Mycologisches Centralblatt, Bd. II, Heft 5.
Ausgegeben am 17. April 1913.

Conjugation in the heterogamic genus *Zygorhynchus*.

By

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(With 2 plates.)

As is well known, sexual reproduction in the *Mucorineae* is brought about by the union of gametes cut off from hyphae which have met and swollen at their points of contact. In most of the forms investigated, these gametes are morphologically essentially equal. In a few hermaphroditic genera, however, there is a marked and constant difference in size between the gametes which unite to form the zygospore. Judging from the conditions in higher forms, the larger gamete in these mucors has been considered the female and the smaller the male. *Zygorhynchus* is the best known form of these heterogamic species and its method of conjugation has been found by the writer as well as by most students of the group, to be in general the same as that characteristic of other mucors except for the difference between the gametes. Recently, however, Gruber [1]) and also Atkinson [2]) have called in question the usual account of of the process of conjugation in *Zygorhynchus*. Since their observations are at variance each with the other as well as with those of the writer it seemed desirable to make a careful restudy of conjugation in *Zygorhynchus*, especially in view of the fact that heterogamic species have been made the basis of a paper on conjugation now in process of publication.

1) E. Gruber, Einige Beobachtungen über den Befruchtungsvorgang bei *Zygorhynchus Moelleri* (Ber. d. Deutsch. Bot. Gesellsch. 1912, **30**, 126—133).

2) G. F. Atkinson, The Morphology of *Zygorhynchus* and its Relation to the *Ascomycetes* (Science N. S. 1912, **25** [26. Jan.], 151). — A letter to the writer from Prof. Atkinson outlines the results of his more recent study of *Zygorhynchus* as follows: "Studies on the cytology of conjugation in *Zygorhynchus heterogamus* and *Z. Moelleri* by the aid of microtome sections, during the past year and which are still in progress have convinced me that I was in error in my interpretation of the sexual process in *Zygorhynchus* published in a note in Science N. S. 1912. This note was to the effect that the sexual apparatus was similar to that of Monascus, a female branch with a trichogyne in conjugation with a male branch I was led to this interpretation because of the fact that the female branch, in the form of *Z. Moelleri* which I studied usually, and in *Z. heterogamus* often, applies its tip to the side of the male branch before any outgrowth or sign of a gamete appears on the latter. I have now found that in such cases the small male gamete grows out from the male branch after contact and pushes the female branch away. I wish therefore to retract the interpretation of the sexual apparatus of *Zygorhynchus* given by me in the note above referred to."

Gruber, in the paper already mentioned, states that he investigated sectioned material of *Z. heterogamus* and *Z. Moelleri* and in addition with the latter species followed the process of conjugation in living material under the microscope. Consecutive figures showing the development of individual zygospores, however, are not given. His figure 1 corresponds to our figure 2 and correctly gives an early stage where the more vigorous zygophore has bent around and applied itself to the more delicate filament. His figure 2 represents a later stage and resembles in appearance the condition shown by our figures 7, 13 and 22. We shall use our figure 22 in explanation of his interpretation. From the delicate filament (fig. 22 *a*), according to his account, a large progamete grows out, pushing away the larger zygophore (*b*) and soon cuts off by a basal cross wall a large female gamete (*c*). At this stage a violent motion of the protoplasm in the conjugative apparatus is reported. The writer, however, has been unable to discover any decided movement of the living protoplasm directly connected with zygospore formation. The swollen end of the larger zygophore (*b*) is considered the male progamete since Gruber believes he has found evidence from sectioned material that fertilization consists in a squirting in of a mass of protoplasm from *b* into his female gamete (*c*). The line separating what are really the gametes (*m* and *f* in our fig. 21), he would hold was usually at least only a partial wall showing itself as a temporary ridge inside his female gamete (*c*).

Our studies on living and stained material of *Z. heterogamus*, *Z. Moelleri*, as well as of *Z. Vuillemini* (which, however, is not figured) have convinced us that Gruber has fallen into error in interpreting the process of conjugation in the genus *Zygorhynchus* on account of the difficulty usually experienced in following the process in living material as well as on account of the difficulty in finding an abundance of the early stages of development.

The writer[1]) has already given the following account of conjugation in *Z. Moelleri* using stained material. The figures given refer, however, to the present paper. "In the simpler case illustrated by the more common made of conjugation, a terminal portion of an erect hypha is distinguished by a septum from the portion below. Immediately beneath this septum is produced a branch which, growing upward, recurves to meet the side of the slender zygophoric filament cut off by the septum already mentioned (figs. 1, 9, 16). The two zygophores are from the beginning unlike in character as well as in origin. While the first, which contains but a small amount of protoplasm that becomes massed at the point of contact with the other, undergoes no further development, the second, which has arisen immediately below it, is from the outset richly supplied with dense protoplasm. Immediately after contact a progamete is developed as a perpendicular outgrowth from the slender erect zygophore, and in juxtaposition to this a progamete is formed by the terminal enlargement of the more vigorous zygophoric branch (figs. 2, 10, 17). In each of these progametes a transverse septum is formed, distinguishing the gametes which are unequal in size, the larger being formed on the side of the vegetatively more vigorous zygophore (figs. 4, 11, 21). This

1) Sexual Reproduction in the *Mucorineae* (Proc. Amer. Acad. Arts and Sciences 1904, **40**, 205—319).

difference in size is always distinct, though in some cases less marked than in others. The contents of the two gametes become united through the disappearance of the intervening wall, and the zygote here formed (figs. 7, 13, 22), by the gradual enlargement of the two cells thus united, assumes the shape of a mature zygospore (figs. 8, 15, 24). The supply of nutrient for this ripening process comes almost entirely by way of the more vigorous zygophoric branch, and, although the stretched wall of the larger gamete makes up the greater part of the outline of the zygospore, still the stretched wall of the smaller contributes to it. Although it may show a certain tendency in this direction, the condition here is thus not comparable to an oogamous fertilization where the male gamete furnishes protoplasm to, but forms itself no essential part of, the mature oospore."

It does not seem necessary at the present writing to change the account of conjugation already given.

In the usual method of obtaining stages of development by taking material from a young culture and examining it stained under the microscope, it is extremely difficult to find the earlier stages. Attempts to follow the development in living material in the ordinary 1 inch VAN TIEGHEM cell culture are difficult from the small amount of nutrient in the hanging drops of agar. Moist chambers made of Syracuse watch-glasses, 2 inches in diameter, and PETRI-dishes, 4 inches in diameter covered with number 2 covers cut from thin sheets of glass, give much better results. Sufficiently numerous drops of the nutrient agar are placed on the under side of the cover to counteract the tendency shown by the aerial hyphae to dry up despite the presence of water in the bottom of the culture dish. The mycelium grows rapidly to the edge of the nutrient drop and out for a short distance upon the glass itself where it produces zygospores in abundance. Many will be found so near the surface that their development can be followed under the 4 mm objective.

In practice, a considerable number of the earliest stages are marked on the glass and camera lucida drawings made of each at various times throughout their development. Some become covered over by other hyphae, while some dry up or touch the drops of condensed moisture on the glass or abort for other reasons If, however, a sufficient number are watched at the same time, there is no great difficulty in securing a complete series of stages for several conjugations from the same culture. Figures 1—8 and 9—15 were thus obtained and show the development respectively of *Z. heterogamus* and *Z. Moelleri*. It will be noted that the process is relatively rapid at first and this fact probably accounts for the difficulty most observers have experienced in finding the early stages of development.

It is needless to say that camera drawings of hyphae in the air at some distance from the cover glass cannot be expected to be as accurate in outline as those made from mounted material. They do, however, show with fidelity the succession of the stages figured.

In living material again it is difficult to be certain of more than surface markings. Mounted material is therefore necessary to determine the completeness of the cross walls, The cover of a large moist chamber culture which shows stages in conjugation may be conveniently inverted and used directly as an object slide. If a mixture of equal parts alcohol and glycerine colored with eosin be added, the protoplasm will be strongly

plasmolized and the cell walls will be rendered more distinct in consequence. It is less easy to induce the protoplasm to withdraw from the wall separating the two gametes than from the other walls of these cells. This is especially true of the smaller gamete. It has been accomplished in a sufficient number of cases, however, to leave no doubt in the writer's mind that there are two gametes which are definitely separated by a continuous cross wall as shown in fig. 20. There seems no doubt also that these two gametes which plasmolize into two separate masses of protoplasm unite later by the dissolution of the intervening cross wall into a single cell the contents of which now plasmolize into a single mass as shown in fig. 23.

Explanation of Plates.

Figures in plates I and II were outlined with the aid of a camera lucida. half were all viewed through a 4 mm objective and have been reduced to about one They in reproduction. Surface sculpturing on mature zygospores is not represented.

Plate I. *Zygorhynchus heterogamus.*

Figures 1—8. Consecutive stages in zygospore development in living material from moist chamber culture. Drawings were made at times indicated.

Plate II. *Zygorhynchus Moelleri.*

Figures 9—15. Stages in zygospore development in living material from moist chamber culture.

Figures 16—24. Stages of zygospore development taken from stained and mounted material. Outlines of cell walls only are represented, except in figures 20 and 23 where plasmolized cell contents are shown in stippling. *a* Branch which has given rise to male gamete; *b* Branch which has given rise to female gamete; *c* Zygote formed by union of male and female gametes; *m* Male gamete; *f* Female gamete.

On the Morphology and Development of *Phoma Richardiae* n. sp.

By

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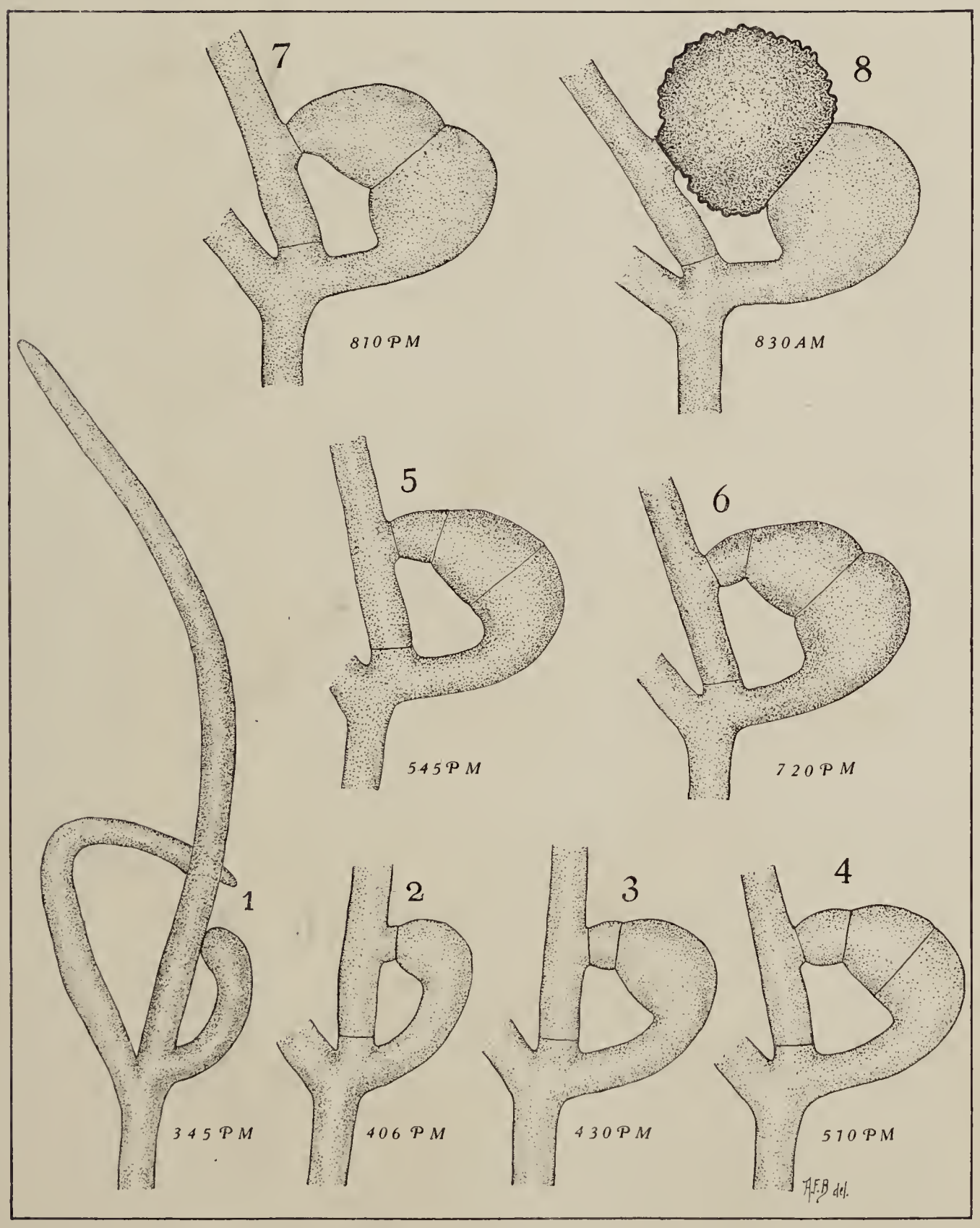
(With 6 Textfigures.)

In a group of *Calla*-plants under glass-house cultivation isolated leaves, partially or wholly in a state of decay, are usually to be found. Sometimes the decaying area is sharply marked off from the rest of the leaf; sometimes the transition from sound to unsound tissue is very gradual. The unsound area is usually situated peripherally; at times however it is represented by a round or oval area in the middle of the leaf, wholly surrounded by healthy tissue. In the examination of such unsound leaves, numerous fungi have been met with, notably species of *Hormodendron*, *Alternaria*, *Macrosporium*, *Penicillium* etc. Together



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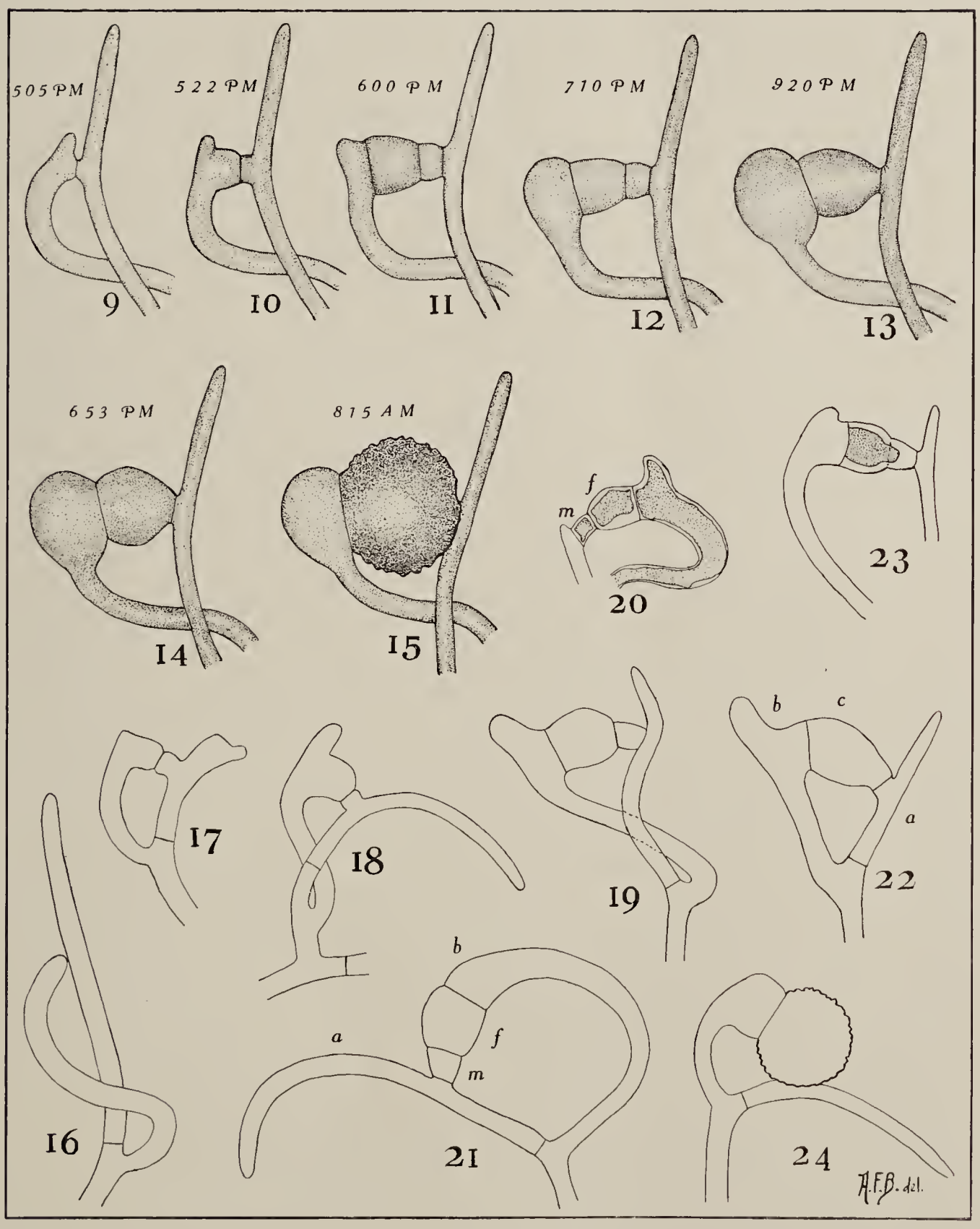

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