# Reproductive systems in the myxomycetes: a review

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A review of reproduction in the myxomycetes reveals that they have a basic one-locus multiplealleleic heterothallic mating system, which controls syngamy between haploid amoeboflagellates to produce the diploid plasmodium. However, each morphologically defined species contains a number of biological sibling species that can't interbreed with each other and are centered in different regions of the world. Also, these morphospecies generally contain numerous nonheterothallic strains that can complete the life cycle from a single isolated spore. While there is information that suggests that some of these strains are homothallic, the majority of the evidence supports an apomictic system derived from a blockage of meiosis during spore formation. Thus, these non-heterothallic strains produce diploid amoeboflagellates that can develop directly into plasmodia without the need for crossing.

**Key words** – apomixis– heterothallism – homothallism – sibling-species

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### Introduction

The basic reproductive pattern described by deBary (1887) has been found to generally apply to every species of myxomycetes that has been examined (Martin & Alexopoulos 1969, Collins 1979, Stephenson & Stempen 1994). Sporocarps are formed from plasmodia and produce spores, these spores then germinate and release uninucleate amoeboflagllates that divide mitotically and produce large clonal populations, which then produce coenocytic plasmodia that grow and fruit to produce more sporocarps. However, information has accumuated over the years, which indicates that there are different nuclear cycles associated with this general pattern, which subdivides this general life cycle into two major categories, heterohallism and non-heterothallism. The heterohallic group has a sexual system with mating types controlling haploid amoeboflagellate fusion to produce the diploid plasmodium, with meiosis occurring during spore formation. In contrast, the non-heterothallic group doesn't display a mating type system. Therefore, the individuals, in this group, are either homothallic (sexual with a ploidal cycle) or apomictic (non-sexual with no ploidal change). The non-heterothallic myxomycetes, following a long mycological tradition, have often been automatically labeled homothallic without any evidence as to a change in ploidy; however, this unsupported practice should not be continued.

# Heterothallism

The first report of heterothallism in the mycomycetes, by Pinoy (1908), was an obvious error, since he concluded that + and – plasmodia needed to fuse before sporulation could occur. As such the first acceptable

reports of heterothallism in a myxomycete were those of Skupienski (1917), working with an isolate of Didymium diforme, and von Stosch (1935) working with an isolate of Didymium iridis (identified as Didymium nigripes); however, the Skupienski isolate was later determined to be non-heterothallic by Schünemann (1930). The work of Dee (1960); using Physarum polycephalum and Collins (1961, 1962) working with Didymium iridis and Physarum pusillum marks the generally accepted beginnings of current heterothallic myxomycete studies. In all of these reports, the mating alleles were of two types (designated + and -); however, further studies with additional isolates of D. iridis (Alexopoulos & Zabka 1962, Collins 1963, Collins & Ling 1964, Mukherjee & Zabka 1964, Collins 1965), *Physarum pusillum* (Collins et al. 1964), and *P*. polycephalum (Dee 1966), indicated that these species possessed a one-locus (matA) multipleallelic mating system (any two amoeboflagellates that differ for the various mating alleles can undergo sexual fusion). Later reports of a second matB (Youngman et. al 1979) and third matC (Kawano et al. 1987) mating locus in P. polycephalum have not been found to occur in other species such as D. iridis (Clark 1983) and the two extra P. polycephalum loci are now generally considered to be mating type modifier genes (Dee 1978); that control cell fusion rates while the mating locus controls nuclear fusion and plasmodial differentiation (Bailey et al. 1990).

### **Syngamy**

The amoeboflagellate is a vegetative stage, which also serves as a gamete in the heterothallic isolates; therefore, it must undergo a conversion from the vegetative to the gametic state so that syngamy can take place. As the amoeboflagellate population increases in density (Shipley & Ross 1978, Pallotta et al. 1979), the cells produce a mating factor (Youngman et al. 1977, Albert & Therrien 1985) which apparently acts as a pheromone that induces the matA controlled amoeboflagellate to plasmodial transition (Nader et al. 1984), since it increases the rate of plasmodial formation not only in heterothallic crosses; but also in non-heterothallic cultures. Heterozygousity at the matB locus in P. polycephalum apparently only increase the rate of cell fusion (a lower rate of fusion occurs without mating typeB heterozygousity), thus providing more opportunities for the matA controlled plasmodial transition (Youngman et al. 1981), while heterozygousity at the matC locus increases the pH range in which cell fusions can take place (Shinnick et al. 1978). Plasmodial color locus segregation (Collins & Clark 1966) and cytophotometric DNA ploidal level (haploid amoeboflagellates and diploid plasmodia) changes (Collins & Therrien 1978) have confirmed that normal syngamy and meiosis occurs in the heterothallic isolates of the myxomycetes.

# Sibling biological species

A study (Henney 1967, Henney & Henney 1968) of nine isolates identified as Physarum flavicomum found that all nine isolates were heterothallic; however, when the mating types of the different isolates were crossed in all possible pair-wise combinations, it was found that the isolates formed three distinct non-interbreeding mating series. Four isolates, which displayed a one-locus multipleallelic mating group were then identified as Physarum rigidum due to slight morphological differences from the rest of the isolates. The other five isolates formed two genetically isolated sibling biological species since they could not be separated on morphological grounds and were not interbreeding. One on the sibling species, contained three interbreeding isolates, and the other sibling species contained two interbreeding isolates. More extensive studies using numerous isolates of the Didymium iridis morphospecies (Collins 1976, Betterley & Collins 1983, Clark & Stephenson 1990, Clark & Landolt 1993, Clark & Mires 1999, Clark et al. 2001) have produced similar results. Ten separate sibling biological species were reported in the D. iridis morphospecies. However, one of them was later assigned to the Didymium megalosporum morphospecies due to sight morphological differences (which overlapped with the *D. iridis* variations). It was also found that the barriers between the different biological species was only partial in that certain crosses between some of the biological species sporadically produced plasmodia which were generally weak and rarely sporulated (Clark et al. 1991). Sibling biological species have also been found within the morphospecies of Arcyria cinerea (Clark et al. 2002), Badhamia gracilis (Clark et al. 2002), Didymium ovoideum (Clark 2004), Didymium annellus (Clark & Landolt 2001), Didymium squamulosum (ElHage et al. 2000), Echinostelium minutum (Clark & Haskins 1998), Physarum globuliferum (Henney 1968), and Physarum pusillum (Clark &Landolt 2001). The sporangial morphology of the D. ovoideum biological species was also found to overlap with the D. iridis morphology (isolated originally identified as D. iridis were later found to belong to the D. ovoideum biological species).

### Non-heterothallism

The first report of non-heterothallism in the myxomycetes was by Jahn (1911), who used an isolate of Didymium squamulosum. This was followed by reports by Cayley (1929) on isolates of Didymium diforme and D. iridis (two isolates, one identified as D. nigripes), and by Schünemann (1939) on isolates of Physarum leucopus, D. diforme, and D. iridis (identified as D. nigripes). Von Stosch (1935) also found that isolates of Didymium iridis, D. difforme, D. squamulosum and Physarum cinereum were non-heterothallic and suggested that they were apogamic on the basis of chromosome studies. The report by Kerr and Sussman (1958) and Kerr (1961) that the amoeboflagellates of a non-heterothallic strain of D. iridis (identified by them as D. nigripes) underwent cell fusion (which they believed to be gamete fusion) and was thus homothallic, was later contradicted by von Stosch et al. (1964), who concluded that the same strain was apogamic on the basis of chromosome studies. Kerr's later report (1967) also found that plasmodia formation in this strain could occur without amoeboflagellate fusion. A number of reports, illustrated by the extensive studies on meiosis, by Wilson and Ross (1955), and syngamy, by (Ross 1957), on a number of species and isolates of myxomycetes could not settle the homothallic versus apomictic question. Thus, while amoeboflagellate cell fusion, is suggestive of syngamy, it is not a reliable criterion of sexual reproduction, since it has been shown (Bailey et al. 1990) that amoeboflagellate cells homoallelic for mating type can fuse and their nuclei can also fuse to form a diploid, but plasmodial formation does not occur. Also, the very small size of myxomycete chromosomes makes accurate chromosome counts and the identification of meioitic figures very difficult and not very definitive (see Collins 1979). Haskins (1976) had to use high voltage electron microscopy to count the 124 very tiny chromosomes in an apomictic isolate of Echinostelium minutum. Synaptonemal complexes (EM evidence of meiotic chromosome pairing) also does not prove that a sexual cycle is present, since isolates of Echinostelium minutum, Didymium iridis and Stemonitis flavogenita in which the complexes were found (Carroll & Dykstra 1966, Aldrich & Carroll 1971, Haskins et al. 1971, Gaither & Collins 1984) where later proven by cytophotometric DNA studies (Therrien & Yemma 1974, Haskins & Therrien 1978, Therrien & Haskins 1981, Collins et al. 1983) to lack a haploiddiploid ploidal cycle. Apparently, these isolates are automictic, with the chromosomes pairing during first division meiosis, but then the first division products fuse to reform the diploid state. Thus, the only method that can determine whether an isolate is homothallic or apogamic is via means of amoebofagellate and plasmodial photospectrometric DNA ploidy determinations. However, this is fairly difficult and has been done for only a handful of species and isolates. Of the 12 non-heterothallic isolates examined by cytophotometry to date, the following results have been obtained: one isolate of Echinostelium minutum (Haskins & Therrien 1978, Therrien & Haskins 1981), four isolates of Stemonitis flavogenita (Collins et al. 1983), and three isolates of Didymium iridis (Therrien & Yemma 1974, Therrien et al. 1977) were apogamic and four isolates of D. iridis (Therrien et al. 1974) were considered to be homothallic. However, one of the homothallic D. iridis isolates was later found to be an apomict that had partially converted to heterothallism (Collins 1980, Collins & Gong 1985). Both haploid and diploid amoeboflagellates were present in the culture, but apparently only the cells having haploid nucleic were considered to be amoeboflagellates. Thus, the occurrence of polyploid amoeboflagellates and plasmodia (Mulleavy & Collins 1979), chromosome elimination (Collins et al. 1978, and mixoploid plasmodia (containing nuclei of several different ploidal levels) (Kubbies et al. 1986) makes even DNA level determinates of apogamic or homogamic cycles difficult in the non-heterothallic isolates.

# **Apomictic-heterothallic conversion**

Three isolates, that were at first designated as non-heterothallic on the basis of plasmodial formation by single spore derived amoeboflagellates, were later found, after a period in culture, to also produce amoeboflagellate clones that could form plasmodia only after crossing with a heterothallic clone displaying a different mating type. In the Phi-1 (Yemma et al.1980) and Pan-4 (Collins 1980) isolates of D. iridis, and the CR-1 isolate of Stemonitis flavogenita (Collins et al. 1983), stable plasmodial producing non-heterothallic lines and stable mating type lines were established. Cytophotometric DNA measurements, in both of the *D. iridis* isolates (Yemma et al. 1980, Collins & Gong 1985) and the Stemonitis flavogenita (Collins et al. 1983) isolate, found that the stable non-heterothallic lines were diploid apomicts, and the crossing lines were haploids that produced diploid plasmodia when crossed. These apomictic-heterothallic conversions would seem to indicate that some apomictic isolated from nature, and therefore probably most non-heterothallic isolates, are created by a meiotic blockage that produces diploid spores containing both mating types.

# Polyploidy and apomixes

The mechanism for the formation of apomictic lines is not known; however, it is likely that polyploidy is somehow involved. Polyploid amoeboflagellates of the myxomycetes occur and can function normally as a vegetative cell and also undergo sexual fusion if it is a heterothallic line with mating type alleles (Collins et al. 1978). Mulleavy and Collins (1979, 1981) have shown that when two compatible diploid clones are crossed, a tetraploid plasmodium is produced that then produces diploid spores when it sporulates. Half of these diploid spores produce amoeboflagellate clones that are heterozygous for mating type and are also capable of producing plasmodia without crossing. However, when these diploid plasmodia sporulate they produce normal haploid lines that must be crossed in order to form plasmodia. Thus, while apomictic clones are produced, they are only temporary, and a mutation to block meiosis would be required to produce permanent apomictic lines. Since apomictic myxomcyetes do not undergo meiosis, they also do not need to have identical chromosome pairs. Thus, chromosome aberrations, including partial polyploidy, could be a mechanism to block meiosis that would produce permanent apomictic lines.

# **Reproductive variations**

While most myxomycete isolates have either heterothallic or non-heterothallic reproductive cycles, there are a number of variations that can depart from these common systems.

# Haploid amoeboflagellate selfing

Heterothallic amoeboflagellate clones, having a single mating type, can occasionally produce plasmodia without crossing to a different clone. While these plasmodia are often weak and have sporulation problems they can sometime complete the entire life cycle in culture. This clonal haploid selfing is generally sporadic and variable and has been shown to occur in D. iridis (Collins 1961, Collins & Ling 1968), P. flavicomum (Henney 1967), P. polycephalum (Dee 1960, Collins 1975, Adler & Holt 1975), and several additional species (Collins. unpublished, but cited in Collins 1979). The available evidence from genetic and cytophotometric studies on D. iridis (Collins & Ling 1968, Yemma & Therrien 1972, Therrien & Yemma 1975) indicates that the resulting plasmodia are haploid and were thus produced apogamously. It has been postulated (Yemma et al. 1974) that selfing is controlled in D. iridis by a cytoplasmic factor which acts on the mating type gene to induce plasmodial formation, and that some mating types are more susceptible to this induction. Wheals (1970), Cooke & Dee (1974), and Anderson et al. (1976) have reported a homothallic clone of P. polycephalum, which is actually a haploid clone that is not only capable of crossing with other mating type clones; but is also capable of producing plasmodia 100% of the time when it is not crossed. Generally, these plasmodia are produced by the formation of a haploid binucleate amoeba by mitosis followed by their fusion to form a diploid nucleus and plasmodial

formation (Anderson et al. 1976). However, it may also occur by the crossing of two amoeboflagellate cells, each carrying the mutant matA-h allele, since Wheals (1970) was able to show mating type and plasmodial fusion gene recombination and segregation. Collins & Tang (1988) studied a D. iridis heterothallic isolate which had selfed in nature, since it displayed only one mating type and could also produce plasmodia without crossing. Thus, rare haploid facultative apomictic lines can occur in nature. A series of papers (Therrien & Collins 1976, Collins et al. 1978, Ritch & Therrien 1987, 1988) investigated the possibility that a polyploid clone could induce the selfing of a haploid strain since it appeared that only haploid plasmodia were produced when crosses were attempted between the two clones. However, closer examination indicated that sexual fusion and plasmodial formation had taken place, but that the polyploidy chromosomes were rapidly eliminated from the plasmodium.

# Mating type mutation

While Collins (1965) reported that he had recovered a mating type mutant in D. iridis, the major examination of mating type and plasmodial formation mutations has occurred in P. polycephalum. The extensive studies (Adler et al. 1975, Anderson et al. 1976, Davidow & Holt 1977, Anderson 1979, Anderson & Youngman 1985, Anderson et al. 1989) on mutants that effect plasmodium development in P. polycephalum have found that most of these mutations are located on the mating type chromosome and fall into three groups. These are (1) the amoebal-plasmodium transition (apt) mutants that block plasmodial formation in clones carrying the Colonia mtA-h allele that allows plasmodial formation without crossing, (2) non-plasmodial forming mutants (npfA, npfB, npfC) that also block plasmodial formation in mtA-h lines, and (3) greater asexual development mutants (gad) that increase plasmodial formation without crossing in all mating types. The interaction between these mutants is complex, but it appears that the gad mutants permit higher levels of selfing by regulating the npfB locus (Anderson et al. 1989) and are thus like the mtA-h allele. Therefore, the mtA-h allele is probably a gad mutant of a mtA-2 allele, since npfB mutants with the

matA-h locus behave like mtA-2 alleles (Anderson 1978). How the other mutant types interact in regard to controlling plasmodial formation is still unknown, as is the exact mechanism of how plasmodial formation is controlled.

# **Sterility**

It was noticed in the early myxomycete reproductive studies (Collins 1961) that some amoeboflagellate clones did not form plasmodia either by themselves (in non-heterothallic isolates) or when crossed (in heterothallic isolates), and while some of these failures were no doubt due to unknown cultural problems such as pH tolerance (Collins & Tang 1973), many of them were likely due to sterility derived from a mutation or a developmental abnormality. Similarly, plasmodia produced in culture or isolated from nature are often found to not sporulate in culture (Clark & Collins 1976, Stephenson et al. 2004). Again many of these cases are due to unknown sporulation requirements (Collins 1979), but apparently some of them are also due to genetic mutations or developmental abnormalities. Thus sterile amoeboflagellate clones and plasmodia lines appear to be produced fairly often in the myxomycetes. This sterility and the fact that both amoeboflagellates and plasmodia can produce resistant structures during poor growth conditions (see Collins 1976) and that amoeboflagellates and plasmodial are potentially immortal (Clark 1984, Clark 1992) would seem to suggest that it is quite likely that sterile amoeboflagellate and plasmodial lines exist in nature.

# Occurrence

All of the known reproductive system reports for myxomycete species are shown in Table 1 except for a few papers with ambiguous results and those dealing with the details of plasmodial developmental regulation using mutations (see Anderson et al. 1989). Some reproductive system reports that have been published for certain species are not recognized as valid, either because the identification was in error or the evidence indicates that the presumed species is a variant of another species. The numerous references to *Didymium nigripes* by Kerr (for example 1961, 1967, 1968) have been incorporated into the *D. iridis* species

### **Ceratiomyxales (Ceratiomyxaceae)**

Ceratiomyxa fructiculosa (Müll.) T. Macbr. 1 non-heterothallic isolate: Costa Rica<sup>a</sup>

#### **Echinosteliales (Echinosteliaceae)**

Echinostelium coelocephalum T.E. Brooks & H.W. Keller

17 heterothallic isolates: 17 isolates (1 Arizona US<sup>b</sup>, 16 California US<sup>b</sup>) with a minimum of 9 mating types in one biological species

Echinostelium minutum deBary

30 non-heterothallic isolates: 30 isolates (various locations in the US<sup>c</sup>)

7 heterothallic isolates:

A1 biological species: 6 isolates (6 California US<sup>d</sup>) with 4 mating types A2 biological species: 1 isolate (Arizona US<sup>d</sup>) with 2 mating types

Semimorula liquescens Haskins, McGuinness & Berry 1 non-heterothallic isolate: Washington US<sup>a</sup>

### Liceales (Liceaceae)

Licea biforis Morgan

1 non-heterothallic isolate: Washington US<sup>a</sup>

#### Physales (Physaraceae)

Badhamia afinis Rostaf.

1 non-heterothallic isolate: Texas USe

Badhamia apiculospora (Härk.) Eliasson & Lundq.

2 non-heterothallic isolate: 1 isolate as *Physarum apiculosporum* (Finland<sup>f</sup>), 1 isolate as *Badhamia semiannulata* (Florida US<sup>g</sup>)

Badhamia follicola A. Lister

1 non-heterothallic isolate: California USh

Badhamia gracilis (T. Macbr.) T. Macbr. (also recognized as B. melanospora Speg.)

61 non-heterothallic isolates: 2 isolates (Canary Islands Spain<sup>i</sup>), 12 isolate (Mexico<sup>i</sup>), 27 isolates (Puerto Rico<sup>i</sup>), 1 isolate (Taiwan<sup>i</sup>), 9 isolates (Arizona US<sup>i</sup>), 2 isolates (California US<sup>i</sup>), 3 isolates (New Mexico US<sup>i</sup>), 5 isolates (Texas US<sup>i</sup>)

5 heterothallic isolates:

A1 biological species: 3 isolates (Arizona US<sup>i</sup>) with 4 mating types

A2 biological species: 2 isolates (New Mexico, USi) with 4 mating types

Badhamia utricularis (Bull) Berk.

2 heterothallic isolate: 1 isolate (Great Britian<sup>k</sup>), 1 isolate (California US<sup>h</sup>), could not be tested for multiple allelism.

Craterium obovatum Peck

1 non-heterothallic isolate: as *Badhamia curtissi* (unknown location<sup>1</sup>)

Fuligo cinerea (Schwein.) Morgan

1 non-heterothallic isolate: Tahiti<sup>m</sup>

Fuligo septica (L.) F.H. Wigg.

6 non-heterothallic isolates: 1 isolate (Alaska US<sup>n</sup>), 3 isolates (Texas US<sup>o</sup>), 1 isolate (West Virginia US<sup>n</sup>), 1 isolate (Wyoming US<sup>n</sup>)

4 heterothallic isolates:

A1 biological species: 4 isolates (Texas US°) with 4 mating types

Physarella oblonga (Berk. & M.A. Curtis) Morgan

2 non-heterothallic isolate: 1 isolate (Venezuela<sup>n</sup>), 1 isolate (unknown location<sup>p</sup>)

Physarum cinerum (Batsch) Pers.

7 non-heterothallic isolates: 2 isolates (Curacao<sup>q</sup>), 1 isolate (Great Britain <sup>s</sup>), 2 isolates (Indonesia<sup>q</sup>), 1 isolate (Taiwan<sup>r</sup>), 1 isolate (Venezuela<sup>q</sup>)

2 heterothallic isolate: 1 isolate (Germany<sup>t</sup>) with two mating types, 1 isolate (Venezuela<sup>h</sup>) with 2 mating types; the two isolates could not be tested for multiple alleles or biological species

Physarum compressum Alb. & Schwein.

48 non-heterothallic isolates: 6 isolates (Australia<sup>u</sup>), 18 isolates (Costa Rica<sup>h,u,v</sup>), 1 isolate (Columbia<sup>u</sup>), 8 isolates (Ecuador<sup>u,v</sup>), 3 isolate (Guatemala<sup>u,v</sup>), 1 isolate (Guyana<sup>u</sup>), 2 isolates (Honduras<sup>h,v</sup>), 1 isolate (Panama<sup>h</sup>), 4 isolates (Puerto Rico<sup>u,v</sup>), 2 isolates (Thailand<sup>v</sup>), 1 isolate (Tennessee US<sup>u</sup>), 1 isolate (West Virginia US<sup>u</sup>)

# Table 1 (Continued) Heterothallic and non-heterothallic reproductive system reports.

2 heterothallic isolates: A1 biological species: 2 isolates (1 Canary Islands Spain<sup>u</sup>, 1 Costa Rico<sup>v</sup>) with 4 mating types Physarum didermoides (Pers.) Rostaf. 1 non-heterothallic isolate: unknown location<sup>w</sup> 2 heterothallic isolates: A1 biological species: 2 isolates (1 Costa Rica<sup>a</sup>, 1Texas US<sup>a</sup>) with 4 mating Physarum flavicomum Berk. 5 heterothallic isolates: A1 biological species: 3 isolates (Texas US<sup>x,y</sup>) with 6 mating types A2 biological species: 2 isolates (1 Philippines<sup>x,y</sup>, 1 Texas US<sup>x,y</sup>) with 4 mating types Physarum gyrosum Rostaf. 5 non-heterothallic isolates: 1 isolate (Brazil<sup>t</sup>), 2 isolates (Guyana<sup>t</sup>), 1 isolate (Puerto Rico<sup>a</sup>), 1 isolate (unknown location<sup>h</sup>) Physarum globuliferum (Bull.) Pers. 3 heterothallic isolates: A1 biological species: 1 isolate (Texas US<sup>z</sup>) with two mating types A2 biological species: 1 isolate (Texas US<sup>z</sup>) with two mating types A3 biological species: 1 isolates as P. bilgramii (Texas US<sup>z</sup>) with two mating types Physarum leucopus Link 1 non-heterothallic isolate: Germanv<sup>aa</sup> Physarum melleum (Berk. & Broome) Massee 21 non-heterothallic isolates: 1 isolate (Costa Ricabb), 1 isolate (Mexicou), 18 isolates (Puerto Ricobb), 1 isolate (South Carolina US<sup>u</sup>) Physarum nicaraguense T. Macbr. 1 non-heterothallic isolate: from US<sup>cc</sup> (probably should be included in *P. compressum*) Physarum nudum T. Macbr. in Peck and Gilbert 1 non-heterothallic isolate: Great Britain Physarum polycephalum Schwein. 7 heterothallic isolates: A1 biological species: 7 isolate (1Russia $^{dd}$ , 1 Indiana US $^{ee}$ , 1 Iowa US $^{ff}$ , 2 Japan $^{gg}$ , 1 North Carolina US $^{ee}$ , 2 Wisconsin US $^{hh,ii}$ , 3 unknown USA origin $^{ee,ii}$ ) with at least 15 mating types Physarum pusillum (Berk. & M. A. Curtis) G. Lister 11 non-heterothallic isolates: 1 isolate (Australia<sup>a</sup>), 6 isolates (Costa Rica<sup>a,q</sup>), 1 isolate (Puerto Rico<sup>q</sup>), 1 isolate (Trinidad<sup>a</sup>), 1 isolate (Venezuela<sup>h</sup>), 1 isolate (Michigan US<sup>h</sup>) 3 heterothallic isolates: A1 biological species: 1 isolate (Arizona US<sup>jj</sup>) with 2 mating types A2 biological species: 1 isolate (Arizona US<sup>ij</sup>) with 2 mating types A3 biological species: 1 isolate (Mexico<sup>jj</sup>) with 2 mating types Physarum rigidium (G. Lister) G. Lister 4 heterothallic isolates: A1 biological species: 4 isolates (1 Costa Rica<sup>x,y</sup>, 1 Philippines<sup>w,x</sup>, 2 Texas US<sup>x,y</sup>) with 8 mating types Physarum roseum Berk. & Broome 1 non-heterothallic isolate: California US<sup>n</sup> Protophysarum phloiogenum M. Blackw. & Alexop. 1 heterothallic isolate: A1 biological species: 1 isolate (Colorado US<sup>ij</sup>) with 2 mating types Wilkommlangea reticulate (Alb. & Schwein.) Kuntze 1 non-heterothallic isolate: Pennsylvania US<sup>II</sup> as Cienkowski reticulata Didymiaceae Didymium annelus Morgan 7 non-heterothallic isolates: 5 isolates (Peru<sup>q</sup>), 2 isolates (Puerto Rico<sup>q</sup>) 2 heterothallic isolates: A1 biological species: 1 isolate (Puerto Rico<sup>q</sup>) with 2 mating types A2 biological species: 1 isolate (Puerto Rico<sup>q</sup>) with 2 mating types Didymium annulisporum H. W. Keller & Schokn. 1 non-heterothallic isolate: from unknown location<sup>mm</sup>

Didymium atrichum Henney & Alexop.

1 non-heterothallic isolate: Texas US<sup>nn</sup>

# **Table 1 (Continued)** Heterothallic and non-heterothallic reproductive system reports.

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Didymium difforme (Pers.) Gray
         10 non-heterothallic isolates: 4 isolates (Germany<sup>.n,s,aa</sup>,), 1 isolate (Great Britain<sup>r</sup>), 2 isolates (California US<sup>f</sup>), 1
         isolate (Minnesota USf), 1 isolate (Michigan USf), 1 isolate (Ohio USf)
Didymium dubium Rosaf.
         2 non-heterothallic isolates: 1 isolate (Indonesia<sup>a</sup>), 1 isolate (West Virginia US<sup>n</sup>)
Didyium laxifillum G. Lister & J. Ross
         2 non-heterothallic isolates: 1 isolate (Curacao<sup>q</sup>), 1 isolate (California US<sup>f</sup>)
Didymium megalosporum Berk. & M.A. Curtis
         2 non-heterothallic isolates: 1 isolate (Costa Rica<sup>oo</sup>), 1 isolate (Thailand<sup>oo</sup>)
         9 heterothallic isolates:
             A1 biological species: 9 isolates with some early reports as D. iridis (1 Curacao<sup>oo</sup>, 3 Mexico<sup>oo,pp</sup>, 2 Thailand<sup>00</sup>,
         1 Georgia US<sup>pp</sup>, 2 Hawaii US<sup>oo</sup>) with 11 mating types
Didymium minus (G. Lister) Morgan
         1 non-heterothallic isolate: West Virginia US<sup>n</sup>
         1 heterothallic isolate:
              A1 biological species: 1 isolate (Louisiana USh) with 2 mating types
Didymium iridis (Ditmar) Fr.
         68 non-heterothallic isolates: 7 isolates (Costa Rica<sup>f,oo,qq,ss</sup>), 1 isolate (Dominica<sup>ss</sup>), 3 isolates (Ecuador<sup>oo</sup>), 2
         isolate (France<sup>f,oo,ss,tt</sup>) with the Kerr D. nigripes isolate being one of them, 4 isolates (Germanyr<sup>s,t,aa</sup>), 1 isolate
         (Guatemala<sup>pp</sup>), 2 isolates (Indonesia<sup>oo</sup>), 1 isolate (Japan<sup>pp</sup>), 4 isolates (Panama<sup>f,ss</sup>), 1 isolate (Philippines<sup>f,ss</sup>), 2
         isolates (Puerto Rica<sup>oo</sup>), 2 isolates (South Africa<sup>f,pp</sup>), 3 isolates (Thailand<sup>oo</sup>),1 isolate (Trinidad<sup>f</sup>), 3 isolates
         (Tanzania<sup>n</sup>), 9 isolates (California US<sup>f,pp,rr,ss</sup>), 1 isolate (Georgia US<sup>f,rr,ss</sup>), 3 isolates (Hawaii US<sup>oo,ss</sup>), 1 isolate (Kansas US<sup>f</sup>), 6 isolates (Minnesota US<sup>f,rr</sup>), 1 isolate (Missouri US<sup>f,rr,ss</sup>), 1 isolate (North Carolina US<sup>pp</sup>), 3
         isolates (South Carolina US<sup>oo</sup>), 2 isolates (Texas US<sup>f,oo,ss</sup>), 2 isolates (Washington US<sup>a,f,rr</sup>)
         36 heterothallic isolates:
              A1 biological species: 20 isolates (10 Costa Ricaf,oo,rr,ss,uu, 1 Guatemalaqp, 3 Hondurasf,m,rr,ss,ww, 4
         Panama<sup>f,pp,rr,ss,ww</sup>, 1 New York US<sup>xx</sup>, 1 Iowa US<sup>f,rr,ss</sup>) with 18 mating types
              A2 biological species: 3 isolates (1 Kentucky US<sup>f,h,rr,ss</sup>, 1 Virginia US<sup>uu</sup>, 1 West Virginia US<sup>oo</sup>) with 5 mating
              A3 biological species: 1 isolate (Washington US<sup>rr,ss</sup>) with 2 mating types
              A4 biological species: 1 isolate (Panama<sup>yy</sup>) with 2 mating types
              A5 biological species: the A5 biological species was found to be D. megalosporum
              A6 biological species:1 isolate (Florida US<sup>zz</sup>) with 2 mating types
              A7 biological species: 1 isolate (Great Britain<sup>zz</sup>) with 2 mating types
              A8 biological species: 1 isolate (Hawaii US<sup>oo</sup>) with 2 mating types
              A9 biological species: 2 isolates (2 Costa Rica<sup>oo</sup>) with 4 mating types
              A10 biological species: 1 isolate (Mexico<sup>oo</sup>) with 2 mating types
         Undetermined biological species (not tested to the other biological species): 3 isolates (1 Australia<sup>aaa</sup>,1
         Honduras aaa,bbb, 1 Hawaii aaa) with uncertain connections to the A1 biological species have 8 mating types and are
         in 2 biological species; 2 isolates (Germany<sup>t</sup>, Georgia US<sup>f,rr</sup>) with no known connections to any of the known
         biological species each with two mating types
Didymium ovoideum Nann.-Bremek.
         9 non-heterothallic isolates: 3 isolates (Costa Rica<sup>oo</sup>,), 5 isolates (Indonesia<sup>oo</sup>), 1 isolate (Tennessee US<sup>oo</sup>)
         16 heterothallic isolates:
              A1 biological species: 15 isolates (1 Tennessee US<sup>oo</sup>, 10 Virginia US<sup>ccc</sup>, 4 West Virginia US<sup>ccc</sup>) with 2
         mating types
              A2 biological species: 1 isolate (South Carolina US<sup>a</sup>) with 2 mating types
Didymium saturnus H. W. Keller
         3 non-heterothallic isolates: 2 isolates (Peru<sup>q</sup>), 1 isolate (Iowa US<sup>mm</sup>)
Didymium squamulosum (Alb. & Schwein.) Fr.
         45 non-heterothallic isolates: 4 isolates (Australia<sup>u</sup>), 26 isolates (Costa Rica<sup>h,u,ddd</sup>), 4 isolates (Germany<sup>h,t,eee</sup>), 2 isolates (Indonesia<sup>ddd</sup>), 3 isolates (Puerto Rico<sup>ddd</sup>), 1 isolate (Thailand<sup>u</sup>),1 isolate (Hawaii US<sup>u</sup>), 1 isolate
         (Michigan US<sup>h</sup>), 1 isolate (Oregon US<sup>u</sup>), 1 isolate (Tennessee US<sup>u</sup>), 1 isolate (unknown location<sup>fff</sup>)
         19 heterothallic isolates:
             A1 biological species: 1 isolate (Costa Rica<sup>ddd</sup>) with 2 mating types A2 biological species: 4 isolates (4 Costa Rica<sup>ddd</sup>) with 6 mating types
              A3 biological species: 1 isolate (Puerto Rico<sup>ddd</sup>) with 2 mating types
              A4 biological species: 3 isolates (3 Costa Rico<sup>u</sup>) with 6 mating types
              A5 biological species: 1 isolate (Mexico<sup>u</sup>) with 2 mating types
              A6 biological species: 9 isolates (6 Australia<sup>u</sup>, 2 New Zealand<sup>u</sup>, 1 Thailand<sup>u</sup>) with 13 mating types
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# **Table 1 (Continued)** Heterothallic and non-heterothallic reproductive system reports.

Didymium vaccinum (Durieu & Mont.) Buchet

1 non-heterothallic isolate: Mexico<sup>a</sup>

#### **Stemonitales (Stemonitaceae)**

Comatricha laxa Rostaf.

4 heterothallic isolates:

A1 biological species: 4 isolates (Arizona US<sup>ggg</sup>) with 2 mating types

Comatricha lurida A. Lister

1 heterothallic isolate:

A1 biological species: 1 isolate Arizona UShhh with 2 mating types

Lamproderma arcyrionema Rostaf.

1 non-heterothallic isolate: Costa Rica<sup>ggg</sup>

Stemonitis flavogenita E. Jahn

7 non-heterothallic isolates: 1 isolate (Costa Rica<sup>h</sup>), 1 isolate (Ecuador<sup>h</sup>), 1 isolate Honduras<sup>h</sup>), 1 isolate (Indonesia<sup>n</sup>), 1 isolate (California US<sup>h</sup>), 1 isolate (Iowa US<sup>h</sup>), 1 isolate (Washington US<sup>h</sup>)

1 heterothallic isolate:

A1 biological species: 1 isolate (Costa Rica<sup>h,iii,jjj</sup>) with 2 mating types

Stemonitis fusca Roth

1 heterothallic isolate:

A1 biological species: 1 isolate (Utah USkkk) with 2 mating types

Stemonitis herbatica Peck

1 non-heterothallic isolate: India<sup>kkk</sup>

#### **Trichiales (Trichiaceae)**

Arcyria cinerea (Bull.) Pers.

10 non-heterothallic isolates: 8 isolates (Costa Rica<sup>III</sup>), 1 isolate (Ecuador<sup>III</sup>), 1 isolate (Mexico<sup>III</sup>)

3 heterothallic isolates:

A1 biological species: 1 isolate (Ecuador<sup>lll</sup>) with 2 mating types A2 biological species: 2 isolates (Ecuador<sup>lll</sup>) with 2 mating types

Perichaena vermicularis (Schwein.) Rostaf. 1 non-heterothallic isolate: Indonesia<sup>a</sup>

#### Table references:

<sup>a</sup>Clark et al. 2004, <sup>b</sup>Haskins et al. 2000, <sup>c</sup>Haskins personal communication, <sup>d</sup>Clark & Haskins 1998, <sup>e</sup>Blackwell personal communication, <sup>f</sup>Betterley 1981, <sup>g</sup>Raub et al. 1979, <sup>h</sup>Clark & Collins 1976, <sup>i</sup>Clark et al. 2003, <sup>j</sup>Liu 1990, <sup>k</sup>Carlile 1974a,b, <sup>l</sup>Ross 1964, <sup>m</sup>Collins 1961, <sup>n</sup>Clark 1995, <sup>o</sup>Alexopoulos & Henney personal communication, <sup>p</sup>Gehenio & Luyet 1950, <sup>q</sup>Clark & Landolt 2001, <sup>r</sup>Shen 1964, <sup>s</sup>Cayley 1929, <sup>l</sup>von Stosch 1935, <sup>u</sup>Clark & Stephenson 2003, <sup>v</sup>Irawan et al. 2000, <sup>w</sup>Polanowski 1973, <sup>x</sup>Henney 1967, <sup>y</sup>Henney & Henney 1968, <sup>z</sup>Henney 1968, <sup>aa</sup>Schünemann 1930, <sup>bb</sup>Clark & Stephenson 2000, <sup>cc</sup>Solis 1962, <sup>dd</sup>Collins & Tang 1977, <sup>ec</sup>Dee 1966, <sup>ff</sup>Collins 1975, <sup>gg</sup>Kawano & Kuroiwa 1989, <sup>bh</sup>Dee 1960, <sup>ii</sup>Kirouac-Brunet et al. 1981, <sup>ij</sup>Collins et al. 1964, <sup>kk</sup>Blackwell 1974, <sup>ll</sup>Therrien personal communication, <sup>mm</sup>Keller personal communication, <sup>nn</sup>Henney et al. 1980, <sup>oo</sup>Clark et al. 2001, <sup>pp</sup>Clark & Landolt 1993, <sup>qq</sup>Collins 1965a,b, <sup>rr</sup>Betterley & Collins 1983, <sup>ss</sup>Collins 1976, <sup>tk</sup>Kerr & Sussman 1958, <sup>uu</sup>Clark & Stephenson 1990, <sup>vv</sup>Collins & Ling 1964, <sup>ww</sup>Collins 1965, <sup>xx</sup>Collins & Tang 1989, <sup>yy</sup>Collins & Gong 1985, <sup>zz</sup>Clark et al. 1991, <sup>aaa</sup>Mukherjee & Zabka 1964, <sup>bbb</sup>Alexopoulos & Zabka 1962, <sup>ccc</sup>Clark & Stephenson 1994, <sup>ddd</sup>ElHage et al. 2000, <sup>eee</sup>Jahn 1911, <sup>fff</sup>Gustafson & Thurston 1974, <sup>ggg</sup>Clark & Haskins 2002, <sup>hhh</sup>McGuinness & Haskins 1985, <sup>iii</sup>Collins et al. 1983, <sup>jii</sup>Collins & Tang 1988, <sup>kkk</sup>Clark 1997, <sup>Ill</sup>Clark et al. 2002.

(Collins 1976), based on a reappraisal of its morphology. While *D. nigripes* is considered to be a valid species, these authors do not believe that it has so far been successfully cultured. On the other hand, *P. bilgramii*, is treated as a variant of *P. globuliferum* due to cultural studies (Henney 1968). Of the 51 species in the table, 38 are in the Physarales, one in the Ceratomyxales, one in the Liceales, two in the

Trichiales, three in the Echinosteliales, and six in the Stemonitiales. This distribution of reports, in regards to taxonomic order, is probably due to a number of factors, including the number of species per order, and the particular interests of individual researchers. However, the overriding factors are probably the abundance, distribution and culturability of a particular species, with the "weedy" species

being much more likely to be cultured (Clark 2004).

### Heterothallism and non-heterothallism

Fourteen of the 51 species in the table have both heterothallic and non-heterothallic reports, eight have only heterothallic reports, and 29 have only non-heterothallic reports. However, only five of the eight species with only heterothallic reports have five or more studied isolates, and only four of the 27 species with only non-heterothallic reports have five or more studied isolates. Therefore, it is likely that investigations involving more isolates, would show that many of these species display both reproductive systems. However, it is possible that some species, such as Echinostelium coelocephalum, are totally or mostly heterothallic, and others, such as *Physarum melleum*, are totally or mostly non-heterothallic.

# Multiple alleles

Thirteen of the sixteen species, having more than two heterothallic isolates in the same sibling species, displayed multiple alleles at the mating locus. Only the *Didymium ovoideum* A2 sibling species, in which sixteen isolates were examined, the *Arcyria cinerea* A2 sibling species, in which two isolates were examined, and *Comatricha laxa*, in which 4 isolates were examined, displayed only two mating alleles. Thus, multiple alleles, at the mating locus, appear to be nearly universal in the myxomycetes.

### Sibling species

Fourteen of the sixteen species (which could be tested) having two or more heterothallic isolates, displayed sibling biological species. Only *Physarum polycephalum* (7 isolates) and *Didymium megalosporum* (9 isolates) seem to lack sibling species. Thus, sibling biological species seems to be the norm in the myxomycetes.

#### **Methods**

The determination of heterothallism and non-heterothallism in the myxomyctes is straightforward once an isolate is in culture, however while many species are relatively easy to culture, others have been resistant to all attempts to date (see Clark & Collins 1976,

Clark 2000, Clark 2004). The recent review article by Haskins and Wrigley de Basanta (2008) on agar culture methods for the myxomycetes should be the starting point for anyone wishing to culture these organisms. Once an isolate is in culture, a fresh well formed sporangium should be used as a source for single spore amoeboflagellate isolations. Using sterile techniques, a spore suspension is produced and diluted to a level that provides a few widely spaced spores when spread on agar. Single spores can be picked up with a needle using a dissecting microspore with 60X magnification and sub-stage lighting (Collins 1963), and then placed in a new plate with the appropriate food organism. The spore will then germinate and grow into a clonal population. Alternatively, the dilute spore suspension can be spread on a plate with a lawn of food organisms and the germinating spores will form plaques, that can be transferred to new plates (McGuinness and Haskins 1985), or the spore suspension, mixed with a food organism, can be spotted on the plates and the germinating spores will then form a population in the spots from which they can be isolated. In all of these procedures, getting a proper dilute is the critical part, since it is necessary to insure that only a single spore is present in each case. Once isolated, the amoeboflagellate clones can be maintained by serial transfers to new plates or slant tubes. Non-heterothallic isolates will produce plasmodia in these clones, while heterothallic isolates, require crossing by mixing two different clones together (Collins 1979). For any particular heterothallic isolate half of the amoeboflagellate clones will be of one mating type and the other half will have a different mating type. You need approximately 15 clones to insure that you can detect heterothallism, and a second generation of clones, derived from a first generation cross, should be produced for both heterothallic and nonheterothallic isolates to insure that the system is stable.

The determination of homothallism or apomictic development is very difficult since normal cytological procedures are not adequate in the myxomycetes. Amoeboflagellate fusion is not proof of syngamy since homoallelic amoeboflagellates can fuse and form diploid nuclei; however, this produces a polyploidy

amoeboflagellate and not a plasmodium (Bailey et al. 1990). These polyploidy amoeolaellates, if heterothallic, can fuse to produce a plasmodium that then undergoes chromosome elimination so that it can have the same amount of chromosomes and DNA as the amoebolagellates (Ritch & Therrien 1988). Also, diploid apomictic lines, after a period in culture, can revert some of the amoeboflagellates back to haploid cells with mating types, therefore, the detection of haploid nuclei in a nonheterothallic culture is support, but not proof for a determination of homothallism (Collins & Tang 1988). Because of these problems, the only sure way of separating the two possibiities (homothallism and apogamy) is by genetic recombination studies (this would occur at high frequency only in homothallism), or microcineatography of the amoeboflagellate transition to plasmodia coupled with cytophotometric determinations of DNA levels in the amoeboflagellates and plasmodium. However, recombination studies require genetic markers which are rarely available, while microcinematography and cytophotometry requires specialized equipment and procedures (see Collins & Therrien 1976, Bailey et al. 1990) combined with careful cultural studies (need to start with a freshly re-cloned culture, to insure that the non-heterothallic behavior examined is not due to a mixed population of apomictic and revertant heterothallic amoeboflagellates). Thus, it is seldom useful to go beyond a non-heterothallic determination for an isolate, unless there is a compelling research need for the information.

#### **Conclusions**

Of the 900 or so recognized species of myxomycetes, 51 of them have had one or more isolates investigated as to their reproductive system. While this would seem to be a reasonable sample size, it is not a random sample, since reproductive systems can only be determined with cultured species. However, whether or not a myxomycete species has been cultured is dependent upon its abundance in nature and its specific requirements. Approximately 30% of the recognized myxomycete species (Schnittler & Mitchell 2000) are known from only a single or a few collections, and until recently, the culture of these collections was seldom attempted. There is also a great

variability in the ease of culture for those species which can be easily obtained for study. While the cosmopolitan and common Didymium iridis is easy to culture, the equally common and cosmopolitan Lycogala epidendrum has never been cultured despite numerous attempts. Except for Cribariaceae, at least one species has been cultured from every family (Clark 1995), although a number of families are poorly represented in culture and four (Cribariaceae, Reticulariacae, Dianemaceae, Clastodermataceae) have no mating system reports. Thus the reproductive systems studied are biased toward common cosmopolitan easily grown species, with a few rare specialized species present due to the particular interests of a number of investigators. The moist chamber method (small substrate samples are placed in a Petri dish with water and monitored for plasmodial formation and sporulation) which is commonly used to produce collections for study is also likely to bias the sample. Since non-heterothallic isolates require only a single spore to produce a collection, they would produce plasmodia and sporangia more frequently in moist chambers even if the number of heterothallic and non-heterothallic spores in the region were identical.

Based on these reproductive system studies, myxomycetes essentially have a heterothallic one-locus multiple-allelic mating system with a number of derived variations. During sporulation, meiosis occurs to produce spores which germinate to produce haploid amoeboflagellate cells that divide mitotically to produce a clonal population of identical cells. As the cell density increases, the amoeboflagellates become competent to act as gametes and fuse with another cell having a different mating type allele. This fusion creates a zygote which then grows into the coenocytic plasmodium that completes the lifecycle by sporulating. This basic system has been frequently modified by the development of nonheterothallic systems. The homothallic system, which is probably rare if it is actually present, retains the sexual reproduction system with haploid amoeboflagellate cells and diploid plasmodia, but has lost the mating types, so that any two amoeboflagellates can form a zygote. On the other hand, the apomictic system, which is probably the most common non-heterothallic form, has dispensed with sex altogether and produces diploid amoeboflagellate cells, by blocking meiosis, that are then capable of producing plasmodia without fusion. Also, a rare selfing (facultative haploid apomixes of heterothallic amoeboflagellates) system is present in a few isolates, where haploid amoeboflagellate cells produce haploid plasmodia apomictically. There are probably also a number of sterile reproductive systems in nature, where amoeboflagellate populations (that can't produce plasmodia) and plasmodia (which can't sporulate) are present and functional.

The driving forces behind the evolution of multiple alleles at the mating locus and nonheterothallic lines are probably identical. The resistant wind borne spores provide the myxomycetes with a very efficient dispersal mechanism, which could very easily produce a situation where an amoeboflagellate population derived from a single heterothallic spore would not have a mating partner. This problem could be partially overcome by multiple alleles, since the greater the number of mating types in the population at large, the greater the probability that amoeboflagellates derived from two random spores deposited on the same substrate would be compatible. And in the case of the non-heterothallic lines this problem is completely eliminated. The formation of biological sibling species is also probably related to dispersal. While the spores are apparently capable of maintaining a widespread morphological species, the great majority of individuals within a particular region would tend to be genetically related, since most spores would not travel great distances. This relatedness would allow the local populations to adapt to the local conditions and could also allow the development of reproductive barriers between distance sexual populations, which would produce the sibling species. Also, since the non-heterothallic lines are completely isolated from the sexual population and from each other, they would be free to adapt to very specific local conditions, and also to fix minor morphological variations.

### **Taxonomy**

This complex reproductive system has taxonomy implications for the myxomycetes.

Apparently many, if not most, of the morphologically defined species consists of a number of sibling biological species that are regionally centered, and also of a large number of genetically isolated non-heterothallic clonal lines. The local biological species can evolve into minor variants of the widely dispersed morphological species, and the clonal non-heterothallic lines could also evolve into a variety of integrating morphological forms. This can be seen in the cluster of recognized morphological species (D. nigripes, D. megalosporum, D. ovoideum, D. bahiense, D. verrucosporum, D. eximium) that centers on Didymium iridis. Culture and genetic studies have shown that a minimum of 20% of the heterothallic isolates identified as D. nigripes on morphological grounds are actually D. iridis, and a similar percentages of D. megalosporum and D. ovoieum isolates are identified as D. iridis. These studies also show that the morphological idenified heterothallic isolates of D. bahiense and D. verrucosporum are common variants that are found in the D. iridis biological species. Didyium eximum, like D. nigripes (all reported cultures were actually D. iridis) has never been cultured (or attempted), and in the opinion of the senior author, it is the same as D. ovoideum (D. ovoideum is the newer name and therefore this species should probably be called D. exium). In terms of the non-heterothallic isolates, they all blend together, and except for D. ovoieum isolates which has a yellow instead of brown plasmodium, only a few morphological distinct forms can be separated from D. iridis (Clark & Mires 1999). A similar cluster of recognized morphological species is centered on Diymium squamulosum, this cluster is proably even more complex, since it appears that there are a large number of recognized morphoogical species that are rare and are separated from D. squamulosum by one or at most a few fairly minor traits. It is quite likely that some of these rare species are local non-heterothallic lines that have evolved a distinct character trait. Other examples of similar, but smaller, species clusters can be seen centered on Physarum flavicomum and Physarum compressum, and others are probably quite common.

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