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Keynote Paper

The poetry of mycological accomplishment and challenge☆

John W. TAYLOR

Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA

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ABSTRACT

Adaptation is the evolutionary process that has given us the remarkable diversity of fungi and I cannot think of a theme better suited to bringing together the wide variety of topics at this congress: pathogenicity, cell biology, genomics, evolution and ecology. It is also a timely theme because the latest biological innovation, genomics, has stimulated new thinking about the types of genetic variation and subsequent selection that enable adaptation. For more than half a century the Modern Synthesis of natural selection and genetics has provided the framework for evolutionary inquiry as reviewed by Kutschera and Niklas (2004). The Modern Synthesis is focused on point mutations as the mode of genetic variation, but genomics has emphasized the role of gene loss and gene gain by duplication or horizontal transfer in generating genetic variation. Likely, the Modern Synthesis can be stretched to accommodate these new processes, as it was to accommodate neutral evolution (Pigliucci, 2007). However, there are those who are calling for a new synthesis and I encourage mycologists to take a look at these proposals because fungi may be the organisms of choice in testing these ideas (Koonin, 2009a, b).

Adaptation in fungi is a theme with an abundance of material due to the efforts of many mycologists and I have time for just three stories that showcase these accomplishments. At the same time, great challenges remain and I aim to highlight these, as well. My title promises more than science, it promises poetry. There being a rich tradition of poetry in Scotland, I'll begin with the most famous stanza of what is possibly the most famous Scottish poem, "To a Mouse."

> But Mousie, thou art no' thy lane,
> In proving foresight may be vain:
> The best-laid schemes 0' Mice an' Men, Gang aft agley,
> An' lea'e us nought but grief an' pain
> For promis'd joy!

I'm ashamed to admit that when I spring this poem on my friends in Berkeley, they ask, as often as not, "It's Shakespeare, isn't it?" That is not a mistake that would be made in Edinburgh because To a Mouse is the work of the bard of Scotland, Robert Burns (Fig. 1).

1. Pathogenesis

Keeping Burn's thoughts in mind, I'll begin with a story of adaptation in pathogenesis. We'll start with flax rust, the

☆ The opportunity to open an International Mycological Congress is a singular honor and I offer my sincere thanks to Nick Read, Chair of the IMC9 program committee, and its membership: Simon Avery, Nicholas Clipson, Geoff Gadd, Neil Gow and Geoff Robson. Thanks also are due to Neil Gow for his thoughtful introduction and Nina Cosgrove and her crew from Elsevier for implementing the committee's vision for this glorious congress.

E-mail address: jtaylor@berkeley.edu

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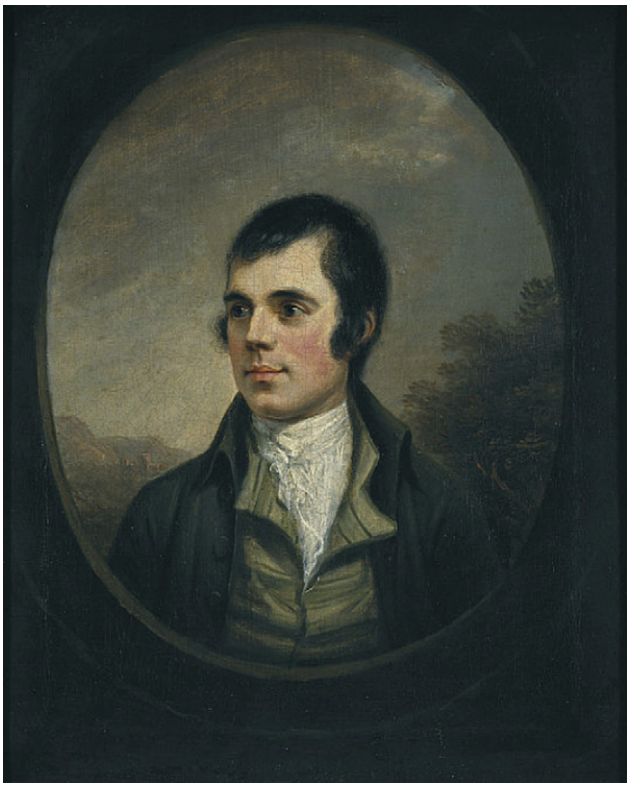

Fig. 1 – Portrait of Robert Burns by Alexander Nasmyth 1787, Scottish National Portrait Gallery.

Fig. 2 – Flax rust, *Melampsora lini* Lev., showing orange uredinia on flax, *Linum* sp. Reproduced from the cover of the Plant Cell, Ellis *et al.* (2007).

disease of *Linum* species caused by the rust, *Melampsora lini* (Fig. 2), and follow it to North Dakota. The gene-for-gene hypothesis to explain host resistance and pathogen avirulence was developed in that state by Flor in mid-twentieth century. It was the first study of the genetics of both host and parasite and represents one of the great accomplishments of mycology (Flor, 1971). For biotrophic pathogens, like rusts and powdery mildews, the recognition of pathogen-produced avirulence gene products (effectors) by plant-produced resistance gene (R genes) products, and the ensuing plant hypersensitive response, has driven a tremendous amount of basic research and practical plant breeding (Chisholm *et al.*, 2006; Jones and Dangl, 2006). We now know that molecules associated with fungi, for example, chitin or ergosterol, collectively known as pathogen-associated molecular patterns (PAMPs), are recognized by plants, which then mount generalized responses, such as, callose deposition to thicken cell walls or the production of reactive oxygen species, in a process termed PAMP-triggered immunity (PTI). Not surprisingly, biotrophic fungi have evolved effectors, which disrupt the PTI. Equally not surprisingly, plants have evolved R genes to recognize effectors and, through subsequent programmed cell death, kill the plant cell harboring the biotroph, thereby starving it (termed effector-triggered immunity, ETI). Studies of the evolution of effectors and R genes document the evolutionary back and fourth (Fig. 3) associated with avoidance of detection by the pathogens and restoration of detection by the plant (Anderson *et al.*, 2010; Stukenbrock and McDonald, 2007). Of course, there is a cost to resistance (Korves and Bergelson, 2004; Tian *et al.*, 2003), and questions about this cost have arisen as plans are made to add stacks of resistance genes to crops (Rausher, 2001; Stuiver and Custers, 2001), although

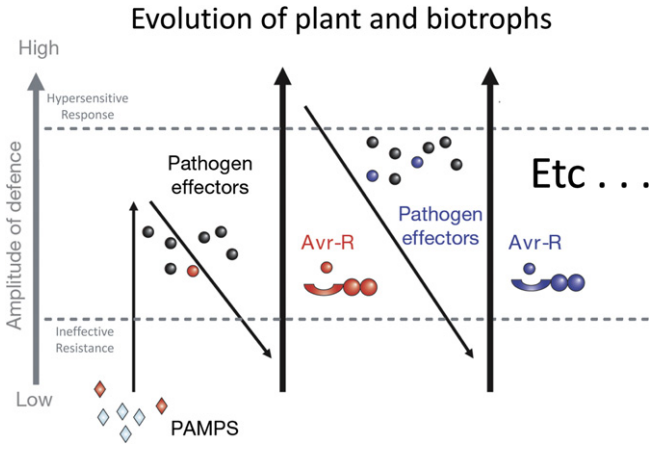

Fig. 3 – Diagram of the evolution of plant immunity beginning with pathogen-associated molecular patterns (PAMPS) that lead to PAMP-triggered immunity and progressing to effectors, plant resistance genes (Avr-R) and effector-triggered immunity. Modified from Jones and Dangl (2006).

farmers seem willing to suffer a known, regular loss to avoid a catastrophic one.

Alas, most of the work on gene-for-gene hypothesis has considered the interaction of just one pathogen and one host, but plants in nature face more than one parasite and not all of them are biotrophs. Consider the necrotrophs, pathogens that kill plant cells with toxins and then feast on the dead cells (Govrin and Levine, 2000). Recently it has been shown that the toxins produced by these fungi, e.g., *Phaeosphaeria nodorum* and *Pyrenophora tritici-repens* (many of them seem to be Dothideomycetes), are akin to elicitors and they target plant resistance genes to turn on the plant hypersensitive response (Friesen *et al.*, 2008). Whereas killing the cell in which the fungus resides is a good strategy for stopping a biotroph, it is exactly the wrong response for a necrotroph. As expected, in this inverse gene-for-gene system there is evidence for evolutionary "arms races" or "trench warfare" with a premium for plants whose resistance genes now fail to interact with elicitors turned toxins, and fungal toxins that keep pace with changes in resistance genes (Stukenbrock and McDonald, 2009). When both biotrophs and necrotrophs are considered, the true cost of extra R genes becomes apparent, because each additional R gene that protects against a biotroph invites a new necrotroph. In fact, the effect of additional R genes is additive for necrotrophs; the more interactions between R genes and effectors that act like toxins, the more disease (Friesen *et al.*, 2007). The emphasis on resistance for biotrophs has contributed, along with changes in tillage and climate, to an emergence of necrotrophs. Clearly, the challenge is to develop crops that resist both biotrophs and necrotrophs. However, current research suggests that it will not be simple. Take oat, for example, where the same R gene acts to protect against the biotroph, *Puccinia coronata* var. *avenae*, and enable the necrotroph, *Cochliobolus victoriae* (Lorang *et al.*, 2007). To sum up the conundrum of protecting against biotrophs and necrotrophs, it is hard to do better than the Scottish Bard, "The best laid plans of mice and men gang aft aglay …"

As a last thought about pathogenesis, could there be relevance of the findings about plant pathogens to medical mycology? The plant R genes are typically cell surface molecules with nuclear binding, lysine rich repeats (Jones and Dangl, 2006). Often, the nuclear binding part is evolutionarily conserved and the lysine rich repeat part is exceptionally variable, a molecular arrangement similar to those produced by the MHC immune region of mammalian genomes. Given that human-associated fungi show variation in their interactions with the host, from commensalism to systemic parasitism, one wonders if there could be similar trade offs in human resistance to the different types of fungal infection.

2. Cell biology

Cell biology is the next topic and the strides made from the combination of computer-enhanced light microscopy, fluorescently tagged molecules and mutation by molecular genetics represent accomplishments that may be unparalleled in recent mycological research. To choose a poem for cell biology, I am going south to England for a poem oft found in American middle-school anthologies, Percy Bysse Shelly's Ozymandias. Shelly and his poet friend, Horace Smith, competed to write a sonnet about the statue of Ramesses II in the British Museum (Fig. 4). Shelly's, below, is viewed as one of the most perfect sonnets ever written.

Ozymandias.

I met a traveller from an antique land
Who said: Two vast and trunkless legs of stone
Stand in the desert. Near them, on the sand,
Half sunk, a shattered visage lies, whose frown
And wrinkled lip, and sneer of cold command
Tell that its sculptor well those passions read
Which yet survive, stamped on these lifeless things,
The hand that mocked them and the heart that fed:
And on the pedestal these words appear:
"My name is Ozymandias, king of kings:
Look on my works, ye Mighty, and despair!"
Nothing beside remains. Round the decay
Of that colossal wreck, boundless and bare
The lone and level sands stretch far away

I chose this poem because it deals with a large, complex structure, the statue of Ozymandias, which I will liken to the large complex fruiting bodies made by Pezizomycotina and Agaricomycotina (Fig. 5). I also like it because it addresses hubris, here defined as a lack of humility before the gods. In ancient Greece, hubris did not go unpunished. Punishment was the task for Nemesis, who, with her lamp and sword, tracked the transgressors. I'll come back to Nemesis because some cell biologists are venturing perilously close to hubris.

Fig. 4 – Colossal bust of Ramesses II, the 'Younger Memnon' (1250 BC) in the British Museum. This statue is said to have inspired Shelly to write the poem Ozymandias. Image from Wikipedia.

Remembering my theme, adaptation, I want to focus on septa and their pores, because these tiny organelles might just hold one of the keys to the development of complex, multicellular structures in fungi. It is widely appreciated that the Ascomycota and Basidiomycota have regular septa whereas most earlier-diverging groups of fungi do not, and also widely known that these septa control cytoplasmic bleeding when hyphae are injured (Buller, 1933; Trinci and Collinge, 1973). However, I think that it is fair to say that the details of the septa have not received the level of attention paid to, for example, the growth of hyphal tips or the formation of spores. Looking at the fungal tree of life, the two clades that produce complex, multicellular structures with differentiated tissue are the Pezizomycotina and the Agaricomycotina and these are precisely the clades that have septa equipped to control hyphal damage (Fig. 6). The connection between these septa and multicellularity may be as simple as the ability to stop a damaged hypha from bleeding being essential to building a complex, multicellular fungus. After all, these large structures invite damage, in the form of mycophagy by invertebrate and vertebrate animals.

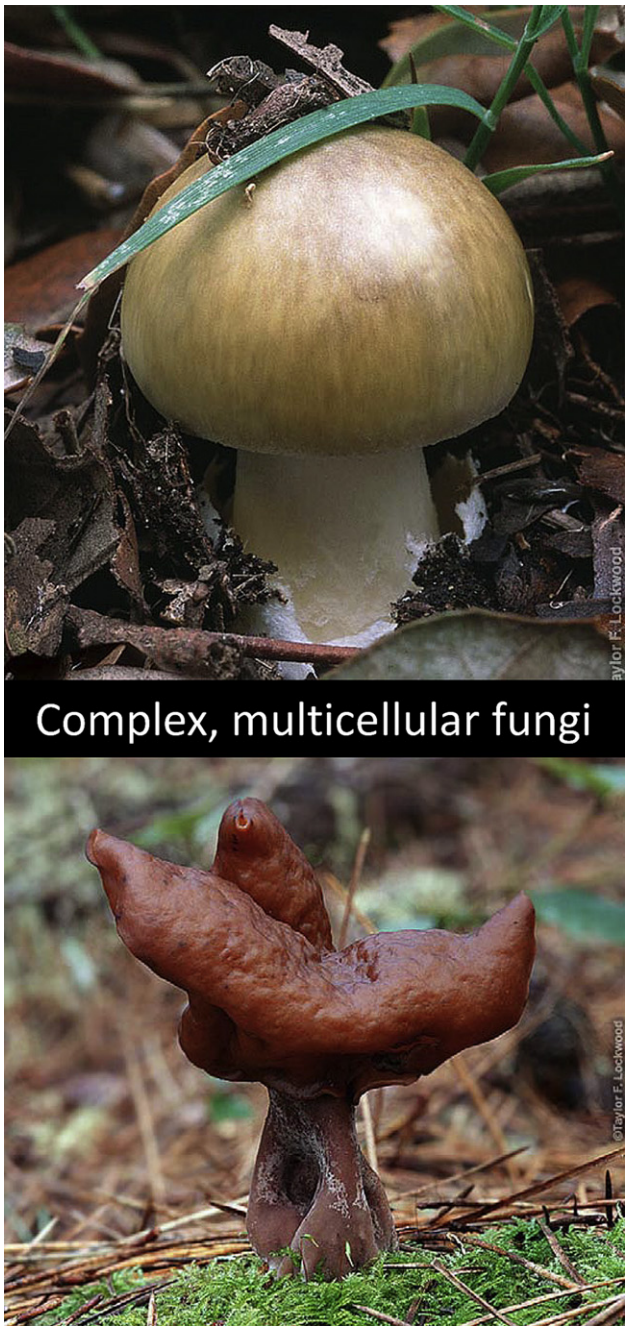

Fig. 5 – Examples of large, multicellular fungi with differentiated tissues. Top, *Amanita phalloides*. Bottom, *Gyromitra influa*. Photos by Taylor Lockwood in Harmon, K. 2009. Slide show of seven deadly mushrooms. Scientific American slide show, June 5, 2009.

Starting with the Agaricomycotina (Basidiomycota) septum, it was Buller who observed that the pore in this septum could be plugged to limit the loss of cytoplasm (Buller, 1933) (p 145) and other famous mycologists helped elucidate the septal structure (Bracker and Butler, 1963; Girbardt, 1957), notably describing the barrel-shaped cell wall surrounding the pore, the dolipore, and the membranous organelle that covers the pore, the septal pore cap (SPC) (Fig. 7). The Netherlands has long been a center for studies of fungal cell walls and recently a center for SPC research has emerged in Utrecht, led by Teun Boekhout and Han Wösten at the CBS and the University of Utrecht. They and their colleagues, Arend van Peer in particular, have found that SPC has a protein core that is covered by a lipid membrane and that both the protein core and the membrane are responsible for the SPC shape, which in *Schizophyllum commune* is a mesh with large, hexagonal pores (van Peer *et al.*, 2009). Their work extends the early experiments of Buller by showing that the SPC functions to control the flow of organelles and that closing of the pore can be affected by changes in nutrition, temperature or osmotic stress (van Peer *et al.*, 2009).

Recently, the Utrecht team has discovered three proteins associated with isolated SPCs, two that are shared by *Schizophyllum* and *Rhizoctonia*, and one unique to *Rhizoctonia*. The *Rhizoctonia* protein, Spc18, is found in the ER and the SPC, and then moves to the plug that closes the pore (van Driel *et al.*, 2008). One of the shared proteins, Spc33, is localized to the membranes of the SPC and when it is deleted, *Schizophyllum* hyphae bleed cytoplasm and fruiting body development is affected (van Peer *et al.*, 2010). This experimental evidence for the role of the SPC in multicellular development provides support for the hypothesis that the ability to control hyphal damage is important to the development of multicellularity. The exciting challenge with the SPC will be to find all the relevant proteins and then, using fluorescent labels, unravel SPC development.

In the Pezizomycotina (Ascomycota), Woronin bodies (Fig. 8) were discovered and named by Buller (Buller, 1933) and their function in stopping hyphal bleeding was demonstrated in 1973 (Trinci and Collinge, 1973). The story of their development begins just a decade ago when Greg Jedd began to work on Woronin bodies (WB), starting at the Rockefeller (Jedd and Chua, 2000) and continuing at Temasek Life Sciences Laboratories in Singapore. In a series of elegant publications he and his colleagues have shown that WBs are a new organelle that is related to other membrane organelles, i.e., the nuclear envelope, the endoplasmic reticulum, endosomes, lysosomes and, particularly, peroxisomes (Fig. 9) (Liu *et al.*, 2008). In this work, they have shown that the core of the WB

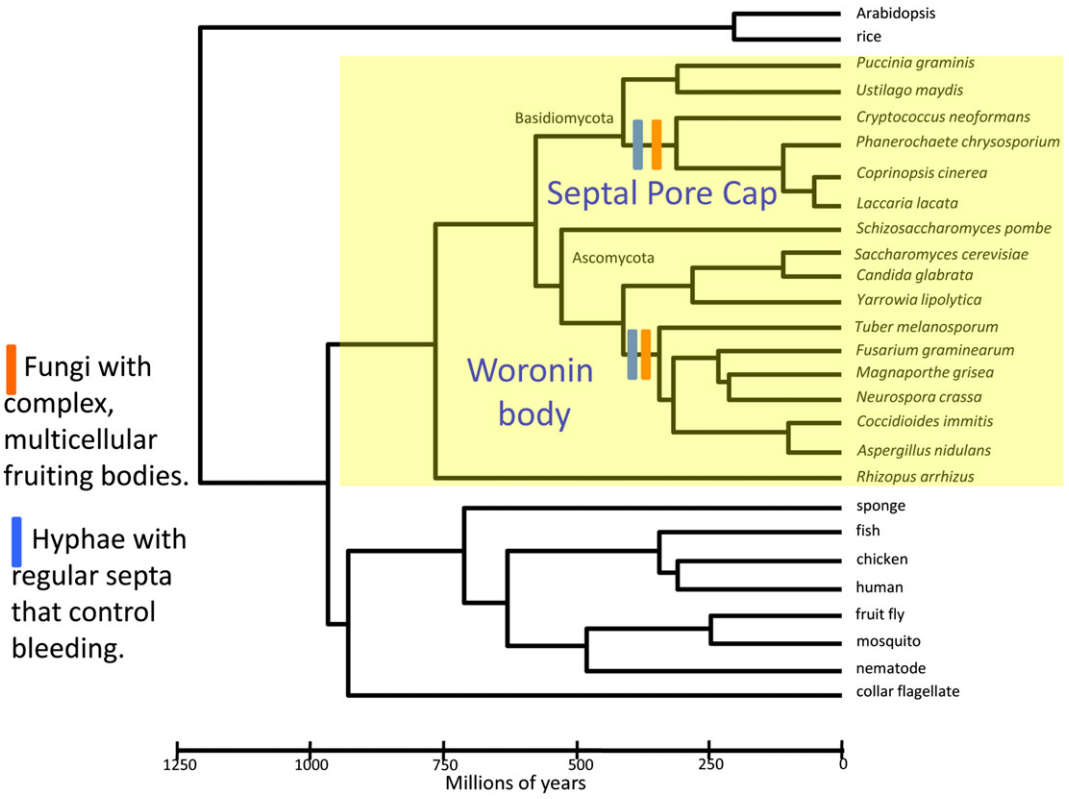

Fig. 6 – Phylogenetic tree showing the identity of clades of fungi that are capable of making complex, multicellular fruiting bodies and that possess hyphae with regular septa that control loss of cytoplasm after injury. Modified from Stajich *et al.* (2009).

is a hexagonal crystal made of Hex, a protein with a peroxisome targeting signal that self-assembles once inside the organelle (Jedd and Chua, 2000). The nascent WB recruits another protein, Woronin sorting complex (WSC), to the peroxisome where it envelops the Hex crystal as both the crystal and its WSC infiltrated, lipid membrane bud from the peroxisome (Liu *et al.*, 2008). A third protein is recruited, leashin, which attaches to both the budding WB and the septum and, afterward, the WB separates from the parental peroxisome and remains tethered near the septum (Ng *et al.*, 2009) (Fig. 10). When the hypha is damaged, the WB plugs the central pore of the Ascomycota septum, stopping the loss of cytoplasm.

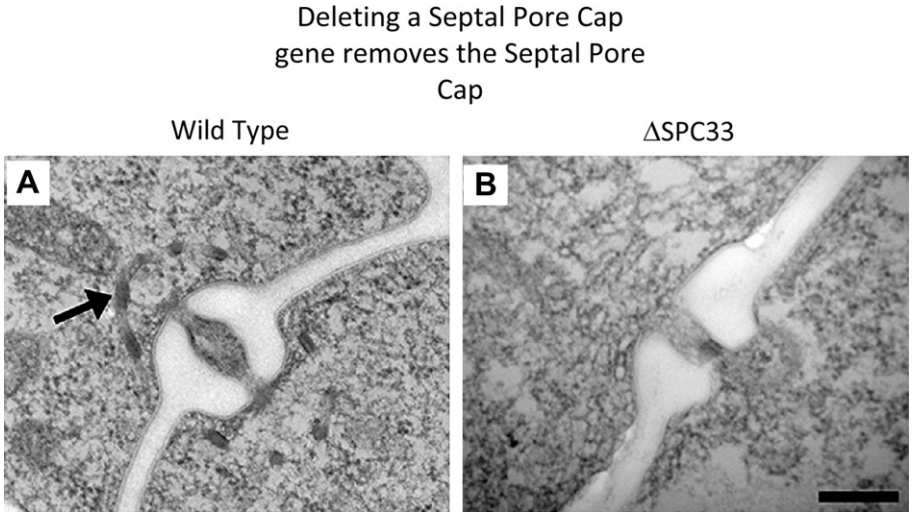

Fig. 7 – Septal pore caps (SPC) of the Basidiomycota, Schizophyllum commune, showing (A) its presence (arrow) in a wild isolate and (B) its absence when the SPC33 gene is deleted. Reproduced from van Peer *et al.* (2010).

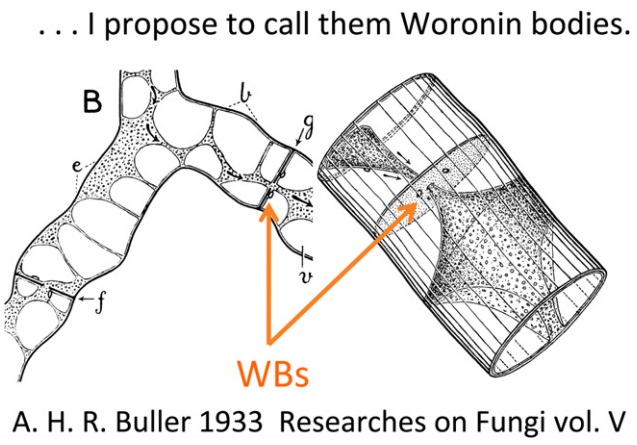

Fig. 8 – Woronin bodies (WBs) in hyphae of Pyrenophora confluans, Figure modified from Buller (1933). V Fig 64 and fig 63.

How can the evolution of a new organelle occur? In a recent review, Jedd points to gene duplication as a key process (Jedd, 2011). For example, the core protein Hex is extremely similar to an mRNA binding protein involved in translation, elongation translation initiation factor (EIF5A). Whereas Hex is found only in Pezizomycotina, EIF5A is found in all domains of life. EIF5A can dimerize, is associated with ER and the nuclear membrane, and just one codon change gives it a peroxisome targeting signal. EIF5A is essential, so duplication of this gene and subsequent evolution of one copy to form Hex must have produced intermediates that showed increasing polymerization and self-assembly. Another protein, the coat protein WSC, is very similar to the peroxisome membrane protein PMP22. WSC appears to have been usurped by the WB without any gene duplication because no fungi are known to have both PMP22 and WSC.

More mysterious is the origin of leashin, which lacks homology with any known protein, in or out of the Pezizomycotina (Ng *et al.*, 2009). The ends of leashin must bind the WB and the septum, but the middle part seems to have fewer

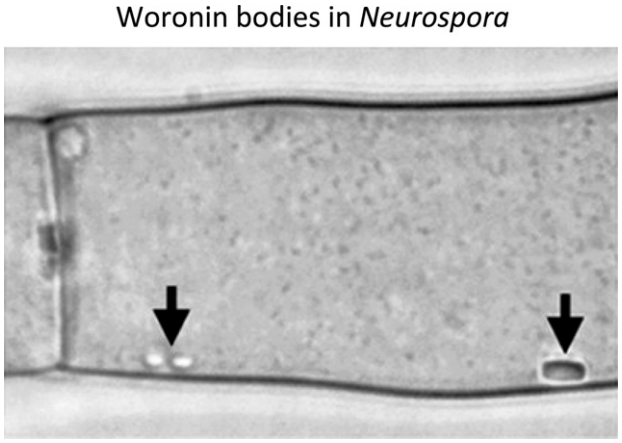

Fig. 9 – Woronin bodies (arrows) in *Neurospora* hyphae. Reproduced from Liu *et al.* (2008).

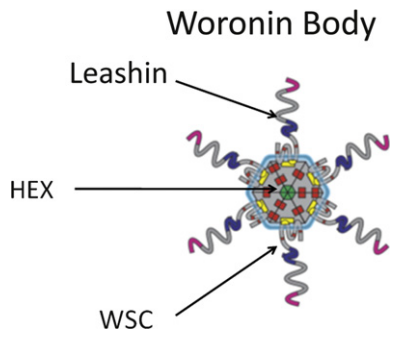

Fig. 10 – Model of the Woronin body showing the core Hex protein, the Woronin sorting complex (WSC) and the protein that attaches the Woronin body to the cortex of the hypha, leashin. Figure adapted from Jedd (2011).

constraints, apart from maintaining an appropriate length and perhaps elasticity. Leashin could have originated through duplication of a known protein, and then evolved so rapidly that sequence similarity has been obscured. In support of this thinking is evidence from *Neurospora* and *Sordaria* that leashin can evolve rapidly. These fungi have two leashins instead of just one. One type tethers the WB to the hyphal cortex and the other type rests near the apex and also at septa (Ng *et al.*, 2009). Examination of the *Neurospora* genome shows that genes for the two leashins originated by cleavage of the ancestral gene (Fig. 11); but why? It has been postulated that rapid cytoplasmic flow associated with the rapid growth for which the large hyphae of *Neurospora* are famed could be involved with the evolution of two leashins (Ng *et al.*, 2009; Plamann, 2009). If rapid growth increases fitness in these fungi, and if growth is limited by inappropriate WB plugging of the septal pore due to overly rapid cytoplasmic flow, one could imagine selection acting to create a solution to the problem. Two leashins provide a solution by keeping the WB away from the septum until needed to staunch bleeding cytoplasm.

Jedd and colleagues tested this hypothesis by patching together the two *Neurospora* leashin genes to make a strain with just one gene and one leashin (Ng *et al.*, 2009). When WBs were examined in this reverse-evolved strain, they were no longer found at the cell cortex, but were tethered to the septa as seen with all other Pezizomycotina WBs. Consistent with the cytoplasmic streaming hypothesis, hyphal growth is slower in this genetically modified strain. Undoing evolution, as Jedd and colleagues have done, must clearly qualify as hubris. Jedd and his group might want to be on alert for a winged god with a lamp and sword.

The challenges in this field are daunting. SPCs and WBs may be necessary for multicellularity, but they cannot be sufficient. There must be many other essential parts and finding them may not be easy. There also is the challenge to explain *Dimargaris*, a zygomycete with no multicellularity but sporting regular septa complete with central pores and plugs, or *Neolecta*, a Taphrinomycotina with a beautiful multicellular ascoma but no known WBs. In both cases, genomes of these fungi might be useful in searching for orthologs of genes known to be important to fungal wound plugging.

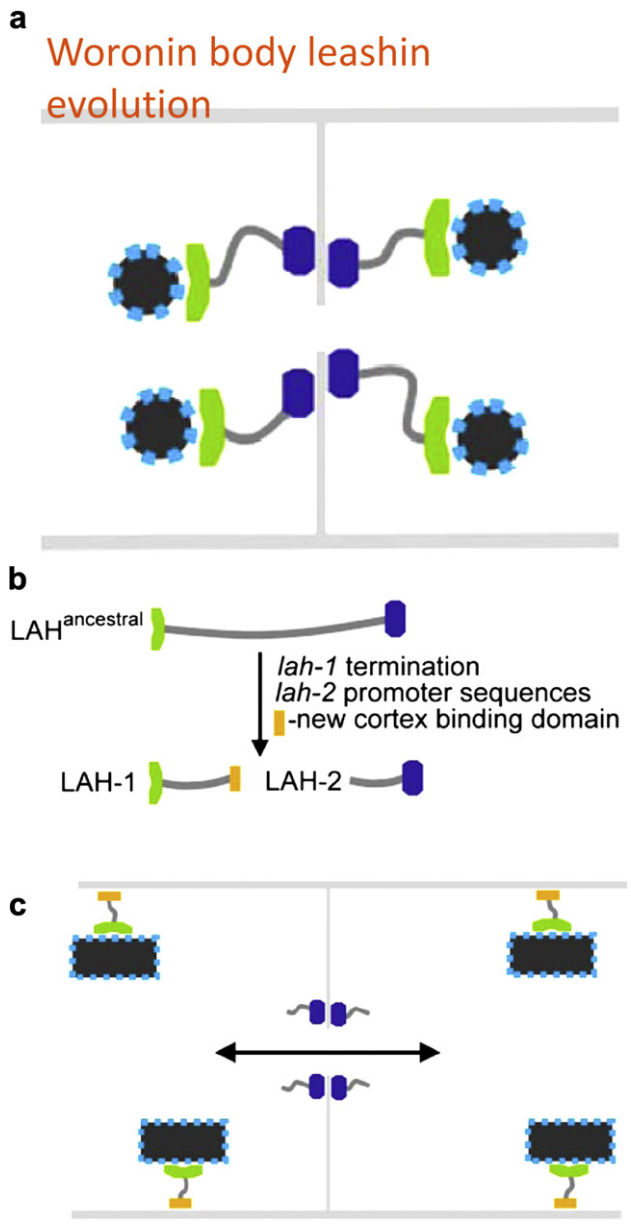

Fig. 11 – Models of (a) the ancestral and widespread Woronin body in Pezizomycotina, of (b) the evolution of a single ancestral leashin molecule to the two leashins, LAH1 and LAH2, found in *Neurospora*, and of (c) the Woronin body and leashins in *Neurospora*. Figure adapted from Ng *et al.* (2009).

3. Genomics, evolution and ecology

Mention of genomes of *Dimargaris* or *Neolecta* provides an introduction to the final segment of my presentation. I am going to combine genomics with the two topics that interest the most IMC9 delegates, evolution and ecology, because I believe that genomics will have its greatest impact on these areas. On the one hand genomics is merely the latest in a series of technological advances, beginning with enzyme electrophoresis, that have revealed more and more about genetics. On the other it seems to offer the ultimate view of the genome and already has highlighted modes of selection that could not have been recognized by the framers of the Modern Synthesis (Koonin, 2009a). These modes include selection for changes in the size of gene families and for the retention of genes that have been horizontally transferred between distantly related taxa. Neither of these modes of evolution are recent revelations, for example, gene duplication was mentioned in the section on Ascomycota WBs and there is a wonderful story about horizontal gene transfer of virulence genes between the wheat pathogens, *Phaeosphaeria* (anamorph: *Stagonospora*) and *Pyrenophora* (Friesen *et al.*, 2006). What comparative genomics has shown is that these non-traditional modes are commonplace and may be more important to adaptation than the slow accumulation of single nucleotide substitutions as featured in the Modern Synthesis (Koonin, 2009b).

For a story about comparative genomics and adaptation I am going to stick close to home and focus on a study centered in Berkeley that was led by Tom Sharpton and Jason Stajich along with a team of researchers from Arizona, California, Texas, Massachusetts and Maryland (Sharpton *et al.*, 2009). This adaptation is a dramatic one, from fungi that eat plants to those that eat meat and it has the added allure of human disease. Naturally, a poem is called for and the one that I have in mind is unusual for two reasons. First, it is a grace and second, although it is attributed to Robert Burns, it was in use a century before his birth. Biology, it seems, is not the only field in which a well known person can be credited with more than he or she deserves.

Selkirk grace.

Some hae meat and canna eat,
and some wad eat that want it,
but we hae meat and we can eat,
and sae the Lord be thankit.

Available data suggests that the first fungi ate plants. When we think about the origin of the fungal kingdom, we concern ourselves with the state of plant life at that geologic epoch (Berbee and Taylor, 2010). However, there are groups of fungi that have adapted to a meat diet and many of them lie in the Onygenales (Ascomycota, Eurotiomycetes). It is not a coincidence that the Onygenales also is home to most of the fungi that bedevil humans as parasites (Taylor, 2006) and it is the connection with human disease that made this comparative genomic study possible. In Maryland, the NIH worked with JCVI (then TIGR) and the Broad Institute to sequence strains from *Coccidioides immitis*, *Coccidioides posadasii* and a close, non-pathogenic relative, *Uncinocarpus reesii*. Added to these three genomes of fungal carnivores was a fourth from *Histoplasma capsulatum*, and 13 genomes from herbivorous species in the genera *Aspergillus*, *Fusarium*, *Stagonospora*, *Chaetomium*, *Podospora* and *Neurospora* that, together, provide the data for comparative genomics. Although *Coccidioides* species are as familiar to some Californians as thistles are to a Scot, I realize that some background is in order because not everyone has an intimate knowledge of these fungi. *Coccidioides* species have been found in the small mammal population of the desert southwestern USA as well as similar environments in Mexico, Central and South America and would have remained in obscurity but for their ability

to infect humans with a potentially fatal disease (Galgiani, 2007; Pappagianis, 1988). The closely related genus *Uncinocarpus* is also associated with small mammals, but does not cause a human disease and the more distantly related fungi, *Neurospora*, *Aspergillus* and the others, are similarly benign for healthy humans and lack a close association with mammals. Phylogenomic comparison was undertaken to shed light on genome changes associated with adaptation to life with animals.

To make their phylogenomic comparison, Sharpton *et al.* (2009) identified protein domains in genes of the 17 genomes and then, with the aid of a multigene phylogeny, searched for significant changes in gene family size from a pool of nearly 2800 gene families (Fig. 12). They found 13 gene families to be significantly smaller in the Onygenales, and two to be significantly larger. The most interesting of the shrunken gene families is the one with cellulose binding domains, which are abundant in the plant-associated fungi but absent in *Coccidioides* or *Uncinocarpus*. Nor were genes harboring cellulose binding domains the only ones associated with plant cell wall digestion to be absent from Onygenales, they were joined by genes coding for tannase, pectic lyase C, pectinesterase and melibiase. It seems sensible to infer that plants have lost importance in the diets of *Coccidioides* and *Uncinocarpus*.

Looking at the expansion of gene families, several of the most dramatic expansions were for genes possessing subtilisin N and peptidase S8 domains, which are involved in digesting animal protein. Judging by these results, it seems that contractions of gene families associated with plant cell wall digestion and expansion of gene families associated with digestion of animal protein helped facilitate the adaptive switch of fungi from a plant diet to an animal one. Having identified gene families with significant changes in gene number, the researchers then built gene geneologies to visualize the history of gene duplications responsible for the expansion. Peptidase S8 provides a clear example with 10 gene duplications on branches leading to the ancestor of *C. immitis*, *C. posadasii* and *U. reesii* (Fig. 13). Documenting the role of gene duplication in gene family expansion raises the question of the role of selection in the process, given that evolution cannot prepare for future events. One possible scenario is that fungal genomes suffer duplications regularly, that most duplications are not adaptive and are lost, and that it is the duplications that increase fitness that are selected and persist. Evidence used to formulate this argument comes from the Institut Pasteur, where gene dosage recovery experiments in Bernard Dujon's lab showed that yeasts experience spontaneous, large, segmental duplications (Koszul *et al.*, 2004) and where subsequent research showed that selection is needed to maintain many of these types of duplications (Koszul *et al.*, 2006). By this scenario, fungal genomes are very dynamic and their apparent stasis must be due to strong purifying selection. Their genome dynamism would be an advantage when confronted by new environments, where

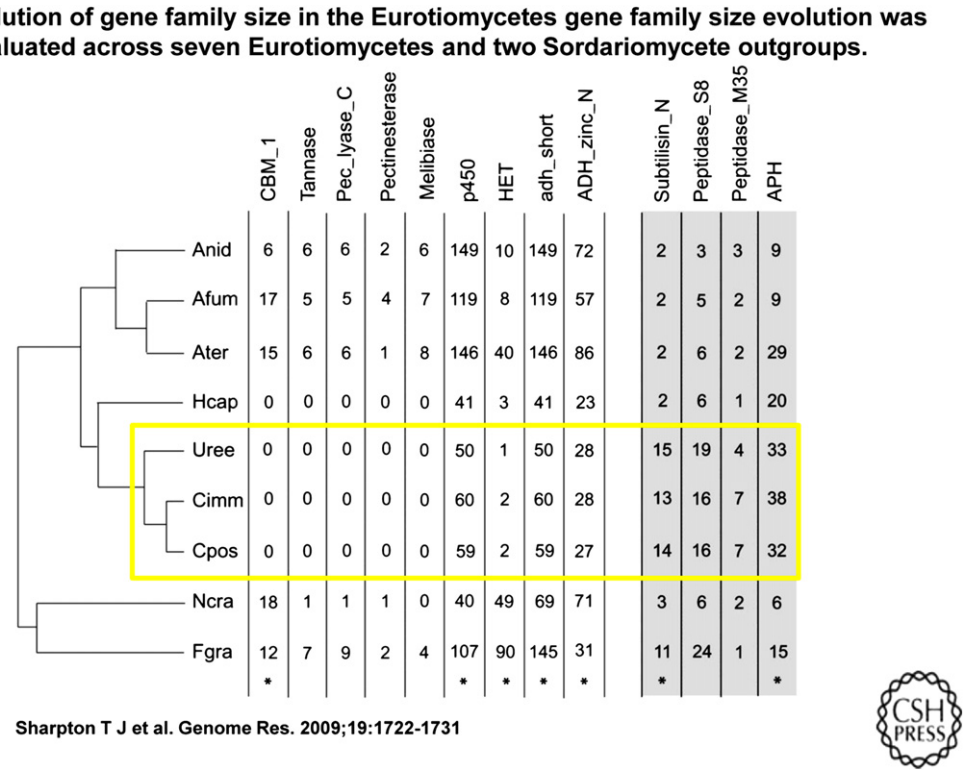

Fig. 12 – Gene family reduction and gene family expansion determined by phylogenomic comparison of Ascomycota. Highlighted area focuses on fungi associated in nature with animals. *Aspergillus nidulans* (Anid), *Aspergillus fumigatus* (Afum), *Aspergillus terreus* (Ater), *Histoplasma capsulatum* (Hcap), *Uncinocarpus reesii* (Uree), *Coccidioides immitis* (Cimm), *Coccidioides posadasii* (Cpos), *Neurospora crassa* (Ncr), *Fusarium graminiarum* (Fgra). Adapted from Sharpton *et al.* (2009).

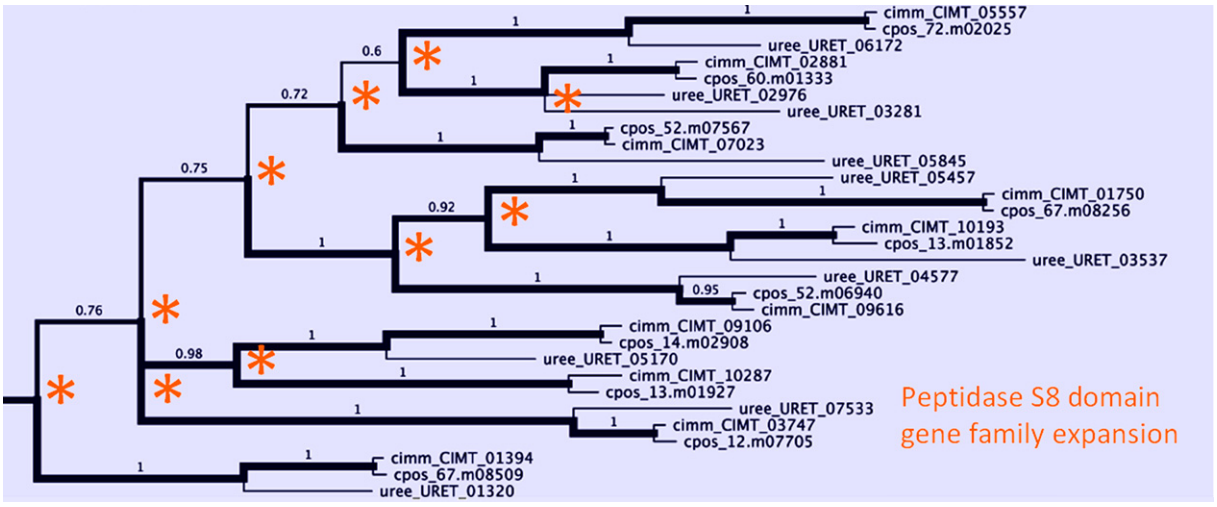

Fig. 13 – Evidence of 10 gene duplication events (*) inferred from a gene genealogy of genes containing peptidase S8 domains. *Coccidioides immitis* (Cimm), *Coccidioides posadasii* (Cpos) and *Uncinocarpus reesii* (Uree). Adapted from Sharpton *et al.* (2009).

selection can act rapidly to retain duplications that increase fitness.

This brief foray into comparative genomics shows that data from fungi support the thinking that the Modern Synthesis needs to expand to accommodate new modes of generating genetic diversity. However, it is too early to declare that these new modes, such as gene duplications or horizontal gene transfer, outweigh the impact of point mutations because these big events are the ones that can most easily be detected with a few, well-diverged genomes. Assessing the impact of point mutations will take genomes from recently diverged populations. Already there are studies with tens of genomes for one species or population (Neafsey *et al.*, 2010), but hundreds and perhaps thousands will be needed to reveal the full range of evolutionary processes. Population genomics at this scale is ideally suited to fungi.

The most ambitious studies associating genetic variation and phenotypic variation are now conducted with humans due to their obvious relationship to health (Durbin *et al.*, 2010), but fungi offer advantages apart from their small genomes that will never be equaled with humans. As simple eukaryotes, fungi can reproduce both clonally and by mating and recombination, as does most of life. Many fungi are haploid, cultivated individuals are immortal, and large collections of cultivated fungi can be kept inexpensively, thus obviating the need for inbred lines, greenhouses, or laboratory animal facilities. In fact, the accelerating decrease in the cost of genome sequencing should make it possible to bring population genomics to any fungus that can be cultivated or collected in sufficient quantity. The limiting factor will be the collection of fungal individuals. I encourage you to "think big" with your favorite fungus.

Some fungi have a rich history of laboratory experimentation and some of them also have population collections that have been scrutinized phylogenetically, *Neurospora* for example (Dettman *et al.*, 2003a, b). Mycologists are notoriously independent, but we might take a cue from plant biologists and pick a few model organisms to span the range of fungal behavior, e.g., a parasitic symbiont, a mutualistic symbiont and a saprobe or two. In each case it wouldn't hurt to pick a fungus of interest to society, perhaps an agent of plant disease, a mycorrhizal fungus, and a saprobe of economic importance. Population genomic studies of yeast (Liti *et al.*, 2009) have begun to show what is possible. The recent exploitation of *Neurospora* in connection with the bioconversion of plant biomass for energy production (Galazka *et al.*, 2010; Tian *et al.*, 2009) and even more recent population genomic analyses of diverging *Neurospora* populations (Ellison *et al.*, 2011) show what is possible with filamentous fungi. Again, as a community, mycology needs to think about model evolutionary organisms and "think big."

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