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The fungal dining habit: a biomechanical perspective

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Invasive hyphal growth allows filamentous fungi to insinuate themselves in the solid materials that serve as their food sources. Hyphae overcome the mechanical resistance of plant and animal tissues, and other substances through the secretion of digestive enzymes and the exertion of force. This force is derived from the osmotically-generated turgor pressure within the hypha and is governed by wall loosening at the growing apex. This article offers a concise description of the biomechanics of this process.

Keywords: appressoria, decomposition, hyphae, invasive growth, rock penetration, turgor pressure, wood decay

Environmental challenges and fungal solutions

When fungi consume leaf litter, fallen timber, and living tissues of plants and animals, they push themselves into their food sources. To consider the physical challenges facing the fungus, a human analogy may be helpful. Apple bobbing was a popular entertainment at village fêtes in the Britain of my youth; I imagine that this has been prohibited in recent years by EU regulations concerning *Salmonella*. Apple bobbing tested the persistence (or degree of inebriation) of the contestant, because it is very difficult to sink one's teeth into a floating apple without the aid of a hand. The bobber's lips brush uselessly against the apple, pushing it away through the salivary water, illustrating the way in which an imbalance of forces results in the acceleration of one object away from another. A similar thing can happen when a fungus encounters an apple, or any other solid food source, though in this case, the predator is much smaller than its prey. If the fungus isn't secured to the surface of its food, or to some nearby platform, hyphal extension will push it away from its meal (Fig 1A). To every action, there is an equal and opposite reaction (as an exponent of other thought experiments involving apples said in the seventeenth century). Penetration only becomes possible after firm attachment (Fig 1B), which suggests that invasive growth could not have evolved without the coincidental development of adhesives.

Besides the need to elaborate methods for sticking to different surfaces, fungi are challenged by the toughness of their food sources. Hyphal penetration is driven by hydrostatic pressure. Hyphae are inflated by a few atmospheres of osmotically-generated turgor, and can exert a proportion of this internal pressure on their surroundings by allowing slippage between cell wall polymers at their apices (Money, 2001; Bastmeyer *et al.*, 2002). But the magnitude of the applied pressure is at

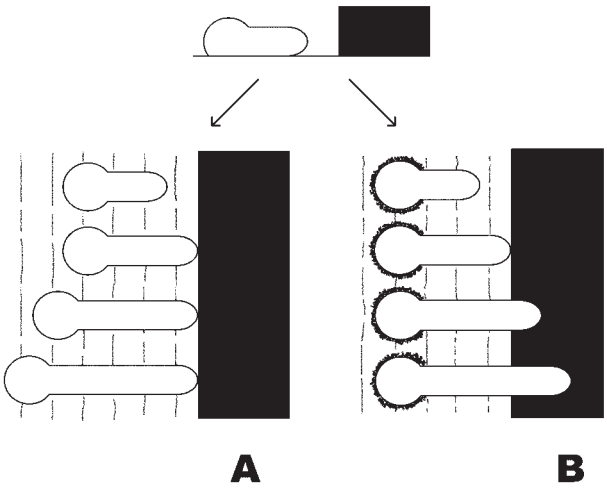

Fig 1 Interactions between fungal germlings and solid obstacles or potential food sources. Top illustration shows germling (in side elevation) extending over horizontal surface toward obstacle. Accompanying sequences show germling in plan view, with obstacle sectioned in B to expose fungal intrusion. Sequence A illustrates unsecured germling (i.e., one that has not fixed itself to the substrate by the secretion of an adhesive). The fungus elongates and pushes itself away from the obstacle. Sequence B shows germling affixed to surface by the production of an adhesive at the spore surface. Once secured to the adjacent substrate, the fungus pushes itself into the obstacle. Germlings often succeed in fastening themselves to surfaces via the secretion of adhesives along the length of the elongating germ hypha; this accessory myco-glue is not shown in the figure.

least an order-of-magnitude too low to overcome the constitutive resistance of many of the materials that fungi consume. To devour plant and animal tissues, fungi use secreted enzymes to bridge the gap between the strength of their hyphae and the otherwise impervious nature of their meals. I'll discuss some numbers shortly.

One thing missed in this glancing introduction to the invasive process is the sensitivity that fungi show to the texture of their environment (Hardham, 2001). Hyphae usually bulge at their apices when they hit something dense, then reorient the direction of tip growth to wind around the obstacle, or form an appressorium (or hyphopodium) to attempt penetration. A formal demonstration of this behavioral complexity was provided by the experiments of Hoch *et al*. (1987). Hoch and colleagues showed that germlings of the rust *Uromyces appendiculatus* were capable of discriminating between ridges of slightly differing height on microfabricated surfaces and on replicas of leaves. They grew over most ridges, but produced appressoria when they detected obstacles of a specific height that mimicked natural cues for invasion found on the surface of their hosts.

How do we measure the strength of hyphae?

Hyphal turgor pressure had not been determined with any confidence before the introduction of the pressure probe, an instrument originally developed by plant physiologists in the 1970s. The device is an infuriating invention. It is difficult to machine, calibrate, and use- the problems including broken pipettes, leaking neoprene washers, and, most of all, uncooperative cells - but does yield data that cannot be obtained by other methods. Before hyphae were stuck with probe pipettes, turgor estimates had rested on experiments that measured the amount of sugar needed to deflate hyphae, or upon measurements of the combined sugariness and saltiness of the cytoplasm. Pressure probing is verging on ancient history now, so I'll skip the details, but the technique did furnish good data on the pressures inside hyphae, particularly for the larger cells characteristic of oomycete water moulds (see references in Money, 2001).

Experiments with the pressure probe led to some interesting discussions about the role of turgor as a component of the hyphal growth mechanism, and what became apparent was that this pressure was of particular importance when hyphae penetrated things. Initial evidence for this was found by rendering the hyphae of some species flaccid during experiments on osmotic stress: surprisingly, the hyphae (again, those of water moulds) continued to grow, but they were disabled by the pressure loss and were limited to crawling over surfaces. Next came the realization that the pressure inside the cell was not the same as the pressure exerted by the hyphal apex upon its substrate. The mechanical principle is simple. A hypha with a rigid cell wall will not apply any of its interior pressure upon its surroundings. But if the wall at the hyphal apex loosens, the cell will apply a proportion of its turgor on the material in contact with its tip. In other words, a measurement of turgor represents the maximum possible pressure that a cell *can* exert, but not the actual pressure it *does* exert. This led to the search for methods to measure the forces operating at the hyphal apex.

The pressures exerted by hyphae have now been quantified using two methods. Neither is perfect, but there is good reason to think that the information obtained from these experiments is sound. I'll begin with the use of miniature strain gauges. Fig 2 shows the instrument and illustrates how it is used. By positioning a tiny strain gauge just in advance of a growing hypha, the strength of the cell is measured when it pushes against the instrument's silicon beam (Johns *et al*., 1999; Ravishankar *et al*., 2001; MacDonald *et al*., 2002). In practice, hyphae can change shape, stop growing, or shift direction a few minutes after touching the beam, but by then, the necessary data have already been collected. When the hypha contacts the beam, the electrical output from the strain gauge immediately changes in proportion to the applied force (μN), and peaks within a few seconds or minutes (Fig 2D). The pressure exerted by the cell is derived by dividing the measured force by the contact area with the beam (because pressure = force/area). Hyphal pressure is conveniently expressed in units of $\mu N\ \mu m^{-2}$, which are interchangeable with MPa; note that $1.0\ \mu N\ \mu m^{-2}$ = 1.0 MPa = 10 bars = 9.9 atmospheres.

The second approach for measuring hyphal force relies upon an optical technique that measures the depth of indentations in a synthetic membrane called a 'waveguide' (Bechinger *et al*., 1999). When appressoria of phytopathogens (see later) are allowed to develop on this membrane, they depress its surface in an attempt to penetrate the impenetrable - the waveguide consists of an aluminum sandwich containing silicone gel. A laser is then used to scan the waveguide and to generate a 3-D picture of the dent caused by the fungus with nanometre accuracy. The waveguide is calibrated with glass needles to determine the relationship between applied force and deformation, so that the dent can be related to the force that produced it. In the same way

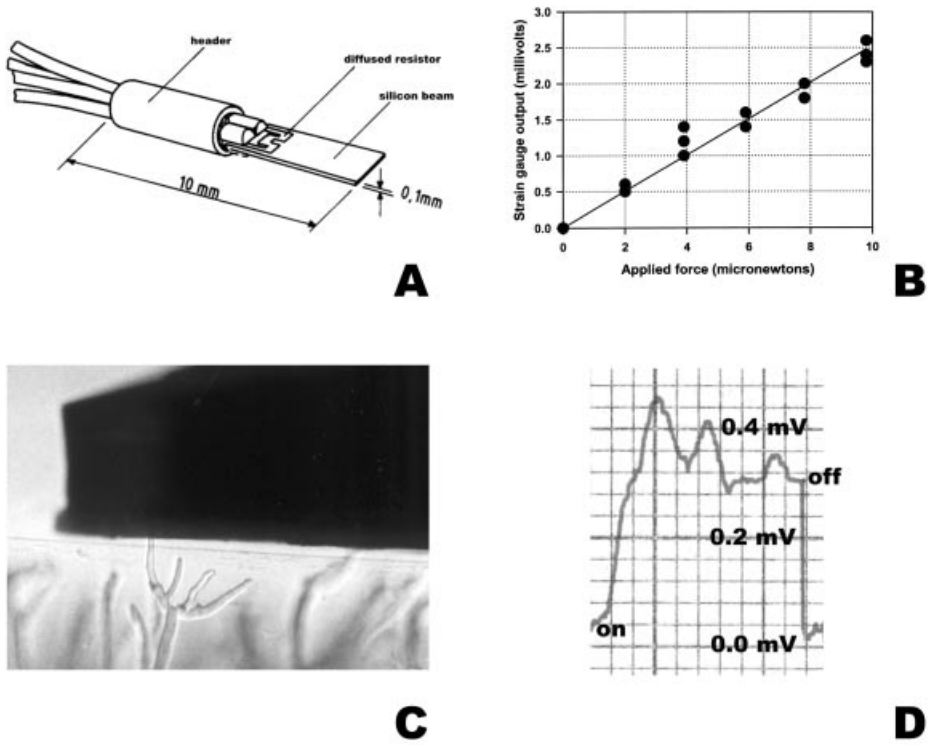

Fig 2 A. Diagram of miniature strain gauge used to measure the force exerted by individual hyphae. Adapted from technical literature from Sensor One Technologies Corporation, Sausalito, CA. B. Calibration curve showing relationship between applied force and strain gauge output voltage. Force is controlled by placing µg and mg masses (F = mass x gravitational acceleration) on the end of the silicon beam of a strain gauge that is clamped in a horizontal orientation. C. Hypha of *Mucor hiemalis* pushing against silicon beam of strain gauge. Single apex of branched cell growing within agar medium extends from an agar shelf into a well filled with nutrient broth. A micromanipulator is used to position the strain gauge in the well, a few µm away from the agar shelf. For scale note that the width of the silicon beam is 0.1 mm. D. Example of force recording from *Mucor* hypha; 'on' marks the time (t = 0) when hyphal apex contacted the silicon beam; 'off' marks the time (t = +1.4 min) when the strain gauge was moved away from the hypha, resulting in the immediate drop in instrument output to the baseline voltage. In this case, the peak strain gauge output was 0.44 mV, which corresponds to an applied force of 1.4 µN (see 2B). Since the area of the hypha in contact with the silicon beam was 20 μm^2, the estimate of applied pressure is 0.07 µN μm^{-2} (= 0.07 MPa = 7/10ths of one atmosphere).

that hyphal pressure is derived from a measure of force made with the strain gauge, the force acting on the waveguide is converted to a pressure by dividing by the contact area between the fungus and its imprint. The method is very elegant, but does not seem suited for experiments with vegetative hyphae (or, perhaps I should say that so far nobody has figured out how to use waveguides for work with these cells).

What do the biomechanical data say?

Most of the initial strain gauge experiments on hyphae concerned water moulds (Kingdom Stramenopila, Phylum Oomycota), including members of the family Saprolegniaceae, and pathogenic species of *Pythium* (Johns *et al*., 1999; Ravishankar *et al*., 2001; MacDonald *et al*., 2002). More recently, basidiomycete, ascomycete, zygomycete, and chytridiomycete fungi (Kingdom Fungi) have been studied with this technique. Data from these experiments will be published elsewhere, but a few useful points can be made without examining the details. The most important finding is that when viewed in terms of their biomechanical behavior, hyphae of species within Kingdom Fungi seem to operate in much the same way as the oomycetes. Table 1 offers a snapshot of the data by comparing the vital statistics of the zygomycete *Mucor hiemalis*, and the oomycete *Thraustotheca clavata*. I find the mechanical similarity between these microorganisms striking. Species that sit in distant parts of the galaxy of biodiversity (Fungi with a capital F, which share a common ancestor with the animals, and water moulds that evolved from some other antediluvian protist) have developed the same mechanical attributes that enable them to utilize an identical nutritional strategy (Latijnhouwers *et al*., 2003). This is a beautiful case of convergence, perhaps comparable to the evolution of swimming in fish and

Table 1. Comparison between biomechanical characteristics of hyphae of a zygomycete fungus and an oomycete water mold. Turgor measurements made with pressure probe and by vapor pressure deficit osmometry, and force measurements made with a miniature strain gauge.

Organism	Turgor pressure (MPa)	Applied force (μN)	Contact area (μm^2)	Applied pressure ($\mu N\ \mu m^{-2}$ or MPa)[c]	Ratio of applied pressure to turgor
Mucor hiemalis[a] Kingdom Fungi Phylum Zygomycota	0.58 ± 0.01 (12)	2.1 ± 0.4 (24)	52 ± 13 (24)	0.05 ± 0.01 (24)	0.09
Thraustotheca clavata[b] Kingdom Stramenopila Phylum Oomycota	0.69 ± 0.02 (9)*	9.8 ± 3.5 (21)	166 ± 42 (21)	0.06 ± 0.01 (21)[NS]	0.09

Notes. Data shown as mean ± standard error, with number of replicates in parenthesis.

[a]Cells cultured in potato dextrose agar and broth

[b]Cells cultured in peptone-yeast extract-glucose agar and broth.

[c]Individual measurements of force were paired with individual measurements of contact area, providing an estimate of applied force for each hypha. Mean values for applied pressure were obtained from these data, not by division of mean force (column 3) by mean contact area (column 4).

ANOVA showed a significant difference (*) in turgor ($P < 0.005$), but no significant difference (NS) between the pressures applied by the two species ($P = 0.37$).

aquatic mammals, only more impressive because the ancestors of the Fungi and oomycete water moulds diverged more than a billion years ago.

Although the level of hyphal turgor and applied pressure varies from species to species, the available data establish that all hyphae must use enzymes to reduce the mechanical resistance of their food sources to facilitate invasion. This conclusion has been an implicit assumption of researchers for many years, but thanks to the biomechanical experiments this is now tested, supported, and appended with numbers. Plant and animal tissues breached by saprobic and pathogenic fungi offer overwhelming resistance to mechanical penetration. Specifically, pressures of 4 to 118 atmospheres are needed to pierce the epidermis of roots and leaves (Miyoshi, 1895; MacDonald *et al.*, 2002), and puncture experiments using skin taken from very fresh human cadavers show that penetration is resisted until the pressure exerted at the tip of a needle exceeds 100 atmospheres (Ravishankar *et al.*, 2001). These data show that the strength of plant and animal tissues must be greatly reduced by prior injury, or through the action of fungal enzymes, before hyphae overcome the remaining obstructions by brute force.

To go beyond this generalized picture and profile the feeding mechanism used by a particular fungus, we need an inventory of its substrate-degrading enzymes. Innumerable experiments with mutants lacking one or more secreted enzymes have failed to furnish the necessary information, but research founded on genome-level inquiries has revived hope that the most important enzymes used by a few of the 'model' fungi will be exposed before too long. Because vegetative hyphae feed as they penetrate, and vice versa, many of their enzymes probably serve to liberate nutrients as they weaken barriers to intrusion.

The degree to which the application of pressure comes into play probably changes from minute to minute when fungi explore their food sources, depending upon alterations in the strength of the substrate on a micrometer scale. It is worth asking, however, whether the biomechanical characteristics of a fungus are important if so much of the work of invasion is done by enzymes. The sensitivity of the invasive process to changes in turgor and substrate resistance (shown by experiments cited in Money, 2001), provides the best evidence that the biomechanical behavior of hyphae is a critical feature of the invasive process. Pure logic is compelling too. Unless the fungus can completely liquify its surroundings, its hyphae must use mechanical force to thrust forward. Even 1% of the original strength of a leaf epidermis is an insurmountable barrier to a fungus that cannot wield invasive pressure.

The exceptional appressorium

Appressorial development has been reviewed in enough places that I need not pursue fresh adjectives to describe the amazing feats of these cells in this essay (Deising *et al*., 2000). But I do think it is useful to explain the ways in which these specialized infection platforms bend the general rules I have outlined for vegetative hyphae. The melanized appressoria of the rice blast fungus, *Magnaporthe grisea*, and various species of *Colletotrichum*, have been studied most intensively. Although the cellular biochemistry of the invasive process in these fungi remains muddled (e.g., identity of the major pressure-generating osmolytes), it is clear that appressoria can generate tens of atmospheres of hydrostatic pressure, or approximately an order-of-magnitude higher turgor than a vegetative hypha. By doing so, and then allowing most of this to act upon the underlying substrate, appressoria overcome the natural strength of the host surface and achieve penetration solely, as far as we can tell, by mechanical means. The tiny hyphae that extend through the cuticle and epidermal cell wall are referred to as penetration hyphae or pegs. They function only in host penetration and probably do not absorb nutrients. As soon as the host envelope is breached, subsequent mycelial development inside the host presumably occurs as I have described above for vegetative hyphae, by a combination of enzyme secretion and the application of force.

The reliance of their invasive apparatus upon extraordinary levels of turgor, sets appressoria apart from the behavior of vegetative hyphae that achieve substrate penetration through a seamless combination of substrate dissolution, nutrient absorption, and the unremitting application of lower pressures. But the fact that fungi can use osmosis to generate enormous pressures in appressoria has made me wonder whether hyphae might behave like these infection platforms from time to time, briefly elevating their turgor to deal with a tougher-than-average obstacle. There is no evidence of this physiological maneuver from any experiments. For energetic reasons, the osmolyte synthesis necessary to elevate pressure to very high levels could be impossible for a cell with a much smaller surface area to volume ratio than an appressorium. Nevertheless, I remain optimistic and look forward to learning that someone has discovered a fungus with hypertensive and hyper-invasive vegetative hyphae.

Rock penetration

Another remarkable example of the invasive activities of fungi comes from the discovery of hyphae that penetrate rocks, including seemingly impenetrable substrates like granite and marble (Burford *et al*., 2003). Some of the fungi that explore these mineral environments are mycorrhizal symbionts that connect with the roots of shrubs. Others are black-pigmented moulds related to fungi that grow in human tissues as opportunistic pathogens. Rock invasion appears to involve a combination of pressurized swelling to pry apart crystalline debris, and the slow solubilization of inorganic nutrients by the secretion of metabolic acids. The role of acid secretion is analogous to the function of enzymes during the invasion of plant and animal tissues, because both succeed in weakening the substrate, easing the way for the growing hyphae. Rock penetration by fungi may be a very significant ecological activity in certain habitats, like arctic tundra, where plant growth is severely restricted by climatic conditions and soil infertility. It almost certainly accelerates the weathering of rocks, including those that have been utilized as building stone.

Future research

At a meeting a couple of years ago, a mycological colleague expressed disappointment in the progress that has been made in understanding fungal cell biology using biomechanical methods. He ventured that real advances could not be achieved until we developed instruments capable of making measurements on a smaller spatial scale and with greater accuracy than the type of strain gauge I have discussed. I mention this, because our mutual friend raised a crucial question about the future of biomechanical research on fungi (as limited as this is). Firstly, I believe that critics of this research backwater overlook the recent headway that has been made. A little more than a decade ago we had no reliable data on fungal turgor, no idea how much pressure a hypha could exert, and only skimpy notions about the nuts-and-bolts of the invasive mechanism discussed in this essay. Beyond this, the desire for better methods is understandable. With more sensitive instruments, we might monitor the fluctuations in force at the hyphal apex, perhaps obtaining insights into the exocytotic process by detecting the quantum events of individual vesicles rupturing at the plasma membrane (this was the first thing I thought of). The atomic force microscope might make these kinds of measurements possible.

In the meantime, there are significant questions that can be explored using the available instrumentation. For example, it would be very

interesting to study the mechanical activities of hyphae when they are bundled in multicellular organs, namely fruiting bodies, strands, and rhizomorphs. Do individual hyphae operate in the same way inside a rhizomorph as they do when they act alone? Can mushrooms be viewed as a straightforward sum of their parts, in biomechanical terms, or do their component hyphae behave differently when they participate in generating larger structures that burst through the soil surface? I'll close here by encouraging you to consider your personal research questions in light of the perspective offered in this contribution to the *Mycologist*. Everything that fungi do has a biomechanical component.

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References

Bastmeyer, M., Deising, H. B. & Bechinger, C. (2002). Force exertion in fungal infection. *Annual Review of Biophysics and Biomolecular Structure* **31**: 321-341.

Bechinger, C., Giebel, K. F., Schnell, M., Leiderer, P., Deising, H. B. & Bastmeyer, M. (1999). Optical measurements of invasive forces exerted by appressoria of a plant pathogenic fungus. *Science* **285**: 1896-1899.

Burford, E. P., Kierans, M. & Gadd, G. M. (2003). Geomycology: fungi in mineral substrata. *Mycologist* **17**: 98-107.

Deising, H. B., Werner, S. & Wernitz, M. (2000). The role of fungal appressoria in plant infection. *Microbes and Infection* **2**: 1631-1641.

Hardham, A. (2001). Cell biology of fungal infection of plants. In *The Mycota*, Volume VIII, *Biology of the Fungal Cell*. Edited by R. J. Howard & N. A. R. Gow, Springer-Verlag, Berlin, Heidelberg, New York, pp. 91-123.

Hoch, H. C. Staples, R. C., Whitehead, B., Comeau, J. & Wolf, E. D. (1987). Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. *Science* **235**: 1659-1662.

Johns, S., Davis, C. M. & Money, N. P. (1999). Pulses in turgor pressure and water potential: resolving the mechanics of hyphal growth. *Microbiological Research* **154**: 225-231.

Latijnhouwers, M., de Wit, P. J. G. M. & Govers, F. (2003) Oomycetes and fungi: similar weaponry to attack plants. *Trends Microbiol*. **11**, 462-469.

MacDonald, E., Millward, L., Ravishankar, J.P. & Money, N.P. (2002). Biomechanical interaction between hyphae of two *Pythium* species (Oomycota) and host tissues. *Fungal Genetics and Biology* **37**: 245-249.

Miyoshi, M. (1895). Die durchbohrung von membranen durch pilzfäden. *Jahrbücher für wissenschaftliche Botanik* **28**: 269-289.

Money, N. P. (2001). Biomechanics of invasive hyphal growth. In *The Mycota*, Volume VIII, *Biology of the Fungal Cell*. Edited by R. J. Howard & N. A. R. Gow, N. A. R. Springer-Verlag, Berlin, Heidelberg, New York, pp. 3-17.

Ravishankar, J. P., Davis, C. M., Davis, D. J., MacDonald, E., Makselan, S. D., Millward, L. & Money, N. P. (2001). Mechanics of solid tissue invasion by the mammalian pathogen *Pythium insidiosum*. *Fungal Genetics and Biology* **34**: 167-175.