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Myxomycete plasmodial biology: a review

Clark J[1] and Haskins EF[2]

[1] *Department of Biology, University of Kentucky, Lexington, Kentucky 40506*
[2] *Department of Biology, University of Washington, Seattle, Washington 98195*

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Abstract

The most characteristic stage of a myxomycete is the assimilative plasmodium, a naked free-living multinucleate motile mass of protoplasm which varies in size and morphological details with species. The plasmodium is formed from the amoeboflagellate stage by either of two methods which can be found within a particular species: by fusion of two haploid cells carrying different mating types to form a zygote, or by conversion of an apogamic diploid cell directly into a plasmodium. The plasmodium, which is generally covered by a slime sheath, is multinucleate and wall-less, and is therefore capable of movement which occurs by means of differential protoplasmic streaming. The larger plasmodial types (aphanoplasmodia and phaneroplasmodia) routinely form a reticulate structure where different regions of the plasmodia undergo a continuous cycle of separation and coalescence; therefore they also have a complex genetic system that prevents the fusion of genetically unrelated plasmodia. These plasmodia engulf bacteria, yeast and other organic matter, which they surround and digest in food vacuoles. Under adverse conditions (cold, drying) the plasmodium can form a resistant sclerotium which can revive and continue growth when conditions improve. However, the end point purpose of the plasmodium is sporulation with the production of spores and their germination to produce the alternate amoeboflagellate stage; which is generally triggered by the mature plasmodium undergoing starvation in the presence of light.

Key words – aphanoplasmodium – coalescence – phaneroplasmodium – protoplasmodium, sclerotium – senescence – sporulation – syngamy

Introduction

The two assimilative stages of the myxomycetes are the plasmodium and the amoeboflagellate; with the plasmodium being the larger multinucleate more conspicuous stage which, during sporulation, produces many small spores that germinate into the small uninucleate amoeboflagellates. The plasmodium is a wall-less (generally slime covered) free-living multinucleate, motile mass of protoplasm which varies in size and morphological detail with age and species. These plasmodia, some of which are occasionally seen in the field, vary from large (several centimeters across) colorful gelatinous masses to minute colorless blobs. They feed on bacteria, fungi, and any other organic matter that is encountered which they can engulf. Plasmodia usually inhabit environments, such as soil, dead wood, and various plant litter and debris, which are moist and support bacteria and other decay microorganisms (Stephenson & Landolt 1996). Some

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Corresponding Author: Jim Clark – e-mail – jimc1939@frontier.com

species, such as *Physarum polycephalum*, seem to prefer to grow on or just under the surface of the substrate; however, the great majority of species probably spend most or all of their active life within the substrate. Under the influence of desiccation, low temperature and other unfavorable conditions, the plasmodium can be converted into a resting stage sclerotium, which reverts to an active plasmodium when conditions again become favorable.

Plasmodial Formation

Plasmodial formation concerns the mechanisms that produce the plasmodium and the limitations imposed on these mechanisms by the environment.

Mechanisms

Plasmodia are produced by the amoeboflagellate stage either by the formation of a diploid zygotic cell by means of sexual fusion, or by the conversion of an apogamic diploid amoeboflagellate into a plasmodium (see reviews by Clark & Haskins 2010, 2013). In the sexual cycle amoeboflagellates differing at a multiple allelic mating system (Collins 1963) undergo syngamy (Bailey et al. 1990) after they have become competent upon reaching a critical cell density (Shipley & Holt 1982); while in the apogamic cycle, the amoeboflagellates are diploid, due to automixis (see Clark & Haskins 2013), and convert to plasmodia when they become competent. This zygotic or diploid amoeboflagellate cell apparently then undergo a number of biochemical changes in its cell membrane (Ross & Shipley 1973) and a series of mitotic nuclear divisions without cell division to produce the multinucleate plasmodium.

Environment

While any condition that prevents amoeboflagellates from achieving competence will prevent plasmodial development, the requirements for plasmodia formation are apparently more stringent than for growth, since plasmodia will often not be produced by vigorously growing amoeboflagellates (Gray & Alexopoulos 1968). The temperature limits for plasmodial formation have been determined for a number of species: 7-30˚ C for *Fuligo septica* (Constantineanu (1907), 14-30˚ for *Physarum didermoides* (Constantineanu (1907), 5-30˚ C for *Didymium squamulosum* (Constantineanu (1907), 10-25˚ C for *Physarum nicaraguense* (Solis 1962), and 12-28˚ C for *Didymium iridis* (Clark unpublished). Recent research on the nivicolous (snow bank) species *Lepidoderma chailletii* and *Physarum nivale* indicate that plasmodia can only form at temperatures of 0-2˚ C in the presence of a water film (Shchepin et al. 2014). The presence of free water also appears to be required for plasmodial formation in some species of the Stemonitales (Alexopoulos 1960, Wollman & Alexopoulos 1964), although it is apparently an enhancer in other species that are capable of plasmodial formation on a moist substrate such as agar (Indira & Kalyanasundaram (1963). The pH of the substrate is apparently a key factor that affects plasmodial formation, which has been examined in some detail in a few species. Collins and Tang (1973) found that *Physarum polycephalum* had an optimum pH 5-6 range for plasmodial formation, and Shinnick et al. (1978) discovered a gene that affected this pH range with two alleles producing upper limits of 5.6 and 6. A pH optimum of 6.2 for plasmodial formation in *Didymium iridis* (as *D. nigripes*) (Kerr 1961) and 5.5 for *Licea alexopouli* (Mock & Kowalski 1976) has also been reported. While some progress has been made concerning environmental factors which affect plasmodial formation, it is obvious that, to date, we lack a comprehensive understanding of this important process. However, an examination of well documented field studies which record substrates, seasonality, and extremes (snow banks, deserts, etc.), could offer valuable clues for directed culture studies on environmental factors affecting plasmodial formation.

Plasmodial Types

Considerable variations in plasmodial morphology were recognized by deBary (1887) in his early studies of myxomycete life cycles; however, most early plasmodial investigators (Cienkowski 1863, Strasburger 1884, Howard 1931) generally confined their studies to the rather large easily

grown reticulated pigmented plasmodia in the Physarales. However, there were reports of non-conforming plasmodia by various authors: a transparent reticulate plasmodium in a number of *Stemonitis* species (Čelakovský 1893, Miller 1898), and a small amoeboid plasmodium in *Licea parasitica* (Zukal 1893). While both Watanabe (1932) and Nauss (1947) had expressed the view that not all plasmodia were alike, the description of distinct plasmodial types did not occur until Alexopoulos' 1960 publication where he defined the protoplasmodial, aphanoplasmodial and the phaneroplasmodial types.

Protoplasmodia

The protoplasmodium is characteristic of the Echinosteliales and possible of the Liceaceae, although the plasmodia found in a number of *Licea* species appear to be greatly reduced phaneroplasmodia. The uninucleate initial plasmodial cell undergoes nuclear division to form the characteristic mature protoplasmodium, which is a microscopic (20-200 micron diameter) multinucleate colorless amoeboid cell with many short pseudopodia on its periphery and a heavy slime sheath (Alexopoulos 1960, Haskins & Hinchee 1974). It migrates very slowly over the substrate exhibiting slow irregular cytoplasmic streaming, and does not produce an advancing fan-shaped region, although it can display an anterior/posterior orientation. Unlike the other two plasmodial types it does not produce a reticulated plasmodium or fuse with either itself or any other plasmodium (Haskins 1978). Upon reaching an upper size limit the protoplasmodium undergoes binary plasmotomy to produce two daughter plasmodia (Haskins 1978), and upon sporulation, each protoplasmodium produces a single sporangium: *Echinostelium minutum* (Peterson 1952, Alexopoulos 1960, Haskins 1971), *Clastoderma debaryanum* (McManus 1961b), *Barbeyella minutissima* (Jarocki 1939, Schnittler et al. 2000). These plasmodia may represent the ancestral form for all of the myxomycetes, and appear to be evolved for residence in ephemeral habitats on the surfaces of plants and plant debris.

Aphanoplasmodia

The aphanoplasmodium is characteristic of the Stemonitales and Ceratiomyxales, although DNA evidence indicates that it is unlikely that the plasmodia of the two orders are closely related (Fiore-Donno et al. 2009). The uninucleate initial plasmodial cell usually forms a short multinucleate strand that soon starts to branch and form a reticulum that grows into characteristic mature aphanoplasmodium (Alexopoulos 1960, Haskins 1974); which is a large multinucleate colorless reticulated structure consisting of numerous flattened tubular strands of various sizes (5 to 35 microns in diameter) which lack a slime sheath (Haskins & Hinchee 1974) and form a complex of large and small meshes with many free ends. The young aphanoplasmodium grows out slowly over the substrate and displays an irregular rapid to slow rhythmic reversible flow inside the strands which have a very thin ectoplasm. Since aphanoplasmodia must fuse with themselves to form the reticulum, they are also capable of coalescence with other genetically identical plasmodia (Haskins 1990). The aphanoplasmodium of *Stemonitis flavogenita* can reach a diameter of 15 centimeter or more, while *Comatricha laxa* rarely exceeds 20 millimeters; although they do not seem to have an obvious upper size limit like the protoplasmodium, their sizes appears to be controlled by a mix of genetic and environmental factors and accidental fragmentation of the larger units. The *Stemonitis* aphanoplasmodia produce a whitish to yellowish pigmented heaped coralloid stage, which produces a heavy slime sheath prior to sporulation, which is highly mobile and usually produces a clump of sporangia (Haskins 1974, 1981). On the other hand the *Comatricha* plasmodia generally produce one to several individual sporangia from whitish mobile pre-sporangial masses. However, the aphanoplasmodium of *Ceratiomyxa fructiculosa* which is very similar to *Stemonitis flavogenita* in morphology, development and size, forms whitish masses of protoplasm that do not undergo extensive migration prior to sporophore formation (Clark et al. 2004, Clark unpublished). The thin reticulate strands and growth in free water; indicate that this plasmodial type probably originated as an adaptation to growth within the cells and connecting pits of dead wet woody materials, where

the slime sheath would not be needed until it migrated to drier conditions outside the wood where it could sporulate and disperse its spores.

Phaneroplasmodium

The phaneroplasmodium is characteristic of the Physarales, Trichiales, and most if not all of the Liceales. Phaneroplasmodia are the most common and the most variable of the plasmodial types; not only varying in size from the tiny protoplasmodium-like plasmodia found in the Liceaceae up to the large (30 centimeters) plasmodium of *Fuligo septica*, but also including the Trichiales plasmodia, which are considered to be a separate type intermediate with the aphanoplasmodia by some (Indira & Kalyanasundaram 1963, McManus 1962, Ross 1967). The plasmodia of *Licea* species are small, sluggish lumpy amoeboid masses that typical produce a single sporocarp, and are therefore classified as protoplasmodial by many researchers; however, the plasmodia are usually pigmented and in some species such as *Licea biforis*, the can be larger branching structures (resembling the early stage of a typical phaneroplasmodium) and produce more than one sporocarp (McManus 1964, Wollman & Alexopoulos 1967, Clark et al. 2004). Thus, it seems likely that these small plasmodia are examples of ancestral or reduced (due to living in an ephemeral habitat) phaneroplasmodia. The phaneroplasmodia of the Physarales are typified by the well known plasmodia of *Physarum polycephalum* and *Didymium iridis*. These plasmodia develop from the small uninucleate initial cell by nuclear division into a small protoplasmodial-like structure, before they form an elongate strand which displays a rhythmic reversal streaming, which soon develops a reticulated fan like region (Alexopoulos 1960). The mature phaneroplasmodium has an anterior fan-shaped sheet of pigmented granular protoplasm, within which channels of streaming protoplasm originate. These channels are orientated from front to back, with shorter connecting channels at right angles and or diagonally to the main channels, with the anterior channels merging into the plasmodium's posterior reticulum of relatively thick strands (Alexopoulos 1960). They also have a noticeable ectoplasm exterior to the streaming center. The phaneroplasmodium observed in the Trichiales is usually a less robust form with a thinner less pigmentation protoplasm than those found in the Physarales. In general it appears that the phaneroplasmodium is adapted to growth in a drier, somewhat exposed habitat, such as on and in the surfaces and structures of plant debris.

Plasmodial Structure

While plasmodial structure has been intensely studied in the phaneroplasmodia of a number of Physarales species, information of other types and species of myxomycetes, except *Echinostelium minutum*, is somewhat limited and sporadic; therefore much of this discussion is derived from the *Physarum* and *Echinostelium* studies.

Slime Sheath

The plasmodium of the myxomycetes lack any form of a cell wall, and are thus their only protection from injury or desiccation is the slime sheath present in the protoplasmodial and phaneroplasmodial forms; however, the aphanoplasmodium also produces a slime sheath when it is briefly exposed during sporulation (Haskins 1981). The sheath is a relatively thin, flexible coating outside of the cell membrane which is produced by the growing and moving plasmodium and is left behind as a collapsed tube (slime track) as the plasmodium migrates over the surface of the substrate. Electron microscopic studies (Rhea 1966, Haskins & Hinchee 1974) of the slime sheath indicate that it is composed of filaments that appear to be attached to the plasma membrane, which could allow it to serve as a sort of flexible exoskeleton. Reports on the biochemical nature of this slime has been inconsistent; with Simon & Henney (1970) reporting a glycoprotein whose carbohydrate was galactose, and McCormick et al. (1970) characterizing the slime as a polysaccharide consisting of galactose, sulfate, and traces of rhamnose.

Protoplasm

The plasmodium has the cell organelles found in the normal eukaryotic cell: plasma

membrane, nuclei, mitochondria, food vacuoles, contractile vacuoles, endoplasmic reticulum, ribosomes, and golgi; as well as pigment granules in the pigmented types. These organelles are typical in their structure and function except for their very small size. The mitochondria have the typical protozoan tubular cristae (Dugas & Bath 1962) and are quite small (0.5 micron) and are thus difficult to identify with a light microscope. Since the plasmodium feeds by engulfing bacteria and other organic material and organisms, small food vacuoles with partially digested bacteria and other debris are common in the active plasmodium.

Pigment Granules, Pigments and Lipids

The pigment granules in the phaneroplasmodia are non-standard protoplasmic structures, which account for the range of colors (white, yellows, and reds ranging to near black) found in these plasmodia. These granules are amorphous spheroids surrounded by a membrane (Lieth & Meyer 1957) and apparently the pigments are localized in these granular structures. The chemical nature of these pigments is not well known, although tetrameric acid derivates (Casser et al. 1987) and carotenoids (Czeczuga 1980) have been reported. While plasmodial color has been reported to be changed by substrate pH (Seifriz & Zetzmann 1935) and ingested bacteria (Kambly 1939), it is, in general, a constant characteristic, although stable color variants within a species do occur which can affect the color of the sporophore (especially stalk color). Alexopoulos (1964) described a white plasmodial form of *Physarella oblonga* which produced a lighter colored stalk than the normal yellow plasmodial form; Anderson (1977) isolated a white mutant of the normally yellow plasmodium of *Physarum polycephalum*, and Collins and Clark (1966) found that a genetically determined cream colored plasmodium in *Didymium iridis* produced lighter colored stalks than the brown pigmented plasmodium.

The plasmodium also contain high levels of lipids, and recent studies indicate that they could be a possible source for the production of biodiesel fuels, since *Physarum polycephalum* can be grown on a low-cost carbon source and the lipids can be easily extracted due to the lack of a cell wall (Tran et al. 2015).

Nuclei and Nuclear Division

The multinucleate condition is the unique and defining feature of a plasmodium. The nuclei in the myxomycete plasmodia, while very small (less than 6 microns in diameter), have a standard eukaryotic structure with a double nuclear membrane, nucleolus, and chromosomes (Aldrich 1966). While early workers considered plasmodial nuclear division to be amitotic (Lister 1893) and sporadic (Schûnemann 1930), later researchers reported synchronous divisions in *Physarum polycephalum* (Howard 1932, Koevenig & Jackson 1966), *Didymium iridis* (Kerr 1976), and *Echinostelium minutum* (Hinchee & Haskins 1980), which were entirely intranuclear and lacked centrioles and asters. When Guttes et al. (1959) reported that *Physarum polycephalum* retained its precise periodic synchronous mitotic division when grown on defined media; a large number of biochemical studies on DNA and RNA synthesis during the different division stages became possible. Readers interested in these biochemical studies should consult Aldrich & Daniel (1982) for an entry into this area of research.

Physiology

Protoplasmic Streaming and Locomotion

Protoplasmic streaming is a universal aspect of myxomycete plasmodial activity, which varies from the sluggish somewhat random movements in the protoplasmodia to an irregular slow shuttle streaming in the aphanoplasmodia to the very rapid shuttle streaming in the channels or veins of the phaneroplasmodia. This streaming serves two purposes: it is a mechanism to move and homogenize the materials and nutritional substances throughout the plasmodium, and is the basic of plasmodial locomotion. This shuttle streaming can be extremely rapid (1,350 microns/second) and moves large amounts of material (Kamiya 1950b), and while streaming may occur in a stationary

plasmodium, it is required for locomotion. Time-lapse sequences of moving phaneroplasmodia and aphanoplasmodia (Haskins & Kerr 1976) show a rhythmic pulsation associated with locomotion, which is apparently connected to the shuttle streaming. This streaming generally has a longer duration in the direction of movement causing a bulging out of the anterior fan, followed by a shorter reverse flow (Vouk 1910), giving a two step forward, and one step black movement seen in the pulsations. However, the duration of streaming in one direction is only an indication of bulk flow, since Kamiya (1950a) found that in some cases a short duration flow in the forward direction could still move larger amounts of protoplasm. The flow in any one direction displays a considerable variation and range from one half to 30 minutes (Steward & Steward 1959) in duration. The apparent motive force for protoplasmic streaming is an ATP fueled (Kamiya et al. 1957) actin-myosin contractile system of fibers (Loewy 1950, Hatano & Oosawa 1964), which can be visualized in the region where contraction takes place (Nagai & Kamiya 1966). This localized contraction apparently produces a localized hydraulic pressure that drives the protoplasmic streaming. Kamiya & Takata (1967) found that the ectoplasm to endoplasm ratio, in different regions of the plasmodium, tended to be balanced by protoplasmic streaming and suggested that this ratio might control the state of actin-myosin fiber activity.

Nutrition

Myxomycete plasmodia are vegetative stages which undergo growth and differentiate; thus they require energy and organic substrates in order to function. The plasmodium seeks out and takes up organic materials from its environment by means of locomotion and engulfment. When the plasmodial membrane meets a food particle it ceases to advance in that portion and the adjacent regions continue to move and form a pocket around the particle that is eventually closed off to form a food vacuole (Camp 1937b). After the nutrients have been extracted, the food vacuole is egested by a reverse process of the ingestion. While any suitable sized organic material seems to be ingested, the major source of plasmodial nutrition appears to be bacteria. While over 74 species of myxomycetes have been cultivated from spore to spore on agar culture (see Collins 1979), almost all of the species have been grown with a live bacteria or yeast food organisms. Although most of these species could be grown in monoxenic culture (a single live food organism), only a few of the species have been cultured axenically (without a live food organism). Generally the plasmodia are grown on water agar with sterilized oat flakes and a microorganism; isolated from the original culture or a common bacterium addition such as *Echerichia coli* as a food source (Haskins &Wrigley de Basanta 2008). The recent finding that plasmodia of some nivicolous species grow only at 0-2˚ C may, in part, be due to a preference for cold adapted microorganism as a food source (Shchepin et al. 2014). However, only twenty of these species have been grown without a live bacterial or yeast food source and of these twenty only ten have been grown on a semi-defined medium containing yeast extract, peptone, glucose and minerals (see Hu & Clark 1986). These ten species also included the four species which have been grown on a defined minimal medium: *Physarum flavicomum* (Henney & Lynch 1969), *P. polycephalum* (Daniel at al. 1963), *P. rigidum* (Henney & Lynch 1969), and *Stemonitis flavogenita* (Clark et al. 1990). These defined minimal media were relatively simple and similar in all four cases; consisting of minerals, several vitamins, amino acids, glucoses and hematin. The growth of unrelated *Stemonitis* and *Physarum* spp. on similar simple minimal media suggests that at least some myxomycete plasmodia have simple basic nutritional requirements. On the other hand, most myxomycetes species have not been cultured even in crude xenic cultures. These conflicting observations are difficult to reconcile, but it may turn out that specific growth parameters such as pH and the presence or absence of particular compounds, such as hematin, are more important for most myxomycetes than the underlying basic nutritional requirements.

Plasmodial Coalescence

Since myxomycete plasmodia do not have a cell wall and the phaneroplasmodial and aphanoplasmodia types routinely undergo fusion during formation of the reticulum, the

maintenance of separate genetic individuals is apparently insured by genetic barriers that prevent coalescence (see review by Clark & Haskins 2012). This system consists of a three tiered polygenic complex with dominant and recessive alleles (Ling & Clark 1988) Thus, plasmodia must be phenotypically identical for approximately 16 loci in order to fuse (CC and Cc are phenotypically identical, but different from cc). The first level of the system (having a minimum of seven Fus loci) controls membrane fusion, and it apparently prevents fusion unless the two plasmodia have identical membrane or slime sheath components. The second level (having a minimum of six Cz loci) produces a rapid lysis of a small mixed region, of the two plasmodia, if membrane fusion has occurred. This lysis is directional in that it targets the recessive phenotype, and it is apparently triggered by some pre-formed substances when they come into contact with a different plasmodium (Clark & Collins 1972). The third level (having a minimum of three Let loci) comes into play if membrane fusion occurs and there is no rapid lysis of the mixed plasmodium; it produces a slow lethal reaction, which targets and degrades the nuclei of the recessive phenotype (Carlile 1976). This reaction occurs over a period of five to twenty hours and requires the synthesis of new RNA and proteins (Schrauwen 1981). Since, this complex system can produce a minimum of 65,536 different incompatibility phenotypes; it is highly unlikely that any two phaneroplasmodia will undergo a successful fusion unless they are very closely related. Species with aphanoplasmodia apparently have a similar system (Haskins 1990) but species with small protoplasmodia do not appear to undergo any type of plasmodial fusion (Haskins 1978). While this complex incompatibility system usually produces a situation in nature where most of the plasmodia in a region have diverse incompatibility phenotypes (ElHage et al. 2000, Irawan et al. 2000), in at least one case a single phenotype has been shown to occur broadly over several square kilometers and to have persisted for several years (Stephenson et al. 2004).

Senescence

The plasmodia of most strains of *Physarum polycephalum* (Poulter 1969) and *Didymium iridis* (Lott & Clark 1980) when maintained by routine serial subculture on agar will undergo a senescence phase and die. The lifespan, from syngamy to necrosis is controlled by the genome of the individual plasmodium and is minimally affected by most environmental factors excepting temperature (Clark & Lott 1981); with a decreasing lifespan with increasing temperatures. Most plasmodia that are isolated from nature display plasmodial senescence (Clark 1984), although some are apparently immortal, and others develop immortality (Hu et al. 1985) or become senescence (Kerr & Waxlaw 1968) when cultured on agar. However, when *Physarum polycephalum* is grown in axenic shake culture it does not display an obvious senescence phase (Poulter 1969, Hu et al. 1985); but when transferred to non-axenic agar culture the plasmodium will undergo senescence and die within a short period of time; which is reversely correlated with the length of time that the plasmodium was maintained in axenic shake culture (Hu et al. 1985). On the other hand, when transferred to axenic agar culture, the senescence phase does not result in death since the plasmodium recovers and undergoes a new growth phase; followed by a recurrent series of short senescence and growth phases while maintained on agar (Hu et al. 1985). During the senescence phase there is a slow loss of function, with plasmodial fragmentation, pigment loss, accumulation of polyploidy nuclei (McCullough et al. 1973, Clark & Mulleavy 1982), and degeneration of mitochondria (Hu et al. 1985, Abe et al. 2000). A possible explanation of the axenic shake culture immortality and recurrent senescence on axenic agar is that senescence is due to degeneration of mitochondrial DNA; with fragmentation of the plasmodium and selection for micro-plasmodia having functional mitochondrial DNA allowing continued growth. In shake culture this fragmentation occurs continuously, and on agar culture it occurs during senescence. However, in non-axenic agar culture, the extremely small fragments apparently do not normally survive the rigors of the mixed organism culture. This possibility is supported by age heterokaryon studies, which are made possible by the vegetative amoeboflagellate stage which also serves as gametes (Collins 1963). A single isolated amoeboflagellate will grow and produce a clonal population that can be maintained indefinitely in culture; therefore two clones can be crossed at different times

(thus being of different ages) to produce isogenic plasmodia that can fuse to form an age heterokaryon (a single plasmodium with nuclei and organelles of different ages). These age heterokaryons, in almost all cases, die concurrently with the oldest plasmodial control (Clark & Hakim 1980), even when the heterokaryon was formed with a much smaller segment of the older plasmodium (Clark & Lott 1989). Thus, the older plasmodial portion apparently controls the lifespan of the age heterokaryon, possibly by means of an infectious mitochondrial degeneration. A study (Nakagawa et al. 1993) of the mitochondrial DNA of an immortal strain of *Physarum polycephalum*, found that it contained several rearrangements associated with a plasmid integration, and that a sub-strain which displayed senescence on agar had a deletion of one of these rearrangements; thus adding support to the mitochondrial theory of aging in the myxomycetes. While mitochondrial degeneration may be the immediate cause of senescence, the evidence for nuclear involvement (McCullough et al. 1973, Clark & Hakim 1980, Lott & Clark 1980) is also strong; however, these results need not be in conflict, since nuclear genes could be involved in the control and modulation of the mitochondrial degeneration system.

Sclerotium

The sclerotium is a resistant structure formed by the plasmodium when it is subjected to adverse environmental conditions. This dormant stage is not required to complete the life cycle, but may be important under certain circumstances, where growth is interrupted due to short or long term unfavorable conditions. This characteristic may be especially valuable to species that inhabit extreme environments such as deserts, snow banks or the bark of trees, where rapid swings in temperature and moisture may occur. Conditions which have been reported to induce the sclerotial state are temperatures below the growth minimum (deBary 1887, Macbride 1922, Jump 1954), slow desiccation (Macbride 1922, Luyet & Gehenio 1944, Jump 1954), certain heavy (Fe, Cu, Zn) metals (Jump 1954), low pH (Jump 1954), and starvation under conditions that prevent sporulation (Daniels & Rusch 1961). Scerotia, under laboratory conditions, may retain their viability for several years (Gehenio 1944, Lonert 1965); however, eight to sixteen months is a more general average for most longevity studies (deBary 1887, Gehenio 1944, Jump 1954). The basic structure of the scerotium is the spherule; a round to ovoid cell of variable size (up to 25 microns in diameter) with an amorphous fibrous wall (Aldrich & Blackwell 1976) and a variable (0 to 14) number of nuclei (Jump 1954). The overall morphology of the sclerotium can be quite variable; however, phaneroplasmodia generally produce a compact mass of spherules with a horny consistency, although a looser more irregular grouping may occur in the same species (Nauss 1943) and aphanoplasmodia generally produce loose strings, clusters or individual groupings of spherules (Alexopoulos 1963, Wollman 1966, Haskins 1974). Each protoplasmodium of *Echinostelium minutum* forms a single cyst (spherule) with a wrinkled single layered fibrillar wall under adverse conditions (Haskins et al. 1985). Once, favorable growth conditions reoccur, these resistant structures rapidly (in 3 to 24 hours) excyst and return to normal plasmodial activities (Lonert 1965, Haskins et al. 1985).

Sporulation

The natural end point of the assimilative plasmodial stage of the myxomycetes is sporulation, which is a necessary step in the life cycle. During sporulation the entire plasmodium is converted to one or more sporophores, depending upon the species and type of plasmodium. However, subcultures of the same plasmodium keep under growth conditions do not sporulate; and thus sporulation must be induced by particular environmental factors. Numerous studies have been conducted to determine these factors, and although the results have often been contradictory and difficult to interpret, there is a general consensus that most plasmodia require an aging and or starvation period prior to a light trigger before they can sporulate. However, other conditions which affect the vigor of the plasmodium, such as pH and temperature extremes (Gray 1938, Collins 1959), can also inhibit sporulation.

Aging and or starvation

Klebs (1900) and Camp (1937a) appears to be the first researchers to conclude that starvation was a primary factor in plasmodial sporulation, since they could maintain the plasmodia of a number of species in the vegetative condition for long periods by means of serial sub-culturing. However, Gray (1938) who studied some of the same species, including *Physarum polycephalum*, concluded that while starvation enhanced and sped up sporulation, it was not necessary, since fed cultures would also sporulate. Ling (1968) also found that progressive starvation produced progressively better sporulation in *Didymium iridis*. On the other hand, Schure (1949) found that *Mucilago crustacean* never sporulated when a nutrient organism (baker's yeast) was present in the culture. The sporulation of *Physarum polycephalum* grown in defined medium was investigated by Daniel & Rusch (1962), who found that optimal sporulation required a mature plasmodium (just prior to nutrient exhaustion), four days of starvation in the dark on a minimum medium containing only salts and niacin or tryptophane, and a light trigger. They concluded that the niacin was somehow involved with sensitivity to the light trigger, and was probably not a metabolic precursor needed for sporulation. The present authors, who have cultured multiple isolates of many species of myxomycetes, have reached certain conclusions concerning aging and starvation from their long history of culturing myxomycetes. A few species or isolates, usually those from ephemeral habitats, sporulate after a short period of active growth even when abundant nutrients are present; since they are difficult to maintain in culture by serial sub-culture. Apparently, these isolates commit to sporulation after reaching a certain growth age. However, the plasmodia of most species and isolates can be kept in the vegetative stage for long periods by means of serial sub-cultures, and do not sporulate until they are starved. We believe that reports of fed plasmodia of these species undergoing sporulation, is due to culture problems which disrupt the growth and nutrition of the plasmodium. These culture problems could be the accumulation of staling products (continuous feeding in the same culture), pH changes, and the growth of adverse microorganisms; all of which could produce a pseudo starvation situation.

Light

It is sometimes suggested that plasmodia become phototrophic just prior to sporulation, and thus migrate to the surface of the substrate where optimal spore dispersal can occur. Whether this is true or now has not been investigated, and since most species sporulate at night (Miller 1898), as anyone who has studied sporulation can attest, it may be correct since the plasmodium usually moves to its sporulation position in the evening prior to sporulation. The requirement of a light trigger to induce sporulation was extensively investigated by Gray (1938). He conclude that plasmodia with colored pigment granules required a light trigger, that plasmodia with white pigment granules may require a light trigger and that unpigmented plasmodia did not require a light trigger; and that those plasmodia that did require a light trigger were stimulated to produce sporangia more quickly with higher light intensities (up to a certain maximum level of intensity). Vouk (1910) had earlier reported that unpigmented plasmodia could sporulate in either light or darkness, and this conclusion has been generally supported by other investigators except for McManus' (1961a) report that *Stemonitis fusca* required a light stimulus to sporulate. The possibility (Straub 1954, Scholes 1962) that some white pigmented plasmodia (and some colored pigmented) did not required a light trigger was quieted by Ling's (1968) finding that in *Didymium iridis* the minimum white light trigger could be as low as five seconds at 30 foot-candles of intensity. Investigations on the action spectrum of sporulation (Gray 1939, Straub 1954, Rakoczy 1967) indicated that blue light was the most stimulatory and green light was inhibitory to sporulation; and while some of the yellow plasmodial pigments have a similar absorption spectrum (Lieth 1954), no one, to date, has been able to positively identify them as the photoreceptor pigment.

Discussion

Multinucleate amoeboid trophic stages (plasmodia) are relatively common in the Amoebozoa super-group to which the Myxomycetes belong (Adl et al. 2012); however, outside of the myxomycetes, they are most common in the protostelid groups (produce stipitate sporophores) which are scattered throughout the super-group, with plasmodia frequently occurring in the Cavosteliida, Protosporangiida (Ceratiomyxales and *Protosporangium*), and Schizoplasmodiida (Spiegel et al. 1995). Some of these plasmodia are reticulate and are capable of fragmentation and possibly they may able to fuse (Spiegel personnel communication). Their life cycles may also include an amoeboflagellate stage, and there are indications of a possible sexual cycle in some species (Adl et al. 2012), besides that found in *Ceratiomyxa fructiculosa*. Thus, the plasmodial state is either an ancestral morphology that has been lost in many taxa, or it is a morphology that has been evolved separately in many different taxa.

The plasmodium is often associated with the production of spores, which generally provides both an efficient means of dispersal and a resistant structure that can withstand temporary adverse conditions. This relationship may be due to the ability of the plasmodium to devote resources to the production of a complex sporophore; that provides access to spore dispersing turbulent air conditions. While some sporophore producing protostelids have uninucleate trophic stages, they are greatly restricted in size and form since the spore requires the great majority of the resources present in the amoeba. Plasmodial protostelids, on the other hand, may produce multinucleate single spores, multispored sporocarps, or a number of individual single spored units (Adl et al. 2012). The only other mechanism for sporophore production available to amoebae is the aggregation and differentiation model of the dictyostelids. Thus, plasmodial size, made possible by the multinucleate state, has apparently allowed the myxomycetes to evolve their large elaborate fruiting structures containing large numbers of spores; which provides the group with an efficient dispersal mechanism not present in most other Amoebozoans. This size advantage may also provide a competitive advantage in terms of habitat and nutritional competition (Haskins 1990). The ability to form a large trophic stage, and to rapidly increase its mass by fusion of genetically identical young plasmodia, can quickly produce a large mobile efficient food gathering structure, that could not only outcompete smaller organisms, but could also ingest many of them. On a final note, Haskins plasmodial film cited in the references can now be viewed on-line by going to AV-Portal: TIB, then opening up the portal up and search for C1220 to view the specific film, or Haskins E F to view his series of films on myxomycetes.

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