Life history strategies of corticolous myxomycetes: the life cycle, plasmodial types, fruiting bodies, and taxonomic orders

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The myxomycete life cycle is reviewed and evaluated based on historic and current evidence, and completely illustrated in detail, including trophic stages (myxamoebae, swarm cells, and plasmodia), resting or dormant stages (spores, microcysts, and sclerotia), and developing fruiting bodies. Most books and journal papers fail to include all life cycle stages or accurately illustrate the morphology and mating of swarm cells. Life history strategies of the corticolous myxomycetes, as related to type of plasmodium and fruiting body, are described and quantified based on the five taxonomic orders. The bark of 30 individual trees, representing six different species, and 30 grapevines of two species, was sampled from the canopy at 3.3 m to 16.5 m in the Berea College Forest and Daniel Boone National Forest in Kentucky and in the Great Smoky Mountains National Park in Tennessee, using the double-rope climbing technique. Moist chamber cultures of bark samples (580) yielded a total of 46 myxomycete species, representing 20 genera, with 4 taxa identified only to genus. Results showed that each taxonomic order is well represented and the majority of corticolous myxomycetes have stalked fruiting bodies and the proto- or trichiaceous plasmodial type. This evidence supports observations that corticolous myxomycetes in the tree canopy are r-selected species, adapted to irregular wet and prolonged dry cycles on the bark surface of living trees and vines by utilizing the resistant, dormant, resting stages of the life cycle. The most abundant corticolous species utilize the plasmodial type with the smallest surface to volume ratio (protoplasmodium), sporulate quickly within 2 to 4 days by producing a single, tiny, stalked sporangium, and efficiently release spores via an evanescent peridium. Species in the order *Echinosteliales* are the best examples of this life history strategy.

Key Words: corticolous myxomycetes, fruiting body, life cycle, plasmodia, r-selected, slime moulds, tree canopy

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Introduction

Myxomycetes (acellular, non-cellular, plasmodial, or true slime moulds) are characterized by an amorphous, multinucleate, protoplasmic mass called the plasmodium, and fruiting bodies (1-200 mm) with internally-borne spores (5-20 µm). They have been known for more than 350 years based on Pankow's figure and description of *Lycogala epidendrum* (L.) Fr. in 1654 (Martin and Alexopoulos, 1969). Heinrich Anton de Bary, often referred to as the father of modern mycology, first demonstrated (Martin, 1958) myxomycete spore germination, and the subsequent stages (myxamoebae, swarm cells, plasmodia) along with details of fruiting body formation (aethalia, sporangia, plasmodiocarps). Martin (1958) further states: "This general sequence has never since been open to question". However, Martin (1958) also noted that de Bary described spore germination as "…giving rise to one or sometimes two swarm-cells, that these divide repeatedly and finally fuse, forming amoebae first, then gradually enlarging into small amoeboid bodies which are essentially small plasmodia". De Bary's observations provided the evidence to remove the Myxomycetes from the Gasteromycetes, then considered fungi, and coined the name Mycetozoa instead of the older name Myxomycetes. The discovery of these micro-

scopic, animal-like stages influenced his publication in a zoological journal and alignment with animals (Martin, 1958).

Myxomycetes have been classified in the Kingdom Plantae (Class Myxomycota) and the Kingdom Animalia (Class Mycetozoa) (Martin and Alexopoulos, 1969), and because myxomycetes are typically found in the same habitats as fungi, they were treated as taxa within the Kingdom Fungi (Class Myxomycetes). Unlike fungi, myxomycetes do not excrete extracellular, digestive enzymes and the role of myxomycetes in the environment is not as decomposers or pathogens (Keller and Braun, 1999). Bauldauf and Doolittle (1997) conducted a phylogenetic analysis of highly conserved, elongation factor 1-alpha (EF-1α) gene sequences and showed that myxomycetes are not fungi. Physiology, morphology, life history, and genetic analysis support classification of myxomycetes in Kingdom Protoctista along with other eukaryotic microorganisms (Spiegel *et al*., 2004). Therefore, the similarity in appearance of slime moulds and fungi is an example of convergent evolution, due to selective pressure on fruiting body structure for effective dissemination of spores (Olive, 1975; Kaiser, 1993). More recently, taxonomic puzzles such as the myxomycete genus *Schenella*, considered a myxomycete for approximately 100 years, failed to produce any of the life cycle stages of a myxomycete, and molecular DNA sequencing techniques demonstrated this taxon to be a gasteromycete, *Schenella simplex* T. Macbr.=*Pyrenogaster atrogleba* (Zeller) L.S. Domínguez & Castellano (Estrada *et al.*, 2005).

Myxomycetes occur throughout the world, with highest diversity documented in temperate forests (Spiegel *et al.*, 2004). Within temperate forests, some myxomycete species inhabit the forest floor only on rotting logs, other species are found on decaying leaf litter, and still others on the bark of living trees and vines and are called corticolous myxomycetes. Corticolous myxomycetes complete the entire life cycle, from spore to fruiting body formation, only on the bark of living trees and vines. For example, *Diachea arboricola* H.W. Keller & M. Skrabal has only been found on the bark of living trees above three meters and is considered a true corticolous myxomycete (Keller *et al.*, 2004). However, some ground species such as *Fuligo septica* (L.) F.H. Wiggers and *Lycogala flavofuscum* (Ehrenb.) Rostaf., have been found on the bark of living trees up to two meters high (Keller and Braun, 1999). For this reason, the tree canopy is here defined as three meters and above, in order to exclude opportunistic ground species and emphasize true corticolous myxomycetes.

More studies have been conducted on ground species than on corticolous myxomycetes in the tree canopy (Snell and Keller, 2003). In comparison, this study on the species assemblages and life history characteristics of the tree canopy corticolous myxomycetes suggests different aspects of the life cycle allow them to persist and survive in the arid bark microhabitat of living trees. The purpose of this study is to highlight details of the myxomycete life cycle, examine life history strategies of the tree canopy corticolous myxomycete, quantify plasmodial and fruiting body types of the taxonomic orders, and discuss the impact of these factors on the life history strategy of the corticolous myxomycetes.

The Life Cycle

Myxomycetes produce spores in fruiting bodies as shown in Fig. 1, N, A. Spore germination will produce one to four haploid, unicellular myxamoebae or swarm cells, together termed the amoeboflagellate, that lack a cell wall and are surrounded only by the plasmamembrane (Gray and Alexopoulos, 1968). The myxamoebae or swarm cells emerge from the spore through a pore or through a fracture of the spore that, in some cases, breaks it into halves (Fig. 1, B) (Keller, 1970; Keller and Schoknecht, 1989a). The myxamoeba is an amorphous cell that divides via mitosis (Fig. 1, C, E.). The swarm cell is comma-shaped, does not divide via mitosis, typically has two, whiplash flagella of unequal length at the anterior end, and fuses (syngamy) only at the posterior end (Fig. D, H). Two myxamoebae or two swarm cells of genetically compatible mating types, heterothallic or homothallic (non-heterothallic), fuse as sexual gametes to form the diploid zygote (Fig. 1, G, H, I) (Clark, 2000). The diploid zygote will begin feeding which initiates synchronous mitosis without cytokinesis to form an assimilative multi-,

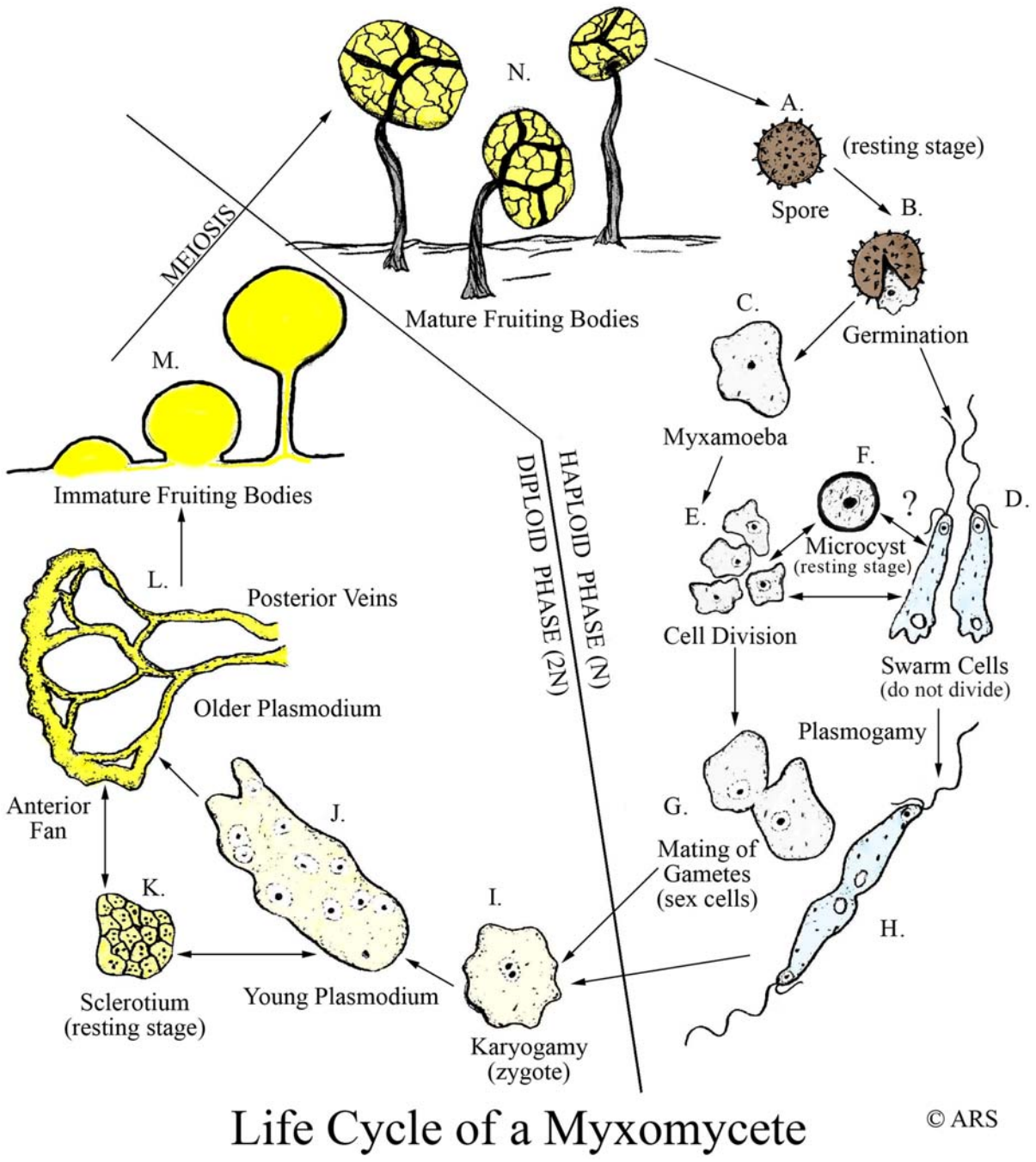

Fig. 1. Life cycle of a myxomycete. Illustration by Angela R. Scarborough.

nucleate, protoplasmic mass. This amorphous mass is called the plasmodium which is the source of the common name "plasmodial slime moulds" (Fig. 1, J, L). The plasmodium varies in size, shape, color, and morphological characteristics (Gray and Alexopolous, 1968).

The myxomycete life cycle has been well studied in the past, but the swarm cell stage lacks accurate morphological and developmental details and in some cases a complete life cycle with all of the resting or dormant stages is not illustrated (Frederick, 1990; Stephenson *et al.*, 1993; Stephenson and Stempen, 1994; Alexopoulos *et al.*, 1996; Keller and Braun, 1999; Novozhilov *et al.*, 2000; Fiore-Donno *et al.*, 2005).

Many flagellate or ciliate organisms divide by cell (binary) division, for example,

the Diplomonads, Euglenas, Foraminiferans, Ciliates, and Heliozoans, among others; but careful myxomycete life cycle observations using time lapse photographic techniques and direct microscopic observations in hanging drop slides or on agar cultures fail to show swarm cell divisions (Koevenig, 1964; Gray and Alexopoulos, 1968; Keller and Schoknecht, 1989a). The myxomycetes are unusual in that the biflagellate stage does not divide by cell (binary) division (Fig. 1, D). This may in part be a question of semantics, because the biflagellate swarm cell undergoes the process of transformation into the myxamoebal stage (flagella are resorbed into the protoplast) then mitosis and cell division occurs. Life cycle diagrams that show the flagellate stage undergoing cell (binary) division, or references in text or figure legends to binary division, perpetuate a longstanding error (Stephenson and Stempen, 1994; Fiore-Donno *et al.*, 2005). In addition, myxomycete swarm cells mate or fuse by their posterior ends, not side by side as in some life cycle illustrations (Stephenson *et al.,* 1993; Stephenson and Stempen, 1994; Fiore-Donno *et al.*, 2005).

Photographic evidence has not shown convincingly that microcysts (the encysted resting, resistant, or dormant stage) form directly from swarm cells, as is clearly the case in their formation from myxamoebae. The detailed developmental study of swarm cells by Koevenig (1964) and Keller and Schoknecht (1989a) showed that culture conditions can cause the swarm cells to round up with an apparent wall, suggesting that encystment (microcyst) occurs before formation of the myxamoebal stage (Fig. 1, F). The inter-conversion between mxyamoebae and swarm cells (the amoeboflagellate stage) is well documented by direct observations and life cycle illustrations (Gray and Alexopoulos, 1968; Frederick, 1990; Stephenson *et al.*, 1993; Stephenson and Stempen, 1994; Alexopoulos *et al.*, 1996, Keller and Braun, 1999; Novozhilov *et al*., 2000; Fiore-Donno *et al.*, 2005; Fig. 1, E, D). The life cycle as shown here includes the three resting or dormant stages (spores, microcysts, and sclerotia) and the swarm cells mating by their posterior ends, but not undergoing cell division and also possibly forming microcysts (Fig. 1, D, F).

Plasmodial types

There are three distinct morphological forms of plasmodia, the phaneroplasmodium, aphanoplasmodium, protoplasmodium, and an intermediate type called the trichiaceous plasmodium (Gray and Alexopolous, 1968). The phanero- and aphanoplasmodia are visible with the unaided eye. The phaneroplasmodium typically has a fan-like plasmodium that exhibits polarity and reversible protoplasmic streaming, and can form hundreds of fruiting bodies or one large aethalium up to 70 cm across (Keller and Braun, 1999). The phaneroplasmodium has a broad, anterior feeding edge, followed by a defined set of reticulate, posterior trailing veins (Fig. 1, L).

The aphanoplasmodium requires the presence of free water, forms a vein-like reticulate pattern that lacks distinct antero-posterior polarity, and exhibits erratic protoplasmic streaming. The characteristically thin and hyaline aphanoplasmodium is adapted to growing in the interstices of wood, flowing through the phloem and xylem (Gray and Alexopoulos, 1968).

The protoplasmodium is microscopically rounded or irregular-shaped, does not develop the vein-like network characteristic of the other plasmodial types, and will give rise to only a single stalked sporangium.

The trichiaceous plasmodium has characteristics of both the aphano- and phanero-plasmodium, requiring free water for development, having a generally appressed appearance and distinct antero-posterior polarity (Keller, 1971).

Under unfavorable conditions (low temperatures, low moisture, overcrowding, lack of food, or accumulation of metabolic products), the plasmodium will either migrate to a new location or desiccate and harden into a durable resting or dormant state that can survive for seven months or longer under controlled conditions (Alexopoulos, 1960b; Gray and Alexopoulos, 1968). This dormant stage is called the sclerotium (Fig. 1, K) and is composed of spherules that are contiguous and enclose one to many nuclei. Once favorable conditions return, the sclerotium will hydrate and the active plasmodium will crawl out, continue feeding, and produce fruiting bodies.

Fruiting body types

Fruiting bodies form one of four general types: sporangium, plasmodiocarp, pseudoaethalium, or aethalium. The sporangium is a generally globose structure, usually less than one mm in diameter and is either stalked (stipitate) or sessile (non-stipitate). Spores are contained as a mass enclosed by a single, acellular layer called the peridium. In some species, the peridium is partially to fully supported by a capillitial network of sterile thread-like structures. The capillitial network branches from the columella, an extension of the stalk inside the peridium. The peridium ranges from evanescent to persistent, as in *Stemonitis* and *Perichaena,* respectively.

The plasmodiocarp is a single mass of spores in a reticulate network that derives its shape from the plasmodial veins that mature *in situ*, and in some cases plasmodiocarp and sessile sporangia are produced by the same plasmodium.

The aethalium is a single, sessile mass of spores that is often large and pulvinate, cushion- or mound-shaped, and the spores are sometimes supported by the pseudocapillitium, thread-like structures analogous to the capillitium. The spores are covered by the cortex, a thickened peridium. Aethalia are characteristic of some members of the *Liceales*, for example the genus *Lycogala* and, in the case of *Fuligo septica* in the *Physarales*, has attained world record proportions greater than 70 cm across (Keller and Braun, 1999).

The pseudoaethalium is a fused mass of sporangia that outwardly looks like an aethalium. The surface is superficially divided by individual sporangial caps that, for example, have thread-like strands hanging from the corners of the caps, as in *Dictydiaethalium plumbeum* (Schumach.) Rostaf.

The fruiting bodies of myxomycetes can be collected in the field or induced to fruit via the moist chamber culture technique (Keller and Braun, 1999). The life cycle, from spore to mature fruiting body development, can be completed in as few as five days for *Didymium eremophilum* M. Blackw. & Gilb. (Blackwell and Gilbertson, 1980), six days for *Didymium annulisporum* H.W. Keller & Schokn., *Badhamia spinispora* (Eliasson. & N. Lundq.) H.W. Keller & Schokn., and *Badhamia rhytidosperma* H.W. Keller & Schokn. (Keller and Schoknecht, 1989a,b,c) and seven days for *Echinostelium minutum* de Bary (Alexopoulos, 1960a). Life cycle times were measured for spores placed on agar medium with optimum moisture, light, and nutritional conditions, and are not typical of myxomycetes on bark substratum placed in moist chamber culture.

In moist chamber culture, the appearance of myxomycete fruiting bodies that develop from a sclerotized, diploid plasmodium requires two days to two weeks, while those that appear after one month are likely to have come from the haploid microcyst or spore (Alexopoulos, 1964; Keller and Brooks, 1976b). Myxomycete fruiting bodies are used in the description of genera and species.

Taxonomic orders

There are five orders currently recognized in the Myxomycetes: *Echinosteliales*, *Liceales*, *Trichiales*, *Physarales*, and *Stemonitales* (Fig. 2). The *Ceratiomyxales* is an order recognized in older texts, but is now placed in the Class *Protostelia* due to the presence of an externally-borne spore (Olive and Stoianovitch, 1971; Olive, 1975; Spiegel *et al*., 2004). The *Echinosteliales* is a small order (19 species, Lado, 2001) characterized by a protoplasmodium, single, tiny sporangiate fruiting body (usually less than one mm), light coloured spores in mass, and are commonly found on the bark of living trees and vines. The *Liceales* (135 species, Lado, 2001) is a heterogeneous assemblage of species that have either a proto- or phaneroplasmodium, fruiting bodies that lack a columella, true capillitium, and calcium carbonate (Fig. 2). The *Trichiales* (156 species, Lado, 2001) are characterized by a trichiaceous plasmodium and fruiting bodies with light coloured spores in mass, the presence of a true capillitium, and absence of a columella and calcium carbonate (Fig. 2). The *Stemonitales* (175 species, Lado, 2001) are characterized by an aphanoplasmodium and sporangia that may appear separate or pseudoaethalioid due to close grouping. The sporangia contain dark coloured spores in mass and are characterized

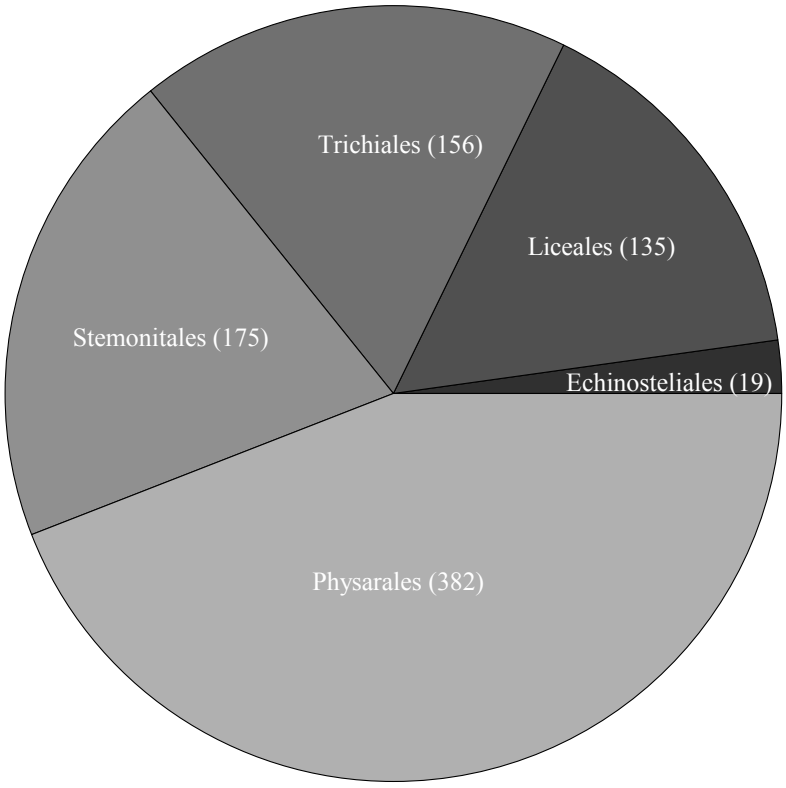

Fig. 2. Number of myxomycete species in each taxonomic order (modified from Lado, 2001).

by presence of a true capillitium and absence of calcium carbonate (Keller and Braun, 1999). The *Physarales* is by far the largest order (382 species, Lado, 2001) and has large phaneroplasmodia and fruiting bodies with dark coloured spores in mass (Fig. 2). Fruiting bodies have a true capillitium and granular or crystalline calcium carbonate (Keller and Braun, 1999).

Phylogenetic relationships of the five orders of myxomycetes based on comparison of EF-1α and small subunit RNA gene sequences (Fiore-Donno *et al*, 2005) showed that the *Echinosteliales* is a basal clade and a sister clade to the other orders. The four other orders branch off in pairs, *Stemonitales* with *Physarales*, and *Liceales* with *Trichiales*. As such, the five orders are divided into three distinct groups, consistent with spore colouration as described by Lister (1925), either colourless, violet-brown to purplish-grey (dark-spored), or non-violet-brown to purplish-grey (clear-spored).

Unfortunately, Lister uses antiquated taxonomic terms for spore colouration and Fiore-Donno *et al.* applied these terms to species that were not described at the time the Lister book was published. More recent treatments of spore colouration, such as the world monograph by Martin and Alexopoulos (1969), should be used in conjunction with additional genetic sequence data, such as distribution and variation in the RNA editing site (Krishnan *et al.*, 2007). Regardless, the growing evidence of a relationship between spore colour and DNA sequence variation suggests that spore characteristics may be useful taxonomic characters. Nevertheless, there are exceptions. The *Trichiales* and *Liceales* are characterized by light coloured spores. However, *Licea parasitica* (Zukal) G.W. Martin has dark coloured spores, and *Licea inconspicua* T.E. Brooks & H.W. Keller has yellowish-orange spores (Keller and Brooks, 1977). The *Liceales* are a paraphyletic assemblage that requires further study.

r- and K-selection

The life history of any organism is the pattern of growth, differentiation, energy

storage, and reproduction, represented by individual size, reproduction, and survival. Examination of links between life histories and habitats reveals strategies used to survive in those habitats. Each strategy is a result of resource trade-offs, where devoting resources to one trait will remove resources from another. For example, increased resource allocation to reproduction will likely decrease survival or rate of growth, and increased number of offspring will likely decrease their individual fitness. Predictions of life history strategies are represented by the r- and K-selection scheme, first proposed by MacArthur (1962), MacArthur and Wilson (1967), and further detailed by Pianka (1970).

The letter *r* is used to refer to the life history strategy that utilizes a high "reproductive rate", whereas the letter *K* refers to a life history strategy that is characterized by a population at or near its "carrying capacity". The concept is based on two types of habitats, r- and K-selecting, in which the different life history characteristics are selected. The r-selecting habitat is unpredictable or short-lived, with a cycle of favorable and unfavorable conditions, and is characteristic of ephemeral habitats; in contrast, the K-selecting habitat is fairly constant and has little variation (Begon *et al*, 2006).

Populations in the r-selecting habitats must be able to rapidly colonize during favorable conditions, but survival of both young and adults is unpredictable and independent of population density, size, or condition of the individuals. Populations in the K-selecting habitats will maintain a constant population size and therefore must be able to compete for resources, resulting in high survival rate of the young. Characteristics of individuals in r-selecting habitats will be smaller in size, have early maturity, larger reproductive allocation and many, small offspring. In contrast, individuals of K-selected populations will be larger in size, exhibit long life, have delayed maturity, have an extended time for reproduction, but have only a few, large offspring (Begon *et al*, 2006).

Among the myxomycetes, the r- and K-selection scheme can be applied to populations within temperate forest habitats, ground site species versus canopy corticolous species on living trees and vines. Relative to one another, the ground habitat retains more moisture within rotting logs and deep in the leaf litter, creating a more constant environment, whereas the canopy corticolous habitat is usually very dry, and wet only for a short period of time after moderate to heavy rainfalls. Indeed, Ing (1994) notes that the most productive woodland environment, in terms of myxomycete collections, is one that has moisture-retaining but not water-logged soils, and has a variety of canopy cover that allows some plasmodia of species to feed in the moist leaf litter under shrubs and move to the upper layers to sporulate.

Life history strategies are evident in various aspects of the life cycle, such as physiological characteristics of the plasmodium and fruiting bodies, and temporal variation in fruiting body development and dormant stages. For example, phanero- and aphanoplasmodial types take longer to develop, have larger plasmodia and a larger surface to volume ratio, and often develop extensive plasmodial tracks, larger numbers and size (stalked 1-2 mm and spore case 0.5 to 1 mm) of fruiting bodies produced in one reproductive cycle. The phaneroplasmodium is the largest of the different plasmodial types. Surface to volume ratios in mature phaneroplasmodia are the largest, especially when the anterior, feeding, fan-like edge and trailing posterior veins cover a large area on decayed logs on ground sites. The bright yellow phaneroplasmodium of *Physarum polycephalum* Schwein. may cover an area on decayed logs of more than two meters and produce thousands of sporangia (Keller and Braun, 1999). However, when phaneroplasmodia are present in the tree canopy corticolous habitat, the plasmodial size is markedly reduced.

Schnittler (2001) has shown that myxomycetes of a winter-cold shrub desert characterizes leaf litter-inhabiting species as K-strategists, that tend to have a phaneroplasmodium and take a longer period of time to develop larger fructifications than bark-inhabiting, corticolous species. Schnittler also noted that species with small proto- or aphanoplasmodia are able to develop rapidly and typically produce fruiting bodies with an

evanescent peridium or lacking a peridium. Furthermore, his results support the possibility of competition between the three most common species with phaneroplasmodia (*Physarum notabile* T. Macbr., *Didymium anellus* Morgan, and *D. difforme* (Pers.) Gray. However, his study and others have not quantitatively examined the life history strategy of corticolous myxomycetes in temperate forests, sampled at different heights from the canopy of living trees, nor have they examined the relationships at the level of taxonomic order.

Materials and Methods

Study areas

Each individual tree and associated vine that was climbed and sampled represented a study site. The Great Smoky Mountains National Park (GSMNP) study sites were confined to Tennessee. The Daniel Boone National Forest (DBNF) and Berea College Forest (BCF) were located in Kentucky. These study areas were selected because of favorable climatic conditions, variety of habitats, and high diversity of tree and grapevine species (Stupka, 1964; Jones, 2005).

Great Smoky Mountains National Park covers an area of approximately 210,545 hectares located on the North Carolina and Tennessee border, at the southern-most range of the Appalachian mountain chain in the United States of America (Keller, 2004). The average yearly rainfall is 140-216 cm (Shanks, 1954). Typical dominant tree species within most of GSMNP are yellow-poplar (*Liriodendron tulipifera* L.), eastern hemlock (*Tsuga canadensis* (L.) Carr.), and a variety of oak species (*Quercus* spp.). Six species of *Vitis* (grapevines) are known to occur in GSMNP (Stupka, 1964).

Daniel Boone National Forest (DBNF) extends northwest to southeast in eastern Kentucky to the Tennessee border, and spans 225 kilometers on the eastern edge of the Cumberland Plateau that includes extensive, rugged areas of over 258,440 hectares. The forest type is within the mixed-mesophytic region of the eastern deciduous forest with tree species such as yellow-poplar, hickory (*Carya* spp.), maple (*Acer* spp.), and eastern hemlock. Precipitation in Kentucky varies from the north to south, from 104-135 cm per year (Martin *et al*., 1993), and is drier than GSMNP.

Berea College Forest (BCF) is privately owned and managed by Berea College, Berea, Kentucky. The forest is located to the east of DBNF, near Berea, Kentucky, also on the edge of the Cumberland Plateau. The physical features and vegetation, encompassing 3,200 hectares, are similar to DBNF. The land was acquired between 1898 and 1960 and was in poor condition due to heavy logging, farming, and grazing, but was reforested by the college (Perry, 2000).

Field methods

Field expeditions were conducted during the summer of 2006 in GSMNP from 1-13 and 25-29 June and 1-9 August, and in DBNF and BCF from 15-23 June and 18-30 July. Forays involved collection of myxomycetes from ground sites and sampling bark from the tree canopy for moist chamber culture. The tree canopy is defined as three meters high to the tops of living trees (Snell and Keller, 2003).

Suitable climbing trees supporting grapevines were difficult to locate. The selection criteria were trees with a minimum diameter at breast height (dbh) of 60 cm and grapevines with a dbh minimum of 4 cm. These selection criteria excluded many trees that allowed bark sampling higher than 16.5 m; many combinations of trees with grapevines were excluded. For example, many suitable climbing trees had grapevines that were too small; many grapevines were large enough but were either not on trees suitable for climbing or were purposely cut at the base and dead. For these reasons, random selection of trees with grapevines was not possible. Any suitable climbing tree with a grapevine, dbh greater than 4 cm, was climbed, and sampling bark from more than two trees with grapevines per day was considered a successful sampling day.

The double-rope climbing technique was used to access the canopy as described in detail by Jepson (2000) and Keller (2004). Only trees and grapevines that allowed sampling in a vertical transect, every 3.3 meters, from 3.3-16.5 meters were sampled. Collection of bark involved evenly prying off bark samples from areas within reaching distance of the climber, avoiding damage to living tissue, and half-

filling a paper bag (ca. 1000 cm^3). Bark was selected from six tree species *Acer saccharum* Marsh. (*Aceraceae*), *Fraxinus americana* L. (*Oleaceae*), *Liquidambar styraciflua* L. (*Hamamelidaceae*), *Liriodendron tulipifera* L. (*Magnoliaceae*), *Platanus occidentalis* L. (*Platanaceae*), and *Tsuga canadensis* (L.) Carrière (*Pinaceae*). Bark from five trees of each species and their corresponding grapevines, *Vitis aestivalis* Michx. or *V. vulpina* L., was used to prepare individual moist chamber cultures.

Laboratory methods

There were ten moist chamber cultures for each tree and each grapevine with two replicates for each site, where each sample height on a particular tree or grapevine is referred to as a sample site (Snell and Keller, 2003). Bark was scanned for presence of myxomycetes using a dissecting stereomicroscope at 70X magnification on days 4, 8, 16, and 32.

Given the ability of plasmodia to fuse and for sporulation of a single individual to occur over a long distance, species were recorded as present or absent for each moist chamber culture. Therefore, the number of observations of each myxomycete species is based on the number of moist chamber cultures positive for the species. For the purposes of this study, each observation may be referred to as an individual, although each may represent one or many true individuals, and fruiting bodies within a single moist chamber culture plate.

A pin was placed near myxomycetes that were immature, and when mature, they were identified using a key to species by Martin and Alexopoulos (1969). Voucher specimens of myxomycetes were made after all measurements were complete, with separate voucher boxes for each species from an individual tree or grapevine. Bark with myxomycete fruiting bodies was removed and glued into the bottom of a standard collection box (4.5 cm x 10.5 cm x 2 cm) (Keller and Braun, 1999). Myxomycete voucher specimens have been sent to the United States National Fungus Collections (BPI), Beltsville, Maryland, USA.

Results

The bark of 30 individual trees and 30 grapevines was sampled, and 580 moist chamber cultures yielded a total of 1324 observed individuals of 46 different myxomycete species, representing 20 genera, with 4 taxa identified only to genus. Species are listed in order of abundance (number of observations) and shown in Table 1. In addition to abundance, the relative occurrence of each species per site is listed, where "site" refers to bark collections from a particular height on each tree and grapevine (Snell and Keller, 2003). This shows that although the most abundant species *E. minutum* was observed 210 times, it constituted only 43.1% of sites among all of the trees and grapevines sampled. Fruiting body and plasmodial type were described and recorded along with the taxonomic order of each species observed in their order of abundance in Table 1.

The proportion of myxomycete species in each taxonomic order is shown in Fig. 2. The largest order based on the number of species is the *Physarales* (44.1%), followed by *Stemonitales* (20.2%), *Trichiales* (17.9%), and *Liceales* (15.6%), in almost equal proportions, and the *Echinosteliales* (2.2%) as the smallest order. In this study, Fig. 3, the *Echinosteliales* (8.7%) represent a much larger proportion of the corticolous myxomycete species, the *Liceales* (23.9%) a slightly larger proportion, and the *Physarales* (30.4%) are a substantially smaller proportion. The *Stemonitales* (19.6%) and *Trichiales* (17.4%) are observed in almost equal proportion in this study as compared to all myxomycete species in Fig. 2.

The number of each fruiting body type observed is given in Fig. 4. Note that nearly three quarters (73.1%) of the individuals in this study have stalked sporangia, over one quarter (25.5%) have sessile sporangia, and the remaining fruiting body types represent 1.4% when combined.

Figure 5 shows that over half (51.2%) of the 1324 individual corticolous myxomycetes observed were protoplasmodia, over one quarter (27.3%) were trichiaceous plasmodia, while aphanoplasmodia and phaneroplasmodia

Table 1. Species abundance with fruiting body type, plasmodial type, and taxonomic order.

Species	No. obs.	% sites	Fruiting body type	Plasmodial type	Taxonomic order
Echinostelium minutum	210	43.1	stalked sporangia	Proto-	*Echinosteliales*
Cribraria violacea	138	27.6	stalked sporangia	Proto-*	*Liceales*
Arcyria cinerea	136	30.0	stalked sporangia	Trichiaceous	*Trichiales*
Perichaena chrysosperma	126	26.6	sessile sporangia	Trichiaceous	*Trichiales*
Comatricha ellae	92	21.4	stalked sporangia	Aphano-	*Stemonitales*
Licea parasitica	51	12.1	sessile sporangia	Proto-	*Liceales*
Calomyxa metallica	50	12.1	sessile sporangia	Trichiaceous	*Trichiales*
Echinostelium coelocephalum	44	9.7	stalked sporangia	Proto-	*Echinosteliales*
Physarum nutans	44	11.7	stalked sporangia	Phanero-	*Physarales*
Clastoderma pachypus	41	10.3	stalked sporangia	Proto-	*Echinosteliales*
Physarum crateriforme	36	9.3	stalked sporangia	Phanero-	*Physarales*
Clastoderma debaryanum	34	9.0	stalked sporangia	Proto-	*Echinosteliales*
Lamproderma biasperosporum	34	8.6	stalked sporangia	Aphano-	*Stemonitales*
Dianema sp.	32	7.6	sessile sporangia	Trichiaceous	*Trichiales*
Cribraria confusa	30	6.9	stalked sporangia	Proto-*	*Liceales*
Licea operculata	30	7.6	stalked sporangia	Proto-	*Liceales*
Macbrideola cornea	26	6.6	stalked sporangia	Proto-*	*Stemonitales*
Diderma chondrioderma	23	5.5	sessile sporangia	Phanero-	*Physarales*
Macbrideola decapillata	20	4.8	stalked sporangia	Proto-*	*Stemonitales*
Enerthenema papillatum	18	4.8	stalked sporangia	Aphano-	*Stemonitales*
Perichaena depressa	16	4.8	sessile sporangia	Trichiaceous	*Trichiales*
Licea pedicellata	15	4.1	stalked sporangia	Proto-	*Liceales*
Licea kleistobolus	12	2.4	sessile sporangia	Proto-	*Liceales*
Licea marginata	9	2.8	sessile sporangia	Proto-	*Liceales*
Licea minima	7	1.7	sessile sporangia	Proto-	*Liceales*
Badhamia rugulosa	5	1.4	plasmodiocarp	Phanero-	*Physarales*
Licea biforis	5	1.4	sessile sporangia	Proto-	*Liceales*
Minakatella longifila	5	1.4	pseudoaethalium	unknown	*Trichiales*
Physarum synsporum	5	0.3	sessile/plasmodiocarp	Phanero-	*Physarales*
Comatricha laxa	4	1.4	stalked sporangia	Aphano-	*Stemonitales*
Cribraria minutissima	4	1.4	stalked sporangia	Proto-*	*Liceales*
Stemonitis axifera	3	1.0	stalked sporangia	Aphano-	*Stemonitales*
Lycogala epidendrum	2	0.3	aethalium	unknown	*Liceales*
Macbrideola scintillans	2	0.7	stalked sporangia	Proto-*	*Stemonitales*
Physarum auriscalpium	2	0.7	stalked sporangia	Phanero-	*Physarales*
Physarum melleum	2	0.7	stalked sporangia	Phanero-	*Physarales*
Stemonitopsis curiosa	2	0.3	stalked sporangia	Aphano-	*Stemonitales*
Badhamia sp.	1	0.3	sessile sporangia	Phanero-	*Physarales*
Diderma effusum	1	0.3	sessile sporangia	Phanero-	*Physarales*
Hemitrichia sp.	1	0.3	stalked sporangia	Trichiaceous	*Trichiales*
Physarum galbeum	1	0.3	stalked sporangia	Phanero-	*Physarales*
Physarum oblatum	1	0.3	stalked sporangia	Phanero-	*Physarales*
Physarum pusillum	1	0.3	stalked sporangia	Phanero-	*Physarales*
Physarum sp. (silver peridium)	1	1.7	sessile sporangia	Phanero-	*Physarales*
Trichia contorta	1	0.3	stalked sporangia	Trichiaceous	*Trichiales*
Willkommlangea reticulata	1	0.3	plasmodiocarp	Phanero-	*Physarales*

*observations of these plasmodia in agar cultures have not been made

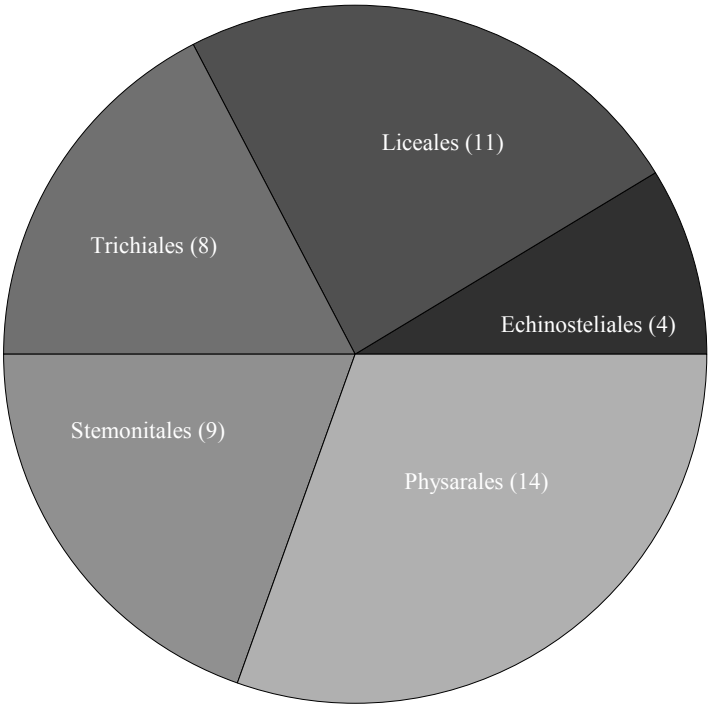

Fig. 3. Number of corticolous myxomycete species in each taxonomic order based on 46 species recorded.

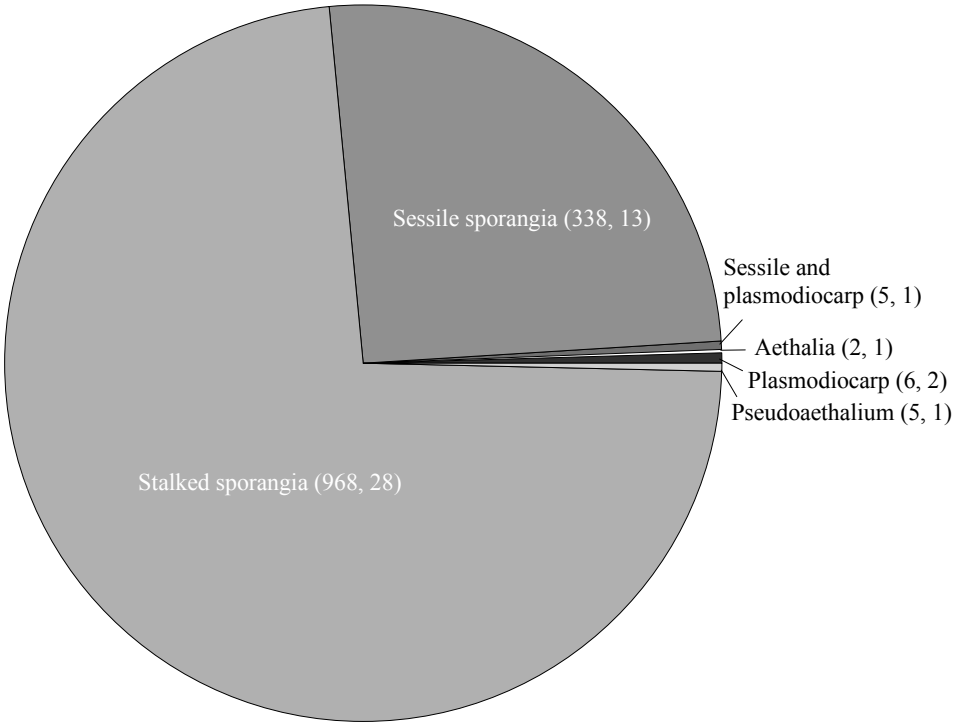

Fig. 4. Number of corticolous myxomycetes with each fruiting body type; number of observations and number of species in parenthesis..

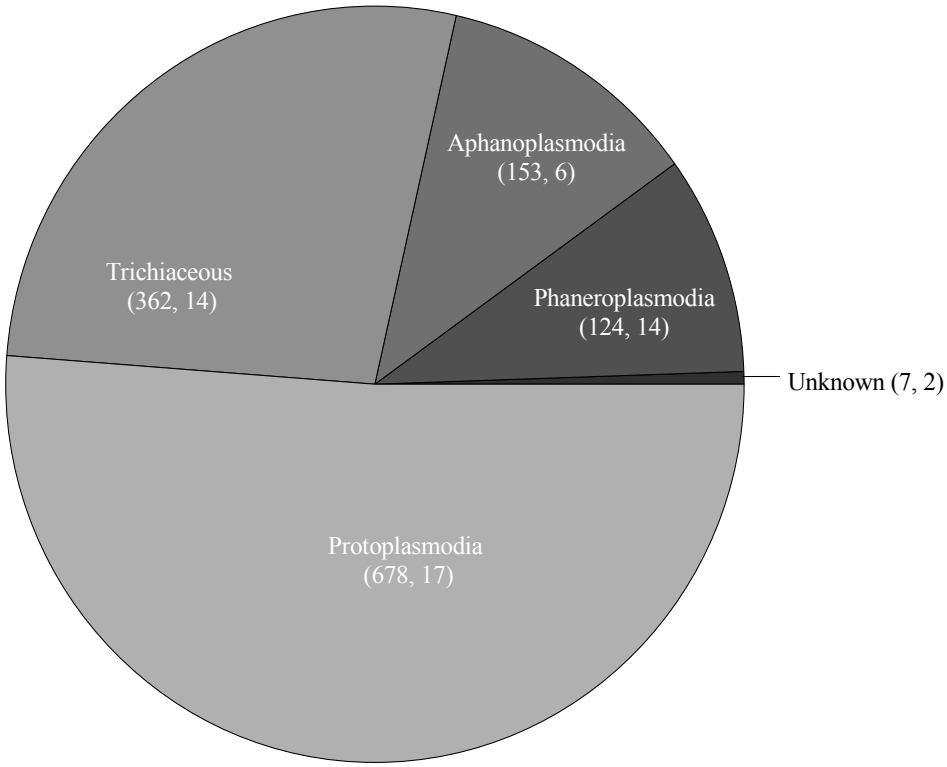

Fig. 5. Number of corticolous myxomycete species with each plasmodial type based on 1324 total individuals; number of observations and number of species are given.

were roughly equal (11.6% and 9.4%, respectively). Only 0.5% of observed species had plasmodial types that were unknown.

Discussion

The fraction of myxomycete species in each taxonomic order showed decreased representation by the *Physarales*, and a greatly increased representation by the *Echinosteliales* compared to those found by Lado (2001). This difference is even greater with respect to individuals observed in each order. In comparison, the fraction of fruiting body types and plasmodial types showed that the majority of corticolous myxomycete species observed had stalked sporangia (73.5%), and protoplasmodium (51.5%) or the trichiaceous (27.5%) plasmodial type (Fig. 4). The stalked habit, small size, and rapid sporulation appear to have a selective advantage on the surfaces of living trees and vines in the canopy (Table 1).

Echinostelium minutum appeared as the most abundant species (210 obs. Table 1; Everhart, 2007) and is probably the most common corticolous myxomycete, forming hundreds of stalked sporangia over extensive areas of bark from living trees with a pH range from 4 to 7 (Snell and Keller, 2003). *Echinostelium minutum* was reported previously in the United States of America by others as the most abundant species on bark (Peterson, 1952; Stephenson, 1983; 1989). Myxomycete species, such as *E. minutum,* which sporulate rapidly in the first 48 hours, develop from encysted plasmodia, since the shortest spore to spore completion of the life cycle occurs in 5 to 7 days (Alexopoulos, 1964; Keller and Brooks, 1976b).

Echinostelium minutum forms the smallest plasmodial type (the protoplasmodium) that is microscopic (100-300 μm) with a circular, plate-like shape that fails to develop vein-like strands with reversible protoplasmic streaming and gives rise to a single, tiny fruiting body. This plasmodial type is more common in drier environments such as living trees and vines in temperate regions or dry, semiarid, desert-like regions. A smaller surface to volume ratio of protoplasmodial mass is selected for survival of the periodic dry-wet or arid environments when moisture is available

for only short periods of time. Furthermore, species in the order *Echinosteliales* are all stalked and usually less than 1 mm in height with a tiny spore case less than 0.5 mm in diameter. This reduction in fruiting body size means that less energy is invested in each spore, and it allows rapid development and spore dissemination.

Examination of the percent occurrence per site shows that even the most abundant and common corticolous myxomycete, *Echinostelium minutum,* was found at fewer than 50% of the sites where bark was sampled. These results support the study by Snell and Keller (2003) that corticolous myxomycetes have a patchy distribution pattern on individual trees in the tree canopy. A patchy distribution pattern of species is characteristic of an r-selecting habitat and would suggest that the dominant corticolous species are adapted to this habitat. Therefore, the individual characteristics of the r-selected, corticolous myxomycetes are all adaptations for survival. For example, protoplasmodia lack the size and motility to migrate and feed over long distances (less feeding time) and cannot compete with larger phaneroplasmodia, therefore sporulate faster and disseminate spores faster and first. This allows protoplasmodial species to avoid competition with phaneroplasmodial species, typical of ground sites, by occupying the niche space provided on the bark surface of living trees and vines.

Species of *Echinostelium* all produce protoplasmodia (Table 1) and also produce spores more rapidly than larger, physaraceous species (Keller and Brooks, 1976b). Echinostelia have a stalked habit, smaller fruiting body size, faster sporulation, and usually evanescent peridia allowing rapid spore dissemination. Species of the order *Echinosteliales* are the best myxomycete examples of the r-selected life history strategy.

Analogous to Ing's (1994) observation that moist, but not wet ground sites are the most productive in terms of myxomycete collections, Keller and Braun (1999) reported that the greatest diversity of corticolous myxomycetes occurred on *Juniperus virginiana* L. (Eastern Red Cedar), *Ulmus americana* L. (American Elm), in orchards of *Malus* (apple), and on species of *Vitis* (grapevines). The bark of these species acts like a sponge, rapidly absorbing water, and staying moist over longer periods of time. This facilitates colonization by more species (Keller and Braun, 1999). In addition to water holding capacity of bark, the general shape of the tree, surface texture of the tree bark, (fibrous, furrowed, ridged, scaly, smooth) along with epiphytic cover of algae, mosses, liverworts, and lichens may also influence the presence of corticolous myxomycetes. For example, *Diderma corrugatum* T.E. Brooks & H.W. Keller, has a watery white phaneroplasmodium often associated with mosses and liverworts (Brooks *et al*., 1977).

Most phaneroplasmodia on living trees and vines generally are much smaller and produce fewer numbers of sporangia than on moist, decayed logs on ground sites (Keller, pers. observ.). In the *Physarales*, *Physarum crateriforme* Petch was the second most abundant species in this study (36 obs., 9.3% of sites, Table 1) and occurs on living trees with a near neutral pH (6.5 to 7.5), especially on *Fraxinus americana* L. (White Ash) and *Juniperus virginiana* (Keller and Braun, 1999; Snell and Keller, 2003; Keller, 2004). This species has a stalked habit with a small (<1 cm) phaneroplasmodium that gives rise to solitary or scattered, small groups of two to six sporangia. Along with the other physaraceous species observed in this study, this represents a trend toward reduced plasmodial size and fewer stalked sporangia on living trees and vines.

Physaraceous species often form sheet-like, diploid sclerotia on the bark surface of living trees (Keller and Brooks, 1976a). Phaneroplasmodia species usually develop in 5 to 7 days, up to 21 days, much later than the Echinostelia. If moist chamber cultures are observed for 2 to 3 weeks, phaneroplasmodia of a few species become much larger (1-2) cm and produce many more sporangia as in *Diachea arboricola*, *Didymium clavus* (Alb. & Schwein.) Rabenh. and *Physarum nutans* Pers. There appears to be a succession of species represented first by protoplasmodia that form tiny, stalked sporangia in 2 to 4 days and later the larger phaneroplasmodia and aphanoplasmodia in 5 to 7 days up to 21 days after wetting bark in Petri dishes. The stalked habit appears

(represented by 73.5% of individuals, Fig. 4) to be an adaptation and selective advantage in the tree canopy. Novozhilov *et al*., (2006) found similar results for bark inhabiting myxomycete species in the arid regions of the Lower Volga River of Russia.

Conclusions

Common corticolous myxomycetes that grow and sporulate on the canopy bark surface of living trees and vines represent a group of organisms that appear adapted to and, in many cases, are known only from this habitat. Sporulation occurs faster for species with small fruiting bodies, in 2-4 days, suggesting that the resting stage is a sclerotium and probably not haploid microcysts that would take longer to complete the life cycle (Keller and Brooks, 1976b). This also suggests that protoplasmodia do not have a long feeding time and can survive and sporulate after light rain showers, because of shorter time intervals of dry-wet conditions.

Canopy bark surfaces of living trees and vines may be subjected to long, dry periods that also alternate with moist or excessively wet conditions of short or long duration. In addition, hot summer temperatures (32-38°C) and cold winter temperature (<-18°C) for several months create extremely harsh environmental conditions that favor organisms with dormant or resistant stages (Keller and Braun, 1999). The myxomycete life cycle stages that survive unfavorable environmental conditions, represented by spores, microcysts, and sclerotia (Fig. 1), allow survival and success of the corticolous myxomycetes.

These results support prior observations that corticolous myxomycete species are adapted to arid conditions and rapid production of a large number of stalked sporangia (Novozhilov *et al*., 2000). In this manner, the corticolous myxomycetes can maximize spore dissemination without the greater investment associated with large sporangia and phaneroplasmodia, as in the *Physarales*. *Echinostelium minutum* was the dominant corticolous myxomycete species well adapted to the tree canopy. It occurs across a wide range of substrata, producing a single, tiny fruiting body, and forming a protoplasmodium that is small and moisture conservative. In contrast, the *Physarales* observed had low abundance, and most were usually identified as typical ground species. Results of this study indicate that the corticolous myxomycetes in the tree canopy exhibit the r-selected life history strategy.

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