FLAGELLAR STRUCTURES OF ZOOSPORES OF SOME FUNGI AND ALGAE

THESIS FOR DEGREE OF MASTER OF SCIENCE
MICHIGAN STATE COLLEGE

BERNARD ELLISON

1944

THESIS

This is to certify that the

thesis entitled

Flagellar structures of zoospores of some fungi and algae

presented by

Bernard Ellison

has been accepted towards fulfilment
of the requirements for

M. S. degree in Botany

Ernst A. Bessey
Major professor

Date July 1, 1944

FLAGELLAR STRUCTURES OF ZOOSPORES OF SOME FUNGI AND ALGAE

by

BERNARD ELLISON

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

THESIS

ACKNOWLEDGEMENT

I should like to express my appreciation to Dr. Ernst A. Bessey for suggesting this research problem and for his valuable aid and advice. His interest and encouragement have been a source of inspiration to me throughout the course of the investigation and his suggestions and criticisms have been most helpful.

I should also like to thank Mr. John M. Roberts for many stimulating and valuable suggestions and for furnishing me with certain material used in the investigation. Likewise I should like to express my thanks to D. J. C. Walker of the Agricultural College of the University of Wisconsin, and to Dr. C. M. Haenseler of the New Jersey Experiment Station who were good enough to furnish me with clubbed cabbage roots from which I obtained the zoospores of Plasmodiophora brassicae. I should like to thank Mr. Robert Hagelstein, Curator of Myxomycetes, at the American Musieum of Natural History, New York, for furnishing me with material and a number of helpful suggestions.

In addition I should like to express my appreciation to all the members of the Botany and Plant Pathology Department staff not only for aid given me from time to time on this problem but also for the consideration and the simulating association I have had during my attendance at Michigan State College of Agriculture and Applied Science.

STUDIES IN THE FLAGELLAR STRUCTURE OF SOME FUNGI AND ALGAE

INTRODUCTION

In those unicellular organisms posessing flagella it has been shown that the latter may fall into two or possibly three types (34, 35). These are: the ciliated flagellum or flagellum possessing tinsels, the whiplash type, and the type having neither whiplash or tinsels. This last type of flagellum is probably a modification of the whiplash type of flagellum. The lash of the whiplash type is merely the extension of the central core of the flagellum beyond the sheath. This extension or whiplash has been found to vary in length considerably on individuals of the same species on the same slide and in one case a living zoospore was seen by Couch (6) to suddenly extend the whiplash enormously. Under the circumstances it is doubtful whether one should consider a flagellum in which the whiplash is very short or not extended at all a distinct type on a par with the other two distinctive types.

Certain botanists use the terms flagellum and cilium interchangeably. It is the author's opinion that this is a mistake in view of the fact that the term cilium when applied to a swimming organelle of a unicellular organism is so well entrenched in zoological literature as referring to those distinctive structures (morphologically quite different from flagella) found on members of the subphylum Ciliophora, Phylum Protozoa. A flagellum as contrasted to a cilium is that relatively long, whip-like, swimming organelle, characterized by having an outer sheath surrounding an inner core; the inner core arising from a blepharo-

oplast which is in turn connected to the nucleus by a rhizoplast. It is in this sense that the terms flagellum and cilium are used in this paper.

It is now considered that the position, number, type, etc. of the flagella are of phylogenetic importance and they are being used more and more by mycologists as a means of classification of certain groups. In most cases there is a consistent correlation between the number and types of flagella and certain physiological characteristics. Chief of these characteristics is the cell wall composition and in some of the algae the nature of the photosynthetic product is also correlated (3, 39).

It was Dr. Bessey's (3) suggestion that probably the primitive or ancestral flagellate of some of the algae and also some of the lower fungi was a unicellular, biflagellate, green alga having both the whiplash and the ciliated flagellum present, as occurs in the algae of the group Xanthophyceae (or Heterokontae). These two flagella come in the course of evolution, to have a lateral position with one directed forward and the other to the posterior. Thus we have, by the loss of one or the other of the flagella a uniflagellate organism with the flagellum directed anteriad or posteriad. From such a generalized organism have arisen other algae such as the heterocont green algae in which the swimming state has been reduced and now represented by zoospores. These algae may in turn produce a parasitic series due to the loss of the chlorophyll and could reasonably be regarded as the ancestral forms of the Chytridiales in the broad use of the name. From this group others arose in turn through

the loss of one flagellum or other. Those retaining both flagella led to the Olpidiopsidaceae; those that lost the posterior, whiplash flagellum led to Rhizidiomyces and relatives, and by the loss of the anterior, tinsel type flagellum arose the whole Olpidium to Monoblepharis series (6). Likewise from the green flagellates arose the Sarcodina in general and the Mycetozoa, Plasmodiophorales, etc. series in particular through the loss of chlorophyll and the progressive modification of the flagellum into the pseudopodium.

Sachs (40) suggested that the Saprolegniales originated from the Siphonales with Vaucheria close to the ancestral form. There are several facts which support this hypothesis one of the chief being that there is a strong serum reaction between Vaucheria and Saprolegnia. (26).

It was Dr. Bessey's opinion that investigation of the flagellar structure of certain groups might yield additional information on the phylogeny of those groups. With this in mind he suggested that flagellar studies be made on the Mycetozoa, Plasmodiophorales, Chytridiales, Saprolegniales and some of the heterokont and coenocytic green algae. It was hoped that the results would lend support to one of the theories of phylogeny and relationship that had been based upon other data i.e. comparative morphology, serum reaction, etc.

MATERIALS AND METHODS

Zoospores were obtained from material collected by me and also from material obtained from other collectors. I shall not go into the generalities of inducing germination in the var-

ious organisms largely because there are a number of comprehensive articles on this subject(10, 11, 12, 14, 30, 37, 41). In the author's experience there are no general methods which are effective in inducing the germination in the different genera. Each species, indeed each specimen, must be treated as an individual and germination must be induced by a laborious process of trial and error. Robert Hagelstein in a letter to the author expressed the opinion that the germination studies based on individual specimens do not establish the germination characteristics or requirements for a whole species by any means. The author is in hearty accord with this opinion.

Some of the various treatments suggested by different authors as a means of inducing germination are as follows: seeding the spores in distilled water; seeding the spores in soil solutions of various concentrations; seeding the spores in dilute nutrient solutions of various types; chilling; incubation at various temperatures; wetting and drying, etc. The author has found most of these methods useful in individual cases but useless as a general procedure for inducing either germination or the formation of zoospores.

Germination was usually carried on in hanging drop cultures in Van Tieghem cells or more effectively in culture slides. The latter method was found to be the most satisfactory in a number of cases for the reasons that when germination takes a period of several days, condensation on the coverglass in a hanging drop culture will cause the drop to spread and sometimes be lost by running down the side of the cell. Furthermore in cases where there seems to be a mass action effect in the ger-

mination the culture slide makes it possible to use a greater number of spores than is possible in the hanging drop. Temperature was controlled when necessary by the use of incubators but the majority of the zoospores were germinated at room temperature. The specific methods of obtaining the germination for each organism referred to in this paper will be discussed farther on.

Zoospores to be stained for flagellar structures were collected by means of a micro pipette and placed on a very clean slide. These zoospores were killed by inverting the slide over the fumes of a two percent solution of osmic acid. The slide was allowed to dry for several hours and was then stained with the Löffler flagellar stain technique (23). The relative time of mordanting and staining was varied according to trial and error to obtain the best results. In general it may be said that treating the smear for a maximum time with the mordant and a minimum time with the stain will produce a sharper staining with less of the stain precipitating on the slide. After staining preparations were allowed to dry over night in a desiccator. They were then mounted in Canadian balsam.

Cytological stains were made in cases where it was necessary to determine whether biflagellate zoospores were abnormal or were in a stage of division. These preparations were stained by an adaptation of the method developed by Cotner (41 42). The strength of the stain was varied somewhat. It was found that zoospores of the Mycetozoa stain more readily than most other zoospores and that a weaker stain was more satis-

factory. Smears were prepared in the same way as those prepared for staining by the Löffler stain. Rather than introducing a drop of stain into the drop of water containing the dead zoospores as Cotner did, it is satisfactory to apply the stain to the dried smear. After staining, the slide is placed in a desiccator for twenty-four hours, cleared with clove oil and then mounted in Canadian balsam.

The slides were studied under high dry and a clearite oil immersion lens. Drawings were made with a camera lucida and then photographed.

OBSERVATIONS

Stemonitis ferruginea Ehrenb. The specimen of S. ferruginea used was obtained from John M. Roberts and was collected by him in Indiana in July 1940. It was stored for approximately two years with no particular attempt to keep the spores viable. When germination of the spores was attempted in January, 1942, they were found to germinate readily in distilled water at room temperature. This germination was carried on in a hanging drop culture using garden hose washers in place of the glass Van Tieghem cell. The first germination was observed after forty-eight hours and the spores had germinated up to eighty percent in fifty-four hours. Zoospores were collected in a micro pipette and tranferred to slides cleaned in hot soapy water and then stored in ninety-five percent alcohol. The importance of having the slides perfectly clean cannot be over emphasized because foreign material will cause the stain to precipitate on the slide. It is likewise important to keep the culture reason-

Fig. 1. Stemonitis ferruginea Flagellar structure of the swarm cells.

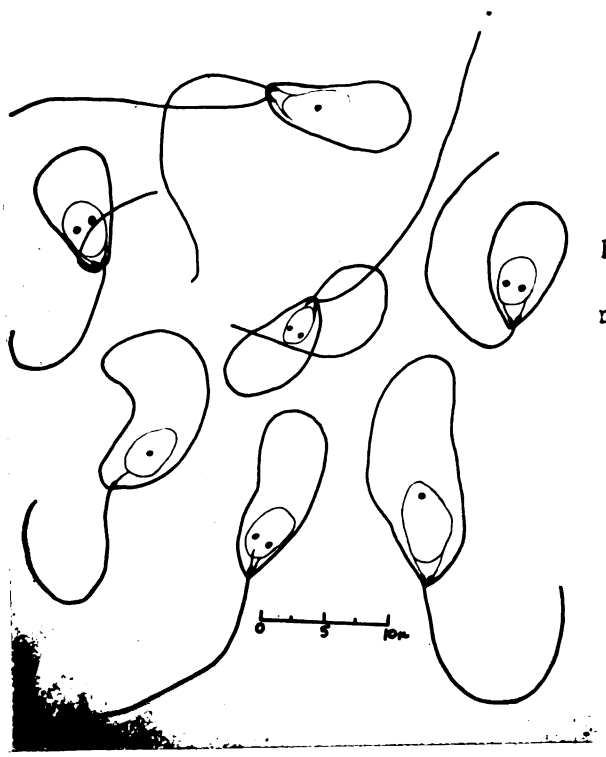

Fig. 2. Cytological and neuromotor apparatus of S. ferruginea.

ably free of bacteria as they may if presant in large numbers cause a precipitation of stain on the slide. The zoospores were killed by exposure to fumes of osmic acid for thirty seconds. The slides were allowed to dry in an inverted position. This has the advantage of allowing the zoospores to become better distributed over the slide because it prevents them from being attracted to the periphery of the drop. It also helps keep the slides dust free. A number of smears were taken for staining. The time of mordanting and staining was varied from one half to one minute for the mordant solution and one to two minutes for the stain. During the mordanting and staining the slide was heated very gently over an alcohol burner with the flame very low. The slides were never allowed to get so warm as to be uncomfortable when placed on the back of the hand. Slides selected as being most satisfactory had been treated with the mordanting solution for three quarters of a minute and stained for one and one half minutes. Cytological stains were made by treating a dried smear for five minutes with a .5 percent aqueous solution of crystal violet. The stain was washed off with distilled water and the slide allowed to dry for forty-eight hours in a desiccator. It was then cleared with clove oil and mounted in Canadian balsam.

Three types or modification of the flagella were discernable on the slides stained with the Loeffler stain. They were as follows: approximately 54 percent were of the ordinary blunt ended type; 44 percent had slender whiplashes of variable length; and 1.3 percent had the knobbed type of flagella. Bi-

flagellate zoospores occurred in the ratio of fifteen biflagellate to seven hundred and sixty uniflagellate or 1.9 percent. Ofthese biflagellate zoospores the flagella were of various types as follows: both flagella stubbed; one stubbed and one whip-lash; one stubbed and one knobbed. Combinations that were not found were those having two whiplash flagella or a whiplash plus a knobbed flagellum. No tinsel type flagella were found.

The cytological stain showed a large, dark staining nucleus present in all cells. It was invariably located in the anterior portion or flagellar end of the cell. A darker staining endosome is usually present and in some cases two are present. The number of endosomes in the nucleus seems to have no correlation with the number of flagella. In no case was there more than one nucleus present in the cell regardless of the number of flagella present. Nor was there any indication that the nucleus was preparing to divide or was in any stage of division.

There is no apparent difference in the neuromotor apparatus of either the uniflagellate or biflagellate cells. Even in the uniflagellate zoospores the neuromotor apparatus is clearly of a potentially dual nature. This supports the investigations of the Japanese mycologists Sinoto and Yuasa who contend that the zoospores of the Mycetozoa, whether uniflagellate or biflagellate, have two bleparoplasts (29, 38).

Two blepharoplasts are present each with a rhizoplast connecting it with the nucleus. Each blepharoplast gives rise to a flagellum in the case of the biflagellate zoospores. There is no apparent difference in the blepharoplasts whether they

produce a flagellum or not.

The zoospores were in various stages of becoming amoeboid. Practically all of the zoospores showed the formation of pseudopodia while still in the actively swimming or flagellated state. Other zoospores were seen that had completely lost their flagella and were carrying on locomotion exclusively by pseudopodia. In these cases the entire neuromotor apparatus had disappeared.

Stemonitis fusca (Roth) Rost.: The specimens of S. fusca used in the flagellar studies were collected in the second college woodlot during the month of April, 1943 and germination studies were carried on immediately after collection in hanging drop cultures. It was found that the spores germinated readily in either sterile river water or distilled water at twenty eight degrees centigrade. Approximately seventy percent of the spores germinated under these conditions within ninety-six hours. Little or no germination was obtained at room temperature.

As in the case of S. ferruginea the zoospores were collected in a micro pipette and transferred to clean slides which had been washed in soapy water and stored in ninety-five percent alcohol. These zoospores were then killed by exposure to the fumes of a two percent solution of osmic acid for thirty seconds. The smears were then allowed to dry upside down in a dust free chamber and were then stained both for flagellar structure and for cytological details. Again it was found that the most satisfactory slide for studying flagellar structure was the one that had been treated with the mordant solution for three

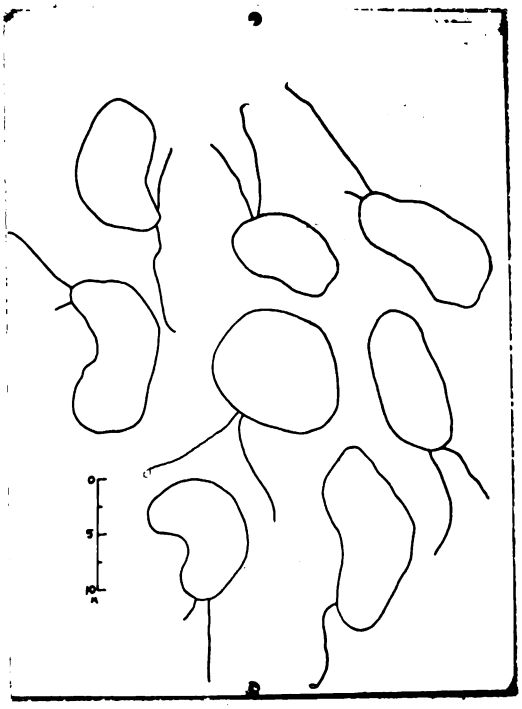

Fig. 3. Flagellar structure of Stemonitis fusca.

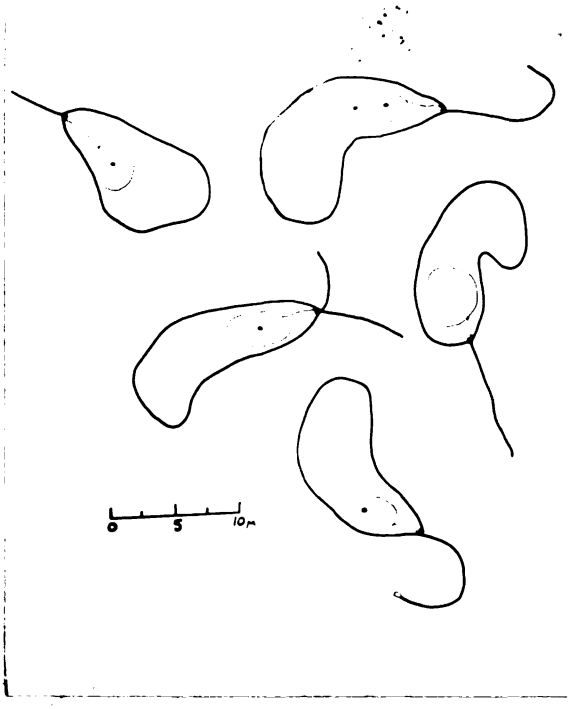

Fig. 4. Neuromotor apparatus of S. fusca.

quarters of a minute and with the stain solution for one and one half minutes. Cytological stains were made by treating the dried smear for five minutes with a .5 percent aqueous solution of crystal violet. The slides were then cleared and destained until satisfactory. The clearing and destaining were carried on while observing under the microscope and the process was stopped by washing the oil of cloves off the slide with xylol. The slides were then mounted in Canadian balsam.

With the Löffler stain three modifications of the flagella were observed. They were as follows: approximately 30.4 percent were of the plain type of flagellum; 14.8 percent were of the whiplash variety, and 46 percent were of the knobbed variety. Biflagellate zoospores occurred as about 8 percent of the total. In the biflagellate zoospores the flagella occurred in the following combinations: plain plus plain; plain plus whiplash; plain plus knobbed; whiplash plus knobbed; whiplash plus whiplash. None were seen in which both flagella were of the knobbed variety. In no case was a flagellum present of the tinsel type. The relative length of the flagella varied all the way from markedly heterokont to isokont. The length of the flagellum seemed to have no correlation with the type.

Zoospores of S. fusca were the best studied as far as the knobbed flagellum was concerned. These zoospores exhibited the condition in a greater number of cases than zoospores of any other genus or species. It is difficult to say what this modification represents. Knobbed flagella have been observed on members of the Chytridiales and interpreted variously as ab-

normal forms due to degeneration or due to immaturity (7, 1, 13). Neither of these explanations can be regarded as applicable in this case. The percentage of zoospores having the knobbed flagellum was seen to be as great in young, newly germinated cultures as in old cultures. The percentage remained approximately the same in cultures germinated in both distilled water and in river water. Also the temperature during germination did not affect the percentage. Spores from other specimens were used to make sure that the condition was not due to a peculiarity of an individual specimen. The knobs on the flagella were observed by Dr. Bessey and myself on living zoospores thus removing the possibility that the feature was due to the treatment the zoospores received during the killing or staining processes.

Without additional investigation it would be difficult to make any authoritative explanation of this type of flagellum. Many authors regard the flagellum and the pseudopodium as homologous. If this is true then the sheath of the flagellum would represent the ectoplasm of the pseudopodium and the central cylinder the more fluid endoplasm. If the cylinder or endoplasm were for some reason more fluid than is usually the case a slight extension of the cylinder instead of remaining as a whiplash would, due to surface tention, tend to collect into a drop or knob at the end of the flagellum. This idea is supported in part by the fact that those species having the knobbed flagellum on some of their zoospores also have whiplashes on others of their zoospores. Thus the knobbed flagellum may

represent a modification of the ~~of the~~ whiplash type and not a third and distinct type of flagellum, and it must not be regarded in all cases as being an abnormality due to age or environment.

It would be interesting to investigate this problem further and determine if the knobbed condition is characteristic of any particular groups, particularly the Mycetozoa.

In S. fusca the dual nature of the neuromotor apparatus is not as evident as it is in S. ferruginea. All zoospores remain potentially biflagellate in that they have the usual two blepharoplasts. However, only one of the bleparoplasts has a rhizoplast connecting it to the nucleus. In the case of the uniflagellate zoospores it is always the blepharoplast which does not have the nuclear connection which produces the flagellum. Exactly the same condition is found in the biflagellate zoospores in regard to the neuromotor apparatus as is found in the uniflagellate ones except that in the former case both blepharoplasts of course produce a flagellum.

A large nucleus is invariably located in the anterior portion of the cell just as in the case of S. ferruginea. This nucleus has a darker staining endosome present and occasionally two. There is no apparent correlation between the number of endosomes in the nucleus and the number of flagella on the zoospore. In no case was there a zoospore seen with more than one nucleus or any condition that indicated that the nucleus or the cell was preparing to divide or in any state of division.

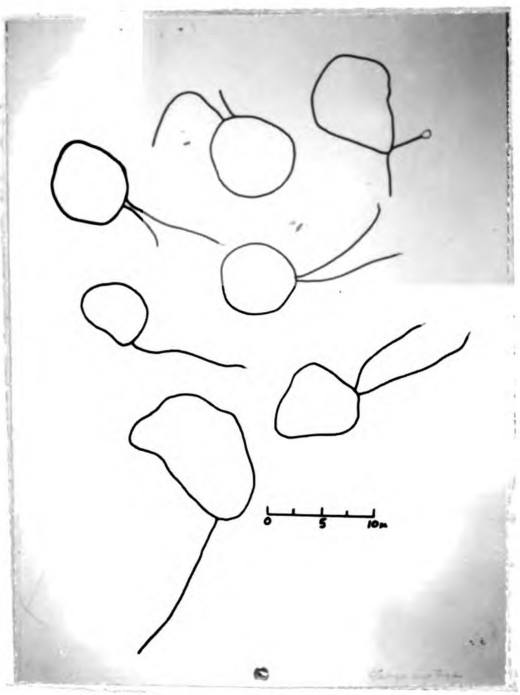

Fig. 5. Flagellar structure of Fuligo septica.

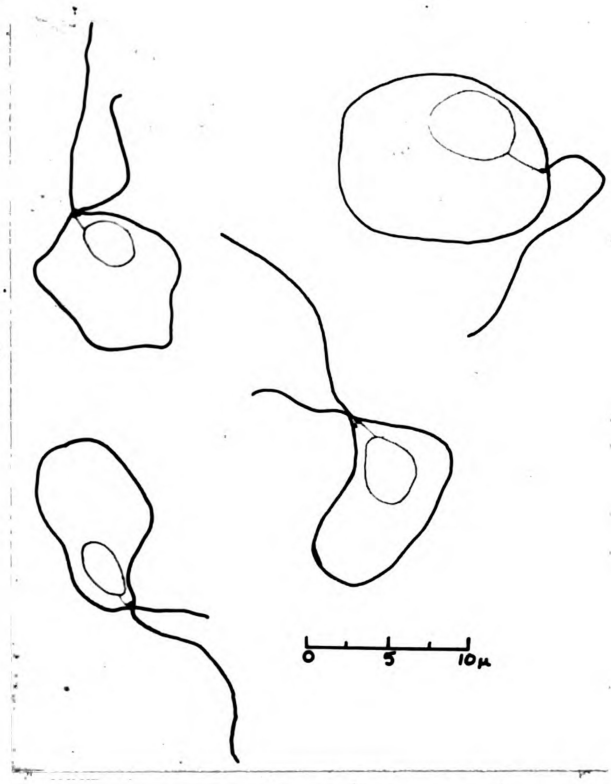

Fig. 6. Neuromotor apparatus of F. septica.

Pseudopodia were present on all zoospores and the zoospores were seen in all stages of becoming amoeboid. In those in which the flagella had been completely absorbed the neuromotor apparatus had likewise degenerated.

Fuligo septica (Linn.) Gmel.: Specimens used in the study were collected shortly after the formation of the sporangium along the railroad track by the "Stores" building. These germinated in four days in both distilled water and in sterile river water at room temperature and also at 28 degrees centigrade. Time of germination was cut down to 48 hours at room temperature by wetting and drying the spores. Germination was carried on in culture dishes. This is the only one of the many slime molds which I germinated or attempted to germinate which was benefitted by the wetting and drying method as recommended by Jahn (14).

Three modifications of the flagellar structure were seen in the following proportions: 52 percent the whiplash type; 47 percent of the blunt ended type; and less than one percent of the knobbed type. Since these are ~~all~~ probably all modifications of the whiplash type it would probably be more accurate to say that one hundred percent of them were of the whiplash type. None of the zoospores had flagella of the tinsel type.

Biflagellate zoospores were common, amounting to about 26 percent of the total. These had the following combinations of flagella: blunt ended plus blunt ended; blunt ended plus whiplash; whiplash plus whiplash; and blunt ended plus

knobbed flagellum. The combination not observed was whiplash plus knobbed. The relative length of the flagella varied all the way from markedly heterokont to isokont.

Like the other members of the Mycetozoa studied two blepharoplasts are always present in both the uniflagellate and the biflagellate zoospores. In the case of the biflagellate condition each of the blepharoplasts produces a flagellum. Only one of the blepharoplasts had a rhizoplast connecting it to the nucleus in the case of the uniflagellate as well as the biflagellate zoospores. In this regard the zoospores resemble those of Stemonitis fusca. In the uniflagellate zoospore the single rhizoplast connects the blepharoplast that does not produce the flagellum.

A rather large but lightly staining nucleus is present in the anterior part of the cell. No endosomes were observed in the nuclei. In no case was there more than one nucleus present in a cell. There was no indication that any of the nuclei were in a stage of division or were preparing to divide.

Plasmodiophora brassicae Woronin 1878: The flagellar structure of Plasmodiophora brassicae is of particular interest at the present time due to a tendency on the part of some authors (17) to place the genus in the family Woroninaceae. This is done largely on the basis of Ledingham's research (19, 20, 21) which showed that some genera of the Plasmodiophoraceae (Plasmodiophora and Spongospora) had two flagella rather than one as was stated in the original description. It has been

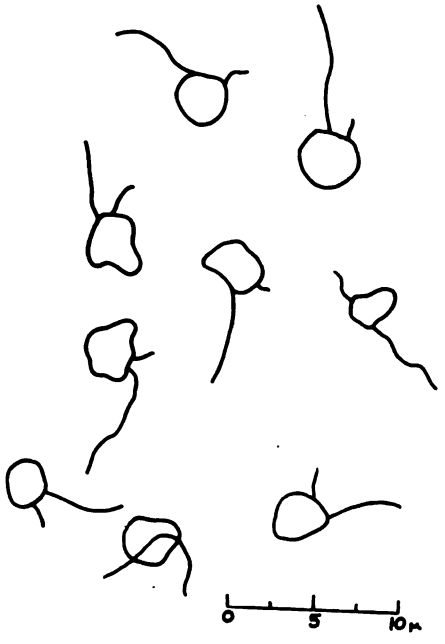

Fig. 7. Flagellar structure of Plasmodiophora brassicae.

shown, however, that the slime molds as a group are potentially biflagellate (29, 38) in that they have two blepharoplasts and so the posession of two flagella should not necessarily make it impossible to consider the Plasmodiophorales as being allied to the slime molds. As some other members of the Woroninaceae have been shown to have a tinsel type of flagellum and the slime molds have only the whiplash or modified whiplash type, the presence or absence of the tinsel flagellum on Plasmodiophora should be a diagnostic character of some importance and would tend to confirm its inclusion in the Woroninaceae. Peronospora line on the one hand or its alliance to the slime mold group on the other.

The zoospores used in this study were obtained by germinating spores from infected roots supplied by Dr. C. M. Haenseler of the New Jersey Experiment Station and by Dr. William J. Hooker of the University of Wisconsin. A number of different methods as recommended in the literature for obtaining germination of the spores were tried with little success. Chupp (4) recommended germinating the spores in a muck soil filtrate (pH not specified) and incubating them at an optimum temperature of 28 degrees Centigrade. He found that germination dropped rapidly as the incubation temperature was lowered and he was able to get little or no germination at room temperature. He was unable to obtain infection of cabbage seedlings in the greenhouse during the winter. He was also unable to obtain the germination of the spores in distilled water. Wellman (37) on the other hand found the optimum temperature for germination

to be not over twenty-five degrees centigrade with the percentage of germination rapidly dropping off as the incubation temperature was raised. He reported germination as being good in distilled water. Ledingham in a letter to the author reported that he found germination to be satisfactory when the spores were wet and dried a number of times and then incubated at room temperature in tap water with a pH of 8. He also used distilled water and obtained satisfactory germination. He did recommend, however, that the spores be germinated in considerable quantity because there seemed to be some mass effect. These ways were tried as recommended including placing sterile, excised root tips into the cultures in hope that the presence of the living host tissue might have a stimulatory effect on the germination of the spores. Rain water and melted snow water were tried. The pH was manipulated by adding small amounts of lactic, acetic and hydrochloric acid to the various media. Minute amounts of hydrogen peroxide were added to the media in an attempt to supply oxygen to the spores. Oxygen and air were bubbled through the cultures in an attempt to induce germination. Results were either entirely negative or the germination was so very slight that it was impossible to obtain the zoospores. A spore was once observed germinating, by both Dr. Bessey and myself, which had become attached to one of the root hairs of an excised root placed in the culture. The spore case was seen to be split and a small amount of protoplasm had oozed out. Attached to this bit of protoplasm two actively beating flagella could be detected. Unfortunately this was lost in attempting to transfer the

rootlet from the culture dish to a slide for staining. Our observation confirms those of Wellman (37) on the germination of the spore. He reports that flagella are produced almost immediately after the first bit of cytoplasm oozes through the break in the spore case and from that time on the partially germinated spore swims about actively. As considerable time may elaps before germination is complete it makes the last stages of germination exceedingly hard to observe. It is not altogether surprising that spores of P. brassicae should vary in their requirements for germination because the existence of biological races in P. brassicae has been shown by Walker (36).

The best germination obtained was obtained under the following conditions: infected roots which had been frozem and thawed several times over a two month period were macerated in a mortar. Distilled water was added and the mixture stirred up to place the liberated spores in suspension. The coarser material was allowed to settle to the bottom and the supernatant liquid with the spores in suspension was decanted off and strained through cheese cloth. It was then placed in the centrifuge and rotated slowly to throw the coarser plant material to the bottom. The spore suspension was then poured into another centrifuge tube and centrifuged again, this time more rapidly so that the spores were thrown down. The water containing most of the bacteria was then poured off. Sterile distilled water was added and the spores stirred up into a suspension again. They were centrifuged once again. This washing was repeated a number of times depending on how numerous the bacteria were originally.

The spores were eventually left to germinate in a Syracuse watch glass containing sterile distilled water made just acid by adding acetic acid with litmus paper as the indicator. The spores were incubated at twenty-five degrees centigrade. On the third day a fair degree of germination had taken place.

Zoospores were collected by means of a micro pipette and placed on clean slides. The zoospores were then killed and fixed by a forty-five second exposure to the fumes of a two percent solution of osmic acid. They were than stained by means of the Löffler technique. Those slides selected as most satisfactory were those that were mordanted for forty-five seconds and stained for one minute.

The zoospores were all of the biflagellate type and in most cases markedly heterokont. In some of them the shorter flagellum was so reduced as to be almost unnoticable. In one zoospore found the two flagella were very nearly isokont. The one flagellum was only slightly shorter than the other. The flagella were in all cases of the blunt ended type. No tinsels or whiplashes were to be found. In regard to the absence of whiplashes this agrees with the work done by Ledingham. In his work he used the Cotner stain. This type of stain is good for showing the whiplash. In none of the zoospores in his photomicrographs is there a whiplash type of flagellum to be seen. No zoospore had a tinsel type of flagellum. No cytological stains were made.

Nowakowskiella sp. Schroter (1892:82): The zoospores used were obtained from Mr. John M. Roberts from a culture that he was

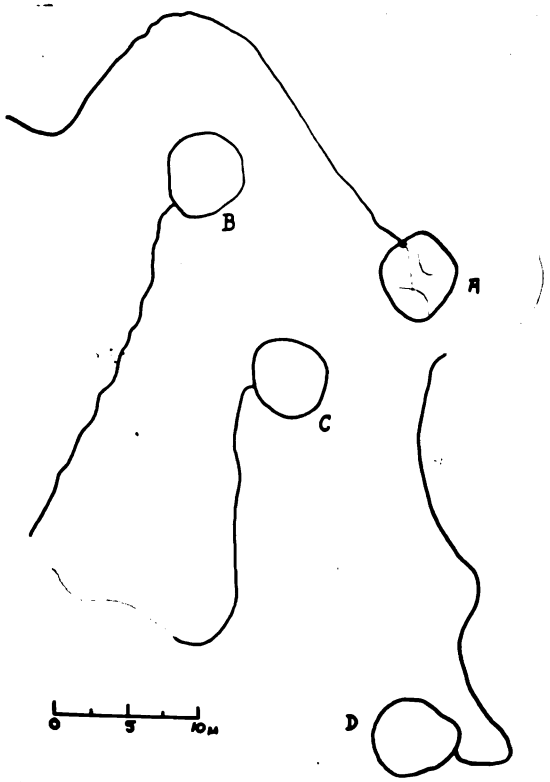

Fig. 8. Flagellar structure of the swarm cell of Nowakowskiella sp.

Fig. 9. Flagellar structure of the swarm cells of Synchytrium decipiens.

using for his research on the general cytology of the fungus. Liberation of the zoospores was induced by mounting the zoosporangia in a hanging drop of distilled water and chilling slightly. This caused almost instant release of the zoospores. Zoospores were collected with a micro pipette and transferred to clean slides where they were killed and fixed by a thirty second exposure to the fumes of a two percent osmic acid solution. The smears were allowed to dry in a dust free chamber and stained with a Löffler stain. These preparations were mounted in Canadian balsam. The best results were obtained by treating the slides with mordant for three quarters of a minute and with the stain for half a minute. Cytologically stained mounts were obtained from Mr. Roberts. They had been stained by the Cotner method.

Two types of flagella were observed on the slides stained by the Löffler method. These were the definite whiplash type and the blunt ended flagellum. The whiplash varied in length from very short to some having the whiplash as long as the flagellum itself. It was found that in the cases where the whiplash was extremely long there was a corresponding reduction in the length of the flagellum proper. Less than one percent of the zoospores had two flagella. These flagella were both of the whiplash type. Mr. Roberts interprets these biflagellate but uninucleate zoospores as abnormal forms.

The cytological stains show that the flagellum arises from a basal granule or blepharoplast located just under the cell membrane. A rhizoplast connects the blepharoplast with

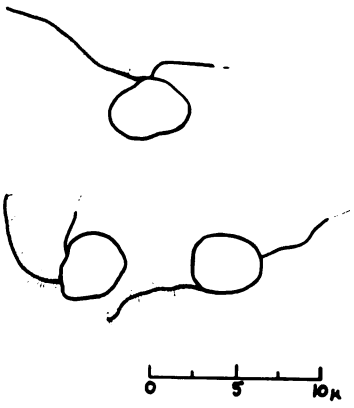

Fig. 10. Flagellar structure of the zoospore of Pythium sp.

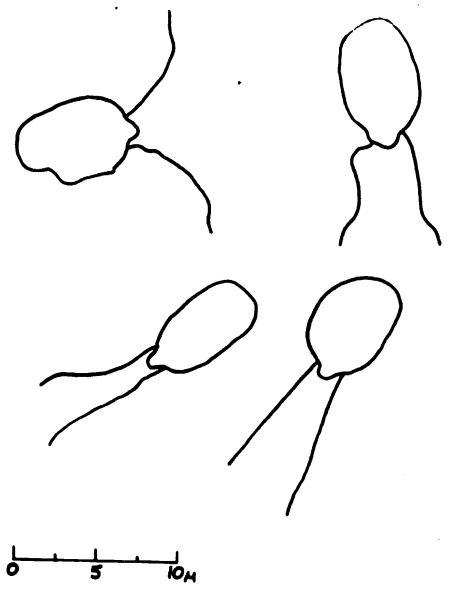

Fig. 8. Flagellar structure of the swarm cell of Clamydomonas sp.

the nuclear cap. The rhizoplast seems to pass near a large refractile vacuole in most cases. The significance of this is not clear. The abnormal biflagellate zoospores have not yet been investigated satisfactoryily.

Synchytrium decipiens Farlow: The specimen used was collected by Dr. Bessey on a leaf of Amphicarpa dioica. The zoosporangia were scraped from the sori on the leaf and transferred to a drop of distilled water in a hanging drop culture. They germinated in three hours at room temperature. The zoospores were collected by a micro pipette and transferred to clean slides where they were killed and fixed by exposing them to the fumes of a two percent solution of osmic acid for thirty seconds, then dried in a dust free chamber for twenty-four hours. These smears were stained with a Löffler stain.

Only uniflagellate zoospores were found to occur. These flagella were all of the whipash or blunt ended type. The whips were relatively short, varying from none at all to about one micron in length. Approximately fifteen percent had no whiplash at all. No cytological studies were made because no biflagellate zoospores were found.

Pythium sp. Pringsheim (1858): The specimen used was collected by placing a hemp seed in a petri dish with water from the Red Cedar River. A pure culture was obtained as far as fungi were concerned by transferring a number of zoosporangia into a petri dish containing some hemp seeds in sterile river water. Zoosporangia were induced to germinate by placing them in distilled water in a hanging drop culture and chilling slightly. Zoo-

spores were produced within an hour. These zoospores were collected by means of a micro pipette and because of their great numbers placed in drops of distilled water on clean slides. These were then exposed to the fumes of a two percent solution of osmic acid for thirty seconds. The smears were dried for twenty-four hours and then stained by the Löffler method.

The flagellar stains showed a very consistant type of flagellation throughout. There was a "flimmer" or tinsel type flagellum densely covered with cytoplasmic filaments and a whiplash type of flagellum. The whiplash flagellum was remarkably constant in having whiplashes of approximately the same length, the length of the whiplash being about .8 of a micron. In no other form studied was the flagellation so constant. No cytological stain was made because the flagellation was consistent in all cases with that described for the species.

Clamydomonas sp. Ehrenberg, 1833: The specimens of Clamydomonas used were collected from a sand culture growing in the greenhouse. These were placed on clean slides and killed by exposure to the fumes of two percent osmic acid for a minute and a half. It was found that in general algae required a longer exposure to osmic acid fumes to properly kill and fix than did the zoospores of fungi. The smears were left to dry in a dust free chamber for twenty-four hours and then stained with the Löffler stain. The treatment which seemed to give the best results was to treat with the mordant for two minutes and with the stain for half a minute. These were then dried for twenty-four hours and mounted in Canadian balsam.

The flagellar stain showed the two flagella to be of the whiplash type. The length of the whiplash varied from none at all to about half a micron in length. Approximately twenty-five percent showed no whiplash at all on either flagellum, thirty percent with a whiplash on one of the flagella, and forty-five percent with whiplashes on both flagella.

No cytological stain was made because the flagellation was consistant with that described for the genus and also because Smith in his text (Cryptogamic Botany Vol. 1) gives a very good description and drawing of the neuromotor apparatus for Clamydomonas.

Cladophora glomerata Kutzing, 1843: The Cladophora used in these studies was collected in late June from rocks in the Red Cedar River. A number of the terminal cells were seen to be swollen and rounded and it was supposed that they were about to produce zoospores. To initiate zoospore production in algae it is suggested that one variously treat the alga by removing it from swiftly running water to still; placing in the dark; placeing in bright light; placing in distilled water, etc. None of these ways seemed to have the desired effect. When the swollen cells were placed in distilled water plasmoptosis took place and the contents of the cells were discharged in an amorphous mass. Finally the cells were left in a hanging drop culture of sterile river water. After three days what were thought to be zoospores were observed to be swimming around in the drop and the enlarged cells were seen to have been emptied through a round hole usually on the side of the cell. The swarmers were

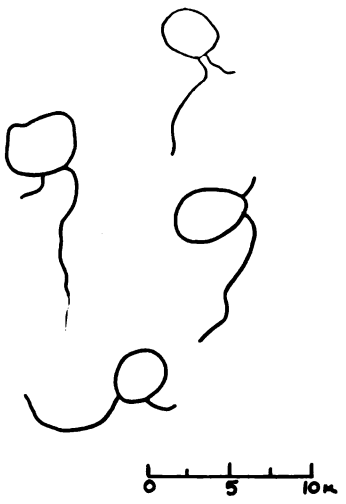

Fig. 12. Flagellar structure of the gametes of Cladophora glomerata.

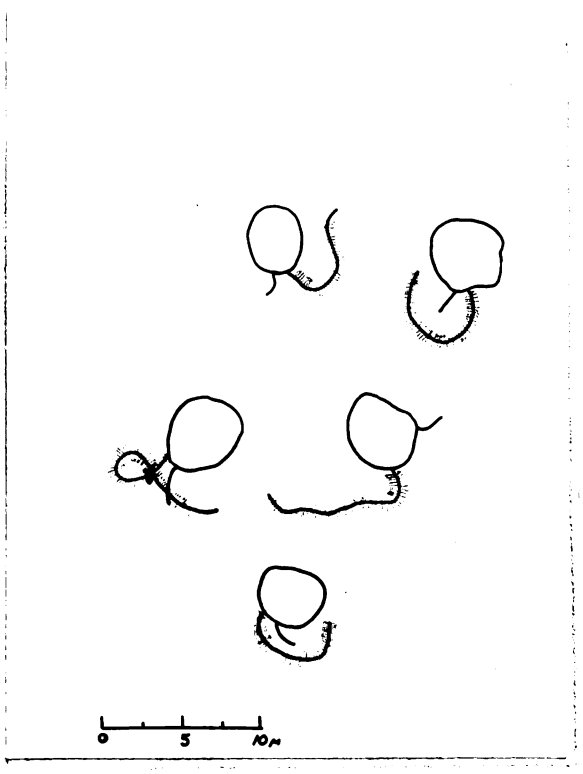

Fig. 13. Flagellar structure of the swarm cells of Botrydium granulatum.

collected in the usual manner with a micro pipette and transferred to clean slides. They were exposed to the fumes of two percent osmic acid for a minute and allowed to dry for several hours and then stained with the Löffler stain. It was found best to apply the mordant for one and three-fourths minutes and the stain for half a minute. Upon examination it was seen that they were gametes rather than zoospores as they had only two flagella. The gametes were markedly heterokont with the longer flagellum about three times longer than the shorter flagellum. One zoospore was seen to have a well developed whiplash on the longer flagellum. In all other cases the two flagella were all of the blunt ended variety. No cytological stains were made. A number of gametes appeared to have only one flagellum, but this may be explained by the fact that the shorter flagellum could very easily be hidden from view by the cell body. None of the gametes had tinsel type flagella.

Botrydium granulatum (L.) Grev: The specimens used in this study were collected by me both in the spring and fall in Field 19. According to the description of the genus and species they are supposed to produce zoospores when flooded with water. I was unable to stimulate zoospore production in this way. Instead aplanospores were produced which probably represented encysted zoospores. It was found that these aplanospores could be sown on culture media made by adding agar to a soil filtrate solution. On this medium the spores germinated to form a germ tube. This eventually grew to form an irregularly shaped body which in about a weeks time produced zoospores. These zoospores escaped

through a hole dissolved in the cell wall at almost any point. These zoospores were collected with a micro pipette, killed and fixed by exposure to the fumes of two percent osmic acid for a minute and a half and then allowed to dry in a dust free chamber for twenty-four hours. The smears were then stained with a Löffler stain. It was found most satisfactory to mordant for a minute and a half and stain for half a minute. The stained specimens were dried for twenty-four hours and mounted in Canadian balsam.

Upon examination it was seen that the zoospores were markedly heterocont. The longer flagellum was invariably of the tinsel type while the shorter flagellum was without tinsels and was of the blunt ended variety. In no case was a definite whiplash seen. No cytological stain was made.

Ulothrix sp. Kutzing, 1833: The Ulothrix used in these studies was found growing in the pool in the botany greenhouse. It was placed in culture dishes filled with distilled water and zoospores formed in many of the cells. Various ways were tried to induce the liberation of the zoospores from the cells but with no success. Finally it was found that by manipulating the filaments vigorously with a teasing needle a number of the gravid cells could be broken and the zoospores liberated.

The zoospores were collected and placed on slides in the usual manner. They were killed by a 60 second exposure to the fumes of a two percent osmic acid solution and dried for twenty-four hours, then stained with a Löffler stain. It was found most satisfactory to mordant for one minute and stain for

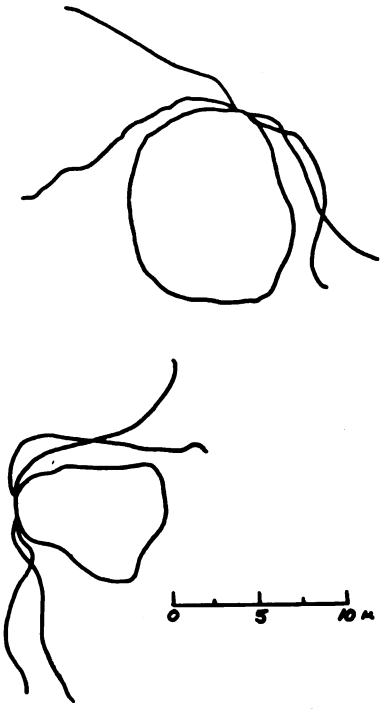

Fig. 14. Flagellar structure of the swarm spores of Ulothrix sp.

one minute. The slides were dried and mounted in Canadian balsam in preparation for examination.

It was found that all four flagella were consistently of the same length and all of the blunt ended variety. None were seen with tinsel type flagella. No cytological stain was made.

SUMMARY AND CONCLUSIONS

Mycetozoa and Plasmodiophora: The work done on this research problem has confirmed the contention of Sinoto and Yuasa (29, 38) that the swarm cells of the Mycetozoa have two blepharoplasts. It shows that the Mycetozoa may be regarded as potentially biflagellate and that as Gilbert (10) and the Japanese authors mentioned above pointed out, actually, two flagella are frequently found. The writer definitely demonstrated their presence in Stemonitis ferruginea, S. fusca and Fuligo septica. It establishes the type of flagellum for these forms and by inference the entire Mycetozoa as being the whiplash type or a modification of that type, i.e. blunt ended or knobbed.

A germinating spore of Plasmodiophora brassicae was seen to have two actively beating flagella thus confirming Ledingham's investigation that indicated that the zoospores of P. brassicae were biflagellate. The investigations of the flagella types of this swarm cell lend support to the view that the Plasmodiophorales are closely allied to the Mycetozoa in that their flagellation is of the Mycetozoa type. Certain authors wish to place the members of the Plasmodiophorales in the family Woroninaceae. Some members of this family however have

been shown to have the tinsel type for one flagellum and the whiplash type for the other. Therefore P. brassicae, the type genus and species of the family and order Plasmodiophoraceae and the Plasmodiophorales, must of necessity be excluded from relationship to the Woroninaceae due to its type of flagellation.

Pythium sp.: The work done on Pythium sp. confirms the work of Vlk and Couch (6, 34, 35) and helps to establish the flagellation of the Peronosporales as being the tinsel plus the whiplahs type.

Synchytrium decipiens and Nowakowskiella sp.: This investigation establishes the flagellar type of these two genera to be of the whiplash type. This would strongly suggest that the flagellar type for the entire Chytridiales (in the narrow sense of the term) should be of the whiplash or modified whiplash type.

Chlamydomonas sp.: Vlk's investigation showing the flagella of Chlamydomonas to be of the whiplash type are confirmed by my results which show them to be of the whiplash or modified whiplash type.

Botrydium granulosum: These investigations show the flagella of this form to be of the tinsel plus whiplash type and helps, with the investigations by Vlk, to establish this as the type of flagellation for the Heterokont algae.

Cladophora glomerata: The zoospores of this genus still remain uninvestigated. The gametes, however, are now seen to be markedly heterokont and to have blunt ended flagella, nei-

ther of the tinsel type.

Ulothrix sp.: The flagella of this genus are seen to be of the blunt ended type by these investigations. This confirms Vlk's findings and helps to establish this as the type for the group as a whole.

Some interesting deductions and observations should be mentioned as follows: Evidence has been presented in this paper that the ordinary blunt ended flagellum as well as the knobbed flagellum are only modifications of the whiplash type. The knobbed flagellum, as described in the literature prior to this paper is described as an abnormal and degenerate condition due to age etc. This is clearly not the case in the organisms reported in this paper. Instead the indication is very strong that the knobbed flagellum is a modification of the whiplash type flagellum and not degenerative in its nature. It is interesting to note that in all the organisms investigated so far in which there are the two types of flagella, tinsel plus whiplash, and which are of different length (heterokont) it is always the longer of the two flagella which is of the tinsel type.

LITERATURE CITED

1. Berdan, H. B. A developmental study of three saprophytic Chytrids. Am. Jour. Bot. 28:422-438. 1941.

2. Bessey, Ernst A. A text-book of Mycology. xv plus 495 pp. 139 figs. P. Blakiston's Son and Co., Inc., 1012 Walnut Street, Philadelphia, Penn. 1939.

3. Bessey, Ernst A. Some problems in fungus phylogeny. Mycologia 34:344-379. 5 figs. 1942.

4. Chupp, C. Studies on clubroot of cruciferous plants. Cornell Univ. Agr. Exp. Sta. Bull. 387. 1917.

5. Couch, J. N. Observations on cilia of aquatic Phycomycetes. Science 88:478 (preliminary report). 1938.

6. Couch, J. N. The structure and action of the cilia in some aquatic Phycomycetes. Am. Jour. of Bot. 28:704-713. 3 pls. 1941.

7. Curtis, K. M. The life history and cytology of Synchytrium endobioticum (Schilb) Perc., the cause of wart disease in potatoes. Phil. Trans. Roy. Soc. London B 210:409-478. 1921.

8. Fischer, Alfred. Uber die Geisseln einiger Flagellaten. Jarb. f. Wiss. Bot. 26:187-235. 1894.

9. Fitzpatrick, Harry Morton. The lower fungi. Phycomycetes. xi plus 331 pp. 112 figs. McGraw-Hill Book Co., New York. 1930.

10. Gilbert, Frank A. On the occurrence of biflagellate swarm cells in certain Myxomycetes. Mycologia 19:244-283. 2 figs. 1927.

11. Gilbert, Frank A. A study of the method of spore germination in Myxomycetes. Am. Jour. Bot. 15:345-352. pl. 1929.

12. Gilbert, Frank A. Factors influencing the germination of Myxomycete spores. Am. Jour. Bot. 16:280-286. 1929.

13. Hillegas, A. B. The cytology of Endochytrium operculatum (de Wildman) Karling in relation to its development and organization. Bull. Torrey Club 67:1-32. 1940.

14. Jahn, E. Myxomycetenstudien Berichte der Deutscher Botanischen Gesellschaft 58:182-199. 1 fig. 1940.

15. Johansen, Donald Alexander. Plant microtechnique. xi plus 523 pp. 110 figs. McGraw-Hill Book Company, Inc., New York and London. 1940.

16. Jones, Philip M. Morphology and cultural history of Plasmodiophora brassicae. Archiv für Protistendunde 62:313-324. Pls. 15-21. 1 fig. 1928.

17. Karling, John S. The Plasmodiophorales. ix plus 174 pp. 17 plates. Published by the Author, New York. 1942.

18. Kudo, Richard Roksabro. Protozoology. xi plus 689 pp. 201 figs. Charles C. Thomas, Springfield, Ill., and Baltimore. 1939.

19. Ledingham, G. A. Zoospore ciliation in the Plasmodiophorales. Canadian Jour. Res. C, 17:38. 1939.

20. Ledingham, G. A. Occurrence of zoosporangia in Spongospora subterranea (Wallroth) Lagerheim. Nature 135: 394. 1934.

21. Ledingham, G. A. Zoospore ciliation in the Plasmodiophorales. Nature 133:534. 1 pl. 1935.

22. Lister, Arthur. A monograph of the Mycetozoa. A descriptive catalogue of the species in the herbarium of the British Museum. Third edition--revised by Gulielma Lister. xxxii plus 296 pp. 223 plates. 56 figs. Trustees of the British Museum, London. 1925.

23. Löffler. Eine neue Methode zum Farben der Mikroorgansimen. Zb. Bakter. 6 & 7. 1889-1890.

24 MacBride, Thomas H. The North American slime-moulds. A descriptive list of all species of Myxomycetes hitherto reported from the continent of North America. xvii plus 347 pp. 33 plates. The MacMillan Company, New York. 1922.

25. Mac Bride, Thomas H. and G. W. Martin. The Myxomycetes. A descriptive list of the known species with special reference to those occurring in North America. The MacMillan Company, New York. 1934.

26. Mez, Carl. Versuch einer Stammesgeschichte des Pilzreichs. Schriften der Königsberger Gelehrten Gesellschaft Naturwissenschaftliche Klasse 6:1-58. 1 fig. 1929.

27. Minchin, E. A. An introduction to the study of the Protozoa with special reference to the parasitic forms. xi plus 511 pp. 194 figs. Edward Arnold, London. 1922.

28. Petersen, J. B. Beiträge zur Kenntnis der Flagellatengeisseln. Bot. Tidsskrift 40:342-389. 1929.

29. Sinoto, Y. and A. Yuasa. Studies in the cytology of reproductive cells I. On the planocytes in five forms of

Myxomycetes. Bot. Mag. (Tokyo) 48:420-729. Figs. 1-19. 1934.

30. Smart, Robert F. Influence of certain external factors on spore germination in the Myxomycetes. Am. Jour. Bot. 24:174-159. 1937.

31. Smith, Gilbert M. The fresh-water Algae of the United States. xi plus 716 pp. 449 figs. McGraw-Hill Book Company, Inc., New York and London. 1933.

32. Smith, Gilbert M. Cryptogamic Botany Vol. I. viii plus 545 pp. 299 figs. McGraw-Hill Book Company, Inc., New York and London. 1938.

33. Sparrow, Fredrick K. Jr. Aquatic Phycomycetes. xxi plus 785 pp. 1 pl. 69 figs. University of Michigan Press, Ann Arbor, Michigan. 1948.

34. Vlk, Wladimir. Uber den Bau der Geissel. Arch. f. Protistenkunde 90:448-488. 1938.

35. Vlk, Wladimir. Uber die Geisselstruktur der Saprolegniaceenschwärmer. Archiv f. Protistenkunde 92:157-160. 1939.

36. Walker, J. C. Physiological races in Plasmodiophora brassicae Woronin. Jour. Agr. Res. 59:815. 1939.

37 Wellman, F. L. Clubroot of Crucifers. U. S. D. A. Tech. Bull. 781. 1930.

38. Yuasa, Akira. Studies in the cytology of reproductive cells III. The genesis of the flagellum in the planocytes of Fuligo septica Gmelin. Bot. Mag. (Tokyo) 49:538-545. Figs. 1-24. 1935.

39. Weston, W. H. The bearing of recent investigations on the interrelationships of the aquatic Phycomycetes. Proc. 5. Internat. Bot. Congr. 1-266. 1935.