A CYTOLOGICAL STUDY OF FERTILIZATION IN
ACHLYA HYPOGYNA COKER AND
PEMBERTON

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The question of fertilization in the Saprolegniaceae has long aroused interest among botanists. Pringsheim (1855), while he did not actually observe fertilization, believed that it took place. Later workers, de Bary (1884), Humphrey (1892), Hartog (1892–1895) studying various genera, came to the conclusion that fertilization did not occur. However, Trow (1895, 1904) disagreed with Hartog and showed without doubt that fertilization does occur in some species of the Saprolegniaceae.

Since Trow’s work further evidence that fertilization does occur has been demonstrated by Claussen (1908), Mücke (1900), Kasanowsky (1911), Carlson (1925), and Couch (1925).

MATERIALS AND METHODS

The fungus used as a basis for this study was secured from dead minnows in Tomahawk Lake, Wisconsin. Cultures were grown on boiled corn endosperm in distilled water. As the early cultural studies were made in the field, it was impossible to make single spore isolations; therefore growth from single sporanges was taken as a basis for classification. Cultural studies were made from time to time, and from Coker’s “Classification of the Saprolegniaceae” (1925) the species was identified as Achlya hypogyna Coker and Pemberton. This determination has been since verified by Mr. J. V. Harvey.

The material was preserved in formol-acetic-alcohol, imbedded by the paraffin method, and sections were cut to 5 microns in thickness. Overstaining with Haidenhain’s iron-alum haematoxylin with light green as a counter stain gave the best results.
OBSERVATION AND DISCUSSION

In Achlya hypogyna Coker and Pemberton, the oogones arise from hyphae on short lateral branches of the mycelium. In the formation of the oogene there is a streaming of the cytoplasm into the hyphal tip, forming in it a marked swelling. The streaming continues until the swelling has caused the hyphal tip to become almost spherical. A cross wall is now laid down at the base of this spherical tip and the young oogene is thus formed. The cytoplasm at this stage is multinucleate; conspicuous vacuoles are present, surrounded by cytoplasm containing granules (mitochondria) of varying size and shape. A small dark body resembling a nucleolus was observed in each of the nuclei. This body seems to be identical with that described by Hartog as a chromatin body, by Trow as a combination of a chromosome and a nucleolus, by Davis, Claussen, Mücke, and Carlson as a nucleolus. Shortly, there appears a definite vacuole near the center of the oogene. As this vacuole increases in size many of the nuclei migrate to the periphery of the oogene and show signs of degenerating. The remaining nuclei are found near the vacuole and vary as to number, from a few to as high as sixteen in some oogones. These nuclei undergo only one mitotic division (fig. 1). Many of the nuclei formed as a result of this division degenerate, leaving seemingly only those that are to function as nuclei of the eggs. These remaining nuclei enlarge slightly and are readily recognized (fig. 2). That there is only one division occurring within the oogene agrees with the conclusions of Davis, Claussen, Mücke, Kasanowsky, and Carlson in regard to closely related species. Trow (1904) describes the occurrence of two divisions in the oogene, the second of which he believed to be a reduction division.

The central vacuole after mitosis enlarges greatly by furrowing the cytoplasm (fig. 2). Further furrowing extends to the periphery of the oogene, leaving apparently small rounded portions of the cytoplasm extending into the vacuole. These partially rounded cytoplasmic masses are known as egg initials and their number depends upon the
number of nuclei that remained following the divisions and subsequent degeneration of some of the nuclei. This is evidently the case, because in each of the initials only one of these nuclei was found. Occasionally, however, an egg was found containing two nuclei. In no instance were extra nuclei found scattered about in the egg, unless they were degenerating (fig. 3). Davis found in these egg initials a body which he termed a coenocentrum and which he believed was instrumental in the rounding up of the eggs. He referred to this rounding up as the "balling" of the cytoplasm. This body acted as a dynamic center which drew the cytoplasm to it and rounded it up into a spheroid mass. Miss Carlson and the writer found what might be termed a coenocentrum, but observed it only a few times. Clausen also mentioned such a body. From continued observation of the formation of the eggs and the infrequency of the finding of the "coenocentrum," the writer is inclined to the belief that the coenocentrum is not the active agent. This body was observed in eggs that had nearly rounded up. It lay partially surrounding the nucleus and several fibrils radiated from it. The agent that is instrumental in the formation of the eggs is the central vacuole, according to the present writer, and by its continual furrowing of the cytoplasm the eggs are cut out. Later, when the vacuolation has gone to completion and the egg initials have rounded up, they come to lie close together so that their sides appear to be in contact. Shortly afterwards they separate and remain suspended in the liquid in the oogene.

During the formation of the eggs in the oogene, antherids have been approaching the oogene from the neighboring hyphae. The name of this species was derived from the fact that the antheridial branch often arises from the hypha immediately beneath the oogene and attaches itself to it. Antherids were observed to attach themselves to the oogones after the formation of the eggs, but more frequently at the stage of the egg initial. The development of the male gametes within the antherid is very similar to that in the oogene. After the cytoplasm has streamed to the tip of the antherid, a cross wall is laid down. The cytoplasm is multinucleate and, as in the oogene, many of the nuclei migrate to the periphery and degenerate. The re-
maining nuclei undergo one mitotic division and many of these nuclei also degenerate. Those nuclei remaining were scattered in the cytoplasm and seem to enlarge slightly.

The antherid, upon coming in contact with the oogone, may indent the oogone wall; whether it finally ruptures the wall and discharges its contents into the oogone was not observed. As is more often the case, the antherid adheres to the oogone and sends one to several fertilization tubes through the oogone wall. These fertilization tubes grow until they come in contact with one or more eggs. However, when a fertilization tube comes in contact with an egg, other fertilization tubes from this same antherid either grow to neighboring eggs or indicate signs of collapse. The fertilization tubes vary as to length and width. Some have walls that are fairly smooth, while others are quite irregular. Several nuclei are often present within them and the nucleus that is most terminal appears slightly larger than the others and is generally the nucleus that functions as the male gamete nucleus. Upon the discharge of the male gamete nucleus into the egg the other nuclei in the fertilization tube soon showed indications of degeneration.

Previous workers observed that the wall of an oogone in a number of species varied in thickness. The oogone wall in surface view, appears to be made up of many circular thickened plates at the intersection of which the areas were thin-walled. In section view, this gave the appearance of thickened areas separated by a thin membrane. It was believed for a long time that the fertilization tube penetrated the thinner areas only, due probably to some chemotactically stimulus. Couch, in his studies with the water molds, has observed the fertilization tubes penetrating the thickened areas as well as the thinner ones, and so believes that there is no ground for the assumption that the fertilization tubes penetrate the thinner areas only.

Before the fertilization tube is in contact with the egg it may contain several nuclei, the more terminal one of which has enlarged. This tube continues to grow until it comes in contact with the egg and the terminal nucleus enlarges still more and in doing so probably ruptures the tip of the fertilization tube (fig. 4). The male gamete nucleus, as it is now recognized, can be seen within the egg (fig. 5). The
female gamete nucleus is also present and is recognized by the fact that it is slightly larger and denser. A nucleolus was not observed in either of the gamete nuclei. It was not uncommon to find the female gamete nucleus not in the center of the egg. In these preparations, while it may not be characteristic of the species, the writer observed that the male gamete nucleus was invariably discharged into the egg at the side farthest away from the female gamete nucleus.

The male gamete nucleus, upon its discharge into the egg, migrates to the female nucleus and evidently unites with it (fig. 6). The nuclei were easily recognized, one slightly over-lapping the other. The penetration path left by the male gamete nucleus as described by Couch in *Leptolegnia caudata*, was not observed. Upon the union of the gamete nuclei the egg becomes a zygote (fig. 6). A wall now appears about the zygote, the cytoplasm becomes finely vacuolate and often several oil bodies of varying size are present. The zygote when mature is slightly contracted.

The results of this cytological investigation seem to definitely establish the presence of fertilization in *Achlya hypogyna* and add, therefore, an eighth species of the Saprolegniaceae wherein fertilization has been observed. Up to the present, fertilization has been observed in three Saprolegniaceae (by Trow (1895) and Claussen (1908); in four Achlyas by Trow (1899, 1904), Davis (1905), Mucke (1908), Carlson (1929), and by the writer; in an Aphanomyces by Kasanowsky (1911); and in Leptolegnia by Couch (1925). Owing to the large number of genera and species in the Saprolegniaceae, there is opportunity for further work to be carried on in this field.

The writer wishes to express to Dr. E. M. Gilbert his appreciation for the advice and encouragement received during the course of the work.

**BIBLIOGRAPHY**


Coker, W. C. 1923. The Saprolegniaceae, with notes on other water molds. Chapel Hill, N. C.

**Explanation of Plate 2**

All figures were drawn with a camera lucida and with a Leitz 4-ocular and a 1/12 oil immersion. Magnification 1500.

Contents of the oogones were drawn with a Zeiss 12 compensating ocular and a 3 mm. aprofychromatic oil immersion.

**Fig. 1.** Young oogone with the nuclei in the metaphase; degenerate nuclei at the periphery.

**Fig. 2.** Central vacuole prior to the formation of the egg initials.

**Fig. 3.** Oogone containing four egg initials; antherids present.

**Fig. 4.** The approach of the fertilization tube containing the male gamete nucleus to the egg.

**Fig. 5.** The fertilization tube has discharged the male gamete nucleus into the egg.

**Fig. 6.** The male gamete nucleus about to be discharged from the fertilization tube. Zygote; gamete nuclei in contact.