GRAVITATIONAL BIOLOGY OF MUSHROOMS: A FLOW-CHART APPROACH TO CHARACTERISING PROCESSES AND MECHANISMS

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Abstract—Flow-charts are presented which systematise recently published work on gravitropic responses of the mushroom stipe of *Coprinus cinereus*. The hypothetical model represented by the charts suggests that the meiotic division is a pivotal point in the gravitational biology of the mushroom fruit body. The unilateral gravity vector seems to be required for formation of the tissues in which meiosis normally occurs, and stipes become gravitropically competent only after onset of meiosis. The gravitropism flow-chart also indicates that two signals emanate from the upper regions of the stipe, one promotes the process of gravitropic bending, and is followed by a second signal which compensates for excess bending and adjusts the stipe apex to the vertical. Formalisation of the various observations into flow-charts, even though comparatively simple at the moment, facilitates comparison with other species and concentrates attention on aspects requiring further experimental analysis.

INTRODUCTION

When a mushrooms is turned on its side, growth of the stem is modulated so that it bends upwards to restore orientation of the cap. Superficially similar things happen in plants, but fungi *are not* plants so graviperception and gravitropism in the two Kingdoms could be very different.

The mushroom is a spore dispersal device which is completely dependent on gravity for efficient functioning. Spore release in these organisms is intolerant of water so the spore producing tissue (the hymenophore) must be protected from the rain; the mushroom cap is, effectively, an umbrella. Evolutionary strategies which have increased the surface area of the hymenophore have produced hanging plates (the 'gills' of agarics), tubes (in the polypores) or spines or teeth (hydnoid forms) lined with spore producing cells. A consequence of such strategies is that spores must fall vertically, frequently for many millimetres and usually with little clearance, between gills or through the tubes to escape from the protecting cap to be dispersed on air currents beneath the fruit body. The fruit body must be positioned absolutely vertically if spore fall is to occur successfully and with the minimum of spore entrapment within the cap. This positioning can only be achieved by referencing morphogenesis to the gravity vector.

Research on gravitropism in mushrooms was in progress at the turn of the century, but because of their confusion with plants for most of this century (and before) little more is known about this aspect of mushroom physiology than that agaric stipes (the mushroom stems) are negatively gravitropic and that the gills of most agarics are positively gravitropic. Only very recently has research been resumed in a way which promises to establish the physiological processes involved in gravity perception and response. In this paper we bring together the most recent research and formalise it into a flow-chart description in the hope that this will focus attention on the mechanisms involved.

2. NORMAL UPRIGHT GROWTH IN *COPRINUS*

A flow-chart depicting normal upright growth of the ink-cap fungus, *Coprinus cinereus*, is shown in Fig. 1. Although this is very much simplified, it is based on the principles discussed by Barlow [1, 2]. The model depicted derives from recent measurements of cell sizes and cell population distributions in microscope sections of fruits bodies whose temporal development was defined by the stage they had reached in meiosis and sporulation [3, 4]. Comparison of lenght/width ratios showed that in pre-meiotic stages of primordium development, stipe elongation was more due to cell proliferation than inflation. Major cell inflation was a post-meiotic event and could account for all the basidiome expansion involved in maturation. Inflation of cells in the pileus was closely correlated with inflation of cells in the stipe indicating that some form of long range signalling system exists.

It is important to emphasise that Fig. 1 is not representative of species other than *C. cinereus*. Eilers [5] found that stipe elongation occurring after meiosis in *C. radiatus* was accompanied by a doubling of the cell number as well as increase in cell length, and cell proliferation in the apical region is considered to
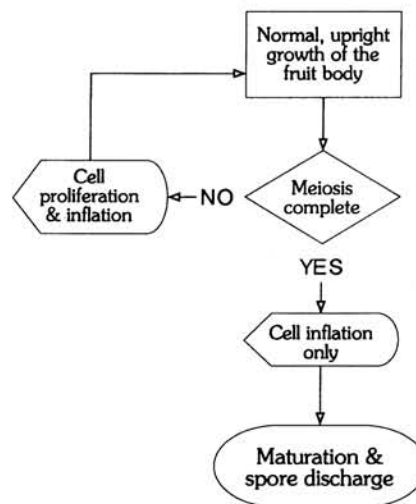

Fig. 1. Simplified flow-chart describing normal upright growth of the mushroom stipe of *Coprinus cinerus*.

provide for stipe growth in other agarics. However, among the fruit bodies examined in *C. cinereus*, the increase in overall size of the primordium was greater than the increase in mean cell length only in pre-meiotic (between 3 and 8 mm tall) primordia. Throughout the rest of the size range examined (up to 83 mm) increase in size of the stipe was easily accounted for solely by increase in cell size. The most remarkable feature, though, is that onset of most rapid stipe elongation corresponds with the ending of meiosis, and this is correlated with a change in tropic response as discussed below.

3. EXPRESSION OF GRAVITROPISM IN
COPRINUS CINEREUS

3.1. Gravitropic competence achieved after meiosis

Very young fruit body initials grow perpendicularly away from the surface on which they arise [6-9], probably due to negative chemotropisms to products of metabolism or to water activity [10]. Subsequently, an initial period of light-seeking growth in the earliest stages of development is followed by negative gravitropism and we have shown that the stipe becomes gravireceptive after completion of meiosis [11]. This is the first test condition (diamond-shaped symbol) shown in the flow-chart of Fig. 2.

3.2. Perception threshold

Analysis of video recordings showed that stem bending first occurred 25.4 ± 13.1 *min* ($n = 18$) after being placed horizontal [11]. By analogy with gravitropic responses in plants, this value is the *reaction time*, which is the time from first reorientation of the organ to the appearance of the tropic growth curvature. In plants this can vary between about 10 *min* and many hours and it covers all of the processes involved in production of the growth response which generates gravitropic curvature. The *presentation time*, which is the minimum time of stimulation required to provoke a gravitropic response, was determined to be 9.6 *min* in preliminary experiments with clinostats [12]. Other observations indicate that the stipe must be more than 10-15° from the vertical for the gravitropic response to be stimulated. These two parameters define the perception threshold which is the second test condition in Fig. 2.

3.3. Gravitropic bending

Stipe bending first occurs in the apical 15% of its length, then the position of the bend moves rapidly towards the base, traversing 40% of stem length in 2.5 *h*. Removal of segments of the apex does not affect the ability of the stipe to respond gravitropically until more than 60% of the stipe length is removed. However, reaction time was directly proportional to the amount of stipe apex removed [13]. Thus, although the physical apex of the stipe is not essential to the gravitropic reaction, the stipe is polarised and the apical regions make a cumulative contribution to the phenomenon. Basal movement of the bend and the significance of the 'apex' are taken to imply basipetal migration of a bending signal. Stipes transferred to a clinostat after some minutes
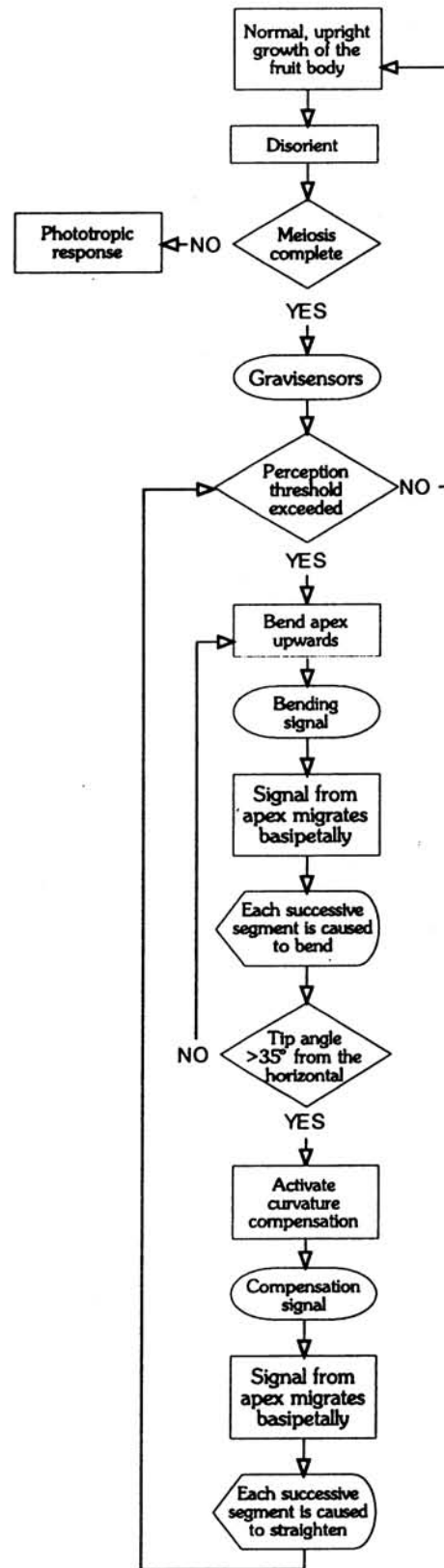

Fig. 2. Flow-chart of the gravitropic response in stipes of *Coprinus cinereus*.

gravistimulation showed degrees of curvature which increased with the length of initial gravistimulation, indicating that continued exposure to the unilateral gravity vector was necessary for continued bending [11] and suggesting that, under normal circumstances, the bending signal is produced for as long as the stipe tip is disoriented.

3.4. Curvature compensation

The bend moves towards the base of the stipe, yet when the apex has returned to the vertical the upper regions (in which the bend first appeared) are straight. This implies that the bending process is followed by curvature compensation. Calculations suggest that 90% of the initial bend is compensated as the stipe brings its apex accurately upright [11]. When the horizontal stipe was pinned through its apex, the base was elevated but always overshot the vertical, often curling through more than 300°. Evidently, the apex must be free to move for curvature compensation to occur. Thus, in the normal disoriented fruit body curvature compensation prevents the apex overshooting the vertical and is a correction system which depends on the apex perceiving the gravitational vector. It is assumed to involve basipetal migration of an organising signal. Most plots comparing tip angle with time since first disorientation are S-shaped curves with an inflection point beyond which the rate of increase in the tip angle is progressively lessened. This inflection is assumed to represent the point at which activation of curvature compensation becomes evident. The average tip angle at which it is observed is 34.8° and this constitutes the third test condition in Fig. 2.

4. MECHANISMS OF GRAVITROPISM IN COPRINUS CINEREUS

Removal of apical segments [13] suggests that most of the apical region (extending to more than half the total length of the stipe) is capable of producing a gravitropic response. Importantly, removal of the apical segments did not impair the ability of the stipe to adjust to the vertical, i.e. curvature compensation, as well as gravitropic bending both occurred in these specimens. The stipe is clearly polarised. There appears to be a gradient of diminishing gravitropic capability in this upper zone which could be accounted for by position-specific variation in gravitropic competence (discussed in ref. 13). A crucial observation, though, is that when a horizontal stipe is secured at the apex, the stipe bends into a full circle [11]. For such a phenomenon to be controlled locally, the cells would have to express negative, dia and then positive gravitropism. Thus, rather than suggesting such complex localised control, we believe that gravitropic bending and the apical polarity of the stipe are controlled by organising signals emanating from the apical zone. When the apex is secured in a horizontal position it continually produces a signal which promotes bending, and so the stipe winds into a circle. When the apex is free to move (i.e. to be elevated by the bending stipe) the bending signal is followed by a second one which promotes curvature compensation and adjusts the apex to the vertical.

The nature and origin of the signals involved is still obscure. Involvement of mechanical factors, like change in the distribution of mass, has been eliminated by the demonstration that the stipe apex is able to return to the vertical, and continue vertical growth, despite sustained application of lateral loads greatly exceeding the normal fresh weight of the fruit body [13].

Control of cell calcium accumulation has been implicated in the gravity perception mechanism of plants but treatment of stipes of C. cinereus with a Ca^{2+} channel blocker, a Ca^{2+} ionophore, a Ca^{2+} chelator, or an inhibitor of calmodulin had no effect on gravity perception [14]. However, the ionophore, chelator and calmodulin antagonist all caused significantly diminished gravitropic bending, so it is thought that Ca^{2+} is not involved in gravity perception, but does contribute to transduction of the gravitropic impulse in the Coprinus stipe. The results would be consistent with regulation of the gravitropic bending process requiring sequestration of Ca^{2+} within a membrane-bound compartment. Interestingly, treatment with the ionophore enhanced extension growth rate of the stipe (by 30%) but decreased (by 43%) the rate of bending, suggesting that tropic bending may result from a different sort of growth from that which is responsible for vertical growth of the stipe. Studies are currently underway on the cellular morphometrics of gravitropic bending.

5. APPLICABILITY TO OTHER MUSHROOMS

Coprinus species, especially the smaller ones like C. cinereus, tend to have delicate, short-lived fruit bodies which develop, mature and senesce more rapidly than other mushrooms. These differences in speed of development seem to be reflected in the rate of gravitropic response. A horizontal fruit body of Flammulina velutipes takes 9 to 24 h to restore its apex to the vertical [15, 16], whereas the same is achieved in only 3 to 5 h by C. cinereus [11]. Another important difference is that meiosis is highly synchronised in Coprinus whereas the hymenium in other agarics contains basidia at all stages of meiosis throughout the life of the fruit body [17].

Perhaps the most significant difference between Coprinus and other agarics is that the gills of Coprini are not gravitropic [18] so it is the stipe alone which is responsible for spatial orientation of these fungi. In contrast, the gills of Agaricus campestris readjust to the vertical providing the cap is not tilted beyond 30°; adjustment of the gills being detectable with a microscope within 1 h, and by the naked eye within 2 h [7]. In these agarics, gravitropism of the stem provides the 'coarse' adjustment and the 'fine' adjustment resides in the spore-bearing tissues themselves. Where both cap and stipe respond, some of their adjustment could be futile unless there is some degree of co-ordination. Evidence for co-ordination of cell expansion in cap and stipe of Coprinus has been presented [3] but is lacking in other species. It is noteworthy that onset of gravitropic adjustment of gills in Agaricus and of curvature compensation in the gravitropically responding stipe of C. cinereus both occur at about the same displacement angle (30 to 35°); perhaps a common strategy is served by different tactics.

As well as the gills of agarics, even the 'teeth' (= spines) of *Hydnum* are positively gravitropic [18] but in poroid forms, the growing rims of the pores (the edges of the *dissepiments* or partitions between the tubes) are able to form a reoriented pore if the fruiting structure is disturbed [8]. This is a morphogenetic rather than tropic response, the dissepiments producing new tissue rather than adjusting the position of existing tissue. There are many aspects of early mushroom morphogenesis which *might* be influenced by gravity, but the clearest association seems to be with meiosis. The progress of meiosis influences the sensitivity of the stipe to the gravitational stimulus [11] and a compilation of results of clinostat and orbital experiments (Table 1) shows that hymenium development is consistently impaired, implying that gravity is a component of the process that commits sporulation to completion [19]. Though meagre, the data in Table 1 suggest that, in some species at least, the unilateral gravity vector is a requirement for maturation of the fruiting body. Thus, the tropic and morphogenetic effects of gravity both seem to revolve around the meiotic division. How the gravitational fields is perceived and how signals are distributed throughout the fruit body remain to be determined.

Table 1. Compilation of recent results from clinostat and orbital experiments with developing mushrooms

Organism	Developmental behaviour	Ref
(a) Clinostat experiments		
Polyporus brumalis	Extremely shallow hymenial tubes	8
Polyporus brumalis	Tubular hymenophore not formed; hymenium aberrant, karyogamy rare, spores infrequent	20
Lentinus tigrinus	Stem often branched, many without caps	20
(as *Panus tigrinus*)	When formed, caps always morphologically abnormal, though did have gilled hymenophore on ventral surface	
Coprinus cinereus	Primordium development initially normal but then aborts prior to spore formation	20
(b) Orbital experiments		
Polyporus brumalis	Cosmos 690. Grown in the dark; stems twist around walls of the test-tube culture vessel	21
Pleurotus ostreatus	Cosmos 690. Fruit body formed in orbit generally much longer than the control	22
Pleurotus pulmonarius	Cosmos 690. No size difference between experiment and control	22
Polyporus brumalis	Salyut 5. Cultures flown with preformed fruit body rudiments. Tubular hymenophore formed in orbit but hymenium limited to base of tube	23
Polyporus brumalis	Salyut 6. Fruit bodies formed in orbit lacked the tubular hymenophore	24

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