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Spore Dispersal of Slime Molds and Higher Fungi via Animal Vectors

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Spore Dispersal of Slime Molds and Higher Fungi via Animal Vectors

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Abstract

Myxomycetes and dictyostelids are Amoebozoans that are cosmopolitan inhabitants of a variety of habitats, particularly forest environments. Both groups reproduce using spores which are primarily dispersed via wind in myxomycetes but this characteristic poses a problem for dictyostelids. The spores of dictyostelids are incased in a mucilaginous matrix that makes wind ineffective except in exceptional cases. It has been suggested that animals such as birds may play an important yet understudied role in the dispersal of these organisms. This study investigated how animals could potentially serve as vectors for spore dispersal of dictyostelids and myxomycetes with some limited data obtained of higher fungi. The ecology of these organisms is understudied and the potential interactions between these and other animals is largely unknown. Animals may disperse spores to different areas by consuming spores or other animals such as insects that have consumed spores, or by moving across areas where myxomycetes and dictyostelids occur. Coprophilous myxomycetes occur primarily on dung and data was collected from the northwest Arkansas area investigating potential differences in the species composition of myxomycetes isolated on the dung of large herbivorous mammals. There is limited previous data indicating that birds, amphibians, small mammals, and bats may disperse the spores of dictyostelids and this study was the first recorded instance where dictyostelids have been isolated from reptiles.

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Introduction

This study investigated the role that animals play in the dispersal of myxomycetes and dictyostelids with some limited data also obtained from higher fungi. All three groups produce spores that can be dispersed by animals such as birds, insects, and large herbivorous mammals via either direct ingestion or when the animal moves across the fruiting body of a slime mold or fungus. These ecological relationships are understudied and may be particularly significant for dictyostelids. Dictyostelids are Amobozoans that are commonly found in the soil/litter layer on the forest floor. The spores of dictyostelids are incased in a mucilaginous matrix, which makes wind dispersal less effective than it is for myxomycetes. Previous studies have indicated that animals such as birds may pick up the spores of dictyostelids from eating insects or from foraging on the forest floor and that the spores can pass through the digestive system unharmed. Prior to this study there had been no published studies investigating whether reptiles can serve in a similar role. In 2014, a series of samples obtained from reptiles was evaluated for the presence of dictyostelids. These samples were collected from Lake Fayetteville, The Ozark Natural Science Center, and Lake Leatherwood in northwest Arkansas. Swabs were taken from lizards, turtles, and snakes and then taken back to the Eumycetozoan Laboratory at the University of Arkansas for processing. It was hypothesized that the species of dictyostelids isolated from these reptiles could have related to the habitats of these animals. Myxomycetes are a much larger group of Amoebozoans that are cosmopolitan and found in virtually every ecosystem investigated to date. Although myxomycetes can disperse their spores via wind a small number of myxomycete are found primarily and in some cases virtually only on the dung of large herbivorous mammals. Coprophilous myxomycetes are the most understudied ecological group of myxomycetes and this association may be particularly important in arid regions where

there are extreme temperatures and alternative substrates are not available. During the fall and winter of 2019 and 2020, a series of dung samples was collected from horses, cows, deer and other animals from Pennsylvania, West Virginia, the University of Arkansas Experimental Farm, and the North Dakota Zoo. Dung samples were air-dried, and several series of moist chambers were prepared. The moist chambers were checked approximately once a week with a dissecting microscope for up to five months. Fruiting bodies that formed in culture were identified and placed in small paper boxes for storage. During the fall of 2019, deer pellets were collected and examined for the spores of higher fungi. Each deer pellet was suspended in a vial of distilled water and agitated to separate any spores present from the substrate. A series of photographs was generated to show fungal spores from some of the samples, but this project could not be completed due to the historic COVID-19 pandemic.

Spore Dispersal of Slime molds and Higher Fungi via Animal Vectors

Abstract

The purpose of this study was to investigate the role that animals play in the dispersal of myxomycetes and dictyostelids. Dictyostelids (cellular slime molds) and myxomycetes (plasmodial slime molds) are protists that are cosmopolitan and reproduce through the production of spores. Animals such as birds have been shown to disperse their spores in previous studies, in some cases over vast distances. The role that animals play as spore vectors is still largely understudied and more research is needed to understand the importance of this dispersal strategy and its potential effects on the ecology and genetics of these organisms. This association may be particularly important in dictyostelids since their spores are encased in a mucilaginous matrix which makes wind dispersal largely ineffective except in exceptional cases.

Introduction

Such unassuming places as dried leaf litter, soil, tree bark, and animal dung reveal a rich diversity of small, amoeboid organisms informally referred to as slime molds or mycetozoans. Although they resemble certain microfungi, slime molds are classified as protists belonging to the domain Amoebozoa. Most people have never heard of slime molds, but there exists a varied world of these organisms that goes largely unnoticed since most are too small to be observed directly with the naked eye. Amoebozoa is extremely diverse and includes important model organisms and significant pathogens. Slime molds are a large group of over 1000 species and were traditionally placed in the Eumycetozoa with three major groups Dictyostelia, Myxogastria, and Protosporangiida (formerly Protostelia). Within Amoebozoa there are two groups. These are the sorocarpic slime molds (dictyostelids, social amoebae, aggregative amoebae, or cellular slime molds) and the sporocarpic myxomycetes (plasmodial slime molds or myxomycetes) along with

the protosteloid amoebae (formally referred to as protostelids now classified as protosproangiids). In sporocarpic organisms a single amoeboid cell develops into a usually stalked, subaerial structure (fruiting body) that supports one to many propagules called spores, a characteristic unique to the Amoebozoa. Sorocarpic amoebae aggregate into a multicellular mass that develops into a subaerial fruiting body consisting of either distinct stalk cells and spores or non-differentiated encysted cells (usually also called spores). Within the Amoebozoa sorocarpic cells are found in two lineages the Dictyostelia (Eumycetozoa) and in Copromyxa (Tubulinea). Sorocarpic taxa are found outside the Amoebozoa among Opisthokonta, Excavata, Stramenopiles, Alveolata, and Rhizaria (Adle et al. 2019: Kang, 2017: Olive 1975: Sheikh et al. 2018: Raper 1984: Spiegel et al. 2017).

The plasmodial slime molds, which are commonly referred to as myxomycetes, "true" slime molds, or acellular slime molds are more familiar since most are macroscopic and some species can form large fruitings that are easily observed. Plasmodial slime molds were first referred to as myxomycetes by Heinrich Link in 1833, and their name is derived from the two Greek words *myxa* (slime) and *myketes* (fungi). He regarded them as fungi, but this view was later challenged by Anton de Bary, who felt that they were more closely related to protozoans and named the group Mycetozoa, from the Greek words *zoon* (animal) and *mykes* (fungus). Olive (1975) named this group Eumycetozoa with the monophyletic taxa Myxogastria and Dictyostelia arising from the paraphyletic taxon Protostelia (Shadwick et al., 2009). Myxomycetes are cosmopolitan and species have been found on every continent, including Antarctica (Olive, 1975). There are over 1,000 species of these organisms that traditionally have been divided into six different taxonomic orders. These are the Ceratiomyxales, Echinosteliales, Liceales, Physarales, Stemonitales, and Trichiales. These groups were based primarily on morphological

characteristics such as spore color, but the advent of phylogenetic analysis has shown that the classical grouping of taxa, especially at the genus, family and order levels were artificial and, in many cases, not monophyletic. The domain Eukaryota was revised several times to better reflect evolutionary relationships among protists. Adle et al. (2005, 2019) divided the Eukaryotes into two domains called the Amorphea and the Diaphoretickes. Amorphea was further divided into the Amoebozoa, Nucletmycea and Holozoa. Amoebozoa is considered the sister super group to Nucletmycea (fungi) and Holozoa (animals). Phylogenetic analysis has revised the class Myxomycetes into bright-spored and dark-spored clades composing the subclasses Lucisporomycetidae and Columellomycetidae, respectively. The bright-spored clade is composed of four orders Cribrariales, Reticulariales, Liceales and Trichiales. The dark-spored clade contains five orders. These are the Echinosteliales (considered the basal group), Clastodermatales and Meridermatales, Stemonitidales, and Physarales (Leontyev et al., 2019). Members of the former group Ceratiomyxales along with some protosteloid amoebae were grouped in the class Ceratiomyxomycetes but are now considered Protosproangiids (Spiegel, 2017).

All three groups of slime molds play an important role in the nutrient cycling within the microhabitats in which they occur by feeding on bacteria and maintaining the nutrient balance that exists with bacteria and other microorganisms. Bacteria are regarded as the main item in the diet of slime molds, and these organisms can affect bacterial population size through predation. Myxomycete amoebae have also been documented to ingest fungal spores and plasmodia have been observed feeding on yeasts, algae and fungal fruiting bodies (Rollins & Stephenson, 2011).

Myxomycetes and dictyostelids vary dramatically in their life cycles and these differences can play a role in their distribution. The myxomycete life cycle consists of two

trophic stages (plasmodia and amoeboflagellate cells) and a reproductive stage (spore-producing fruiting bodies). Most of the myxomycete life cycle is spent as microscopic amoeboflagellates; the plasmodium is a macroscopic slimy mass of protoplasm that creeps like an amoeba over surfaces and engulfs bacteria and small pieces of organic matter. Plasmodia are usually too inconspicuous to be noticed by the casual observer, but the reproductive structures called fruiting bodies are usually macroscopic and, in some cases, can reach a considerable size. Myxomycetes began their life cycle as a single microscopic spore that is released by the fruiting body (see Figure 1B) In unfavorable environmental conditions such as when low temperatures or limited moisture occur, amoeboflagellate cells can form a resistant structure called a microcyst that can remain dormant for significant periods of time (See Figure 1D). Under favorable environmental conditions two compatible amoeboflagellate cells fuse to form a diploid zygote from the fusion of the protoplast (plasmogamy) and nuclei (karyogamy). After fusion the zygote feeds and grows by repeated mitotic divisions, which leads to a giant multinucleated cell not delimited by cell walls. The plasmodium can form another resistant structure, called a sclerotium, during adverse environmental conditions (See Figure 1I). The mature plasmodium will eventually form one or more fruiting bodies that will sporulate and begin the cycle anew. It is not completely understood what causes a plasmodium to form fruiting bodies. but factors such as pH, the exhaustion of available food supply, and changes in moisture are believed to affect sporulation. The fruiting body is formed from the whole plasmodium or from fragmentation of the latter into smaller multinucleate units. Once the fruiting body is fully formed the multinucleate protoplast usually cleaves into uninucleate cells that develop into spores with spore walls. Meiosis in sexual strains takes place after the spore walls are mature. Some myxomycetes never undergo meiosis and

spend their entire life cycle in the diploid condition and are referred to as apomictic (Clark, 1984, Olive, 1975).

Dictyostelids are a much smaller group than myxomycetes with only about 160 species, many of which cosmopolitan They are common inhabitants of the humus-leaf litter layer on the forest floor. Like myxomycetes dictyostelids have a life cycle with both unicellular and multicellular structures. Most of the dictyostelid life cycle is spent as free-living one-celled myxamoebae that feed on bacteria, growing and multiplying until their food source is exhausted (Figure 2). Chemical attractants are then excreted causing the myxamoebae to stream together into an aggregation center. The aggregation center eventually forms a slug-like structure called a pseudoplasmodium that may remain stationary or moves a small distance across the substrate, often towards a light source. Each individual amoeboid cell remains distinct but no longer acts independently and moves in unison. Either immediately or after some movement across the substrate the pseudoplasmodium forms a fruiting body called a sorocarp where spores develop at the tip. Remarkably, each myxamoeba differentiates into a different part of the sorocarp to form the stalk and sorocarp. Cells near the anterior or the pseudoplasmodium begin to secrete cellulose and rise upward to form the stalk (sorophore) while cells near the posterior end are lifted off the surface on the end of the extending stalk and begin to differentiate into a mass of spores (sorus). This is an asexual process, but sexual reproduction also occurs in the microcyst state, resulting from the fusion of compatible mating types. Dispersal in dictyostelids is particularly problematic due to the morphology of the sorocarp. Wind dispersal is regarded as the most common method of distributing myxomycete spores, but this is not the case with dictyostelids. Unlike the fruiting bodies of myxomycetes the spores of a dictyostelid sorocarp are encased on a mucilaginous matrix that usually makes wind dispersal ineffective. Wind can only

disperse dictyostelid spores in instances when the soil is dry and blown into the atmosphere as dust (Cavender, 1973). Although dictyostelids will sometimes migrate across the substrate during the pseudoplasmodal stage the distance traveled is limited, probably no further than several cm over a 3-4 d life cycle. How is it possible for dictyostelids to effectively distribute their spores? It is possible that animal vectors play an important but understudied role in the dispersal of these organisms (Landolt et al., 2009: Raper, 1984: Stephenson and Landolt, 1992). Keller and Smith (1978) observed that the spores of myxomycetes can be picked up by insects and mites crawling over herbarium specimens and that the spores can survive passage through the digestive system unharmed. A later study by Suthers (1985) indicated that dictyostelid spores can also survive the digestive system of birds for up to ten days which indicates that their spores could be transported over vast distances. Studies of earthworms by Huss (1989) and of small mammals, birds, moths and salamanders by Stephenson and Landolt (1994) have also indicated that animals can potentially disperse the spores of dictyostelids either by ingestion (either directly such as in insects or indirectly when an animal such as a bird ingests an invertebrate that has fed on dictyostelids) or by picking up spores when passing over fruiting bodies. Dictyostelids have also been cultured from the boots of humans which indicate that anthropogenic dispersal may also influence dispersal (Perrigo et al., 2012).

The protosteloid amoebae (formerly considered protostelids) are a non-monophyletic assemblage within Amoebozoa characterized by sporocarps that bear one or a few terminal spores. This is a much smaller group of about 100 species, with roughly 40 that are formally named. Protosteloid amoebae were not formally described until the 1960's and there have been few studies on this group due to a lack of interest. Protosteloid amoebae have a more inconspicuous morphology than myxomycetes and dictyostelids and this may have resulted in

fewer studies even though protosteloid amoebae appear to be cosmopolitan and share the same environments as other Amoebozoans. Like myxomycetes and dictyostelids, protosteloid amoebae have an ameboid trophic stage and a spore-producing sporocarp stage. During the amoeboid stage, the amoebae consume bacterial and fungal cells while moving through decaying vegetation. The amoeboid states of this group vary widely in morphology and many species have life cycles that include both amoeboflagellates and obligate amoebae. Protosteloid amoebae are found primarily in primary (leaves, stems and inflorescences) or secondary (wood or bark) decaying vegetation but can occasionally be found in herbivore dung and the humus layer of the soil. Most species of protosteloid amoebae form single-spored sporocarps but some species contain two, four or eight spores. Most species appear to be cosmopolitan with fewer occurring in high latitudes. Species assemblages of protosteloid amoebae in different habitats may be similar but differences often exist for different microhabitats in the same habitat. Some species are present on bark or on dead aerial plant material but not on both, although this distinction seems less pronounced in tropical forests. Protostelid-like organisms have been suggested as the progenitors of myxogastrids and dictyostelids since their sporocarps are simpler. Originally referred to as protostelids, phylogenetic studies have shown that these organisms belong to seven clades that contained protostelids but did not appear be related to one another and were scattered among groups of amoebae that were never observed to fruit. For this reason, a monophyletic Eumycetozoa was rejected and protostelids were renamed to protosteloid amoebae (Shadwick et al. 2009: Stephenson, 2010: Spiegel et al. 2007).

The varied life cycles of slime molds pose a major challenge to biodiversity assessments. Unlike larger organisms, describing a “population” and delimiting patterns of species occurrence are difficult since most of a slime molds life cycle is spent as inconspicuous single-celled

myxamoebae. Even macroscopic structures such as fruiting bodies tend to be ephemeral and seldom last for an extended period. It can be difficult to define an "individual" and it is not possible to observe and record all trophic stages. Even within a single species there can be marked differences in appearance due to morphological plasticity. Furthermore, species occurrence is affected by a variety of biotic and abiotic factors including seasonality, prevailing weather conditions, microclimate variability, substrate pH, vegetation gradients, variation in food sources, and competitive interactions (Walker & Stephenson, 2016).

Although slime molds have traditionally been viewed as cosmopolitan, more recent studies suggest that slime molds show some biogeographical patterns. Temperature, moisture, pH, and the presence of decomposing plant material are regarded as the main factors that cause these patterns. Slime molds have been divided into four major ecological groups depending upon what substrate they occur lignicolous, corticolous, litter-inhabiting, and coprophilous species. Lignicolous species are found on dead and decaying wood and are regarded to be the most abundant group although this could in fact be partly because many lignicolous myxomycetes produce conspicuous fruiting bodies. Changes in species composition have been observed depending on the level of wood decay, moisture levels and the amount of bark still present (Rollins & Stephenson, 2011, Stephenson & Rojas, 2017). Corticolous species occur on the bark of living trees and were virtually unknown until the moist chamber technique was introduced. Factors such as surface texture, epiphyte load, water holding capacity, and pH impact what species occurs on the living bark microhabitat. In general, the highest numbers of species occur on bark with circumneutral pH and decreases with a lower pH. Litter-inhabiting species occur on dead plant material such as fallen leaves and twigs, and the species assemblage is still poorly documented. It has been demonstrated that some species of myxomycetes prefer the litter of

either broadleaf or coniferous trees. The depth of the litter layer has also been shown to produce clear patterns of species diversity. Of the four ecological groups coprophilous myxomycetes are the least studied and much remains unknown on the ecology of slime molds that inhabit this substrate. In general, coprophilous myxomycetes seem to be more abundant in arid climates such as deserts and grasslands, less common in temperate forests and extremely rare in tropical forests. In addition to these four ecological groups, myxomycetes have also been found in soil, aerial litter, twigs, bryophytes and associated with alpine snowbanks. There is also some indication that myxomycetes exhibit seasonal trends. For example, in temperate regions they are found from early summer until late fall and are usually not present in the winter. In tropical regions temperature and moisture affect when species are visible. Unlike many other organisms that follow the latitudinal species concept with species richness increasing with decreasing latitude myxomycete species richness is highest in temperate areas and decreases as one travels north or south. Tropical, Arctic and boreal forests tend to have lower species diversity while deserts and grasslands have surprisingly high species diversity (Rollins & Stephenson, 2011).

Several noteworthy ecological associations have coevolved between myxomycetes and animals, most notably insect-myxomycete associations and myxomycetes occurring on the dung of herbivorous animals (coprophilous myxomycetes). Many of these associations are poorly understood but there is some evidence that these associations help with the dispersal of myxomycetes. Birds may further aid in dispersal of myxomycetes via ingestion of insects associated with slime molds. These insect-myxomycete associations have been widely recorded, but more research is needed to further investigate these relationships. Several insects, especially members of the order Coleoptera (beetles) will often consume slime mold spores and may exert a considerable influence on dispersal. Consumption of slime mold spores and plasmodia by beetles

has evolved independently in at least six Coleopteran phyletic lines. Spores can survive passage through the gut, which may increase germination success due to pH shock. Some species of Coleoptera feed exclusively on slime molds while others are accidental feeders who happen to share the same microhabitat of rotting forest litter with the mycetozoans. The plasmodia of slime molds also serve as a substrate for some species of to lay their eggs, acting as an incubator and a food-rich nursery for larvae (Ing, 1967, Russell, 1979). Beetles are incredibly numerous and widespread throughout the world in a variety of habitats and make up over 25% of all described animal species (Stephenson et al., 1989). There is clearly evidence that some groups of Coleoptera are strongly associated with myxomycetes, in some cases being obligate spore or plasmodial feeders. The dependence of myxomycetes on beetles for dispersal is less clear, and more research is needed to understand this association. There is evidence that spores can adhere to the exoskeleton of beetles and can be left behind, such as in the study by Blackwell & Laman (1982), which was the first study indicating the possible role Coleoptera played in spore dispersal of myxomycetes. The authors observed the lathridiid beetle *Enicmus* feeding on the spores of the myxomycete *Fuligo septica* and successfully grew colonies on plated agar where the beetles had crawled. In one Petri dish, bacteria and yeast from the beetle's interior also formed colonies on which *Fuligo septica* fed, indicating that the myxomycete could make use of food sources from the beetle's digestive tract. Fecal samples of the beetles were also plated on agar but most of the spores were cracked and flattened and none germinated. The authors concluded that spores could attach to the beetle's exoskeleton but could be lost by contact with the agar, thus distributing the spores to a new area. Wheeler (1984) suggested that the pH shock of going through the alimentary canal might aid in spore germination although this hypothesis needs more research. Species of mycetophagous beetles have demonstrated different

morphologies based on their feeding preferences although some groups contain both spore and plasmodial feeders (Lawrence and Newton, 1980). Morphological adaptations in some beetles such as the dorsal mandibular cavities of *Sphinus* increase the success of spore adherence and subsequent dispersal. Plasmodial feeding by beetles may be more common than observed because plasmodia are cryptic and can escape detection underneath tree bark, leaf litter and other substrates. Many species of beetles have been found associated with *Fuligo septica*, which is the largest species of North American myxomycete. The large size of this species could allow for more observations than more cryptic species, creating an inaccurate indication of what species are consumed. (Olive, 1975; Wheeler, 1984).

It is unlikely that myxomycetes rely on animal vectors to disperse their spore because wind is an effective dispersal agent (Alexopoulos 1963, Grey and Alexopoulos 1968, Ing 1994, Kamano et al., 2009). However, animals may act as an important aid to dispersing spores to areas where conditions are more favorable and may be particularly important for dictyostelid spore dispersal. Huss (1989) examined the gut contents of earthworms (*Aporrectodea calignosa* and *Octolasion tyrtaeum*) and pillbugs (*Armadillium nasatum* and *A. vulgare*) and found high abundances of living dictyostelids. The author hypothesized that earthworms may disperse the spores of dictyostelids to better microsites, since earthworms avoided drought, heat, and cold. The author also suggested that such dispersal could aid in increasing genetic diversity through the dispersal of different mating strains. Several species of flies are also commonly found on myxomycetes including members of the family Mycetophilidae, Sciaridae and Drosophilidae and some species are only known to breed on the fruiting bodies and/or plasmodia of myxomycetes. Although data on slime mold flies is extremely limited it has been observed that when the larvae of these flies pupate they come into contact with the spores of fruiting bodies which adhere to

their exoskeleton and are carried away when the fly leaves the mature myxomycete, indicating that flies as well as beetles may play a role in myxomycete spore dispersal (Russell, 1979, Wheeler, 1979).

Myxomycetes that occur predominantly on dung are referred to as *coprophilous* or f*imicolous*. These myxomycetes are considered one of the least studied and most specialized of myxomycetes, with over 100 species occurring on this substratum and 16 species are considered obligate coprobionts. Four species of obligate coprobionts (*Licea alexpoulii, Kelleromyxa fimicola*, *Perichaena taimyriensis* and *Trichia brunnea*) have distinctive thick-walled spores that may be an adaptation to passing through the intestinal tract of herbivorous animals (Eliasson, 2013, Eliasson and Keller, 1999). It is believed that coprobiont myxomycetes evolved because dung was the only suitable substrate on which they could grow on due to extreme temperatures. It has also been observed that the pH of desert substrates tends to be higher than other types of substrates. Dung tends to have a higher pH than bark or litter and this may be less of a limiting factor in environments with higher substrate pH values.

Dictyostelids are common in many types of soil (forest, cultivated, prairie, desert, and marshland), dung, decaying plant material, wood, and decaying fungi (Raper, 1984, Stephenson, 2010). They were once considered primarily coprophilous since they were originally isolated from dung, but later studies indicated that they are common inhabitants of the leaf litter decomposition zone on the forest floor. In addition to forests, dictyostelids have been isolated from the soil of cultivated areas, grasslands, the Arctic, alpine regions, and “canopy soil” the soil associated with epiphytes on trees. They appear to be more common in soil/humus layer on the forest floor than in the soil in other areas. More species of dictyostelids are found at lower latitudes than higher latitudes and at given latitude more species are found at lower elevations

than higher elevations. Dictyostelids also appear to prefer moist to dry soil but are rare where the ground is saturated. Species competition has also been found to vary with forest composition and in some cases, populations can be affected via competitive exclusion between species dependent on the same kind of bacteria. Some species of dictyostelids occur solely in tropical regions, others are strictly temperate and others that are considered cosmopolitan are more abundant in temperate regions than in tropical ones (Stephenson and Feest, 2012).

Romeralo et al. (2010) conducted the first large-scale investigation of the genetic diversity of a single species of cellular slime mold using sequences from the rDNA internal transcribed spacer (ITS1 and ITS2) and the 5.8S gene for *Dictyostelid rosarium* and found little difference between specimens from Europe, the United States, Mexico, Pakistan, Hawaii and New Zealand. This result was not expected since populations separated by vast distances would be expected to show more diversity due to divergence and samples have frequently been isolated in caves that are separated by considerable distances (Stephenson et al., 2006). Animal vectors could be one possible explanation for how spores travel from cave to cave. Five species of dictyostelids were isolated from six of twelve cave crickets (*Ceuthophilus gracilipes* (Haldeman)) by Stephenson et al. (2007). These invertebrates forage outside of caves during the night and may introduce spores to these environments when they return from feeding. A survey of over 100 caves in Alabama, Arkansas (17 caves sampled), Indiana, Missouri, New York, Oklahoma, South Carolina, Tennessee, West Virginia, Puerto Rico, and San Salvador in the Bahamas by Stephenson et al. (2006) revealed about 17 species. Although dictyostelids were not found in all caves there was high diversity and density in the caves where they were detected. *Dictyostelid rosarium* was common in sampled caves, even though this species does not commonly occur above ground and may indicate introduction by animal vectors, especially bats. Bats could verve as vectors by

consuming insects that were feeding on dictyostelids. Previous studies by Huss (1989) and Suthers (1985) have shown that the spores of dictyostelids can survive passage through the digestive tract of animals and form fruiting bodies once deposited in fecal matter. During the summer of 2011, the senior author isolated the spores of *D. rosarium* from the droppings of an Eastern Pipistrelle (*Pipistrellus subflavus*) from a private cave in Benton County, Arkansas, further indicating that there may be a correlation between this species and animal vectors. Prior to this study there had been little data on the species diversity of dictyostelids in Arkansas. Cavender and Raper (1965b) reported species from only one area in western central Arkansas. Waddell (1982) recorded eight species from Blanchard Springs Caverns including *Dictyostelium caveatum*, a species new to science that was isolated from bat guano. A survey of over 100 caves in Alabama, Arkansas, Indiana, Missouri, New York, Oklahoma, South Carolina, Tennessee, West Virginia, Puerto Rico, and San Salvador in the Bahamas by Landolt et al. (2006) reported 17 species from over 25 caves. In Arkansas eight species were isolated from 17 caves including *Dictyostelium sphaerocephalum*, *D. mucroroides*, *D. rosarium*, *D. giganteum*, *D. discoideum*, *D. purpureum*, *D. caveatum*, *Polysphondylium violaceum* and *P. pallidium*. *Dictyostelium caveatum*, which had been isolated by Weddell in 1982, was not found and this species has not been isolated either above ground or in caves since this one record from Blanchard Springs Caverns. Apart from *D. rosarium* which is common in cave studies but rare in other environments, the general pattern observed was that caves contained similar species diversity when compared to samples collected outside of caves.

The most complete study of dictyostelid diversity in Arkansas was from a study conducted from 2003-2008 by Landolt et al. (2009) who isolated 13 species from the six geographic regions of Arkansas to bring the total species count for the state to 16. *Polysphondylium pallidum* was

the most abundant species, making up 47% of isolated clones. Another 30% of species identified included *Dictyostelium minutum*, *P. violaceum*, and *D. purpureum*. The other nine species were considered uncommon to rare. There did not seem to be any distinct patterns of species richness and density observed for the different geographic areas and each area yielded at least eight species. The species isolated in this study were similar to those reported from other studies in North America and other temperate regions of the Northern hemisphere. Compared to Ohio where dictyostelid diversity is best known there are differences in species composition that may be ecologically significant but are still not fully understood. Cavender and Vadell (2006) reported that *D. mucoroides* was the most common followed by *P. pallidum.* In contrast, five other species of dictyostelids were more common in Arkansas than *D. mucoroides* (*P. pallidum, D. minutum, P. violaceum, D. purpureum, D. giganteum*). Another pattern observed was that *D. discoideum* was common in Ohio but made up less than 1% of clones. Landolt (1986) reported that *P. pallidum*, *D. murcoroides* and *D. purpureum* being the most abundant species in southern Oklahoma and north central Texas with *P. violaceum* and *D. minutum* also occurring as a common isolate.

In contrast to slime molds, ecological associations of higher fungi and animals are more fully understood. Mycophagy, the consumption of fungi, is well documented in a variety of animals such as deer, squirrels, mice, voles, and other small mammals. This ecological association may be particularly important in the dispersal of ectomycorrhizal fungi. Ectomycorrhizal fungi form symbiotic relationships with trees and other plants and are crucial to the health of a forest by increasing surface area on plant roots and consequently increasing nutrient and water intake. Surface area is increased via hyphae, threadlike fungal projections that compose most of the fungal body and serve as extensions of plant roots (Gehring et al., 2002). There is a second type

of mycorrhizal fungi, the arbuscular fungi (AM or VAM). Ectomycorrhizal fungi form a sheath on the outside of plant roots without entering the cell. In contrast, arbuscular mycorrhizal fungi form a network consisting of pouch-like vesicles that store lipids and branching structures called arbuscules that exchange material between the fungus and the host plant. AM fungi disrupt the cell interior although the cells seldom die from it. Most ectomycorrhizal fungi belong to the phylum Basidiomycota, but some are found in Ascomycota. The endomycorrhizal fungi belong to a different phylum, the Glomeromycota.

Like myxomyctes, the spores of fungi are widely regarded as wind dispersed but animals may aid in introducing fungi to favorable environments via mycophagy. Seedling and plant establishment during primary succession depends on inoculation of mycorrhizal spores to form this relationship and animals may be an important aid in dispersing mycorrhizal spores. If no mycorrhizal inoculum is introduced the seedling will eventually die (Ashkannejhad & Horton, 2006). Mammals such as mice, squirrels and deer have been commonly demonstrated as vectors of mycorrhizal fungi by feeding on toadstools and dispersing them in fecal material (Cázares & Trappe, 1994: Shchipanov et al. 2006: Terwilliger & Pastor, 1999).

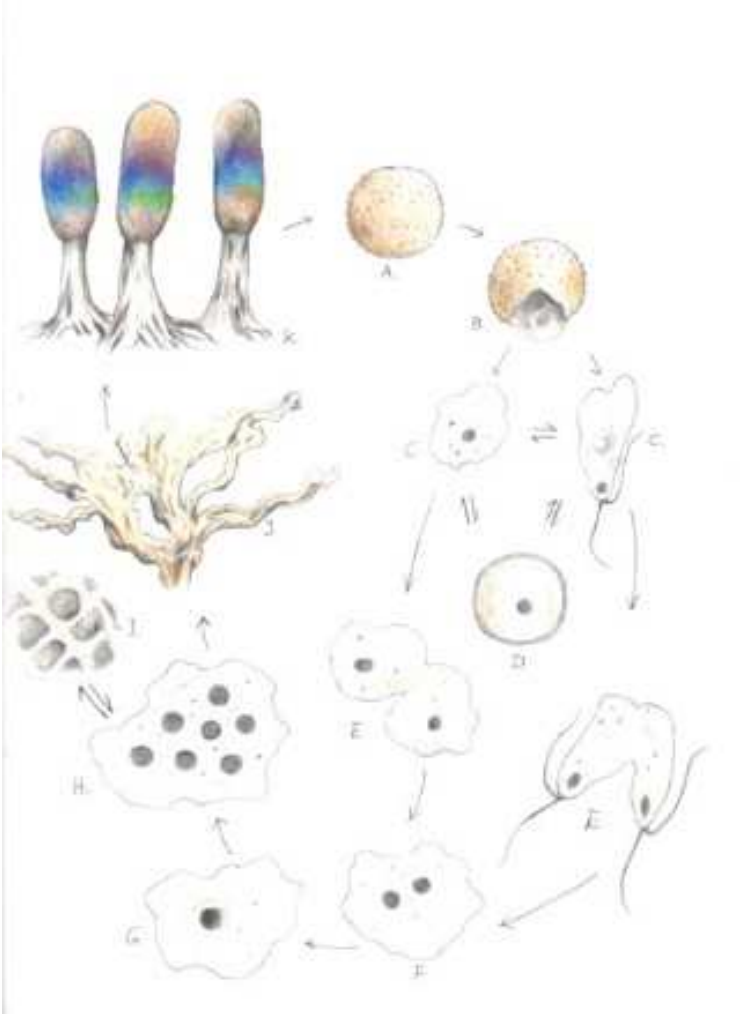

Figure 1: Typical life Cycle of Myxomycetes (species illustrated is *Diachea leucopodia*): A. Spore. B, Germinating spore. If conditions are favorable, then the spore germinates by cracking open or a small pore forms in the cell wall to release one to four haploid protoplasts. Protoplasts may be flagellated (swarm cells) or amoeboid (myxamoebae) and are also called amoeboflagellate cells since the two forms are convertible. Flagella can develop after germination and the flagellated form is more commonly found in wet conditions. Both forms divide by binary fission with environmental conditions affecting what stage forms next. C. Unicellular stage as a myxamoeba (left) or swarm cell (right). D, Microcyst. E-F, Fusion of compatible amoeboflagellate cell to form a single multinucleated cell. G, Zygote. H, Early plasmodium. I, Sclerotium. Consists of irregular masses of small cell-like units called macrocysts. The sclerotium will revert into a plasmodium once conditions become favorable. J, Mature plasmodium. K, Mature fruiting bodies with spores enclosed. Illustration by the senior author.

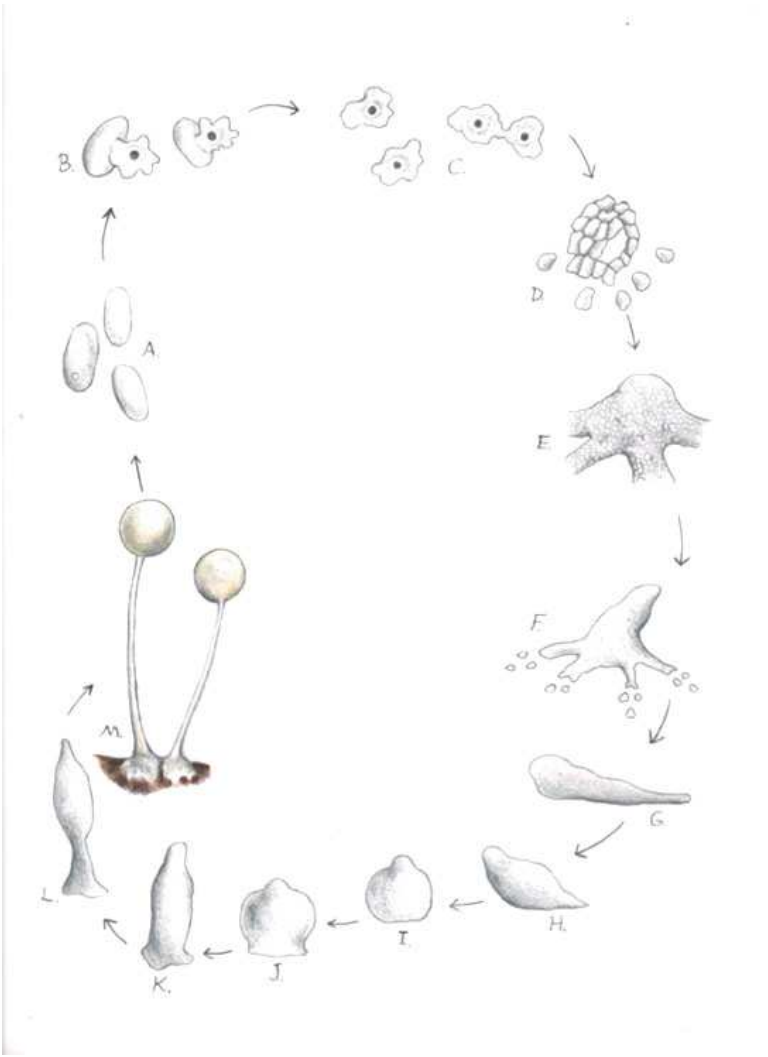

Figure 2: Typical Life Cycle of Dictyostelids (Illustration shows *Dictyostelium Discoideum*). A, Spore. B, Germinating spore. C, myxamoebae. D, Beginning of cell aggregation. E, Streams of aggregating myxamoebae. F, Late aggregation. G, Pseudoplasmodium. H-L, Progressive stages in the formation of the sorocarp. M, Mature sorocarp bearing a spore mass. Illustration by the senior author.

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Dispersal of Dictyostelid Spores by Reptilian Vectors

Abstract

A study of reptilian vectors for the presence of dictyostelids (cellular slime molds) spores was carried out as part of a broader study of animal vectors to determine if reptiles can aid in spore dispersal. Dictyostelid spores cannot easily be dispersed by wind and there is evidence that animal vectors may aid in dispersal and perhaps even impact their genetic diversity. Dictyostelid spores have been shown to survive passage through the digestive tract of birds for up to ten days and may consequently be dispersed over vast distances during that time. The effect of reptile and amphibian vectors on dictyostelid spore dispersal is largely unknown and more research is needed.

Introduction

Dictyostelids or cellular slime molds are Amoebozoans that are commonly associated with the humus-litter layer of the forest floor. Dictyostelids have both unicellular and multicellular trophic stages. Most of their life cycle is spent as free-living myxamoebae but their food supply becomes depleted individual cells will aggregate to form a slug-like structure called a pseudoplasmodium, which in some cases will move a short distance. Ultimately, a spore-producing fruiting body is formed called a sorocarp (Chapter 1, Figure 2). Unlike the fruiting bodies of myxomycetes the spores of a dictyostelid sorocarp are encased in a mucilaginous matrix that makes wind dispersal less effective although it may occur under extraordinary circumstances. Although dictyostelids will sometimes migrate across the substrate during the pseudoplasmodum stage the distance traveled is limited, probably no further than several cm over a 3-4 d life cycle. A small number of studies have demonstrated that animals may play a

role in the dispersal of these organisms (Raper, 1984, Suthers, 1985, Stephenson and Landolt, 1992).

The first published study demonstrating mycetozoan spore dissemination via digestion was observed in *Tyrophagus putrescentiae*, an acarid mite (Keller & Smith, 1978). A film segment by Koevenig in 1961 had already shown that myxomycete spores can be picked up by insects and mites crawling over herbarium specimens (Keller & Smith, 1978). *Tyrophagus putrescentiae* was observed feeding on an undescribed species of *Didymium* (a plasmodial slime mold) ingesting mature spores, which passed unharmed through the mites' digestive tracts. Fecal pellets were collected and mounted in clear lactophenol. Spores germinated, demonstrating that myxomycete spores can survive passage through another organism's digestive system (Keller & Smith, 1978).

Suthers (1985) conducted the first study to investigate what possible role animals play in dictyostelid spore dispersal. She isolated spores from fecal samples and foot swabs of seventy species of migratory and breeding birds in overgrown fields and nearby forests in New Jersey and the rainforest of Central America and found a correlation between birds that were ground feeders and the presence of dictyostelid spores. Ground-feeding finches and sparrows had the highest concentration of spores, followed by thrushes and ground-feeding warblers. Occasional ground feeders had a lower percentage of positive samples, and dictyostelids were virtually absent from arboreal species. The author also hand-raised starling, robin, grackle and pigeon fledglings and fed them different forms of dictyostelid propagules to test spore viability after digestion. Amoebae, spores and macrocysts all survived passage through the digestive tract for up to ten days, a significant find since birds could potentially disperse spores over vast distances during migration. Rare species not found in the soil of the study area were isolated from birds.

The rare dictyostelid *Polysphondylium filamentosum*, which had first been described in Switzerland and Ohio the previous year, was found unexpectedly on an Ovenbird *(Seiurus aurocapillus)* and a Rufous-sided Towhee *(Pipilo erythrophthalmus)*. Suthers indicated that such rare species could serve as markers, indicating where migratory birds had traveled and birds with known habitats could indicate where rare dictyostelids may occur. Spores of dictyostelids could have been picked up by birds through contact with the forest litter or through ingestion of invertebrates. Beetles and other invertebrates eat dictyostelids and may also serve as vectors. When larger animals consume these organisms, the spores are consequently ingested and may be dispersed in the fecal matter (Blackwell & Laman, 1982; Russell, 1979).

Only one published paper exists on dictyostelid spore dispersal by reptiles and amphibians, and only one species of amphibian was sampled. Reptiles were not sampled due to the scarcity of species found at the Mountain Lake Biological Station in southwestern Virginia, where the study was carried out. Mountain Lake occurs at an elevation where there are very few reptiles (samples were collected between 1127.76 and 1249.68 meters (3,700 to 4,100 feet)) (Stephenson, personal communication). Thirteen out of fourteen Red-Backed Salamanders (*Plethodon cinereus*) sampled in the study by Stephenson and Landolt (1992) yielded dictyostelid spores. Dictyostelids were also found in the droppings of a big brown bat (*Eptesicus fuscus*), an eastern woodrat (*Neotoma floridana* Ord), a white-footed deer mouse (*Peromyscus leucopus* Rafinesque), a pine vole (*Pitymys pinetorum* Le Conte) and an eastern chipmunk (*Tamias striatus* Linnaeus). Of the three species of birds sampled, dictyostelids were isolated from the two that were ground feeders. These were a wood thrush (*Hylocichla mustelina* Gmelin) and a slate-colored junco (*Junco hyemalis* Linnaeus). *Dictyostelium discoideum* was isolated from a woody angle moth (*Semiothisa aequiferaria* Walker), which spends the pupal

stage of its life cycle in leaf litter. The results of this study indicated that a wide variety of organisms can disperse the spores of dictyostelids and there appears to be a strong correlation between contact with the ground litter and the presence of dictyostelid spores.

Dictyostelid spore dispersal by humans was first investigated by Perrigo et al. (2012) who isolated 6 samples of dictyostelids from 18 pairs of boots. Four species were recovered including *Dictyostelium minutum*, D. *sphaeroceph- alum*, *D. leptosomopsis* and a new species, *Polysphondylium sp*. Nearly every sample larger than 5 g. yielded samples and myxomycete amoebae and plasmodium-like aggregations were also observed. The authors concluded that anthropogenic dispersal could play a role in some dispersal patterns since some species of dictyostelids with limited ranges have been found in unexpected areas. The authors also speculated that anthropogenic dispersal has been found to be a factor in aquatic protist species so this preliminary study could be an indication of significant anthropogenic roles in human dispersal. The authors also speculated that the morphology of different species such as height, number and size of sori and branching patterns could also play a role in dispersal patterns. Cosmopolitan species such as *Dictyostelium sphaerocephalum*, which was isolated more than once from boots in this study, have been found to have a larger surface area and produce a larger number of spores. Dictyostelids have also been isolated from earthworms and pillbugs with spores surviving passage through the digestive tract (Huss, 1989). Blackwell & Laman (1982) observed colonies of the myxomycete *Fuligo septica* in Petri dishes where the lathridiid beetle *Enicmus* had been allowed to crawl after it was observed feeding on myxomycete spores. On one of the petri dishes yeast and bacteria also formed colonies on which *Fuligo septica* fed, indicating that myxomyctes could utilize food sources also found on the beetle's exterior.

Digestion of dictyostelid spores by turtles may also provide a suitable habitat for a similar increase in growth (Murray et al. 1985).

Methods and Materials

This study was carried out in the Ozark Plateau region of northwest Arkansas, an area primarily composed of oak-hickory forests and consisting of low plateaus and valleys with an average annual temperature between 14° and 17° C and year-round precipitation between 104-152 cm. Historically northern red oak (*Quercus rubra*), white oak (*Quercus alba*), pignut hickory (*Carya alba*), mockernut hickory *(C. tomentosa*), shortleaf pine (*Pinus echinate*) and eastern red cedar (*Juniperus virginiana*) were the dominant trees in this ecosystem (Ley et al., 2019).

Figure 2 Swabbing a Three-toed Box Turtles (*Terrapene carolina triunguis*) at the Ozark Natural Science Center, Huntsville Arkansas.

Figure 3 Swabbing a Little Brown Skink (*Scincella lateralis*).

Samples were collected on 4 April 2012 at Lake Fayetteville from two Three-toed Box Turtles (*Terrapene carolina triunguis*) one Western Rat Snake (*Pantherophis obsoletus*) and two Eastern Fence Lizards (*Sceloporus undulates*). On 10 April, a sample was taken from one Timber Rattlesnake (*Crotalus horridus*), one Eastern Gartersnake (*Thamnophis s. sirtalis*), one Little Brown Skink (*Scincella lateralis*) (Figure 3), one Western Rat Snake (*P. obsoletus*) and one Three-toed Box Turtle (*T. Carolina triunguis*) (Figure 2) at the Ozark Natural Science Center. Animals sampled at Lake Leatherwood on April 24 included one Eastern Hognosed Snake (*Heterodon platirhinos*) and Four Eastern Collard Lizards *(Crotaphytus collaris)*. Sampling consisted of dipping both ends of a Q-tip in a vial of distilled water and swabbing the feet, plastron (if present) and cloaca of the animal and placing the swab in a sterile sandwich bag

(Figures 2-3). Individual swabs were used for each body part sampled. Once collected, the swabs were taken back to the lab and plated out according to the method of Cavender and Raper (1965a). Petri dishes filled with hay infusion agar were labeled, inoculated with three drops of *Escherichia coli* (a standard food source for dictyostelids), the swab was brushed across the streak and three drops of distilled water were added to aid in dispersing any propagules present. The Petri dishes were stacked and left to incubate for four days, after which they were examined for the presence of dictyostelids with the aid of a dissecting scope (Figure 4). The plates were then checked twice a week for four additional weeks. Six additional samples from Three-toed Box Turtles (*Terrapene carolina triunguis*) were collected by a fellow graduate student and yielded samples although identification was hindered by contamination in the lab.

Results

Of the five samples collected at Lake Fayetteville, four (excluding one Eastern Fence Lizard (*Sceloporus undulates*) sample) yielded at least one species of dictyostelid, a success rate of 80%. Both swabs from the Three-toed Box Turtle (*Terrapene Carolina triunguis*) were positive for dictyostelids. *Dictyostelium discoideum* was detected after one week from one sample and *Polysphondylium violaceum* was found after four weeks from the other sample. *Dictyostelium discoideum* was detected after one week from the Western Rat Snake (*Pantherophis obsoletus*) swab, *P. violaceum* and *D. mucoroides* were both detected from one of the two Eastern Fence Lizard (*S. undulates*) samples after four weeks. No results were obtained from the samples collected at the Ozark Natural Science Center. Of the five samples collected at Lake Leatherwood, two had positive colonies, a success rate of 40%. Colonies of *D. sphaerocephalum* were found from two of the Eastern Collard Lizards *(Crotaphytus collaris)*, one male and one

female and *Dictyostelium mucoroides* was isolated from an Eastern Hognosed Snake (*Heterodon platirhinos*).

Figure 4. Checking for clones with a dissecting microscope.

Discussion

This study is the first recorded incidence of dictyostelids being isolated from reptiles and demonstrates that this group of vertebrates apparently plays a role in the dispersal of these organisms. There was a high success rate (80%) of samples collected from Lake Fayetteville and samples from Lake Leatherwood showed a relatively high success rate of 40%, indicating that reptiles can serve as vectors of dictyostelids. The samples in this study took up to four weeks to appear which is not typical of dictyostelids, which appear within a week on average. The reasons for this may have been due to spore germination being inhibited by soil factors or certain soil bacteria or perhaps bacterial supply was too low. If there is a poor supply of bacterial food then it would take longer for amoebae to proliferate to an aggregating population. It is also possible that the spores could have been incased in a material from the lizard's skin that retards germination or

that the dictyostelids isolated were microcyst producers, since macrocysts are sometimes reluctant to germinate releasing amoebae (John Landolt, personal communication). Contamination of samples by spores in the lab is unlikely because plates were kept tightly closed and the lid was not removed after inoculation. Prior to this study there had been very little data on the species diversity of dictyostelids in Arkansas. In this study the most common species isolated was *Dictyostelium mucoroides* (3), followed by *D. discoideum* (2), *Polysphondylium violaceum* (2), and *D. sphaerocephalum* (1). Compared to the study of dictyostelids in Arkansas by Landolt, et al. (2009) the species isolated in this study, although they do occur in Arkansas are considered less common. The previous study indicated that *P. pallidum* is the most abundant (47% of cultures isolated) with three others (*D. minutum*, *P. violaceum* and *D. purpureum*) making up 30% of remaining species. Of the four species isolated from reptiles only one (*P. violaceum*) was considered common in the previous study with 205 clones observed. Of the 2,082 clones recovered in the previous study *D. murcoroides* yielded only 96, *D. discoideum* 24 and *D. sphaerocephalum* 16. The four species isolated in this study were also found in caves by Landolt et al. (2006) who isolated two clones each of *D. sphaerocephalum* and *D. murcoroides*, one clone of *D. discoideum* and four clones of *P. violaceum* Although the number of samples collected in this study were small this was the first recorded instance of dictyostelids to be isolated from reptiles and indicated that this group of organisms, along with other animals may play a role in the dispersal of these organisms. Three of the four species isolated were considered rare in the survey of Arkansas dictyostelids by Landolt et al. (2009). One possibility for these findings might be due to the micro and macro habitat preferences of these reptiles. Three-toed Box Turtles (*Terrapene Carolina triunguis*) are commonly found in grasslands in late spring and early fall but prefer forested habitats during the summer, early spring and late fall. During these

times they frequently come into contact with leaf litter because they will dig just beneath the surface to make shallow burrows called “forms”. Three-toed Box Turtles are omnivores and may also pick up spores when they ingest invertebrates that contain spores both internally or externally or when they consume leaves, twigs or roots. Western Rat Snakes (*Pantherophis obsoletus*) are primarily woodland species and will climb trees to find fledglings and eggs (Trauth et al. 2004). *Dictyostelium discoideum* has been primarily isolated from rotting leaves, old wet leaves and the humus layer of forest soils (Cavender & Raper, 1965b). It is not surprising that this species was isolated from these two reptiles since both animals spend much of their time on the surface of the forest floor. *Dictyostelium mucoroides* has been isolated from a variety of habitats including the humus layer of the forest floor (Cavender & Raper, 1965b). Eastern Hognosed Snakes (*Heterodon platirhinos*) are primarily found in dry mixed deciduous forests or pine woods and feed almost exclusively on toads in the genus *Bufo*. It is possible that this species could have acquired the spores of *D. mucoroides* either via contact with the forest floor or by the ingestion of toads which feed primarily on insects. Northern Fence Lizards (*Sceloporus undulates*) are commonly found at forest edges and are good climbers. In contrast, the Eastern Collared Lizard (*Crotaphytus collaris*) is strictly a saxicolous (rock dweller) species and is found in rocky, sandstone or limestone cedar glades rather than forested habitats (Trauth et al. 2004). A study of dictyostelids in grasslands by (Rollins, et al. 2017) showed that *Dictyostelium sphaerocephalum* was particularly common in grassland habitats compared to other cosmopolitan species. The fact that this species was isolated from the Eastern Collared Lizard (*Crotaphytus collaris*) may be due to its preference for drier less forested habitats. Although more data would be needed it is possible that reptiles may help disperse species that are less

common in Arkansas such as *D. sphaerocephalum* and impact species diversity in ways that are still not fully understood.

This study showed that dictyostelid spores can be found on reptiles and that these animals may play a role in dispersal but the sample size taken was too small to make any assumptions other than the fact that dictyostelid clones can be isolated from the bodies of reptiles. Going forward I would need to increase the number of organisms swabbed and make more detailed observations of differences in body parts swabbed (feet, cloaca, etc.) to see how these factors might influence the species diversity isolated. It might be interesting to sample stomach contents of swabbed reptiles to see what organisms the animal consumed since dictyostelid spores are a food source for some arthropods. A more detailed analysis of other non-fruiting protists on isolated samples could indicate whether or not fruiting has any effect on dispersal by reptiles. The samples in this study took an atypically long time to germinate for dictyostelids and this observation could be further studied by doing a series of isolations comparing swabs from reptiles to isolates from the soil/leaf litter in the surrounding habitat to see if the body/surface of the reptile may have some factor that could inhibit growth or if the difference may have been due to variations in the bacterial microfauna of both habitats (soil surface vs. reptile body). To investigate this the bacteria found in each habitat would need to be identified and compared as well as the chemical composition of both swabbed animals and the soil material collected.

Table 1. Presence/Absence of Dictyostelids in Reptile Samples

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Animal Species	Unknown	*Dictyostelium discoideum*	*Dictyostelium mucoroides*	*Polysphondylium violaceum*	*Dictyostelium sphaerocephalum*
Three-toed Box Turtle (*Terrapene Carolina triunguis*)		+	+	+	
Western Rat Snake (*Pantherophis obsoletus*)		+			
Eastern Fence Lizard (*Sceloporus undulates*)			+	+	
Eastern Hognosed Snake (*Heterodon platirhinos*)			+		
Eastern Collard Lizard (*Crotaphytus collaris*)					+
Three-toed Box Turtle (*Terrapene Carolina triunguis*) 023P0	+				
Three-toed Box Turtle (*Terrapene Carolina triunguis*) 024FL	+				
Three-toed Box Turtle (*Terrapene Carolina triunguis*) sample 025CH	+				
Three-toed Box Turtle (*Terrapene Carolina triunguis*) sample 026P0	+				
Three-toed Box Turtle (*Terrapene Carolina triunguis*) hybrid sample 021P0	+				
Three-toed Box Turtle (*Terrapene Carolina triunguis*) sample 016CP	+				
Eastern Collard Lizard (*Crotaphytus collaris*)					+

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Large Herbivorous Mammals as Vectors of Coprophilous Myxomycetes

Abstract

The objective of this study was first to document what species of myxomycetes are associated with the dung of large herbivorous mammals and then to evaluate the effect of differences in pH on the species diversity of these organisms in this microhabitat. Although wind is the main dispersal mechanism for myxomycete spores, animals may play an important although little studied role in this process. Myxomycetes that occur on dung are considered coprophilous and this substrate may be particularly important in arid regions such as deserts and grasslands due to the lack of alternative substrates. A series of moist chambers was prepared with dung samples collected from cows, horses, deer and several other species of herbivores and observed over a period of several months for the occurrence of fruiting bodies. The pH and any corresponding differences were noted and recorded.

Introduction

Myxomycetes occur in a wide variety of habitats, especially decaying wood, bark and leaf litter. Many species appear to prefer growing in specific microhabitats and myxomycetes that occur predominantly on dung are referred to as *coprophilous* or f*imicolous*. A small number of species are considered truly coprophilous since they have been recorded only on this substrate (Eliasson, 2013, Eliasson and Keller, 1999). In arid areas such as deserts and steppes, there are myxomycetes that occur on weathered dung and are considered coprobionts. These myxomycetes are considered one of the least studied and most specialized of myxomycetes, with over 100 species occurring on this substrate and 16 species are considered obligate coprobionts. It is believed that coprobiont myxomycetes evolved because dung contained adequate moisture for this substrate to serve as an ideal microhabitat (Vlasenko et al. 2017).

Dung is a very complex substrate, and its physical and chemical properties vary depending on the animal it came from, remaining plant fragments, age, grade of decomposition, and the variety of bacteria and fungi present. The occurrence of myxomycetes on dung may be related more to what bacteria are present than the physical properties of the substrate although more research is needed to explore this relationship. This high microbial content along with high nutrient richness and moisture create a favorable substrate and microhabitat for myxomycetes (Eliasson, 2013, Hudson, 1986, Stephenson, 2011). Compared to bark and other woody substrates on which lignicolous myxomycetes commonly occur, dung has a much higher nitrogen content (up to 4%) and also has a pH much higher than most of the other substrates that myxomycetes usually grow on (Stephenson, 1989). The pH of a myxomycete substrate is one of the most important factors in determining what species will grow on them and can be used to divide different species into groups (Eliasson, 2013). Although many species of myxomycetes can tolerate a broad range of pH this can become a limiting factor in some cases. Previous studies have shown pH to affect species diversity on bark, with the most acidic bark containing the lowest species richness (Liu et al. 2015). Most of the species observed on dung occur on other types of substrates and their occurrence of dung is considered accidental. For example, many species that usually grow on decaying wood occur on other substrates as well. Examples include *Comatricha nigra, Cribraria cancellata, C. microcarpa, Diderma radiatum, Lycogala epidendrum, Physarum album, Trichia botrytis* and *T. varia*. Other coprophilous myxomycetes include such species as *Cribraria violacea* and *Echinostelium minutum* that both inhabit the bark of living trees as well as *Didymium nigripes*, which usually grows on leaf litter (Eliasson, 2013). Although it is believed that there are no truly obligate coprophilous myxomycetes in the strictest sense, there are 16 coprophilous species that usually and in a few cases have been found only on

dung. These include *Badhamia apiculospora, B. rhytidosprema, B. spinispora, Didymium annulisporum, D. rugulosporum, Kelleromyxa fimicola, Licea alexopouli, L pescadorensis, Macbrideola indica, Perichaena liceoides, P. luteola, Trichia brunnea, T. elaterensis, T. fimicola*, and *T. papillata*. Among these the most common species to occur on dung include *Badhamia apiculospora, B. spinispora, Licea alexpoulii, Perichaena liceoides* and *P. luteola*. Of these 16 species some have been shown to have thick-walled spores. This is the case for: *Licea alexpoulii, Kelleromyxa fimicola*, *Perichaena taimyriensis* and *Trichia brunnea*. This morphology may be an adaptation for passing through the intestinal tract of herbivorous mammals and the action of intestinal juices may be required for germination to take place (Eliasson, 2013). All four species with thick-walled spores have been found exclusively on dung. *Licea alexpoulii* was first described by Blackwell (1974), who isolated cultures from horse and cow dung. This species was set apart from other members of its genus by its smooth shiny black peridium and its hemispherical to subglobose sporangial shape. Eliasson *et. al*. (1991) proposed a new genus *Kelleromyxa*, renaming the myxomycete *Licea fimicola* to *Kelleromyxa fimicola*. This species was set apart from other members of the order Liceales by the presence of a true capillitium composed of short unbranched threads and occasional longer branched threads; a phaneroplasmodial plasmodium type giving rise to many fruiting bodies per plasmodium; clustered fruiting bodies, dark spores with uniformly thickened walls; and the presence of calcium in the peridium. This species was found only on the dung of herbivorous animals and has been isolated from horse, cow, bison, pronghorn and rabbit in the New World and from dik-dik, cow or yak in the Old World. The evenly thick spore walls and lack of a thin region where germination occurs also sets *Kelleromyxa fimicola* apart as unique. Prior to this study, there was evidence that Liceales was an unnatural taxon defined by the lack of a characteristic (a

capillitium) rather the presence of one and these differences marked *Licea fimicola* apart as an anomaly in need of reclassification. *Trichia brunnea* was first described by Cox (1981) in his survey of coprophilous myxomycetes of the western United States.

Myxomycetes have also been found on the droppings of birds that feed on seeds, buds and other plant parts. This is significant because in theory a bird could transport spores across vast distances. Myxomycetes have been isolated from the droppings of the Capercaillie (*Tetrao urogallus*) (*Arcyria cinerea, Physarum confertum, Didymium difforme*); grouse (*Lagopus*?) (*Physarum bitectum, Didymium difforme*); black grouse (*Lyrutus tetrix*) (*Didymium diffome*); goose (*Anser*?) (*Perichaena corticalis*); and rock dove (*Columba livia*) (*Physarum compressum*) (Eliasson, 2013). The myxomycete species assemblage was investigated in a colony of great cormorants (*Phalacrocorax carbosinensis*) and compared to the surrounding pine forest in western Lithuania (Adamontytė et al. 2013). The authors found that the lowest species richness was found in the most active part of the colony with the newest and most abundant nests as well as the largest difference in species composition compared to the surrounding pine forest. They also observed species of myxomycetes that were not present in the surrounding forest, several of which were considered rare (*Comatricha mirabilis* and *Arcyria leiocarpa*). The authors cited previous studies that piscivorous birds act as vectors in the transfer of material between aquatic and terrestrial environments and influence the biogeographical cycling of nitrogen and phosphorous that occurs in guano. Compared to the surrounding forest soil collected from the colony had a higher pH due to cormorant guano and also contained species resistant to a higher pH. This study indicated that myxomycete species composition could change dramatically in hypertoficated environments. Coprophilous myxomycetes were first described in 1803 by Schumacher, who collected *Physarum fimetarium* from cow dung. There were relatively few

studies on this group of myxomycetes until Eliasson and Lundqvist (1979) whose study aimed to show what species of myxomycetes occurred on dung. This monograph is significant due to the large variety of animal species sampled and the fact that samples were left to culture for at least several months (Eliasson, 2013). Over 160 samples from 25 species of animals were set up using the moist chamber technique. It was found that myxomycetes growing on this substrate always developed late, after at least three weeks and concluded that at least a month was probably needed to complete a life cycle. Of the samples collected, cow dung was the most favored substrate (55 species collected), followed by the dung from hares (5 species) 23, horse 21, rabbit 18, elk and moose 14, roe deer 7, goat 5, sheep 5, capercaillie 3, donkey 3, camel 2, lemming 2, and grouse 2. The authors admitted that these distribution patterns were partially reflective of what substrates are more readily available than others but did find some patterns of substrate preference for some species. About 80% of positive samples for *Perichaena liceoides* were found on cow dung and this species favored the dung of domesticated animals (cow, horse, sheep, donkey; 32 samples) to wildlife such as deer. *Didymium difforme* was also common on cow dung (38% of samples) but had a wider host range of nine species. *Arcyria cinerea* was found on 50% of horse dung samples and a total of 6 host species. *Stemonitis fusca* was found only on the dung of forest animals (elk, roe deer, hare; 10 occurrences). A similar pattern can be found in fungi. Lundquist (1972) found that some fungi favored the dung from certain species and divided his findings into three groups: (1) fungi with a wide ecological range and low substrate preference; (2) those with high substrate specificity and a wide ecological range; and (3) a small number of fastidious species that were restricted to certain substrates. Among these it was found that all common Nordic species of *Sordariaceae* sampled were found only on the dung of three species out of a total of 100 (Richardson, 2001).

Since the number of studies focusing on coprophilous myxomycetes has been limited, a number of coprophilous species have been discovered fairly recently. *Perichaena taimyriensis* was not discovered until 2000 when Novozhilov & Schnittler (2000) described a species of myxomycete with thick-walled spores from reindeer (*Rangifer tarandus*) dung collected from the Arctic tundra. The species was placed in the genus *Perichaena* and was described as an intermediate species between the genus *Perichaena* and *Licea* since it lacked a capillitium. Since reindeer dung was the only substrate from which this species was isolated, the authors suggested that reindeer might play an important role in the dispersal of this species due to the large number of distances this animal travels (as much as 10,700,000 sq. km annually). Adamonyte (2003) isolated a new species of *Trichia* from the dung of hare (*Lepus* sp.) and roe deer (*Capreolus capreolus*) in southern Lithuania. The species was named *Trichia papillata* and differed from similar species of *Trichia* by having a yellow plasmodium and a papillate peridium divided by yellow bands into angular segments.

A comprehensive study of myxomycetes by Stephenson (1989) revealed that different species of myxomycetes preferred different pH levels as well as different microhabitats. The study took place in the Mountain Lake area of southwestern Virginia, where a series of moist chambers were made sampling the bark of living trees, leaf litter and deer dung. Bark was collected from twenty-two species of trees and one species of tall shrub. Prior to this study there had been very little research on myxomycetes that occur on the bark of living trees, with most studies focusing on decaying bark as a substrate. The author observed that species composition patterns were related to bark acidity and texture. These findings complemented an earlier study by Harkonen (1977) who observed patterns of pH optima in different groups of myxomycetes. Members of the Stemonitales preferred more acidic conditions than members of the Physarales

and Trichiales. Stephenson (1989) observed similar mean pH values for myxomycetes on litter and the bark of living trees and concluded that pH is an important factor in determining distribution patterns of species of myxomycetes, even when different microhabitats are involved. One hundred twenty-nine moist chambers of deer dung samples yielded 44 samples of five species (34%) which was a much lower species diversity than for bark or leaf litter. The author concluded that myxomycetes do not appear to be common on this substrate in temperate forests and that truly coprophilous species were rare or nonexistent. In addition to this study there have been very little investigations into myxomycete dispersal by mammals in temperate forests. A later study by Stephenson and Landolt (1992) primarily investigated the role of animal vectors of dictyostelids, but the authors were able to isolate plasmodia from the droppings of white-footed mice (*Peromyscus leucopus*) and big brown bats (*Eptesicus fuscus*). Although their efforts to induce fruiting were unsuccessful, this did indicate that the spores of myxomycetes were able to survive passage through the digestive tract of animals.

In contrast to temperate regions, coprophilous species of myxomycetes may be more widespread in arid climates. Cox (1981) completed a survey of coprophilous myxomycetes in the western United States and identified a total of 26 species, seven of which were considered rare or new state records. *Trichia brunnea*, isolated from cow dung was the first description of this species. *Perichaena minor*, also isolated on cow dung, was a new record for the state. Another species collected from cow dung was *Calomyxa metallica* and this was the first time it was recorded on this substrate. Normally this species is found on the bark of dead and living trees. *Physarum spinisporum* was isolated from cow dung for the first time in the Western hemisphere. This is an extremely rare species that had only been reported previously from the Canary Islands and Ethiopia; in all instances they have only been isolated from dung. *Physarum ovisporum*,

isolated from cow dung was another rare species isolated and was the first record for Arizona and New Mexico and the first instance it was found on dung. *Physarum gyrosum* was isolated from deer dung and was the first report on dung for this species and for the Western United States. *Didymium ovoideum*, isolated from cow dung was an additional new record for California and the first instance of it being coprophilous. Another rare species isolated was *Didymium karstensii*. Novozhilov et al. (2003) conducted a survey of the Colorado Plateau and collected samples from rabbit (*Lepus sp.*), mule deer (*Odocoileus hemionus*), pronghorn (*Antilocarpra americana*), red deer (*Cervus elaphus*), cattle and horse. The authors found that the most common coprophilous species were *Badhamia cf. apiculospora*, *Fuligo cinereal*, *Perichaena liceoides*, and *Licea tenera*. A later study by Novozhilov et al. (2006) surveyed the desert and steppe regions of the lower Volga River Basin in Russia and Kazakhstan revealed 23 species with *Fuligo cinerea* and *Perichaena liceoides* being most common. The authors noticed a marked difference in species composition between coprophilous species collected from different habitats. *Didymium difforme*, *Physarum bivalve*, and *Leocarpus fragilis* were more common in the northern taiga and tundra while *F. cinerea* was uncommon in these habitats but numerous in desert samples. A higher diversity of coprophilous myxomycetes in desert habitats may be due in part to the higher pH values of available substrates. Blackwell and Gilbertson (1984) found that Sonoran Desert myxomycetes grown on the pith of cacti tolerated higher pH values than previous studies showed, as high as 8.7-10.4. Although dung tends to be higher in pH than other types of substrates this may be less of a limiting factor in desert habitats where pH of alternative substrates is also high.

A set of bison and cow dung collected in a temperate prairie in North Dakota yielded 6 species of myxomycetes from 30 samples. There was a higher species frequency mean for bison

dung and a lower mean pH. The authors observed that species in bison samples (*Arcyria cinerea, Didymium difforme and Kelleromyxa fimicola*) prefer a lower substrate pH than those found on cow dung in this study (*Perichaena depressa, Perichaena liceoides* and *Physarum* sp.). Moist chambers were observed for three months, with 55% of myxomycetes appearing in the first month, 35% in the second month and 10% in the final month. *Didymium difforme* and *Perichaena liceoides* were found throughout the time period and *Arcyria cinerea* did not appear until the third month. Both bison and cow dung had the same species richness of 4 with *Arcyria cinerea, Didymium difforme, Kelleromyxa fimicola*, and *Perichaena liceoides* being present on bison dung and *Didymium difforme, Perichaena depressa, Perichaena liceoides* and *Physarum* species occurring on cow dung. Species frequency was higher on bison dung, with the genus *Didymium* being the most frequent (Onduso et al. 2019). An earlier study by Rollins et al. (2010) indicated that fewer clones of dictyostelids would grow from culture in grasslands that are grazed vs. ungrazed grasslands. The authors attributed lower abundance to soil compaction from grazing herbivores, fewer microhabitats due to a decreased amount of dead plant material being deposited on the ground and possibly a negative impact on local soil bacteria. It was also suggested that dung deposited by grazing herbivores might combat these impacts by serving as a refugia due to its high moisture and bacterial content. In this study a series of moist chambers were made using cow and horse dung to evaluate differences in pH and any corresponding differences between these two microhabitats were recorded.

Methods and Materials

A total of 49 samples of horse and 44 samples of cow dung were collected in September 2019 from four pastures at the University of Arkansas Experimental Farm in Fayetteville, Arkansas (Figures 2 and 3). The collection site was in Northwestern Arkansas in the Ozark Plateau region. This ecosystem is primarily composed of oak-hickory forests and consists of low plateaus and valleys with an average annual temperature between 58° and 64° and average year-round precipitation between 104-152 cm. The forests were historically dominated by northern red oak (*Quercus rubra*), white oak (*Quercus alba*), pignut hickory (*Carya alba*), mockernut hickory *(C. tomentosa*), shortleaf pine (*Pinus echinate*) and eastern red cedar (*Juniperus virginiana*) (Ley et al., 2019). Each sample collected was placed in a paper bag and brought to the Eumycetozoan Laboratory at the University of Arkansas for processing. A series of moist chambers were prepared according to the method of Stephenson (1985). Moist chambers consisted of 90 mm plastic disposable Petri dishes lined with filter paper on which individual samples were placed and distilled water was poured into each moist chamber until the sample was submerged. The moist chambers were left for approximately 24 hours and then a pH reading of the water in each sample was taken and the excess water was poured off. Differences in pH, animal dung (either horse or cow) and level of deterioration were recorded on the Petri dish (dung was either fresh, intermediate in decomposition or old). Each sample was checked for myxomycetes after a week, then approximately every one to two weeks for the following six months. When a myxomycete was observed, specimens were identified with the aid of a dissecting microscope and some bodies were removed with forceps and glued in a paper box. A compound microscope was used to examine spore morphology to confirm species identification. To estimate the average number of fruiting bodies a grid was made on a Petri dish using graph

paper (See Figs. 3-4). Three additional sets of moist chamber cultures were also started in December 2019 and January 2020. Twenty samples of weathered cow dung (at least 6 months old) were collected from a pasture in West Virginia by Dr. Steve Stephenson; thirteen samples of deer pellets from white-tailed deer (*Odocoileus virginianus*) were collected in Pennsylvania by Dr. Autumn Coffey and 17 dung samples were collected form the North Dakota Zoo by Dr. Francis Onduso. The latter included the dung of mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), highland cattle (*Bos taurus*), bighorn sheep (*Ovis canadensis*), reindeer (*Rangifer tarandus*) and camels (*Camelus sp*.). Species diversity (alpha-diversity) was calculated using Shannon's diversity index $H' = -\sum P_i \log P_i$, where P_i is the relative abundance (the proportion of the total number of individuals or records of a species (Shannon and Weaver, 1963, Magurran, 1988).

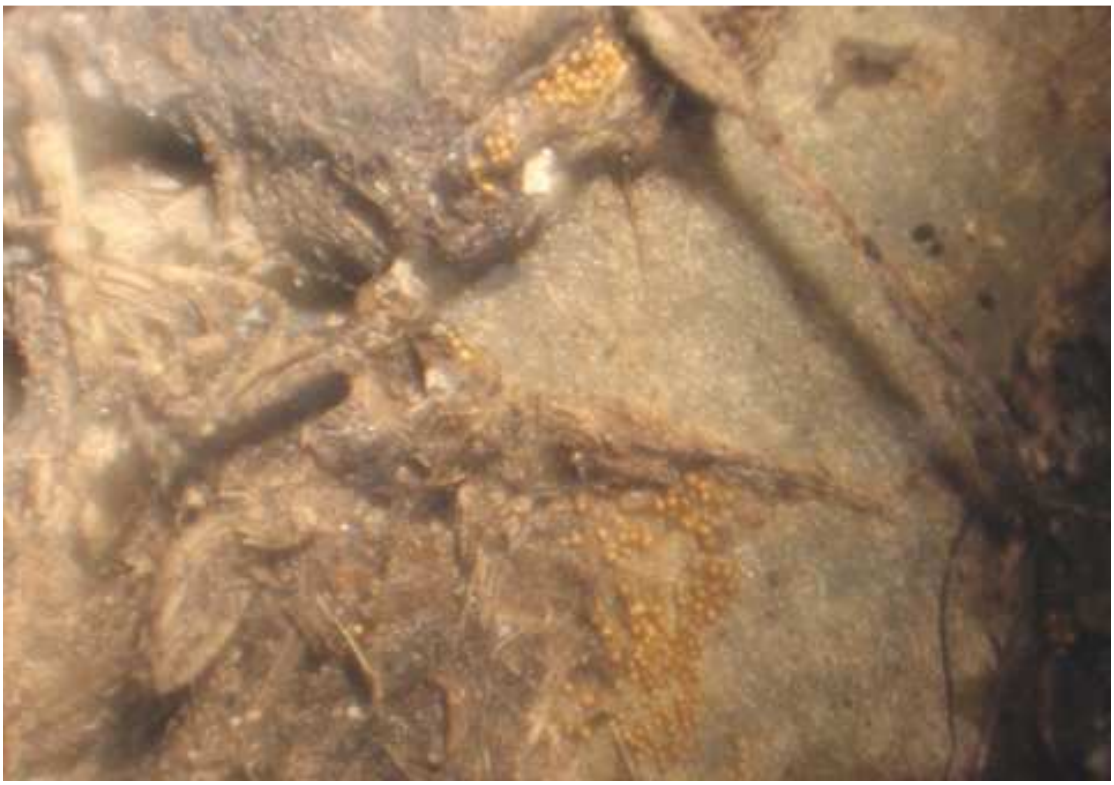

Fig 1. *Perichaena liceoides* in a moist chamber culture of horse dung. Photo courtesy of Apulu Ndotimi

Figure 2. Study site at the University of Arkansas Experimental Farm, Fayetteville, Arkansas.

Figure 3. Study site at the University of Arkansas Experimental Farm, Fayetteville, Arkansas.

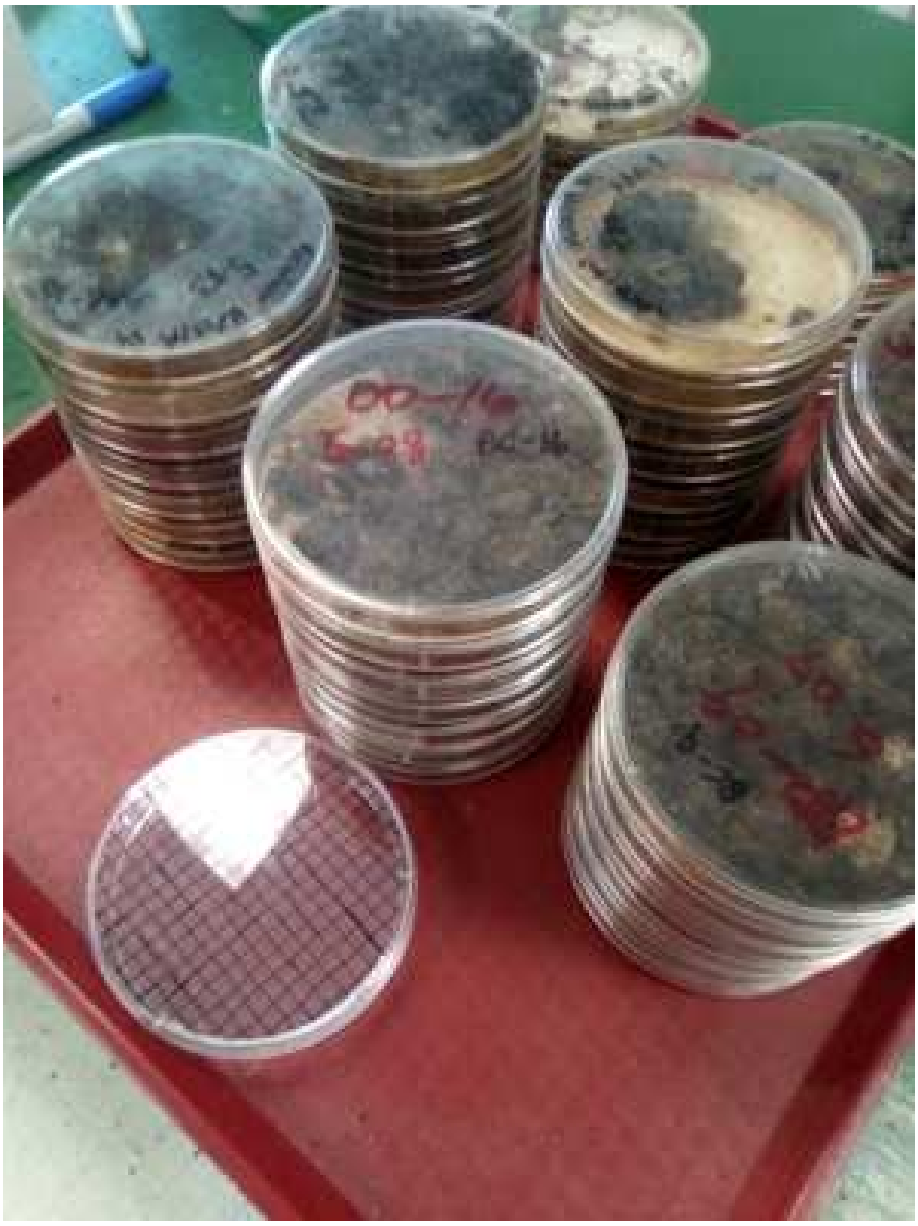

Figure 4. Moist chamber cultures in the Eumycetozoan Laboratory at the University of Arkansas. Graph paper was used to mark a Petri dish in order to estimate the relative abundance of fruititng bodies.

Figure 5. Estimating the number of fruiting bodies with the aid of a dissecting microscope.

Results

Twenty-seven of the 93 moist chambers prepared with samples from the University of Arkansas Experimental Farm yielded myxomycetes a success rate of 29%. A total of four species (*Perichaena liceoides, Physarum* sp., *Perichaena depressa Libert,* and *Perichaena vermicularis* (Schw.) Rost.) were isolated, with *Perichaena liceoides* being by far the most common (See Table 1). The other moist chamber series started in December 2019 and January 2020 did not

yield cultures, including the weathered cow dung samples. The moist chamber cultures from the University of Arkansas Experimental farm did not yield cultures for the first two months, with most appearing after three months. These observations are consistent with previous studies indicating that coprophilous myxomycetes take at least three weeks to appear in moist chamber cultures and many species will not yield cultures until the third month (Eliasson and Lundqvist, 1979). One species of coprophilous fungus, the Dung-Loving Birds' Nest Fungi (*Cyathus stercoreus* Schweinitz), was also observed.

Although there were slightly more samples of horse than cow dung (49 and 44, respectively) there were more positive cultures in horse dung compared to cow dung. Eighteen horse samples yielded specimens, a success rate of 37%. Only 8 cow dung samples produced cultures, an 18% success rate (Figure 9). Cow dung had slightly higher diversity values (H' = 0.22) than horse dung (H' = 0.20) due to species richness values (3 for cow dung and 2 for horse dung). Horse dung contained a higher average number of fruiting bodies than cow dung (Table 1).

Table 1. Species recorded by substrate.

Cow Dung	**Average Number of Fruiting Bodies**	**Horse Dung**	**Average Number of Fruiting Bodies**
Perichaena liceoides	79	*Perichaena liceoides*	100
Physarum sp.	1	*Perichaena vermicularis*	15
Perichaena depressa	2		

Table 2. Average number of fruiting bodies related to pH of the substrate

	pH	Average Number of Fruiting Bodies on Cow Dung		pH	Average Number of Fruiting Bodies on Horse Dung
Average	7.59	59.5	Average	7.23125	105.65
Max	8.08	320	Max	7.73	560
Min	6.69	1	Min	6.75	1
Richness	3		Richness	2	

Table 3. Number of positive cultures related to the age of the substrate.

Substrate Age	Horse	Cow
Fresh	8	5
Intermediate	10	3
Weathered	_	0

Table 4. Values of species diversity.

Species diversity by substrate (H')	
Horse	Cow
0.20	0.22

Discussion

Eliasson and Lundqvist (1979) reported that *Perichaena liceoides* prefers the dung of domesticated animals with cow dung being the most preferred. More positive cultures on horse dung in this study may be due to the slightly lower pH in horse dung (Figure 8). The pH values for this first set showed slightly higher averages for cow dung than for horse dung; the mean pH for horse dung was 7.3 and the mean pH of cow dung samples was 7.70 (See Table 2). A comparison of substrate age (either fresh, intermediate or weathered) indicated that older substrate might be less productive. Both fresh and intermediate substrates collected from the

University of Arkansas Experimental Farm yielded cultures (see Table 3) and there were slightly more fruiting bodies formed on horse dung that was in an intermediate stage of decomposition compared to cow dung. The pH values of dung tend to be higher than most other substrates and has been regarded in previous studies as a limiting factor to what species occur on dung (Eliasson and Lundquist, 1979, Stephenson, 1989). The lack of success in the other moist chamber cultures may have been that the samples from North Dakota came from caged animals and the deer pellets and weathered cow dung samples were smaller sets of samples. Time may also have been a factor in this study. Data collected from the University Experimental Farm did not begin to yield cultures for several months, with many specimens not appearing until the third, fourth and in some cases five months from when the moist chambers were started. Although moist chambers for the other data sets were checked periodically for the first two months closure of the University due to the historical COVID-19 pandemic hindered additional investigation. Based on previously published data, deer pellets in temperate forests are not rich sources of myxomycetes (Stephenson, 1989). Perhaps the abundance of alternate substrates in this sort of habitat is a factor in low numbers of myxomycetes. In contrast, dung collected from the University of Arkansas Experimental Farm was from open grassland with scattered trees. There are some recent preliminary data that coprophilous myxomycetes may be abundant on dung in grassland habitats and perhaps the habitat of animal vectors of coprophilous myxomycetes may be one of the most important variables in determining species abundance and diversity of coprophilous myxomycetes (Onduso et al 2019).

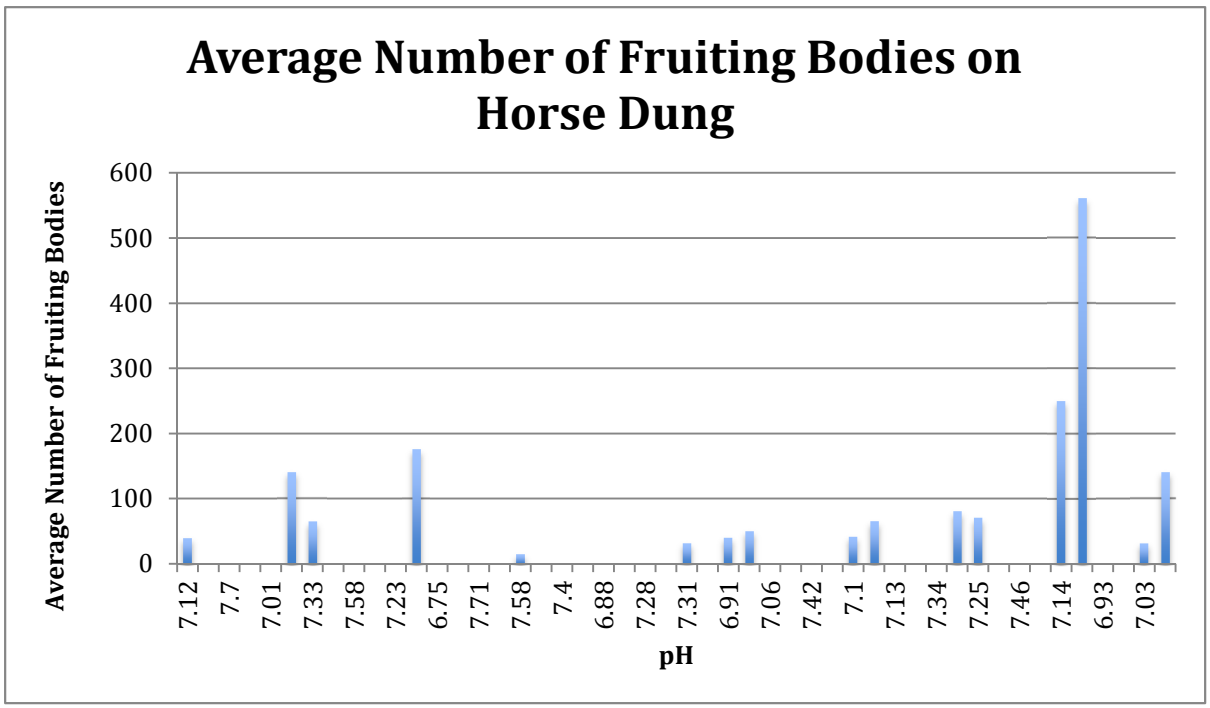

Figure 6. Average number of fruiting bodies on horse dung

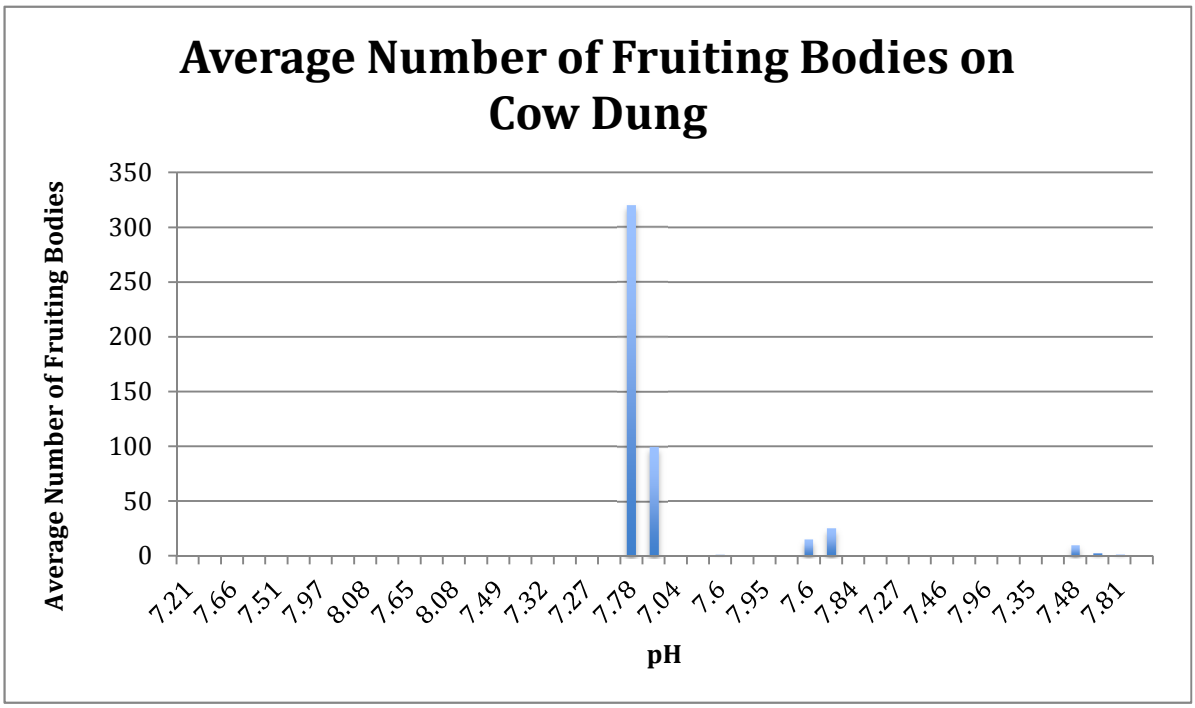

Figure 7. Average number of fruiting bodies on cow dung

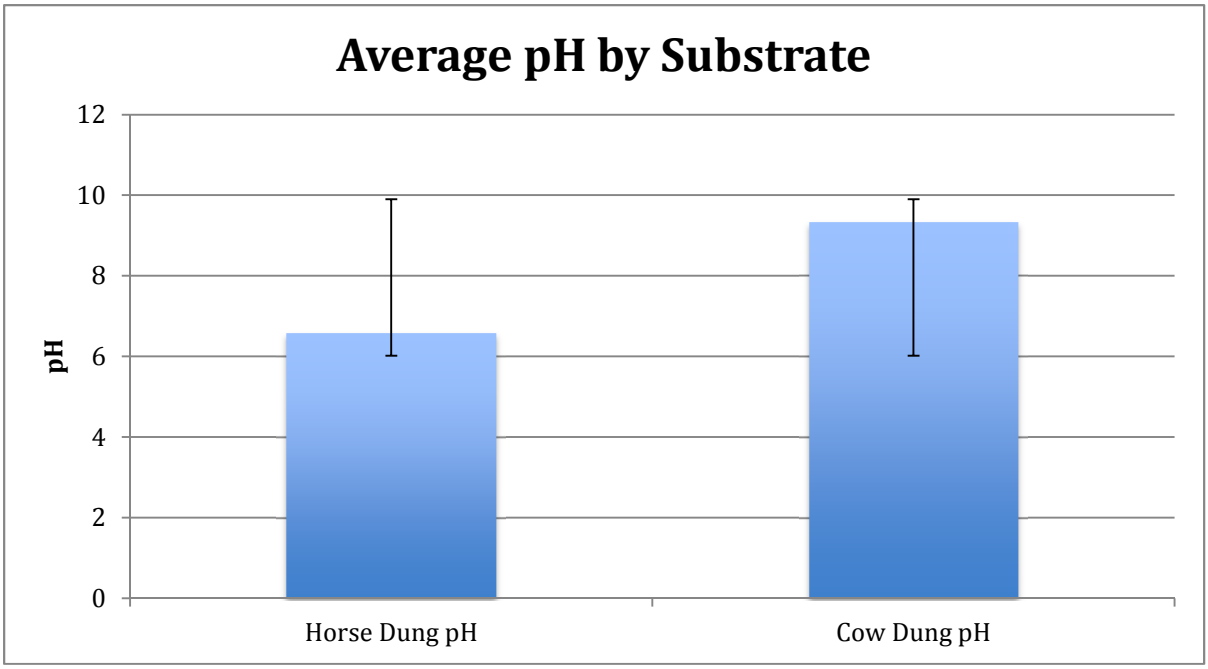

Figure 8. Average pH by substrate

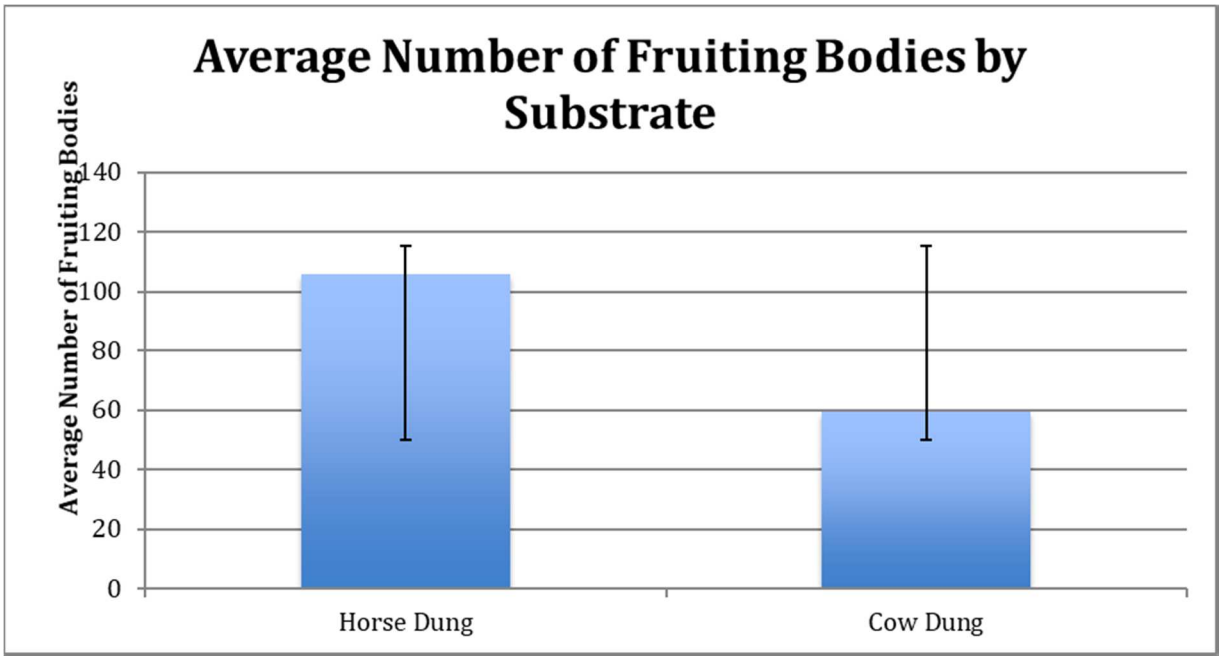

Figure 9. Average number of fruiting bodies by substrate.

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Conclusions

This is the first published report that dictyostelid spores were isolated from reptiles and indicates that these animals may aid in dispersal like birds have shown in previous studies (Suthers, 1985). Swabs that were taken from reptiles in 2014 revealed four species of dictyostelids: *Dictyostelium mucoroides* (3 isolates), *D. discoideum* (2), *Polysphondylium violaceum* (2), and *D. sphaerocephalum* (1). Of the five samples collected at Lake Fayetteville, four (excluding one Eastern Fence Lizard (*Sceloporus undulates*) yielded at least one species of dictyostelid, a success rate of 80%. From the five samples collected at Lake Leatherwood two had positive colonies, a success rate of 40%. Of these species isolated only one of them was considered common from a previous survey of Arkansas dictyostelids. These findings were surprising since the reptiles sampled were either primary or secondary carnivores and these results may be related to the macro and microhabitats of these animals. Three-toed Box Turtles (*Terrapene Carolina triunguis*) and Western Rat Snakes (*Pantherophis obsoletus*) are primarily woodland species and may have picked up spores moving across the forest floor or from climbing trees. Eastern Hognosed Snakes (*Heterodon platirhinos*) are also primarily found in dry mixed deciduous forests or pine woods and feed almost exclusively on toads in the genus *Bufo*. It is possible that this species could have acquired the spores of *D. mucoroides* either via contact with the forest floor or by the ingestion of toads which feed primarily on insects. Northern Fence Lizards (*Sceloporus undulates*) are commonly found at forest edges and are good climbers. In contrast, the Eastern Collared Lizard (*Crotaphytus collaris*) is strictly a saxicolous (rock dweller) species and is found in rocky, sandstone or limestone cedar glades rather than forested habitats. The fact that *Dictyostelium sphaerocephalum* was only isolated from the Eastern Collared Lizard (*Crotaphytus collaris*) may be due to its preference for drier less forested habitats. Previous

research indicates that *D. sphaerocephalum* is more common than other species of dictyostelids in dry grassland habitats. Although more research is needed these preliminary results indicate that animals such as reptiles can carry the spores of rare species of dictyostelids and may possibly exert an impact on species distributions.

Of the moist chambers started with the dung of herbivorous mammals four species of myxomycetes were isolated from horse and cow dung: *Perichaena liceoides, Physarum* sp., *Perichaena depressa Libert,* and *Perichaena vermicularis* (Schw.) Rost. *Perichaena liceoides* was by far the most common species sampled. Horse dung samples contained a higher average number of fruiting bodies then cow dung and this difference may have been due to the lower average pH of horse dung. This differs from previous data indicating that *Perichaena liceoides* is more common on cow then horse dung. A minimum of two months was required to yield any fruiting bodies, with some not appearing until five months. These results are consistent with previous research that coprophilous myxomycetes require a much longer than average growing time and may be the reason that the other series of moist chambers started in December and January did not yield any data (Eliasson, 2013). Deer pellets collected in the fall of 2019 did appear to have fungal spores present in some of the samples although further identification was not possible due to the closure of the University of Arkansas due to the 2020 COVID-19 pandemic. The role that animals play in the dispersal of myxomycetes, dictyostelids and higher fungi is still poorly understood but may play significant ecological roles that effect the distribution and genetics of these organisms. Further research may show new additional associations as this study indicated from reptiles.