

Chapter 1

Inside the developing mushroom – cells, tissues and tissue patterns

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Summary

Major tissues of the mushroom (cap, stem, veil, basal bulb, etc.) are established very early in development. This Chapter offers a generalised overview of mushroom morphology, morphogenesis and mechanics, with a view to identifying the controlling events which determine tissue differentiation and distribution. Currently we face a major problem of insufficient information on the detailed anatomy of the fruit body. Knowledge of the seat of growth which drives morphogenesis and appreciation of the balance between physical and biological phenomena are also often lacking. Application of simple numerical methods to microscopic analyses of the stem in *Coprinus* has produced a quantitative account of the dynamics of hyphal changes which sculpture even this, apparently simple, structure. In tissues of the cap, there is *prima facie* evidence for control of patterning by diffusion of chemical morphogens in morphogenetic fields. The generation of the agaric gill can be understood in terms of organising centres which interact with one another by production of (and response to) freely diffusing activator and inhibitor molecules. Recent analyses show that (i) agaric gills grow at their base, not their margins and (ii) agaric gills are initially convoluted, being stressed into their regular radial arrangement later.

Pattern developments in theory

The shape, form and structure of an organism arise through its morphogenetic development, but the basic ‘body plan’ of the developing structure (whether fungal, plant or animal) does not appear all at once. Instead, its shape and form are assembled through a series of distinct acts of

differentiation by cells already specified by earlier differentiations to belong to a particular morphogenetic pathway.

Cells differentiate in response to chemical signals from other regions of the developing structure and/or under the influence of suitable environmental triggers. These chemicals (none yet identified) may be termed organisers, inducers, morphogens, growth factors or hormones and seem to inhibit or stimulate entry to particular states of differentiation (Chapter 7). For example, morphogens may contribute to a morphogenetic field around a structure (cell or organ) which permits continued development of that structure but inhibits formation of another structure of the same type within the field. This can produce a pattern in the distribution of the structure over the tissue as a whole. If the inhibition caused by the morphogen is strong and extends (through its diffusion or active transduction) over a wide area, then the structures will be widely spaced. On the other hand, if the inhibitory influence of the morphogen is localised (perhaps because it decays rapidly) then the structures will be formed close together.

The morphogen is providing the cells which respond to it with positional information, which allows those cells to differentiate in a way characteristic of their position in the morphogenetic field. Positional information seems to be imparted by concentration gradients of one or more morphogens emitted from one or more spatially distinct organisers. Effectively the cell moderates its behaviour on the basis of the nature and strength of the incoming morphogen signals, consequently adjusting its response in accord with its position relative to the controlling organisers.

Phenomena such as these generate the spatial pattern of cell and tissue distributions which characterises the 'body plan' of the developing organism. They have been described most effectively in various aspects of animal and plant morphogenesis but evidence does exist that such mechanisms contribute to the development of fungal structures. Development, differentiation and morphogenesis involve change with time, and the dynamics of cell and tissue interactions in time contribute to the form and structure of the organism which results.

Morphogenesis has a time dimension

As fungal fruit bodies develop a complete change occurs in the behaviour of hyphae and hyphal branches. The invasive growth which characterises the vegetative mycelium (Chapters 2, 3), and which enables it to act 'as a mould' is drastically modified. Instead of diverging and avoiding contact with their

fellows, hyphae of what will become a fruit body grow towards one another contributing, with their branches, to a population of co-operating hyphal systems which makes up the undifferentiated tissue described as the fruit body initial.

Regions of recognisable cap and stem can be resolved in microscope sections of even very small (mm size range or below) fruit body initials. Such images provide *prima facie* evidence for the pattern forming processes outlined above because formation of histologically distinct regions of these sorts requires that some organisation is imposed upon the homogeneous undifferentiated tissues. Over many years, Reijnders (1948, 1963, 1979) stressed the importance of: (i) the manner of development and nature of the veil and the 'epidermis' of the cap (called the pileipellis) in relation to protection of the developing cell layer which is responsible for eventually producing the (basidio)spores, (the hymenophore which carries the hymenium or spore-producing tissue); (ii) the sequence of development of the stem, cap and hymenophore, which are the major functional zones of the mushroom fruit body; (iii) the mode of development of the hymenophore. The terminology has been discussed by Watling (1985) and the taxonomic, phylogenetic and ontogenetic significance of these features are discussed by Watling & Moore (1994) and in Chapter 8.

Evidently, the major tissue regions of a mushroom fruit body are demarcated at a very early stage in development. For example, in *Coprinus cinereus*, cap and stem are clearly evident in fruit body initials only 800 μm tall (Moore, Elhiti & Butler, 1979) though this size represents only 1% of the size of a mature fruit body.

The most highly differentiated cells are found at the boundaries of tissue regions in such young fruit body initials (Williams, 1986). In the youngest specimens these perimeters are occupied by layers of parallel hyphae (called meristemoids by Reijnders, 1977) with short cell compartments, suggesting that these hyphae are involved in rapid septum formation (Chapter 2). But these are not meristems like those which occur in plants. Meristems do not occur in fungi. Nor do meristemoids imply that the tissue borders are actively growing outwards. Of course, the meristemoids are growing actively; at this stage in development, all regions are growing actively. But the most significant point is that the driving force for growth of the developing fruit body primordium is its internal expansion; growth at the boundaries of tissue layers takes place to compensate for increase in the area of the boundary layer which occurs because internal growth increases the volume of each tissue region.

Using *C. cinereus* as an example again, as a typical fruit body grows from

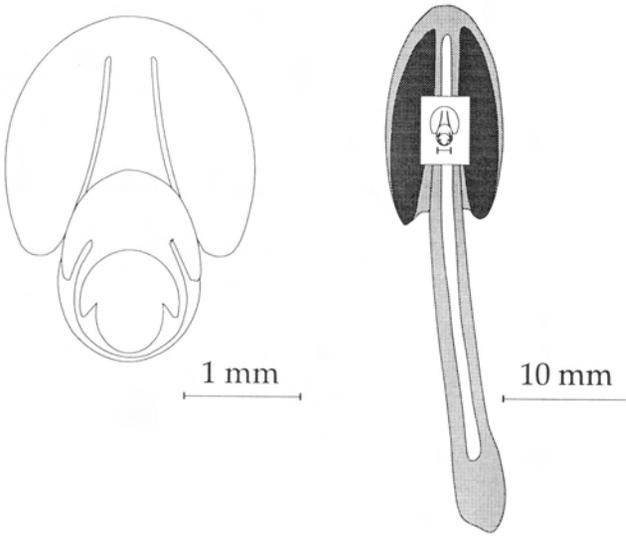


Fig. 1. Scale diagrams showing the size relationships of primordia and fruit bodies of *Coprinus cinereus*. On the left a set of three 'nested' outlines of vertical sections of primordia illustrate the steady outward expansion of the tissue layers. On the right, these same diagrams are superimposed (to scale) onto a median diagrammatic section of a mature basidiome to demonstrate the full extent of the outward movement of tissue boundaries. (Redrawn after Moore, 1995).

1 to 34 mm in height (i.e. a vertical linear change of $34 \times$), the circumference of the stem in section increases $9 \times$, the outer circumference of the cap in section increases $15 \times$, but the volume increases more than $3000 \times$ (Fig. 1).

The differential growth which generates primordium enlargement exerts enormous mechanical effects on relationships between tissue layers which are often concentrically arranged. Mechanical forces themselves generate many of the patterns which characterise the form and structure of the mature mushroom fruit body. Some of these will be described in the following sections.

Co-ordination of cellular activities across the fruit body

The changes in shape and form which characterise fruit body maturation in basidiomycetes is usually described as 'expansion' because, clearly, the fruit body increases greatly in volume as it matures. Two basic strategies seem to be used to achieve fruit body expansion. The polypores and their close relatives (including those, like *Lentinula*, which have gills rather than pores)

tend to produce more hyphal branches continuously; so they show no significant overall increase in cell size, but large increase in cell number. On the other hand the agarics depend on cell inflation for fruit body expansion and it is usually possible to show that the extent (and often the rate) of fruit body expansion far outstrips the production of new hyphae and/or branches.

There are two types of cell inflation. A slow process is usually found in young primordial stages, with a more rapid one being involved especially in stages of cap maturation. These sorts of cell inflation are distinct aspects of cell differentiation, representing functional specialisation of the cells concerned (Reijnders & Moore, 1985). Reijnders (1963) showed that the different parts of fruit bodies enlarge in proportion. The different tissues of the fruit body consequently develop and mature without being impeded or distorted by the growth of other parts. If fruit body primordia do enlarge proportionally the implication is that there may be some sort of co-ordinating mechanism operating over distances of many millimetres. On the other hand, an alternative interpretation might be that 'co-ordination' is more apparent than real because events may be arranged in a consequential series - one (secondary) event being instigated by the initiation or completion of an earlier (primary) event.

Cell inflation in agarics (as represented by various aspects of 'fruit body growth') has been the subject of a good deal of research, and reference should be made to Gooday (1974, 1982, 1985), Moore *et al.* (1979) and Rosin, Horner & Moore (1985) for work with *Coprinus cinereus*; Bret (1977) on *C. congregatus*; Hafner & Thielke (1970) on *C. radiatus*; Wong & Gruen (1977), Gruen (1982, 1991), and Williams, Beckett & Read (1985) on *Flammulina velutipes*; Bonner, Kane & Levey (1956) and Craig, Gull & Wood (1977) on *Agaricus bisporus*.

All of these studies examined individual processes in the fruit bodies concerned. There has only been one holistic account of inflation over the whole fruit body which enables an assessment of the correlation between cell behaviour in widely separated parts of the same fruit body (Hammad *et al.*, 1993a). The aim of this study was to determine how different tissues of the fruit body of *Coprinus cinereus* expanded both along the developmental time scale and in relation to the other tissues of the fruit body. Any such effort to examine co-ordination of events requires an objective time base. Many earlier studies have relied (quite satisfactorily within the context of the experiments undertaken) on morphological aspects of fruit body development to define the developmental stage of each specimen (Madelin, 1956; Takemaru & Kamada, 1972; Matthews & Niederpruem, 1973;

Table 1. Designation of developmental stages of fruit body development in *Coprinus cinereus*

Stage	Time	Predominant feature (reference a)	Other designations in the literature			
			b	c	d	e
1	0-12h	Basidial differentiation	Stages 1-2	Stages 1-3	Day 8	
2	13-24h	Karyogamy and meiotic prophase	Stage 2	Stage 2		-2 Days
3	25-36h	Meiotic divisions and spore formation	Stages 3-4	Stage 4	Day 9	-1 Day
4	37-48h	Stem elongation and spore release	Stage 5			Day 0

References: (a) Pukkila *et al.*, 1984; (b) Moore, Elhiti & Butler, 1979; (c) Morimoto & Oda, 1973, 1974; (d) Kamada, Kurita & Takemaru, 1978; (e) McLaughlin, 1982.

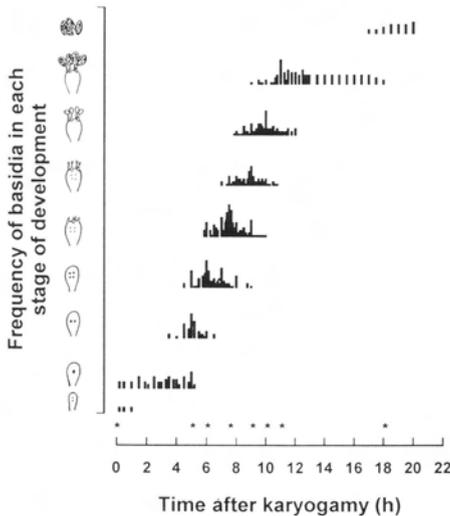


Fig. 2. Time course of progress through meiosis and sporulation in *C. cinereus*. The abscissa shows elapsed time. Samples were removed from fruit bodies at regular time intervals and sample populations of hymenial cells observed in squashes were quantitatively categorised into the developmental stages represented in the cartoons on the ordinate. Time 0h is arbitrarily set to the start of karyogamy. Other timings emerged from the observations (as the medians of the distributions shown): meiosis I occurred at 5 h, meiosis II at 6 h, sterigmata appeared at 7.5 h, basidiospores started to appear at 9 h, began pigmentation at 11 h and mature basidiospores were being discharged from 18 h. The asterisks on the abscissa emphasize these stages. (Redrawn after Hammad *et al.*, 1993a).

Morimoto & Oda, 1973; Moore *et al.*, 1979). Pukkila, Yashar & Binninger (1984) introduced nuclear staining to define the stage of development of the whole fruit body in terms of progress through meiosis and spore formation in the hymenium (Table 1). Hammad *et al.* (1993a) extended this approach by examining a sufficiently large sample of fruit bodies to establish the exact timing of major meiotic and sporulation events, creating a time base to which any other process can be referenced simply by microscopic examination of a sliver of cap tissue (Fig. 2). The resultant time base is objective because it depends on observation of processes which are endogenously controlled and reliable because the processes concerned are central to the functioning of the fruit body. Time courses could be established with individual fruit bodies because such small amounts of tissue were required for the staining technique used (Pukkila & Lu, 1985) that repeated samples could be removed at known time intervals.

Hammad *et al.* (1993a) performed a morphometric analysis by measuring cell sizes in microscope sections of fruit bodies whose stage of development was defined by the stage they had reached in meiosis and sporulation. Measurements were made of cells in different positions in the stem (base, middle and apex) and of a range of hymenial cell types (basidia, spores, paraphyses, cystidia and cystesia) and veil cells. The resultant sets of measurements were then analyzed for information about stem elongation and cap expansion; stem structure in longitudinal sections and the co-ordination of cell expansion.

Stem elongation and cap expansion

In the early phase of slow stem growth the rate was in the region of $10 \mu\text{m min}^{-1}$; in the rapid elongation phase the growth rate was $110 \mu\text{m min}^{-1}$. During the most rapid phase of elongation, an average stem may elongate 80 mm in less than 12 h (Moore & Ewaze, 1976). Rapid stem elongation in *C. cinereus* occurred in a phase which occupied most of the 5 h prior to spore discharge, starting 8 h after karyogamy. Cap expansion started as spores matured, about 14 hours after karyogamy (Fig. 3). Stem elongation was significantly greater when the cap was left attached than when it was removed (Table 2) and this was true whether the caps were removed before (10 h after karyogamy) or after (12 h after karyogamy) onset of the phase of rapid elongation. Leaving a half cap in place was sufficient to ensure normal elongation. These stems curved away from the side with the half-cap on it during the 8 h immediately following treatment (height range 80-85 mm),

Table 2. *Effect of the cap on stem elongation in Coprinus cinereus*

Treatment	Initial length (mm)	Final length (mm)	Elongation (mm)
(a) caps removed 10 h after karyogamy (just before onset of rapid elongation phase)	35.8 ± 4.9	109.2 ± 12.2	73.3 ± 8.8
(a) control	36.0 ± 5.5	130.4 ± 2.99	94.4 ± 6.3
(b) caps removed 12 h after karyogamy (just after onset of rapid elongation phase)	35.5 ± 5.11	23.0 ± 22.9	87.5 ± 19.3
(b) control	37.6 ± 5.4	155.6 ± 16.0	118.0 ± 13.7
(c) half of cap removed 12 h after karyogamy	47.0 ± 9.4	121.5 ± 14.2	74.5 ± 9.2
(c) control	48.0 ± 10.8	124.0 ± 12.8	76.3 ± 3.9

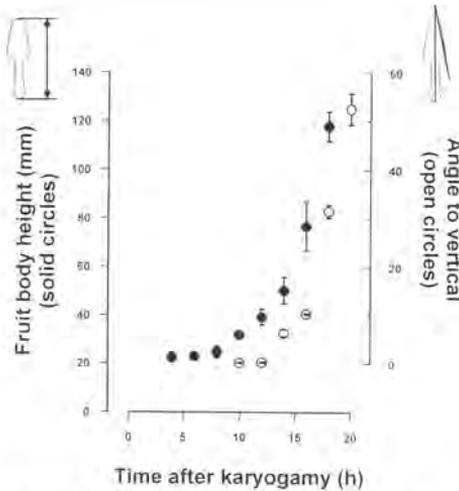


Fig. 3. Correlation of stem elongation and cap expansion with meiosis in *C. cinereus*. (Redrawn after Hammad *et al.*, 1993a).

but in the next 4 h, during which they grew to 104-137 mm in height, growth was strictly vertical.

The kinetics of stem elongation has attracted a great deal of attention. Buller (1924) examined *C. sterquilinus*; *C. radiatus* has been studied by Borriss (1934, using the name *C. lagopus*), Hafner & Thielke (1970) and Eilers (1974); *C. congregatus* by Bret (1977); and *C. cinereus* by Gooday (1974), Cox & Niederpruem (1975, using the name *C. lagopus*) and Kamada

Table 3. Sizes of stem cells (in terms of sectional area) measured in longitudinal sections of stems and the length/width ratios of the cells

Fruit body height (mm)	Section	Mean stem cell area (μm^2)	Length/width ratio
3	Middle	148	1.9
8	Middle	211	2.0
25	Apex	292	1.8
	Middle	3857	11.5
	Base	2705	6.2
48	Apex	3184	9.0
	Upper middle	6813	12.7
	Lower middle	5735	10.3
	Base	3449	10.6
55	Apex	9243	11.5
	Upper middle	9496	18.1
	Lower middle	10522	19.9
	Base	6533	13.8
83	Upper apex	6258	12.6
	Lower apex	11960	22.0
	Middle region 4	12894	26.0
	Middle region 3	11672	30.2
	Middle region 2	10448	35.1
	Middle region 1	5538	20.4
	Upper base	4785	14.1
	Lower base	2681	8.8

Each entry represents the mean of 50 measurements

& Takemaru (1977, using the name *C. macrorhizus*). In all these species the upper half, and generally the upper mid-region, has been shown to be the most active zone of elongation, and this part of the stem showed the most dramatic increases in cell size (Table 3). The species represented in the literature differ in the degree of stem autonomy and the relative parts played by cell division and cell elongation in the process of stem elongation. In *C. congregatus* stem elongation was dependent on both the parental mycelium and the cap during the whole period of development of the fruit body (Bret, 1977). In *C. radiatus* elongation occurred after separation from the parent mycelium, but was dependent on the cap only until the final phase of rapid elongation was reached: after the stem had reached 25% of its final size decapitation did not impair elongation (Borriss, 1934; Eilers, 1974). In contrast, Gooday (1974) showed that stem elongation in *C. cinereus* had no requirement for connection either with the cap or the parental mycelium. This was confirmed by Cox & Niederpruem (1975) who extended the observation by showing that primordia about 5 mm in height (which is

equivalent to between 5% and 10% of final size) were able to elongate after excision and decapitation. Hammad *et al.* (1993a) demonstrated another aspect of cap/stem interplay by showing that the *C. cinereus* stem does not depend on the presence of the cap, but stem elongation certainly benefits considerably from the presence of the cap (Table 2).

Stem structure in longitudinal sections

The data in Table 3 show that there was little increase in the areas of cells in longitudinal sections between a 3 mm fruit body and an 8 mm tall fruit body, both of which were at pre-meiotic developmental stages. Since the mean cell length does not change while the fruit body more than doubles in length, it is presumed that stem elongation at these stages is due primarily to cell division rather than cell elongation. It is difficult to see how hyphal apical growth could contribute to this throughout the stem, and remembering that here we are discussing a structure made up of fungal hyphae, it might be speculated that 'cell division' in this context could take place by intercalary septum formation in the hyphae, followed by elongation of the daughter compartments to the size of their mother compartment. In contrast, there was a large increase in longitudinal sectional area of stem cells between the stems of the 8 mm fruit body (pre-meiotic) and that of a 25 mm fruit body (which was undergoing meiosis). Initially it was the cells in the basal and middle regions of the stem which inflated. The apical cells did not expand to the same extent. Even in a fully elongated fruit body apical cells were considerably shorter than cells in the other regions of the stem. The most elongated cells were found in the upper mid-region of the stem.

Cell length/width ratios did not change much in pre-meiotic stems (3 mm and 8 mm fruit bodies) but, particularly in the upper middle regions of the fruit bodies, increased greatly after meiosis. In the 48 mm fruit body stem cells were approximately 10 times longer than they were wide, in the 55 mm fruit body the cells in the upper middle region had a length/width ratio of approx. 20 and in the 83 mm tall fruit body the cells in the middle region of the stem this ratio approached 35 (compare with a ratio of 2 for cells in the same regions of 3 mm and 8 mm fruit bodies).

Stem elongation in *C. radiatus* was accompanied by a 6- to 8-fold increase in cell length and a doubling of the cell number (Eilers, 1974). In contrast, in *C. cinereus*, although the DNA content of the stem has been found to increase abruptly just before the most rapid phase of elongation (Kamada, Miyazaki & Takemaru, 1976) and stem cells become multinucleate (Lu, 1974; Moore *et al.*, 1979; Stephenson & Gooday, 1984), stem elongation has

been attributed solely to cell elongation (Gooday, 1975; Kamada & Takemaru 1977). The data of Hammad *et al.* (1993a; see Table 3) support this view. Only between the 3 and 8 mm tall primordia was the increase in overall size greater than the increase in mean cell length. The increase in size of stem throughout the rest of the size range was easily accounted for by the increase in cell size.

Co-ordination of cell expansion

Hammad *et al.* (1993a) measured the sectional areas of cells of the hymenium in the same longitudinally sectioned specimens on which the stem cell measurements were made (Table 4). Large scale expansion of stem cells commenced at or just after meiosis (25 mm fruit body), this being reflected in the observation on the macro-scale that rapid stem elongation was correlated with the ending of meiosis (Fig. 3). Remarkably, expansion of the different cell types in the fruit body cap as well as inflation of cells of the stem began immediately post-meiotically (Table 4). Expansion of the cells of the stem is necessary to elevate the cap into the air for more effective spore dispersal; expansion of the different cell types in the gill tissue is also necessary for effective spore dispersal and co-ordination with stem expansion is clearly advantageous.

Hammad *et al.* (1993a) clearly demonstrated that the major phase of cell inflation was a post-meiotic event throughout the fruit body and could account for all the growth involved in fruit body maturation of *C. cinereus*. Also, inflation of cells in the cap was closely correlated with inflation of cells in the stem.

It is possible that the different tissue types are co-ordinated only by being independently synchronised at some early stage of development to the same external timing signal. Their shared time scale being maintained through all of their differentiation processes. An alternative explanation would be that co-ordination was achieved by some sort of signalling system 'reporting' the end of meiosis to spatially distant parts of the fruit body. The route such a signal might take is not clear, but since primary gills are attached to the stem, with their central (tramal) regions in full hyphal continuity with the central stem and cap context (shown by Reijnders, 1963, 1979; Moore, 1987 and diagrammed in Fig. 1), the connection between tissues undergoing meiosis and the upper (most reactive) regions of the stem may allow direct transmission of signals for co-ordination of development in every part of the fruit body.

Table 4. Sectional areas (μm^2) of hymenial cells measured in longitudinal sections together with pooled data for stem cells from the same specimens

	Fruit body height (mm)							
	3	6	8	25	27	48	55	83
Stem apex				292 \pm 25				
Stem middle	148 \pm 7		211 \pm 13	3857 \pm 194		3184 \pm 174	9243 \pm 548	9109 \pm 390
Stem base				2705 \pm 181		6274 \pm 214	10009 \pm 434	10138 \pm 308
Veil cells	238 \pm 14	242 \pm 11	276 \pm 16			3449 \pm 200	6533 \pm 474	3733 \pm 182
Basidia					151 \pm 3	181 \pm 3	177 \pm 3	138 \pm 3
Paraphyses					193 \pm 7	244 \pm 9	253 \pm 7	215 \pm 7
Spores					39 \pm 1	48 \pm 1	43 \pm 1	45 \pm 1
Cystidia					1194 \pm 28	1423 \pm 44	2495 \pm 93	1391 \pm 42
Cystesia					305 \pm 7	303 \pm 11	387 \pm 17	260 \pm 8

Each entry for the hymenial cell types is a mean of 50 measurements. The data for the stem cells are means of 50 to 400 measurements.

Cellular structure and patterning in the stem of Coprinus

Although the genus *Coprinus* has been one of the most keenly studied, the structure of the stem has been essentially ignored. The *Coprinus* stem is commonly described as being composed of greatly inflated and elongated cells and little else. Although Gooday (1975) mentioned the presence of narrow hyphae, the most comprehensive description of stem structure in *C. cinereus* is: 'The stem includes a central column of dikaryotic hyphae and a cortex of giant multinucleate cells' (Lu, 1974). This observation refers to a pre-meiotic fruit body and implies that the cortex is made up exclusively of inflated cells, but this is not so. The cortex comprises both narrow hyphae and inflated cells but although the cortical narrow hyphae may have been observed before no record of them appears in the literature and they tend to be dismissed as fragments of the generative (undifferentiated) hyphae which constituted the young primordium (A. F. M. Reijnders, personal communication).

Hammad, Watling & Moore (1993b) carried out a detailed morphometric analysis, counting and sizing cell profiles in transverse sections of the stem tissue using computer-aided image analysis of light microscope images. They demonstrated that the stem contains both narrow and inflated hyphae. Narrow hyphae (cross-sectional area $< 20 \mu\text{m}^2$) always comprise a significant numerical proportion (23% to 54%) of the cells seen in microscope sections of the stem tissue, although they only contribute 1% to 4% to the overall cross-sectional area of the stem (Table 5).

Overview of stem structure

Low magnification images of transverse sections of stems of any fruit body more than a few mm tall are dominated by the profiles of highly inflated cells (Hammad *et al.*, 1993b) though even in these images a scattering of very much narrower hyphal profiles is evident and narrow hyphae are clearly visible in longitudinal sections and scanning electron microscope images. Many histological dyes stained narrow hyphae selectively, but all staining reactions were differential in the senses that (i) only some of the narrow hyphae were stained in any one transverse section, and (ii) adjacent compartments of the same hyphal filament in longitudinal sections sometimes stained differently (for photomicrographs refer to Figs 6-9 in Hammad *et al.*, 1993b).

Table 5. Comparison of the numbers of narrow hyphae and the area they contributed to the total area of cells in the transect for fruit bodies of *Coprinus cinereus* at different stages of development

Stem Length (mm)	Stage of development	Narrow hyphae (% of total hyphae)	% area contributed by narrow hyphae
6	Pre-karyogamy	47.2	4.2
27	Sporulation in progress	43.6	3.4
45	Sporulation completed	35.2	2.2
70	Sporulation completed, elongation in progress	33.2	1.9

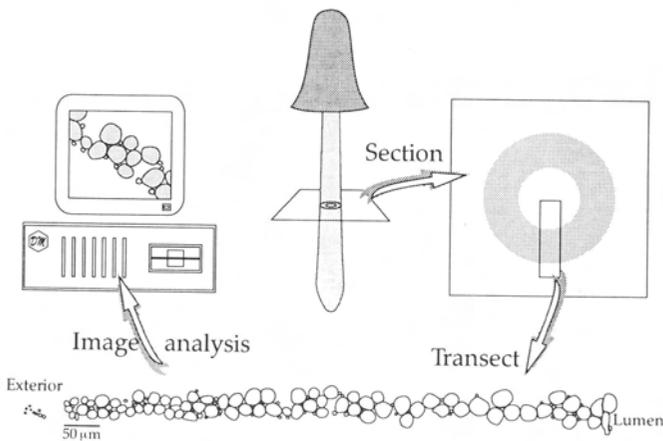


Fig. 4. Determination of cell population distributions. The diagram shows how glycolmethacrylate sections of the stem were used for image analysis of cell cross sectional areas. Transects were routinely 12 μm wide; a wider transect is shown here for illustrative convenience. (Redrawn after Hammad *et al.*, 1993b).

Size spectrum of stem cells in transverse sections

Measurements of the cross-sectional areas of hyphal profiles in 5 μm thick sections were made in transects of transverse sections of stems of various ages. Fig. 4 shows a typical transect and Figs 5 and 6 the graphical plots derived from it to analyse the spatial distributions of stem cells. All of the transects showed the sort of distribution represented in Figs 5 and 6, namely hyphae in the 0–10 and 10–20 μm^2 categories made up the largest classes of the hyphal population. Inflated hyphae had a very dispersed

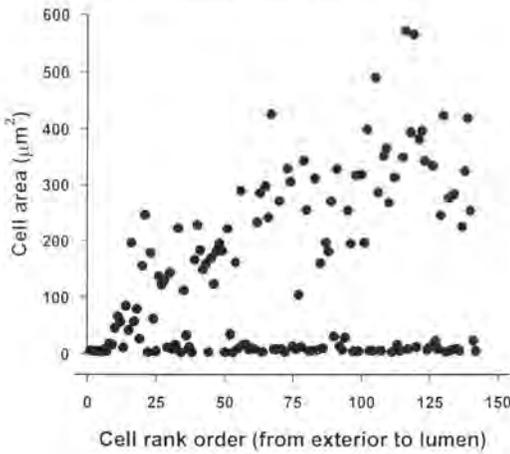


Fig. 5. Plot comparing cell cross sectional area with position of the cell in the transect (= rank order) for the transect diagrammed in Fig. 4. (From Hammad *et al.*, 1993b).

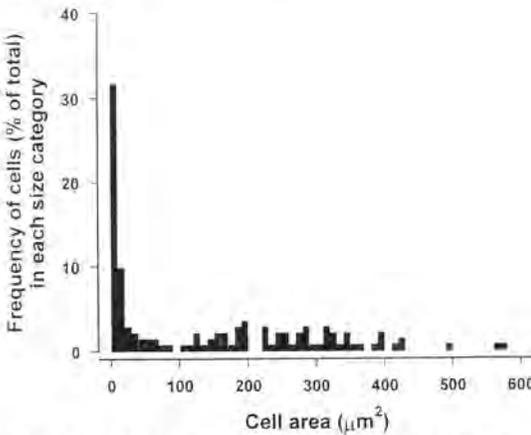


Fig. 6. Plot showing the cell size distribution of the transect diagrammed in Fig. 4. (From Hammad *et al.*, 1993b).

distribution in terms of cell area, no particular cell size being predominant. Very obviously there are two distinct populations of hyphae: narrow hyphae with cross-sectional area less than $20 \mu\text{m}^2$, and inflated hyphae of cross-sectional area greater than or equal to $20 \mu\text{m}^2$ (Table 5).

No single function can be assigned to narrow hyphae, all available evidence points to diverse functions. Narrow hyphae tend to be particularly concentrated at the exterior of the stem and as a lining to the lumen. On the

outer surface they may serve as an insulating layer, like an epidermal layer of hairs. At the lumen they may excrete material into the cavity [Cox & Niederpruem (1975) referred to a brown gel in the lumen which disappeared as the stem extended] or merely represent the remnants of the initially central core of dikaryotic hyphae.

Narrow hyphae stained densely with a number of stains but not all narrow hyphal profiles in a transverse section and not all hyphal compartments belonging to any one narrow hypha in longitudinal sections stained equally. The reason for this differential selectivity of staining is not known, but it might reflect differential function within the population of narrow hyphae. Narrow hyphae may be important in translocation of nutrients through the stem, so differential staining may simply reflect inhomogeneities in the distribution of cytoplasmic materials during translocation. The narrow hyphae seem to form networks independent of the inflated hyphae. Narrow hyphae were branched and interconnected laterally with other narrow hyphae, but inflated hyphae were neither branched nor associated in networks. Thus narrow hyphae may be important in translocation both longitudinally (as for the supply of nutrients to the cap) and transversely (as for communication and co-ordination across the radius of the stem).

Spatial distribution of stem cells in transverse sections

Narrow hyphae were particularly evident as an outer coating of the stem and lining the lumen but they were also interspersed throughout the rest of the tissue. In sections where there was no lumen present in the stem the central region (presumptive lumen) was occupied exclusively by narrow hyphae. This was true both for extremely young fruit bodies (e.g. 6 mm tall) in which the lumen had not yet developed, and for the extreme apex of mature fruit bodies (e.g. 70 mm tall) in a region above a well developed lumen.

Statistical analysis showed that the distribution of inflated hyphae departed significantly from a non-random distribution, and tending towards evenness regardless of the age of the fruit body or position of the section within the stem. On the other hand, the spatial distribution of narrow hyphae differed significantly from randomness in only upper middle and upper apical regions of 27 mm tall and 70 mm tall fruit bodies, where there were slight tendencies towards even distributions.

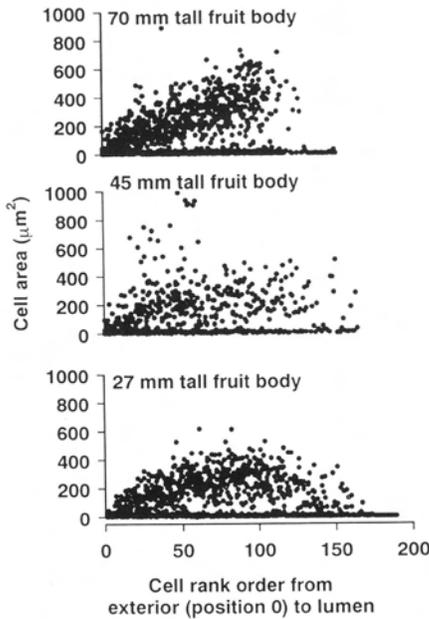


Fig. 7. Cumulated rank order plots of 27, 45 & 70 mm stems. Note how the cell size distribution across the radius of the stem changes with increasing stem length. Sizes (areas) of individual cell profiles in radial transects of transverse sections are collected in the scatter plots for each size of fruit body (1147 measurements for the 27 mm stem, 617 measurements for the 45, 1147 measurements for the 70 mm stem). (From Hammad *et al.*, 1993b).

Changes in the pattern of hyphal distribution

Narrow hyphae were interspersed with inflated hyphae across the full radius of all stems at all positions along the length of the stem and irrespective of the developmental age of the stem. However, there was a progressive change in the distribution of inflated hyphae (Fig. 7).

In 6 mm and 27 mm tall fruit bodies the inflated hyphae increased in cross-sectional area up to a point halfway across the cortex but their size declined again towards the lumen so that cells of the greatest cross-sectional area were found in the middle of the cortex. In a 45 mm tall fruit body the cross-sectional area of inflated hyphae increased gradually from the exterior to the lumen. In the 70 mm tall fruit body this pattern of increase in size right up to the margin of the lumen was even more pronounced, the peak cell area being adjacent to the lumen rather than in the mid-cortex (Fig. 7).

These observations give a very dynamic view of the way the internal

cellular structure of the stem changes during its development. The measurements demonstrate that expansion of the stem involves an initial increase in size of inflated hyphae in the mid-cortex. During further development of the fruit body the cells between this zone and the lumen show the greatest expansion. Inflated hyphae around the periphery of the stem do not enlarge much. Importantly, while these changes are going on, narrow hyphae are present at all positions in the fruit body (from base to apex) and at all stages of development. The population of narrow hyphae was reduced by about 25% as size increased from 27 to 70 mm, presumably due to narrow hyphae (approx. 25%) becoming inflated. This fraction might therefore be considered to be that fragment of the primordial generative hyphae preserved as a reserve of hyphal inflation capacity to support final maturation growth; but it is only a minority fraction of the narrow hyphal population. Other members of that population have other functions. If inflated hyphae do arise by expansion of the randomly distributed narrow hyphae, the even (i.e. non-random) distribution of inflated hyphae implies that a pattern forming process determines their differentiation.

The mechanical consequences of this pattern of cell inflation are simple. Increase in cross-sectional area of inflated hyphae in the middle of the cortex will (i) result in the central core being torn apart, leaving its constituent cells as a remnant around the inner wall of the lumen so created; and (ii) stretch, reorganise and compress the tissues in the outer zones of the stem (Fig. 8).

Thus, formation of the mature stem as a cylinder with outer tissues under tension and inner tissues in compression (the optimum mechanical structure for a vertical cylindrical support) is entirely a result of the pattern of cell inflation within the stem as the stem develops. This specific pattern of inflation must be organised by signalling molecules which determine differential cell inflation across the stem radius.

What makes some of the hyphae differentiate into inflated and multinucleate structures while the narrow hyphae remain morphologically similar to the vegetative mycelial hyphae is not known. This differentiation occurs at an extremely early stage as both narrow and inflated hyphae were seen in 3 mm tall primordia.

Cell distributions in other fungal fruit bodies

The studies of Hammad *et al.* (1993a, b) on *Coprinus cinereus* remain the only thorough quantitative analyses of cell distributions in any fungal fruit body. All other studies in the literature are traditional observational ones.

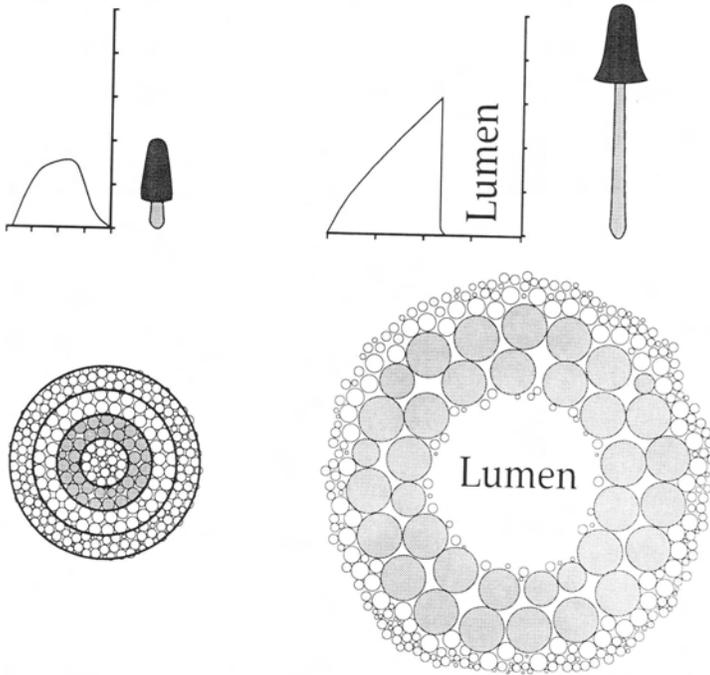


Fig. 8. Interpretation of the geometrical consequences of the cell size changes during development revealed by the data of Fig. 7. The graphs in the upper part of the figure show the lines of best fit for the scatter plots (Fig. 7) of 27 and 70 mm stems, together with scale drawings of the fruit bodies. The diagrams in the lower part of the figure are transverse sections of the stems, drawn to scale. The diagrammatic transverse section of the stem of a 27 mm tall primordium is (on left) is composed of solid tissue which is divided into four zones corresponding to the zones in the radius in the graph above. The central (zone 4) and outermost (zone 1) zones are comprised of rather smaller cells than the two cortical zones. During further growth the most dramatic cell inflation occurs in the cells of zone 3 which are here shown shaded. Growth from 27 mm to 70 mm in height is accompanied by a 3.6-fold increase in cell area in zone 3 and a 1.6-fold increase in zone 2. The cells in the other zones have to be rearranged to accommodate the inflation of zone 3 and a major consequence is that a lumen appears in the centre of the stem. Changes in area are shown accurately to scale in these diagrams, though only a total of 327 cell profiles are illustrated. As this is only a tiny proportion of the cells involved *in vivo*, the diagrams inevitably distort the apparent relationship between cell size and stem size. Narrow hyphae have been ignored in these diagrams, though they are distributed throughout the tissue and their conversion to inflated hyphae contributes to stem expansion.

Corner (1932) introduced hyphal analysis as a procedure to encompass descriptive studies of hyphal systems in polypore fruit bodies (for review see Pegler, 1996). Corner introduced the terms monomitic, dimitic and trimitic to describe tissues consisting of one, two or three kinds of hyphae, and

hyphae in these different categories have been referred to as generative (because they ultimately give rise to the basidia and directly or indirectly to all other structures), skeletal (thick walled with narrow lumen, but lacking branching and septation) or binding hyphae (which have limited growth and irregular often repeated branching). Corner (1966) later coined the terms sarcodimitic and sarcotrimitic to describe particular hyphal systems where there are two or three types of hyphae of which one is inflated and thickened. Redhead (1987) was able to recognise a whole group of closely related agarics with such structures but this included neither the Coprinaceae nor the Russulaceae. Fayod (1889) had already demonstrated that the trama in Russulaceae consists of a mixture of swollen cells (sphaerocysts) and filamentous hyphae, which he called 'fundamental hyphae', but it was Reijnders (1976) who showed that there is a developmental pattern in the tissue with some of the filamentous hyphae playing a very important role. He has described aggregations of hyphae, termed hyphal knots (Reijnders, 1977) in a wide range of species (Reijnders, 1993). The common features of Reijnders' hyphal knots seem to be a central hypha (which remains hyphal) and an immediately-surrounding family of hyphae which differentiate in concert. Such structures have also been observed in *Lactarius* (Watling & Nicoll, 1980). Thus, swollen cells in a ring or cylinder around a central hypha may be formed by many species (another example is described in Section 7.5) and may be the basis of longer range positional control of patterns (see Section 7.7).

The cystidial morphogenetic field

The hymenium of *Coprinus cinereus* contains four cell types: basidia, paraphyses, cystidia and cystesia. The paraphyses expand to become the major structural members of the gill lamellae, but they arise secondarily as branches from sub-basidial compartments and when first formed, the hymenium consists of a carpet of probasidia with a scattering of cystidia (Rosin & Moore, 1985a). According to Smith (1966), cystidia '. . . occur haphazardly in the hymenium, depending on the species, and vary from abundant to absent . . .'; a description of a cell distribution pattern which, in terms of developmental biology is totally useless. Much of the literature dealing with cystidia is similarly blinkered, considering them from the taxonomic viewpoint (e.g. Lentz, 1954; Price, 1973), reflecting assiduous attention to fine distinctions of nomenclature at the expense of appreciation of the remarkable developmental plasticity revealed by their occurrence and form. Brefeld (1887, cited in Buller, 1910) concluded that cystidia are

metamorphosed basidia, a view summarised by Corner (1947) in the phrase '...cystidia represent sterile basidia which become overgrown...' Certainly, young basidia and young cystidia both originate as the terminal compartments of branches from the hyphae of the sub-hymenium, but cystidia are NOT overgrown basidia. The mature cystidium is a cell that is highly differentiated for its particular function (see below).

Fuzzy logic in fungal differentiation

Development of a cystidium represents expression of a perfectly respectable pathway of differentiation and commitment of a hyphal tip to the cystidial as opposed to the basidial pathway of differentiation. The commitment must occur very early in development of the hymenium because young cystidia are recognisable in the very earliest stages (Rosin & Moore, 1985a, b). The controls which determine formation of a cystidium, instead of a basidium, by a particular hyphal apex need to be established. It is certainly the case that the basidial developmental pathway (in *Agaricus bisporus*) can be interrupted to allow this cell type to serve a structural rather than spore-producing function (Allen, Moore & Elliott, 1992), though this is clearly arrested meiosis, not sterility. Similarly, it is also evident that cells which are undoubtedly cystidia on morphological criteria can occasionally show evidence of entry into meiosis (Chiu & Moore, 1993; and see Chapter 5), which suggests that entry to the cystidial pathway of differentiation does not totally preclude expression of at least part of the meiocyte differentiation pathway. Similarly, the fact that a large fraction of the *in situ* basidial population of *A. bisporus* remains in arrested meiosis indicates that entry to the meiotic division pathway does not guarantee sporulation; a fact also demonstrated with excised gills of *C. cinereus in vitro* (Chiu & Moore, 1990a).

Further examples can be found in the literature. Watling (1971) observed some cystidia bearing hyphal outgrowths looking like sterigmata in a spontaneous fruit body variant of *Psilocybe merdaria*, while Schwalb (1978) reported that basidia of a temperature-sensitive mutant of *Schizophyllum commune* not only aborted meiosis but also produced elongated sterigmata at the restrictive temperature. A spore-deficient mutant of *Lentinula edodes* (= *Lentinus edodes*) produced some abnormal basidia bearing both a hyphal outgrowth and basidiospores (Hasebe, Murakami & Tsuneda, 1991).

Similar abnormalities in basidia have been induced in *Coprinus cinereus* by transplanting gills to agar medium containing some metabolic inhibitors (Chiu & Moore, 1988a & b; 1990a; and see Chapter 6). These explantation

experiments have been discussed mainly for their value in understanding commitment to the basidium differentiation pathway, but it is equally important that all other cells of the hymenium and hymenophore showed no commitment; immediately reverting to hyphal growth on explantation. This implies that all differentiated cells except the meicyte have an extremely tenuous grasp on their state of differentiation; so tenuous that when removed from their normal tissue environment they revert immediately to the vegetative hyphal growth mode. That these cells do not default to hyphal growth *in situ* implies that their state of differentiation is somehow continually reinforced by some aspect of the environment of the tissue which they comprise. Interestingly, although cystidia of *Coprinus* reverted to hyphal growth when excised, cystidia of excised gills of *Volvariella bombycina* were arrested and did not show reversion to vegetative growth suggesting they are another differentiated hymenial cell type (Chiu & Moore, unpublished).

Chiu & Moore (1993) discuss the possibility that fungal differentiation pathways exhibit what would be described as 'fuzzy logic' in cybernetic terms. Instead of viewing fungal cell differentiation as involving individual major 'decisions' which switch progress between alternative developmental pathways which lead inevitably to specific combinations of features, this idea suggests that the end point in fungal differentiation depends on the balance of a number of minor 'decisions'. So, rather than rigidly following a prescribed sequence of steps, fungal differentiation pathways are based on application of rules which allow considerable latitude in expression. Developmental decisions between pathways of differentiation seem to be able to cope with a degree of uncertainty, allowing fungal cells to assume a differentiation state even when all conditions of that state have not been met.

Distribution of cystidia

Descriptions of cystidium distribution commonly encountered in the literature feature adjectives like 'scattered', 'haphazard', 'fairly uniform'. The only statistically valid description of cystidial distribution has been published by Horner & Moore (1987).

Cystidia are found 'scattered' in fair number over the hymenium of *C. cinereus*. Visually, cystidial density-distribution on the face of the gill is fairly uniform but at the gill edge the density of cystidia is locally increased. When sufficiently developed, cystidia span the gill cavity, their apices adhering to cystesia in the opposite hymenium. At early stages in growth of

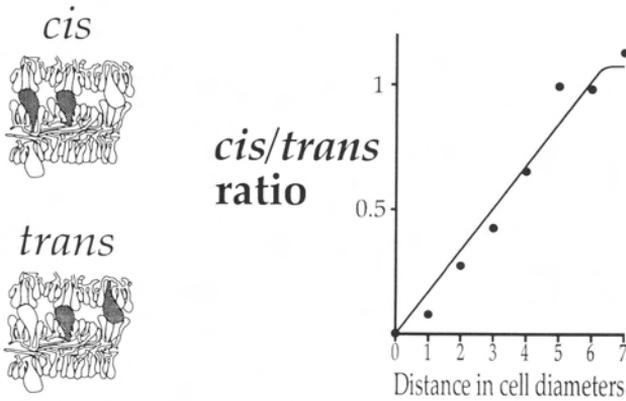


Fig. 9. Cystidium distribution in the *Coprinus cinereus* hymenium. The drawings on the left of the graph show the categorisation of neighbouring pairs of cystidia in micrographs as either *cis* (both emerge from the same hymenium) or *trans* (emerging from opposite hymenia). The plot compares the frequencies of these two types over various distances of separation and shows that closely-spaced *cis* neighbours are less frequent than closely-spaced *trans* neighbours, implying some inhibitory influence over the patterning of cystidia emerging from the same hymenium. (Redrawn after Moore, 1995).

the cystidium the cell(s) with which it will eventually come into contact in the opposing hymenium are indistinguishable from their fellow probasidia. However, when the cystidium comes firmly into contact with the opposing hymenium, the hymenial cells with which it collides develop a distinct granular and vacuolated cytoplasm, more akin to that of the cystidium itself than to the neighbouring probasidia. This suggests that a contact stimulus sets in train an alternative pathway of differentiation leading to an adhesive cell type called the cystesium.

About 8% of the hyphal tips in the protohymenium of *C. cinereus* become cystidia (Rosin & Moore, 1985b). Cystidia may arise from either of the two hymenia which are on opposite sides of each gill cavity and they can be seen, counted and measured easily in sections cut for light microscope observation (details in Horner & Moore, 1987). Demonstration of the cystidial morphogenetic field relied upon measurements made of such sections. The argument was based on the expectations that cystidia spanning the gill cavity may be 'distant', having other cells separating them, or 'adjacent', with no intervening cells; and, in either case, both cystidia may emerge from the same hymenium (described as '*cis*') or from opposite hymenia ('*trans*'). *A priori*, one would predict that if the distribution of cystidia is entirely randomised then the frequency of adjacent pairs will

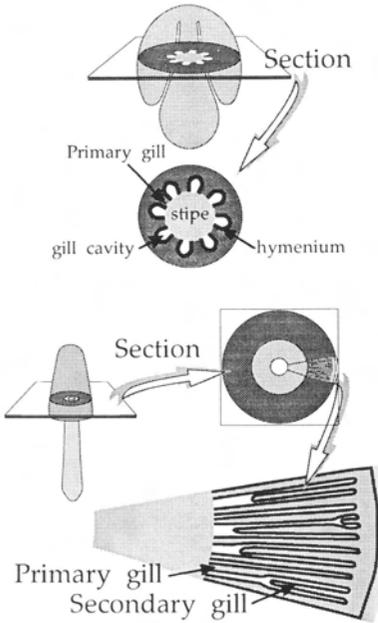


Fig. 10. Diagrammatic cross section of primordium (top) and older fruit body (bottom) showing primary and secondary gills. (Redrawn after Moore, 1995).

depend on the population density only and there will be an equal number of cis and trans in both the distant and adjacent categories. However, quantitative data from serial sections showed that there was a positive inhibition of formation of neighbouring cystidia in the same hymenium (Fig. 9).

The interpretation of data shown in Fig. 9 is that formation of a cystidium actively lowers the probability of another being formed in the immediate vicinity. The extent of the inhibitory influence extends over a radius of about $30\ \mu\text{m}$ and is strictly limited to the hymenium of origin. For the activating component of the system it could be suggested that differentiation leading to cystidium formation is activated by the concentration of a constituent of the atmosphere in the gill cavity immediately above the developing hymenium. Many cystidia seem to be secretory (Chiu & Moore, 1990c) so their metabolism may well react to the local gaseous environment. The distribution pattern of cystidia is thus interpreted as being dependent on interplay between activating and inhibiting factors which define the cystidial morphogenetic field.

Forming gills into patterns

Most of the patterns discussed so far are particular cell distributions which are evident only on the microscopic scale. The most obvious pattern in an agaric fruit body, though, is the distribution of the gills. The gills are essentially tissue plates suspended from the underside of the fruit body cap. In *Coprinus*, the cap of the fruit body primordium encloses the top of the stem and gills are formed as vertical plates arranged radially around the stem (Fig. 10).

There are two types of gill (diagrammed in Fig. 10): primary gills which, from formation, have their inner (tramal) tissue in continuity with the outer layers of the stem, and secondary (and lesser ranked) gills in which the hymenium is continuous over the gill edge (Reijnders, 1979; Rosin & Moore, 1985a; Rosin *et al.*, 1985; Moore, 1987).

The direction of growth

Crucial to a proper understanding of the morphogenesis of fruit bodies is knowledge of the direction of growth of the constituent parts. Agaric gills are suspended from the flesh of the cap and the intuitive expectation is that gills extend at their free edge. This direction of growth (i.e. radially inwards towards the stem) seems to be assumed as an unassailable truism in most of the earlier literature. Logic seems to be in its favour. After all, fungal hyphae characteristically show apical growth, and the free edge of the gill is composed of hyphal apices; should they not extend the gill?

Unfortunately, this thought immediately raises a problem. The hyphal tips which make up the free edge of the gill are terminally differentiated into hymenial cells; they have lost their capacity for extension growth. Primary gills of *Coprinus* spp. present another problem because they are connected with cap tissue at their outer edge and with the stem at their inner edge. How does a gill without a free edge extend? The observations and experiments discussed below show that while it is not unreasonable nor illogical to assume that the direction of growth is radially inwards towards the stem, it is, in fact, diametrically wrong.

During gill development in *Volvariella bombycina* it has been demonstrated that the free edges of the gills remain essentially intact. This was done by painting black ink marks on the tissues in a primordium (Chiu & Moore, 1990a). During further fruit body development, ink marks placed on the cap margin and those placed on the edges of the gills remained at the margin or the gill edges respectively. The growth increment in these

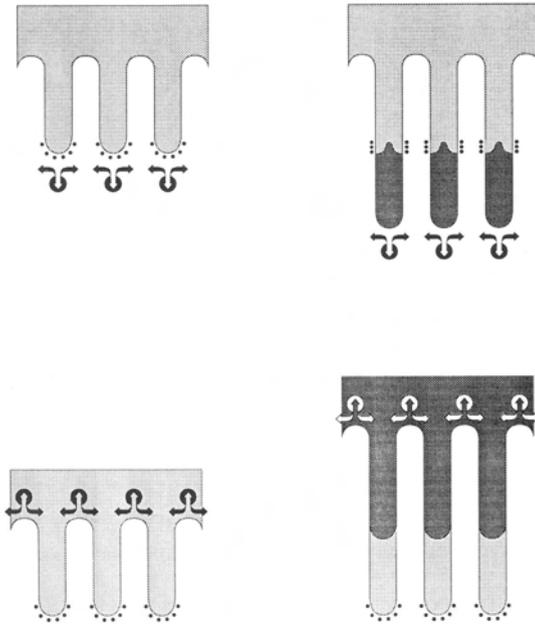


Fig. 11. Gill formation in *Volvariella bombycina*. Line drawings illustrating the outcome of marking experiments (Chiu & Moore, 1990a) and what they reveal about the direction of gill development in this organism. The diagrams show diagrammatic sections of primordial gills on the margins of which ink particles have been placed (diagrams on the left). The diagrams on the right illustrate alternative outcomes from further growth depending on whether growth occurs at the gill margin (gill organiser located at the gill margin; top right) or at the gill base (gill organiser located at the foot of the gill; bottom right). In the experiments reported by Chiu & Moore (1990a), ink marks painted on the gill margins of primordial fruit bodies were still clearly visible on the gill margins of mature fruit bodies, demonstrating that agaric gills grow by extension at their roots, and not by extension from the free margin. (Redrawn after Moore, 1995).

experiments was quite considerable, the radius of the cap increasing from 0.5 to 2.5 cm and the depth of the gills from 1.5 to 5 mm. If growth of the cap and gill margins resulted from apical growth of the hyphal tips which occupied the margin, then ink particles placed on those hyphal tips would be left behind as the hyphal apices extended which would consequently have resulted in the ink marks being buried beneath 4 to 20 mm of newly formed tissue by the end of the experiment (Fig. 11). It follows, therefore, that the hyphal tips which first form the cap margin, and those which form the gill margin, always remain at the margin. They do not continue to grow apically to extend the margin radially, nor are they overtaken by other

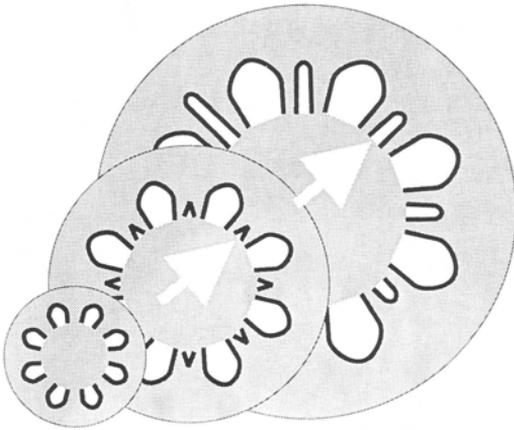


Fig. 12. Diagrammatic transverse sections of developing primordia of *Coprinus* illustrating that because primary gills are attached to the stem, their thickness must increase as the stem circumference increases. To avoid formation of excessively thick gills, new gill cavities must arise within the central (tramal) tissue of primary gills (arrows). Their progression outwards identifies the prime direction of development in this fruit body. (Adapted and redrawn after Moore, 1995).

hyphae; instead they are ‘pushed’ radially outwards by the press of fresh growth behind, and they are joined by fresh branches appearing alongside as the circumference of the margin is increased (Fig. 11).

There seems to be an exactly homologous process in *Coprinus*. Gills in young *Coprinus* primordia are not open to easy manipulation, so in this case the argument depends upon observation of particular gill structures during development. The crucial observation derives from the fact that primary gills of *Coprinus* spp. are connected with cap tissue at their outer edge and with the stem at their inner edge combined with consideration of the geometrical consequences of this arrangement as the tissues expand (as depicted in Fig. 1). The gills are attached to the stem circumference, but the stem circumference must increase greatly during development. If the gills are attached to a stem of increasing circumference, why does gill thickness at the point of attachment not increase in proportion? The tendency to widen as the stem circumference increases is compensated by gill replication, and specifically by formation of a new gill cavity and its bounding pair of hymenia within the trama of a pre-existing gill (Moore, 1987). This clearly sets the direction of development as outwards from the stem; i.e. gills in the *C. cinereus* fruit body grow radially outwards, their roots extending into the undifferentiated tissue of the pileus context (Fig. 12).

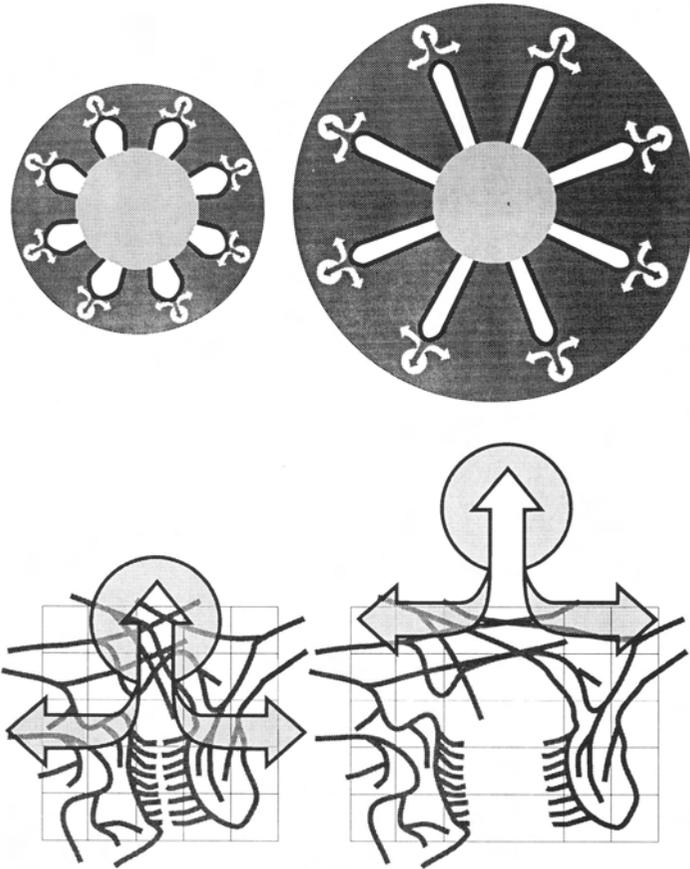


Fig. 13. The outward progression of gill cavities (upper diagrams) is managed by gill organisers which migrate radially into newly-produced undifferentiated tissue. As they pass through an undifferentiated region, gill organisers promote the branching pattern that generates the two opposing hymenia of neighbouring gills (bottom left). Subsequent expansion of the inner tissues separates the two hymenia (depicted in the 'stretched' co-ordinates of the diagram at lower right) to produce the gill cavity. (Adapted and redrawn after Moore, 1995).

Two different sources of information consequently demonstrate that growth of an agaric gill occurs by outward progression of gill tissue at its foot. Gill growth proceeds radially outwards; differentiation of gill tissue extending into the undifferentiated tissue of the outwardly expanding cap tissue (Fig. 12).

Embryonic gills are convoluted

In both *C. cinereus* and *V. bombycina* gills are formed as convoluted plates (Chiu & Moore, 1990b). A sinuous, labyrinthiform hymenophore appears to be a normal 'embryonic' stage in fruit body development in agarics, yet a regular radial arrangement of the gills is characteristic of the mature basidiome. How this is achieved is a function of the expansion of the maturing primordium generating stresses between tissue layers which stretch or inflate the convoluted gills into strict radii. Stretching is the effective force in *C. cinereus*; the cystidia being critical elements in the communication of the formative stresses around the fruit body. Inflation occurs in *V. bombycina*: the tightly appressed cells of the hymenium forming a tensile layer containing the compression generated by cell inflation in the gill trama.

The gill organizer

A fundamental 'rule' during the very earliest stages of agaric gill formation seems to be: if there is room, make a gill; without reference to the exact spatial orientation of the gill tissue so formed. The mechanics of fruit body expansion will compensate for any meandering in the direction taken during gill formation. The formative element which directs the development of undifferentiated tissue of the cap into gill tissue is an organiser in the tissue at the extreme end of the gill cavity (Fig. 13).

The gill organiser is responsible for the progression of the gill cavity radially outwards, away from the stem. It directs the 'undifferentiated-to-differentiated' transition. Presumably this is largely an increase in branch frequency to produce branches of determinate growth which are mutually 'attracted' so that they form the opposing young hymenia on either side of what will become the gill cavity. Cap expansion separates the two protohymenia, thus extending the gill cavity deeper into the tissue of the cap. Since they are progressing radially outwards, neighbouring organisers become further and further separated from one another as development proceeds and as the distance between neighbouring organisers increases a new one can arise between them (diagrammed in Fig. 13; micrographs shown in Rosin & Moore, 1985a); when a new gill organiser emerges, the margin of a new (but 'secondary') gill is formed. It is extended not by growth of its margin, but by continued radial outward progression of the two (mother and daughter) gill organisers which now straddle either side of its root.

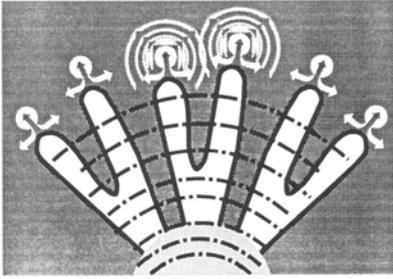


Fig. 14. Continued outward progression of gill organisers could be their response to a radial gradient within the inner tissues (which might be an outwardly diffusing signal or a metabolic concentration gradient). In addition, each gill organiser could control its own morphogenetic field by producing an inhibitor that prevents a new gill organiser arising within its diffusion range. As existing gill organisers move radially away from each other the inhibitory effect diminishes and a new organiser can be produced in response to the primary signal from the centre. (Adapted and redrawn after Moore, 1995).

In the origin of the gills we see operating two classic components of theoretical morphogenesis – activation and inhibition by diffusing morphogens. First, we can assume that diffusion of an activating signal along the fruit body radius assures progression of the gill organiser along its outward radial path. Second, each organiser can be assumed to produce an inhibitor which prevents formation of a new organizer within its diffusion range (i.e. the gill organiser uses this inhibitor to control its morphogenetic field) (Fig. 14). As radial progression into the extending (undifferentiated) cap tissue causes neighbouring organisers to diverge, a region appears between them which is beyond the range of their inhibitors – at this point a new organizer can arise in response to the radial activating signal. Interactions between the diffusion and decay characteristics of the activator and the inhibitor are all that is necessary to control gill spacing, gill number, gill thickness, and the radial orientation of the gill field.

The origin of space

The internal structure of the *Coprinus* primordium is uniformly solid at the time that gills begin to arise, so gills and gill space arise together. Similarly, as is argued above, as development proceeds gills, hymenia and the gill spaces between them emerge together as the gill organisers migrate outwards into undifferentiated cap tissue.

Lu (1991) has claimed that the gill cavities in *Coprinus* arise as a result of

'programmed cell death' (originally 'cell disintegration' (Lu, 1974)) after observing multivesicular and membranous bodies in cells of the gill cavities on chemically fixed and processed transmission electron microscope specimens. He interpreted these observations to indicate that cell disintegration accounts for formation of the gill cavity, proposing a selective and programmed cell death as a part of tissue remodelling during gill formation. The hypothesis of programmed cell death is attractive but there is not enough evidence for it. In animal cell systems, cell death (apoptosis) is an essential component of embryogenesis as well as a continuing strategy for control of the dynamic balance of tissues in the body (Sen, 1992). Apoptosis is a very specific cellular process which exhibits a number of characteristic cytological and molecular processes. None of these have been observed in fungi. Further, cell disintegration has not been reported in any earlier studies on *Coprinus* (Reijnders, 1963, 1979; Rosin & Moore, 1985a, b) although similar vesicular aggregations have been ascribed to fixation artifacts (Waters, Butler & Moore, 1975a, b). The balance of evidence, therefore, is that the case has not been made for apoptosis generating the spaces which occur in fungal fruit bodies.

Moore (1995) pointed out that cell degeneration, whether by necrosis or apoptosis, was unnecessary to create spaces within a fruit body which is continually expanding. All that is needed is that branching patterns are organised to form 'surfaces' which can separate when the fruit body expands. He called the process *cavitation* and suggested that spaces can be formed in the following way. When branches of determinate growth (which are mutually 'attracted') are formed opposing one another as a pair of palisaded cell plates (like the opposing hymenia of neighbouring gills), they form an incipient fracture plane. This plane can be opened out into a cavity when the expansion of the underlying tissue puts tension across the 'fracture' and pulls the palisades apart. The argument applies to cavitation in all differentially expanding cellular structures. Variations on the theme can be imagined in other regions and other organisms. If the 'fracture planes' form an annulus around the top of the stem (one tissue layer might be the stem apical meristemoid, the other the hymenophore meristemoid), then an annular cavity could arise before gill formation.

Conclusions

Even the most casual observations confirm that co-ordination of developmental processes is successfully achieved in fungal multicellular structures, but there is little convincing evidence for chemicals able to perform the

signal communication involved (Chapter 7). Thus, there are no clues to the nature of the growth factors involved in these phenomena. Also, given that lateral contacts between fungal hyphae are rare in comparison with the lateral interconnection by plasmodesmata, gap junctions and cell processes in cells of plant and animal tissues, any morphogens responsible for coordinating the activities of different hyphal branching networks are likely to be communicated exclusively through the extracellular environment (Reijnders & Moore, 1985).

Perhaps the ultimate morphogenetic regulatory unit in multihyphal fungal structures is the Reijnders hyphal knot - a little community comprising an induction hypha (or hyphal tip, or hyphal compartment) and the immediately surrounding hyphae (or tips, or compartments) which can be brought under its influence. Larger scale morphogenesis could be co-ordinated by 'knot-to-knot' interactions (Moore, 1995).

Many of the phenomena described in this chapter are poor candidates for experiment but the chapter shows how careful application of essentially conventional but quantitative observational methods can extricate valuable information. Direct experiment requires a morphogenetic model system. This, too, seems to be available: in one case through the use of explantation experiments (Bastouill-Descollonges & Manachre, 1984; Chiu & Moore, 1988a & b, 1990a; and see Chapter 6) and also through the use of tropisms which are simple developmental pattern forming processes able to generate a particular morphogenetic change on demand in a specific location (Moore, 1991; Moore *et al.*, 1996).

Significant advances in fungal developmental biology will only be achieved if more use is made of quantitative methods. The science is doomed to stagnation if, in the days of computer-aided image analysis and virtual reality, the practitioners of mycology remain satisfied with hand drawn line art and subjective verbal description to communicate their observations. Fungal developmental biology must get into the 20th century before the 21st brushes it aside.

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