

## CHAPTER 10. Morphogenesis: The Oospore

Nuclear fusion in the oosphere of water molds followed by the accretion of a thick wall and accompanied by certain internal cytoplasmic and reserve material adjustments creates the oospore or mature zygote in species of Saprolegniaceae. Ultrastructural analyses by Beakes and Gay (1978a), however, suggest that the usual concept of an oospore must be modified to some degree. It is evident from their work that the transition from oosphere to oospore now can be more precisely defined than in the past.

Oospore maturation progresses through four phases (Beakes and Gay, 1978a), and the definition of this structure is to be found by tracing events in that progression. The oosphere stage (two phases) begins with the onset of cytoplasmic furrowing (Chapter 9) and ends with the acquisition of an outer wall and the initiation of an inner one. At this point, some 8-15 hours after cleavage begins, fertilization (Chapter 12) takes place. The inner wall then is completed, the central ooplast vacuole (Howard, 1971; Howard and Moore, 1970) develops, and the cell is now properly termed an oospore (third phase). The fourth phase is a period of unchanging internal organization -- afterripening, perhaps? -- terminating in germination.

Two salient points regarding oospore structure emerge from the foregoing sequential definition offered by Beakes and Gay (1978a) and from earlier studies by Howard (1971) and Howard and Moore (1970). The first point is that the oospore wall is not a single layer. De Bary (1884) maintained that spores had three wall layers: the exospore (derived from condensed periplasm), the episporium (initial wall layer of the zygote), and the endospore (secondary wall layers deposited during zygote maturation). However, in a review paper on the Saprolegniales, Dick (1973) stated that the oospore wall in these fungi had only two layers. The outer layer consists of the zygote membrane together with the oosphere membrane (if any) and any accompanying deposits. The endospore or inner wall (Dick, 1973) is thick and usually stratified. Electron microscopy applied to the oospore investiture shows it to be much more complex than ordinary light microscopy reveals. Beakes and Gay (1978b) have demonstrated that in *Saprolegnia furcata*, at least, the oospore has an outer wall, and an inner one consisting of an inner and an outer layer separated by an electron dense accretion.

The second point concerning oospore structure also emerges from ultrastructural studies. Mature oospores of *Saprolegnia terrestris* contain an ooplast. Howard and Moore (1970) reported that this structure is in fact a large vacuole, but which has been traditionally defined and illustrated as the protoplasm of the zygote! According to their interpretation of the oospore, the cytoplasm proper (containing the organelles and reserve globules) surrounds or partially encompasses the ooplast. Beakes and Gay (1978a) also recognized the ooplast ultrastructurally.

Using *Saprolegnia diclina*, J. Fletcher (1978) has explored very thoroughly the appearance and timing of events in the process by which oospheres mature into oospores. He found that the occurrence of the stages in maturation was quite variable, ranging from one-quarter to twice the calculated mean times. The extremes in variation

in time could occur among oospores in a single oogonium as well among those from different oogonia. The mean time elapsed between the delimitation of the oogonium and the appearance of identifiable oosphere initials was 9 hours 25 minutes; discrete oospheres appeared 10 hours and 55 minutes after oogonium delimitation. On the average, 12 hours and 46 minutes after discrete oospheres were visible in the oogonia of *S. diclina*, an identifiable oospore wall was produced. The central ooplast was formed at 45 hours 52 minutes (mean time) after condensed, spherical oospheres were present, and the reserve bodies were aligned peripherally -- signaling oospore maturation -- at 66 hours and 47 minutes after the oosphere was completed. There are several recognizable stages in the maturation process in *S. diclina*. Following the condensation of the oosphere initial into the oosphere an oospore wall appears. Subsequently, the granular protoplast separates from the wall leaving a clear zone. In time, this clear, peripheral area seems to be occupied, at least in part, by the reserve globules that develop as maturation reaches the climax. The ooplast becomes visible in the maturing zygote first as a number of well-defined globose structures; these eventually coalesce into one body, the ooplast proper. The final stage in maturation is the peripheral alignment of the small reserve globules.

The study by J. Fletcher (1978) should be repeated with other species to determine if the sequential events -- and the timing of them -- are of general occurrence among the water molds or only an unusual instance in one isolate. Such explorations might well begin with the dioecious *Achlyas*, taking into account the procedure developed by Rozek and Timberlake (1979) to induce synchronous oospore production in *Achlya ambisexualis*. Encysted spores from the oogonial (female) thallus and from the antheridial (male) thallus were mixed, and the resulting mycelial growth analyzed for oogonium and oospore production. The number of oogonia produced was found to be directly related to the proportion (in the original inoculum) of "female" spore cysts. A ratio of seven oogonial thallus spore cysts to three from the antheridial thallus was optimum for the formation of oogonia with oospores.

## THE TYPES OF OOSPORES

Classically, three types of oospores have been recognized based upon what is ordinarily thought of as the arrangement of oil globules in the cytoplasm. Because these deposits are chemically more complex than lipid alone, Dick (1969b) substituted the term reserve globule; we shall refer to this material by this latter designation or simply as a refractive material.

In view of the study by Howard and Moore (1970) it is necessary to modify the more familiar definitions of oospore types to take into account the ooplast. A centric oospore (Fig. 16) possesses peripherally in the layer of cytoplasm that surrounds the ooplast one or two complete concentric spheres of small reserve globules. In a subcentric oospore (Fig. 16) the cytoplasm only partially encompasses the ooplast, and contains two or three peripheral partial spheres of oil reserve globules in one area, or, in some cases, a full peripheral sphere of bodies in addition. In optical section, such an oospore generally is seen to have two or three rows of refractive bodies at one side

(gathered in a lunate configuration), and only one row on the opposing side. In some cases, particularly where the oospore is ellipsoidal and is viewed in optical section, two or three “lines” of globules may be present at each end of the cell, with a single “layer” connecting them. Such oospores are also subcentric. The reserve substance of an eccentric oospore is a single refractive globule situated laterally to the cytoplasm, with the ooplast contiguous to it on one side, and the plasmalemma bounding it on the opposite side (Fig. 16). Thus what has been universally looked upon as a “cap” of cytoplasm over the single large reserve globule in an eccentric oospore is in reality the ooplast. Dick (1969b) introduced a new terminology to characterize an eccentric oospore, referring to its parts as phases or fractions. The ooplast is evidently what he refers to as an optically inactive clear matrix (Dick, 1969b: fig. 3).

It has been suggested (Newby, 1948a) that the eccentric nature of an oospore is favored if the zygote matures slowly. Drechsler (1954a: 212) was prompted to include fine particles of maize in plated media since these bits of solid substratum appeared to him to enhance development of the oogonia of *Aphanomyces cladogamus* thus insuring that the oospores would have “... the correct internal organization necessary for longevity.” The implications in Newby’s and Drechsler’s comments warrant exploration.

Howard and Moore (1970) described a fourth type of oospore, the expersate one. Such a zygote possessed a single reserve globule as did the eccentric one, but in contrast to the latter, the refractive material was contained entirely within the matrix of the cytoplasm (not just bounded by the plasmalemma as in the case of the eccentric type), and there was no ooplast. Species of *Aphanomyces* were alleged to have expersate oospores. Howard (1971) reported that the surface of the refractive globule in oospores of *A. laevis* was irregular as if membrane-bound, and might not be lipid. At one point in the germination of an eccentric oospore, however, the refractive reserve body becomes convoluted as it changes chemically.

On the basis of brightfield observations, Dick (1971b) concluded that each oospore of *Aphanomyces stellatus* and *A. cladogamus* in fact has an ooplast although this structure is optically inactive. The absence of supportive illustrations (Dick, 1971b) notwithstanding, we agree with Dick that a configuration interpreted as expersate cannot be recognized as a separate entity. It must be admitted, however, that the description by V. D. Matthews (1927) of the oospores in *Leptolegnia eccentrica* surely recalls the “expersate type”.

In 1960(c) Dick introduced the term subeccentric for oospores of a watermold which he at that time identified as *Saprolegnia asterophora*, but later (Dick, 1969a) placed in *Scoliolegnia*. In a subeccentric oospore (the position of the ooplast with respect to the other fractions in the cell has not been determined) the reserve deposit consists of several globules of various diameters clustered together at one side of the cytoplasm (Fig. 16) or, in some cases (Dick, 1969a), on opposing sides. Such oospores have been detected in *Pythiopsis cymosa* (de Bary, 1888; Coker, 1923; Dick, 1960c), *Calyptralegnia achlyoides* (Dick, 1960c<sup>1</sup>), *C. ripariensis* (Höhnk, 1953b), and *Isoachlya eccentrica*<sup>†</sup> (Dick, 1960c). It must be recorded that during maturation some eccentric oospores may show temporarily a subeccentric configuration as the reserve globules coalesce. Hence, the

concept of subeccentric (Dick, 1960c<sup>1</sup>) as a valid oospore type rests solely on the assumption that this condition represents a fully mature zygote.

The subcentric Type III oospore configuration described and illustrated by T. W. Johnson (1956b: pl. 1, fig. 6f; pl. 6, figs. B, E) for two isolates of *Achlya treleaseana*<sup>†</sup> has not again been reported. It clearly is an aberrant form undeserving of continued recognition. The oospore structure described for *Leptolegnia subterranea* by Coker and Harvey (J. V. Harvey, 1925b: 158, pl. 19, figs. 6-8) as "...a cup of oil globules on one side..." is best considered as subeccentric.

On the basis of oogonium and oospore morphogenesis Dick (1969b) proposed that the Oomycetes can be distributed into three groups. The Saprolegniaceae constitute a distinct group characterized by centrifugal oosporogenesis without the formation of periplasm, and aplerotic oospores containing a peripherally disposed reserve. Species of *Leptolegnia* perhaps constitute a cluster of taxa to be excluded from the Saprolegniaceae, Dick suggests, because in these, oosporogenesis is said to be centripetal.

The reserve globules, by whatever name one chooses to apply, have figured inextricably in taxonomy of the water molds. Traditionally, the oospore type has been held to be inviolate within a given species. This is not an entirely acceptable conclusion since in some taxa -- *Saprolegnia ferax* and *Achlya racemosa*, to name two -- centric and subcentric oospores may occur within the same oogonium. Instances of eccentric oospores and centric or subcentric ones in the same oogonium (or species) have not been reported.

## OOSPORE GERMINATION

With some very scattered exceptions, instances of oospore germination in members of the family have been noted largely by chance. It has long been thought that zygote germination was accompanied by meiosis, but current evidence points to the contrary. Two main aspects of germination have been explored: the factors triggering or influencing the events, and the morphological products of the process.

In 1899, Trow recorded some details of oospore germination in *Achlya americana* var. *cambrica*<sup>†</sup>, and although later studies by others contradict his account in a few minor ways, his treatment still describes reasonably correctly morphogenesis of germination. At least three events appear to take place as germination commences in an eccentric oospore (light microscopy; see Chapter 15, and Beakes and Gay, 1978a, b for details of germination at the ultrastructural level). The single, large, reserve globule first becomes embedded in the matrix of the cytoplasm (the complex deposit of lipids is already within the plasmalemma, but exterior to the ooplast, so that the refractive

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<sup>1</sup> Centric oospores were described for this species by Coker and Couch (1923), not by Coker (1923) as Dick cites. The illustration by Coker and Couch depicting a "centric" oospore of *C. achlyoides* (as a *Thraustotheca*) in optical section clearly portrays a subcentric type.

material appears to migrate into the center of the oospore). Once within the matrix, the globule begins to diminish in diameter, and as it does so, its outline becomes irregular (Weston, 1918, for an isolate of *Thraustotheca*). Trow simply said that the globule was absorbed. Concurrently, the oospore wall becomes progressively thinner, and the zygote itself increases in diameter. Both Trow (1899) and Weston (1918) reported that during these events the protoplasm actually increased in volume, but precisely how this occurs and what internal changes make it possible have not been determined. In any case, prior to emergence of the germ hypha (more than one may develop in some species) the cytoplasm becomes centrally vacuolate. As the young hypha elongates, its vacuole becomes confluent with that in the oospore. With continued growth, the hypha eventually breaches the confining oogonial wall (if that structure is still intact), but the details of this process are obscure. It has been firmly established that mitotic divisions of the somatic nuclei accompany germ tube growth.

So far as we are aware, the path of germ tube egress from the oogonium described by F. R. Jones and Drechsler (1925) for *Aphanomyces euteiches* has not again been observed. In the germination of some oospores in this species the germ tubes breached the fertilization tubes and grew through them, thence into the antheridial cell. Subsequently, these young hyphae ruptured this cell and became extramatrical. The fertilization tube in these cases functioned as a channel for the emerging germ hypha.

At what point in its existence is the oospore capable of germination? Clausz (1968) demonstrated that oospores of *Achlya hypogyna*<sup>†</sup> matured in eight days, but there followed a lengthy afterripening period at the end of which the irreversible events in germination commenced. This "ripening" sequence is not necessarily universal in the water molds if Latham's (1935) observations are correct; he reported that the oospores of *A. recurva*, were capable of germination immediately after they were formed (in about three days time). In one of the first recorded comments on oospore germination, Pringsheim (1873-74) remarked that there was no fundamental difference between a fertilized oospore and one matured parthenogenetically, except that the latter germinated more readily than the former.

The one study of oospore germination in water molds that is still accepted as the most extensive of its kind was that by A. W. Ziegler (1948a, b). He recognized four patterns of germination among the several species he examined, namely, the production of (1) a single germ hypha (of variable length) bearing an apical sporangium, (2) a sparingly branched mycelial system forming sporangia at the tips of the main branches, (3) a branched mycelium lacking sporangia, and (4) a single, unbranched hypha. In addition to these modes of germination a fifth may be added (though it is evidently quite uncommon and may be abnormal). De Bary (1881, *Saprolegnia ferax*), and F. R. Jones and Drechsler (1925, *Aphanomyces euteiches*) among others (see Table 22) described oospores which simply converted directly into sporangia without an intervening hyphal system. Trow (1899), on the contrary, had concluded that such a pattern of germination never occurred.

Little is known about the segregation of sexual strains in the germination of zygotes in dioecious species, but experimental work by J. N. Couch (1926b) with germ hyphae from oospores of *Dictyuchus monosporus* is pertinent. He succeeded in

germinating the oospores of this species, cut off segments of the resulting mycelium, and propagated colonies from these. The results of his experiments, in terms of mating strains produced, demonstrated that the developing mycelium apparently consisted of certain sexual or nonsexual regions. Some parts of the mycelium were pure male, some pure female, and others were undifferentiated sexually. As growth proceeded, the "sexes" became intermixed. (Couch's own data suggest that the mycelium from oospores produced as the result of successful crosses in *D. monosporus* was diploid.)

Precisely what products oospores form in nature when they germinate, and how those products are generated is not known. Presumably motile spores produced by germinating oospores provide the inoculum for substrates when dried soil is flooded and baited. This being so, all functional oospores -- including those with the nonsporangiate germination patterns recognized by A. W. Ziegler -- must at some point be able to develop planonts. The types of zygote germination as they are now known are products of the laboratory, and the process may bear little resemblance to reality.

Some work on *Aphanomyces euteiches* by F. R. Jones and Drechsler (1925), Scharen (1960a, b) and Olofsson (1968), and on *A. raphani* by Ghafoor (1964) bears directly on "natural" germination of oospores. By embedding oospores in plant debris in cellophane bags, and positioning these containers near pea roots, Scharen found that oospore germination was stimulated. Pea root extracts, horse dung infusion, and pea leachate in sand also provided a stimulatory effect. Oospores did not sprout in distilled water, and unsterilized soil had a distinctly fungistatic effect (there were significantly higher germination percentages in sterile than in nonsterile soil). Using oospores of *A. euteiches* harvested from naturally infected pea roots Olofsson (1968) demonstrated that pH was a significant factor in germination in these species. Oospores in a medium at or about neutrality (6.5-7.0) were not stimulated to germinate by heat (60 °C), subjecting them to below freezing temperatures (-4.0 and -20 °C), or alternately freezing and thawing them. Similarly, there was no germination of oospores submerged in tap water adjusted to a pH below 3.2 or above 5.1. Germination took place, however, in water in the range of pH 4.5-4.8. Oospores produced germ hyphae more readily if they had been harvested from decayed roots (tissue about four months old) than if taken from undecayed roots (about four weeks old), Olofsson suggested that at a low pH fungistatic substances in pea roots were inactivated, and oospore germination was then no longer inhibited. When F. R. Jones and Drechsler (1925) placed oogonium-bearing hyphae from 15-day-old cultures (*A. euteiches*) in Van Tieghem cells oospore germination occurred. In these cases, therefore, pH evidently was not a contributing factor. Ghafoor (1964) found that the germination percentage of oospores of *A. raphani* was more than doubled when radish seedlings were grown in autoclaved soil containing these oospores than when the host was not present in the sterile soil.

It is evident that much variation exists among the species of water molds with regard to the germinability of their oospores under laboratory conditions. Moreover, the time required for the process to begin once the "aged" oospore is capable of germinating is vastly different among the representatives of the family. Table 22 records the pertinent information on oospore germination in a number of species in the

family; the list is by no means exhaustive, and there are noticeable gaps in the data since not all investigators have provided full accounts.