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Biosystematics of the myxomycete *Badhamia gracilis*

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Abstract: Sixty-four isolates that conformed to the general morphological description of *Badhamia gracilis* were isolated from several arid regions in the southwestern USA, northern Mexico, Puerto Rico, and the Canary Islands. These isolates were then subjected to a biosystematic study in which reproductive systems, culture characteristics, and morphology were examined. Five of the isolates were heterothallic and were divided into two separate biological species with multiple allelic mating systems: A1 consisting of three isolates (Arz 4, Arz 5, Arz 6) displaying four alleles, and A2 consisting of two isolates (NM 3, NM 4) also displaying four alleles. The remaining 59 isolates were nonheterothallic and presumably apomictic. All of the isolates had similar culture characteristics in that they had white (rarely with a yellowish tinge) plasmodia that sporulated at a relatively small size. While all of the isolates generally conformed to the standard species description, there were several variations from the norm that occurred at a high frequency. The sporotheca was often laterally flattened instead of globose or ovate, the spores generally averaged 10 μm instead of 14 μm in diam, and the capillitium often appeared physaroid instead of badhamoid. This study indicates that *Badhamia gracilis* is probably a widespread species complex consisting of a number of local sexual populations and numerous asexual clones that are adapted to arid conditions.

Key Words: apomixis, biological species, heterothallism

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INTRODUCTION

Despite our accumulated knowledge on the life cycle and physiology of the myxomycetes (Collins 1979, Clark 2000), classification in this group is still based almost entirely upon the characteristics of the fruiting stage, which is relatively constant, readily observable, and can be preserved indefinitely. However, the inherent genetic and environmental variations in these microscopic eukaryotes can sometimes make a precise morphological description difficult. In fact, in the myxomycetes and other eukaryotic microorganisms, a combination of relatively limited morphological traits, widespread distribution of the morphospecies, and frequent asexuality can make species definitions especially difficult (Sonneborne 1957, Clark 1995). Different subpopulations of a morphospecies can be genetically isolated apomictic clones, incompatible sibling species, or part of a single panmictic species (Clark 1995, Clark and Haskins 1998).

Reports on a number of species, especially the *Didymium iridis* (Ditmar) Fries complex, indicate that there is considerable variation in the extent of genetic exchange occurring within the morphologically defined species of this group. Within each morphospecies, there can be nonheterothallic and heterothallic isolates, with the heterothallic isolates being divided up into a number of geographically based sibling species that may have overlapping ranges (Betterley and Collins 1983, Clark and Stephenson 1990, Clark and Haskins 1998, Haskins et al 2000, El-Hage et al 2000). Nonheterothallic isolates, on the other hand, can complete their life cycle from a single isolated myxameoba and are therefore homothallic (crossing without mating types, not documented in the slime molds), automictic [the products of meiosis I fuse to produce a diploid nucleus, Sherman and Mims (1985)], or apomictic [no crossing or ploidy changes, Haskins and Therrien (1978)]. This variability in reproductive systems and its effect on how genes are transmitted raises questions concerning the relationships of these genetic groups to the often-cosmopolitan morphospecies of current usage. As such, there is a need for further biosystematic studies in the myxomycetes (Alexopoulos 1969, Collins 1979, Clark 2000).

Badhamia gracilis (Macbr.) Macbr. is generally found in arid regions and is one of the most common

TABLE I. Origin of isolates used in this study

Isolate	Origin[a]
Arz 1	cactus skeleton, Saguaro National Park, Tucson, Arizona
Arz 2 to 6	cactus skeletons, near Willcox, Arizona
Arz 7 to 12	cactus skeletons, Organ Pipe Cactus National Monument, Arizona
Ca 1 to 2	cactus skeletons, near Hell's Gate, Death Valley National Park, California
CI 1 to 2	cactus and agave skeletons, Las Mercedes, Tenerife, Canary Islands
Mex 1	cactus skeleton, Picanate Biosphere Reserve, Puerto Peñasco, Mexico
Mex 2 to 12	cactus skeletons, Playa la Jolla, Puerto Peñasco, Mexico
NM 1 to 4	cactus skeletons, near Carlsbad Caverns National Park, New Mexico
NM 5	cactus skeleton, near Alma, New Mexico
PR 1 to 3	cactus skeletons, site 1 Magueyes Island, Puerto Rico
PR 4 to 10	cactus skeletons, site 2 Magueyes Island, Puerto Rico
PR 11 to 26	cactus skeletons, site 3 Magueyes Island, Puerto Rico
Tex 1 to 5	*Opuntia* pads, Big Bend National Park, Texas

[a] All isolates were derived from moist chamber cultures by E. Haskins except for the CI isolates, which were grown from sporangia collected by J. Mosquera (TFC Mic 7318, 7354).

and prolific myxomycetes in the desert environment (Blackwell and Gilbertson 1980). For example, more than half of all field collections obtained over a four-day period in Big Bend National Park, Texas were specimens of this species (Stephenson unpubl). This species has been grown from spore to spore in culture several times [Alexopoulos in Gray and Alexopoulos (1968)] and a single isolate from Taiwan has been found to be nonheterothallic (Liu 1990). However, a detailed biosystematic study of *B. gracilis* in terms of reproduction, culture, and morphology has only just become possible with the recent accumulation of a large number of isolates from various geographical areas.

MATERIALS AND METHODS

The isolates (TABLE I) were derived from mature sporangia produced in moist chamber cultures of cactus skeletons, or as field collections from nature. Spores from the original sporangia were placed on CM/2 agar plates (8 g corn meal agar and 8 g plain agar per L distilled water) to produce amoebal populations and eventually plasmodia, which were then freed of filamentous fungal contamination by migration over water agar (16 g plain agar per L distilled water) and re-isolated. These plasmodia, grown on water agar and fed sterile rolled oats, grew to a relatively large size and were induced to sporulate by transferring them to water agar without the oats. These clean sporangia could then be used as a source for reproductive, cultural, and morphological studies.

For reproductive studies, the spores were plated onto CM/2 plates and the resulting amoebae were then dilution-plated in order to produce clonal populations derived from a single amoeba. If these clonal plates produced plasmodia by themselves, the isolate was classified as nonheterothallic; however, if they required crossing among themselves in order to produce plasmodia, they were then classified as heterothallic. Tester clones from each heterothallic isolate were then crossed to each other in all possible pairwise combinations in order to determine the presence of multiples alleles and biological species.

Morphological variations were compared against species descriptions in "The Myxomycetes" (Martin and Alexopoulos 1969) and specimens from the National Fungus Collections (BPI). Six *Badhamia gracilis* specimens (743544—Kansas, 743547—Kentucky, 743548—Mexico, 743554—Iowa, 743558—Kansas, 743559—Colorado), four *Badhamia affinis* Rost. (743156—Kansas, 743159—Texas, 743161—Iowa, 743163—New York), four *Badhamia macrocarpa* (Ces.) Rost. (743668—Utah, 743677—Venezuela, 743678—Turkey, 806078—Illinois), and four *Physarum straminipes* Lister specimens (805480—England, 810294—England, 810295—Netherlands, 810302—England) were examined and their variations compared to the cultured isolates.

RESULTS

Five of the isolates (Arz 4, Arz 5, Arz 6, NM 3, NM 4) were found to be heterothallic, while the other 59 isolates were nonheterothallic and presumably apomictic. The five heterothallic isolates each displayed two mating types for which tester mating type clones were selected for each isolate. These ten tester clones were then crossed in all possible combinations (TABLE II) in order to determine mating type multiple alleles and biological species. There were no plasmodia produced in any of the crosses of the three Arz isolates with the two NM isolates; therefore, the Arz and NM isolates belong to two different biological species: A1 (the Arz isolates) and A2 (the NM isolates). Crosses amoung the six tester clones of the three Arz isolates indicated that there were four different mating types distributed in the six testers. Similarly, the four testers of the two NM isolates also displayed four different mating types.

TABLE II. Multiple mating type alleles and biological species

	Arz 4		Arz 5		Arz 6		NM 3		NM 4	
	1	4	1	3	1	2	1	2	1	8
Arz 4-1[a] A1[1]	−[b]	+	+	+	+	+	−	−	−	−
Arz 4-4 A1[2]	+	−	+	+	+	+	−	−	−	−
Arz 5-1 A1[3]			−	+	−	+	−	−	−	−
Arz 5-3 A1[4]			+	−	+	−	−	−	−	−
Arz 6-1 A1[3]					−	+	−	−	−	−
Arz 6-2 A1[4]					+	−	−	−	−	−
NM 3-1 A2[1]							−	+	+	+
NM 3-2 A2[2]							+	−	+	+
NM 4-1 A2[3]									−	+
NM 4-8 A2[4]									+	−

[a] Clonal designations: Arz 4-1 is clone one of the Arz 4 isolate; and mating type designations: A1[1] is mating type one (the superscript) of the A1 mating series.

[b] Plasmodial formation: (+) indicates crossing and plasmodial formation, and (−) indicates non-crossing and no plasmodial formation.

All 64 isolates had very similar culture characteristics. The white plasmodia (isolates PR 18, Tx 2, and Tx 3 were very light yellow) grew normally but did not generally produce large plasmodia, since they sporulated at a relatively small size prior to the utilization of all available nutrients. The myxamoebal stage, on the other hand, was typical for the myxomycetes in terms of growth and behavior.

The morphology of the cultured isolates generally conformed to the standard description in Martin and Alexopoulos (1969); however, there were some consistent minor variations from the standard and some isolates were consistently at the extremes. In the information given below, the standard description will be given first and this will be followed by the variations from this standard that were found in the cultured isolates. Habit gregarious or clustered—most (54 of 64) of the isolates and all of the herbarium specimens conformed to this standard, but a few (10) of the isolates were multi-headed with fused stalks. Sporotheca globose or ovate and 0.5–0.7 mm in diameter—although globose and ovate sporotheca could be found in all of the isolates, the most common shape in the majority (41 of 64) of the isolates and specimens (5 of 6) was a laterally compressed kidney shape that was seldom over 0.5 mm in diameter. Peridium thin, translucent and sparsely flecked with white calcareous nodules—the peridium of the majority (53 of 64) of isolates and all of the specimens was covered with a rugose calcareous crust that was reduced to scattered nodules on low lime sporangia. Stalks sessile to long (usually short), pale straw-yellow to pinkish, weak, sulcate, and more or less twisted and with an umbilicate attachment—the stalks of most (45 of 64) of the isolates and all of the specimens had a light orangish tinge and a number of the isolates (19) had fairly robust upright stalks. Capillitium delicate and of uniform-diameter calcareous tubes (some massed in center)—the isolates displayed considerable variation in this trait, with 27 being completely badhamoid; 28 mostly badhamoid, seven mostly physaroid, and two completely physaroid, however, the specimens were less variable, with 5 completely badhamoid and one mostly badhamoid capillitial forms. Spores dark brown, globose (may appear angular due to the reticulate ridges), closely and irregularity warted, 12–16 μm in diameter, and having a course reticulum of ridges—a few (6 of 64) isolates had lighter brown-colored spores with very faint to no reticulation, and spore diameters seldom exceeded 12 μm in all of the isolates. The six herbarium specimens conformed to the spore description except for sightly smaller diameters of 9–12 μm.

DISCUSSION

The reproductive system of *Badhamia gracilis* is fairly typical for the myxomycetes (Clark 1995). This morphospecies consists of numerous asexual clonal lines and relatively rare heterothallic isolates that are probably divided into geographically separated biological sibling species with multiple allelic mating types.

In culture, the myxamoebal stage did not display any obvious adaptions (e.g., rapid growth or plasmodial production at low densities) to an arid habitat. However, the plasmodial stage sporulated at a relatively small size before all of the nutrients were utilized. This is an atypical behavior for most myxomycete species, for which starvation acts as a sporulation trigger (Gray and Alexopoulos 1968). The only variation detected in the vegetative stages of the 64 isolates was the occurrence of a few light yellow-tinged

plasmodia instead of the pure white color found in the rest of the isolates. Blackwell and Gillbertson (1984), who studied the distribution and sporulation phenology of some of the more common species of myxomycetes occurring in the Sonoran desert of Arizona, reported that *Badhamia gracilis* developed in moist chamber cultures prepared with samples of dead cactus tissue with a pH (8.7–10.4) which is much higher than is typical for the substrates upon which most myxomycetes usually develop. Moreover, spore germination in the one isolate they tested under such conditions was not significantly reduced after the spores had been incubated for four months at 30 C. Both of these characteristics are obvious adaptations to a warm desert environment, but neither substrate pH nor spore germination was examined in the present study.

While the morphology of the isolates we examined was quite variable, it was coherent enough to form a single entity. However, the standard description in Martin and Alexopoulos (1969) needs to be somewhat expanded to include some of these variations. The occurrence of laterally flattened (often kidney shaped) sporotheca is the most obvious omission, but the rugose lime-covered peridium is also more common than is the presence of nodules as mentioned in the description (nodules appear in sporangia with less lime). Minor variations are the orangish brown-tinged, instead of straw-yellow, stalks and the occasional physaroid capillitium. The dark brown spores with abundant warts and a lax reticulate ridge are quite distinctive. We have found that the spores are generally somewhat smaller (9–13 μm instead of 12–16 μm) and have distinctive thick walls, not mentioned in Martin and Alexopoulos (1969). Also, a few isolates have thinner walls, and the reticulate ridge is missing or greatly reduced. None of our isolates conformed to the *B. gracilis* var. *melanospora* (Speg.) Castilla, Moreno & Illana description (Castillo et al 1996) which has larger (15–18 μm), usually darker spores. However, in the light of the spore variations that we did see, such variations would not be unexpected and are therefore most likely to form an integrated continuum with the rest of the species complex.

When compared to other species, *Badhamia gracilis* is generally distinct; however, some of the variation extremes can be confused with related species. The generally sessile, or short dark stalked, discoid sporangium of *Badhamia affinis* is covered with a thin rugose incrustation of lime, and its general appearance can overlap that of *B. gracilis*. However, the generally larger size, dark stalks, delicate capillitial network and dark brown spores densely covered by small spinulose ornamentations adequately separate these two species. The generally globose, sessile (may have short light-colored stalks) sporangia of *Badhamia macrocarpa* has a peridium covered with a thin rugose lime coat, and its appearance can also overlap with the generally smaller *B. gracilis* sporangium. However, in this case, the capillitia are indistinguishable and the spores can not be completely separated, since a few *Badhamia gracilis* isolates lack the distinctive thick walls and reticulate ridges of the species and some *B. macrocarpa* specimens have thick-walled spores which may have a faint reticulate ridge. While electron microscope studies have shown that the spores of *Physarum straminipes* and *Badhamia gracilis* are nearly identical (Scheetz and Alexopoulos 1971, 1976), the generally larger obovoid sporangium of *P. straminipes* has a short, lax, pale stalk merging into the sporotheca (no umbilicus) and displays a distinct physaroid capillitium. However, some of the smaller, sessile, globose sporangia of *Physarum straminipes* appear very similar to those *Badhamia gracilis* isolates that produce the more physaroid capillitial forms.

Nonheterothallism (presumptive apomixis) is a common reproductive system in the myxomycetes and occurs along with heterothallism in the same morphospecies (Clark 1995, 2000). Different species vary from mostly nonheterothallic to mostly heterothallic, and *Badhamia gracilis* is in the mostly nonheterothallic class, with a small number of heterothallic biological sibling species. The rapid sporulation of plasmodium prior to the utilization of all available nutrients is apparently an adaption to the short wet periods of arid environments where this species is most common. While *Badhamia gracilis* has a variable morphology that can overlap with *Badhamia macrocarpa* and *Physarum straminipes* variations, it is generally distinct. Therefore, the existence of a single *B. gracilis* morphospecies (albeit consisting of a complex of several sibling heterothallic species and numerous apomitic lines) is corroborated by this biosystematic study.

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