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Which way is up? Gravity sensing in basidiomycetes

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Summary

During the course of development of a basidiomycete fruit body different tropisms predominate at different times. The youngest fruit body initials grow perpendicularly away from their substratum though the nature of this tropism is unknown. The fruit body primordium then becomes first positively phototropic but later negative gravitropism predominates. The switch between predominance of the two tropisms has been associated with the onset of sporulation in a number of studies.

The major adjustment of the direction of growth in response to a tropic stimulus is made by the mushroom stem. Gravitropic growth curvatures are limited to the normal growth zones of the stem and seem to depend on re-allocation of available growth resources. The hymenophore (gill, tube or tooth) is positively gravitropic and responds independently of the stem. Bracket polypores do not show tropisms but exhibit gravimorphogenetic responses such that gross disturbance leads to renewal of growth to produce an entirely new fruiting structure.

One experiment performed on an orbiting space station suggests that, in the absence of a light stimulus, gravity may be required for initiation of fruiting in *Polyporus brumalis*. Otherwise, the indications from both clinostat and space-borne experiments are that the basic form of the mushroom (overall tissue arrangement of stem, cap, gills, hymenium, veil) in agaric and polypore alike is established independently of the gravity vector although maturation, especially commitment to the meiosis-sporulation pathway, requires the normal unilateral gravity vector. The nature of the graviperception mechanism in fungi is unknown.

Introduction

Gravitropism is commonly observed in fungi and has been the subject of investigation for more than a century, yet only *observations* of the phenomenon can be described - very little of any substance can be said about the *mechanisms* involved which is a contrast with the other two major Kingdoms of eukaryotes. Among the reasons for this is that although research on gravitropism in mushrooms was in progress at the turn of the century, the most acute analytical work has been done more recently on tropisms in structurally simpler fungi, particularly on the sporangiophore of *Phycomyces*, and no serious attempt has been made to unite the two bodies of observations. Further, fungi have been confused with plants for most of this century (and before) and this has led both to diminished effort on fungal research and to the misguided expectation that similar effects should be revealed in plants and fungi. Inevitably, the problem of understanding graviperception and gravitropism is far more acute, and further from solution, in fungi than in either animals or plants.

The most characteristic spore distribution structures of the basidiomycete fungi are the mushrooms and toadstools. Spore release in these organisms is intolerant of water so the hymenophore must be protected from the rain. Effectively, the mushroom is an umbrella and the spore-bearing surface must be on the lower side of the cap. Spores must fall vertically between the spines (hydnum) and gills (agarics) or through the tubular pores (polypores) to escape from the cap for dispersal on air currents. All of these deceptively simple descriptive

statements depend upon detection of gravity and modulation of growth processes by reference to the direction of the gravity vector. The ‘umbrella’ fruit body must grow *upwards*; the hymenophore must be on the *lower* surface; and spines, gills or pores must be *vertical*.

The most striking gravitropic reactions were reported by Schmitz (22, 23) and Sachs (20, 21); establishing the basic facts that agaric stipes are negatively gravitropic and the gills positively gravitropic. More experimental work was done in the early years of this century (3-6, 12, 25) and there have been a number of studies since then. Most of the research bears literally on tropic responses to gravity, rather than on morphogenetic responses.

Gravitropism in hymenomycetes

Different groups of hymenomycetes exhibit different gravitropic responses (10). In most agarics and polypores the hymenophore may be the main centre of gravitropic growth. On the one hand the hymenophore has its own orientation mechanism assuring positively gravitropic growth, and on the other hand there is evidence that the hymenophore may influence growth of the stem. Species of *Coprinus* exhibit a different pattern - the gills are not gravitropic and the stem alone is responsible for spatial orientation. Finally, bracket fungi are probably best considered to show gravimorphogenetic rather than gravitropic reactions. After their position is changed the direction of growth changes in the meristemoid zones and only the newly formed parts of the fruit body are vertical. The fruit body as a whole, though, is not gravitropic.

Gravitropism develops as the hymenomycete fruit body grows. The very youngest fruit body initials grow perpendicularly away from the surface on which they arise independently of the direction of light or gravitational signals (3, 4, 18, 24), a reaction which may be analogous to the avoidance reactions of *Phycomyces* sporangiophores (8, 14, 17).

As they develop further, the stems of ground agarics (gilled mushrooms) are generally non-phototropic but show a marked negative gravitropism whereas lignicolous and coprophilous hymenomycetes are often both phototropic and gravitropic (18). Usually, an initial period of light-seeking growth in the earliest stages of development is followed by negative gravitropism. Gravitropism of the stem provides the ‘coarse’ adjustment of fruit body position, the ‘fine’ adjustment is done by the positively gravitropic hymenophore.

Buller (4, [pp. 51-52]) showed that all the gills of *Agaricus campestris* could readjust to the vertical providing the cap was not tilted beyond 30E. These responses can be quite rapid. Buller (4, 5) detected the gravitropic adjustment of the gills of *Agaricus* within an hour and states: ‘If one turns a [*Coprinus*] fruit-body from a vertical to a horizontal position, the stipe begins to turn up the pileus within about three minutes after first receiving the geotropic stimulus’ (4, [pp. 69-70]). At the other end of the spectrum, the responses can be maintained for very long periods. In bracket polypores the tubes often extend vertically downwards for considerable distances and as many of these fruit bodies are perennial, this gravitropic growth may continue for many years (5, [p. 112]).

Gravimorphogenetic effects

Polarity of the primordium, the relative positions of stipe, hymenophore and cap tissues and the initial distribution of agaric gills, are all aspects of early mushroom morphogenesis which *might* be influenced by gravity, but these could all equally well be controlled by non-gravitational cues. The obvious experimental approach is to remove suspected cues and study the progress of events in their absence. Unfortunately, the influence of gravity can only be escaped in orbital laboratories, although a number of earthbound devices can help. The clinostat distributes the effect of gravity around its axis of rotation, to some extent simulating hypogravity - a reduced gravitational field (though actually being an omnilateral as opposed to the usual unilateral gravity exposure), whilst centrifuges permit increase in the apparent

gravitational field (actually centripetal acceleration) - the hypergravity experience.

Clinostat experiments

Hasselbring (12) investigated the effect of growth on a clinostat on development of *Polystictus* (= *Pycnoporus*) *cinnabarinus*, *Schizophyllum commune* and *Coprinus* spp and Reijnders (19, [p. 316]), Plunkett (18) and Badham (1) also report clinostat experiments. Unfortunately, most of these are of very limited value because the authors report only selected results ('... only a few gave results ...' (12); 'The results given relate to cases where no obvious arrest to development occurred' (18). However, Hasselbring reports hymenophore formation over the whole surface of clinostated fruit bodies of *Pycnoporus cinnabarinus* and *S. commune* and Plunkett (18) states: 'In the two fruit-bodies studied extremely shallow hymenial tubes, 1 mm. deep, were produced but developed no further.' These are the first reports of morphogenesis being disturbed by absence of a unilateral gravity vector.

The most recent, and undoubtedly the most carefully executed, clinostat experiments by Gorovoj, Kasatkina & Laurinavichius (11) used *Polyporus brumalis*, *Panus* (= *Lentinus*) *tigrinus* and *Coprinus cinereus*. These showed a consistent absence of the tubular hymenophore in fruit bodies of *Polyporus brumalis* arising on cultures grown on the clinostat. A series of five experiments were performed, each with 4 replicate cultures, but in no case was any sign observed of the typical tubular hymenophore, or even of the network of dissepiments which initiates it. In some experiments Gorovoj *et al.* (11) placed samples on the clinostat with fruit bodies in different stages of development, clinostating being continued until mature fruit bodies were obtained. In those in which cultures consisted only of mycelium at the start, the mature fruit bodies had a smooth hymenophore surface; in the second series of cultures which bore fruit body rudiments up to 5 mm in size when transferred to the clinostat, the mature fruit bodies had the lower surface of the caps covered with blebs (the very earliest stage in formation of dissepiments) and in some places the cap even had a small network of dissepiments; and in the third series, comprising cultures which initially bore fruit bodies whose caps had begun to differentiate, the mature fruit bodies had a network of dissepiments on the lower surface of the cap. However, formation of the normal tubular hymenophore did not occur in any of the samples. These results parallel those obtained by the earlier workers and imply that the unilateral gravity vector is required for formation of the tubular hymenophore. Microscopically, there were no major anatomical differences in the structure of fruit bodies grown on the clinostat compared with control but hymenial cells in clinostat cultures stopped development at the dikaryon stage. Karyogamy was rarely observed and basidia with spores were infrequently seen.

The situation was quite similar in *C. cinereus* grown on the clinostat, as no normal, spore-yielding, fruit bodies were obtained. However, in *C. cinereus* clinostat growth did not impair progress through the initial stages of development; all the main parts of the fruit body - cap, stem, hymenophore, and veil - were formed, but all remained underdeveloped. As in *Polyporus*, it seems that the unilateral gravity vector is required for normal progress through meiosis and sporulation; in its absence *Coprinus* fruit body development stops prior to spore formation.

In contrast, the greatest influence of the clinostat on *Lentinus tigrinus* was on morphogenesis of the stem. This formed numerous branches rather than a cap. Thus, it seems that in this organism absence of a unilateral gravity stimulus results in loss of polarity and apical dominance.

Experiments in orbit

Test-tube cultures of *Polyporus brumalis* were flown during an orbital space flight aboard the unmanned Soviet 'biosputnik', Cosmos 690 (26). The Cosmos 690 cultures were in orbit for 20 days during which time they were in the dark. In the absence of illumination, fruit body

caps were not formed but the stems of the fruit bodies grew well so this experiment demonstrated that at least the early stages of fruiting could occur in zero gravity. Further experiments were conducted, again with *Polyporus brumalis*, during flights (in 1977-78) of the manned space stations Salyut-5 and Salyut-6 (15, 16). In the experiment conducted aboard Salyut-5 cultures bearing fruit body rudiments up to 2 mm tall were taken aboard the space station and some were incubated with illuminated while others were in darkness. All formed fruit bodies, those in the dark failed to form caps, as expected; those grown in the light were similar to normal and had a tubular hymenophore, although the hymenium was restricted to the bottom of the tubes (16). In the experiment on Salyut-6, cultures were delivered to the space station bearing mycelium only. Again, cultures were incubated in weightless conditions either in the light or dark. Fruit bodies were formed in the light, but most of the fruit bodies had no hymenophore and little hymenium. No fruit bodies were formed in the dark although in dark-grown controls on earth fruit body rudiments were formed in the dark. The Salyut dark-grown cultures were shown to be able to fruit when illuminated after recovery so it seems that some aspect of the fruit body initiation process in the dark requires the gravity vector.

The limited experiments done in orbit seem to support the conclusion from clinostat experiments that it is hymenophore development which is most dependent on the gravity vector. They also suggest that a clinostat of the design used by Gorovoj *et al.* (11), with a rotation speed of 2 r.p.m., is a good analogue of the 'zero' gravity state.

Hypergravity - enhanced gravitational fields

The device used by Gorovoj *et al.* (11) incorporated a centrifuge able to apply centrifugal accelerations of 1 and 4.5 g. Hypergravitation, though, did not greatly affect fruit body morphogenesis either macroscopically or microscopically and the normal gravitropic reactions occurred.

Perception of the gravitational impulse

Perception of gravity is potentially quite different from perception of other stimuli because there is no gravitational gradient. The perception system must depend on gravity establishing an asymmetric distribution of matter within the organism. Gravity-induced asymmetries might include (a) differences in hydrostatic pressure between 'top' and 'bottom'; (b) differences in forces of compression or extension between the 'top' and 'bottom' of rigid structures; (c) changes caused by movement of extracellular or intracellular structures relative to immovable parts of the cell or tissue.

Little work has been done to establish the location and nature of the structures responsible for perception of gravity in hymenomycetes. Borriss (2), described '... particles of plasma, ... situated in about the centre of the cell ...' in *Coprinus lagopus* (= *C. cinereus*?). Gooday (9) states that 'The cytology of geotropism of stipes of *Coprinus cinereus* was investigated by the late G. H. Banbury (personal communication) ... light and electron microscopy ... showed that when horizontal, the distribution of cell contents was displaced so that the vacuole occupied most of the upper part, and the cytoplasm ... the lower part.' These two accounts appear to be *the only* reports of any cytological examination of gravitropism in hymenomycetes.

Much more attention has been devoted to the sporangiophores of *Phycomyces* but the cytology has also been neglected. Dennison & Shropshire (7) provide the only evidence (but only at the light microscope level) of an intracellular reorganization in response to reorientation of the sporangiophore, concluding 'The existence of protoplasmic asymmetry is a plausible first step in the gravireceptor stimulus-response chain...' and '...the gravireceptor mechanism ... must involve the rearrangement of intracellular liquid phases of differing

density, specifically the protoplasm and vacuole'. Channelling of chitosomes and other peripheral vesicles into the lower part of the cell would indeed be an attractive explanation for greater expansion growth on one side of the hypha rather than the other side in relation to current models of wall growth. However, because of the astonishing absence of cytological observation and experimentation it remains the case that no sensory apparatus has been identified in any fungus. Furthermore, Lilian Hawker (13) wrote in 1950: 'It is desirable that research should be directed towards an interpretation of tropisms in fungi based on the study of growth-regulators. At present nothing is known of any mechanism in fungi comparable to the redistribution of auxins in the higher plants.' Sadly, *this is still true* 40 years later, so we are also totally ignorant of the growth co-ordination processes which respond to the gravity-perception system.

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