CHAPTER 20. The Physiology of Reproduction: Sexual

Certain generalities regarding sexual reproduction in the Saprolegniaceae emerged from work centering on the Klebsian principle (Chapter 18), and are yet to be contradicted by modern methodology. Among the more direct facts to come to light are the following. (1) The presence in a growth medium of particular inorganic salts favors development of the sex apparatus. (2) A noteworthy relationship exists in some watermolds between mycelium weight and abundance of oogonia and antheridia (early workers with these fungi said to the contrary). (3) Some combinations of carbon and nitrogen sources (Pieters, 1915a) stimulate antheridial production in excess of oogonium formation while others are antagonistic to the male element. (4) The concentration of constituents in the medium may (Horn, 1904) effect one or more structural changes in the sexual apparatus. (5) The addition of inorganic salts to a nutrient-rich medium does not necessarily stimulate oogonium production (Obel, 1910a). (6) Age of the mycelium has some effect on when the sexual apparatus will appear -- the structures are more likely to develop on older than on younger hyphae (Klebs, 1899; Obel, 1910a) -- but it does not necessarily follow that when sporulation ceases the sexual reproductive processes commences.

THE OOGONIUM AND ANTHERIDIUM

Most published reports on the physiology of sexual reproduction in the family treat the sexual apparatus as a single unit. There are, however, conditions in which either the oogonia or the antheridia are favored, as in the case of hormonal control in the dioecious Achlyas (Chapter 21). Such factors as medium constituency, temperature, light, and pH, in particular, are associated with the physiology of sex cell development. With the exception of O’Sullivan's (1965) ecological study of some soil-inhabiting Saprolegniaceae, work on the effects of various environmental parameters on initiation and development of the sexual apparatus in the watermolds has been carried out through laboratory experimentation. O’Sullivan immersed young colonies of watermolds in nonsterile soil and found that some species (*Saprolegnia ferax*, for example) repeatedly exhibited early formation of oogonia in the soil under certain moisture levels. The precise factors contributing to this accelerated sexual development were not determined.

CHEMICAL FACTORS: SAPROTROPHIC SPECIES

A broad spectrum of compounds has been added to culture media in attempts to discover what effects they have on the initiation and maturation of the sexual apparatus in members of the family. Moreau and Moreau (1936a), for example, added glycerin to media in which they propagated *Achlya colorata, A. flagellata* (=*debaryana*), and *A. conspicua* (=*debaryana*). The oogonia and antheridia on mycelium immersed in concentrations of glycerin up to 4% were normal. Above this amount, oosphere cleavage in these fungi was inhibited, and development of the sex cells was at first retarded then inhibited. Within particular concentrations, ions of calcium, strontium, and magnesium (among others), the Moreaus (1938) discovered, stimulated oogonium formation. Ions such as those of chlorine, bromine, or iodine were toxic in various degrees, resulting in oogonium degeneration, or, in some concentrations, failure of mycelium to produce the sexual apparatus at all.

In a parallel to the study on the effects of glycerin on sexual reproduction, Moreau and Moreau (1936b) also subjected watermolds to increasing concentrations of maltose, glucose, fructose (as levulose), and saccharose. In high concentrations, these carbohydrates simply retarded growth, but at the same time were said to increase antheridial branching, and to reduce the prominence of wall ornamentations (among other effects). It may be noted in this connection that Reischer (1949c) found temperature, not nutrition, to be the dominant factor in determining the size and abundance of wall ornamentations in *Achlya colorata*, and as Clausz (1968) has shown, *A. hypogyna* (=*Protoachlya hypogyna*) responds in a similar fashion (*see* also section on temperature).

The constituents in media that provide energy and a nitrogen source for watermold growth appear to have some control over the production of oogonia as well, especially when the supplying compounds are in particular combinations. Data provided by Whiffen (1945) showed the amount of glucose in relation to the level of peptone to be a factor of consequence in oogonium development. Using an isolate of *Saprolegnia diclina*, Tingle (1972) demonstrated that the carbon:nitrogen ratio was not solely stimulative to oogonium production; rather, the amounts of each compound supplying these elements were critical. For example, oogonia were produced on mycelium grown in a synthetic medium having a C:N ratio of 3:2. When the glucose and glutamate constituents of the substrate were increased ten-fold but the ratio between them maintained at 3:2, vegetative growth was greatly amplified while sex cells were not formed. Tingle also called attention to the influence on sex cell development of the particular source of carbon and nitrogen supplied to the mycelium. The range in concentration of glutamate and methionine that supported oogonium formation by hyphae of *S. diclina* was much narrower than that in which hyphal growth only was stimulated. This quite properly recalls the Klebsian principle of the relation between nutrient level and reproduction. Tingle recognized that a lowering of phosphate, glucose, and total mineral elements in a chemically defined medium [Lee's (1965) modification of the medium employed by Scott, Powell and Seymour (1963)] reduced the abundance of oogonia in *S. diclina*. This and accompanying data in Tingle's account appear to support the notion that a substantial increase in mycelium production is accompanied by a decrease in development of the sex cells.

By modifying the constituents of Machlis' (1953b) Medium B, Barksdale (1962a) devised a synthetic broth that promoted oogonium formation in *Achlya ambisexualis* (Table 35). Her experimental work during the formulation of the medium incidentally provided some detailed insights into sex cell production in relation to specific kinds and levels of ingredients. Methionine, cystine, and cysteine were suitable sulfur sources for supporting oogonium-producing hyphae of this species and calcium, magnesium, and phosphorus were critical. If the concentration of any one of these three elements was even slightly below that required for maximum growth, oogonium development was sharply reduced (Obel, 1910b, had reported much earlier that phosphorus was essential for oospore formation). However, when glucose was the limiting factor, the diminution of numbers of oogonia relative to the reduction in amount of growth was least. When sulfur or phosphorus were limiting, oogonium production declined. Mycelium of *A. ambisexualis* in Barksdale's chemically defined medium with nitrogen in a concentration beyond 8 mg 100 mL^{-1} showed a sharply curtailed level of oogonium production. When glucose was supplied in excess of "demand" for growth, the magnitude of oogonium formation was lowered noticeably. Barksdale (1962a) demonstrated, then, that nutrient deficiency definitely becomes a restrictive factor in oogonium production. It also was nutrient deficiency, not culture age, that Reischer (1949b) earlier had suspected was involved in the production of longer antheridial branches than was typical in *A. sparrowii* (=*racemosa*).

Calcium has been singled out as having a specific role in such diverse aspects of watermold development as sporangium initiation and the ultrastructure of the sexual apparatus (J. Fletcher, 1979a). This mineral also appears to influence quantitative aspects of oogonium expression, at least in one species of the family, *Saprolegnia terrestris*. Although J. Fletcher (1979c) found that varying the concentration of calcium chloride in the growth medium did not cause variations in the dry weight of mycelium, the number of oogonia per unit area of that mycelium was influenced by calcium (and other minerals). As the $CaCl_2$ concentration in the medium was decreased, the range of the number of oospores per oogonium increased. Fletcher also noted that the oospore abortion rate in his isolate of *S. terrestris* rose slightly as the calcium concentration in the medium was reduced, and the frequency of abortion in oogonia producing only one or two oospores was higher than that in oogonia having three or more zygotes. There is a suggestion in the results which Fletcher obtained that a calcium deficiency in the medium inhibits the disintegration of supernumerary nuclei in the developing oogonia of this watermold. However, he concluded that survival of an abnormally large number of nuclei in an oogonium was unlikely to lead directly to the development of a large number of oospores in that cell. In sum, Fletcher's data may be interpreted as suggesting that the number of oospores in relation to oogonium diameter may be calcium-dependent. In *S. terrestris*, at least, there appears to be a calcium-specific effect on oospore number.

Compounds other than the usual sugar, nitrogen, and sulfur sources incorporated into media also have been tested for possible physiological roles in sexual reproduction by watermolds. Hendrix (1965) reported that sex cell development in three species of Saprolegniaceae -- *Achlya flagellata, Isoachlya* (=*Saprolegnia*) *unispora*, and *S. ferax* -- was not influenced by an exogenous supply of cholesterol. Glucan, however, can be used as an energy source in *Achlya ambisexualis* and *A. heterosexualis* if no exogenous carbon is added to the medium (Faro, 1972b). During genesis of the sex cells in these two species glucan consumption exceeds its synthesis, but as differentiation proceeds, glucan accumulates. Prior to the formation of oospores, the mycelia of these species once again consume glucan. The sequential fluctuation of this compound (probably a β-1, 3-glucan) appears to be correlated with a possible suppressive effect of sex hormones on glucose metabolism (Faro, 1972b). Dimethylsulfoxide (DMSO), Faro noted, prevented the formation of the sexual apparatus by the watermold isolates he tested. This was accompanied by cessation of glucan consumption. Calcium added in excess to the medium counteracted the effect of the DMSO.

It has been demonstrated by R. F. Elliott (1967a) that *Saprolegnia australis* is responsive to certain growth regulators -- kinetin and 6-benzylamino purine (BAP) -- and to the purines adenine and hypoxanthine. Indole-3-acetic acid and gibberellic acid elicit no sexual response in this species. Adenine and hypoxanthine stimulate oogonium production, while kinetin and BAP inhibit it. The effect of kinetin, however, can be suppressed if adenine is added to the medium. Elliott suggested that natural purines in *S. australis* might promote the action of sex hormones (although these are yet to be demonstrated in species of *Saprolegnia*). It is known, of course, from the work of Timberlake (1976) and others, that the protein inhibitors actinomycin D and *p*-fluorophenylalanine prevent or severely retard antheridial branch formation in antheridiol-induced thalli of *Achlya ambisexualis* (Chapter 21).

Salinity may be a factor in the production of sex cells by watermolds, as would be expected (*see* Duniway's 1979 review of water relations). Studies by J. L. Harrison and Jones (1975) with seventeen species of Saprolegniaceae demonstrated clearly that salinities in excess of 20% seawater (v/v) suppressed sexual reproduction. Species that evidenced some oogonia at zero and 10% seawater formed only vestiges of such cells at 20% and 30%, or failed to form any at all. *Protoachlya paradoxa, Isoachlya toruloides* (=*Saprolegnia torulosa*), and *I.* (=*Saprolegnia*) *eccentrica* were the only ones to develop oogonial initials in media containing a concentration of seawater. [Bremer (1976) has summarized the 1972 dissertation by J. L. Harrison on salinity responses by some watermolds. The publication by J. L. Harrison and Jones (1974), however, evidently incorporates most if not all pertinent information on salinity tolerances reported two years earlier by J. L. Harrison in that dissertation.]

Much of the pioneering work on the relation of salinity to sexual reproduction by members of the Saprolegniaceae is attributable to Höhnk. In 1952(d), he reported that mycelium of *Saprolegnia ferax* produced oogonia only in nonsaline water, and this reaction, coupled with the fungus' growth pattern in relation to salinity, led him to conclude that this species was a freshwater one. Colonies of *Aplanopsis terrestris*, on the contrary, formed oogonia when submerged in water at salinities of 0-18 ppt (Höhnk, 1952d, 1953a), but these cells did not mature in salt water (Höhnk, 1953a). Wu's (1979) data on a single isolate of *S. diclina* show that for this one representative, at least, "optimum" (he did not define this term) oosphere production occurred at a higher salinity (6 ppt) at 15 °C incubation than at either 10 ° or 20 °C.

It is known (T. W. Johnson, 1956b) that *Achlya intricata* (Beneke, 1948a, b) produces the sexual apparatus only in a narrow zone of mycelium adjacent to the substratum, but a reason for this unusual locational pattern has not been found.

Kauffman (1921) reported that oogonium position on the mycelium of *Isoachlya toruloides* was influenced by certain nutrients in the surrounding medium. Torulose oogonia appeared in colonies grown in hemoglobin and peptone, but these sex cells were often in pairs on mycelium propagated in hemoglobin and fructose (as levulose). In solutions of leucine, on the contrary, hyphae bore only nontorulose oogonia. Undisturbed colonies of *S. ferax*, Lee and Scott (1967) reported, expressed a quantitative distribution pattern that changed as the mycelium aged. The account of this pattern is not clear; the data in their paper seem to show that as the mycelium aged, the number of oogonia increased, and were distributed into zones in the "circular" colony.

It is relevant to the systematics of the Saprolegniaceae to assay the influence of nutrients and other constituents in the medium on the expression of those characters used in identification. Kauffman (1908), Pieters (1915b), and others, have reported environmentally, and nutritionally-induced variations in a number of species, but for the most part the information from the respective publications is of a qualitative rather than a quantitative nature. Are the sexual reproductive structures that can be measured -- particularly the oogonia and oospores -- modified substantially, if at all, by the nature of the growth medium? The information at hand is somewhat contradictory. Reischer (1949b) reported that there was no significant correlation between oogonial diameter and number of oospores in *Achlya sparrowii* (= *racemosa*). In *Saprolegnia australis*, R. F. Elliott, 1967a) found quite to the contrary, as did J. Fletcher (1979c) working with *S. terrestris*. Definitive answers to the foregoing question are wanting, but critical work such as that by J. Fletcher (1979c) on calcium and sex cell sizes represents an approach that is certain to provide taxonomically applicable information.

Ordinarily, antheridial branch size is not determined in the characterization of species of watermolds. One fungus, *Dictyuchus pseudodictyon*, however, produces such abundant antheridia in clusters (umbratiform) that measurements can be made conveniently, as Linthicum and Ziegler (1954) have done. They propagated the fungus on various seeds and grains in water containing vitamins and other growth promoting substances. The grains and seeds supported colonies in which the diameters of the antheridial branch clusters attendant to the oogonia were extremely variable. Of the other compounds used, only thiamine and IAA had a stabilizing effect on the size of the clusters of branches. Mycelium of *D. pseudodictyon* grown in the presence of adenine, guanine, and uracil developed antheridial branch clusters with the narrowest range of size variation. Temperature, but not light, also influenced the size of antheridial branch clusters in this species: the size range decreased with an increase in incubation temperature.

CHEMICAL FACTORS: PARASITIC SPECIES

The plant pathogenic species of *Aphanomyces* have been studied quite extensively with respect to the physiology of the sexual reproductive process. An account of Kanouse's (1932) pioneering work on nutrition and the inducement of sex cells of *Saprolegnia parasitica* (=*diclina*) already has been recorded (Chapter 18).

Whiffen (1938) succeeded in propagating *Aphanomyces phycophilus* in culture in the absence of its alga host. The fungus would not grow on hempseed, but on a maltose-peptone medium the sexual apparatus developed. Oospores did not mature in oogonia on mycelium in this medium; zygotes did so, however, when hyphae bearing oogonia were transferred to water. No other alga-inhabiting species in the genus has been cultured.

Aphanomyces euteiches produces oogonia[1] in synthetic media (Papavizas and Davey, 1960a), but evidently does not do so if peptone (J. E. Mitchell and Yang, 1966; Geach, 1936) or small amounts of sodium nitrate or ammonium sulfate are incorporated into growth media (Geach, 1936). Kotova (1971) found that certain concentrations of ammonium nitrate and calcium phosphate, for example, reduced or prevented oogonium production by *A. euteiches*. The factors affecting sexual reproduction in this species were explored in detail by Papavizas and Davey (1960b), using a basic mineral salts medium with *d*-glucose, thioglycollic acid, and a vitamin mix. Three isolates were tested, and to some extent the specimens reacted differently to certain constituents. Oogonia were produced on hyphae grown in the presence or absence of *dl*-methionine, yet this sulfur source was necessary for oospore production. Thioglycollic acid supported oospore production by two other isolates; *l*-cystine·HCl in the basal medium promoted oospore formation in all three isolates. Data accumulated by Papavizas and Davey indicate that the optimum carbon:nitrogen ratio for oospore production depended upon the concentration of amino nitrogen in the medium. Vitamins were not essential for oogonium development. In general, sexual reproduction in *A. euteiches* occurred when three elements in the medium were in proper balance: a carbon-contributing sugar, amino nitrogen, and a suitable sulfur source. The latter is extremely important for this species (Davey and Papavizas, 1962). Elemental sulfur, and any reduced form with valences of -2 (cystine, methionine, or Na_2S, for example) supported oogonium-producing mycelium. By contrast, oxidized sulfur compounds such as Na_2SO_4 or Na_2SO_3 did not do so. Strain differences in *A. euteiches* also appear with respect to the ability of the mycelium to use various forms of sulfur in the formation of oogonia. For example, some isolates produced oogonia and oospores when sulfide sulfur or thiosulfate was supplied, whereas others did not. Both organic and inorganic sulfur sources, in the proper oxidation state, supports functional oogonium development in the pea root pathogen (Davey and Papavizas, 1962).

Of 43 carbon sources (mono-, di-, tri- and polysaccharides; pectic substances and derivatives; carbohydrate derivatives, and TCA cycle intermediates) tested by Papavizas and Ayers (1964) for ability to support sexually reproductive mycelium of *Aphanomyces euteiches* only 17 functioned in this regard. Maximum oogonium

---

[1] Literature on the pathology of species of *Aphanomyces* nearly always refers to oospore production when dealing with sexual reproduction by these fungi. Such a reference to oospores ignores the fact that the development of the oogonium must precede the formation of the oospore in the sexual sequence.

production occurred when mycelium of the fungus was grown in a synthetic medium with *d*-glucose or *d*-fructose as the carbon source. The oligosaccharides *d*-mannose, *l*-rhamnose, cellobiose, melibiose, sucrose, raffinose; the polysaccharides glycogen, dextrin, xylan, inulin, carboxymethylcellulose, and soluble starch, and the carbohydrate derivatives glycerol, mannitol, and sorbitol also supported sexually-reproducing colonies. Neither pectic substances nor pyruvate served as suitable carbon substrates for sex cell development by *A. euteiches*. Even though some carbon sources would support little mycelial growth of the isolates, functional oogonia could still develop and mature (Papavizas and Ayers, 1964) in spite of reduced vegetative growth. It may be mentioned in passing that the two sugars most prominent in root exudates from *Pisum sativum* -- glucose and fructose (Rovira, 1956) -- also were supportive of the best production in *A. euteiches* of oogonia and oospores.

That some knowledge is at hand dealing with the interaction of physiology and sexual reproduction in the radish blackroot fungus, *Aphanomyces raphani*, is attributable to experimental work by Ghafoor (1964). He found that cultures of this species would produce mature oogonia on colonies in radish root extract, but not on maltose-peptone, glucose-yeast extract, or oatmeal agar, or on extracts such as those from peas, hempseeds, or leaves of *Raphanus sativa* L. On the other hand, glutathione, cystine, and *l*-cysteine·HCl supported mycelium of *A. raphani* in which oogonia were produced in abundance. Colonies of this same species could form oogonia in concentrations of peptone as low as 0.005 and 0.001%, according to Herold (1952).

The experimental data on the physiology of oogonium development by the plant pathogen *Aphanomyces cochlioides* comes primarily from three sources. The attempts by Schneider and Yoder (1973) to secure maximum yield of oospores as possible inoculum units give some insight into the effect of nutrition of this species. They propagated *A. cochlioides* in a variety of natural product decoctions and homogenates, and determined the numbers of oogonia (oospores) produced. The highest density (classes of 0-4) of sexual reproductive cells occurred when the mycelium was grown in extracts of oatmeal, sugar beet seedlings, pearled barley, and buckwheat; no oogonia developed on colonies in decoctions of hempseed, lentil, raisins, soybean seedlings, and sugar beet seeds. If the medium supporting oogonium-producing hyphae was replaced by fresh nutrient, oogonium density was augmented; younger cultures (five days) of the species responded more readily to the replacement medium than did older ones (19 days).

Some unpublished data on the physiology of *Aphanomyces cochlioides* appear in a detailed review published by Papavizas and Ayers (1974). Oogonia of this species developed when mycelium that had grown in a nutrient broth was transferred to a salt solution of calcium nitrate, magnesium sulfate, and potassium nitrate, or to well water. Few oogonia were produced on hyphae in distilled water. For *A. cochlioides*, at least, a nutrient concentration about one-half of that optimum for vegetative growth is suitable for maximum oogonium production (data cited in Papavizas and Ayers, 1974). The results of some experimental work by Herold (1952) seem to support this apparent "Klebsian response" in this species. He found that oogonia with oospores could be produced on hyphae in peptone solutions of very low concentrations, namely, 0.005-0.0005%. Oogonia without oospores developed in a peptone concentration of 0.5%. *Aphanomyces cochlioides* has been reported also (Fowles, 1976) to form its sexual apparatus on mycelium that had been placed in media lacking sulfur or nitrogen, and the nonparasitic *A. stellatus* evidently does likewise in the absence of the latter element.

In connection with his experiments on the effect of exogenous chemicals on growth and asexual reproduction by *Aphanomyces euteiches*, Grau (1975) reported that the dinitroaniline herbicide dinitramine was more effective in preventing oogonium formation by colonies of this fungus than was trifluralin. The latter, in fact, stimulated oogonium development in one isolate, yet inhibited sexual reproduction in another specimen of the same species.

PHYSICAL FACTORS

Temperature: -- Temperature as a factor involved in the development of the sexual reproductive cells in saprolegniaceous fungi has long been recognized, but often only in passing as observers directed their attention to other aspects of the culture environment. Inasmuch as members of the family are able to grow within a wide range of temperatures, it might be expected that sexual reproduction likewise occurs in rather broad temperature regimes. This may be true for some watermolds, but it is by no means universal. According to some investigators, optimum production of sex cells often occurs only within somewhat narrow limits, and higher temperatures rather than lower ones are likely to restrict sharply the numbers of oogonia, or to prevent their development altogether. For some species, such as *Saprolegnia diclina*, it has been reported that there are no single temperatures that prove to be optimum for supporting oogonial- and antheridial-forming mycelium. That the composition of the growth medium may have an influence on sexual apparatus production at particular temperatures seems to be indicated (Chong, 1973).

A compilation of the scanty available data on temperature ranges and oogonium formation by watermolds (Table 36) simply emphasizes how few species have been tested for the temperature tolerance of their sexual reproductive capacity. Beyond the fact that some specimens -- those isolated from fish notably -- have very low temperature optima for the development of the sexual apparatus, there is but one trend evident in these data. With perhaps the exception of (Table 36) *Achlya americana* and *A. flagellata*, sexual reproduction by some Saprolegniaceae seems to be favored by a relatively cool environment.

Temperature influences not only the production of the sex cells but also their maturation. Tingle (1972) found that for every 2° incremental rise in temperature within the range of 15-23 °C there was a corresponding decrease in the incubation time required for mature oogonia to appear on the hyphae of *Saprolegnia diclina*. At 15 °C, for example, mature oogonia appeared at 168 hours; whereas at 23 °C, they were present by the 96th hour of incubation.

Light: -- The effect of illumination -- or its absence -- on the production of the sexual apparatus in species of the Saprolegniaceae has been little investigated. Light did not influence sex cell formation in *Leptolegnia caudata*, J. N. Couch (1932) reported, but earlier (1926a) he had discovered that *Aphanomyces exoparasiticus* was particularly sensitive to illumination. Cultures of this species grown in the dark did not produce oogonia; in alternating light and dark, however, colonies formed sex cells in concentric bands or "rings" corresponding to periods of exposure to light. According to Fowles (1976), a species of *Aphanomyces* isolated from dolphin would not form oogonia under any regimes of light and temperature (some innate factor, probably, is the limiting one in this isolate). *Aphanomyces cochlioides*, however, produced approximately four times more oogonia when incubated in the dark (at 10 ° and 17 °) than in the light, and this has been confirmed by Schneider and Yoder (1973). In every light intensity tested, oogonium development by *A. cochlioides* was inhibited. Illumination also suppressed oogonium development in *A. stellatus* (Fowles, 1976).

Saprolegnia ferax has been used most widely in experiments on the effect of light on sexual reproduction (Etzhold, 1960; Krause, 1960; Lee, 1965; Lee and Scott, 1967; Neish, 1975a). Etzhold noted that oogonia developed on mycelium of this species exposed to light filtered to yield 75–92% of its intensity in the wavelength range of 590-740 nm. Although in colonies of *S. ferax* exposed to white light of decreasing intensities there were increasing numbers of oogonia, Krause (1960) found that monochromatic light in the orange and red bands was particularly stimulatory to oogonium development. Blue and green wavelengths, on the contrary, suppressed the development of the sexual apparatus in *S. ferax*. In part, an identical response was elicited in experiments by Tingle (1972) on *S. diclina*. In this latter species, however, there was no light-induced inhibition in wavelengths beyond 660 nm. Krause (1960) related light quality to the stages in oogenesis and oosporogenesis in *S. ferax*, and discovered that once oogonial initials had been initiated illumination was no longer influential. He naturally concluded that light affected initiation rather than maturation of oogonia. According to Lee (1965) and Lee and Scott (1967), the largest numbers of oogonia are produced by *S. ferax* in the dark, fewer in red light, and the least in blue wavelengths (cellophane filters over fluorescent tubes). These observations essentially parallel Krause's findings.

Oogonium production by *Isoachlya* (=*Saprolegnia*) *luxurians* and *Brevilegnia subclavata* is inhibited if the mycelium is incubated in the dark, but is favored by blue light (Prabhuji, 1979); light of other wavelengths has no effect.

A standard germicidal lamp (253.7 nm) was used by Lee (1965; *see* also, Lee and Scott, 1967) to irradiate various watermolds. Mycelium of *Saprolegnia ferax*, *S. parasitica*, and *Achlya americana* was photoreactivated after exposure to UV. In such instances the magnitude of oogonium inhibition was less if cultures were incubated in the light after UV treatment than if they were placed in the dark. Photoreactivation in *Isoachlya luxurians* and *Brevilegnia subclavata* was reported by Prabhuji (1979). Lee (1965) also has stated that exposure of a plated culture medium to ultraviolet light prior to inoculating it with a fungus resulted in fewer oogonia being produced by the isolate than if the substratum had not been illuminated.

Evidently light and temperature can function coordinately in their effects on the development of sex cells of watermolds. Szaniszlo (1965) incubated colonies of *Saprolegnia diclina* in continuous light at 21 °C, but then found that the sexual apparatus was suppressed. Mycelium grown in the dark at the same temperature reproduced sexually. The lowest light intensity that suppressed oogenesis in this species was 17-22 ft. c., at 20 °C. *Saprolegnia diclina* also responds photoperiodically. No sex cells are formed by colonies of *S. diclina* in a photoperiod of 10 hours light and 14 hours darkness, but oogonia are produced abundantly on mycelium incubated in a regime of two hours light and 22 hours of darkness (Szaniszlos, 1965). Lee (1965) observed an unusual response in *Achlya americana* to the combined influence of light and temperature, although this species reproduces sexually either in the dark or light. In quantitative terms, *A. americana* produced more oogonia when colonies were illuminated and held at 15 °C than when incubated in the dark at the same temperature. At 25 °C, however, the results were in direct opposition (also reported in Lee and Scott, 1967). In the study on this species, no attention seems to have been given to the effect of light in raising temperatures within the confines of culture vessels.

<u>pH</u>: -- Surprisingly little has been done on possible relationships between hydrogen ion concentration and sexual reproduction in the watermolds (Table 37). From the information available, it appears that a slightly acid medium favors sex cell formation in these fungi. One additional important fact seems to emerge from existing literature, namely that while pH may not (within reasonable limits, of course) significantly influence the formation of oogonia, it can have considerable impact on maturation. In *Achlya tuberculata* (=*crenulata*), for instance, it has been reported that 3% of the oogonia produced mature oospores at a pH of 8, but at pH 4.5, 94% did so (A. W. Ziegler, 1950; A. W. Ziegler and Linthicum, 1950). *Achlya flagellata* responds in a somewhat similar fashion, if one may judge from Barbier's (1969) observations. The number of oogonia produced by the mycelium of this species increases very little as the pH is raised from 4.5 to 5.5, but the proportion of "fertile"oogonia (to undeveloped ones) rises even within this narrow range. A six-fold increment obtains in the proportion of fertile to immature oogonia as the pH of the culture medium is raised to 6.0-6.7. If Lounsbury (1930) was correct in his observations, not all species of Saprolegniaceae respond so expressively to this factor. He reported that neither increasing nor decreasing the acidity of the growth medium for *Protoachlya paradoxa* induced the mycelium to form sex cells hence some factor other than pH was influential in this regard.

<u>Oxygen</u>: -- The literature harbors essentially no precise information about the influence of oxygen on production of the sexual apparatus by representatives of the Saprolegniaceae. From some unrefined experimental work with colonies of *Protoachlya paradoxa* grown in various quantities of water, Lounsbury (1930) decided that the sexual

expression was in some way related to free oxygen because oogonia were produced in what he believed were conditions of reduced oxygen tension. Salvin (1942a), on the other hand, showed that the relative abundance of antheridial branches decreased in colonies of *Brevilegnia* sp. as the depth of water in which the mycelium was grown was increased. Sexuality in *Achlya flagellata* seems not to be unduly influenced by oxygen concentration, although Barbier (1969) stated that oxygen on the order of 60% saturation was required if this species was to reproduce. Stationary cultures were found by Chong (1973) to be superior to aerated ones for the production of oogonia by *Saprolegnia diclina.*

THE OOSPORES

For many watermolds, aging (or afterripening) is a necessary prerequisite to oospore germination. The physiological changes associated with aging, however, remain to be uncovered, and thus essentially all that is known of the physiology of the zygotes relates to conditions at the time of their germination. It is to be emphasized that factors involved in promoting germination are still not identified (Lasure, 1972, among others), and consistently effective methods of inducing the process have escaped definition.

TEMPERATURE

To be sure, temperature may have some very slight influence on the number of oospores, as Reischer (1949b) found for *Achlya sparrowii* (=*racemosa*) -- predominantly 3-4 zygotes in oogonia on colonies incubated at 15 °C, and 2-3 in those on mycelium held at 25 °C -- but certainly this is not a universal effect. At 15 °C, oogonia of *A. hypogyna* are reported (Clausz, 1968) to produce 2-6 oospores, but at 25 °C incubation the usual number per oogonium is one or two. Temperature does not affect the mean number of oospores in *Saprolegnia australis* (R. F. Elliott, 1967a), and we could not find a relationship between temperature and oospore number (or size) in some unpublished data gathered during our study (T. W. Johnson and Seymour, 1974a) of several forms of *A. americana*. The influence of temperature, then, is primarily on oospore germination, at least insofar as the existing literature on the family indicates.

Trow (1899), who reported that oospores nearest to fertilization tubes in oogonia of *Achlya americana* var. *cambrica* were first to germinate, simply stated that a combination of bright light and warm temperature induced germination in this species. The most extensive work on oospore germination was that of A. W. Ziegler (1948b), but he said little of temperature effects (germination was inhibited at room temperatures above 29.4 °C. The percentage of germination of the oospores in *A. hypogyna* was reported by Clausz (1968) to rise as the incubation temperature was increased to 20 °C, and then to drop to zero at 35 °C. In 1925 F. R. Jones and Drechsler reported that the product of oospore germination (and not necessarily the process itself) was influenced by temperature. Oospores of *Aphanomyces euteiches* from colonies incubated at 6.5-31 °C produced either mycelium or spores at germination, but the zygotes formed only spores at 14-28 °C. These obviously overlapping ranges seem inconsistent, and certainly the report is in need of confirmation. It may be noted that W. A. Sherwood (1966b) could not determine if temperature was influential in germination in some species of *Dictyuchus* because oospore disintegration was so common.

One aspect of the relation of temperature to oospore germination, that of temperature shock as a triggering factor, hardly has been explored. Schlösser's (1929) study of several species of Saprolegniaceae suggests that shock treatment may at least in some instances substantially reduce the usually lengthy time spans (of afterripening?) required for germination to begin. He subjected cultures to -22 °C, then brought them quickly to 20 °C. This treatment shortened the germination times of some species from 6-8 weeks to periods from ten hours to three days. Less obvious but nonetheless indicative results were also obtained by Wemmer (1954). He exposed cultures of *Saprolegnia ferax* for three weeks to temperatures within the range of 2 to 30 °C, then brought the fungi to room temperature for further incubation. More oospores germinated in cultures exposed to lower temperatures (for example, 2° and 9-10 °C) than in those held for three weeks at 23°, 25–26 °, or 30 °C. The oospores of *Aphanomyces euteiches* certainly do not seem to respond to temperature shock. Olofsson (1968) subjected them to 60 °C, freezing at -4 ° and -20 ° followed by thawing, and to alternating freezing and thawing at –20 ° and 20 °C, respectively. None of these treatments triggered germination.

OTHER FACTORS

The literature is contradictory on the matter of illumination as a factor in oospore germination in watermolds. Trow (1899) found bright light enhanced the process (if combined with high temperature), but Wemmer (1954) saw no effect on the oospores of *Saprolegnia ferax*. Exposure to light was necessary for maximal oospore germination in three test species used by A. W. Ziegler (1948b): *Achlya recurva, Protoachlya hypogyna,* and *Thraustotheca primoachlya* (=*A. primoachlya*). Germination of very low numbers of oospores produced by these species also occurred in cultures in the dark, hence light is not a mandatory prerequisite for this process to occur.

In general, acidity at pH 4.9 and 5.9, A. W. Ziegler (1948b) reported, retarded germination or at least modified the nature of the product in some watermolds. Only the oospores of *Achlya recurva* would germinate at pH 7.9, and none did so in cultures held at pH 8.8.

Olofsson (1968) has explored rather fully the possible factors influencing or triggering oospore germination. He reported that if oospores of *Aphanomyces euteiches* were removed from decomposed roots of *Pisum sativum* and subjected to tap water adjusted to pH 4.5-4.8, up to 40% of the zygotes germinated. The effective range of pH was very narrow since there was no germination at any pH below 3.2 or above 5.1. Olofsson suggested that in acid conditions fungistatic substances in the pea roots might be inactivated, and the oospores embedded in the cortical tissues might then germinate.

His observations demonstrate that the conditions for germination in this species are far more precise and particular than F. R. Jones and Drechsler (1925) had found. They induced oospore germination in *A. euteiches* simply by transferring oogonium-bearing hyphae from cultures 15 days old to drops of water in Van Tieghem cells.

Although Trow (1899) stated that a rich supply of oxygen was very likely an enhancing factor in oospore germination, he offered no supportive evidence. In a paper appearing in 1927, P. M. Patterson wrote that he was able to stimulate germination in 3-month-old oospores of *Achlya colorata* by centrifuging them for 45 minutes. However, the oospores germinated naturally when they were about 3.5 months old, so the efficacy of centrifugation is certainly suspect in this instance. Oxygen, nitrogen, and $C0_2$ did not stimulate oospore germination in species of *Dictyuchus*, nor did enriching the medium or incorporating cellulase into it, W. A. Sherwood (1966b) has reported. He found germinating oospores in oogonia on colonies propagated in filtered, nonsterile, raw creek water, but only after an average incubation time of 32 days. Scharen (1960b) observed that glucose, fructose, and galactose in the medium did not stimulate oospore germination by *Aphanomyces euteiches* nor did a variety of common amino acids. He proposed that two edaphic factors possibly operated to maintain viable oospores of this pathogen in the absence of host plants. Unsterilized soil, he suggested, is fungistatic, and a combination of some degree of anaerobiosis combined with chemical stimulation was necessary to trigger germination. Olofsson (1968) was unable to induce oospore germination in *A. euteiches* either by exposing oogonium-producing colonies to vitamins, amino acids, sugars, or to nucleic acid derivatives (he tested 75 such compounds). It would in any case appear that when oospores of watermolds are properly “aged” -- with whatever implications that might have -- nutrients can at least influence the percentage of germination. This supposition seems to be borne out by Clausz’s (1968) study.