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THE MYXOMYCETES II[1]

CONSTANTINE J. ALEXOPOULOS[2]

Plant Research Institute and Department of Botany
University of Texas, Austin, Texas

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INTRODUCTION

More than twenty years have passed since G. W. Martin (208) summarized our knowledge of the Myxomycetes. Much has been learned concerning their structure, development, life-history, and physiology during this period and some of our ideas have changed regarding them. Fundamentally, however, the Myxomycetes remain a remarkably homogeneous group and must now be considered separate from the Acrasiales and the Plasmodiophorales with which they have often been grouped (7, 30, 292).

Few biologists seriously believe any more that the Myxomycetes and the Acrasieae are closely related. The researches of Raper, Bonner, Sussman, Wilson, and others during the last twenty years, summarized by Bonner (34) in his recent treatise on these organisms, have focused attention on the Acrasieae and have widened rather than narrowed the gap between them and the Myxomycetes. Biologists, on the other hand, have been somewhat more reluctant to deny absolutely any relationship between the Myxomycetes and the Plasmodiophorales. The strongest

argument for such supposed relationship is furnished by the belief that the swarm cells of both groups are heterokont (84, 85, 86, 169, 180) possessing two anterior flagella of the whip lash or modified whip lash type (86). Nevertheless, the discovery of zoosporangia in a number of Plasmodiophorales (157), the absence of a fruiting body, and the peculiar protozoan type of nuclear division which they exhibit and which has not been found in the Myxomycetes, constitute perhaps even stronger arguments against a close relationship. Sparrow, who has for a long time included the Plasmodiophorales in the biflagellate Phycomycetes (304), now separates them completely, giving the Plasmodiophorales class rank under the name Plasmodiophoromycetes (305). In this reviewer's opinion, in the present state of our ignorance, this is the most realistic view.

Martin has expressed the opinion in the past (207) that the Myxomycetes are closely related to the Fungi and has included them as a class, coördinate with the Phycomycetes, Ascomycetes, and Basidiomycetes, in his Division Fungi (212, 213). In his latest dissertation on the systematic position of the Myxomycetes (216) he reviews recent findings and their impact on phylogenetic considerations and reiterates his former views concerning their relationship to other organisms, further strengthening his arguments with the results of newer research.

Our knowledge of the Myxomycetes is still too meager to allow more than mere speculation concerning the phylogeny of the forms within the class itself. The studies of Ross (265) on the development of the life history of 19 species lead him to suppose that the Reticulariaceae, Stemonitaceae, and Trichiaceae are primitive, and the Physarales more advanced. He states, however, that many more species will have to be studied before evolutionary trends become seriously evident. Dennison (75) was of the opinion that *Comatricha* is ancestral to *Stemonitis* and *Lamproderma* and Ross (264) supports this view. At the IX International Botanical Congress in Montreal, Ross (266) also speculated on the possible relationship of the Ceratiomyxales to the Stemonitales on the basis of certain similarities in the initial developmental pattern of the fruiting structures in the two orders. Recent work (12, 13, 14, 222, 223, 224) on the morphology of the plasmodium opens a new line of investigation which may eventually throw additional light on relationships within the class. This paper deals with the Myxomycetes *sensu strictu* and is based largely on literature published between 1940 and 1962.

THE SPORES

STRUCTURE AND DISSEMINATION

The gross morphology of the spores of most species of Myxomycetes is fairly well known, for mycologists have for a long time considered it to be a major character in the differentiation of species. In general, spore walls of Myxomycetes are smooth, spiny, verrucose, or reticulate, or exhibit various combinations of these characters as do some species of *Badhamia, Stemonitis,* and *Reticularia.* A type of spore occasionally encountered is that of *Comatricha martinii* (17) characterized by prominent clusters of spines or warts scattered unevenly over the generally spiny or verrucose surface. What appears to be a unique type of spore is that of the small genus *Echinostelium.* The spore wall in three of the four described species of this genus is thin over most of its surface, but thickened in a few more or less evenly distributed areas (10, 13, 15, 249). In the fourth species the thickenings of the spore wall are described (237) as being "absent or so very inconspicuous as to appear so." Culture work on *Echinostelium minutum* has shown (13) that this spore character is constant when the sporangia are developed on different substrata under different conditions.

Spore markings, however, are not constant in all species of Myxomycetes. For example, a taxonomic study of three species of *Trichia,* which are differentiated to a large extent on the basis of spore characters, reveals (92, 94) a complete gradation in markings from those of *T. favoginea,* whose spore surface is generally described as completely and conspicuously reticulate, to those of *T. persimilis* in which the spores are "marked by elongate warts forming an irregular or fragmentary reticulation" (212). Such variation may be detected even in the spores from a single specimen in some instances.

Whether the electron microscope will help in the study of spore structure remains to be seen (194). Certainly such studies should be based on ultra-thin sections, for studies based on whole mounts (52, 353), although useful, have, up to now, added little to our knowledge of spore structure.

There are very few extant studies on the chemical structure of the myxomycete spore wall. The older literature on the chemical composition of fungal membranes (328) indicates that chitin is not present in the Myxomycetes except possibly in the capillitum of *Stemonitis fusca.* Cellulose, on the other hand, is said to be characteristic of the Myxo-

mycetes (except *Fuligo septica?*) and the Oomycetes. Proteids having the characters of keratins, have been reported in the capillitum, the spore wall, and the cysts of the Myxomycetes, which are unique among the fungi in this respect (328). Chitin has been reported in the Plasmodiophorales as well as in the majority of the higher fungi. Such reports, if valid, constitute additional evidence against a close relationship between the Myxomycetes and the Plasmodiophorales. More recently, the presence of cellulose was demonstrated (117) in the spore walls as well as in the capillitium and stalk of three species of *Comatricha,* both with the I_2KI/H_2SO_4 and the chlor-zinc-iodide reagents. A chitin test gave negative results. It must be emphasized, nevertheless, that until more modern methods are employed in such research our knowledge of the chemical composition of the spore wall of the Myxomycetes will remain so meager that it will continue to be hazardous to draw any conclusions on this topic.

Very little is known concerning the effect of the environment on the size of myxomycete spores. The results obtained by Solis (303) with *Physarum nicaraguense* point to the need for such data. Solis obtained plasmodia from spores sown on corn meal agar and transferred them to sterilized elm bark where they produced fructifications. Comparing the measurements of 200 spores produced on elm bark with those of an equal number developed on corn meal agar under the same conditions of temperature and light, she found that on the natural substratum the range was 7–12.5μ and the median 10μ, whereas on agar the range was 8.5–14.5μ and the median 13μ.

Our ideas concerning dissemination of myxomycete spores are based mostly on field observations; on our knowledge of the structural features of fruiting bodies; and on knowledge obtained from other organisms which possess structures resembling, in a general way, those of the Myxomycetes. In *Trichia persimilis,* after the sporangia split open and expose the spores and capillitial elaters, the latter twist and untwist according to the humidity of the air around them (147). Strains are set up between closely placed elaters which may be relieved when the ends spring free from the mass, flinging adhering spores into the air. Brodie and Gregory (35), studying the action of wind in the dispersal of spores from cupulate plant structures, observed comparative wind action on spore dispersal of *Badhamia utricularis, Comatricha typhoides, Craterium leucocephalum, C. minutum, Fuligo septica, Physarum nutans,* and *Trichia affinis.* In all experiments, they found that

wind of 0.5 meter per second blew spores from myxomycete fruiting bodies but no more easily from cupulate than from non-cupulate structures.

That wind is the primary factor in the dispersal of myxomycete spores has been taken for granted, but reports in the literature of air-borne spores are virtually non-existent. Van Overeem (330) cultured *Physarum nutans* from rain water collected in sterile vessels and Pettersson (256) reported *Stemonitis fusca* and *Arcyria denudata* spores in rain water, but none of the investigators who have been engaged in aeromycological studies has, to my knowledge, reported capturing myxomycete spores directly from the air on coated slides, agar, or other spore-traps used. The lack of such data may be due, at least in part, to the difficulty of recognizing myxomycete spores when they are divorced from the fructifications in which they were developed. The unpublished results of Brown (37), with regard to this topic, are therefore of considerable interest. In connection with a study of airborne algae, mosses, and ferns, still in progress, Brown exposed Petri dishes, containing synthetic agar, to the air in various ways and in various parts of the United States, but chiefly in Austin, Texas, and found plasmodia of Myxomycetes developing not infrequently on the agar. *Physarum gyrosum* and *Didymium iridis* are among the species that have fruited in his cultures. Although spores were not actually observed in the dishes in which the plasmodia appeared, the size of such plasmodia when first detected makes it seem almost certain that they originated from spores in all cases. The only other possible explanation is that plasmodia developed from very minute wind-blown sclerotial fragments.

Insects and other animals have also been shown to carry the spores of some myxomycetous species (168), but how important they are as dispersing agents is not known.

Germination

Germination of myxomycete spores has been studied extensively by a large number of investigators in the past (208), but data regarding optimum conditions for germination are conflicting. It appears that the time required for germination of spores, as well as the percentage of germination, varies not only with conditions and with the age of the spores but probably with the particular strain of the organism as well. For example, Smart (290), in his often quoted paper, reported the shortest average time for spore germination under the conditions

of his experiments, to be 8 days for *Arcyria cinerea,* 3 days for *Didymium iridis*[3], and 5 days for *Physarum viride.* In our laboratory spores of *Arcyria cinerea* (from sporangia developed in moist chamber culture on bark from a Florida palm) germinated in less than 24 hours; of *Didymium iridis* in less than 17 hours, and of *Physarum viride* in less than 74 hours. On the other hand we have found it very difficult to germinate spores of *Arcyria cinerea* from many freshly collected field specimens.

Jahn (149) found that wetting and drying increased the percentage of spore germination in *Reticularia lycoperdon.* Extracts from spruce wood and from certain fungi were also effective. However, the spores of this organism have long been known for the ease and rapidity with which they germinate and these findings add little to our knowledge. Abe (3), studying the effect of pH on the germination of the spores of *Fuligo septica* var. *rufa, Reticularia lycoperdon,* and *Physarum gyrosum,* observed that free acids, even in very weak concentrations, inhibit spore germination. In buffered solutions the spores germinate above pH 4 and the swarm cells swim actively. In a solution of somewhat lower pH no germination occurs, and if active swarm cells are transferred into it they rapidly sink to the bottom. For *Fuligo septica* and *Reticularia lycoperdon* the optimum pH for spore germination was 6.5 and for *Physarum gyrosum* it was 7.3. The effect of cold and heat shock on 6-month-old spores of *Fuligo septica* was studied by Scholes (271) who found that both treatments greatly reduced or prevented germination. Scholes also studied the effect of spore concentration on germination and found that in a range of samples containing approximately 26,000 to 3,200 spores/mm^3 the lower concentration fostered earlier and higher percentage of germination than the higher concentration.

Elliott (84, 85), in one of the most extensive surveys of spore germination made in recent years, worked with 59 species of Myxomycetes. By using sodium taurocholate as a wetting agent, he induced the spores of all species examined, except *Echinostelium minutum,* to germinate. He attributed his one failure to the minuteness of the sporangia of this species which prevented him from obtaining sufficient material to handle by the method employed successfully for other species. Alexopoulos (13) recently succeeded in germinating the spores

[3] It seems best to use currently accepted names for all taxa mentioned in this review. The names used are those recognized by Martin (208).

of *E. minutum* taken from sporangia which had developed on a dead leaf of *Ulmus americana* in moist chamber. He found that germination would often take place in as short a time as twenty minutes when the spores were placed in a drop of water on corn meal agar. He also observed spores germinating on moist agar in the absence of free water. Alexopoulos experienced the same difficulty as Elliott with spores from sources other than the elm leaf, but Olive (249) subsequently germinated spores of this species which had developed on old milkweed pods and dead mullein plants in moist chamber.

It is generally conceded that age markedly affects spore germination in the Myxomycetes. It has been equally well established, however, that some spores remain alive for very long periods of time. Martin (208), reviewing the old literature, mentioned Smith's success in germinating some spores 32 years after their collection. Elliott (85) reported that, although age reduced markedly the percentage of germination in all species he investigated, some spores germinated even from collections which had been stored in the herbarium for as long as 61 years.

Germination of single spores is said to be more difficult than germination in mass sowings (290). Nevertheless, Gehenio and Luyet (109) were able to germinate single spores of *Physarella oblonga;* Kerr and Sussman (165) had considerable success with *Didymium nigripes* when a spore dilution was poured over agar to allow for wide separation of spores; Collins (56) met with little difficulty in germinating single spores of *Fuligo cinerea*; Olive (249) obtained 33 monosporous cultures of *Echinostelium minutum;* and Solis (303) reported 90% germination when she sowed 200 single spores of *Physarum nicaraguense* on corn meal agar. In all these organisms, monosporous cultures gave rise to plasmodia and eventually to fruiting bodies. Single spore germination has also been obtained in recent years for *Physarum polycephalum* (72, 73) and for *Didymium iridis* (19, 56, 57). On the other hand, Koevenig (166) reports that single spores of *Physarum gyrosum* failed to germinate, but that in mass sowings some germination was obtained.

That the source of spores may be a factor influencing spore germination has long been suspected. Alexopoulos' experience with *Echinostelium minutum* has already been mentioned. In attempting to establish clonal cultures of *Didymium iridis,* Collins (56) selected 13 sporangia developed on agar from which he isolated and sowed a

total of 256 single spores. The percentage of germination varied from 0% in two sporangia from which 25 and 30 spores were taken respectively, to 100% in one sporangium from which 12 spores were sown. Sporangia taken from the same Petri dish culture gave similar results, but inasmuch as all the cultures were grown under the same laboratory conditions Collins could offer no explanation for the difference in germination. Such difference in behavior of spores taken from different sporangia may explain the disagreement among different workers concerning time and percentage of germination for spores of the same species.

Our knowledge concerning method of spore germination in the Myxomycetes has not advanced since Martin (208) reviewed the literature 23 years ago. McManus (221) states that spore germination in *Ceratiomyxa fruticulosa* involves the softening and gradual disappearance of the spore wall. However, the "spore" of *Ceratiomyxa* is, in reality, a sporangium (114) and is not homologous with the spores of the Myxogastromycetidae.

SWARM CELLS AND MYXAMOEBAE

Up to 1948, students of Myxomycetes had regarded the swarm cells as uniflagellate with an occasional cell—considered to be atypical—possessing two flagella. Evidence was beginning to accumulate, however, in favor of a biflagellate or potentially biflagellate condition (208). In 1948 and 1949 Elliott (84, 85) reported that the swarm cells of some 58 species, representing 30 genera, possessed two flagella each, which, though difficult to demonstrate in some cells because one flagellum is often very short and is appressed to the protoplasmic membrane, were nevertheless present. Elliott found that the swarm cells of most species studied were heterokont and could be placed in three groups depending on the relative length of the two flagella, but concluded that, because of considerable variation within the same species, the length of the flagella is of doubtful taxonomic significance. Locquin (192), using cinephotomicrography and phase-contrast optics, and as yet unaware of Elliott's findings, found "indisputable" evidence of the presence of a short, immobile flagellum at the base of the long active one, in all 58 species of Myxomycetes which he studied. The second flagellum was recurved on the surface of the swarm cell. McManus (221) confirmed Elliott's findings for *Ceratiomyxa fruticulosa* in which, she stated, one of the two flagella

is so small that "special staining methods are required to see it." Ross (265) referring to the swarm cells of 19 species in 6 families states: "Examination of living cells with the phase microscope . . . reveals that the apparently uniflagellate cells are biflagellate; the second flagellum is extremely short (1–2 μ) and usually closely appressed to the cell membrane." Although the work of Elliott and subsequent workers appeared to have established beyond reasonable doubt the biflagellate condition of myxomycete swarm cells, Cohen (51) reopened the question in 1959 at the IX International Botanical Congress in Montreal, reporting the flagellation of the swarm cells is not necessarily the same for all species. He believes that flagellum-like pseudopodia ("pseudoflagella") which he adequately demonstrated by electron microscopy, have been mistaken for flagella by Elliott and others. Subsequently, Alexopoulos (13) reported biflagellate cells, with flagella of almost equal length, in Echinostelium minutum, but Olive (249) found uniflagellate cells in this organism to be the rule. McManus (222) stated that the swarm cells of *Stemonitis fusca* are uniflagellate, but that most swarm cells of *Clastoderma debaryanum* (223) possess a very short, recurved, second flagellum.

In an attempt to resolve the question of flagellation Koevenig used cinephotomicrography (167) as well as standard fixing and staining procedures (166). He confirmed Cohen's discovery of pseudoflagella, which he found in all the 14 species he studied, but also demonstrated true second flagella in from 1% to 50% of the swarm cells, this percentage varying with the species, the age, and the condition of the swarm cells. Kerr's work (161) with *Didymium nigripes,* demonstrating that newly flagellated cells are uniflagellate but may become biflagellate after several hours, provides at least a partial explanation of the disagreement among various workers.

In view of the consistent presence of two basal bodies in the myxomycete swarm cell (161, 166, etc.) perhaps the most accurate statement that may be made at present is that myxomycete swarm cells are potentially biflagellate, but that uniflagellate swarm cells occur and may even predominate at times in any given population.

The structure of the myxomycete flagella as well as their number ought to be investigated further. Ellison (86) found a knobbed flagellum in some Myxomycetes and theorized upon its nature. Knobbed flagella have been noted by other students of these organisms, although the myxomycete flagella are usually designated as whiplash. Electron

microscope studies should elucidate the nature of the knob and should reveal as well whether, in common with those of many other flagellated cells, the myxomycete flagella exhibit the 9 + 2 strand pattern.

Swarm cells change into myxamoebae by withdrawing their flagella and myxamoebae in turn become flagellated under certain conditions. In the laboratory, optimum conditions for the development of flagella by the myxamoebae of *Didymium nigripes* prevail when the latter are incubated in 0.05 M $NaHCO_3$ at pH 9.1 (161). Flagellum formation seems to be independent of cell concentration and light appears to have no effect. Streptomycin inhibits flagellum formation in proportion to its concentration. What the stimulus is for formation of flagella by the myxamoebae is not known. It appears that a liquid environment is sufficient to stimulate their development.

That myxamoebae divide and build up large populations in a short time is well known. Blickle (31) has shown that when sulfhydryl is added, as 2.6×10^{-7} S in parathiocresol, to the culture medium, the swarm cell and myxamoebal population increases more rapidly than in the control cultures. The addition of 2% (w/v) glucose or 0.2% (w/v) brucine to the culture medium prolongs the myxamoebal stage and prevents plasmodium formation in *Didymium nigripes* (165). Plasmodium formation may be delayed for 24 hours by the addition of 0.1% (w/v) of sulphanilamide. Brucine may act as a chelating agent, removing from the medium certain multivalent cations necessary for plasmodial formation (162).

The myxamoebae of *Physarella oblonga,* according to Gehenio and Luyet (111) survive (2–48% survival) immersion in liquid nitrogen for one minute if previously treated with 2–4 M ethylene glycol. Untreated myxamoebae do not survive.

Whether swarm cells as such ever divide is still a somewhat controversial question. It is believed at present that divisions take place only after the flagella have been withdrawn and the swarm cell has changed into a myxamoeba.

THE NUCLEAR CYCLE

PLASMOGAMY

Reports concerning syngamy in the Myxomycetes are conflicting. Some writers have reported gametic fusion for certain species whereas others claim that these same species are apogamic (208). Moewus (228), reviewing in 1943 the pertinent literature on sexuality of the

Myxomycetes, pointed out the necessity for exact cytological observations, particularly for species for which apogamy had been claimed. Luyet (200, 201) in 1950 questioned the conclusions of all those who had reported gametic fusions in the Myxomycetes and was clearly of the opinion that sexuality is lacking in these organisms. Gehenio and Luyet (109) obtained plasmodia and sporangia from monosporous and monamoebal cultures of *Physarella oblonga* and presented these results as evidence for lack of gametic fusion in this species. In cultures in which a large population of myxamoebae had developed through successive divisions, "one or more larger amoebae appeared quite suddenly in the culture . . . The actual formation of these larger amoebae . . . was never observed." In view of myxamoebal fusions which have since been observed (265) in this species, the more probable conclusion to be drawn from the work of Gehenio and Luyet is that *Physarella oblonga* is homothallic.[4] McManus (221) observed fusion of swarm cells in *Ceratiomyxa fruticulosa*, but not karyogamy. Ross (265) working with 19 species distributed in six families found gametic copulation and karyogamy in all. In all but three, syngamy occurred through fusion of swarm cells. In the other three *(Didymium squamulosum, Physarum oblatum,* and *Physarella oblonga)* the swarm cells changed to myxamoebae without copulating, and fusion occurred between pairs of myxamoebae. Koevenig (166) observed plasmogamy in *Stemonitis flavogenita, Physarum gyrosum, Reticularia lycoperdon, Didymium iridis,* and *Fuligo cinerea.* In *R. lycoperdon* fusion was seen only between swarm cells; in *D. iridis* and *F. cinerea* only between myxamoebae; and in *S. flavogenita* and *P. gyrosum* sometimes between myxamoebae and at other times between swarm cells. In the last species both types of fusion were film-documented (167). Kerr (163) reported both types of plasmogamy in *Didymium nigripes.* McManus (222) failed to establish gametic fusion in living material of *Stemonitis fusca*, but states that in stained preparations there is some evidence of fusion between one flagellated and one amoeboid cell. It is interesting to note here that Benedict (27) observed such copulation and stated that this is the only method which *S. fusca* employes in

[4] The terms homothallic and heterothallic, as applied to the Myxomycetes, refer to the ability or lack of it of monosporous cultures to form zygotes and plasmodia. Inasmuch as myxomycete spores are characteristically uninucleate and haploid, zygote formation resulting in plasmodial development occurs in monosporous cultures of homothallic but not of heterothallic species.

plasmogamy. The myxamoeba, he writes, flows into the swarm cell, the union producing a flagellated zygote. McManus in another paper (223) reported fusion of flagellated cells in *Clastoderma debaryanum.* Thus, although the possibility that apogamy may operate in some species cannot be denied, the evidence for the occurrence of gametic fusion in all species investigated is overwhelming. Genetic experiments, to be described in a subsequent paragraph, prove beyond doubt that plasmogamy is a critical phase of the myxomycete life cycle.

The method by means of which plasmogamy takes place is still controversial, as can be seen from the above discussion, but it is probable that many species employ either swarm cell or myxamoebic copulation depending on circumstances.

KARYOGAMY

As mentioned above, Ross (265) reported karyogamy in the zygotes of 19 species of Myxomycetes he investigated. His conclusions were based on stained preparations which showed nuclei in close proximity and presumably fusing in the same protoplast. Koevenig (166) actually observed and, by means of phase cinephotomicrography (167) conclusively demonstrated karyogamy in the zygote of *Physarum gyrosum.* Dee (73), however, states that her densitometer measurements of DNA in the nuclei of the amoebae and the plasmodium of *Physarum polycephalum* show these nuclei to have the same ploidy. She suggests that karyogamy in this organism takes place just before meiosis at the time of spore delimination.

COMPATIBILITY

The question of homothallism and heterothallism (the existence of an incompatibility mechanism) in the Myxomycetes has only recently been clarified, for, in spite of claims to the contrary (46, 208, 283, 284), heterothallism, although strongly suggested, had not been clearly established in any myxomycete prior to the work of Dee (72) and Collins (56). These two investigators, working independently, proved beyond doubt that *Physarum polycephalum* and *Didymium iridis* respectively are heterothallic, i.e., require the fusion of two compatible gametes of opposite mating types for the formation of a zygote and, consequently, of a plasmodium.

Kerr and Sussman (165) using dilution techniques, observed myxamoebal populations in the vicinity of empty spores of *Didymium ni-*

gripes on agar media, and assumed that these populations and the plasmodia formed from them represented clones. The cultures fruited, as had Cayley's (46) single spore cultures of this species. Assuming that gametic fusion is a prerequisite to plasmodium formation in *D. nigripes,* as indeed it has been shown to be by Kerr (163), Kerr and Sussman's cultures, if clonal, constitute proof that this organism is homothallic. Olive (249) has obtained plasmodia and sporangia from single spore cultures of *Echinostelium minutum.* This may indicate that the organism is homothallic. However, syngamy has never been reported for this species and the possibility of apogamy cannot be ruled out. In the same paper in which he reported heterothallism in *Didymium iridis,* Collins proved that *Fuligo cinerea* is homothallic[5]. Solis (303), using the same techniques, showed that monosporous cultures of *Physarum nicaraguense* invariably produce plasmodia and fruiting bodies with viable spores under favorable culture conditions.

Thus, of seven species investigated in recent times, two *(Physarum polycephalum* and *Didymium iridis)* are heterothallic, three *(Didymium nigripes*[6], *Fuligo cinerea and Physarella oblonga)* are homothallic, and two *(Echinostelium minutum* and *Physarum nicaraguense)* are either homothallic or apogamic depending as to whether gametic fusion is necessary for the formation of plasmodia.

Of considerable significance are the results obtained by Collins (57) working with two races of *Didymium iridis,* one from Honduras and one from Panama. He found that although both races are heterothallic, either mating type of each race would freely cross with either mating type of the other. He interpreted these results as indicating the existence of multiple alleles governing mating type and marked these *a, b, c,* and *d.* Working independently with two Honduras races of the same species we have obtained the same results in our laboratory.

Hybridization

Hybridization in the Myxomycetes has, up to now, been accomplished only between races of the same species. Dee (73) in England

[5] Although Collins did not report gametic fusion in this species, Koevenig, working at the same time in the same laboratory had observed plasmogamy in this organism (166).

[6] According to Kerr (160) and Kerr and Sussman (165), clonal cultures of *Didymium nigripes* produce plasmodia and fruiting bodies; according to von Stosch (56, 208) however, this is not the case. It may be that different races of *Didymium nigripes* vary in this respect.

and Alexopoulos and Zabka (19) in the United States were the first to produce myxomycete hybrids, the former working with *Physarum polycephalum,* the latter with *Didymium iridis.* Dee crossed an emetine-resistant race with an emetine-sensitive race and by analysis of the progeny showed that emetine-resistance is controlled by a pair of alleles. Alexopoulos and Zabka crossed two races whose plasmodia would not inter-fuse and obtained hybrid plasmodia which would not fuse with those of either parent.

MEIOSIS

Martin (208) and Olive (248) have discussed the controversy concerning the place in the life-cycle at which meiosis occurs. Martin concluded that from the evidence at hand it is clear that the diploid phase lasts at least until the formation of the spores. In *Physarum polycephalum,* Dalleux (63) found that the nuclear divisions coinciding with sporulation displayed no evidence of meiosis. However, she refused to draw general conclusions regarding the life-cycle of the organism from these results because her cultures produced multinucleate, giant spores. In some maturing spores of *Didymium clavus,* Dangeard (64) observed two or even four nuclei. In other spores he saw two resting nuclei, side by side, with a metaphase plate of 8 chromosomes. He drew the conclusion that this is evidence of meiosis taking place in the spore. According to Schure (272) the first meiotic division in *Mucilago spongiosa* takes place in the protoplasm of the aethalium before cleavage, and the second after cleavage, the immature spores containing a variable number of nuclei. In *Physarum didermoides* only a single division was observed before spore formation. Wilson and Ross (347) in their extensive investigation of meiosis in the Myxomycetes have gone a long way toward resolving this controversy of long standing. They agree with Gilbert (114) in finding that in *Ceratiomyxa* meiosis occurs in the "spore." In all species of Myxogastres studied, meiosis occurs in the fructifications just preceding delimitation of the spores according to these authors. Ross, in two subsequent papers (267, 268), reported his studies of meiosis in five additional species. Again he found meiosis to occur at the time the spores are delimited, the divisions being similar to those in higher organisms. In contrast to Howard (146) and to Guttes, Guttes, and Rusch (131), who found but a single division in the sporangia of *Physarum polycephalum,* Ross described two divisions in this as well

as all other organisms he studied. Koevenig's figures (166, 167) of *Physarum gyrosum* also indicate that meiosis takes place at the time the spores are delimited, but inasmuch as chromosome counts could not be made, his results are not conclusive.

The genetic experiments reported earlier (56, 57, 72, 73) leave no doubt that the spores of Myxomycetes are characteristically haploid. However, in all experiments with heterothallic species, in which normally a monosporous culture does not yield plasmodia unless mated witl. another compatible clone, a certain percentage of single spore cultures always produced plasmodia. This could be accounted for by assuming that the spores from which such cultures developed were binucleate or quadrinucleate and gave rise to two or more amoebae of opposite mating types. However, in *Didymium iridis* at least, no spore was ever seen to produce more than a single swarm cell or myxamoeba (56). This might indicate that some spores were diploid and that, either meiosis occurred sometime during the multiplication of the myxamoebae after spore germination or that the diplophase was carried through to the next sporulation. A mutation from one mating type to another occurring in the myxamoebal population, as suggested by Collins, would also explain these results. It is important to add here that spores obtained from fruiting bodies developed from monosporous cultures were all haploid, none yielding plasmodia in single spore culture.

CHROMOSOME NUMBERS

Up to 1935, various investigators were in agreement that the chromosome numbers in Myxomycetes were small ($n = 4$–8). In that year Von Stosch (in 347) counted 24 chromosomes in *Didymium nigripes* and 80 in *D. iridis (xanthopus)*. Wilson and Ross (347) in 1955 confirmed in a general way Von Stosch's work, reporting very high chromosome numbers for the Myxogastromycetidae (up to 90 ± 3 for *Hemitrichia vesparium*). Continuing this work, Ross published (267) an additional series of excellent photomicrographs which leave no doubt about the very large number of chromosomes in 5 additional species. Accordingly, the chromosome numbers in the Myxogastromycetidae range from $n = 25 \pm 2$ in *Dictydium cancellatum* to $n = 90 \pm 3$ in *Physarum polycephalum*. The same year (1961) Guttes, Guttes, and Rusch (131) reported seeing about 20 chromosomes during mitosis in the plasmodia of *Physarum polycepha-*

lum, and "more than 8" in the division preceding spore formation, but the evidence they present in support of their count is not convincing.

It should be noted parenthetically here that Wilson and Ross (347) confirmed Gilbert's work with *Ceratiomyxa* (114) both as to the place of meiosis in the life cycle and as to the low chromosome number ($n = 8$). This strengthens Gilbert's view concerning the homology of the *Ceratiomyxa* "spore" with the sporangium of the Myxogastromycetidae.

PLASMODIUM FORMATION

The method by which the Myxomycetes form their plasmodia is not entirely clear. Because definite information is lacking on this point for the majority of species, discussions of the general life cycle of these organisms must of necessity either generalize on the basis of very few facts or be vague on this part of the life cycle. Martin (208), in his concluding remarks on the life cycle of these organisms stated: "Growth of the zygote into the plasmodium is accomplished by nuclear division and the plasmodium may increase by fusion with others . . ." Bessey (30) wrote: "The zygote . . . grows in size, which accompanying mitotic divisions of the nucleus. This multinucleate structure is called a plasmodium. Zygotes or small plasmodia may fuse with other zygotes and plasmodia so that growth is both internal and by accretion." Alexopoulos (7) described the formation of the plasmodium as follows: "Growth of the zygote is accompanied by a series of mitotic nuclear divisions resulting in a multinucleate plasmodium with diploid nuclei. A plasmodium may also be formed by the coalescence of many zygotes . . ." Smith (292) does not mention coalescence of zygotes to form a plasmodium, but states that "the diploid nucleus in a myxamoeba resulting from gametic union divides and redivides mitotically for an indefinite number of nuclear generations as the myxamoeba grows into a plasmodium." Bonner (34) also supports the view that a plasmodium is derived from a single zygote through the successive divisions of its nucleus. None of these authors gives the source of his information. A recent contribution to our knowledge of the mechanics of plasmodium formation is that of Ross (265). On the basis of his observations Ross correlates swarm-cell behavior, syngamy and plasmodium formation in the 19 species he studied, and concludes that three life-history patterns are evident: "1. the briefly flagellate type, in which the amoeboid state is predominant, syngamy

occurs in the amoeboid state, and plasmodia form by the coalescence of numerous zygotes; 2. the flagellate type, in which the flagellate state is predominant, syngamy occurs in the flagellate state, and plasmodia develop from single zygotes; 3. the completely flagellate type, in which there is no haploid, amoeboid state, syngamy occurs in the flagellate state, and plasmodia develop from single zygotes." Thus, Ross has made considerable progress in clarifying the situation in a number of species. Nevertheless, all doubts have not been dispelled. In his briefly-flagellate type of life cycle, for example, he found no evidence of plasmodium formation from single zygotes, but did not actually isolate zygotes to see whether they might grow into plasmodia. Kerr (163), on the other hand, working with *Didymium nigripes,* isolated fusing pairs of swarm cells and proved that single zygotes develop into plasmodia in this species.

Koevenig (167), through cinephotomicrography, has proved that in *Physarum gyrosum* plasmodia form both from single zygotes and from successive coalescence of zygotes. His film shows two successive nuclear divisions in a zygote resulting in a four-nucleated plasmodium and constitutes the first unquestionable proof of plasmodial formation by this process. All previous evidence had been drawn from killed material in which two mitotic divisions were demonstrated in a single protoplast, but without knowledge of how the protoplast originated or what its subsequent development would be. In another sequence of the same film the fusion of a zygote with a two-nucleated plasmodium is shown. Koevenig (166) states that he has seen zygotes merging to form two-nucleated plasmodia. Thus, his results do not support Ross' conclusions. On the contrary, they show that a single organism may form its plasmodia by at least two methods. Nevertheless, when Ross and others before him wrote of the "coalescence of numerous zygotes" they described a phenomenon which occurs commonly in certain species. When a large population of amoeboid cells builds up in a culture, there is evidence of mass aggregation from which plasmodia emerge (265, fig. 7). It is presumed, but has not been proved, that this represents aggregation and multiple fusion of zygotes.

Kerr (163), in a study of plasmodium formation in *Didymium nigripes,* concluded that the optimal concentration of amoebae for plasmodium formation was 2.5×10^5/ml. He based his conclusions on the number of "small clonal plasmodia free from myxamoebae." He does not explain, however, how he can be certain that the plasmodia

were indeed "clonal," i.e., that each plasmodium represented the product of but a single zygote. It is well known that small plasmodia of the Physarales fuse instantly upon coming in contact. Zygotes also fuse one with another and with small plasmodia (167). Kerr also found that $SrCl_2$ added at 0 time to suspensions of 1×10^5/ml myxamoebae in phosphate buffer stimulated formation of plasmodia. This time he actually observed numerous fusing pairs of swarm cells in the presence of $SrCl_2$ whereas in the absence of the salt he rarely found them. Among 9 divalent cations tested, only Sr^{2+} and Ca^{2+} were stimulatory. All others inhibited plasmodium formation to a greater or lesser degree.

THE PLASMODIUM

General Morphology

The use of *Physarum polycephalum* as an experimental organism has yielded much significant information, but it cannot be emphasized too strongly that generalizations for the whole group of Myxomycetes based on this one species alone should be avoided. Familiarity with this and a few other species of the Physarales which can easily be grown in culture has led, for example, to the general concept of the myxomycete plasmodium based on the physaraceous type. Early workers signalled the existence of other types of plasmodia many decades ago, and in more recent years Watanabe (341) and Nauss (241) have again called attention to the fact that not all plasmodia are alike, but without describing major differences in detail.

At least three distinct types of plasmodia have now been described (14). The phaneroplasmodium is characteristic of the order Physarales. Its chief characters are: 1. the granular nature of its protoplasm, which makes this plasmodium easily observable even at an early stage, 2. the conspicuous differentiation of its thick veins into streaming endoplasm and stationary, jellified ectoplasm, and 3, the thick fleshy fan which, in a well developed plasmodium, spreads as a perforated or solid sheet of protoplasm with many channels in which the endoplasm streams. The aphanoplasmodium, characteristic of several of the larger members of the Stemonitales, is known to be formed only by members of this order. It has now been described for *Stemonitis flavogenita* (12, 14), *S. herbatica* (14), and *S. fusca* (222). *Comatricha irregularis* and *C. typhoides* probably possess this type of plasmodium also (14). Its chief characteristics are: 1. the non-granu-

lar nature of its protoplasm which results in a transparency and flatness which renders it difficult to see until it is well developed and approaching the fruiting stage, 2. the almost complete absence of detectable jellified ectoplasm, except in the largest veins, and 3. the nature of its advancing fan which often consists of an open network with very delicate hypha-like strands. The protoplasmodium, characteristic of the order Echinosteliales (14, 15) and of certain minute species, such as *Licea parasitica* and *Clastoderma debaryanum* (223) belonging to other orders, remains microscopic until it fruits and then gives rise to but a single sporangium. Its protoplasm is granular. It exhibits no differentiated advancing fan, no channels, no veins, no reticulation whatsoever, and no rapid, rhythmical, reversible streaming. Protoplasmic streaming, which is sluggish and irregular, can be detected only under high magnification. Thus the protoplasmodium resembles, even in its maturity, the initial microscopic stages of a phaneroplasmodium retaining juvenile characteristics throughout its existence. No evidence of merging between protoplasmodia which were in intimate contact for several hours could be detected by Alexopoulos (13) in his study of *Echinostelium minutum*, or by McManus (223) in *Clastoderma debaryanum.*

In addition to the three types of plasmodia described above, a fourth type may be representative of the order Trichiales as suggested by Alexopoulos (14) from a brief study of *Arcyria cinerea* and by McManus from a more extensive study of *Hemitrichia vesparium* and *Hemitrichia serpula.* McManus (224) states that the plasmodia of these two species "have some characteristics in common with each of the other three types of plasmodia which have been described. They usually grow as fans, and they have an abundance of dense granules, making them readily visible. These are characteristics of the phaneroplasmodium of the Physarales. But, like the stemonitoid aphanoplasmodium, they have no stationary outer gelified layers in any veins excepting the very largest; all the protoplasm within the channels moves in the reversible flow. The random movement of the granules in the protoplasmic sheet and in the lateral extensions of the isolated veins is reminiscent of 'irregular' type of streaming in the third plasmodial type, the protoplasmodium." Balloon-like vesicular processess are often formed by the plasmodia of the Trichiales at the margin of the plasmodial fans, much like those described for *Stemonitis herbatica* (14). Thus the plasmodium of the Trichiales may prove to represent a

fourth type intermediate between the phaneroplasmodium and the aphanoplasmodium.

Stewart and Stewart (309), in a recent discussion of their cinephotomicrographic study of the plasmodium of *Physarum polycephalum,* describe three reversible states of the plasmodial protoplasm: the flowing protoplasm (State I) in which the suspended particles exhibit a strong Brownian movement; the non-flowing protoplasm (State II) which confines and directs the flow of State I in channels and in which vigorous Brownian movement, indistinguishable from that of State I, also occurs; and a more rigid gel (State III) which forms in response to a variety of mildly unfavorable agents. State III is devoid of both flow and Brownian movement and is more rigid and tough than State II. State III upon recovery from the effect of the agents which induced its formation, reverts spontaneously to States II plus I. Very small plasmodia (a few tens of microns in diameter) show no definite channel structure. Reversal-like changes occur occasionally, but not regularly in such plasmodia.

Of unusual interest is the plasmodium of *Reticulomyxa filosa* described by Nauss (242). Inasmuch as it has never been seen to fruit, however, it cannot be included in the Myxomycetes with any degree of certainty. Nauss thinks it may belong to some group intermediate between the Myxomycetes and the Foraminifera.

That environmental conditions influence the color of the plasmodium is well known (208). This is especially true of certain species in which the plasmodium varies from yellow to almost white. Particularly interesting in this connection is the plasmodium of *Hemitrichia vesparium* which is described as deep red, purple red, or deep red to black, by various monographers (185, 205, 212). Gray (121), who obtained plasmodia from spores on agar media, had stated that the plasmodium is pearly-white during the active vegetative growth, turning red only after sporangial delimitation has begun. Nauss (240), in a rather extensive study of the plasmodium of this species, states that black is the only color she observed either in the field or in the laboratory. She grew some of her plasmodia from spores on blotting paper. McManus (224), in her recent study of this same species, describes the color of the plasmodium as black from pH 5 through pH 8. As the pH increased the color changed through brown and red-brown to dark brick-red at pH 13; as the pH decreased the color changed through brown to orange to lemon yellow at pH 1.

Cytology

THE PLASMODIAL MEMBRANE. The plasmodium is often described as acellular and naked (215) but the existence of a plasmodial membrane or envelope, as it is sometimes called, is recognized by all. The nature of this membrane is not well understood. A recent attempt to elucidate its chemical nature gave only negative results (362).

Electron microscope studies by Stewart and Stewart (310) revealed a large number of "vesicles" in the cytoplasm of the plasmodium of *Physarum polycephalum.* When a plasmodial vein is punctured by a needle, the protoplasm flows out and forms a globose droplet which assumes a firm consistency within a few seconds. When such a droplet is fixed soon after it is formed a very great diminution of the "vesicles" in the outer layers of protoplasm is revealed by the electron microscope. Stewart and Stewart suggest that the new plasma membrane may be formed by coalescence of the vesicles from their limiting membranes and postulate that the vesicles contain precursors of additional slime.

NUCLEI AND NUCLEAR DIVISION. The nuclei in the myxomycete plasmodium were described many years ago and the older literature contains many references to them. Newer observations unfortunately do not add much to our knowledge of their structure. Dalleux (63) describes the nuclei of *Physarum polycephalum* as spherical with a diameter of 3–4.5μ. Each nucleus is surrounded by a membrane and contains a nucleolus over 1μ in diameter. All nuclei appear to be identical in an active plasmodium and to exhibit a certain plasticity, inasmuch as more or less elongated forms can be seen. The nuclei of *Fuligo septica* are described as being similar to those of *P. polycephalum* in structure, but smaller. Nuclear divisions are typically mitotic and remarkably synchronous throughout the plasmodium. The chromosome number is large. Lewis (181), in a very meager description of the nuclei of *P. polycephalum,* says they are small, and round. Each contains a large, gray nucleolus. Dangeard, (64) studying *Didymium clavus,* found that all of the nuclei in an active plasmodium are of one type. In the developing sporangia, however, he found two types of nuclei: large, vesicular nuclei, and small nuclei which appeared to be degenerating. The latter type first appears at the very beginning of sporangial formation when the fruiting body is nothing more than a small elevated cushion on the surface of the plasmodium. As the sporangium develops, more and more of these nuclei can be observed.

Dangeard believed the large nuclei to be diploid and the small ones haploid. How this fits into the life cycle of the organism, he did not explain. Locquin (193) studied the nuclei in the plasmodium of *Licea biforis,* using phase contrast cinephotomicrography. He records two conclusions from his study: 1. The nuclei exhibit an extreme plasticity; 2. A relatively rigid connection unites the nuclei to their surrounding cytoplasm. Andersen and Pollock (21) found the nuclei of *P. polycephalum* to be spherical, with a diameter of 4–7μ. These dimensions are larger than those given by Dalleux (63) and other previous observers. The nuclei, according to these writers, contain from one to as many as 5 nucleoli, the size of the latter varying inversely with their number. No nuclear divisions were observed. Guttes, Guttes, and Rusch (131) grew plasmodia of *Physarum polycephalum* in axenic, liquid culture. In interphase, the nuclei were uniformly 4–5μ in diameter and each contained a single nucleolus. A few nuclei were observed with a diameter 1.5 times the usual. A few degenerating nuclei were also present.

Nuclear divisions in myxomycete plasmodia have been described by many workers (63, 64, 146, etc.) and it has long been accepted that such divisions within a single plasmodium are synchronous and rhythmical. Very precise synchronization was achieved (130, 131) when plasmodia of *Physarum polycephalum* were grown axenically in liquid culture in a partially defined medium under controlled conditions. In the strain under study, nuclear divisions took place every 15.4 hours as measured by the doubling of total DNA. Nuclear divisions of different plasmodia in the same culture were not synchronized. When the plasmodia were allowed to coalesce, however, mitosis became synchronous within the entire protoplast after a period of 6–7 hours, or half the average time for interphase. In starved plasmodia, nuclei continued to divide, but at longer intervals and with less synchrony. All stages of mitosis could be found within one plasmodium at the same time. When nutrients were added at early interphase the onset of mitosis was advanced. On the other hand, when nutrients were added less than 7 hours prior to the next mitosis, mitosis was delayed as compared to starved controls (129).

Of great interest are the studies of Nygaard, Guttes, and Rusch (247) which show a great increase in DNA synthesis in a plasmodium starting immediately after each nuclear division and lasting for 1–2 hours, but that RNA synthesis is continuous. It is unlikely, there-

fore, that DNA synthesis acts as a trigger for nuclear division in *Physarum polycephalum.*

Whether heterokaryosis occurs in the Myxomycetes is a very interesting question which cannot be answered as yet. Bonner (34) touches the subject with his categorical statement that "all parts [of a plasmodium] are the product of one zygote nucleus and there is no possibility here of the aggregation of numerous, genetically different nuclei." This is a surprising statement for, although some writers do not admit that a plasmodium may originate in ways other than by the growth of a single zygote, the ability of plasmodia to coalesce is easily demonstrable in many species. It remains to be seen, therefore, whether only genetically identical plasmodia fuse. It has been demonstrated (9, 19, 122, 286, 287) that some species consist of physiological races whose plasmodia will not intercoalesce. Nevertheless, until it is proved that all nuclei of compatible plasmodia are genetically identical, the possibility of heterokaryosis cannot be ruled out. In species like *Echinostelium minutum,* in which plasmodial fusion does not take place readily (13), heterokaryosis may not be a common phenomenon.

VACUOLES. Vacuolation appears to vary considerably with the age and development of the plasmodium, but we have very little definite information recorded concerning such variation. Here again, most studies have been made on the plasmodia of the Physarales and particularly *P. polycephalum* and may not hold true for plasmodia of other groups.

There seems to be some controversy both as to the types and the position of the vacuoles in a plasmodium. Lewis (181) states that vacuoles containing granules persist in the streaming endoplasm of *P. polycephalum.* He observed numerous contractile vacuoles throughout the gel layer. Dalleux (63) in her report of a cytological study of the living plasmodia of *P. polycephalum* and *Fuligo septica* stained with vital stains, writes that all vacuoles she observed contained food particles and that no contractile vacuoles were ever seen. Dangeard (64), however, who studied the living plasmodium of *Didymium clavus* stained with neutral red, observed two types of vacuoles. The food vacuoles are relatively large and contain heavily stained particles, presumably food residues. These vacuoles occur in the streaming portions of the plasmodium. Dangeard also observed much smaller vacuoles, often not exceeding 1μ in diameter, found not only in the

streaming cytoplasm, but also in the ectoplasm. These, according to him, have no relation to ingested material. They appear to be homogeneous and stain uniformly. Anderson and Pollock (21) describe 4 types of vacuoles in the plasmodium of *P. polycephalum*: vacuoles containing food material, vacuoles containing debris, empty vacuoles, and contractile vacuoles. They calculate that 18.1% of the total volume of a plasmodium is occupied by vacuoles, emphasizing that this is a very rough estimate.

OTHER PROTOPLASMIC INCLUSIONS. For many years cytologists have used the plasmodium of the Myxomycetes to study the submicroscopic structure of protoplasm.

In addition to the granules which are included in the vacuoles, Lewis (181) noted numerous granules throughout the gel layer and the endoplasm of the plasmodium of *P. polycephalum.* These he believed to be mitochondria. Dalleux (63) and Dangeard (64) have described structures they term chondriosomes in their respective organisms. In *P. polycephalum* Dalleux found that the chondriosomes are identical in all stages studied (vegetative plasmodia, sporulating plasmodia, and mature spores). They are punctiform bodies whose diameter varies between 0.3 and 1μ. In *Didymium clavus,* Dangeard observed innumerable grains, which he presumed to be chondriosomes, in all stages of the plasmodium preceding sporangial formation. These structures accumulate in great quantities in the young sporangia and eventually in the spores which are rich in chondriosomes. Andersen and Pollock (21) found two types of granules in *P. polycephalum*: pigment granules make up 5% of the total volume of the plasmodium; mitochondria 1–2%; and all inclusions about 30% (nuclei 7%, vacuoles 18%, pigment granules 5% and mitochondria 1–2%). Guttes, Guttes, and Rusch (131) also found mitochondria and pigmented granules in this organism. The former measured 1μ and the latter from 0.2 to 2.0μ in diameter. In addition, they found a third type of granule, measuring about 0.5μ in diameter, which they assume to be polyphosphate.

Among the most recent researches on the mitochondria of the Myxomycetes are those of Sponsler and Bath (306, 307) and of Niklowitz (244). The first mentioned authors studied the plasmodium of *Physarum polycephalum* with the electron microscope. They detected ellipsoidal structures 6,000 Å x 12,500 Å in size, which contained heavily electron-scattering particles in a lighter matrix. These parti-

cles are arranged in a somewhat regular pattern. Attempts to determine their composition gave inconclusive results. A number of other particles, some sphaeroidal, some rod-like, are also described. The possibility is mentioned that certain protoplasmic fibrillae detected may be related to the actomyosin-like proteins extracted by Loewy (198). The work of Loewy and others on this subject is cited later in this review in the section on *Protoplasmic Streaming*. Niklowitz (244), in an electron microscope study of ultra-thin sections of the plasmodium of *Badhamia utricularis,* found that the mitochondria are bordered by double-contoured membranes which separate them from the surrounding cytoplasm. While the central region of the mitochondrium exhibits a finely granular structure, the remaining space is filled with numerous saccate granules which are also surrounded by double membranes and which, in suitable sections, appear to have an open connection with the surrounding cytoplasm. Niklowitz thinks that these granules may be identical to the structures described by Sponsler and Bath (306, 307) and believes that they probably consist of condensed phosphates. As will be pointed out later, mitochondria have been associated with enzymatic activity in *P. polycephalum*. Lieth and Meyer (184) examining thin sections of the plasmodium of *Didymium nigripes* with the electron microscope observed many small (1–2μ) oval structures, which they interpreted to be pigment granules. These were surrounded by an osmiophilic membrane, and contained a strongly electron-scattering kernel.

PHYSIOLOGY

Martin (208) included a section on physiology in his earlier review. Hawker (138) in 1952 brought the literature up to that date. The reader is, therefore, referred to these two reviews for the earlier papers on general physiology.

PROTOPLASMIC STREAMING AND LOCOMOTION. In recent years ever-increasing numbers of biochemists and biophysicists have discovered that the plasmodium of the Myxomycetes is an excellent experimental tool for the study of the motive force responsible for protoplasmic streaming. Mainly because of the ease with which the plasmodium of *Physarum polycephalum* may be maintained in culture, most studies have been based on this organism alone.

Much of the literature dealing with this subject, in the realm of biochemistry and biophysics, is far removed from this reviewer's fields

of competence. It is most fortunate, therefore, that Kamiya (156) has recently published a very comprehensive article on protoplasmic streaming in which he reviews all the literature up to 1959, much more thoroughly and critically than would have been possible here. The reader is referred to Kamiya's review for the pertinent literature on this subject.

Among the most significant findings relative to the problem of protoplasmic streaming in a plasmodium are those of Loewy (197, 198), Ts'o and coworkers (82, 323-327) and Takeuchi and Hatano (in 156). In 1952 Loewy (198) extracted a system from the plasmodium of *P. polycephalum,* the viscosity of which is delicately controlled by at least two substances. The viscosity of the system is rapidly lowered by ATP and more slowly raised by muscle adenylic acid. Inasmuch as it was found that the lowering of the viscosity by ATP was followed by a slow rise, it was postulated that the plasmodium is able to form adenylic acid from the ATP provided. The author believed that the viscosity changes measured in the plasmodial extract may be related to the motive force. Ts'o, Eggman, and Vinograd (82, 323-327) isolated a cytoplasmic contractile protein, myxomyosin, from the plasmodium of *P. polycephalum.* They found that the addition of ATP affected the gel structure and the rate of protoplasmic streaming, and that myxomyosin is involved in this response. When ATP is added to myxomyosin, changes in viscosity similar to those in the actomyosin-ATP system are manifested. Myxomyosin is a rod-shaped molecule, 4000–5000 Å long with a molecular weight of 6 million. The properties of this protein are further discussed by Nakajima (236) in a more recent article. Takeuchi and Hatano (in 156) demonstrated the presence of ATP in the plasmodium of *Physarum polycephalum* and showed that "ATP obtained from the slime mould contracts the glycerinated muscle *(psoas)* of a rabbit as conspicuously as muscle ATP does." In a later article, Hatano and Takeuchi (137) found that the plasmodium contained 0.4 micromole of ATP/g wet wt. and that the changes in the ATP level paralled the generation of motive force when the material treated with 2,4-dinitrophenol, but not when the plasmodium was under the influence of low 0_2 tension and 10^{-3} M monoiodoacetic acid.

Stewart and Stewart (309), after a critical discussion of protoplasmic structure as revealed by cinephotomicrography, suggest that myxomyosin may function as a labile structural protein controlling the

relation between the flowing and non-flowing portions of the protoplasm and thus control flow pattern without being the direct source of the motive power. They explain motive force generation in a plasmodium on the basis of the diffusion drag-force hypothesis, postulating that "metabolic processes in plasmodial protoplasm establish concentration gradients of some particular compounds, with appropriate magnitude, direction and distributions. The drag forces associated with diffusion down these gradients then constitute the proposed motive force for protoplasmic flow."

The question of the relationship between streaming and locomotion in a plasmodium is an interesting one. Streaming has long been considered to be the mechanism of plasmodial movement. Seifritz (273) made a sharp distinction between streaming and locomotion, and later with Urbach (277), in a series of experiments, showed that streaming may continue actively even though spreading, locomotion, and growth of the plasmodium are at a standstill. Whereas they do not deny that locomotion of a plasmodium depends on streaming, Seifritz and Urbach conclude that the primary function of streaming is circulation and that only secondarily is streaming an aid to locomotion. Kamiya (156) relates the movement of the plasmodium to the volume of protoplasm transported by protoplasmic streaming, pointing out that it is the net difference between the quantities of protoplasm carried in two opposite directions which contributes to the locomotion. In an earlier paper (155), he had stated that the flow toward the direction of movement of the plasmodium does not necessarily last longer than the flow in the opposite direction. This indicates that duration of flow in one direction is not necessarily correlated with the volume of protoplasm transported in the same direction.

Locomotion of the plasmodium is described at length by Lewis (181) who bases his discussion on Camp's well known paper (43). Anderson (22) found that the plasmodium of *Physarum polycephalum* migrated toward the cathode and that the effect of the current was inhibitory toward anodal migration rather stimulatory toward cathodal. In a later paper (23) he reported a greater potassium concentration in the anterior over the posterior regions of a migrating plasmodium. In a plasmodium whose migration is electrically oriented, this difference amounted to 30%. In the control plasmodia the difference was 5%. The same potassium concentration was found in the anterior

regions of both experimental and control plasmodia. Polarity, thus, seems to be closely associated with potassium concentration.

Rakoczy (260), working with *Didymium iridis,* confirmed and expanded upon Skupienski's (289) observations on the regeneration powers of a plasmodium. He found that even a slight contact with the edge of a razor blade caused an immediate cessation of streaming for a distance of up to 1.5 cm on either side of the point of contact. The ability of plasmodial fragments to regenerate appears to be independent of the size of fragments or their origin from various regions of the plasmodium, but depends to an extent on the age of the plasmodium. The most regular growth and migration was observed when fragments were taken from 8-day-old plasmodia.

TOXINS, ANTIBIOTICS, PIGMENTS, ENZYMES. Seifriz (274), studying *P. polycephalum,* observed that when two plasmodia approach each other under favorable conditions, they will meet and fuse, but when the approach is gradual they halt a short distance from each other, establishing a zone of definite width between them, and never fuse. This is interpreted to mean that exotoxins are secreted by the plasmodia. Locquin and Prevot (195), Locquin (191), and Sobels (295-298) have reported the production of antibiotic substances by various species of Myxomycetes. Some are antibacterial and some are effective against yeasts and filamentous fungi. Texera (316), on the other hand, found no antibiotic activity when he tested aqueous and other extracts from plasmodia of *Physarum polycephalum* and *Fuligo septica* against bacteria.

Buchberger (38) reports a *Fuligo* plasmodium to be rich in vitamin B_1. Sobels (299) found the pigment of the orange-yellow plasmodium or her strain II of *Badhamia utricularis* to be readily soluble in 96% alcohol. Results from paper chromatography and absorption spectra indicated that the plasmodium contains two pigments of a flavon nature. Wolf (349, 350) extracted two yellow fluorescent pigments from the plasmodium of *Physarum polycephalum* and identified both as pteridines. Dresden (80), on the other hand, concluded that the yellow color of the plasmodium of this species is due to a peptide-type pigment. Kuraishi et al. (176) disagree with both the above, and state that the pigment is neither a pteridine nor a peptide-type pigment. Obviously, more work needs to be done on this subject! Gray (126) postulated riboflavin synthesis by the plasmodium of *P. poly-*

cephalum and mentions the probability that the yellow pigment in this plasmodium is partially caused by the presence of riboflavin. Allman (20) extracted the yellow pigment from plasmodia of this species with methyl alcohol and found that the optical density of the extract as determined by a spectrophotometer bears a linear relationship to the mass of the plasmodium or sclerotium. He proposes this as a reliable method of quantification. As we shall see in a subsequent section (Sporulation) of this review, some of the plasmodial pigments appear to be photoreceptors involved in the fruiting process. Emanuel (87) isolated a sterol with the formula $C_{30}H_{52}0_3$ from *P. polycephalum* and named it physarosterol.

The study of myxomycete enzymes has received some attention in recent years. Holter and Pollock (145), using a centrifiguration technique, investigated the distribution of peptidase and succinic dehydrogenase in the plasmodium of *P. polycephalum.* When these are correlated with distribution of particulate components of the cytoplasm, it appears that succinic dehydrogenase may be linked with the nuclear and mitochondrial stratum whereas the peptidase is apparently free of any particular granular component. Johnson and Moos (151) also link succinoxidase activity with the mitochondria of the plasmodium in the same organism. Haas (132) injected the following enzymes into the plasmodium of *P. polycephalum* and studied their effects: papain, trypsin, pepsin, collagenase, pectinase, hyaluronidase, and ribonuclease. The proteinases accelerated streaming and produced numerous protrusions of the hyaline layer. The polysaccharases, pectinase, and hyaluronidase also increased the rate of streaming, but depressed the forward motion of the plasmodium as a whole. Ribonuclease in addition caused the breakdown or prevented the formation of channel walls. Haas' tentative conclusions are that "the hyaline layer seems to consist chiefly of non-conjugated proteins, while the plasmagel contains proteins in combination with other substances, such as polysaccharides and nucleic acid. Solution and gelation may be interpreted as enzymatic processes involving the loosening and reestablishment of these connections." Ward (335-337) working with the same organism, found evidence of the presence of a flavin enzyme which promotes atypical oxidation of ascorbic acid with H_2O_2 as an end product. He also demonstrated the presence of cytochrome oxidase and succinic dehydrogenase in the plasmodium and theorizes on their role in the succinoxidase system. Younggren (355), after extracting 100 samples

of the plasmodium of *P. polycephalum* and analyzing the materials spectrophotometrically, failed to find evidence of the presence of cytochromes. In view of Ward's findings, however, Younggren's results must be questioned.

RESPONSES TO EXTERNAL CONDITIONS. Much of the literature pertaining to this subject deals with protoplasmic streaming and is reviewed by Kamiya (156).

Kinoshita (in 156) found that the plasmodium of *Didymium nigripes* is positively chemotactic to low concentrations of IAA (.04–0.63 mg/l), but negatively so to higher concentrations (5mg/l). Coman (58) reported the plasmodium of *P. polycephalum* to be positively chemotactic to dextrose and negatively to saccharose, and to H and OH ions in adequate concentrations. Winer and Moore (348) state that plasmodia of this species tend to maintain an approximately constant difference between their own osmotic pressures and that of the substrate. They also found that plasmodia with different osmotic pressures fused readily. Studying the effect of pressure on the plasmodium, they report that sudden application of pressure or sudden release from slowly applied pressure results in gelation of the flowing material for about 0.5 mm on either side. Pressures up to 166 gr/cm^2 cause reversible gelation of the flowing material. Luyet and Gehenio (202) discovered no evidence for any action of gravity on plasmodia migrating on plain agar. Smith and Grenan (291) found that the spreading of the plasmodium is affected by the osmotic pressure of the substratum. Seifritz (275, 276), studying anaesthesia of protoplasm, found that among the agents which produced it were: 30–50 v., 0.2–1 amp. of electricity, which caused a reversible gelation of the protoplasm; low temperature (3° C); high temperature (35–42° C); and ethyl chloride vapors. Voltage above 60 v., 5 minutes exposure to 0° C, and temperatures above 45° C killed the protoplasm.

Gehenio and Luyet (108) and Gehenio (106) studied the effect of low temperatures on the plasmodium of *P. polycephalum.* The results indicate that the average freezing point of the living protoplasm is somewhere around –0.25° C as compared with an average of –0.37° for the dead. Flow ceased between 5° and 0°; at 3° and below finely granular vesicles were formed; at 0° internal protoplasmic disorganization took place. This was followed by a breakdown of the pigment granules. Death occurred after 5 seconds at 0°. Rapid cooling was more injurious than slow. Death was preceded by the gelation of the

protoplasm. Progress of death could be halted by raising the temperature; the portions not yet affected recovered completely.

In an attempt to find electrolytic solutions which are compatible with the protoplasm of *P. polycephalum,* Chambers (47) injected a number of solutions directly into the plasmodium. The most compatible of all solutions tested was of the following constitution: KCl, 0.120 M; NaCI, 0.013 M; $CaCI_2$, 0.003 M. Abbott (1) reported no effect of DDT on the plasmodium of *Physarella oblonga.* Concentrations as high as 12 mg/ml of sodium monofluoracetate permitted plasmodia to develop and survive for a week. Hg^+, Ba^+, Va^+, Cu^+, Mn^+, Mg^+, Fe^+, and Ca^+ ions were found to exhibit various degrees of toxicity from Hg^+ and Ba^+ which were most toxic to Mn,$^+$ Fe^+, and Ca^+ which were non-toxic. Elliott reported (83) 20 hours exposure to paradichlorobenzene fumes killed the plasmodium of an unidentified myxomycete on the surface of agar.

Setälä et al. (278-281) were able to produce tumor-like proliferations in *Physarum polycephalum* by growing the plasmodium in a nutrient agar medium to which polyethylene glycol 400, zephirol, Na dodecyl sulfate, or any one of several carcinogenic hydrocarbous has been added. Such plasmodia generally failed to differentiate. The tumor-like proliferations appeared 4–9 days after inoculation. Tween 60, on the other hand, produced a premature and excessive differentiation of the protoplasm with a gigantic, capillitial-vacuole system containing protoplasmic sacs with a large number of attached granules. There was little differentiation of the sporogenous protoplasm. An abundance of pyroninophilic particles was observed in the tumor-like proliferations caused by either the carcinogenic hydrocarbous or by Tween 60. It is believed that these particles may contain RNA.

The effects of ionizing radiation on mitosis in the myxomycete plasmodium were studied recently by Nygaard and Guttes (245). The conclusion is that "DNA synthesis per se is not sensitive to radiation and that mitosis and DNA-synthesis can be dissociated by irradiation."

SCLEROTIZATION

Sclerotization of a plasmodium is generally considered to be a response to unfavorable conditions, as sclerotia may often be found in the winter under logs or leaves in the woods. When brought into the laboratory and placed under favorable conditions, sclerotia grow out

into typical plasmodia. Sclerotization is often induced in the laboratory and the sclerotia stored for future use.

Hodapp (143) states that viable sclerotia of *P. polycephalum* may be obtained by desiccating plasmodia slowly at 25° C, taking 25 to 70 hours for the process. Aggregation of plasmodial strands is the first macroscopic sign of sclerotization. No sclerotia dried in less than 4 hours were viable. When the temperature was varied with the time of desiccation, the degree of germination, as well as the number of germinating sclerotia, increased with increasing length of the desiccation period at each temperature, up to a certain limit. Germination of sclerotia was reduced after 8–13 months storage. The water content of viable sclerotia was higher (12.3% of weight) than in non-viable ones (7.1% of weight). Gehenio (107) reports that, of initially viable sclerotia, 70% germinated after 1 year, 10 % after 2 years, and none after 3 years of storage. In some instances up to 50% of the germinating power of the sclerotia was lost during the first 3 months of storage at room temperature (18–40° C). Sclerotia produced under identical conditions differed considerably in initial viability as well as longevity. Luyet and Gehenio (203, 204) found a complete loss of viability when sclerotia were dehydrated over desiccants or in a vacuum for several days. Water loss is very rapid at first, proceeding at a gradually lower rate later. Only 1% of the total water loss takes place between the 3rd and 24th hour of desiccation. Death occurs after the sclerotium has been maintained at a low water content for a long time. Resistance of sclerotia of *P. polycephalum* to heat is said to be lower than in most other forms of dormant protoplasm. Sclerotia immersed in water are killed in 10 seconds at 70° C. In water vapor, 5 seconds at 70° C are required to kill them.

Jump (154), in one of the best studies of sclerotia, found that all sclerotia of *Physarum polycephalum* are constant in their structure, in being composed of small "cells" which he terms macrocysts. These vary in size from 10 to 25μ and in the number of nuclei they contain (0–14). Each macrocyst is surrounded by a membrane. The process of sclerotization goes through the following phases: 1. cessation of streaming, 2. gelation of the whole structure, 3. distribution of nuclei, 4. depositing of wall, 5. completion of macrocyst formation, 6. hardening of sclerotium, 7. shrinkage of nuclei to ½ diameter. The reconstitution of the plasmodial phase from the sclerotial reverses the above process. The structure regains its plasticity a few minutes after

it is wet, rehydration of the contents follows, the nuclei regain their original size, the macrocysts merge and disappear, and streaming begins. Jump also studied the conditions which induce sclerotization, of which the most important are: gradual desiccation, low temperatures, high osmotic pressure, sub-lethal doses of heavy metals, low pH. Rapid desiccation tends to form pseudosclerotia in which the nuclei are distorted and the macrocysts have either no or only incomplete membranes around them. Sullivan (314), studying sclerotization in the same organism, found that the amount of total bound water decreases during the initiation of dormancy. There is also a decrease in the content of water-insoluble polysaccharides and in the glycogen content, and a loss of mucoproteins. The lipid content increases. Inasmuch as there appears to be a direct correlation between lipid increases and carbohydrate losses, it is probable that the glycogen is converted into lipids.

An interesting phenomenon which may be observed in certain living cultures is the apparent "sclerotization" of a plasmodium on the surface of agar even under a layer of water. Nauss (240) described the sclerotium of *Hemitrichia vesparium* as consisting of many isolated globules of resting protoplasm. In our own laboratory (14), plasmodia of a number of species—notably *Stemonitis fusca* and *Arcyria cinerea*—have been observed to break up into a large number of spherical, oval or sometimes irregular cysts, much resembling Jump's macrocysts, which remain arranged in the plasmodial pattern, having been formed along the main strands of the plasmodium by the concentration of protoplasm. Such "sclerotization" most often occurs in cultures whose surfaces have been permitted to dry. By adding an excess of water it is usually possible to induce reconstitution of the plasmodium to its original form. In some cultures, however, macrocysts are formed while the plasmodium is under water, and no manipulation appears able to induce reconstitution of the plasmodium.

SPORULATION

Temperature, pH, light, nutrients, and moisture have all been linked in one way or another to the fruiting process by different investigators (138, 285).

Continuing his earlier studies on the fruiting of the Myxomycetes, Gray (121) found that ultra-violet irradiation delayed fruiting in non-pigmented plasmodia, but not in pigmented. *Physarella oblonga*

(yellow plasmodium) fruited 48 hours after exposure for 1–10 minutes to U-V irradiation, whereas *Physarum didermoides* (white plasmodium) required from 28 to 35 days to fruit after similar treatment. Later, studying the effect of monochromatic light on *P. polycephalum*, he found (125) that the shorter the wave length the more rapid the fruiting and the greater the percentage of cultures which fruited. Sobels and Van Der Brugge (301), working with pure and two-membered cultures of *Badhamia utricularis* and with *Physarum polycephalum* strains with orange-yellow plasmodia, found that both species required a much longer time to fruit during the winter months when grown for 6–12 days in darkness and were then transferred to daylight. In the summer, *B. utricularis* fruited after 5–19 days and *P. polycephalum* after 20 hours, but in the winter up to 35 days and 1–3 days, respectively, were required for fruiting. The summer-winter time differential for fruiting was attributed to a difference in light intensity. Gehenio and Luyet (110) reported that light influences sporulation of *Physarella oblonga* and believed it may be necessary for fruiting. Straub (312), working with *Didymium nigripes*, found that plasmodia would fruit under white, red blue, or U-V (350–390 mμ) illumination, but not under green light or in darkness. The time of illumination varied with intensity within certain limits. The time of illumination required for fruiting could be reduced by feeding the expermental plasmodia on previously illuminated plasmodia. This is interpreted to mean that the effect of light is transmitted through the protoplasm. Lieth (182) showed that four pigments can be separated electrophoretically from the reddish-brown plasmodia of this organism. A study of their absorption spectra led him to believe that the pigments cannot be directly responsible for absorbing the necessary energy for sporangium formation. In a later paper (183) Lieth reported that plasmodia of *D. nigripes* sporulated under violet, blue, and red light, but not under green or infrared, thus confirming Straub's results on this point. However, when red light, sufficient to induce sporulation, was supplemented with increasing amounts of green light, the percentage of sporulating plasmodia diminished greatly showing, according to Lieth, that green light actually inhibits fruiting. Schure (272) induced plasmodia of *Mucilago spongiosa* to fruit in culture by placing them inside clean, moist, flower pots and feeding them a suspension of baker's yeast in tap water. Collins (55) working with *Fuligo cinerea* found that both temperature and pH affected fruiting to a

considerable degree. At 30° C no fruiting occurred regardless of the pH value of the substratum. At 25° C, 75% of the cultures fruited at pH 3, 90% at ph 4 and 5, and diminishing percentages up to pH 8. At 20° C, pH seemed to have relatively little effect on fruiting, at least 80% of the cultures fruiting at all pH values with all cultures sporulating at pH 4 and 5. Scholes (271) noted that age of culture was the most important factor influencing sporulation in *Fuligo septica.* Various nutrients, type of substratum, light, temperature, starvation, or desiccation, failed to induce sporulation. There was a significant difference, however, in the percentage of sporulating cultures between two groups, one subcultured every four weeks, the other not subcultured until sporulation occurred. In the former, 4% of the cultures sporulated within 28 days; in the latter 48% of the cultures sporulated.

As a result of Gray's work and that of others, we now know that pigmented plasmodia require light in order to sporulate whereas non-pigmented plasmodia often do not. Seeking a biochemical explanation of the role of pigments in the fruiting of *P. polycephalum* Wolf (349, 350), as was stated in a previous section, extracted two pigments from plasmodia of this species. Component 1, in acid solution, shows a large amount of absorption in the lower limits of the visible spectrum, which is greatly reduced in neutral or alkaline solution. Thus it possesses characteristics which offer a reasonable explanation to Gray's findings (119, 121, 125) concerning the effectiveness of both blue light and acid conditions for fruiting. Component 2, on the other hand, has its longest wave-length absorption maxima deep in the ultra-violet and, according to Wolf, cannot possibly be the photoreceptor of blue light which activates fruiting.

The problem of morphogenesis in *P. polycephalum* is being investigated in a number of laboratories. A copper-containing oxidase different from those occurring in other organisms was found by Ward and Havir (339, 340) to participate in the production of melanin when sporulation occurs. A year later Ward (338) reported a shift in oxidases during sporulation. He found about 3 times as much cytochrome-oxidase activity in the spores as in the plasmodia, and about 6 times as much ascorbic acid oxidase activity in the plasmodium as in the spores. Phosphorylation reactions producing high energy compounds have been demonstrated to occur in the cytochrome system. The increase in cytochrome oxidase activity in the spores may be

linked with energy requirements for nuclear divisions and spore wall formation according to Ward. This line of investigation appears to be promising.

Unfortunately, most of the above investigations appear to have been based on contaminated plasmodia and are subject to criticism. Of particular significance, therefore, is the work being carried on by Daniel and Rusch (66, 68, 69) who work with liquid, shake, bacterium-free cultures of *P. polycephalum.* Plasmodia were grown in a tryptone-yeast extract-glucose medium for three days, harvested, and allowed to fuse into a single plasmodium. After incubation for 4 days at 21.5° C in the dark on a $CaCo_3$-buffered salts medium, containing 0.01% niacin and 0.01% niacinamide, they were exposed for 2 hours to light and returned to darkness. Sporulation was completed 12 to 16 hours after illumination. Certain conditions were necessary for sporulation: 1. an optimal growth age, 2. a dark incubation period of 4 days, and 3. exposure to light of wavelengths between 350 and 500 mμ. Tryptophane could be substituted for niacin. Certain other compounds, such as DPN and TPN, could also be substituted.

Inasmuch as the literature seems to indicate that pigmented plasmodia require light for sporulation, but that colorless or white plasmodia do not, McManus' (222) observations on *Stemonitis fusca* should be checked. She noted that plasmodia of this organism kept in complete darkness would not fruit whereas those exposed to light fruited when they had matured sufficiently. She emphasizes that the plasmodia remain white up to the time of fruiting. If, indeed, light is required for fruiting, substances other than yellow pigments are involved in photoreception in this species.

With the discovery of techniques whereby pure cultures may be obtained, physiological work on Myxomycetes will undoubtedly proceed at an ever-increasing pace. However, Sobels and Van Der Brugge (301) find that pure cultures of *Badhamia utricularis* require an abnormally long time for fruiting. Lazo (178) has observed the same phenomenon with pure cultures of *Physarum polycephalum,* and particularly with *P. didermoides,* and *Fuligo cinerea* which, in crude culture, fruit regularly and in a relatively short time. Rusch and his coworkers (68, 69, 131) experience no difficulty in inducing pure cultures of *P. polycephalum* to fruit. However, they do not mention whether the spores produced in the pure culture are viable.

THE SPOROPHORE

It is well known that certain species of slime molds sometimes produce their spores in sessile or stalked sporangia, at other times in plasmodiocarps. Single plasmodia will often give rise to all three types of fruiting bodies. In numerous large colonies as observed in the field, there seems to be a distinct suggestion of response to rate of drying. In *Badhamia gracilis* and *Physarum notabile* for example, portions of a fruiting colony on the upper surface of a log may be strongly plasmodiocarpous, whereas as one proceeds to less exposed situations the procession may be to sessile, sporangium-like fruitings and to stalked sporangia. Cohen (50), presenting his own observations on the variability of the fructifications of *Didymium squamulosum, Physarum polycephalum,* and *Badhamia foliicola,* theorizes on the factors responsible for such variation. He explains that the shape of the fructification is probably due to a difference in surface tension of the plasmodium. A low surface tension would tend to produce plasmodiocarps; a higher surface tension, sessile spheres. "If the material is secreted preferentially at the substrate-protoplasm interface, we should expect a stiped form" of fructification. His explanation appears to be valid for many species. Nevertheless, it is undeniable that some forms, such as *Hemitrichia serpula,* seem always to produce plasmodiocarps.

Gray (127) noted that sporangia of *Physarum flavicomum* developed in culture are somewhat variable in appearance. Sporangia which develop under dry conditions tend to have a limy peridium whereas those which develop under humid conditions have a thin, limeless peridium. Gray also observed that umbilicate, reniform, and lobed sporangia—all atypical for this species—appear more frequently under humid conditions. Solis (303), in her experimental studies on the morphology of *Physarum nicaraguense,* found that color and height of stalk, and number and size of sporangial lobes were influenced by temperature and type of substratum, whereas the formation of a pseudocolumella and the attachment of the capillitium to the peridium—both of taxonomic significance—were stable characters independent of temperature and type of substratum.

Locquin (188, 189) investigated the ultra-structure of the capillitium of *Hemitrichia serpula* and of *Calomyxa (Margarita) metallica.* He found the former to consist principally of chitin. Structurally the main fibers are extremely complex, consisting of sinistrorse spirals and ultra-spirals. Their structure becomes more simplified toward the

center, passing from netted-spiral micelles, to spiral, to parallel. The capillitium of *C. metallica,* examined under polarized light reveals the presence of strongly dichroic and birefringent, more or less elliptical inclusions with a spiral structure. In view of Ulrich's assertion (328) that chitin has been found in the capillitium of *Stemonitis fusca* and nowhere else in the Myxomycetes, Locquin's report of this material in *Hemitrichia serpula* is of considerable interest and should be checked carefully. Goodwin (117) failed to demonstrate chitin in any portion of the fructification of *Comatricha nigra, C. fimbriata,* and *C. elegans,* but obtained a cellulose reaction in the spore walls, the stalk, the columella fibers, and the capillitial threads of all three species. Certainly a very thorough study, using modern techniques, of the chemical composition of capillitium and spore walls of representative species of Myxomycetes is very much needed.

The most recent investigations on stalk and capillitial development in the Myxomycetes are those of Welden (345) on *Badhamia gracilis* and *Didymium iridis,* of Ross (264, 268) on some of the Stemonitaceae, and of Goodwin (117) on three species of *Comatricha.* Welden found that the capillitium of both *B. gracilis* and *D. iridis* is formed by the coalescence of tubular invaginations originating from the peridial walls and from vacuoles in the protoplasm of the developing sporangium, and, in *D. iridis,* from the columella as well. This system of tubes becomes filled with calcium carbonate and other excretory substances which, in *B. gracilis,* form the calcareous capillitium, whereas in *D. iridis* they are transported to the exterior of the sporangium or to the columella. Thus the formation of capillitium in these two species of Physarales follows in its initial stages the pattern which had been established for two species of Trichiales 41 years before by Harper and Dodge (208), with this difference: that in *Hemitrichia clavata* and *Trichia* sp. capillitial material is deposited on the walls of the vacuolar tube system, whereas in *B. gracilis* and *D. iridis* capillitial material is deposited within the vacuolar tubes.

Ross (264, 268) investigated the stalk and capillitial formation in four species of *Stemonitis,* in *Comatricha typhoides,* and in two species of *Lamproderma.* He found that the hypothallus is laid down underneath the fruiting plasmodium in all these species and that the stalk, consequently, is formed inside the protoplasmic primordium. Thus, stalk development in *C. typhoides* appears to be similar to that

of *C. nigra* as reported by Jahn in 1931 (148), and as observed later by Goodwin (117) in *C. nigra, C. fimbriata,* and *C. elegans.*

In contrast to the manner in which capillitium is formed in the species of Trichiales and Physarales which have been investigated, Ross (264, 268) finds no invaginations or vacuolar tubes in the seven species of Stemonitaceae he studied, the capillitial threads in these species blending gradually into the protoplasm without evidence of any membrane surrounding them. Ross further reports some interesting differences in capillitium formation between *Comatricha typhoides* and two species of *Lamproderma* on the one hand, and four species of *Stemonitis* on the other. In *C. typhoides* the stalk is composed of a bundle of hollow tubes which elongate upwards forming the columella. The outer tubes bend outward from the columella, grow, branch, and form the capillitium. Thus the capillitium in this species originates entirely from the columella. In the species of *Stemonitis* investigated, the capillitium originates both from the apex of the columella and independently from the surrounding protoplasm. Capillitial tubes which originate in the cytoplasm branch down the periphery of the sporangium and form the surface net. Ross thus speaks of the *Stemonitis* type of capillitial development and of the *Comatricha* type. Goodwin's study (117) of *C. nigra, C. fimbriata,* and *C. elegans* confirms Ross' findings concerning capillitial development in *Comatricha. Lamproderma arcyrioides* and *L. arcyrionema* also develop their capillitia in the same manner as *Comatricha* (264, 268).

One of the interesting problems connected with the developmental morphology of the myxomycete sporophore is presented by the evanescent peridium of the Stemonitales and the Echinosteliales. In most species of *Stemonitis* and *Comatricha* and in *Echinostelium minutum,* the mature sporangia are devoid of a peridium. An evanescent peridium had been postulated for these species (185) but no one had traced its development. We now know that in *Echinostelium minutum* (13), *Comatricha nigra, C. fimbriata,* and *C. elegans* (117), a very delicate peridial membrane actually does form in the early stages of sporangial development, but soon dissolves away.

LABORATORY CULTURE AND NUTRITION

Crude Cultures

Plasmodia of Myxomycetes, found in the field, or developed on natural substrata brought into the laboratory and incubated in moist

chambers, will usually grow well when transferred to artificial media (321). Such cultures often sporulate, producing typical fruiting bodies and spores. The difficulties encountered in growing the Myxomycetes in culture on artificial media starting with spores, however, are well known by students of these organisms. Of the 400-odd known species only a relatively few have been induced to complete their entire life-cycle on artificial media, all in crude or two-membered culture. Even so, the vast majority of these belong to the Physarales, only 8 species in other orders having been cultivated successfully up to now. The following reports of myxomycete cultures from spore to spore on artificial media, though not necessarily the first for the species mentioned, are cited as an aid to those who are interested in this phase of the subject:[7]

ORDER PHYSARALES

Fuligo cinerea (55)
Fuligo septica (59)
Mucilago spongiosa (272)
Physarum compressum (119)
Physarum didermoides (59)
Physarum flavicomum (127)
Physarum gyrosum (166)
Physarum leucophaeum (104)
Physarum nicaraguense (303)
Physarum nucleatum (Alexopoulos, unpublished)
Physarum oblatum (Alexopoulos, unpublished)
Physarum polycephalum (146)
Physarum pusillum (Alexopoulos, unpublished)
Physarum tenerum (119)
Physarella oblonga (113)

Family Didymiaceae

Diderma effusum (59)
Didymium difforme (283)

[7] In many publications, plasmodial cultures of certain species are mentioned without reference to their origin (field collection or spore sowings) or to their ability to sporulate on artificial media. Such reports have not been interpreted in this review as constituting proof that the species in question has completed its life cycle in artificial culture and, consequently, are not cited here.

Didymium iridis (46)
Didymium nigripes (46)
Didymium squamulosum (59)

ORDER STEMONITALES

Family Stemonitaceae

Clastoderma debaryanum (223)
Stemonitis flavogenita (13)
Stemonitis fusca (225)

ORDER ECHINOSTELIALES

Family Echinosteliaceae

Echinostelium cribrarioides (15)
Echinostelium minutum (13)

ORDER TRICHIALES

Family Trichiaceae

Arcyria cinerea (14)
Hemitrichia serpula (224)
Hemitrichia vesparium (119)

Corn meal agar and Knop's agar (12, 13, 59, 124) appear to be the agar media most favorable for starting cultures from spores. When plasmodia are formed they may be maintained on the same media until they fruit, or they may be transferred, to partially purify them, and fed pulverized oats or rice on which most species thrive. Little difficulty is generally experienced in ridding the cultures of fungi, although *Trichoderma* is often difficult to eliminate. Bacteria present a much more difficult problem. Gray (119) has obtained plasmodia of *Lamproderma arcyrionema* by sowing spores on decayed wood, but was unable to duplicate this on artificial media. McManus (224) grew *Hemitrichia serpula* on filter paper. She was unable to induce plasmodium formation on agar media. Strugger (313) has cultivated *Didymium nigripes* from spores to fruiting bodies in the presence of vital stains showing that both cytoplasm and nuclei are vitally stainable without interfering with the normal development of the life cycle.

PURE CULTURES

No myxomycete has been induced to complete its entire life-cycle in the absence of microorganisms. Locquin (190) describes a method for growing Myxomycetes in pure culture from surface-sterilized spores on a synthetic medium, but it is apparent that his is a suggestion

rather than a successful experiment, for he does not claim to have achieved such culture. Repeated attempts in our own laboratory to induce plasmodial formation by sowing surface-sterilized spores of *Fuligo septica* and *Physarum pusillum* on various agar media, including corn meal and Knop's, have failed. The spores germinated, typical myxamoebae were formed, but no further development took place. Non-treated spores carrying normal bacterial flora sown at the same time on similar media yielded plasmodial cultures in six to seven days. Parker (250) reports that myxamoebae of two strains of Myxomycetes when grown singly or in masses in the absence of bacteria failed to form plasmodia. However, successful plasmodial formation occurred when a minimum of 8 myxamoebae grew with bacteria. Kerr (164) grew *Didymium nigripes* in pure culture starting with spores by suspending them in 1% peptone broth to which formalin-killed *Aerobacter aerogenes* had been added. Autoclaved bacterial cells could not be substituted for formalin-killed cells, nor would the organism grow on a variety of tissue culture media in the absence of bacterial cells.

Pure cultures of myxomycete plasmodia were reported several decades ago (208), but Cohen (48) was the first to subject his cultures to critical tests which failed to indicate the presence of bacterial contaminants. Dalleux (63) was unable to purify plasmodia of *Physarum polycephalum* and *Fuligo septica,* but expressed the belief that pure cultures of Myxomycetes were possible. At about the time her paper was submitted for publication, Cohen's paper appeared in print. Cohen used two methods for purifying plasmodia: the migration method, and the enrichment culture method which he described in detail. Dangeard (64) claims to have purified plasmodia of *Didymium clavus,* but offers no proof of the absence of bacteria from his cultures. Sobels (297, 298) and Hok (144), using Cohen's methods, succeeded in purifying the plasmodia of a number of species. Sobels (298) explains that some species are "both parasites and saprophytes" and their plasmodia, therefore, can be easily purified and maintained in pure culture, whereas others are "parasitic" and their plasmodia invariably decline once they are purified. Sobels (294) appears to be the first to have used antibiotics as an aid in purifying plasmodia. Locquin (190) and Hok (144) report similarly successful results with a number of antibiotics. Excellent results were achieved by Lazo (178) who established and maintained pure cultures of plasmodia of a number of species on sterile oats in sterile agar. Daniel and

Rusch (65, 67) were the first to have successfully cultured a myxomycete plasmodium *(Physarum polycephalum)* in pure culture in a liquid medium. Their cultures have been maintained for several years by successive transfers.

The plasmodia of the following species have been reported in pure culture: *Badhamia foliicola* (48), *B. utricularis* (48, 298), *Physarum polycephalum* (48, 65, 67, 178), *P. didermoides* (178), *P. gyrosum* (178), *Fuligo septica* (48, 178, 179, 271), *F. cinerea* (178), *Didymium squamulosum* (48), *D. nigripes* (164), *Stemonitis axifera* (48), *Licea variabilis* (294). Lazo (178) stated that plasmodia which have been freed from bacteria seldom fruit. *Physarum polycephalum* (131) is an exception, but as mentioned before, the viability of the spores produced by this organism in bacterium-free media has not been reported.

The reader must bear in mind that the terms "pure culture" and "axenic culture" have been used in the literature to indicate any culture in which only a single living organism is present. Many of the so-called pure cultures listed above were maintained with killed bacteria or yeasts. To my knowledge, bacterium- and yeast-free cultures of myxomycete plasmodia have been maintained for a relatively long time only by Sobels (297), Daniel and Rusch (67), and Lazo (178).

Two-membered Cultures

The growth of various myxomycete plasmodia in two-membered culture has been reported by Cohen (48, 49), Sobels (293, 297), Cohen and Sobels (53), Sobels and Cohen (300), Hok (144), Lazo (178), and Kerr and Sussman (165). Some of these investigators, however, initiated their cultures from spores and do not mention either surface-sterilizing the spores or testing them for the absence of surface contamination. It is not certain, therefore, that other organisms were not present also in such cultures.

Two-membered cultures of myxomycete plasmodia are usually in association with a bacterium or yeast. The experiments of Lazo (178), are, therefore, of especial interest. Starting with bacterium-free plasmodia of *Physarum didermoides* and *Fuligo cinerea,* Lazo induced them to grow in two-membered culture with three species of *Chlorella.* The algal cells are incorporated in the plasmodium which, under adequate illumination, becomes vividly green. Transfers of small bits

of the association on suitable media (sterile oat flakes in agar) grow into large green plasmodia, both associates increasing, the plasmodium in size, the alga in number of individual cells. Zabka and Lazo (356) reported later that when *Fuligo cinerea* and *Chlorella xanthella* were separately permitted to accumulate sodium radiophosphate from an agar medium and were then grown together with a non-radioactive culture of the other organism, there was a transfer of radioactivity from either associate to the other. These authors pointed out the possible significance of their results in view of previous work (357) indicating that no radioactivity was transferred between plasmodia of two different Myxomycetes which remained in intimate contact for 24 hours.

In summary, it may be stated that most species of Myxomycetes have not been grown in culture on artificial media under any circumstances; about 30 species have been induced to complete their life-cycle on artificial media in the presence of contaminating organisms; the plasmodia of 11 species have been purified and some have been maintained in pure culture with killed microorganisms or on natural media for considerable periods of time; the plasmodium of one species (*Physarum polycephalum*) has been grown in pure culture in a soluble, defined medium; no myxomycete has been induced to complete its entire life-cycle in pure culture without bacterial or yeast cells.

Nutrition

Older literature as well as many of the papers already cited in this review show that plasmodia may feed on a number of microorganisms —bacteria, yeasts, filamentous fungi—and that at least one (*Licea variabilis*) can grow on washed agar to which asparagin and sugar (preferably trehalose) have been added. Abbott (2) in a series of very short notes on *Physarella oblonga* records the following observations: The plasmodium grows well on oatmeal agar, but the culture dies out in about 20 days without fruiting, first undergoing a series of color changes from yellow through orange, to orange-red. Abbott has determined that this color change is due to an increased acidity and calls the process "acid-aging." The richer the medium the more rapid the acid-aging and the shorter the life duration. Studying the effect of substratum pH on this organism, Abbott finds that plasmodia grow best on buffered media of pH 4.4 and 5.8; reasonably well at pH 6.6 and 7.1, but very poorly at pH 8.0 and 9.1. Attempted culture

on synthetic media with various sugar and nitrogen sources failed, none of the cultures surviving more than 8 days. Cohen and Sobels (53) found that certain mineral salts must be present in the medium before two-membered cultures of Myxomycetes on living yeast, or pure cultures with autoclaved yeast, would thrive. Bodkiewicz (32) reported that volatile toxic substances emitted by ground onions retard the development of spores of *Didymium nigripes* in culture and exert a lethal effect on zoöspores and myxamoebae. Skupienski (288) observed that the development of *Didymium nigripes* and *Physarum polycephalum,* was stimulated by the addition to the nutritive medium of small quantities of *Saccharomyces cerevisiae.* The action of the yeast was not nutritive. Growth of *D. difforme* was checked by the yeast. Locquin (190) gives the formula for a synthetic medium on which he claims to have cultured plasmodia of several species of Myxomycetes, but gives no details. Rusch and his coworkers (67, 159), have discovered a chemically defined liquid medium, containing various amino acids, glucose, vitamins, a mixture of salts, and hemin, which will support the growth of pure cultures of *Physarum polycephalum.* This work has opened the way for exact nutritional studies (69a).

The question of whether Myxomycetes may absorb nutrients in solution from the substratum of whether they feed exclusively by phagocytosis had remained unanswered until recently. Some authors (227) maintained that living organisms (bacteria or fungi) were absolutely necessary for the nutrition of plasmodia. Until pure cultures were secured the problem was impossible to resolve. Cohen (48) had difficulty maintaining his pure cultures unless he provided them with autoclaved yeast cells, but he did report having grown *Badhamia foliicola* on agar containing yeast autolysate. Sobels (293, 298) also, as mentioned previously, showed that plasmodia of *Licea variabilis* attained considerable size on agar in which soluble food had been incorporated, and Cohen and Sobels (54) reported some success in growing a strain of *Badhamia utricularis* on a cell-free medium. Even so, the agar itself is a solid substratum and, inasmuch as the agar on which a plasmodium migrates often becomes eroded, it may be argued that the plasmodium ingests solid food. The work of Daniel and Rusch (65, 67) cited previously is, therefore, of particular significance in this connection also. Nevertheless, the fact remains that no one has succeeded as yet in inducing the formation of plasmodia from myxamoebae in the absence of microorganisms. The latter may be

presumed, therefore, to supply some as yet unknown factor necessary for the formation, growth, or aggregation of zygotes.

Physiological Races

Skupienski (286, 287) was the first to signal the existence of physiological races in the Myxomycetes. He isolated different races of *Didymium iridis* and *Didymium squamulosum.* Gray (122) found 3 physiological races in *Physarum polycephalum* differing in the ability of their plasmodia to fuse one with the other. Farr (93) and Thind and Rehill (318) independently reported a white (gray) form of *Physarella oblonga,* the former from Jamaica, the latter from the Mussoorie Hills of India. Alexopoulos reported at the American Mycological Society meeting in Bloomington (9) that he was able to culture the white form from Jamaica from spores. It produced a white plasmodium, in contrast to the yellow plasmodium of the usual form of *Physarella oblonga,* and fruited into white fruiting bodies. The color of the fruiting bodies in this organism is mostly due to the color of the lime (yellow or white) deposited in the trabeculae and on the peridium. Lazo (177) has produced a new strain of *Physarum polycephalum* by subjecting plasmodia of this organism to prolonged exposure to streptomycin hydrochloride. The new strain grows much more vigorously, particularly in pure culture, but seldom fruits. Dee (73) has found a physiological race of the same organism in which the myxamoebae are emetine-resistant.

Alexopoulos and Zabka (19) studied two physiological races of *Didymium iridis* from Honduras and found that, whereas the plasmodia of the two races would not merge under any conditions to which they were subjected, their swarm cells were compatible and would fuse to produce zygotes and plasmodia. Thus, the haploid protoplasts of the 2 races were compatible, but the diploid protoplasts were incompatible. The same situation was reported by Collins (57) to prevail between one of the above Honduran races and a race from Panama.

The inability of the plasmodia of various races of the same species to fuse one with another again raises the question of the existence of heterokaryosis in a plasmodium. Inconceivable as it may be that all the nuclei in a large plasmodium are and always must be genetically identical, the fact remains that up to now no evidence of hetero-

karyosis has been obtained in any myxomycete and all attempts to produce a heterokaryon in the laboratory have, to my knowledge, failed.

ECOLOGY

The last twenty years have brought us little knowledge concerning the ecology of the Myxomycetes, and nothing of great importance can be added at this time to the general conclusions on this subject which were drawn by Martin (208) in 1940. There are a few papers which purport to be ecological studies, but most are not extensive enough to warrant serious conclusions. A note by Buchet (40) on a collection he made of *Physarum pezizoideum* is quite interesting. He collected this usually tropical or subtropical species in Central France in 1938, on *Auricularia mesenterica* and calls attention to the fact that at least five other collections— the type collection in Java, two previous ones in France, one in Germany, and one in Australia—have also been found on or "in the company" of auriculariaceous fungi, and that "Brandza speaks of 'Tremellaceae' (and) Saccardo of 'Jelly fungi' " in this connection. Buchet believes this association to be more than accidental. Nevertheless, the literature reveals at least as many instances of *P. pezizoideum* on other substrata as Buchet cites on auriculariaceous fungi.

Frei (103) records a "mass appearance" of Myxomycetes in a small (100 $\times$ 120 meters) conifer forest in Switzerland where in less than two days he collected 81 plasmodia and 93 sporophore colonies. He lists 24 species and varieties with the substrata on which they were found. Johnson and Andrews (150) collected about 500 specimens representing 33 species and 4 varieties of slime molds in Mississippi in June, 1939. They record the following observations: The pine forests were usually devoid of slime molds; the west and east central swamps were fruitful; the northeastern prairie belt was fairly barren. The east-central region of the state was best for slime mold collecting. Thirteen species "seem major elements of an ecological society to be associated with the June display of Myxomycetes in Mississippi." All are of world wide geographical distribution, and all except one *(Physarum pulcherripes)* very common species. Karsen (158), commenting on her collection of 80 species and 33 varieties of Myxomycetes from Hardanger, Norway, made in September of the years 1933–1939, finds that the following forms are decidedly alpine or sub-alpine: *Diderma alpinum, D. niveum, Lepidoderma*

granuliferum, Lamproderma Sauteri, L. carestiae, L. cribrarioides, and *Trichia alpina.* Of these, *Diderma alpinum, Lepidoderma granuliferum,* and *Lamproderma carestiae* have never been found outside alpine or subalpine regions whereas the others have been recorded also from lover levels. Of interest is Urries' list of Myxomycetes (329) from Navarra, Spain, in which is included a collection of *Ceratiomyxa sphaerosperma* hitherto known only from the tropics. Yanagita (354) reports some observation on the habits of six species of slime molds growing at the entrance and up to 4.2 meters inside a cave and concludes that *Comatricha longa, Dictydium cancellatum* and *Physarum viride* prefer conditions of higher moisture and less light than *Cribraria microcarpa, Stemonitis flavogenita* and *Ceratiomyxa fruticulosa.* His methods, however, leave much to be desired and the validity of his conclusions is questonable. Thirteen common species of Myxomycetes are listed from the Savannah River project area in South Carolina in a report by Batson entitled "An Ecological Study..." (26). All those reported are cosmopolitan species and the list is therefore of no ecological significance, especially since little information is given on the area except for the statement that "no dearth of these Myxomycetes exists in and around the extensive swamplands so common to the projected area." *Stemonitis webberi* Rex. was found by Routien (269) on the bottom and sides of wooden beams in the ceiling of a rather humid room. It was rarely seen in the summer but frequently observed in the winter. It occurred in spots a foot apart over about 500 sq. ft. of ceiling. When washed off with phenol, formaldehyde or a 50% caustic solution, the organism always reappeared.

Peterson, (255) employing the technique which Gilbert and Martin (115) first used to study the species of Myxomycetes which develop on the bark of living trees in moist chamber culture, attempted to correlate the species of slime molds with: 1) species of tree, 2) position on tree (compass direction and distance above the ground), 3) habitat of tree, and 4) season. The study comprised 2500 pieces of bark from 29 different species of trees in nine habitat categories. Almost 60% of all bark specimens developed myxomycete fruiting bodies in moist chamber. These were distributed among 42 species, 10 of which were belived to be undescribed. The author interprets his results to indicate that no significant correlations exist between development of slime mold fructifications in general and position on the tree from which bark was taken or season of collection. Correla-

tions between slime mold development and species of trees or tree habitat were inconclusive because of the method of sampling. Nevertheless his results may be indicative of the existence of some degree of correlation between individual species of slime molds and some of these factors, which a more detailed study might bring out. Warcup (334) in a study of ecological distribution of fungi in soil found myxomycete plasmodia which had developed in his plates. His method consists of placing small amounts of soil in sterile Petri dishes, pouring 8–10 ml of cooled agar (Chapek-Dox + 0.5% yeast extract agar, acidified with phosphoric acid to pH 4.0) over the soil, and dispersing the soil particles by rotation of the dish before the agar solidifies.

Continuing ecological studies on microorganisms in forest litter and soil, Krzemieniewska and Badura (175) found 27 species of Myxomycetes in the beech forest of Muszkowice in Poland. Nine species were isolated from soil or litter in culture on rabbit dung media. In another paper (172), Krzemieniewska reports her collections of Myxomycetes in the Swietokrzyskie Mountains, in the Western Carpathians, and in Lower Silesia, and draws certain general ecological conclusions as follows: 1. Most species of Myxomycetes are independent of the substratum on which they fruit. Among species which show "remarkable ability of adaptation" in this respect are *Physarum bivalve, P. virescens, Leocarpus fragilis, Diachea leucopodia, Mucilago spongiosa,* and *Didymium melanospermum.* 2. Some species fruit more often on wood from broad-leaved trees than on conifer wood. Examples are: *Fuligo rufa, Physarum psittacinum Comatricha typhoides, Lycogala conicum, Arcyria ferruginea, A. denudata, Trichia persimillis, T. scabra,* and *Hemitrichia vesparium.* 3. *Cribraria* and *Dictydium* are more often associated with conifer wood. 4. Most species found in Poland fruit most abundantly in July and August ending their season in September. Autumn species that begin fruiting in August or September and appear in great abundance in October are: *Cribraria rufa, Trichia pusilla, T. scabra,* and *T. varia.* Skulberg (282) found *Didymium nigripes* in a trough in which flowed a mixture of 9 parts well water and 1 part effluent. He cultivated it on mannitol agar to which he added algal material from a culture of *Oscillatoria rubescens.* The sporangia invariably formed at night. Evenson (91), in her paper on Myxomycetes of Southern Arizona, lists 63 species collected in an area within a radius of 90 miles from Tucson. She made collections in five vegetation areas and tabulated them in an

effort to establish some correlation between habitat and species. A summary of her findings is as follows: Forest, 52 species, Chaparral, 6; Desert grassland, 7; Desert shrub, 16; Landscaped city areas, 3. Many of her specimens were developed in moist chambers in the laboratory, on material (bark of living trees, desert debris, pine needles, etc.) collected in various areas.

TAXONOMY

General Taxonomy

The major taxonomic work on the Myxomycetes which appeared during the 20-year period under review is G. W. Martin's "The Myxomycetes" (212). This is now the definitive work on North American slime molds. Martin describes his Division Fungi and recognizes the Myxomycetes as a class coördinate with the Phycomycetes, Ascomycetes, and Basidiomycetes. In general, he follows the lines laid down by Macbride and Martin (205) recognizing one order (Ceratiomyxales) in the sub-class Exosporeae, and four (Physarales, Stemonitales, Trichiales, and Liceales) in the sub-class Myxogastres. Excellent, workable, dichotomous keys to all taxa known up to 1949 in North America are included as well as a very complete taxonomic bibliography.

In a paper read before Section G3 of the A.A.A.S. at the Chicago meeting in December 1959, Martin (216), revised his classification somewhat, dividing his Division Mycota (Fungi) into two Sub-Divisions: Myxomycotina and Eumycotina. The Myxomycotina include the single class Myxomycetes with two sub-classes Ceratiomyxomycetidae (Exosporeae) and Myxogastromycetidae (Myxogastres), the former with the single order Ceratiomyxales, the latter with five orders: Physarales, Stemonitales, Echinosteliales, Trichiales, and Liceales.

Another extensive monograph of the Myxomycetes published during this period is Hagelstein's "The Mycetozoa of North America" (134) based on the extensive collection of slime molds in The New York Botanical Garden. Hagelstein follows the classification of the old Lister monograph, the keys, in most instances, being taken directly from Lister (185). Unfortunately, no attempt has been made to organize the keys into a dichotomous scheme, leaving them as cumbersome as in the Lister monograph.

Other less extensive taxonomic works which appeared during the last two decades are Dennison's revision of the genus *Lamproderma*

(75), Nannenga-Bremekamp's revision of *Reticularia* (237), and Hertel's revision of the genus *Comatricha* (142). Nannenga-Bremekamp, on very logical grounds, combines *Enteridium* and *Reticularia* under the latter name, recognizing now five species (one new) and describing a new variety. Hertel splits *Comatricha* into no fewer than four genera on the basis of characters which are at least as artificial as those which now separate *Comatricha* from *Stemonitis*. Certainly the relative length of the columella (not exceeding ½ sporangium, or reaching the "apex of the sporangium or nearly so"), the length of the stipe in relation to the total height of the fructification, and the evanescence of the peridium, characters which are now used to differentiate between species of *Comatricha*, are no less artificial when used to separate genera. Species like *C. nigra* and *C. laxa*, which have been regarded as close relatives by other authors (185, 205), are placed by Hertel in different genera. On the other hand, characters such as structure of the stipe which might well be regarded as natural, are disregarded altogether, *Comatricha fimbriata*, and *C. martinii* for example, being retained in the same genus *(Paradiacheopsis)*. Hertel transferred *C. typhoides* to the genus *Stemonitis*. Yet it was subsequently found (117, 264) that the capillitial development in *Comatricha* is fundamentally different from that in *Stemonitis*. Whereas this reviewer agrees absolutely with Hertel in his view that the genera *Comatricha* and *Stemonitis* should be thoroughly reexamined, he also believes that splitting the two genera into five on the basis of artificial characters complicates rather than simplifies the situation without in any way reflecting possible relationships. Morphological studies, such as those by Ross (264) and by Goodwin (117), should be extended to other species to give us a firmer basis for a better classification of this interesting group.

Hagelstein (133) continued his taxonomic comments during this period, publishing 4 additional "Notes" (IV–VII) before his death. These deal with observations on various species collected, record variations found, and evaluate characters used in the differentiation of species. Martin also published four "Taxonomic notes" (209) with critical comments about unusual species. In the first of these, the genus *Licea* is discussed and emended; in another of these notes, Martin proposes a number of additional revisions in the Liceales. Very justifiably, he combines *Alwisia* with *Tubifera* and, pointing out that *Tubifera* appears to be closely related to the genera of the Reticulariaceae,

transfers it to this family, thus reducing the Tubulinaceae to synonymy. This leaves the Liceaceae with *Licea* as the only genus. A revised key to the three families of Liceales, a key to the recognized genera of the Reticulariaceae, and revised diagnoses to the Liceaceae and the Reticulariaceae are included. Attention is called to the validity of *Calomyxa* Nieuwl. over *Margarita* A. List. In other papers, Martin defends the name *Hemitrichia* over *Hyporhamma* (211) and recognizes the genus *Schenella* Macbr. as valid (217). He redescribes it to include the type species, *S. simplex* Macbr., and a new species, *S. microspora* Martin.

Alexopoulos (15) described a new *Echinostelium (E. cribrarioides)* which made it necessary to emend the ordinal description to include all four species which now comprise the order Echinosteliales. He included, in his paper, a key to the four species.

Nannenga-Bremekamp, in her notes on Myxomycetes V (237), combined the genera *Dictydium* and *Cribraria* under the latter name on the basis of the presence of a *Cribraria*-like net in the upper part of many specimens now referable to *Dictydium*. Whereas it cannot be denied that the two genera are closely allied and that intermediate forms exist, it seems to this reviewer that they should be retained until experimental evidence of their identity can be obtained.

It is on the species level, as might be expected, that most of the taxonomic work on the Myxomycetes needs to be done.

A case in point is *Didymium nigripes* (Link) Fries. Lister (185) recognizes this species and two varieties, var. *eximium* (Peck) Lister, and var. *xanthopus* (Ditm.) Lister; Hagelstein (134) recognizes all three as distinct species; Martin (212) considers var. *eximium* (Peck) Lister as a synonym of *D. megalosporum* Berk. & Curt., and recognizes var. *xanthopus* (Ditm.) Lister as a distinct species under the name *D. iridis* (Ditm.) Fries. Skulberg (282) found a form of *Didymium* which combines some characters of *D. nigripes* and *D. iridis* and exhibits some others not typical of either. He refers to *D. nigripes* s. lat., to include all four forms, and points to the need for experimental work which will enable us to evaluate the validity of the characters used in differentiating between species. This, of course, in view of what has been emphasized in previous sections of this review, is now possible with only the few species which can be grown in culture. *Didymium nigripes* and *D. iridis,* both have been induced to complete their life cycles on artificial media and this technique could well be applied as a taxonomic tool.

Farr (92, 94), after an extensive study of a large number of collections of 3 species of *Trichia (T. affinis, favoginea, persimilis),* and 2 of *Arcyria (A. cinerea* and *A. pomiformis)* unites the Trichias under the name *T. favoginea,* but finds the Arcyrias to be distinct. In a later paper (97) she reinstates *Badhamia decipiens* in the genus *Physarum* and concludes that it may be an environmental variant of *P. serpula.* Here again the difficulty of growing these organisms in artificial culture is the main obstacle in evaluating the stability of taxonomic characters.

There is little reason to doubt that most species of Myxomycetes are made up of a number of stable genotypes whose existence can be proved only by controlled culture work. As he does with the other fungi, so with the Myxomycetes, the taxonomist would recognize many observable phenotypic variations, morphological and physiological, to be different strains within a single species complex if their genetic stability could be established. Lacking experimental evidence he now either describes them as varieties or species, or, more often, dismisses such small variations as ecological. Neither view is on a sound basis. In this connection, the question of hybridization is a very interesting one. There does not appear to be a single report in the literature of proved interspecific hybridization in the Myxomycetes, but recent work (19, 57, 73) on hybridization of physiological races opens up a vast new field of investigation.

New Taxa

New taxa described between 1940 and 1949 were incorporated by Martin in his monograph (212) if they had been found in North America. Those named after that date, or for other reasons not included by Martin in his monograph, are listed below:

SUB-DIVISION:

Myxomycotina Martin (216)

ORDER:

Echinosteliales Martin (216)

GENERA:

Comatrichoides Hertel (142)
Paradiachea Hertel (142)
Paradiacheopsis Hertel (142)

Species:

Arcyria assamica Agnihothrudu (5)
Arcyria carletae Hertel (139)
Arcyria corymbosa Farr and Martin (99)
Arcyria fonsecai Hertel (139)
Arcyria stellfeldi Hertel (139)
Ceratiomyxa morchella Welden (344)
Comatricha acanthodes Alexopoulos (10)
Comatricha aggregata Farr (93)
Comatricha longipilla Nannenga-Bremekamp (237)
Comatricha martinii Alexopoulos and Beneke (17)
Comatricha mirabilis Benjamin and Poitras (29)
Comatricha solitaria Nannenga-Bremekamp (237)
Comatricha synsporos Alexopoulos (10)
Craterium auronucleatum Nannenga-Bremekamp (237)
Diacheopsis pieninica Krzemieniewska (171)
Didymium floccosum Martin, Thind, and Rehill (219)
Didymium labyrinthiforme Martin, Lodhi, and Khan (218)
Didymium nigrum Krzemieniewska (174)
Didymium ovoideum Nannenga-Bremekamp (237)
Didymium verrucosporum Welden (344)
Echinostelium cribrarioides Alexopoulos (15)
Echinostelium elachiston Alexopoulos (10)
Echinostelium fragile Nannenga-Bremekamp (237)
Fuligo licenti Buchet (39)
Hemitrichia paragoga Farr (93)
Lamproderma verrucosum Martin, Thind, and Sohi (220)
Licea didermoides Martin (209)
Paradiacheopsis curitibana Hertel (140)
Physarum dictyosporum Martin (209)
Physarum mennegae Nannenga-Bremekamp (237)
Physarum mucosum Nannenga-Bremekamp (237)
Physarum retisporum Martin, Thind, and Rehill (219)
Physarum tesselatum Martin and Farr (95)
Reticularia intermedia Nannenga-Bremekamp (237)
Schenella microspora Martin (217)
Stemonitis brasiliensis Farr and Martin (99, 367)
Stemonitis curitibensis Hertel (141)
Stemonitis musooriensis Martin, Thind, and Sohi (220)

Stemonitis travancorensis Erady (90)
Trichia craterioides Martin (209)
Tubifera papillata Martin, Thind, and Sohi (220)

VARIETIES AND FORMS:

Arcyria denudata (L.) Wettst. var. *rosea* Forstner (102)
Badhamia lilacina (Fries) Rost. var. *megaspora* Nannenga-Bremekamp (237)
Comatricha typhoides (Bull.) Rost. var. *cinerea* Hertel (141)
Cribraria languescens Rex fa. *magnigranosa* Thind and Sohi (319)
Reticularia lycoperdon Bull. var. *americana* Nannenga-Bremekamp (237)

GEOGRAPHIC DISTRIBUTION

In the period under consideration, a great many studies have extended our knowledge of the distribution of the Myxomycetes throughout the world. The moist chamber technique, first introduced by Gilbert and Martin (115) to the study of the Myxomycetes, has since been used with considerable success by a number of investigators (8, 11, 15, 17, 29, 93, 115, 209, 237, 254, etc.) and has shown that some species, hitherto considered to be rare, are actually widely distributed and of common occurrence. Such species, because of the very small size of their fructifications, are generally overlooked in the field.

Many check lists, considered to be of local interest only, are published in journals of limited circulation and are difficult to discover. The list of references that follows is bound, therefore, to be incomplete:

Argentina (76, 77); Australia (101); Austria (322); Brazil (96. 99, 136, 139-142); Canada (70, 71, 209, 342); Canary Islands (95); China (39, 315); Ceylon (254); Costa Rica (344); Denmark (229, 233); East Africa (45); England (186, 261); France (40, 41, 78, 187, 206, 252); Galápagos (210); Germany (79, 257); Greece (10, 11, 15); Hungary (24, 25); India (4, 5, 90, 112, 219, 220, 234, 253, 317-320); Iraq (6); Ireland (308); Jamaica (17, 18, 93); Japan (88, 89, 235, 351, 352, 354); Krakatau (33); Liberia (95); Lower Silesia (100); Netherlands (105, 237, 238, 239); Norway (158); Pakistan (196, 218); Panama (214, 344); Poland (171-175, 243); Pomerania (226); Pyrenees (81); Rhodesia (127); Roumania (102); Spain (329); Switzerland (103); Sweden (135, 270, 302); Transyl-

vania (259); United States: Alaska (44), Arizona (91, 209), California (258), Florida (8, 346), Hawaii (251), Illinois (28, 29, 311), Indiana (120, 123), Iowa (231), Kansas (36, 170), Louisiana (98, 199), Michigan (16, 116, 209, 255), Mississippi (150), Mt. Shasta (60), North Carolina (152, 153), New York (232, 331-333), Smoky Mts. (343), South Carolina (26), Virginia (118), Washington (61), Wyoming (230); Venezuela (74, 262, 263).

GENERAL CONCLUSIONS

The Myxomycetes constitute a homogeneous, natural group of organisms which are probably related neither to the Acrasiales nor to the Plasmodiophorales with which they have often been grouped.

Most species are probably cosmopolitan, but a few are strictly confined to the tropics or to the temperate zones. One or two have been found only in alpine regions. Whereas abundant moisture appears to be the chief factor controlling the profusion of Myxomycetes in any given locality, no species is strictly aquatic and many have been found in or isolated from desert regions. Some species appear to be seasonal in their sporulation. Some show a distinct preference for certain types of substrata, fruiting on coniferous wood, for example, in preference to deciduous tree wood, or on leaves in preference to wood or other substrata. Such information, however, is gleaned almost entirely from field observations and impressions, and accurate data, if recorded, have not been compiled to give us an ecological picture of the distribution of Myxomycetes and to enable us to draw conclusions concerning the factors which govern such distribution.

Wind is undoubtedly the chief agent of spore dissemination, with insects and other agents probably playing a lesser role in myxomycete spore dispersal.

The spore wall of the Myxomycetes probably contains cellulose as well as other materials. Any further advance in our knowledge concerning the structure and composition of the spores will come from the study of ultra-thin sections with the electron microscope and from the application of modern techniques (X-ray diffraction patterns, etc.) to the investigation of the spore wall. There is some evidence that the size of spores, hitherto considered to be fairly stable, is appreciably affected by the environmental conditions under which the spores develop.

Spores are highly resistant to adverse conditions and may be germinated after long periods of storage, but both time and percentage

of germination are drastically affected by age, by various conditions of the environment, and by the fruiting body in which spores were formed. Spores of many species germinate as easily when sown singly as when sown in mass. Moisture, but not necessarily free water, is required for spore germination.

When a spore germinates it liberates one or two, rarely up to four, myxamoebae or swarm cells. In an aqueous environment myxamoebae form flagella. In the absence of free water, flagella are withdrawn and swarm cells change into myxamoebae. Flagellated cells are not an essential part of the life cycle of all Myxomycetes. Some species will complete their life cycle without flagellated cells ever being formed if free water is not available during spore germination and zygote formation.

On the basis of our present knowledge it appears that the myxomycete swarm cells are typically and potentially anteriorly biflagellate, and heterokont. One flagellum is usually long and active and one very short, recurved, and appressed against the amoeboid protoplast, or both flagella may be of nearly equal length. Both flagella may be whiplash or one may have a knobbed tip the significance of which is not clear. In certain species or races, the majority of the swarm cells at any given time may be uniflagellate, but two basal granules are invariably present. The fine structure of the myxomycete flagellum awaits investigation.

Myxamoebae divide mitotically and produce large populations of haploid cells before plasmodium formation begins. The myxamoebal stage of the life cycle may be prolonged indefinitely—at least in some species—by the addition of brucine and possibly other substances to the medium. Brucine appears to act as a chelating agent, binding multivalent cations necessary for plasmodial formation.

Syngamy is a prerequisite to plasmodial formation in all species which have been investigated critically. The possibility of apogamy in some species, however, cannot be ruled out. Plasmogamy may occur between two myxamoebae, two swarm cells, or, possibly, between a swarm cell and a myxamoeba. The first two methods, at least, may be employed by one and the same organism, but some species preferentially or obligately employ one or the other.

Bipolar heterothallism has been established for two species of Myxomycetes and mutiple alleles at the incompatibility locus have been found in one. Several species are known to be homothallic.

No interspecific hybrids have been produced experimentally, but hybridization between physiological races has been induced in two species. The question of the occurrence of heterokaryosis in the Myxomycetes has not been answered.

Karyogamy occurs in the zygote, immediately after plasmogamy. Meiosis takes place in the fruiting bodies at the time the spores are delimited. In *Ceratiomyxa,* however, meiosis occurs at spore germination giving added support to the theory that the spore of *Ceratiomyxa* is homologous to the sporangium of the Myxogastromycetidae.

In most species of Myxomycetes investigated chromosome numbers are large, ranging from $n = 25$ to $n = 90 +$. In *Ceratiomyxa fruticulosa* the chromosome number is $n = 8$.

The mode of origin of the plasmodium is still somewhat controversial, but cinephotomicrographic proof has been obtained for the growth of a zygote into a plasmodium and for the coalescence of zygotes successively to form and enlarge a plasmodium. Whether many zygotes come together and form a multinucleate plasmodium by one multiple fusion is still a debatable subject.

At least three general types of plasmodia are now recognized. The protoplasmodium, which retains what might be thought of as juvenile characteristics throughout its existence, is probably the most primitive. It is typically formed by the Echinosteliales, but also by minute species which belong to other orders. The aphanoplasmodium is characteristic of *Stemonitis* and probably of related forms. The phaneroplasmodium is typically formed by members of the order Physarales. As our knowledge increases other types of plasmodia, intermediate between these three basic types, are being discovered.

Physarum polycephalum has figured prominently as a tool for biochemical, biophysical, and cytological research. Nuclear division in this plasmodium, and probably in those of many if not all other species of Myxomycetes, is precisely synchronous when the plasmodium grows in axenic culture under controlled environmental conditions. DNA synthesis begins immediately after mitosis and lasts for 1 or 2 hours. It cannot, therefore be the trigger mechanism for mitosis.

Myxomyosin, a contractile protein with a molecular weight of 6 million, probably plays an important role in the streaming of the plasmodial protoplasm, through its interaction with ATP whose presence in the plasmodium has been adequately demonstrated. Whether myxomyosin ultimately provides the motive force for streaming is

not certain. Polarity in a plasmodium seems to be closely associated with potassium concentration.

Sclerotization may be induced by slow desiccation of a plasmodium at a moderate (25° C) temperature. A sclerotium consists of many, usually multinucleate, sphaeroidal macrocysts 10–25μ in diameter. Sclerotia may retain their viability for 1 to 3 years in storage.

The basic causes of sporulation have not been discovered. A shift in oxidases at the time of fruiting has been recorded. Light is necessary for the sporulation of pigmented plasmodia and may also be essential for at least some plasmodia which appear to lack pigments. The role which the pigments play as photoreceptors is under investigation in several laboratories. The conditions necessary for sporulation of *Physarum polycephalum* in the laboratory are: 1) A medium containing niacin or certain substitutes thereof, 2) an optimal age, 3) a minimum dark incubation period, and 4) subsequent exposure to light of wavelengths between 250 and 500 mμ.

One of the main problems yet to be solved in the study of the Myxomycetes is their artificial culture. Few species have been grown even in crude culture on artificial media. Two-membered cultures are not difficult to obtain with species that can be cultured readily. Pure cultures, though achieved with some plasmodia more than tweny years ago by Cohen, cannot be employed as yet for studies of all parts of the life cycle. Furthermore, in many of the pure cultures which have been established, plasmodia thrive only if killed bacterial or yeast cells are available for food. The success of Rusch and his coworkers in growing the plasmodium of *Physarum polycephalum* in bacterium-free culture, in a chemically defined, liquid medium, represents a major contribution and has opened the way for exact physiological and cytological studies with this organism. Even so, the completion of the entire life cycle has not been achieved under these conditions. Until methods are discovered for growing many species of Myxomycetes in pure culture, free of microorganisms living or dead, in chemically defined media, from spore to spore, studies in morphogenesis and physiology of the Myxomycetes are bound to be greatly hampered and progress will be very slow.

The general taxonomy of the Myxomycetes is more or less stabilized. This does not mean it is perfect. It means that we have probably reached the best system that can be devised without experimental verification of the stability of the characters which are now considered

to be taxonomically important, and without considerably more knowledge of the developmental history of both the plasmodia and the sporophores of a great many species. Enough specimens of most known species have now been collected from many parts of the world under many conditions, and are available for study in the three major world collections and in many smaller ones, to give us a good concept of the range of variation of species under natural conditions. Experimental work is now possible with many species which may be grown in two-membered culture in a partially controlled environment. With most species, however, experimental work will have to await the discovery of practical culture techniques. In the meantime, revisionary work on the basis of field collected specimens should be undertaken on a larger scale. In the laboratory, studies of the plasmodial stage and morphogenetic studies of fructifications are yielding data of considerable taxonomic value.

The Myxomycetes are no longer the private concern of a few devoted specialists. Their value as tools in the study of fundamental biological processes common to all living organisms is now widely accepted. Many young minds are now being attracted to the study of these organisms and new and exciting discoveries may be confidently expected in the years to come.

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