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Professor ***

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The Evolution of Fungi (Basidiomycota) Spore Dispersal: Loss of Ballistospory in Gasteromycete Fungi

Once considered part of the plant kingdom, fungi have long since been placed closer to animals on the tree of life. Fungi play an important role in many aspects of the ecosystems they inhabit, as well as the lives of humans. Not only are they incorporated into many food and beverage making processes utilized in modern society, they are also involved in the production of many commonly used antibiotics such as penicillin (Elder 1944). Additionally, as one of the few organisms that can break down cellulose, they are vital as decomposers and recyclers of nutrients (Baldrian and Valášková 2008). Recent studies suggest that they may even act as pathogens that lead to negative frequency selection which promotes plant and animal diversity (Gadd *et al.* 2007).

Despite the many contributions of fungi to the modern world, humans have been hard-pressed to learn a significant amount about this group of organisms. Dedicated mycologists throughout history have devoted their lives to the study of this intriguing kingdom, yet there is still a great deal of knowledge to be gained. Fungi have varying life cycles and unique natural histories—many of which are far from well understood. An onset of problems has arisen from the fact that not all fungi can be cultured and studied in a lab. Additionally, for decades scientists have been identifying the asexual and sexual fruiting bodies of the same mushroom as separate species (Hibbett and Taylor 2013). Considering that most of these fruiting body forms are

seasonal and that the fungal taxonomic system changes so much, this mistake is understandable. However, with the advent of new DNA sequencing methods, the progression of this field has been positively advanced and will continue to be benefitted in the future. As this new technology becomes more readily available and affordable, scientists can now interpret the evolutionary relationships among fungi lineages with greater confidence. With this data as a foundation, evolution within the fungi kingdom can be better understood. Uncovering the factors responsible for the wide range of fruiting body forms seen in fungi as well as other unique adaptations these organisms possess will be crucial in determining why such a vast range of diversity exists within this group.

One particular aspect of mycology that has been increasingly clarified with molecular technology is the evolution of spore dispersal mechanisms of fungi in the phylum, basidiomycota. Basidiomycetes are fungi that are characterized by sexual reproduction via specialized, club-shaped end cells called basidia (Miller and Miller 2006). These basidia usually produce four basidiospores that are forcibly discharged from the tip—or hilar appendix—of the basidia by a complicated mechanism called ballistospory (Money 1998). This mechanism involves a water droplet called Buller's Drop that coalesces on the hilar appendix and launches the spores a good distance away from their place of origin (Money 1998). While the intricacies of this particular mechanism aren't necessary to spell out, it is important to note the complexity of morphological features and the exploitation of ecological aspects involved in the evolution of this particular spore dispersal method.

Not surprisingly, a number of other spore dispersal mechanisms have also been noted among various basidiomycetes. So many, in fact, that in 1824, Elias Magnus Fries, a Swedish mycologist and botanist, felt comfortable creating a new class for those who had lost the ability

of ballistospory and the forcible discharge of their spores (Fries 1824). This group was titled the Gasteromycetes, meaning “stomach fungi”. Some of the fungi that were classified into this new group were the stinkhorns (Phallaceae), puffballs (Lycoperdaceae), earth stars (Geastraceae), and bird’s nest fungi (Nidulariaceae) (Miller and Miller 2006). They all possess the unifying features of statismospory—meaning they lack forcible discharge of their spores—and an enclosed hymenium (Miller and Miller 2006). The hymenium is the fertile surface where sexual spores are produced. Instead of producing spores on an exposed surface like gills, however, the spores in this group of fungi are produced internally inside a fruiting body. Though these fungi still possess basidia and produce basidiospores, they lack the hilar appendix and, therefore, the complicated evolutionary equipment that necessitates forcible spore discharge. These fungi have evolved other simplistic but unique methods of spore dispersal in lieu of losing their ballistosporic capabilities.

Though the gasteromycetes classification system was utilized well into the twentieth century, it soon became evident that the class was polyphyletic. In a 1995 study of British gasteromycete species, Pegler *et al.* noted that "these fungi represent a heterogeneous assemblage, a mixture of forms which are derived from various lineages…[they] can be collectively referred to as gasteroid fungi, but they cannot be classified as a single group" (Kotlaba and Pouzar 1996). Likewise, Webster (1980) called the gasteromycetes an “unnatural assemblage of basidiomycetes.” The word “gasteroid” is now more widely accepted as a morphological descriptor rather than a valid cladistic term. Although the fungal phylogenetic tree has always been far from static, mycologists have taken this information in full stride. Many recent papers have been published concerning the true positions of these gasteroid forms within the basidiomycota clade based on molecular data. The simplistic body forms of gasteromycetes

made classifying these organisms difficult in the past, but modern DNA sequencing technology has helped in providing key information for the accurate phylogenetic organization of these fungi.

A recent phylogeny concerning the evolution of gilled mushrooms and puffballs based on rDNA data was presented by Hibbett *et al.* (1997). The researchers were able to support the claim that gasteromycetes are a polyphyletic group. They found a correlation between the repeated loss of forcible spore discharge and the origination of gasteroid forms from fungi with exposed spore-bearing structures. Fungi with enclosed spore-bearing structures evolved at least four times, as opposed to originating from a single ancestor. They also noted that fungi that possess ballistospory have never been secondarily derived from gasteromycetes, which suggests the possibility of statismospory being an evolutionary dead end. Once the forcible discharge spore mechanism is lost, it appears that natural selection favors simplicity and ballistospory cannot be regained. Another paper published by Matheny *et al.* (2006) provided similar results to those of Hibbett *et al*. concerning the placement of these gasteromycetes in a phylogeny and was based on rRNA data (Matheny *et al.* 2006).

In the paper by Hibbett *et al.* (1997), the researchers also confirmed that diverse fruiting body forms and spore dispersal mechanisms have evolved among the gasteromycetes. This becomes evident when the spore dispersal mechanisms of bird's nest fungi and puffballs are juxtaposed. While both rely on rain drops to expel their spores, puffballs release puffs of spores from an operculum, or tiny hole, at the top of their fruiting body sack (Miller and Miller 2006). Conversely, bird's nest fungi are comprised of an upturned, concave fruiting body "nest" that holds tiny spore packages resembling eggs (peridioles) (Brodie 1975). These "eggs" are splashed out of the cup upon impact by rain drops. These alternatives to ballistospory, however, were

found to have only evolved once (Hibbett *et al.* 1997). This finding provides further support for the loss of forcible spore discharge being an evolutionary dead end for gasteroid forms.

One question that is impossible to ignore when discussing gasteromycetes and the evolution of statismospory is what may have caused these fungi to lose the ability to forcibly discharge their spores. Unfortunately, the research surrounding this question is not as concrete as that which supports gasteromycetes as a polyphyletic group. Undoubtedly there are multiple factors involved in the answer to this question and it could potentially become much clearer given more research and cross-analyses of ecological, morphological, and phylogenetic data.

Hibbett *et al.* (1994) discuss one potential reason for the evolution of an enclosed hymenophore that must occur in the transition from a nongasteroid to a gasteroid fruiting body. In the gilled mushroom, *Letninus tigrinus*, a naturally occurring mutant has been found (Hibbett *et al.* 1994). This developmental mutant possesses a recessive allele at a single locus that codes for an enclosed hymenophore like that of gasteroid forms. This data suggests that the evolution of gasteromycetes could be conferred by a small number of mutations in genes with impactful genotypic effects. Although the genetic underpinnings of gasteromycetization in other lineages aren't well understood, this is just one potential cause. There is room for a number of potential studies on the genetic bases underlying the loss of the hilar appendix and other morphological features that permit ballistospory to occur.

Theories regarding the evolutionary pathways of gasteromycetes have also been discussed in today's literature. In a paper by Wilson *et al.* (2011) the researchers explore the concept of secotioid forms being a "stepping stone" to gasteroid forms (Wilson *et al.* 2011). Secotioid forms produce their spores internally, but in many cases have not lost ballistospory.

These intermediate forms have been described in multiple fungal lineages of mushrooms that possess forcible spore discharge. In this paper the researchers produced models that suggest gasteroid forms will eventually become more prolific than nongasteroid forms in the clades in which they have arisen. Additionally, they suggest that the low frequency of gasteroid forms, overall, could indicate the recent evolution of many gasteroid lineages. The data presented in this study leads to many questions. If gasteroid forms are evolving so quickly, why haven't they already taken over? Additionally, why haven't gasteroid forms evolved until fairly recently in various fungal lineages?

Nevertheless, before concluding that the entire basidiomycota phylum will soon be comprised of gasteromycetes, there are a few clarifications to be made regarding this study. It appears that the researchers only focused on highly specialized gasteromycetes to make the models for this paper (Wilson *et al.* 2011). These fungi already possess complex nonballistosporic spore dispersal mechanisms and have had time to become well adapted to their environments. If all newly evolved gasteroid forms had these attributes, gasteromycetization would most likely be favored. However, secotioid forms exist as an intermediate stage most likely in the transition state from nongasteroid to gasteroid. They lack ballistospory and specialized adaptive spore dispersal mechanisms. This leaves them at a selective disadvantage and puts them at risk for extinction. This evolutionary reasoning is more consistent with the lower frequency of gasteromycetes seen in today's fungal lineages. Therefore, the models generated in this study are not necessarily representative of the likely evolutionary pathways of all potential gasteroid taxa. The gasteromycetes used in this study are exceptionally successful forms that have already overcome the secotioid threshold and learned to thrive. To this day their evolution matches—if not succeeds—that of their nongasteroid relatives. Most secotioid forms

will probably never reach that point, but those that become the most successful will likely continue to flourish in the lineages in which they have appeared.

As for the proposed recent evolution of gasteroid forms, it seems that other factors such as habitat and ecology should be considered. However, these, too, are understudied areas of gasteromycete research. While the life history and habitat preferences of a few popular species have been researched, virtually no information exists for other gasteroid forms. The bird's nest fungi, for example, belong to the Nidulareaceae family. One mycologist, Harold Johnston Brodie, wrote a book about this group in 1975 and it remains to be one of the only sources of information regarding bird's nest fungi to date. In his book, Brodie discusses the possibility of the peridioles, or spore sacks, of the bird's nest fungi being secondarily herbivore dispersed (Brodie 1975). He conjectured that, because he found certain genera within the family growing on livestock feces, they might require a heat shock by passing through an intestinal system in order to germinate. If this is true, this secondary dispersal method could potentially support the evolutionary dead end of statismospory found in the Nidulareaceae family. Perhaps the possession of the more complicated dispersal method was too energetically costly and was not conducive for regaining the ballistospory mechanism. More research needs to be conducted concerning the potential influence of environmental factors on the evolution of spore dispersal mechanisms and fruiting body types in multiple lineages of fungi in order to further explore this concept.

Regarding recent papers on gasteromycete phylogenies, it might be too presumptuous to assume that the inability to re-evolve ballistospory once it is lost is contingent upon the complicated morphological features involved in the mechanism rather than the influence that the environment may have on these fungi. For the Nidulareaceae, their reliance on an herbivore may

have proved more beneficial than maintaining ballistospory considering the environments they occasionally colonize. However, once again, this argument could be strengthened with more studies regarding the interplay of environment and spore dispersion for various gasteroid fruiting body forms. It could also help provide some insight into why the evolution of gasteromycetes has been a fairly recent phenomenon. Perhaps environmental changes are spurring the evolutionary shift from ballistospory to statismospory.

Although the discipline of mycology has come a long way since fungi were first grouped among plants, there are still major gaps in the collective knowledge of the evolution, life cycles, and natural histories of the organisms that comprise this family. Fairly recent introductions of molecular technology methods have helped immensely in clarifying questions regarding phylogenetic relationships and speculative evolutionary pathways of various fungal lineages—especially within the gasteromycetes. Though the exact evolutionary causes behind gasteromycetization are still being explored, it is clear that a number of basidiomycetes have undergone a unique evolutionary pathway with regard to their spore dispersal methods. This area of mycology would greatly benefit from more studies on the role environmental factors have in the evolution of spore dispersion, possible genetic causes that may be rooted in the transition of nongasteroid forms to gasteroid forms, and more cross-analyses of the potential ecological and morphological benefits that come with gasteromycetization. Grasping a better understanding of the involvement of these factors in the evolution of ballistospory to statismospory might also give some insight into other adaptive aspects of fungi such as the diversity of fruiting body forms. Regardless, there is still an exciting amount of knowledge to be uncovered about this unique and vital group and modern scientific techniques should be directed toward the furthering of dedicated research efforts concerning multiple aspects of the fungi kingdom.

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