



Commentary

Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection?

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Abstract

Fungi are relatively rare causes of life-threatening systemic disease in immunologically intact mammals despite being frequent pathogens in insects, amphibians, and plants. Given that virulence is a complex trait, the capacity of certain soil fungi to infect, persist, and cause disease in animals despite no apparent requirement for animal hosts in replication or survival presents a paradox. In recent years studies with amoeba, slime molds, and worms have led to the proposal that interactions between fungi and other environmental microbes, including predators, select for characteristics that are also suitable for survival in animal hosts. Given that most fungal species grow best at ambient temperatures, the high body temperature of endothermic animals must provide a thermal barrier for protection against infection with a large number of fungi. Fungal disease is relatively common in birds but most are caused by only a few thermotolerant species. The relative resistance of endothermic vertebrates to fungal diseases is likely a result of higher body temperatures combined with immune defenses. Protection against fungal diseases could have been a powerful selective mechanism for endothermy in certain vertebrates. Deforestation and proliferation of fungal spores at cretaceous–tertiary boundary suggests that fungal diseases could have contributed to the demise of dinosaurs and the flourishing of mammalian species. © 2004 Elsevier Inc. All rights reserved.

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1. Introduction

Of the more than 1.5 million estimated fungal species (Hawksworth, 2001), only about 150 cause disease in mammals and of these species only a few are common pathogens (Kwon-Chung and Bennett, 1992). With the exception of the dermatophytes, which are common causes of skin infection and disease in many mammalian species, systemic fungal diseases are relatively rare in intact mammals compared to those caused by bacteria and viruses. In humans, systemic fungal diseases were considered a rarity until the mid-20th century, when advancements in medicine produced antimicrobial

drugs, indwelling venous catheters, and immunosuppressive therapies. In fact, fungal diseases such as cryptococcosis, blastomycosis, and histoplasmosis were not described until the late 19th century when advances in laboratory science led to more detailed pathological descriptions in unusual cases of disease. For example, *Cryptococcus neoformans* was not associated with human disease until 1894 (Knoke and Schwesinger, 1994). In contrast, tuberculosis, smallpox, plague, and many other infectious diseases were known since antiquity. Presumably, this reflects the relative infrequency of systemic fungal diseases in human populations such that the occasional cases that must have occurred before modern times were not recognized as a distinct disease entity worthy of description. Today systemic fungal diseases are common but most occur in individuals with

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impaired immunity as a consequence of HIV infection, immunosuppressive drugs, integument compromise by catheters and surgery, and disruption of host commensal bacterial flora by antimicrobial drug use. Hence, humans as a species appear to be remarkably resistant to fungal diseases except in conditions where host defenses are impaired.

Comparisons of the relative frequency of fungal disease across species are difficult because we lack incidence and prevalence data for the overwhelming majority of animal species. However, there is sufficient information emerging in the literature to begin to assemble generalizations and consider hypothesis that are potentially testable. In common with the rarity of human mycoses, systemic fungal diseases appear to be relatively infrequent in other mammals such as rabbits, rodents, cats, and dogs, although some dog breeds are susceptible to blastomycosis and cryptococcosis occurs frequently in Koala bears (Canny and Gamble, 2003; Connole et al., 2000; Kerl, 2003; Krockenberger et al., 2003; Pollock, 2003). Some birds appear to be susceptible to systemic fungal diseases and *Aspergillus fumigatus* is a relatively common pathogen. Approximately 20% of immature loons succumb to respiratory fungal disease (Sidor et al., 2003) and aspergillosis is common in turkeys and stitchbirds (Cork et al., 1999; Lair-Fuller et al., 2003). In contrast to mammals, fungi are frequently associated with disease in ectothermic organisms such as plants, insects, fish, and amphibians. For example, chytridiomycosis has been implicated in the worldwide decline in amphibian populations (Berger et al., 1998; Daszak et al., 1999) and fungi are common pathogens of mites (van der Geest et al., 2000). Fungal diseases may also be critical contributors to the worldwide decline in coral reefs (Rosenberg and Ben Haim, 2002). Two questions emerge from these observations: (1) Why are some fungi virulent for mammals? and (2) Why are most mammals relatively resistant? In pondering these questions some insights emerge that could be relevant to the unsolved problems of the origins of vertebrate endothermy and the causes of past extinctions.

2. Virulence and pathogenicity

Before analyzing the specific attributes of fungi as pathogenic microbes it is worthwhile to consider the definitions of virulence and pathogenicity, since these are central elements in any discussion of fungal pathogenesis. Virulence is a microbial attribute that is expressed only in the context of a susceptible host, and consequently, it is not an independent microbial property (Casadevall and Pirofski, 2001). Although various definitions for virulence have been proposed over the years, I believe that virulence is best defined as the relative capacity of a microbe to cause damage in a host (Casa-

devall and Pirofski, 1999). This definition is broadly inclusive of the enormous diversity of host–microbe interactions and grounds the term virulence on host damage, a relevant outcome to the host. Host damage can result from microbial processes, the host immune response or both, and disease occurs when host damage interferes with homeostasis (Casadevall and Pirofski, 1999). The realization that damage is the relevant host-related outcome in the host–microbe interaction led to the development of the ‘damage–response framework’ of microbial pathogenesis (Casadevall and Pirofski, 1999, 2000, 2003). This framework is grounded on three observations: (1) microbial diseases result from the interaction of two entities, a host and a microbe; (2) host damage is the relevant outcome of the host–microbe interaction; and (3) host damage can ensue from the direct action of microbial factors, the immune response, or both (Casadevall and Pirofski, 1999, 2000, 2003). When damage is considered as a function of the immune response for host–microbe interactions it is apparent that the basic relationship is usually parabolic with maximal damage occurring in situations of weak or strong immune responses. The ‘damage–response framework’ of microbial pathogenesis describes six basic types of interactions that allow the grouping of human pathogenic microbes into six Classes based on the average outcome of their interaction with certain hosts rather than phylogenetic differences (Casadevall and Pirofski, 1999). Since human systemic fungal diseases occur primarily in hosts with impaired immune function most human fungal pathogens such as *Candida albicans*, *Cryptococcus neoformans*, and *Blastomyces dermatitidis* have been categorized as Class 1 and 2, categories that include many other non-fungal pathogens (Casadevall and Pirofski, 1999). However, some fungal diseases occur as a consequence of strong host responses and *Histoplasma capsulatum* and *Aspergillus* spp. were classified as Class 3 and 4, respectively, which also include bacteria and viruses (Casadevall and Pirofski, 1999). Hence, the ‘damage–response framework’ makes no distinction among pathogenic microbes based on phylogenetic origin and shows no fundamental difference between fungi and other types of pathogens.

3. The rarity of life-threatening fungal diseases in immunologically intact mammals

Fungal pathogens can be divided into two broad groups depending on their source for infection. The first group is endogenous to mammalian hosts, is considered part of the commensal flora, and is presumably acquired by transmission from other hosts. This group includes *Candida* spp. and a few other fungal species such as *Malassezia* (Ashbee et al., 2002). Commensal fungi are adapted to their hosts and systemic disease is almost

always associated with an alteration in the ecology and/or immune status of the host. For example, systemic candidiasis occurs in states of immunosuppression, antibiotic-mediated bacterial suppression, and the placement of intravenous catheters (Edwards, 1991). *Malassezia* spp. rarely causes systemic disease unless the patient is receiving intravenous lipid therapy, which provides essential nutrients for fungal growth. However, this organism can cause skin disease, such as seborrhoeic dermatitis, which is believed to result from the immune response to fungal antigens (Gupta and Kogan, 2004). The second group of fungal pathogens is acquired from the environment and includes *B. dermatitidis*, *C. neoformans*, *Coccidioides* spp., *H. capsulatum*, *Paracoccidioides brasiliensis*, and *Sporothrix schenckii*. Most of these organisms live in soils and none requires an animal host for replication or survival. When humans live in areas where these organisms are found in the environment there is often a high prevalence of infection and a very low prevalence of disease. For example, serological assays have shown a high prevalence of *C. neoformans* infection humans despite a relatively low incidence of disease (DeShaw and Pirofski, 1995; Goldman et al., 2001). However, fungal disease can follow exposure to large inocula even in apparently normal mammalian hosts. Examples of events whereby large inocula presumably lead to disease are the outbreaks of histoplasmosis following tree cutting activities (Ward et al., 1979), cryptococcosis following heavy exposure to contaminated birds (Fessel, 1993), and coccidioidomycosis following earthquakes (Fisher et al., 2000). Outbreaks of histoplasmosis in otherwise healthy humans following cave explorations provide clear examples of the ability of *H. capsulatum* to cause disease in situations where large inocula are expected. A recent report documents two outbreaks of histoplasmosis among visitors to a cave in Costa Rica that affected 72 and 64% of individuals in two separate groups (Lyon et al., 2004). Hence, most human infections are probably asymptomatic and disease is rare unless the individual has impaired immune function or infection follows exposure to large inocula.

Since life-threatening fungal diseases are relatively rare one must conclude that mammalian hosts are remarkably effective at controlling fungal infections, except possibly in situations of massive exposures. The relative paucity of invasive fungal diseases in a world where fungi are ubiquitous suggests that certain characteristics of mammals were selected in evolution for defense against fungi. The mammalian host is a hostile environment for the overwhelming majority of environmental fungi because of a sophisticated immune system and physical characteristics that include a high basal temperature and slightly alkaline serum chemistry. The high basal temperatures in mammals almost certainly constitute a non-permissive environment for the over-

whelming majority fungal species that grow best at ambient temperatures of 25–35 °C (Kwon-Chung and Bennett, 1992). Similarly, the alkaline nature of mammalian fluids must be unfavorable to most fungal species which grow best in acidic conditions such that most mycologic media have pH 6–6.8 (Kwon-Chung and Bennett, 1992). In addition to baseline high basal temperatures mammals have the capacity for fever responses, which must further reduce the proportion of fungal species that can survive in the mammalian host. Fever is defense mechanism used by animals in defense against many microbes (Kluger et al., 1998). The value of high temperature as a defensive antifungal mechanism is evident from studies with ectothermic vertebrates that produce a fever response by seeking warmer ambient temperatures and sunlight. Frogs can be cured of the chytrid pathogen *Batrachochytrium dendrobatidis* by raising body temperature to 37 °C for 16 h (Kluger et al., 1998). The desert locust can raise its body temperature to 38–40 °C by behavioral modification and this results in growth inhibition and killing of the fungal pathogen *Metarhizium anisopliae* var. *acridum*, which grows best and is most pathogenic in ambient temperatures (Elliot et al., 2002).

Studies of cryptococcal pathogenesis in rabbits provide additional insights into the synergistic effects of mammalian temperature and immune system in protecting against fungal diseases. *C. neoformans* grows well at 37 °C and some strains can survive and grow at temperatures in the 40–41 °C range (Martinez et al., 2001). Normal rabbits have basal temperatures of 39–40 °C and are resistant to systemic *C. neoformans* infection, even when the organism is injected into the cerebrospinal fluid (Perfect et al., 1980). Nevertheless, it is possible to induce cryptococcal disease in rabbits by testicular injection, which presumably reflects permissiveness for growth as a result of lower testicular temperature, and perhaps a less effective local response (Bergman, 1966). Hence, immunity alone does not protect against direct infection in a relatively cool organ. However, lethal cryptococcal meningitis can be induced in rabbits if animals are first treated with corticosteroids and then yeast cells are inoculated into the central nervous system (Perfect et al., 1980), implying that temperature alone is not sufficient to control a disease when the fungus is thermotolerant. In humans, cutaneous cryptococcosis is associated with serotype D *C. neoformans* strains (Dromer et al., 1996) which are less resistant to high temperatures than serotype A strains (Martinez et al., 2001). An example of the remarkable resistance of certain mammals to *C. neoformans* is provided by the rat, which mounts a vigorous immune response to infection with as many as 10⁷ yeast cells inoculated directly into the lung, clears the infection, and heals the lung (Goldman et al., 1994). However, administration of corticosteroids to rats makes them susceptible to cryptococcosis

(Graybill et al., 1983). These observations suggest that the relative resistance of mammals to systemic and invasive fungal diseases is likely to reflect a combination of high body temperatures and layered immune defenses that include innate and adaptive immune systems each with humoral and cellular components.

4. Origins of fungal virulence

When entertaining the possible origins of fungal virulence for mammals one possible first step is to consider the diversity of the pathogenic subset at the species and genetic level. If the pathogenic fungi were a closely related group it might suggest origins from a phylogenetic branch that evolved to utilize mammalian hosts for its own purposes. However, if the pathogenic fungi belonged to diverse groups then one might gain insight into the origin of virulence by comparing the estimated phylogenetic branch dates to the fossil record for vertebrates. The mammalian pathogenic fungi are a diverse group of organisms that include ascomycetes and basidiomycetes. Comparative analysis of the 18 S rRNA sequences of *B. dermatitidis*, *H. capsulatum*, *Coccidioides immitis*, and *Trichophyton rubrum* to seven closely related non-pathogenic relatives revealed that both groups were interspersed consistent with the view that the capacity for human virulence emerged independently several times within these pathogens (Bowman et al., 1992, 1996). The major fungal lineages are ancient and have been estimated to have emerged approximately one billion years ago (Hedges et al., 2004). In contrast, animals are more recent with the estimated divergence of mammals, reptiles, and amphibians occurring 300–400 million years ago (Hedges et al., 2004). Hence, the origins and branch nodes in the lineages of pathogenic fungi are significantly older than their vertebrate animal hosts.

Unlike the dermatophytes and commensal yeasts like *Candida* spp., the endemic fungi are pathogenic for mammals despite no obvious requirement for an animal host in their survival. In fact, human pathogenic fungi such as *C. neoformans* can be recovered from the environment by inoculation of environmental samples into mice, which then eliminate most, if not all, other microbes but cannot resist cryptococcal infection. For a microbe to persist in a host it must survive in the physical environment of the host and attach to host tissues in some manner. The ability to survive at mammalian body temperatures and slightly alkaline conditions is a complex phenotype that must reflect contributions from the inherent thermal and pH stability of microbial components as well as the stress responses. Thermal and alkaline tolerances probably originate from physical selection in harsh environmental conditions. Some filamentous fungi manifest invasive hyphal growth during animal pathogenic processes that result from both tissue

enzymatic digestion and the exertion of biomechanical force (MacDonald et al., 2002; Ravishankar et al., 2001), a phenomenon that may have origins in the requirements for food acquisition in the environment (Money, 2004). In contrast, the ability to attach to mammalian tissues and survive immune attack is unlikely to have been selected by direct interactions with mammalian hosts since these organisms are usually soil dwellers that infrequently come into contact with mammals. However, soil fungi must frequently come in contact with bacteria, plants, fungi, viruses, protozoa, and small animals. In the 1970s Bulmer and colleagues carried out landmark studies with amoebae and *C. neoformans* that showed protozoal predation of yeast cells in vitro (Bunting et al., 1979; Neilson et al., 1978). Specifically, they reported that certain amoebae like *Acanthamoeba polyphaga* rapidly phagocytosed and killed encapsulated yeast forms with each trophozoite killing an average of 84 yeast cells each day (Bunting et al., 1979). Interestingly, hyphal forms were resistant to amoebae suggesting that the yeast–hyphal transition could represent a biological ‘escape hatch’ for survival against predation (Bunting et al., 1979). In another classic study evaluating biotic factors that affect *C. neoformans* survival in the environment, it was demonstrated that bacteria, amoebae, mites, and sow bugs each contributed to control the persistence of this fungus in pigeon excreta (Ruiz et al., 1982).

The finding that *C. neoformans* is a facultative intracellular pathogen in mammals with a unique pathogenic strategy (Feldmesser et al., 2001), combined with the observation that predation by amoebae is probably likely in the environment (Ruiz et al., 1982), suggested that the capacity for animal virulence could arise without a need for a close relationship with the affected host (Casadevall et al., 2003). Comparison of the interaction of *C. neoformans* with *Acanthamoeba castellanii* and macrophages revealed a remarkable similarity between the fungal intracellular pathogenic strategy in each cell type (Steenbergen et al., 2001). *C. neoformans* traits associated with virulence for mammals such as the capsule, phospholipase activity, and melanin synthesis were each found to be important for fungal growth in the interaction with amoeba (Steenbergen et al., 2001). Encapsulated *C. neoformans* strains resisted phagocytosis and killed amoeba while the amoebae rapidly killed non-encapsulated fungal cells. In contrast, amoebae killed *C. albicans* cells, which presumably lacked mechanisms for surviving the interaction since this fungus is a human commensal and is therefore not under soil amoeba selection pressure. Similar results were observed with amoeboid cells of the slime mold *Dictyostelium discoideum*, suggesting another potential host–microbe interaction for this organism that could drive the selection of virulence traits (Steenbergen et al., 2003). Other studies revealed that the worm *Caenorhabditis elegans*

avidly ingested *C. neoformans* and that associated cryptococcal virulence factors for mammals were also relevant in this interaction (Mylonakis et al., 2002).

Blastomyces dermatitidis, *H. capsulatum*, *S. schenckii*, and *C. neoformans* var. *gattii* have also been shown to interact with *A. castellanii* in the laboratory (Malliaris et al., 2004; Steenbergen et al., 2004). Like the observations with *C. neoformans*, each of these fungi killed *A. castellanii* but there were major differences in the type of interaction observed. For example, relatively few *B. dermatitidis* and *C. neoformans* var. *gattii* yeast cells were ingested whereby both *H. capsulatum* and *S. schenckii* were readily internalized, yet all four organisms killed *A. castellanii* (Malliaris et al., 2004; Steenbergen et al., 2004). Interestingly, co-incubation of *B. dermatitidis*, *H. capsulatum*, and *S. schenckii* with *A. castellanii* at 37 °C resulted in a marked increase in hyphal forms suggesting that these organisms sensed the presence of amoeba and this triggered the yeast to hyphal transition even at the elevated temperature of 37 °C. Given the earlier observation that hyphal cells of *C. neoformans* were relatively less susceptible to amoeba than yeast cell (Bunting et al., 1979) it is tempting to speculate that fungal dimorphism may have emerged as an escape mechanism against certain types of predators or hostile hosts.

The link between interactions with soil amoeboid predators and mammalian virulence was strengthened by the demonstration that in vitro passage of avirulent fungal strains with amoeboid cells increased the virulence of the fungi for mice (Steenbergen et al., 2003, 2004). Co-incubation of a relatively avirulent *C. neoformans* strain with *D. discoideum* resulted in a significant increase in virulence as measured by shorter survival time (Steenbergen et al., 2003). Similarly, passage of an avirulent *H. capsulatum* strain in *A. castellanii* resulted in the re-acquisition of virulence as measured by the ability of this strain to persist in mouse lungs and to elicit an inflammatory response (Steenbergen et al., 2004). The mechanism by which passage in amoeba increases virulence has not been elucidated. Nonetheless, this phenomenon provides strong experimental support for the view that fungal interactions with soil predators can be a mechanism for the emergence and maintenance of characteristics necessary for animal virulence without the necessity for regular interactions with mammalian hosts.

On the basis of these observations we have formally proposed that the capacity for virulence in certain environmental fungi is a result of selection by interactions with other microorganisms, including amoebae, slime mold, and worms (Casadevall et al., 2003; Steenbergen and Casadevall, 2003). The environmental selection theory for virulence provides an explanation for the observation that many pathogenic fungi can be recovered from their niches with the capacity to cause disease in animals. This phenomenon has been called 'ready-made virulence' to denote the fact environmental isolates express the

necessary attributes for animal virulence when recovered directly from soils (Casadevall et al., 2003).

Environmental selection by predatory organisms cannot be the only mechanism responsible for the emergence and maintenance of virulence among soil microbes. The overwhelming majority of soil-dwelling microbes are not pathogenic for animals, yet all are presumably in a constant struggle with other microbes for survival in their ecologic niches. Since fungi must be under constant selection by different types of predators that range from unicellular microbes to small animals it is likely that different species have evolved different strategies for intracellular survival. Furthermore, it is very likely that not all strategies selected in soil are adaptable (or transferable) for survival in animal hosts. In this regard, comparisons of pathogenic and non-pathogenic *Cryptococcus* spp. are instructive. Among *Cryptococcus* spp. only *C. neoformans* is consistently pathogenic for mammals, despite the fact that other close relatives are occasionally isolated from clinical samples. A comparison of laccase activity between *C. neoformans* and other *Cryptococcus* spp. isolated from clinical samples revealed higher activity in *C. neoformans* (Ikeda et al., 2003), suggesting that quantitative differences in virulence factor expression could contribute to differences in pathogenesis. Furthermore, a comparison of capsule antigenic composition including *C. neoformans* and usually non-pathogenic cryptococcal spp. revealed both common antigens and differences in serological reactivity, suggesting that qualitative differences in the polysaccharide capsule may contribute to differences in virulence (Ikeda et al., 2000). Another instructive study compared the ability for growth at 37 °C, *CNLAC1* expression (gene encoding laccase) and *CAP59* expression (capsule gene) in 21 Heterobasidiomycetous yeast genera including *C. neoformans* (Petter et al., 2001). Polysaccharide capsules were observed in strains belonging to *Filobasidiella*, *Cryptococcus*, *Bulleromyces*, *Bullera*, *Filobasidium*, *Tremella*, and *Trimorphomyces* species but homologs of the *CAP59* gene of *C. neoformans* were not found in any of the other genera (Petter et al., 2001). Although *CAP59* is required for capsule expression its function is not known, but recent studies suggest a role in transport of capsular polysaccharide and possibly secretion of certain enzyme (Garcia-Rivera et al., 2004). Many of these yeasts made melanin pigment when cultured in the appropriate substrate agar but only *Cryptococcus podzolicus* had both a capsule and a close homolog of the laccase encoding gene *CNLAC1* (Petter et al., 2001). However, *C. podzolicus* lacked thermotolerance and was unable to grow at 37 °C (Petter et al., 2001). In fact, none of the heterobasidiomycetous yeast genera grew well at mammalian temperatures (Petter et al., 2001). Hence, *C. neoformans* was unique among these fungi in having a capsule, the capacity to melanize, *CAP59*, and thermotolerance.

Based on these studies with *C. neoformans* and related yeasts one could posit that a minimal combination of capsule synthesis, ability to make melanin and thermotolerance is required for fungal pathogenicity among cryptococcal species. However, other traits are undoubtedly necessary since avirulent strains of *C. neoformans* have been described that possess each of these characteristics (Franzot et al., 1998). Hence, it is likely that environmental pressures can select a broad palette of factors that enhance the likelihood of microbial survival in the environment but only a small fraction of these attributes are suitable for establishing mammalian infection. In the case of *C. neoformans*, the capsule, melanin synthesis, and phospholipase are each common virulence factors for mammals and amoebae. However, for a microbe to successfully infect a host it must also attach and persist in tissue. In this regard adhesins have been described for many of the major fungal pathogens including *B. dermatitidis* (Klein, 2000), *C. albicans* (Hostetter, 1999; Sundstrom, 2002), *C. neoformans* (Merkel et al., 1995), and *H. capsulatum* (Long et al., 2003). The origin and role of these adhesins in the soil fungi is uncertain but they are structurally diverse. For example, the major adhesin of *B. dermatitidis* is a protein known as BAD1 (Klein, 2000), whereas in *H. capsulatum* a heat shock protein mediates adhesion to mammalian cells (Long et al., 2003). Consequently, the presence of surface molecules that can mediate adhesion to mammalian cells is almost certainly a requirement for fungal pathogenesis. The function of fungal molecules that mediate adhesion to mammalian cells in the environment is unknown but it is curious that several interact directly with the complement receptor (Klein, 2000; Long et al., 2003), suggesting the possibility of adhesion function through serendipitous molecular mimicry.

Hence, the capacity of fungi to be pathogenic in mammals appears to occur only when certain set of attributes exist in combination, that include the ability to survive at mammalian temperatures, grow at slightly alkaline pH, attach to host tissues, and survive attack by immune effector cells. In fact, one can envision how the requirement for each attribute reduces the number of potentially pathogenic fungi such that only a very fraction of the total fungal species is capable of virulence in mammalian hosts.

5. Consequences of animal passage for virulence-capable fungal species

The ability of certain soil fungal species to infect and survive in mammals suggests additional considerations that could have important consequences for the ecology and pathogenicity of these organisms. In this regard it is interesting that many of the soil fungi with pathogenic potential in mammals have the capacity for establishing

latent infections in which the organism can persist in the host without eradication. For example, *H. capsulatum* and *C. neoformans* are each capable of establishing asymptomatic infections that can reactivate at later times, particularly in conditions where there is subsequent immune impairment. Furthermore, practically all fungal diseases are characterized by chronicity. The ability to reactivate implies persistence of live fungal cells in tissues that have the capacity to return to the environment upon the death of the host. One can imagine situations where a fungal population lives in the soil for a prolonged period of time under strong selection by soil organisms, then a small subset is acquired by a animal host which is then exposed to the selection pressures of survival high temperature and immunological attack. If these organisms kill the host they return to the soil relatively rapidly and may have acquired differences from immune selection that in turn could add to the diversity of the fungal species and may enhance its likelihood for survival in the environment. If the fungal cells are contained by the immune system in a latent infection their return to the soil is delayed, but animal mobility would bring the advantage of settling in new environments. In this regard, analysis of microsatellite genetic loci in North and South American isolates of *C. immitis* suggests that the introduction of this fungus into South America dates from human migrations (Fisher et al., 2001). In this instance, one scenario is that *C. immitis* infections acquired by Amerindians in the North American southwest may have been carried in their tissues or their livestock as they migrated southwards and returned to the soil when the host died and decomposed.

In addition to the potential for mobility there is some evidence that persistence in an animal host is associated with the emergence of rapid genetic and phenotypic changes. Infection of mice with *C. neoformans* has been associated with the recovery of variants that arise by phenotypic switching, suggesting that variants which arise in vivo are selected for by the immune response and in vivo conditions (Fries et al., 2001). Furthermore, passage of *C. neoformans* strains in mice can result in the emergence of new variants with chromosome polymorphisms illustrating the capacity of animal passage for selection of genetic changes (Fries et al., 1996). In fact, immune selection during occasional animal passage may lead to the emergence of strains with different surface structures by antigenic selection. These in turn could provide the fungus with variants that create new possibilities with regard to the inevitable interactions with soil organisms when the strain returns to the soil upon the death of the host. Hence, the ability for survival in an animal host may arise from soil-related environmental pressures yet its occurrence could confer the microbe with the capacity for mobility and additional selection pressures that could increase its diversity and consequently, enhance its ability to survive environmental change.

6. Fungi and the rise of mammals

The extinction of the dinosaurs and many other species at the end of the cretaceous has been attributed to various processes including the impact of an extraterrestrial bolide approximately 65 million years ago (Alvarez, 1987) and/or increased volcanism (Glasby and Kunzendorf, 1996). The bolide hypothesis is supported by the finding of a global soot-layer at the cretaceous–tertiary (K–T) boundary which could be residue of global wildfires ignited by the collision event (Melosh et al., 1990) and the discovery of a Chicxulub crater in the Yucatan peninsula (Alvarez et al., 1992; Smit et al., 1992). However, more recent analysis suggests that the Chicxulub crater predates the K–T boundary extinction event by approximately 300,000 years raising the questions about a direct and immediate causal relationship between these events (Keller et al., 2004). In fact, it is possible that several concurrent events were responsible for the K–T extinction events and that the great die-off of terrestrial fauna occurred over some time. Although the inciting event and the time line for extinction remain uncertain and controversial, there is widespread agreement that the K–T events were temporally related to the death of many terrestrial species including dinosaurs but spared mammals and birds. There is fossil evidence at the K–T boundary for large scale global deforestation (Vajda et al., 2001), which should have produced a rich nutritional source for vegetation-decaying fungi. In fact, evidence for massive fungal proliferation at the K–T boundary was recently reported and attributed to decreased sunlight and cooler global temperatures (Vajda and McLoughlin, 2004). Fungal proliferation would have resulted in a large increase in fungal spores in the environment, which would have undoubtedly been aerolized. Airborne fungal spores are known to be capable of continental and global dispersal (Brown and Hovmoller, 2002). Consequently it is likely that massive proliferation for the fungal biomass would have resulted in large concentrations of airborne spores that would have delivered large inocula to living organisms.

The evolutionary origin for the emergence and maintenance of endothermy in mammals and birds is poorly understood. Maintenance of elevated body temperature is achieved at a high energetic cost, since whole organism metabolic rates increase exponentially with temperature (Gillooly et al., 2001). Some have proposed that high basal temperature permits higher activity through enhanced aerobic capacity (Bennett and Ruben, 1979). However, it is possible that protection against infectious diseases, and in particular fungal diseases, could also have provided a powerful selection mechanism for endothermy. Massive fungal proliferation feeding on decaying vegetation could have contributed to the extinction of some animal and plant species. Fungi are common pathogens for cold-blooded animals and increases in

fungal biomass would have translated larger densities of fungal spores in the air and environment that would have presented higher inocula for exposed animals and plants. Given that microbial inoculum is a critical parameter in determining the outcome of infection and that high inocula can overwhelm intact immune systems, cold-blooded animals may have been particularly susceptible to fungal diseases resulting from massive exposures. Host susceptibility could have been further increased by the environmental stresses experienced by surviving animal species, which could have impaired immunity as a consequence of nutritional deficits and climatic changes. In this landscape mammals could have been better poised to survive massive fungal challenges as a result of higher body temperatures that are not permissive for growth of many fungal species. Survival of ectothermic animals may have been compromised by a reduction in global temperatures and reduced sunlight, which could have precluded behavioral thermoregulation to induce fever. Consistent with this hypothesis is the fact that birds, the only extant descendants of dinosaurs are also warm blooded (Schweitzer and Marshall, 2001). Birds would have also enjoyed relative immunity to massive fungal infection by virtue of higher basal temperatures.

7. Closing thoughts

The origins of virulence for the soil fungi are probably intimately related to microbial and physical environmental pressures that select for their capacity to survive, proliferate, and cause damage in an animal host. Animal passage may not be necessary for the life cycle of soil fungi, but survival in animals could confer virulent-capable fungi with mobility and expose these microbes to immune selection pressures that may generate additional diversity. Although the origins of fungal virulence for animals cannot be inferred from the fossil record current experience with the interaction of microbes and their hosts would suggest that fungal–animal interactions must have occurred since animals have been extant. Hence, animal evolution may have been driven in part by pressure from fungi, as well as other pathogens. Given that fungi are the primary decomposers of organic matter in soils their biomass is likely to be a function of the available food supply. Cataclysmic changes in earth history that resulted in death of plant life are likely to have increased the fungal biomass by providing a rich supply of nutrients. Increases in fungal biomass could in turn have provided powerful selection pressures on surviving animal species and facilitate the survival of fungal-resistant organisms. Considering that reptiles, amphibians, and mammals emerged at roughly the same time in geological history it is peculiar that mammalian lineages did not prosper until after the K–T event yet underwent a

remarkable expansion in the tertiary to become the dominant land animal forms. Although difficult to prove, a circumstantial case can be made that endothermy provided a significant survival advantage in a fungus-rich world despite its enormous energetic costs.

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