

1961

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McManus, Mary Annunciata and McDade, Ferrell (1961) "Cytological Studies of the Thread Phase of Ceratiomyxa," *Proceedings of the Iowa Academy of Science*, 68(1), 79-85.

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Cytological Studies of the Thread Phase of *Ceratiomyxa*¹

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Abstract. The protoplast which emerges from the spore of *Ceratiomyxa fruticulosa* produces eight swarm cells. The published accounts of the life cycle differ in their descriptions of the steps between germination of the spore and formation of the swarm cells. The thread stage is described in only one previous account. A further description of this stage and illustrations of it are presented here.

Ever since it was first described by Micheli in 1729, *Ceratiomyxa* has held the interest of mycologists. It is found abundantly in most parts of the world, and has been studied since early times. With the exception of Rostafinski's [Cooke (1) says that Rostafinski appeared to be annoyed because it upset the unity of his system of classification], all the important monographs on the Myxomycetes have devoted considerable space to this organism, and the whole or a part of its life cycle has been studied and reported on at least five times (2, 3, 4, 5, 6). The most complete and detailed account is that of Gilbert (5).

Ceratiomyxa is distinguished from the great majority of species of Myxomycetes in bearing its spores externally (Figure 1B) on the pillars which constitute the fruiting body (Figure 1A). All others bear their spores enclosed within a sac-like peridium (Figure 1C) and are therefore placed in the subclass Myxogastromycetidae. *Ceratiomyxa* is the only genus in the subclass Ceratiomyxomycetidae. Each spore is attached to the pillar by its own individual stipe, and Gilbert (5) therefore refers to the spores as "single-spored sporangia".

The life cycle of *Ceratiomyxa* differs at several points from the usual cycle for the Myxogastromycetidae. One major point of interest is at the germination of the spore. Soon after it emerges from the spore case, the protoplast is divided into 8 portions, which for a time remain close together as an "octette". Then each member of the octette develops a flagellum and becomes a swarm cell. Descriptions of the steps between spore and octette differ at least slightly in most of the published accounts.

PREVIOUS ACCOUNTS OF THE GERMINATION OF THE SPORE

Famintzin and Woronin's Account. Famintzin and Woronin (2) reported that after the spores have been in wood extract for

¹ This research was supported in part by a grant from the Iowa Heart Association.

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about 30 hours the protoplast emerges and remains quiet for some time, showing only intermittent amoeboid movements. It then constricts to form two parts. Each of these then divides, and a tetrad is formed. Another division of each member of the tetrad forms the octette. Each member of the octette becomes a swarm cell, which later loses its flagellum and becomes a myxamoeba.

Olive (4) implies that his own observations of the germination process agree with those of Famintzin and Woronin.

Lister's Account. Lister (7) describes the mature *Ceratiomyxa* spore as containing 4 nuclei. Almost immediately after placing spores in rain water, he saw the membranous and colorless spore wall slip free from the protoplasmic contents, often with a sudden jerk. The naked spore remained unchanged for 6 to 9 hours and then began an amoeboid movement, sometimes with the projection of pointed pseudopodia. At this stage, Lister says the 4 nuclei divide by karyokinesis to form 8 nuclei. The protoplast then divides into two parts.

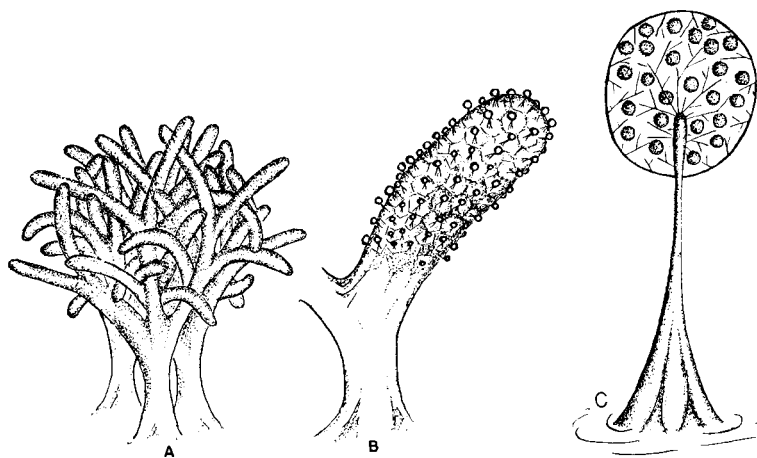


Figure 1. A. Fructification of *Ceratiomyxa fruticulosa*. B. Pillar bearing sporophores externally. C. Sporangium of one of the Myxogastromycetidae. Spores enclosed by peridium. All diagrammatic.

His illustrations then show what appears to be a 3-part condition of the protoplast, which the text does not mention. This is followed by a second and a third division, so that about an hour after movement was first observed the protoplast has divided into 8 equal segments, each containing one nucleus. He remarks that the members of the octette may remain attached to each other by narrow bridges before the flagella develop, but each eventually becomes detached from the group and swims away. He observes that swarm cells can change to myxamoebae.

Gilbert's Account. The account of Gilbert (5) differs on several points from those just discussed. He described the germination process as being of the autocatalytic or enzymatic type, in which a pore is formed in the spore wall. The protoplast slowly makes its way out of the spore through this pore; it is not released by a sudden cracking and snapping off of the spore wall. The globose protoplast then remains quiet or slightly amoeboid for from a few minutes to an hour.

The following stage of development as described by Gilbert has not been found in any other study of the process until now. He remarks,

Curiously enough, I have found no reference in the literature to the next phase in the process of swarmcell formation. It was the thing that I observed, and it is always the easiest phase to see in a culture. For convenience, I have termed it the "thread" phase. Following the globose inactive condition at the conclusion of germination, the protoplast slowly elongates and starts a slow irregular bending movement. During elongation a contraction and renewed elongation may occur several times. Finally, the thread condition is reached and is usually maintained for several hours. The movement of this thread is bending, contraction, or expansion but not at all a flowing or amoeboid movement. The nuclei are usually evenly spaced along the thread. No nuclear changes are apparent during this phase of development. The nuclei are of the type found in the mature spore. One thread with eight nuclei was found, showing that it is possible for a binucleate protospore to mature and germinate normally.

After completion of the thread phase, Gilbert describes a resumption of the globose condition. The protoplasm cleaves around the four nuclei to produce a tetrad. He remarks that the four-nucleate mass never forms a two-parted condition. The nuclei lie against the outer wall in each segment, and after only a minute or two, constriction and cleavage of each segment of the tetrad begins and an octette is formed. Mitosis is concomitant with this division and he was able to count eight chromosomes at metaphase. The nuclei of the octette are likewise appressed to the outer wall. Formation of tetrad and octette occurs in about one half hour. Each member of the octette then becomes flagellate and swims away.

McManus' Account. Much of the information obtained by the above studies was from fixed and stained materials. McManus (6) reported observations on living material from specimens collected in 1957 near St. Louis, Missouri. These confirmed many of Gilbert's findings, but differed in some details. She agreed in finding no splitting of the spore case or jerking of it to free the protoplast, and no division of the protoplast into two parts, as had been reported to occur by previous writers. Division of the protoplast into tetrad and octette and formation of swarm cells was found essentially as Gilbert had reported them, but no thread stage was found. The absence of the thread stage from

the sequence was noted. Spores used in this study were spherical rather than ovoid and germinated in 12 to 24 hours until they were 6 months old.

Because of the discrepancies in the various accounts of the steps from spore germination to swarm cell formation, further investigation of this part of the life cycle was undertaken.

MATERIALS AND METHODS

In 1959 and 1960, freshly formed spores from 15 separate fruitings of *Ceratiomyxa fruticulosa* from various localities in Iowa (all near Cedar Rapids, Iowa City, or Waterloo) were germinated in hanging drops of dilute elm bark extract. They were examined at frequent intervals in the effort to find the thread stage. When suitable stages were found, material was transferred from the hanging drop to a plain slide with a wire loop. This was then either sealed under a cover slip with vaseline and examined further at high magnification or by phase contrast illumination, or was stained. Some such preparations were stained with Giemsa's without fixation, and some were fixed in osmic acid vapor and then stained with Giesma's or with gentian violet. Destaining was usually with clove oil.

RESULTS AND DISCUSSION

Spores from two freshly collected specimens did not germinate at all, in five others germination was less than 50%, and in all the rest germination was nearly 100%. Some of the specimens had fruited in moist chamber in the laboratory, and spores from these often germinated within an hour.

In no case was the spore wall seen to split or to separate from the protoplast with a jerk, and the division of the emerged protoplast into two parts was never observed.

In about 50% of the preparations, the thread stage was found. An attempt was made to correlate the presence of the thread stage with the age of the spores, the shape of the spores, the conditions of relative humidity under which fruiting must have occurred, season of the year, and gross morphology of the fructification. No positive correlation could be found.

The living threads are exactly as Gilbert described them (Figure 2,A). They lengthen and shorten, twist and bend, with a slow worm-like movement. They do not move from one place to another on the slide, but simply twist and turn and bend without moving from one field.

In some cases, the protoplast which emerged from the spore case remained quiet and globose for a time before assuming the thread shape; in others, it was a thread which emerged

from the spore. On some occasions when six preparations had been made at the same time from the same specimen, threads were found in several and were never found in the others. Threads were formed from spores which were only a few hours old and also from spores which were more than six months old. Some of the spores in preparations in which threads were found seemed to have germinated without passing through the thread stage. Since development is not absolutely synchronous, and since it was not possible for us to observe the preparations continuously, these may have passed through the thread stage quickly while not under observation.

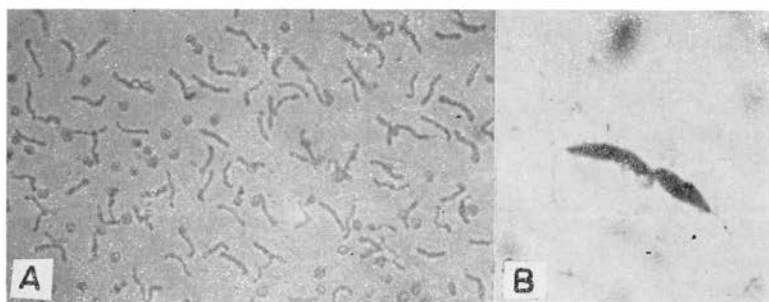


Figure 2. A. *Ceratiomyxa fruticulosa* threads and spores, unstained, X 200. B. Swarm cells in the process of fusion. Osmic acid vapor fixation, gentian violet stain, X about 900.

All the published descriptions of the germination of *Ceratiomyxa* spores, other than Gilbert's, have mentioned the amoeboid movements of the protoplast before it separates into segments. These movements are rather slow and sinuous, not unlike the worm-like movements of the thread. Lister (7) describes a "slow, amoeboid change of outline, sometimes accompanied by the projection of numerous pointed pseudopodia". Olive (4) says that the "naked protoplasmic contents of the spore remain quiet for some time in one spot, showing slight amoeboid movements". McManus (6) speaks of the protoplast within the spore case moving about slightly and says, "Even during this stage one gets a definite impression of four-partedness of the spore contents as they change position. . . . There is no progressive movement of the whole protoplast, but after a time the periphery becomes indented in one place, then pushes outward very slightly in another."

In the present investigation these movements were again noted. We also noted quite marked active movement of the spore before germination. These movements were of great interest to us, and we interpreted them at first as a passive response to the active movements of bacteria attached to the spore wall or in the surrounding medium. But prolonged ob-

servation led us to conclude that they were unmistakably active.

Considering together these observation and those of the various investigators who have not found threads, it seems possible that under certain conditions the protoplast may undergo whatever changes are occurring in the thread while still within the spore wall or while still globose after emergence. The thread might not be a constant part of the life cycle. Environmental conditions, physiological state of the protoplast, or the chance position of the nuclei at the beginning of their division, as explained below, might determine whether or not the plasma membrane stretches out to the elongate form.

We have observed newly formed threads under phase contrast illumination, and have not been able to see the four bodies which stain deeply after osmic acid fixation. These bodies do not stain with hematoxylin or with gentian violet in threads that have just formed; the stainable material seems to be scattered and peripheral (Figure 3,A). In older threads, the bodies stain readily and have the typical appearance of nuclei

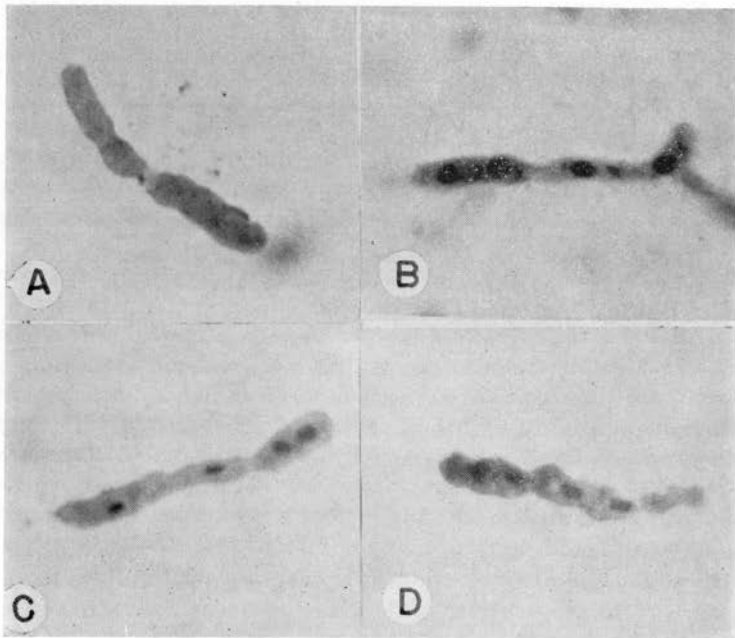


Figure 3. *Ceratiomyxa fruticulosa* threads fixed in osmic acid vapor and stained with gentian violet. Destained with clove oil. A. Thread which had just formed. Chromophilic material was scattered in tiny masses peripherally. B. Older thread. Four chromophilic bodies with the typical appearance of nuclei. C. Thread in which the chromophilic bodies are uneven in size, shape, and position. D. Thread in which the chromophilic masses are somewhat reminiscent of mitotic figures. All X about 900.

(Figure 3,B). This is rather strange behavior for nuclei, unless the nuclear material is scattered and gradually condenses in the thread stage, or unless a chromophilic material such as a protein is synthesized during the thread stage, and gradually accumulates.

The bodies must move about within the thread, because as seen when stained, they are not always evenly spaced nor always of the same size and shape (Figure 3,C). In some preparations their appearance resembles a mitotic figure (Figure 3,D), but we have never found a thread with eight nuclei. Gilbert figures a thread with eight nuclei, and Lister said that the four nuclei divide by karyokinesis before any plasmakinesis. It is possible that the bendings of the thread may be caused by the kinetic activities of the nuclei during division, when the thread stage occurs. When it does not occur, nuclear division might be occurring during the amoeboid movements before the first division of the protoplast. Formation or non-formation of the thread might depend on the relative positions of the four nuclei when their division begins. The physical attractions and repulsions which develop during karyokinesis might produce a thread if all nuclei were aligned when division began, or a sphere if they were at the points of a tetrahedron.

Fusion of the swarm cells as previously reported on *in vivo* studies by McManus (6) was confirmed here in stained material (Figure 2,B). The pulling apart until only a thread joins the fusing partners was again observed, and also the pulling together and apart again until gradually the substance of one swarm cell has passed into that of the other. The resulting amoeboid structure at first has two long flagella.

The number of flagella seen on the swarm cells was inconsistent; some had one and others appeared to have two, one long and the other very short, each with its own blepharoplast.

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