THE MYXOMYCETES

Hanno Koppel

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1. Classification

There is some confusion as to the limits of the class Myxomycetes. There are four orders within this class:\textsuperscript{1}

1) The Myxomycetes - the true (acellular, noncellular, syncitial, plasmoidal) slime molds which will be the topic of this paper.

2) The Acrasiales. These are the cellular slime molds which resemble Myxomycetes in the production of myxamoebae, but do not produce true swarm cells or true plasmodia, and the aggregated amoebae can be easily shaken apart in water. The Acrasiales are the slime molds which are providing biologists with very interesting material for the study of differentiation.

3) The Plasmodiophorales. These include several genera, all parasitic on plants or fungi. They are similar to Myxomycetes but they lack a secreted sporangial wall and capillitial threads, they form zoosporangia and they have a distinctive type of nuclear division.

4) The Labyrinthulales. The taxonomic position of these organisms is uncertain. The assimilative stage consists of spindle or oval shaped cells gliding on or within slime tracks or tubes. The slime tubes are joined to form a network (the filoplasmodium) which is why these organisms are often known as the net slime molds. The filoplasmodium is unrelated to the plasmodium of the Myxomycetes.
2. Life Cycle of the Myxomycetes

The spores germinate releasing from one to four uninucleate, haploid protoplasts. In water these develop flagella and are then called swarm cells. Where free water is lacking they become amoeboid. The amoeboid-phase organisms are called myxamoebae. The myxamoebae can, if they reach free water, develop flagella and become swarm cells. Conversely, the swarm cells can resorb their flagella to become myxamoebae. If the food supply is insufficient or when other adverse conditions prevail, both phases can encyst, the swarm cells becoming myxamoebae before encystment. Both the swarm cells and the myxamoebae divide by binary fission, the swarm cells first resorbing their flagella. The swarming phase may last from several hours to many days.

When a certain critical concentration of cells is reached, compatible myxamoebae or swarm cells behave as gametes and copulate in pairs. It is not known whether copulation of myxamoebae as against swarm cells is a characteristic of individual species. Cell fusion is followed by nuclear fusion and diploid zygotes are formed. Heterothallism has now been adequately demonstrated, but as yet not widely observed.

The zygotes feed and grow. The nucleus divides but the cytoplasm does not, transforming the zygote into a plasmoidium. Nuclear division is synchronised within a single plasmoidium. Plasmodia fuse with one another, and with zygotes, enlarging
by accretion as well as by growth. This phenomenon provides an interesting system for the study of genetics (whether two genetically different plasmodia can fuse, and if so which nuclear type becomes dominant and why) and the synchrony of nuclear division.

When the conditions are right the plasmodium develops into a fruiting body (sporophore), the morphology of which is species specific, accompanied by cleavage of the protoplasm into uninucleate portions. These become enveloped by walls and mature into spores. The network of thread-like structures within the sporophore, the capillatum, usually develops at this point. Meiosis either occurs in the sporophore during spore cleavage or in the spore after its formation; there is some controversy over this point. It may be that meiosis does not occur at the same point in the life cycle in different species, or even the same isolate of the same species.

There is a genus of Myxomycetes whose life cycle differs from that described enough for it to be put into its own subclass; this is the genus Ceratiomyxa. The main difference is that it is exosporous; its spores are born on individual stalks. On germination each spore gives rise to 8 swarm cells. Its plasmodium is more conspicuous than those of the other (endosporous) Myxomycetes.

The life-cycle of the endosporous Myxomycetes can be summarized diagrammatically:
Diagrammatic representation of the life cycle of the Myxomycetes

(not to scale)
3. Morphology and Physiology

**Spores.** The spores are usually spherical. They show a wide range of colors, from hyaline to yellow, pink, brown and black. The wall may be smooth or show one of many distinct surface textures. The average size is 10 µ though they may be as small as 4 µ (Stemonitis smithii) or as large as 20 µ (Fuglio megaspora). These characters are constant and are useful means of identification.

**Swarm cells and myxamoebae.** One to four of these arise from the germinating spore. What happens in nature is unknown, but in culture if the spore is germinated in water the protoplasts released are flagellate, if it is germinated on a moist surface devoid of free water they are amoeboid. The amoebae are up to 30 µ in diameter. They show no polarity and in general show similar characteristics to other amoebae e.g. they phagocytose and pinocytose and divide by mitosis and cytokinesis. The swarm cells are now believed to be biflagellate. After germination a 10-15 µ long flagellum develops, followed later by a very much shorter, recurved flagellum ⁵,⁶. The long flagellum has been demonstrated to be constructed on the typical 9 + 2 pattern⁵. Flagellum-like pseudopodia have been seen to originate at the base of the flagellum and migrate toward the posterior end⁷. Streaming of the swarm cell is easily observed, and the stickiness and pseudopodial activity of the posterior end have been
demonstrated. The swarm cell absorbs food from its surrounding medium\textsuperscript{8,9} and ingests bacteria with the pseudopodia at its posterior end.

It has been reported that myxamoebae are produced by the advancing fan of the plasmodium of \textit{Arcyia cisserea} and \textit{Stemognathus herbatica}\textsuperscript{10}. This was later confirmed in a species of \textit{Physarum}\textsuperscript{11}. The myxamoebae are cut off, and migrate away from the plasmodium.

**Microcysts.** In unfavorable conditions such as overcrowding, lack of food, accumulation of various metabolites, drought or overabundance of water, myxamoebae encyst. The conditions which cause encystment have not been studied precisely, but a reversal of the conditions that cause encystment often initiate a resumption of activity. As described for \textit{Fuglio cinerea} and \textit{Physarum gyrosum} encystment occurs as follows:

A swarm cell resorbs its flagellum and rounds up. The cytoplasm darkens and the cell volume decreases, indicating a condensation of the cytoplasm. The nucleus occupies a central position and also decreases in volume. Mitochondria accumulate around the nucleus. The contractile vacuole ceases to function and disappears. Food vacuoles disappear. Finally, a thick, refractile membrane forms around the cell\textsuperscript{12}.

**Plasmodia.** The simplest view of plasmodium formation is that two haploid myxamoebae or swarm cells, either homothallic or heterothallic, fuse. The plasmogamy is followed
by karyogamy to form a diploid zygote which can then develop into a plasmodium by nuclear divisions without cell divisions. It has been observed that many zygotes may coalesce, but not with sufficient frequency to say whether it is accidental or a result of active aggregation. The existence of apogamy in Myxomycetes is controversial.

There is little work on the factors affecting plasmodium formation. Some very early work investigated various physico-chemical criteria. More recently, ways of inhibiting plasmodium formation have been found; 2% brucine (or glucose) added to SM/5 medium has inhibited plasmodium formation of Didymium nigripes. The brucine and glucose may act by preventing fusion, or, if fusion occurs, by preventing the plasmodia from enlarging. The latter is less likely because plasmodia are easily distinguishable from myxamoebae and it is assumed that they would have been detected if present. The optimal concentration of myxamoebae for plasmodium formation in a strain of Didymium nigripes has been stated to be $2.5 \times 10^5 / \text{ml}$. Work has been done on the effect of temperature and light on plasmodium formation in a species of Physarum. It showed that light has no effect but an increase in temperature shortens the time required for plasmodium formation to a minimum of 3 days at $25^\circ C$ (the maximum being 27 days at $15^\circ C$). However the number of cultures forming plasmodia was less dramatically affected. It is possible that the pronounced effect of temperature on the time required for plasmodium formation was also due to a retardation of spore formation.
Free water may or may not be necessary for plasmodium formation.

The plasmodium itself is a naked, free living, multinucleate, motile mass of protoplasm, usually colorless or some shade of yellow. For studies on plasmodia it has been pointed out that for most purposes the best myxomycete is *Physarum polycephalum*.16

There are three types of plasmodium:

1. **Protoplasmodium.** This is produced by the small myxomycetes such as *Echinostelium minutum* and *Licea parasitica*. It has no veins and a very slow, irregular protoplasmic streaming.

2. **Aphanoplasmodium.** This is produced by *Stemonitis fusca* and *Comatricha laxa*, for example. It may live within the woody substratum on which its fruitings are found. However it is flat, transparent and not easily detectable, and may be on the surface. It forms a mesh of hypha-like branches which develop from the original protoplasmodium-like mass. The mesh may be up to 400 μ in diameter. There may be regular reversible protoplasmic streaming. As it gets older one or more fans with definite margins may be formed. It remains very flat and transparent and therefore almost invisible to the naked eye.

3. **Phaneroplasmodium.** This is the best known of plasmodia and are reported in all species of Physarales studied. They are usually a few cm. square but plasmodia of *Fuglicella septica* have been reported with an area of up to 1 sq. ft.
The development is via a minute plasmodium which shows streaming when it is at a size of 40 μ. The plasmodium elongates and forms a channel in which the protoplasm begins a rhythmic reversible streaming. The plasmodium develops into a fan-shaped structure which shows a distinct differentiation between an outer, jellified, non-streaming region and an inner, more fluid, streaming region. The fan thickens and streaming channels develop. The main channels run from front to back and are cross connected by smaller channels. The phaeroplasmodium has a three-dimensional appearance.

Phaneroplasmodia can be subcultured by transferring bits of plasmodium (as small as 0.01 mg) to fresh substrate. The plasmodium can overcome its injury provided that care is taken. The plasmodium and propagules survive best if fragments are taken during the period of optimum growth (8-day-old cultures on oat agar). The good survival of plasmodia after such operation is due to the rapid gelling of the plasmasol, and the speed of membrane formation. It has been suggested that new plasma membrane may be formed from the limiting membranes of the many vesicles\(^{17}\). The plasmodium is surrounded by a sheath, and it is the discarded sheath, left behind by the plasmodium as it crosses its substrate that is the "slime track" that gives the Myxomycetes their name. It has been determined that the sheath has a fibrillar structure and it is suggested it might be a sort of exoskeleton\(^{18,19}\).
Plasmodial nuclei of *P. polycephalum* grown axenically in liquid medium were found to be 4-5 μ in diameter, and contained a single nucleolus. There are a large number of nuclei in the plasmodium. In *P. polycephalum* there are approximately $8 \times 10^5/\text{mm}^3$, which means that 7% of the protoplasm consists of nuclei, as compared with 6% in the amoeba *Chaos chaos*.

The protoplasm of the plasmodium streams, and because the direction of streaming varies rhythmically it has been named shuttle streaming. The rate of flow is very high, 1350 μ/sec for *P. polycephalum* as compared with flow rate of 250 μ/sec. in a fungus hypha and 6 μ/sec. in a plant. There are at least thirteen hypotheses to explain the mechanisms of streaming: surface tension, hydration, osmosis, sol-gel reversibility, myelin processes, coacervates, autonomous propulsion of particles, kinetic energy, magnetism electrical forces, contractility, diffusion drag and contraction-hydraulic.

**Sclerotia.** In unfavorable conditions the plasmodium may become a sclerotium. This is a dry horny irregular mass which is considerably more resistant than the naked plasmodium and may remain viable for up to a year. Sclerotia of *P. polycephalum* may be produced in the lab by overfeeding plasmodia with rolled oats and drying at between 18°-32° C. The stages of sclerotium formation are as follows:
cessation of streaming

gelation of the whole structure
distribution of nuclei
depositing of wall
completion of macrocyst formation
hardening of the sclerotium
shrinkage of nuclei to half-size.

Sporulation and the Sporophore. The factors responsible for the cessation of assimilation and the initiation of sporulation, although well studied are not completely understood. Studies include the effect of light - continuous versus intermittent, intensity, wavelength, the effect of u.v., the effect of temperature and pH, and genetic control. It is concluded that:

1) Light is necessary for the induction of sporulation in yellow pigmented plasmodia.

2) Niacin is essential for the induction of light sensitivity.

3) The nutritional history is a very important factor in determining whether or not sporulation occurs, and the time of sporulation.

4) the presence of -SH groups prevents a plasmodium from sporulating.

The shape of the sporophore is species specific and is the basis of myxomycete classification. There are four types of sporophore:
1) the sporangium, - sessile or stalked, may have a capillitum system.

2) the plasmodiocarp - similar to a sessile sporangium but elongated and lying flat on the substrate

3) the aethelium - large, thought to be fused sporangia.

4) the pseudoaethelium - intermediate between sporangia and aethelia.

The capillatum is a network of threads within the fruiting bodies of many Myxomycetes. These threads are intermingled with the spores but not connected to them since they have been formed by a completely separate developmental system.
4. **Culture of Myxomycetes**

1. **Crude culture.** Phaneroplasmodia may be grown on Knops agar or CM/2 agar at 20-35°C. The plasmodium is fed by sprinkling it with sterile pulverized rolled oats. Vigorous plasmodium fans may be cut and transferred to new petri dishes to propagate or maintain the culture. Some Myxomycetes prefer oat flake agar.

2. **Spore-to-spore culture.** Spores are spread on CM/2 agar and the surface is flooded with sterile distilled water. After about 3-6 days at 20-25°C plasmodia should have formed (check by microscopic examination). When this has occurred, feed with sterile pulverized rolled oats. With some species the transfer of the plasmodium to non-nutrient agar is sufficient to induce sporulation (*Fuligo cinerea*, *Didymium squamulosum*, *D. iridis*, *D. nigripes*, *Physanum pusillum* and *P. didermoides*.) Other Myxomycetes require other factors to induce sporulation, and these have not yet been defined.

3. **Monaxenic culture.** The procedure is as follows: A spore suspension is made by crushing one or more sporangia in 10 ml sterile distilled water. 3 drops of spore suspension are placed near the edge of a CM/2 agar plate which is tilted, allowing the drops to run across to the other side. The dish is incubated at 25°C and examined periodically for a myxamoebal population which should migrate away from the bacteria growing along the inoculation streak. With a flamed needle cut out a block of agar bearing several myxamoebae, and free from bacterial colonies as determined by at least X90
microscopical examination. Transfer the block to a petri dish of sterile CM/2 agar and add 0.5 ml of an E. coli suspension. When plasmodia form, feed with sterile pulverized oats. When fruiting occurs new monaxenic should be started from the spores. To check the purity of the cultures 5 agar blocks in the vicinity of the first should be cut. One should be placed in each of the 5 tubes containing

1) Nutrient broth
2) Difco Ac broth
3) Yeast extract broth
4) Thioglycolate broth
5) Glucose broth

These tubes should be incubated at 25°C and examined periodically for turbidity. If any turbidity is found in any tube within 30 days, it should be assumed that the myxamoebae isolated were contaminated, and the culture should be discarded. If the tubes remain clear it is safe to assume that a monaxenic culture has been established.

Axenic Culture. The phaneroplasmodium should be rid of fungus by allowing it to migrate on CM/2 agar and transferring a piece of fungus-free fan to oat-flake agar. This is very difficult as often the fungi outgrow the plasmodium. If it is accomplished the plasmodium should be allowed to grow until large, vigorous fans are produced (3-7 days). A piece of fan should then be transferred to a 2% agar plate containing potassium penicillin (2 x 10³ units/ml) and
streptomycin (2 mg/ml) and allowed to migrate. Six hours is sufficient. Test for bacterial contamination as described for monoxenic cultures.

Physarum polycephalum has been grown on a semi-synthetic medium containing chick embryo extract. Subsequent analysis of the organism's requirement for chick embryo extract showed that this could be replaced by haematin or certain haemato-proteins. The partially defined medium for P. polycephalum could be replaced by three completely defined synthetic media which gave cell yields of approximately 70% of those obtained in a partially defined medium. Some modifications were made so that the plasmodia of Physarum flavicomum and Physarella oblonga and the myxamoebae of Badhamia curtisii could be grown in this medium. The medium was further modified to allow the growth of plasmodia of Physarum rigidum.

Myxamoebae of Didymium nigripes have been grown in the presence of bacteria killed with formalin and also bacteria disintegrated by treatment with ultrasonics or a Waring blender and filter sterilized. Didymium nigripes and Physarum cinereum have also been grown in the presence of heat-killed bacteria.

* No. 9 in appendix
5. **Maintenance and Storage**

**Myxamoebae.** These can be stored on CM/2 agar slants with a few ml bacterial suspension at 10-15°C. With occasional transfers such clones have remained viable for several years[^15]. For longer storage lyophilization or liquid nitrogen storage is recommended[^44]. Myxamoebae of *Didymium nigripes* have been maintained (i.e. prevented from forming plasmodia) by growing them on SM/5 agar with a 48 hour culture of *Aerobacter aerogenes* and the addition of 2% glucose or brucine to the medium. The myxamoebae survived and continued to multiply until the plate was cleared of bacteria. On subculture to similar media this process was repeated[^13].

**Plasmodia.** Monoxenic or axenic cultures may be maintained for many years on oatmeal agar in the dark at 10-15°C. They should be transferred once a month to keep them in vigorous condition. They can also be kept as sclerotia for one or two years at 10-15°C and placed on moist agar to reconstitute the plasmodia.

**Spores.** These should be stored dry in the sporangia in which they were formed. If collected in the field they should be stored with an insecticide (e.g. PDB (paradichlorobenzene) to protect from destruction by insects. Spores do not usually survive for more than 3 or 4 years. If the spores are lyophilized they may survive for more than 5 years[^45].
6. Media

1) CM/2 Agar: Cornmeal Agar 8.5 g
Bacto Agar 12.5 g
Distilled water 1000.0 ml

2) Knop's solution Agar: Ca(NO_3)_2 \cdot 4H_2O 0.8 g
KNO_3 0.2 g
KH_2PO_4 0.2 g
MgSO_4 \cdot 7H_2O 0.2 g
FeSO_4 "a trace"
Bacto Agar 20.0 g
Distilled water 1000.0 ml

3) Oat Flake Agar: Place enough oat flakes (rolled oats) preferably of the slow-cooking type to form a 5 mm layer in 100 x 20 mm Petri dishes. Pour enough hot melted Bacto agar in each dish to just cover the oats. Autoclave for 1 hour + at 115 lb/sq. in.

4) Nutrient broth: Bacto nutrient broth 8.0 g
Distilled water 1000.0 ml

5) AC broth: Bacto-AC medium 34.0 g
Distilled water 1000.0 ml

6) Yeast-extract broth: Yeast extract 1.0 g
Distilled water 1000.0 ml

7) Thioglycolate broth: Bacto-thioglycolate medium 24.0 g
Distilled water 1000.0 ml

8) Glucose broth: Glucose 1.0 g
Bacto peptone 1.0 g
Distilled water 1000.0 ml
9) Daniel & Rusch's semi-synthetic medium:

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<tr>
<td>glucose</td>
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<td>CaCl$_2$·2H$_2$O</td>
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<td>MgSO$_4$·7H$_2$O</td>
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<tr>
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<td>CaCO$_3$</td>
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<td>chick embryo extract</td>
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10) SM/5 Medium:

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<tr>
<td>Bacto peptone</td>
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<td>MgSO$_4$·7H$_2$O</td>
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<td>Bacto agar</td>
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<tr>
<td>Distilled water</td>
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7. Some Recent Studies on Myxomycetes

1) Genetic: Plasmodia may or may not fuse depending on whether they have genetic similarities in certain loci. This phenomenon has been studied and two explanations have been proposed. For *Didymium iridis* a polygenic system with simple dominance at each locus has been postulated\(^46\), \(^48\). For *Physarum polycephalum* there is some disagreement; either the system is polygenic as in *D. iridis*\(^49\), or it is a one locus, multiple allelic system\(^59\), \(^51\).

2) Biochemical: The plasmodium of *Physarum polycephalum* has been used for the study of the effects of dithiocarbamates with the result that respiration is strongly inhibited at \(285\, \text{mg/l}\)\(^52\). It was also found to be effective as a screen for 95 out of 144 antitumor agents tested at a concentration of less than 100 \(\mu\text{g/ml}\)\(^53\). Synthesis of DNA in the plasmodium occurs immediately after telophase (there is no G1 phase), and simultaneously in \(99\%\) of the nuclei\(^54\), \(^56\). Mitosis can be induced in nuclei as soon as DNA synthesis has been completed, as has been demonstrated by fusing small plasmodia, whose nuclei have just completed DNA synthesis, with a large plasmodium whose nuclei are in prophase. When the nuclei from the large plasmodium undergo mitosis, the nuclei from the small plasmodium do so, too\(^57\). This result, plus the fact that it appears unlikely that a high degree of mitotic synchrony would be able to occur if mitosis was governed by nuclear stimulation, suggests that the trigger for nuclear division resides in the cytoplasm\(^58\).

Further biochemical events in the nuclear cycle are documented.
in greater detail elsewhere\textsuperscript{59}. Ribonucleases and nucleases have been isolated from \textit{P. polycephalum} and studied\textsuperscript{60-62}. The reasons given for choosing a myxomycete were that no myxomycete ribonuclease had previously been studied, that the knowledge of the properties of such an enzyme would be useful when characterising \textit{Physarum} RNA and as \textit{Physarum} is highly synchronous with respect to nuclear fussion it is suitable for studies of the biochemistry of the cell cycle. Other enzymes studied in \textit{Physarum} are a protein kinase which was found to be inhibited by cyclic AMP\textsuperscript{63}; a glutamate decarboxylase (g.d.) which was found to be similar in some respects to g.d. of mouse brain but an optimum pH nearer to the optimum pH of g.d. of higher plants, and a thermal stability greater than that reported for the g.d. of any other organism\textsuperscript{64}. Isocitrate dehydrogenase glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, acid phosphatase, phosphodiesterase, \textbeta-glucosidase and histidase were studied in growing plasmodia of \textit{P. polycephalum}. 6 of the enzymes showed a continuous increase in activity during the mitotic cycle, showing that the mRNA for all 7 enzymes is stable during the G2 phase\textsuperscript{65}.\vspace{2cm}
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