1977

Cytology of Chaetomidium fimeti

David D. Kimmel
Eastern Illinois University

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Cytology of Chaetomidium fimeti

(TITLE)

BY

David D. Kimmel

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1977
YEAR

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ACKNOWLEDGMENTS

I would like to thank Dr. Smith and Dr. Darding for their helpful review of the manuscript.

I would also like to sincerely thank my advisor, Dr. Speer, for his assistance and guidance throughout the course of this study.

At last I would like to acknowledge the willing help of Dr. Whiteside for his valuable suggestions and time given throughout the research of this problem.

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INTRODUCTION AND LITERATURE SURVEY

Previous cytomorphological studies of the Chaetomiaceae have been primarily concerned with the development of the ascocarp. Few species of this family, generally assigned to the Pyrenomycetes, have been subjected to a detailed cytological study. It is the purpose of this study to elucidate the life cycle and describe a definitive haploid chromosome number for Chaetomidium fimeti Fuckel, the type species for the small and rarely isolated genus Chaetomidium. Originally Chaetomidium fimeti was treated as a species of Chaetomium. It was Zoph (1881) who divided the genus Chaetomium into two subgenera, Euchaetomium for those species possessing an ostiole and Chaetomidium for those lacking an ostiole. Saccardo (1882) later elevated Chaetomidium to genus status in his important listing of all known species of fungi, Sylloge fungorum omnium hucusque cognitorum. This controversial decision has been accepted by Bainier (1910), Chivers (1915), Ames (1961), and Seth (1970) but refuted by von Arx and Muller (1954) and Munk (1957).

The life cycle of the majority of the Ascomycetes involves the formation of asexual conidia. The typical sexual cycle of the Ascomycetes involves gametangial contact of antheridia and ascogonia and subsequent passage of antheridial nuclei through the trichogyne into the ascogonium. This process

stimulates the ascogonium to produce papillae which ultimately become the ascogenous hyphae. Cells of the ascogenous hyphae are generally considered to contain two paired nuclei. Cells of the ascogenous hyphae may mature into croziers with one pair of nuclei located in the crook or penultimate cell of the crozier and single nuclei located in the tip or ultimate cell and in the basal or antepenultimate cell. Karyogamy occurs in the penultimate cell. The resulting diploid nucleus undergoes meiosis, typically with a subsequent mitotic division to produce eight ascospores (Alexopoulos, 1952).

Although a number of cytomorphological studies have been conducted on species of Chaetomium, the only study devoted to Chaetomidium was that of Whiteside (1962) who described ascocarp development of Chaetomidium fimeti as initiating from a hyphal branch. The subsequent irregular coiling originating at the apex of this hyphal outgrowth, which he referred to as the ascogonium, is covered over by branches originating near the base of the outgrowth on the main hyphal branch. These basal branches form the perithecial wall; the ascogenous hyphae originate in the basal region of the centrum and he considered these as derived from the coiled ascogonium. The ascogenous hyphae were described as uninucleate and lacking croziers.

Chaetomidium, as illustrated in Whiteside's study, does not follow the normal morphological development typical of Ascomycetes. The asexual cycle which forms conidia is lacking. Furthermore, he observed no evidence of antheridial

or trichogyne formation during the development of the ascogonia and the subsequent ascogenous hyphae.

MATERIALS AND METHODS

Cultures of Chaetomidium fimeti were supplied by Dr. Wesley Whiteside of Eastern Illinois University. The cultures were grown on 10% agar. The agar was autoclaved and poured into sterile Petri dishes containing sterilized flakes of oatmeal. The fungus was incubated at room temperature and perithecia were harvested between the twentieth and twenty-fifth day of growth and fixed in Carnoy's fluid (three parts absolute ethanol to one part glacial acetic acid). After a twenty-four hour fixation period the perithecia were transfered to a propionic acid-carmine solution for nuclear staining. It was found that storage of perithecia at 4° C. in the stain solution would preserve cellular integrity for an indefinite period of time.

The perithecia were removed from the stain solution, placed on a glass slide and ruptured with fine needles in order to remove the contents. The perithecial walls were then discarded. The propio-carmine stain solution and Hoyer's medium were then added to the ascogenous hyphae and the slide was coverslipped. Gentle pressure and heat were applied in order to facilitate nuclear staining. Cytological observations of various phases of mitosis were examined and photographed using a Nikon microscope and Microflex model AFM camera. Kodak Plus X pancromatic film was used for all photomicrographs.

RESULTS AND DISCUSSION

Mature perithecial formation is evident after approximately a twenty-day incubation period at room temperature. At this time the perithecia have produced the characteristic long, dark-brown, basal appendages and the somewhat shorter, golden-brown appendages (Figure 1).

Although the purpose of this study was to elucidate the life cycle of Chaetomidium fimeti, I was unable to completely demonstrate the process. There was difficulty encountered in establishing the time and location of karyogamy and meiosis. Typically, in the Ascomycetes, karyogamy, followed by meiosis, occurs in the ascus. In the course of this study no asci were observed in a state of nuclear division. Therefore, one may consider three possible ways that the life cycle of Chaetomidium fimeti might be completed with respect to the location of karyogamy and meiosis.

The first possibility is that the life cycle is completely haploid. There may be no fusion of nuclei; therefore, meiosis would not be necessary for ascospore production. The haploid nuclei would simply migrate into the ascus and undergo three mitotic divisions to produce the characteristic number of eight ascospores.

The second possibility is that an imcomplete mitotic division has taken place without cytokinesis, thus resulting in a diploid nucleus within the ascogenous hyphae. This

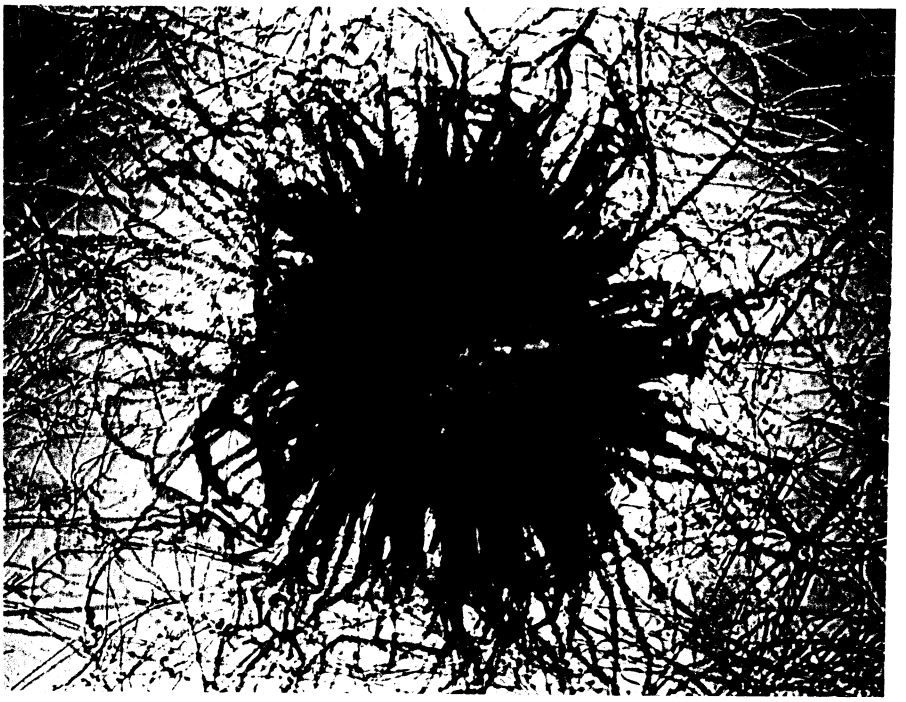

Figure 1. Mature perithecium with a dense network of appendages (x100).

diploid nucleus would then migrate into the ascus and undergo meiosis followed by mitosis to produce eight ascospores.

The final possiblity may be that a haploid nucleus migrates into the ascus and undergoes mitosis, with a nuclear membrane forming around the two sets of haploid chromosomes, resulting in a diploid condition in the ascus. This diploid nucleus would then undergo meiosis and mitosis to produce eight ascospores.

CONCLUSION

Of the three possibilities outlined, only the second possibility is supported by this study. Figure 2 shows a young ascogenous hypha prior to mitosis. Figure 3 illustrates an ascogenous hypha with synchronized mitosis having seven chromosomes. The next mitotic event within cells of the ascogenous hyphae results in a chromosome number of fourteen due to incomplete mitosis (Figures 4, 5, and 6). Cells of the ascogenous hyphae containing fourteen chromosomes might then give rise to young asci. The diploid nucleus containing fourteen chromosomes would then migrate from the ascogenous hyphae into the ascus where meiosis would take place. Each haploid nucleus would then undergo mitosis to produce eight haploid ascospores (Figure 7). However, no nuclei containing fourteen chromosomes were observed in developing young asci. And as stated earlier, no asci were observed undergoing meiosis.

Concerning the remaining possibilities the author discounts the first in that it would represent such an atypical departure from the life cycle of known Ascomycetes. The third possibility was also discounted since no asci were observed undergoing nuclear division.

Other cytomorphological investigations within the genus Chaetomium were conducted by Gries (1941). Through observations of sectioned material containing young asci, he

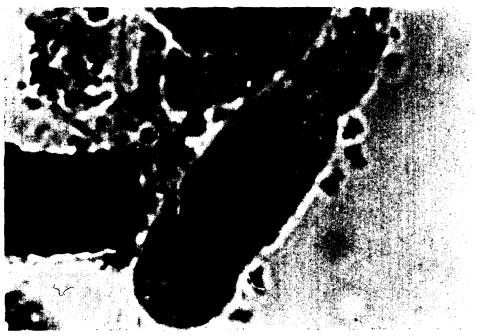

Figure 2. Young developing ascogenous hypha (x500).

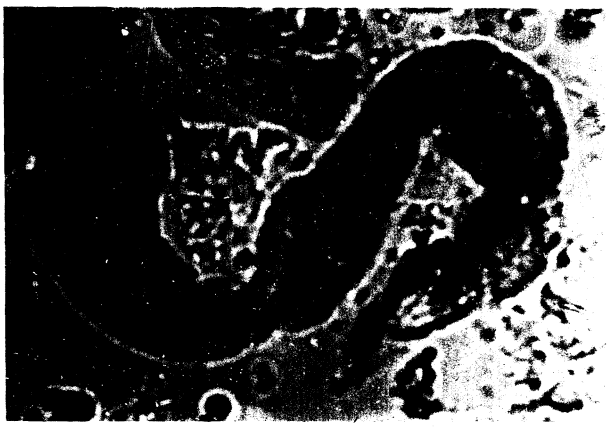

Figure 3. Metaphase of mitosis in ascogenous hypha showing mitotic synchronization. Each set of 7 chromosomes are separated by a septum (x500).

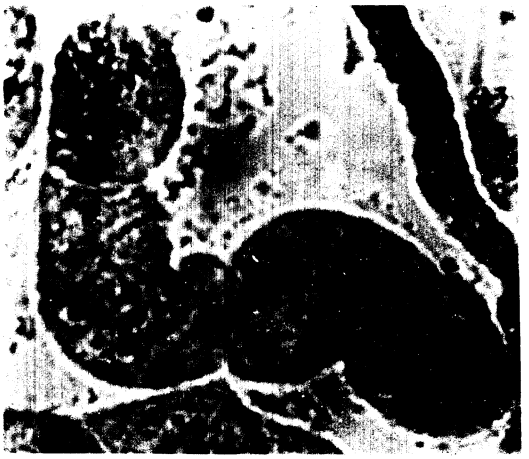

Figure 4. Incomplete mitosis in ascogenous hypha. Note the center cell is still synchronized in mitosis; each contains 14 chromosomes (x500).

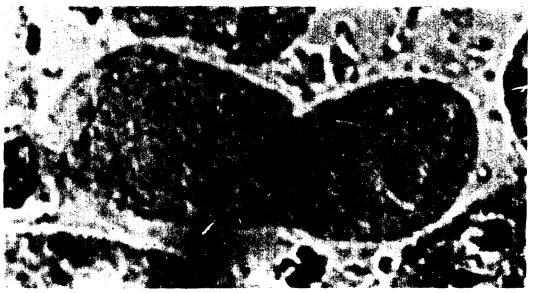

Figure 5. Ascogenous hypha possessing 14 chromosomes (x500).

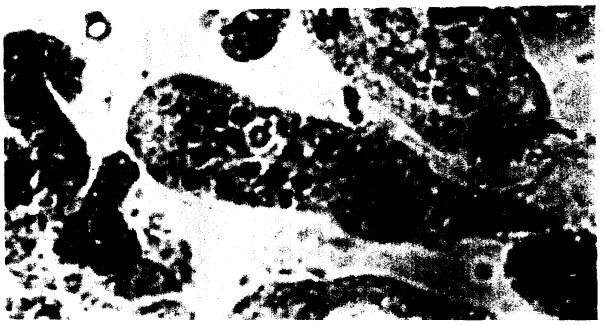

Figure 6. Ascogenous hypha possessing 14 chromosomes. Note elongation of hypha (x500).

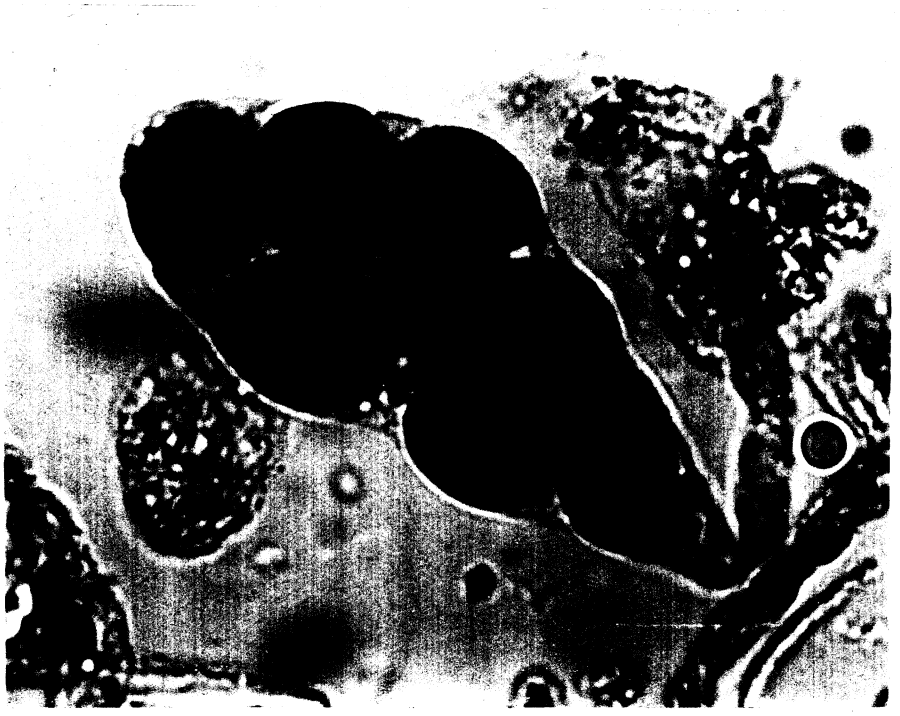

Figure 7. Ascus with 8 mature ascospores (x500).

was able to deduce the haploid chromosome number of Chaetomium kunzeanum Zoph (=globosum Kunze) as being eight. Through similar investigations Gries (1941) also found Chaetomium bostrychoides to possess a haploid chromosome number of six. A more recent study conducted by Brewer and Duncan (1968) involved observations of immature asci from squash preparations of Chaetomium cochliodes Palliser. The haploid number of chromosomes for this species is thought to be seven.

In the course of the author's study, a technique was developed for preserving perithecia for an indefinite period of time in propio-carmine solution without disrupting cellular integrity. Although the sexual cycle was not completely demonstrated through the inability to observe asci undergoing nuclear division, it can be reported for the first time, that the haploid chromosome number of Chaetomidium fimeti is seven.

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