Could insect phagocytic avoidance by entomogenous fungi have evolved via selection against soil amoeboid predators?

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The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* are ubiquitously distributed in soils. As insect pathogens they adhere to the insect cuticle and penetrate through to the insect haemocoel using a variety of cuticle-hydrolysing enzymes. Once in the insect haemocoel they are able to survive and replicate within, and/or evade, phagocytic haemocyte cells circulating in the haemolymph. The mechanism by which these soil fungi acquire virulence factors for insect infection and insect immune avoidance is unknown. We hypothesize that insect phagocytic cell avoidance in *M. anisopliae* and *B. bassiana* is the consequence of a survival strategy against soil-inhabiting predatory amoebae. Microscopic examination, phagocytosis assays and amoeba mortality assays showed that these insect pathogenic fungi are phagocytosed by the soil amoeba *Acanthamoeba castellanii* and can survive and grow within the amoeba, resulting in amoeba death. Mammalian fungal and bacterial pathogens, such as *Cryptococcus neoformans* and *Legionella pneumophila*, respectively, show a remarkable overlap between survival against soil amoebae and survival against human macrophages. The insect immune system, particularly phagocytic haemocytes, is analogous to the mammalian macrophage. Our data suggest that the ability of the fungal insect pathogens *M. anisopliae* and *B. bassiana* to survive insect phagocytic haemocytes may be a consequence of adaptations that have evolved in order to avoid predation by soil amoebae.

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INTRODUCTION

Insect pathogenic fungi, such as *Metarhizium anisopliae* and *Beauveria bassiana*, are considered to be facultative pathogens, since they survive within the soil, do not require an insect host for propagation, and yet maintain virulence (Roberts & Hajek, 1992; Bidochka *et al.*, 2000). Once isolated from soil, these fungi readily infect insects, and one of the most common methods of isolating them is to 'bait' the soil with an insect (Zimmermann, 1986; Bidochka & Khachatourians, 1987). Virulence factors in these insect pathogenic fungi are not constitutively expressed and there are subsets of virulence-related genes upregulated during the various stages of insect infection (Wang *et al.*, 2005). A critical unanswered question arising from the ecological niche and pathogenic nature of these fungi is how virulence is selected for and maintained in the environment. This issue is further complicated by the fact that most isolates of *M. anisopliae* var. *anisopliae* and *B. bassiana* demonstrate

little or no insect host specificity. They are able to infect >1000 different insect and other arthropod species from various orders (Roberts & Hajek, 1992; Bidochka *et al.*, 2001, 2002).

Infection by *M. anisopliae* and *B. bassiana* begins when the fungal conidium contacts the insect cuticle, initiating the expression of genes involved in cuticle adhesion and degradation (Holder & Keyhani, 2005; Cho *et al.*, 2006a, b; Holder *et al.*, 2007). The conidium germinates and the fungal hyphae transgress the insect cuticle, gaining access to the insect haemolymph. Here, another subset of virulence-related genes is upregulated, particularly those involved in differentiation and insect immune avoidance (Wang *et al.*, 2005). Similar to the mammalian innate immune system, certain insect haemocytes are analogous to macrophages in that they encapsulate invading pathogens. However, *M. anisopliae* and *B. bassiana* have evolved mechanisms by which they avoid haemocyte encapsulation (Hou & Chang, 1985; Gotz & Boman, 1985; Bidochka & Khachatourians, 1987; Pendland *et al.*, 1993; Wang & St. Leger, 2006). In an *in vitro* model system, these fungi can

Abbreviations: DIC, differential interference contrast; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

be phagocytosed by host cells, within which the fungal cells can survive and grow, eventually erupting from the host cell (Kurtti & Keyhani, 2008). Human pathogenic fungi such as *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Sporothrix schenckii* appear to have evolved similar traits, being able to survive mammalian macrophage phagocytosis (Steenbergen *et al.*, 2004).

There is evidence to suggest that the survival and growth of mammalian fungal pathogens within a macrophage evolved as a result of environmental pressures from soil-borne predators, particularly phagocytic amoebae (Steenbergen *et al.*, 2001). The mammalian pathogenic fungus *C. neoformans* has been shown to survive phagocytosis, and to grow within the amoeba *Acanthamoeba castellanii* (Malliaris *et al.*, 2004). Amoebae, human macrophages and insect haemocytes share common properties. They phagocytose their host and digest them with lysosomal enzymes and superoxidases (Davies *et al.*, 1991; Bergin *et al.*, 2005; Scully & Bidochka, 2006). Here, we hypothesize that the mechanisms by which insect haemocyte avoidance evolved in insect pathogenic fungi are analogous, if not directly similar, to the evolution of avoidance of soil-borne phagocytic amoebae by mammalian pathogenic fungi. Using green fluorescent protein (GFP)-transformed *B. bassiana* and *M. anisopliae* isolates, the interaction between *A. castellanii* and the fungi was examined. In this study, we show that amoebae actively internalize the fungal conidia; however, the fungi are able to survive, germinate and grow within the amoebae, eventually erupting from the host cells.

METHODS

Strains and culture conditions. Stock cultures of *M. anisopliae* ARSEF 2575 and *B. bassiana* ARSEF 252 and ATCC 90517 were maintained at -80°C in 25% (v/v) glycerol. In one set of experiments we used two additional fungal species, *Alternaria alternata* UAMH 10047 and *Penicillium chrysogenum* UAMH 6742. Cultures were grown on potato dextrose agar (PDA; Becton, Dickinson and Company) at 27°C for 10 days to obtain conidia. Conidia were dislodged from the culture with 10 ml 0.01% Triton-X/PBS, and the suspension was poured through a glass funnel stuffed with glass wool to remove mycelial fragments from the conidial suspension. A stock culture of *Saccharomyces cerevisiae* was maintained at -80°C . Cultures were grown for 4 days in peptone/yeast extract/glucose (PYG) broth in a shaker at 250 r.p.m. at 27°C .

The construction of GFP-expressing plasmids and transgenic fungal lines has been described previously (Fang *et al.*, 2006).

A. castellanii strain 30234 (ATCC) was maintained in 100 ml tissue-culture flasks (Falcon; Becton Dickinson Labware) with 10 ml PYG broth at room temperature on a shaker at 45 r.p.m. The amoeba cultures were maintained by diluting the amoeba medium by one-half with fresh medium every 5–7 days.

The extracted conidia, yeast and amoebae were washed by centrifuging the suspensions at 320 g for 10 min. The supernatant was decanted and cultures were suspended in PBS. The process was repeated twice with the final suspension of *M. anisopliae*, *B. bassiana*, *Alternaria alternata*, *P. chrysogenum* or *S. cerevisiae* suspended in

0.01% Triton-X/PBS, and *A. castellanii* suspended in PBS. The suspensions were diluted and then counted using a haemocytometer.

Amoeba killing assay. The fungal killing assay was based on a previously described protocol (Malliaris *et al.*, 2004), with modifications. *A. castellanii* was diluted to 1×10^4 cells ml^{-1} and counted using a haemocytometer. Amoebae were added to wells of a 96-well plate at 200 μl per well and allowed to adhere to the well for 1 h at 27°C before 200 μl of 1×10^4 cells ml^{-1} of *M. anisopliae*, *B. bassiana* or *S. cerevisiae* was added to produce a 1:1 effector-to-target ratio. The 96-well plates were then incubated at 27°C for 0, 8, 16 and 24 h. *S. cerevisiae* was used as the positive control, since it has been found to be phagocytosed and rapidly killed by *A. castellanii* (Steenbergen *et al.*, 2001).

The viability of *A. castellanii* grown under the experimental conditions was determined using a Trypan blue exclusion assay at each time interval. Fifty microlitres of a 1:20 dilution of Trypan blue stain was added to wells and the percentage of dead amoebae was determined by counting the number of amoeba cells unable to exclude the dye per 50 amoebae counted. The control for the Trypan blue counts consisted of wells containing *A. castellanii* with 0.01% Triton/PBS instead of fungi or yeast.

In the remaining wells the contents were pulled through a 27-gauge syringe needle five to seven times to completely lyse the amoebae and release the contents. The contents of each well were then plated onto PDA agar for *M. anisopliae* and *B. bassiana*, and YPD agar for *S. cerevisiae*, so that approximately 10^2 cells were placed on each plate. The plates were then incubated at 27°C for fungal c.f.u. determination. The control for c.f.u. determination consisted of wells containing *M. anisopliae*, *B. bassiana* and *S. cerevisiae* with PBS. In this experiment, conidia of *P. chrysogenum* and *Alternaria alternata* were also used as controls to determine whether survival of amoebic phagocytosis is a general property of soil-borne fungi.

***A. castellanii* phagocytosis of fungi.** *A. castellanii* was diluted to 1×10^6 cells ml^{-1} with PBS. Ten microlitres of the *A. castellanii* suspension was placed on a microscope slide. *M. anisopliae* and *B. bassiana* were diluted to 1×10^6 cells ml^{-1} using a haemocytometer and 0.01% Triton-X/PBS. Ten microlitres of the fungal solution was placed on the slide with *A. castellanii* and the cell suspensions on the slide were mixed using a micropipette. The slide was placed within a Petri dish, which was wrapped with Parafilm (to prevent desiccation) and placed in an incubator at 27°C . At 2, 4, 6, 8, 10 and 12 h, the microscope slide was taken out of the Petri dish and the percentage of amoebae which had engulfed fungi was recorded. Samples for microscopic examination were prepared by seeding the amoebae in four-well LabTek chambered coverglass systems (Nunc). In some instances, extracellular conidia were separated from amoeba-internalized conidia by Centricoll density-gradient centrifugation. Cultures of co-incubated fungi and amoebae were harvested and immediately applied on top of a step gradient of 25 and 50% Centricoll. Centrifugation was performed in sterile 2 ml tubes, at 10 000 g for 10 min at 4°C using a table-top centrifuge. Amoebae (including those harbouring fungal conidia) were separated from external fungal cells and collected within the top portion (25% step) of the gradient (fungal conidia formed a pellet at the bottom).

***Galleria mellonella* (Wax Moth) larvae bioassays.** *A. castellanii* was diluted to 1×10^6 cells ml^{-1} with PBS. *M. anisopliae* and *B. bassiana* were diluted to 1×10^6 cells ml^{-1} with 0.01% Triton-X/PBS. Five-hundred microlitres of *A. castellanii* or PBS was placed into an Eppendorf tube labelled 24, 48 or 72 h. Five-hundred microlitres of *M. anisopliae* or *B. bassiana* was placed into the Eppendorf tube containing the amoebae or PBS. The Eppendorf tubes were stored at 27°C for 24 h. After 24 h, the tubes were removed from the incubator and the tubes containing *A. castellanii* were lysed using a

vortex at full speed for 30 s. The Eppendorf tubes, at 24 h, were plated onto PDA agar so that approximately 10^2 cells were plated. After 48 and 72 h, the tubes were centrifuged for 10 min at 320 g. The pellet was resuspended with *A. castellanii* or PBS to a volume of 1 ml and stored for another 24 h at 27 °C. The process was repeated twice more until the 72 h sample was plated and stored at 27 °C. The fungal c.f.u. after 24, 48 and 72 h was recorded after 5 days of incubation at 27 °C. The c.f.u. counts were used to dilute solutions of *M. anisopliae* and *B. bassiana* to 1×10^4 cells ml⁻¹ to inject into *G. mellonella*. *G. mellonella* was injected with 3 µl PBS, original conidial suspension, conidia passed through PBS for three 24 h cycles, or conidia passed through *A. castellanii* for three 24 h cycles. The *G. mellonella* were individually placed in snap-lid containers and kept at 27 °C, and mortality was checked daily.

Microscopy. Fixed-cell, live and time-lapse imaging was obtained using a Zeiss Axiovert Pascal LSM5 inverted microscope equipped with a laser scanning unit (for GFP, excitation 488 nm, emission 505 nm). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed using Hitachi S400 and Zeiss EM10-CA electron microscopes, respectively.

RESULTS

Uptake of *M. anisopliae* by *A. castellanii*

Fluorescence microscopy was used to examine the interaction between GFP-transformed *M. anisopliae* and *A. castellanii* (Fig. 1). Superposition of differential interference contrast (DIC) and fluorescence microscopic images of the same sample showed co-localization of fungal cells within the amoebae, with increasing numbers of fungal conidia co-localizing within the amoebae over time. 3D (Z-stack) imaging was consistent with internalization of the conidia within the amoebae. Time-lapse imaging showed predation

Fig. 1. *M. anisopliae* GFP-labelled conidia incubated with *A. castellanii* at 1–3 h. Bar, 25 µm.

of the fungal cells by the motile amoebae, and in several instances multiple conidia could be seen in different planes of focus within the amoebae. Experiments using infection ratios ranging from 0.1 to 10 fungal conidia per amoeba gave essentially the same results, with greater numbers of internalized conidia per amoeba visible as the fungal:host cell ratio or m.o.i. increased. Interestingly, qualitative observations of the amoebae indicated that amoebae that were actively preying upon the fungal cells tended to take up multiple conidia.

To show the internal nature of the fungal cells, SEM and TEM were employed. SEM images of the interactions between fungal cells and the amoebae indicated initial adhesion and subsequent engulfment of the fungal conidia by the amoebae, with pseudopodial interactions evident (Fig. 2). Internalization of fungal conidia by the amoebae was confirmed by TEM, in which samples were examined 3 h post-inoculation of fungal conidia with the amoebae (Fig. 3). In some instances, internalized conidia appeared to be enclosed within an endosomal compartment, although this was not always apparent. Qualitative analysis indicated that uptake increased over time, with amoebae often taking up multiple conidia. Some fungal conidia were clearly in large vacuoles, although a membrane surrounding all internalized fungal conidia was not always evident. To examine whether germination of internalized fungi could be observed by TEM, amoebae were allowed to prey upon the fungal conidia for 3 h, after which external fungal conidia were removed by density-gradient centrifugation, and the amoebae were replaced in media for 18–20 h, before samples were prepared for TEM analysis as described in Methods. Using such a protocol, fungal germination within the amoebae was observed (Fig. 3e, f).

Quantification of uptake and amoeba killing by *B. bassiana* and *M. anisopliae*

To quantify and measure the rate of uptake of the fungal conidia by the amoebae, GFP-expressing *B. bassiana* and *M. anisopliae* were employed to follow the time-course of amoebic phagocytosis. Fungal conidia were phagocytosed by amoebae within 2 h of co-incubation and this increased up to 8 h (Fig. 4). After 8 h, the percentage of amoebae containing fungal conidia decreased. Between 8 and 16 h we observed a decrease in amoebae containing fungal conidia. This concurs with the onset of germination by fungi and an increase in amoeba mortality (see below).

Trypan blue exclusion assays were performed to determine the percentage of amoebae killed by the fungi. These data showed that amoebae were killed when incubated with *B. bassiana* and *M. anisopliae* but not *S. cerevisiae* (Fig. 5). When the assay was initiated, >97 % of the amoebae were alive and excluded Trypan blue. After 24 h incubation in PBS, ~90 % of the amoebae remained viable. There was no statistical difference in amoeba mortality between the control (Ac) and amoebae incubated with *S. cerevisiae* (*t* test, *P*>0.05). After 24 h, *B. bassiana* and *M. anisopliae*

Fig. 2. SEM images of internalization of *M. anisopliae* by *A. castellanii* at 3 h incubation. Bars, 3 μ m.

killed 20–22 % of the amoebae, significantly greater than the control mortality (t test, $P < 0.05$) or incubation with *S. cerevisiae* (t test, $P < 0.05$). Cell-free extracts of *M. anisopliae*

and *B. bassiana* incubated for 48 h in PBS then transferred to an *A. castellanii* culture in PBS did not result in increased amoeba death over the control.

Fig. 3. TEM images of internalization of *M. anisopliae* by *A. castellanii*. (a–d) Samples fixed after 3 h co-incubation; (e, f) co-incubation for 3 h, followed by harvesting and removal of external conidia, culture replaced at 28 °C, and fixed after 18 h incubation. Bars, 6 μ m.

Fig. 4. Time-course of the proportion of amoebae containing *B. bassiana* (white bars) and *M. anisopliae* (black bars). For each time point, 200 amoebae were counted, with $n=5$. Error bars, SD. Data are representative of three separate experiments.

Fungal growth in amoebae

When *B. bassiana*, *M. anisopliae*, *S. cerevisiae* or the amoeba *A. castellanii* was incubated in PBS little growth or replication was noted. Both *B. bassiana* and *M. anisopliae* are dimorphic fungi able to grow as mycelia or, in the presence of nutrient-rich medium, they can evolve single-cell propagules termed blastospores. TEM images of fungal conidia internalized by the amoebae and allowed to incubate overnight revealed fungal germination within the host cell (Fig. 3e, f). Experiments performed using GFP-transformed *M. anisopliae* allowed for dynamic visualization of the fungal growth within the amoebae (Fig. 6). Hyphal emergence from amoebae containing the fungi was apparent, and in many instances several germlings could be seen growing within a single amoeba (Fig. 6).

To assess the extent of fungal growth in the presence of the amoebae, the number of fungal c.f.u. was determined over time during co-incubation experiments (Fig. 7). These data showed that when *M. anisopliae* or *B. bassiana* was incubated in the presence of *A. castellanii*, the total c.f.u. remained stable (t test, $P>0.05$). In contrast, a significant decrease in total *S. cerevisiae* c.f.u. was noted under similar conditions (t test, $P<0.05$). Here, we also showed a significant decrease in total *P. chrysogenum* and *Alternaria alternata* c.f.u. (t test, $P<0.05$). Microscopic examination showed that amoebae phagocytosed these fungi.

It should be noted that a germinated conidium undergoing hyphal growth would record as 1 c.f.u.; therefore, to obtain a better representation of the potential growth of the fungi in the presence of the amoebae, samples were vigorously vortexed to shear the fungal hyphae as well as the amoebae, and the resulting suspension was incubated with a fresh amoeba culture (Fig. 8). Under such conditions, increased growth, as seen by increased c.f.u., for *M. anisopliae* and *B. bassiana* was observed after three cycles of shearing and incubation (t test, $P<0.05$). In contrast, a decrease in *S. cerevisiae* c.f.u. was noted under identical conditions (t test, $P<0.05$), suggesting that *S. cerevisiae* is killed by the phagocytic amoebae, whereas *M. anisopliae* and *B. bassiana* are able to survive and grow.

B. bassiana and *M. anisopliae* virulence is not enhanced by passage through *A. castellanii*

We surmised that if *B. bassiana* and *M. anisopliae* survive insect haemocytic phagocytosis as a consequence of avoidance of amoebae in the soil, then virulence could also be affected by passage of fungal cells through amoeba cultures. To examine this possibility, fungal cultures were passaged three times through the amoebae as well as through PBS as a control. Insect bioassays using the larvae of the Wax Moth, *G. mellonella*, were subsequently performed by injection of 3 μ l of fungal material ($\sim 10^7$ c.f.u. ml^{-1}). From these experiments, median lethal time (LT_{50}) values for *B. bassiana* non-passaged, passaged through PBS and passaged through amoebae were determined to be 3.6, 3.3 and 3.2 days, and the LT_{50} for *M. anisopliae* non-passaged, passaged through PBS and passaged through amoebae was 2.1, 2.5 and 2.2 days. In each case, no significant differences in virulence were noted under the conditions tested.

DISCUSSION

Our data have led to the hypothesis that the selective pressure of soil amoeboid predation on *M. anisopliae* and *B. bassiana* may be critical in maintaining virulence factors that allow these fungi to survive insect phagocytic haemocytes. Using GFP-transformed strains of *M. anisopliae* and *B. bassiana* to investigate internalization, survival

Fig. 5. Time-course of *A. castellanii* mortality, using a Trypan blue exclusion assay, during incubation with *S. cerevisiae* (Sc), *M. anisopliae* (Ma) and *B. bassiana* (Bb). Ac, control mortality. For each time point, 200 amoebae were counted, with $n=5$. Error bars, SD. Data are representative of three separate experiments.

Fig. 6. DIC and fluorescence microscopy images. A low inoculum of *M. anisopliae*-GFP of 1 : 50 (*M. anisopliae* : amoeba) was employed, and the sample was incubated for 18 h at 28 °C. Single- and multi-plane Z-stack images are shown. (a) and (b) show two separate examples. Bars, 10 μm.

and growth within the amoeba *A. castellanii*, our results show that *M. anisopliae* and *B. bassiana* are phagocytosed by amoebae but that these fungi are able to survive and grow within the amoebae, ultimately resulting in the death of the amoebae. After phagocytosis, some of the fungi were localized in membrane-bound vacuoles, and our data are consistent with the ability of *M. anisopliae* to survive phagocytosis by tick cell lines (Kurtti & Keyhani, 2008). In contrast to the entomopathogenic fungi examined, the yeast *S. cerevisiae* was killed by amoebae, and earlier reports examining filamentous soil fungi including *Aspergillus niger*, *Fusarium* spp., *Rhizoctonia solani*, *Sordaria fimicola* and *Stachybotrys atra* have demonstrated that these organisms are unable to avoid amoebic phagocytosis (Old & Darbyshire, 1978). We also showed that two common soil fungi, *P. chrysogenum* and *Alternaria alternata*, were also unable to avoid amoebic phagocytosis. Indeed, the phenomena of survival of amoeba predation may be restricted to zoopathogenic fungi, including the invertebrate pathogens noted in this study, since several species of phytopathogens (i.e. *Gaeumannomyces graminis* var. *tritici*, *Phytophthora cinnamomi* and *Cochliobolus sativus*) do not survive amoebic phagocytosis, potentially

consistent with the lack of macrophage-like cells in host plants that could reinforce such an adaptation (Chakraborty & Old, 1982; Chakraborty *et al.*, 1983).

Several human pathogenic fungi also share the ability to survive and multiply within amoebae, and similarly it has been hypothesized that this interaction allows these fungi to survive human phagocytic cells (Levitz, 2001). The encapsulated fungus *Cryptococcus neoformans* is able to survive phagocytosis by amoebae, and replicate within and eventually kill the amoebae, a process similar to its interaction with mammalian macrophages (Levitz, 2001; Bergin *et al.*, 2005). Other human pathogenic fungi, *Blastomyces dermatitidis*, *Sporothrix schenckii* and *Histoplasma capsulatum*, have also been reported to be ingested by amoebae, with phagocytosis resulting in amoeba death and fungal growth (Steenbergen *et al.*, 2004). Interestingly, larvae of the Wax Moth, *Galleria mellonella*, have been employed as a model system to study *C. neoformans* pathogenesis, and although the fungus is encapsulated by insect phagocytic haemocytes, infected insects eventually die, suggesting that *C. neoformans* is also able to evade insect haemocyte encapsulation (Mylonakis *et al.*, 2005). Furthermore, some bacterial pathogens that are

Fig. 7. c.f.u. at 0 h (grey bars) and after 72 h incubation (hatched bars) of *B. bassiana* (Bb), *M. anisopliae* (Ma), *S. cerevisiae* (Sc), *P. chrysogenum* (Pc) and *Alternaria alternata* (Aa) with *A. castellanii* (Ac) or in PBS. Data are representative of three separate experiments, each with $n=5$. Error bars, SD.

Fig. 8. c.f.u. for three 24 h cycles (with physical disruption between each cycle) of fungi [*B. bassiana* (Bb), *M. anisopliae* (Ma) and *S. cerevisiae* (Sc)] incubated with *A. castellanii* (Ac) or in PBS. Data are representative of two separate experiments, each with $n=5$. Error bars, SD.

able to survive uptake by macrophages have also been shown to be able to survive amoebic phagocytosis. The interaction with amoebae, both as a replication niche and evolutionary force in adaptation to human pathogenesis, has been noted for *Legionella pneumophila*, *Mycobacterium avium*, *Listeria monocytogenes*, *Chlamydia pneumoniae* and *Burkholderia cepacia* (Cirillo *et al.*, 1997; Essig *et al.*, 1997; Ly & Muller, 1990; Marolda *et al.*, 1999; Swanson & Hammer, 2000).

Overall, our data support the idea that facultative insect pathogenic fungi such as *B. bassiana* and *M. anisopliae* that do not require an insect for survival can maintain certain virulence factors under selective pressures that operate in the soil via predatory amoebae. The interaction with soil amoebae is likely to be only one facet of the multi-dimensional dynamics of pathogenesis. There are a plethora of additional soil interactions with other fungi, nematodes, protists, bacteria and plants that provide an extremely rich diversity of interactions that we are just beginning to appreciate, and which may contribute to the evolution of virulence factors and the broad insect host ranges of these fungi.

That mammalian as well as insect pathogenic fungi are able to survive amoeba predation is a novel finding. Avoidance of amoeba predation is not just a factor for human pathogens but may be a much more general lifestyle adaptation. The environmental niches, lifestyles and pathogenic consequences of these organisms are radically different, yet they have evolved a similar mechanism for thwarting the amoebae which, consequentially, can be used as a virulence factor for their respective hosts.

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