one. This is inconsistant with the theory of changing sex in the lateral hyphae. Nielsen is of the opinion that the most natural explanation is that the hyphae contain both sexes, perhaps the one sex at first, but the two at a later stage. He is unable to say whether the terminal hypha turns bisexual, as he had been unable to find cases where there had been a sexual reaction between two terminals.

Satina and Blakeslee (1930) summarised their work to date, and recorded that in the homothallic Zygorhyncus heterogamus the lateral zygophore with the larger gametangium is really the (-)ve, and that the (+)ve is very much smaller, a reversal of the usual occurrence. In consequence they come to the conclusion that the terminal and lateral zygophores in those species which they have investigated, were bisexual.

In Rhizopus sexualis the production of zygophores appears at first sight to agree with the Burgeff theory, even /

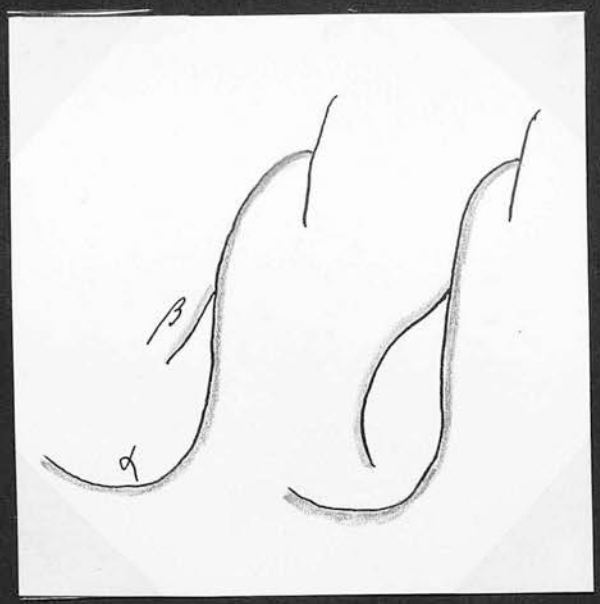


Fig. 25.

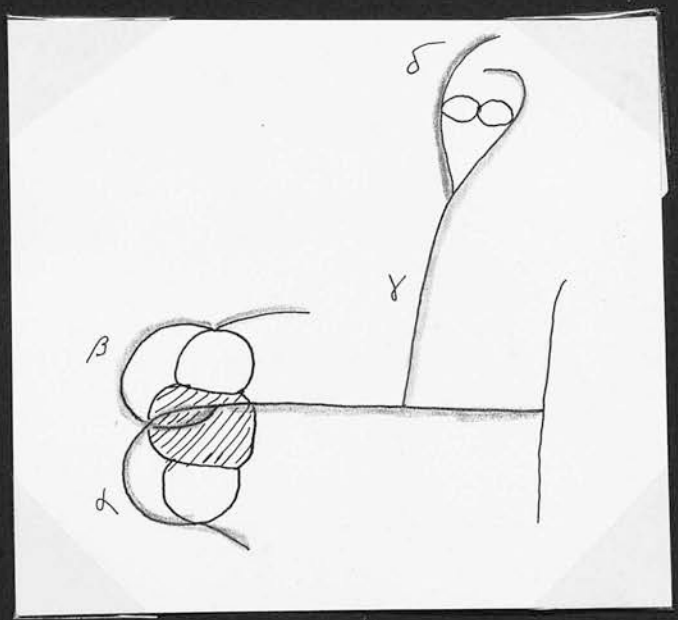


Fig. 26.

even when several zygospores are produced on one branched pair of zygophores as has been observed on many occasions, but on closer examination quite a number of cases crop up which cannot be explained by that theory.

Let us take a simple case first of all. The hypha α (fig.25), which has grown up into the air, has given rise to a lateral β. According to Burgeff, α has a (-)ve sexuality, whilst β has a (+)ve sexuality. β curves round to meet α, and in due course a zygospore would be formed between them. In fig.26, the case is also quite simple. $\alpha + \beta$ have formed a zygospore between them, and α has given rise to a second lateral γ further down, which will therefore be (+)ve. This lateral has in its turn given rise to a lateral δ, which will be (+)ve and γ will have turned (-)ve. There has been a sexual reaction between $\gamma + \delta$, and altogether the case is quite straightforward according to the theory. In fig.27. /

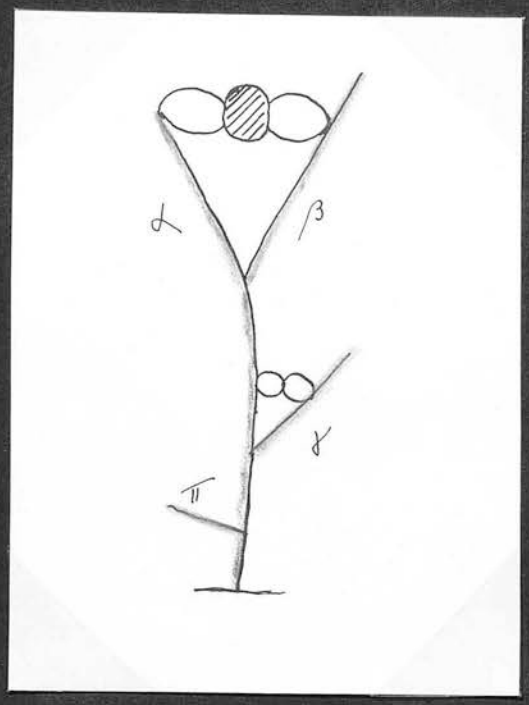


Fig. 27.

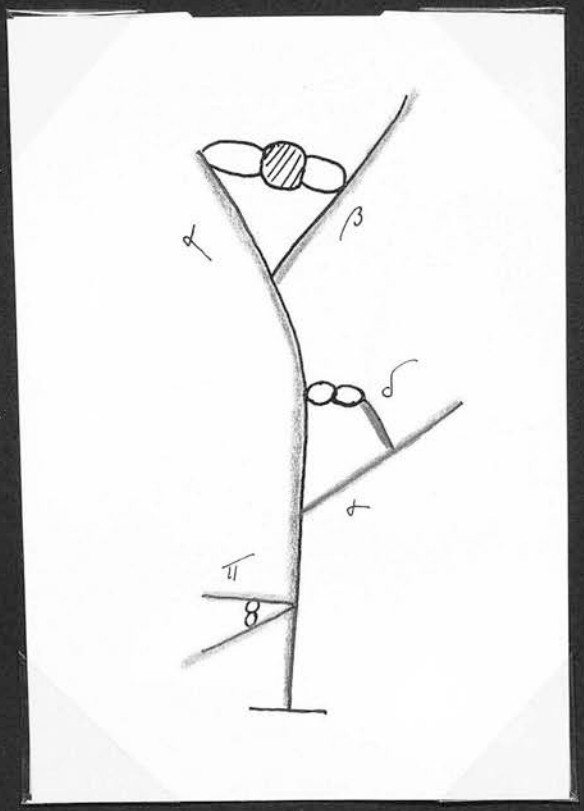
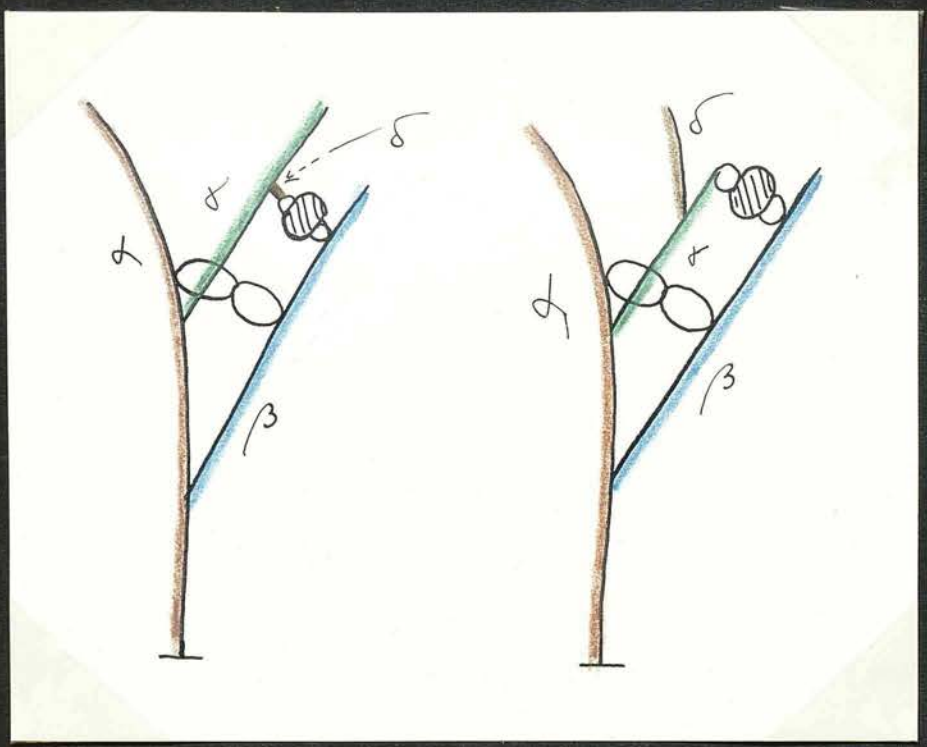


Fig. 28.

In fig. 27 we have a case of one zygophore giving rise to two zygospores, and it may be added that from the nutritional point of view, the mycelium seems well able to do so in this species. The first zygospore has been formed between the terminal α (-ve) and the lateral β (+ve). After its formation, α gave rise to a second lateral γ (+ve) which then formed a zygospore with α. α has given rise to yet a third lateral π, which according to Burgeff will have a (+)ve sexuality. In fig. 28 we have a very similar case to fig. 27. Here the the first zygospore has been formed as usual between α & β, but the lateral γ has developed further than in fig. 27, and has given rise to a lateral δ which means that γ has changed from (+)ve to (-)ve, and δ has assumed the (+)ve sexuality. There has been a sexual reaction between α & δ. α has given rise to a third lateral, π, but in this case π has branched right at its point of origin, though apparently one is (+)ve and the other (-)ve, according to the theory, as there has been a sexual reaction between them.

Several features of interest are presented by the figs. 27 and 28, chief of which is the production of laterals by the aerial hyphae (zygophores) below the forking α/β, each successive one further down towards the base. The formation of zygospores between γ & α in the region of /



a FIG. 29. b

of α designated as non-reacting by Burgeff, suggests that these regions are only non-reacting, as there is little chance normally for them to show they are capable of reacting sexually. This point will be raised later in connection with an imperfect sexual reaction. The lateral π has as yet not been observed to copulate with α, but only to produce its own lateral. There seems to be no reason however why it should not react with α. The production of two branches from the same point, as is the case in fig. 28, is of great interest and will be referred to later.

The case of fig. 29 is less easily explained however. As in the two previous cases, it is assumed that the larger zygospore is the older. With this assumption, it is a relatively easy matter to determine which are the bases of the two original zygophores, but which is α and which is β in the further tangle ? Fig. 29a appears to be the correct interpretation of the hyphae from a careful examination of the branchings. This gives the impossible result according to Burgeff's theory, of the first zygospore being formed between β & δ, two (+)ve hyphae, though the second zygospore between α & β would be normal. There is a possibility that one might interpret the γ & δ hyphae differently, namely, if one assumed that the branches were as in fig. 29b, /

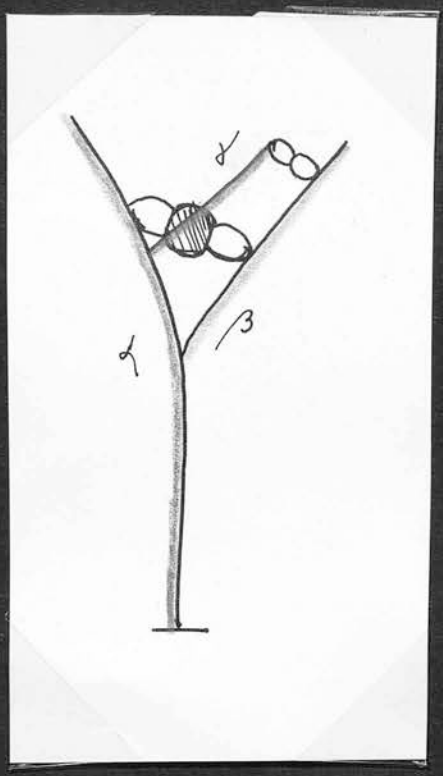


Fig. 30.

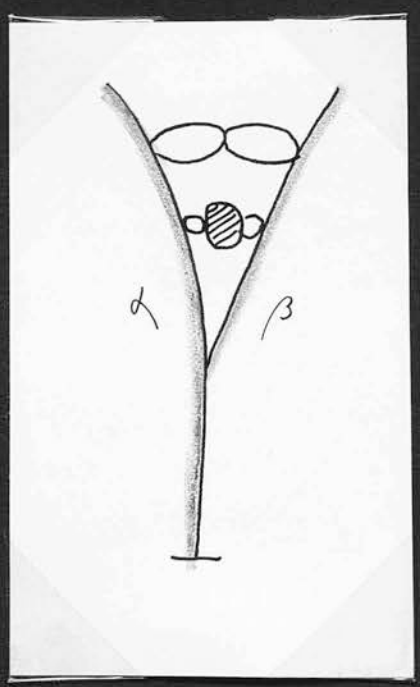


Fig. 31.

fig. 29*b*, and that δ had been formed before the zygospore, then we should have a (-)ve γ copulating with the (+)ve β. It is not very likely however, as there is a great possibility that δ has been formed after the zygospore, and a reexamination of the forking $\gamma\delta$ makes fig. 29*b* seem improbable.

Fig. 30 is a case rather similar to that of fig. 29, and the two figs. 29*b* and 30 bear a close resemblance. In this case however the zygospore formed between α & β is the older, and is quite normal according to the Burgeff theory. But the second one is certainly not normal, as we have a sexual reaction between β & γ, both (+)ve hyphae. The development of this set of zygophores was observed at intervals over a period of ten hours, and there is no doubt whatsoever that it is the β hypha which has produced the two zygospores. It was manifestly impossible to determine with any certainty which was the α hypha and which the β hypha at the end of the ten hours. Even if one did call the α hypha the β, and the β hypha the α, the case could only be explained by the Burgeff theory by assuming that the zygophore bearing the first zygospore had changed its sex to (-)ve. This would make an explanation of the next phenomenon to be mentioned an impossibility.

Of great interest are the cases (fig. 31) where the /

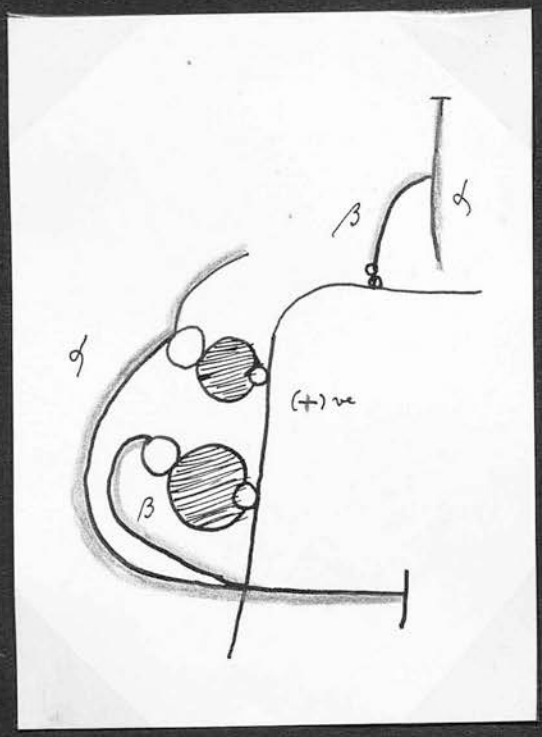


Fig. 33 a

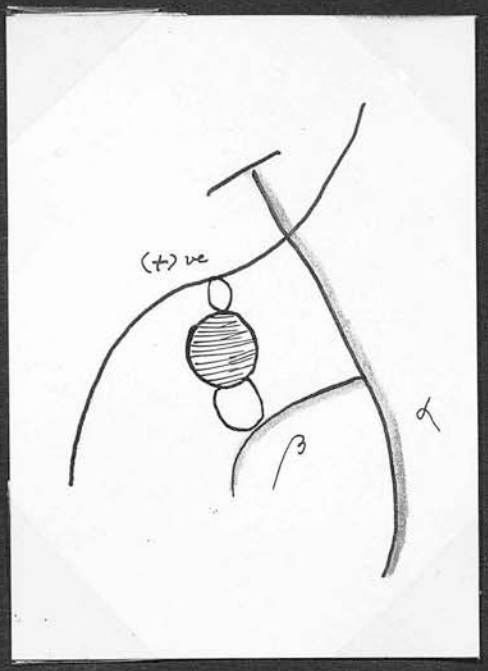


Fig. 32.

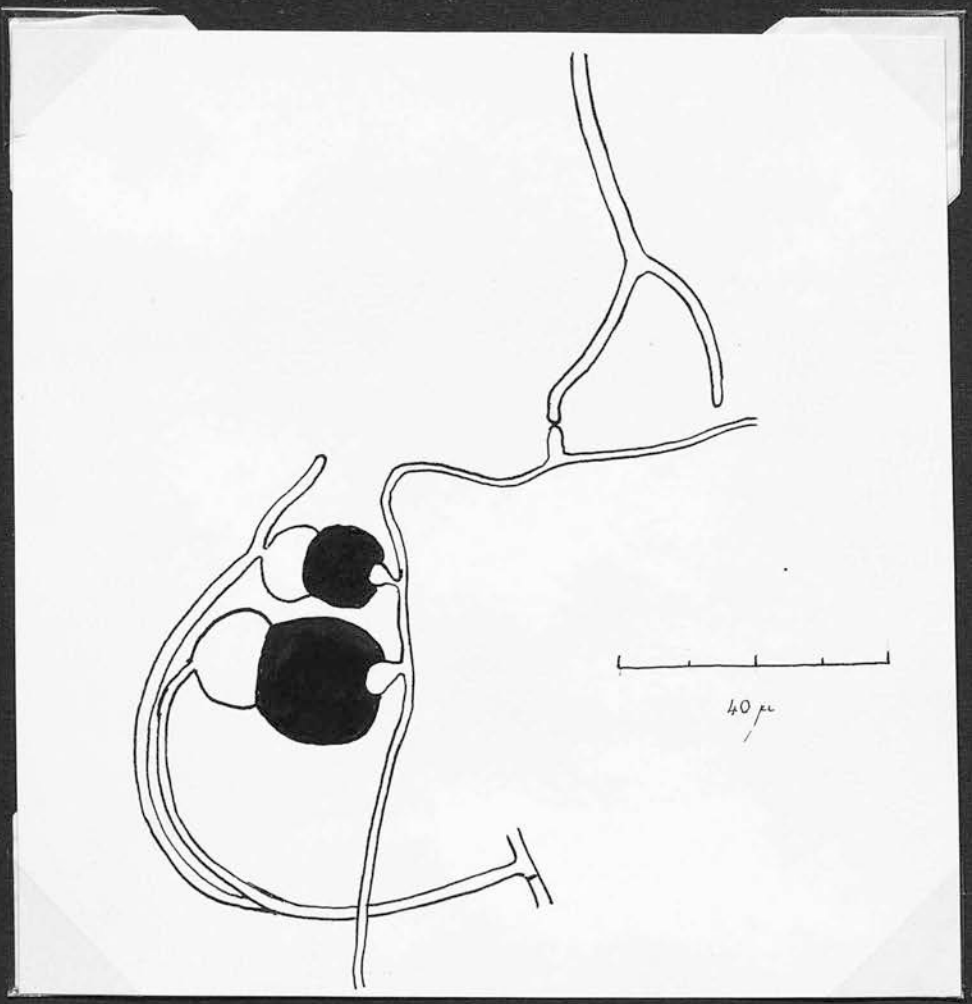


Fig. 33 b

the zygophores α & β have formed one zygospore and then a second one, ladderwise in relation to the first one. As this type of zygospore formation has been unknown in the homothallic Mucorineae (up till the present), according to Burgeff (verbally), he has not given an explanation of it. It would appear to be quite simply explained by the theory however, as, judging by the other cases, the zygophores do not change their sexuality after the formation of a zygospore. This would be a logical conclusion, though the laterals assume a (-)ve sexuality when they themselves give rise to a lateral (in this case a progametangium and a gametangium). If we were to assume this latter supposition as being correct, fig. 30 could be explained by the theory, but the case of fig. 31 would be further confused.

Several cases have also cropped up amongst the hybrids which contradict the theory. In the cross Rh. sexualis X Rh. nigricans (+)ve (fig. 33), a cross was observed where the two zygophores α & β of the homothallic species had both formed zygospores with the same zygophore of Rh. nigricans, and in addition the β hypha (+ve) of a second zygophore is in process of reacting with the same hypha of Rh. nigricans as the previous two. In a second case, fig. 32, Rh. nigricans (+)ve has formed a perfect zygospore with the β hypha (+ve) /

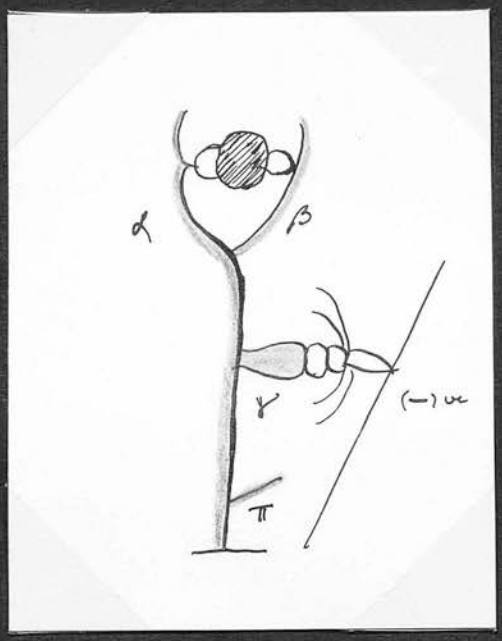


FIG. 34 a

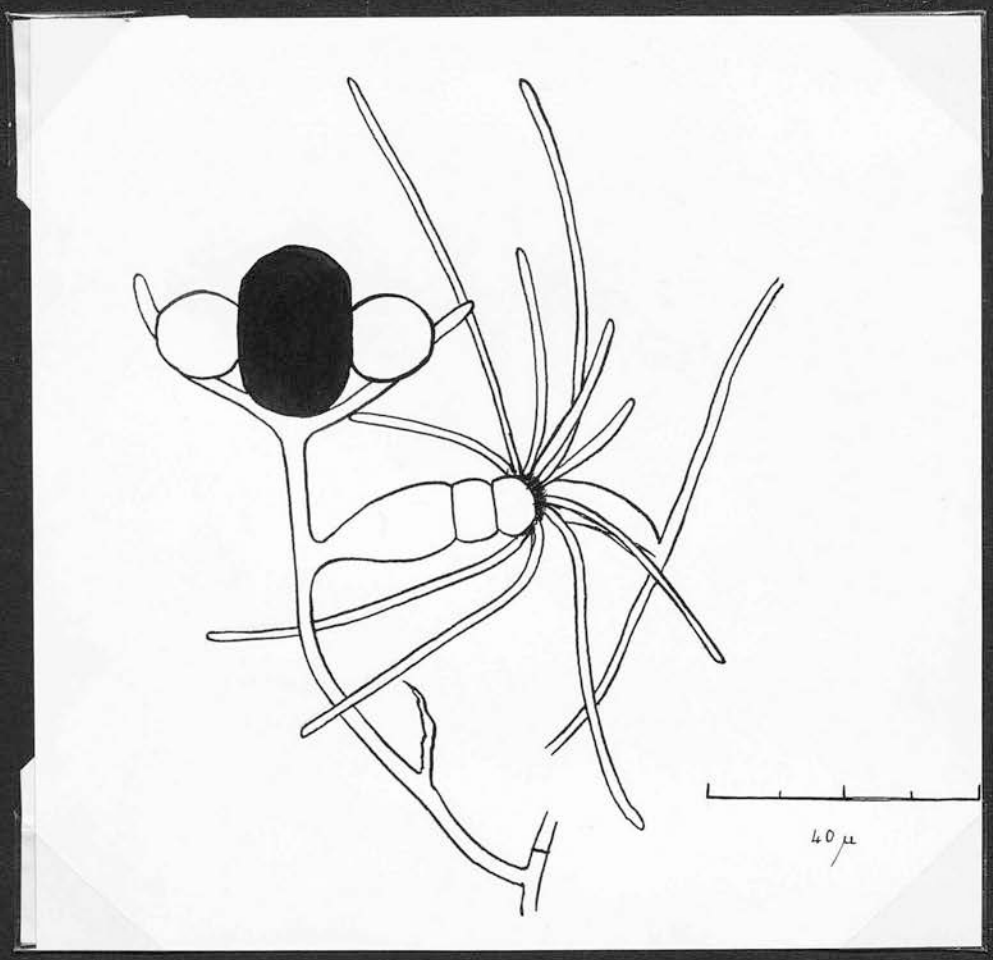


FIG. 34 b.

(+ve) of a Rh. sexualis zygophore. It is almost unnecessary to remark that the culture was not contaminated, infact at the time when these cultures were made in Würzburg, the (-)ve race of Rh. nigricans had already died out, and from experiments carried out, only the (+)ve race was obtained from the air there.

Another interesting case is that figured in fig. 34 in which there has been an imperfect sexual reaction between Rh. sexualis and Absidia glauca (-)ve. Here the Absidia has reacted directly with the zygophore α below the fork $\alpha/3$. According to Burgeff this is a region which should be either non-reacting or of (-)ve sexuality, as the hypha α changes its sexuality from (+)ve to (-)ve on the production of the lateral $/3$. Considering all the cases of this type, namely figs. 27, 28 and 34, we come to the conclusion that in Rh. sexualis at any rate, any part of the zygophores may react sexually, and that there is no passive region.

Nielsen, Blakeslee and Burgeff had never observed a case where two terminal hyphae (zygophores) had reacted together and formed a zygospore between them. In Rh. sexualis however, this has been observed on a number of occasions, either between the unbranched terminal hyphae, or between the α hyphae of branched zygophores. In the case of zygospore formation between two unbranched zygophores /

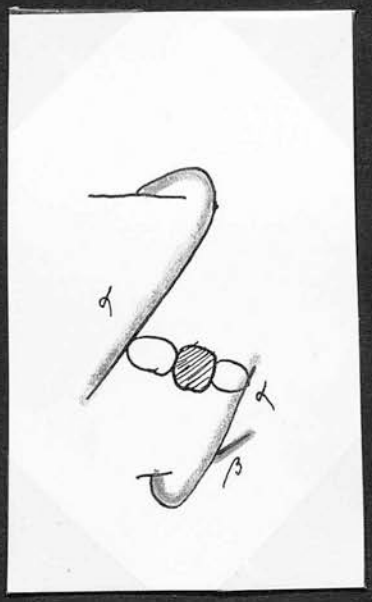


Fig. 35.

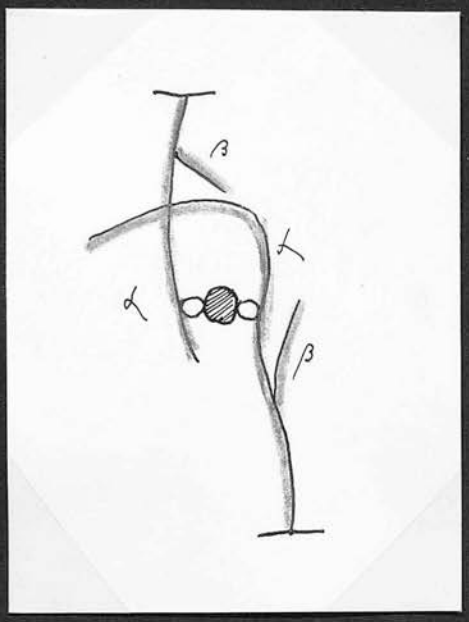


Fig. 36.

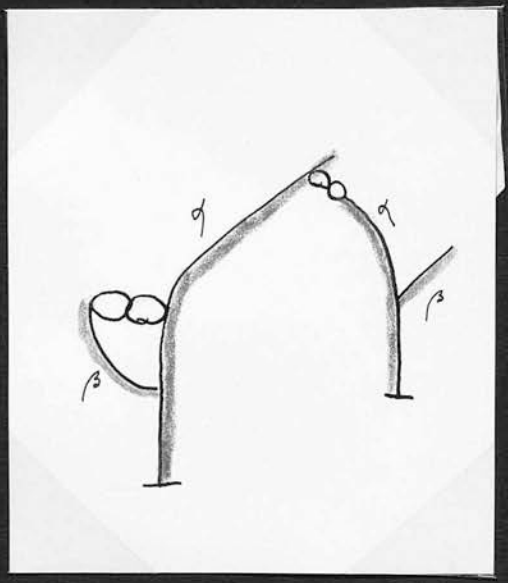


Fig. 37.

zygophores (fig. 4), Burgeff explained personally that this is quite in keeping with his theory, as the hyphae were of different length, and thus presumably of different age, consequently therefore of different sex. One must conclude therefore that he now assumes that the α hypha can change its sex before giving rise to a lateral, that it changes its sex when it reaches a certain age or length as a matter of course. This assumption is not compatible with the working of the theory however, as will be shown presently.

In the cases where one of the zygophores has given rise to a minute lateral (fig. 35) are quite in keeping with the theory, but when in two branched zygophores, the α hypha react together as in fig. 36, we come up against the fact that two hyphae which are (-)ve have formed a zygospore between them. In fig. 37 we have another case of the α hyphae reacting together and there was no mistaking which was the α and which the β hypha of the first zygospore, as the position of the β hypha in relation to the α , and the fact that it was thicker, marked it out as the lateral at once.

In the case of the laterals of a stolon reacting together, we again find that the Burgeff theory is insufficient to explain all the cases. Fig. 38 is quite straightforward /

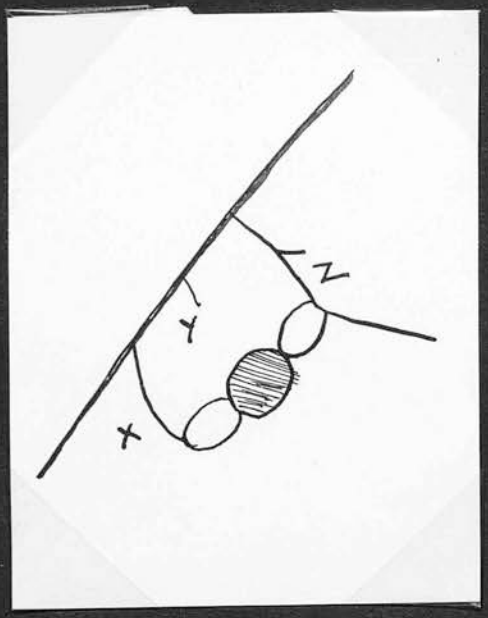


Fig. 38.

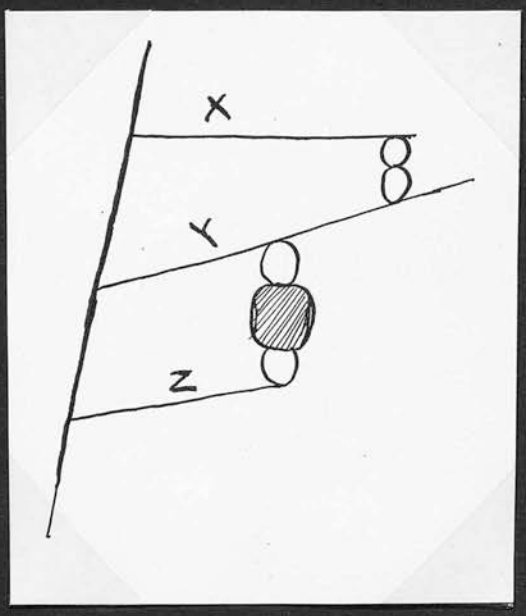


Fig. 39.

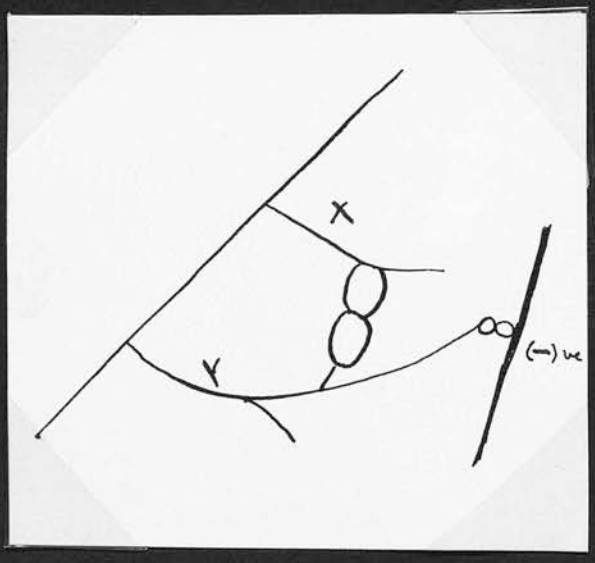


Fig. 40.

straightforward. If we assume that the lateral Z had branched before fusing with X, then we have a (+)ve and (-)ve reacting together. In fig. 39 are three laterals which have grown out side by side, and all of different lengths, and presumably of different ages. If we assume for the sake of argument that Z, the shortest, is (+)ve, then according to Burgeff, Y must be (-)ve. Then we find that Y and X are reacting together, with X the longest and possibly the oldest lateral, and thus (-)ve according to Burgeff. It has become clear from previous work, that the continuation of the zygophore beyond the zygospore does not change its sex, so that the sexual reaction between X and Y cannot be explained by the theory.

In fig. 40 there are only two laterals, Y very much longer than X, and as if to emphasise that Y is (-)ve, it has produced a lateral. This fusion is straightforward, but when we observe that the continuation of the (-)ve zygophore is reacting with Absidia glauca (-)ve, then we must find another explanation.

It is therefore quite impossible to reconcile the theory by Burgeff with the actual facts in Rhizopus sexualis. Blakeslee, with his co-workers (Satina, Cartledge and Welch), had reached the same conclusion with regard to the species he had investigated. Nielsen /

Nielsen (1927), who carried out a re-investigation of one of the species used by Burgeff, came to the tentative conclusion that there was no change of sex in the lateral zygophore, but that they were unisexual at first, and later bisexual. The very nature of the nuclear content of these true homothallic species precludes such a conclusion. All the work carried out on the present homothallic species confirms the findings of Satina and Blakeslee (1930), that the zygophores are bisexual the whole time. But not only the zygophores, the progametangia are bisexual as well. It is impossible at this stage to be certain whether the actual gametangia are unisexual. It would appear however that the actual nuclei present in the gametangia when it is first formed are of the homothallic type.

Taxonomy.

There has apparently only been one species of Rhizopus known to be homothallic. It was described by Raciborski (1900) as Rh. artocarpi on the male inflorescence of Artocarpus incisa from Java. In his attempts to grow it in pure culture, he obtained zygospores in only one culture out of many, after one weeks growth, and he admits to that culture being contaminated. Taking the measurements he gives into consideration, and his description of how the zygospores with their suspensors formed an obtuse angle (einen flachen Bogen), it seems probable that he was dealing with Rhizopus nigricans (p.50). The size of the suspensors and their shape seems to bear this out. The length of time taken for the zygospores to make their appearance is also typical of Rhizopus nigricans, and quite unlike any homothallic species. Zycha (1935) believes this fungus is merely a homothallic race of Rhizopus nigricans.

Sartory and Sydow (1913) isolated Rh. artocarpi from the male inflorescence of Artocarpus integrifolia, and found that their culture was identical with that of Raciborski. They only described the sporangia, and did not even so much as mention that zygospores occurred. There is no evidence to lead one to believe that their fungus was homothallic.

Naumov /

Naumov (1916) was the next to describe a homothallic Rhizopus species, isolated from Ficus Carica obtained in the Caucasus. He believed his fungus to be identical with that of Raciborski, though regretting that Raciborski did not describe the method of zygospore formation. In Naumov's opinion this is an essential point, as it is the sole means of distinguishing Rh. nigricans, which is heterothallic, and his own fungus which is equally definitely homothallic.

Kniep (1928) included Rh. artocarpi in his list of homothallic species, attributing the name to Raciborski, but in brackets: "Naumov 1916", probably referring to the fact that the previous descriptions lacked convincing evidence that the species is homothallic.

Rh. artocarpi appears to have been further studied by several Americans, including Harter, Weimer and Lauritzen (1921), and Weimer and Harter (1923), But they were only concerned with its pathology, and do not mention zygospores or their formation.

A comparison of Naumov's Rh. artocarpi and Rh. sexualis shows many similarities. The figures of the zygospores given by Naumov (1916) could be those of Rh. sexualis, and his measurements are within the limits of those given in this paper. Altogether the description /

description of the sexual phase of Naumov's fungus agrees fully with that of the present species, but scarcely at all with that of Raciborski. His description of the sporangial reproduction, and his measurements do not agree with the present species however, and rather doubtfully with those of Sartory and Sydow.

It is quite possible that Naumov was dealing with the identical species to the one under review, but as his fungus appears to differ considerably from that of Raciborski, there is no justification for calling it Rhizopus artocarpi. The name therefore given by Smith (1939) stands, The present species is obviously a Rhizopus, as it produces stolons, at the nodes of which rhizoids and bunches of sporangiophores are produced. Further, the sporangia have a distinct apophyses, which clearly distinguishes it from the genus Mucor. The fungus must therefore now be called:

Rhizopus sexualis (Smith) comb. nov.

Rh. sexualis is closely allied to Rh. nigricans, though by no means identical with it.

Discussion.

In homothallic species, it is generally assumed that there must be a sexual differentiation before nuclear fusion. In Rhizopus sexualis the suspensors have proved to be homothallic, so that there is no sexual differentiation at the stage when the progametangia are cut off. This differentiation must therefore take place at a later stage.

No definite evidence of nuclear fusion has been obtained, even in zygospores several months old, but the nuclei are found to be associated in pairs, starting at the time of gametangium fusion. Fusion may ultimately take place, possibly at the time of zygospore germination and immediately followed by the reduction division. Up to the present however it has been impossible to bring the zygospores to germination, even when using nineteen month old zygospores. It is therefore not possible to state what happens on germination.

The question naturally arises, do paired nuclei ever fuse ? A case might quite easily be conceived, where there was no fusion of paired nuclei, and that they separated after being associated for a while in pairs, as has been suggested for Endomyces Lindneri by Mangenot (1919, 1920) and Kniep (1928), and for several of the Uredineae (Kniep, 1928).

Obviously /

Obviously further investigation is necessary before any definite conclusions can be drawn with regard to this species.

Summary.

Rhizopus sexualis (Smith) comb. nov. is fully described morphologically. The most interesting points have proved to be (1) the production of zygospores ladderwise on one pair of zygophores; (2) that the regeneration of single suspensors has given only homothallic mycelium; (3) that both auxiliary ("Nebensporangien") and normal sporangia are produced; (4) that plasma excretion occurs.

The general cytology is described. No chromosomes are differentiated. No fusion of nuclei has been observed, even in zygospores several months old. Nuclei associate in pairs from the time of gametangium fusion, though it does not appear necessary that each of a pair must come from a different gametangium. This association is still evident in the zygospores after several months, practically no nuclei being single. Some of the nuclei entering the zygospore degenerate. Germination of zygospores up to nineteen months old has not been obtained.

Perfect hybrid zygospores have been obtained with Rhizopus nigricans (+)ve and (-)ve, Mucor hiemalis (-)ve and Absidia cylindrospora (-)ve, and imperfect sexual reactions with a number of other species. Rhizopus sexualis appears to be of a predominantly (+)ve tendency /

tendency,; only one other such homothallic is known.

Burgeff's theory of changing sex is shown not to be consistent with events in Rhizopus sexualis. The conclusion is drawn, that the zygophores and progametangia are bisexual, though the sexuality of the gametangia has not been determined.

It is finally suggested that fusion and reduction of the paired nuclei may take place on germination, though the possibility is envisaged, that the nuclei never fuse, and that there is only an association of nuclei.

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