Tissue Formation

By David Moore

School of Biological Sciences, Stopford Building, The University, Manchester M13 9PT

Chapter 21, pp. 423-465, in ***The Growing Fungus*** (1994). Edited by N. A. R. Gow & G. M. Gadd, published by Chapman & Hall: London.

1 Introduction

During particular stages in the life history of many fungi, hyphae become differentiated and aggregated to form tissues distinct from the vegetative hyphae that ordinarily compose a mycelium. The mycelium, of course, is a diverse, dynamic population of hyphae which is a fascinating study in its own right (Boddy & Rayner, 1983a, b; Gregory, 1984; Rayner & Webber, 1984; Rayner, 1993), but this chapter will deal specifically with the patterns which result in formation of defined tissues in multihyphal fungal structures.

2 Multihyphal structures

The majority of the macroscopic fungal structures are formed by hyphal aggregation into either linear organs -- strands, rhizomorphs and fruit body stipes -- or globose masses -- sclerotia and the familiar fruit bodies, as well as less-familiar sporulating structures, of the larger Ascomycotina, Deuteromycotina and Basidiomycotina.

2.1 Basic vocabulary and basic principles

The general term **plectenchyma** (Greek *plekein*, to weave, with *enchyma* = infusion, meaning an intimately-woven tissue) is used to describe organized fungal tissues. There are two

types of plectenchyma: **prosenchyma** (Greek *pros*, toward & *enchyma*; i.e. approaching or almost a tissue) is, visually, a rather loosely organised tissue in which the components can be seen to be hyphae; and **pseudoparenchyma** (Greek *pseudo* = false with *parenchyma* = a type of plant tissue) which, as seen in microscope sections, is comprised of tightly packed cells resembling plant tissue. In pseudoparenchyma, the hyphae are not immediately obvious as such, though the hyphal nature of the components can be demonstrated by reconstruction from serial sections or by scanning electron microscopy.

The majority of the lower fungi have coenocytic hyphae, but lower fungi do not form multicellular (multihyphal) structures. Read (1983, 1993) and Read & Becket (1985) have argued for a simple classification of fungal tissues and suggested that the term **cellular element** should be used in preference to 'cell' because fungal cells are always hyphal compartments and consequently different from the concept of the cell which emerges from elementary biological education. However, a case can be made for using the term 'cell'.

Primary septa in fungal hyphae are formed by a constriction process in which a belt of microfilaments around the hyphal periphery interacts with microvesicles and other membranous cell organelles (Girbardt, 1979). Girbardt (1979) emphasised the correspondence between fungal septation and animal cell cleavage. The completed septum has a pore which may be elaborated with the parenthesome apparatus in most basidiomycetes or be associated with Woronin bodies in ascomycetes; in either case the movement or migration of cytoplasmic components between neighbouring compartments is under effective control. So, the cellular structure of the hypha extends, at least, to its being separated into compartments whose interactions are carefully regulated and which can exhibit contrasting patterns of differentiation. Further, Griffin, Timberlake & Cheney (1974) pointed out that, by increasing the number of growing tips, mycelial branching is the equivalent of cell division, and the kinetic analyses of Trinci (1974, 1984) show clearly that fungal filamentous growth can be interpreted on the basis of a regular cell cycle. Thus, fungi can quite reasonably be considered to be cellular organisms producing differentiated tissues composed of cells that are the progeny of an initial cell or cell population, which is induced to start multiplication and differentiation. But there are fundamental differences between this cell concept and one which might be applied to plants; one is the way in which proliferation occurs and the other is the nuclear ploidy-cytoplasm relationship.

Plants, animals and fungi are distinct eukaryotic Kingdoms (Whittaker, 1969; Margulis, 1974; Cavalier-Smith, 1981), which are thought to have separated at some protist level, prior to the establishment of the multicellular grade of organization in any of them. These three Kingdoms are very different from one another in ways that are critical to determining the morphology of multicellular structures as well as in their nutrition (animals engulf, plants use radiant energy, fungi absorb) was part of the original definition of the Kingdoms (Whittaker, 1969). Among the crucial evolutionary steps leading to organised multicellularity were probably the development of mechanisms for dividing a cell, together with a mechanism for controlling the placement of the plane of cell division in particular relation to the orientation of nuclear division. A key characteristic of embryology throughout the animal Kingdom is the movement of cells and cell populations. Plant cells have little scope for movement and their morphogenesis depends upon control of the orientation and position of the daughter cell wall, which forms at the equator of the mitotic division spindle. Fungi are also encased in walls but their basic structural unit, the hypha, exhibits two features which cause fungal morphogenesis to be totally different from plant morphogenesis. These are that hyphae grow only at their tip and that **cross walls form only at right angles to the long axis of the hypha**. The consequence is that fungal morphogenesis depends on the placement of hyphal branches. To proliferate a hypha must branch, and to form a structure the position of branch emergence and its direction of growth must be controlled.

A relevant contrast is the development of the protonema following fern spore germination. Germination produces a uniseriate filament of cells -- very similar to outgrowth of a hyphal germ tube from a fungal spore. Eventually, and usually in the apical cell, the division plane becomes reoriented so that the new cell walls are formed obliquely or parallel to the long axis of the filament and a flat plate of cells (the gametophyte prothallus) is formed (Miller, 1980; and see Fig. 1).

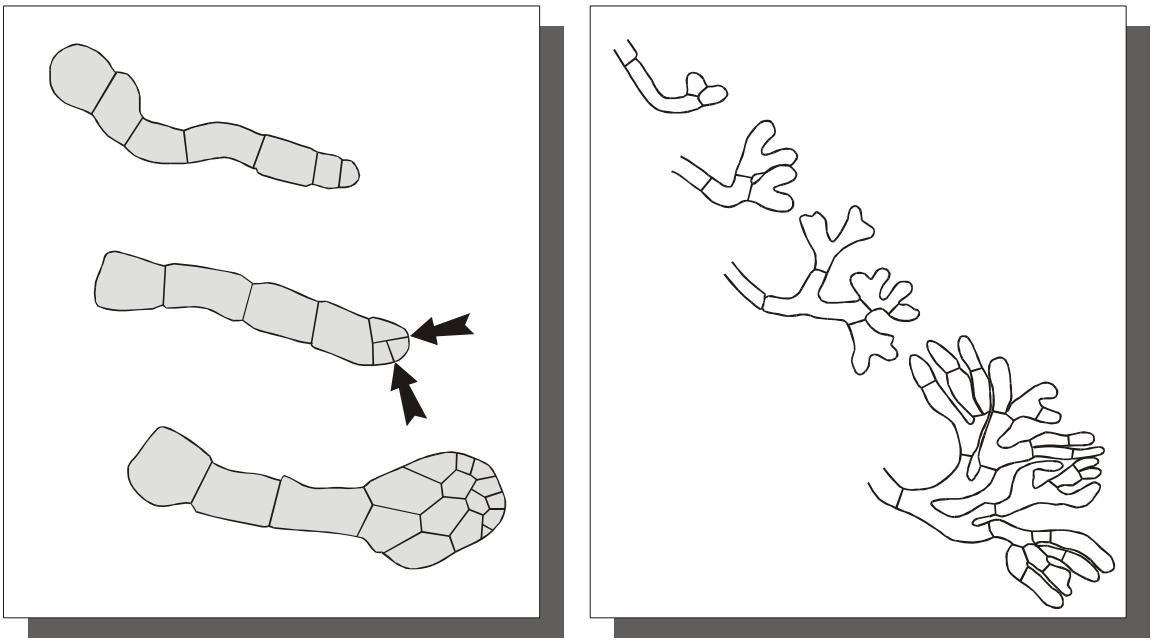

Fig. 1. Transforming a filament into a two-dimensional plate. A comparison of the cell proliferation strategy employed by a fern (left) and a filamentous fungus (right). Left-hand panel: change in the orientation of the plane of division in the apical cell (arrows) of the protonema of the fern *Onoclea sensibilis* converts it into a meristematic cell which can give rise to the planar gametophyte by further regulation of the mitotic division plane (drawings made from photographic illustrations in Miller, 1980). Right-hand panel: orientation of mitotic division spindles is irrelevant in the fungus *Botrytis allii* as cross-walls are always formed at right angles to the long axis of the hypha. A two-dimensional plate can only be formed by controlling the position, orientation and direction of growth of new hyphal tips formed as lateral branches (redrawn from Townsend & Willetts, 1954).

This transition epitomises the importance of the mitotic orientation in plant morphogenesis and serves to emphasise how totally different is the fungal approach to solution of the same problem. Cross-walls in fungal hyphae are formed at right angles to the long axis of the hypha; the only exceptions are in cases of injury or in hyphal tips already differentiated to form sporing structures. Hyphal tip cells are not subdivided by oblique cross-walls, nor by longitudinally oriented ones. Even in fission yeasts producing irregular septation patterns under experimental manipulation, the plane of the septum is always perpendicular to the longest axis of the cell (Miyata, Miyata & Johnson, 1986). In general, then, the characteristic fungal response to the need to convert the 1-dimensional hypha into a 2-dimensional plate or 3-dimensional block is the formation of lateral branches (Fig. 1).

Thus, the regulatory target in fungal morphogenesis is the machinery, presently completely unknown, which is involved in generating a new apical growth centre (to become the hyphal tip of the new lateral branch) and the determination of its position, orientation and direction of outgrowth from the parent hypha. Origin of the branch seems to be the formal equivalent of determination of morphogenetic growth by orienting the plane of division and the new crosswall, as occurs in plants, and directional growth of the new hyphal apex has much in common with the morphogenetic cell migrations that contribute to development of body form and structure in animals. Septation in the main hypha is in some way defined by the position of the dividing nucleus (Talbot, 1968; Girbardt, 1979), but, for branch formation, there does not seem to be any dependence on orientation of the nuclear division spindle. Cytoplasmic vesicles are crucial to the growth of hyphal apices (Grove, 1978; Bartnicki-Garcia, 1990) and distribution of microvesicles may be closely connection with branch initiation. During initial outgrowth of clamp connections of *Schizophyllum commune* vesicles are displaced in the direction of curvature of the clamp cell soon after its emergence (Todd & Aylmore, 1985) and localised accumulation of microvesicles may be a cause of branch initiation (Trinci, 1978). Differential ion fluxes could direct this. Applied electrical fields affect the site of branch formation and the direction of hyphal growth in young mycelia of several fungi

(McGillivray & Gow, 1986). Studies with *Achlya* (Kropf *et al*., 1983, 1984) revealed an ion current caused by influx of protons at the hyphal tip (as an amino acid symport in this organism) and that a new zone of proton influx often preceded and predicted the emergence of a branch. However, the causality of this process in branching is doubtful, as it seems to be more related to nutrient uptake than tip growth (Harold & Caldwell, 1990; de Silva *et al*., 1992).

The information we have is sparse, and it all derives from experiments with vegetative hyphae where the connections between nuclear division, cytokinesis and branch formation may well be relaxed. In the pseudoparenchymatous (or prosenchymatous) 'generative' tissues, which precede final differentiation in developing fungal multicellular structures, the constituent cells are generally smaller and less vacuolated than typical hyphal cells. Small size and dense cytoplasmic content are also often associated with rapidly dividing cells in animals and plants. In the fungi, a consequence of rapid karyogamy and frequent branching might be that a much closer correlation is maintained between nuclear/cell division and branch formation. However, the higher fungi do seem to have a looser connection between cell differentiation and nuclear number and ploidy than is usual in plants and animals. The cells of *Agaricus* mycelia have 6-20 nuclei per cell (Colson, 1935; Kligman, 1943) and cells of the mushroom fruit body have an average of six nuclei (Evans, 1959). Conversely, in a basidiomycete with the most classically regular vegetative dikaryon, *Coprinus cinereus*, cells of the fruit body stipe can become multinucleate by a series of consecutive conjugate divisions, a peculiarity exhibited by other agarics (Stephenson & Gooday, 1984; Gooday, 1985). As *Armillaria* species have diploid tissues in the fruit body, it is clear that the ploidy level and the number of nuclei are both variable, being controlled by factors other than those imposed by the need to assemble multicellular structures.

Although the orientation of the branch tip decides the initial direction of the new growth, the hyphal tip is an invasive, migratory structure. Its direction of growth after initial emergence must be under precise control as it determines the nature and relationships of the cells the hypha will form. This is clearly seen in hymenial layers which are constructed from branches of determinate growth in a precise spatial array (Fig. 9), in the behaviour of binding hyphae in fruit body stipes (Williams, Beckett & Read, 1985), tendril hyphae in mycelial strands (Butler, 1958; and see Fig. 2) and generally in structures constructed from closely appressed axially-arranged hyphae such as strands (Fig. 2), rhizomorphs (Fig. 3) synnemata (Fig. 4), and necks of ascomata (Rayner *et al*., 1985; Read, 1993).

Very little is known about the mechanisms involved but since even the most open fungal tissues appear to be filled with an extracellular mucilaginous material, reactions akin to the cell-matrix interactions of animal tissues may guide and co-ordinate hyphal growth in tissues. A highly hydrated extracellular matrix, comprised predominantly of glucans, fills the interhyphal spaces within sclerotia (Willetts & Bullock, 1992), similar to that found in fruit bodies (Williams *et al*., 1985) and rhizomorphs (Rayner *et al*., 1985) and may be important in providing an environment in which morphogenetic control agents can interact. Different tissues may synthesise and secrete specific polysacccharides and/or glycoproteins to provide a local environment within which hyphae and growth control factors interact with the specificity associated with the notion of 'control by context' where the response of the cell to a growth factor is influenced by the extracellular matrix within which the interaction takes place (Nathan & Sporn, 1991). In animal systems it is becoming increasingly apparent that the extracellular matrix regulates transcription directly, probably through integrin-mediated signalling (Damsky & Werb, 1992; Hynes, 1992; Streuli, 1993). If applicable to fungi, this raises the possibility of a hypha directly influencing the gene expression of its neighbours through the extracellular matrix molecules it secretes.

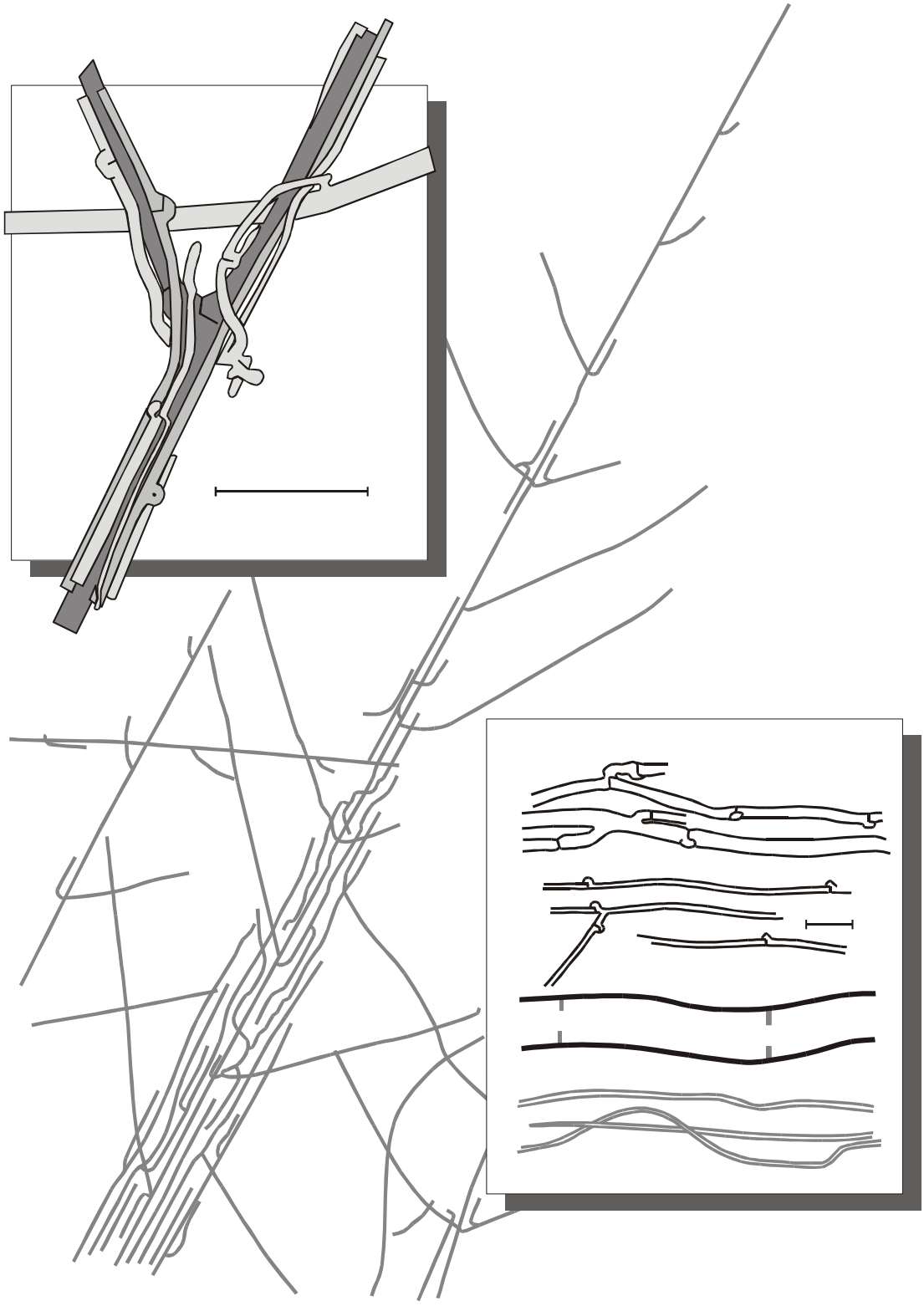

Fig. 2. Hyphal strands of *Serpula lacrymans*. Strands originate when branches of a leading hypha form at an acute angle to grow parallel to the parent hypha which also tends to grow alongside other hyphae it may encounter. Anastomoses between the hyphae of the strands consolidates them and narrow hyphal branches ('tendril' hyphae) from the older regions of the main hyphae grow around the main hyphae and ensheath them. The strand is shown in a general habit sketch running diagonally across the figure and the top panel shows tendril hyphae intertwined around main hyphae (redrawn after Butler, 1958, 1966). The bottom panel shows some of the cell types encountered in strands, with undifferentiated hyphae at the top then tendril hyphae, a vessel hypha and fibre hyphae (redrawn after Jennings & Bravery, 1991), scale bar = 20 μm.

2.2 Linear organs -- strands, cords, rhizomorphs and stipes

Formation of parallel aggregates of morphologically similar hyphae is common among Basidiomycotina, Ascomycotina and Deuteromycotina. Mycelial strands and cords provide the main translocation routes of the mycelium, developing under circumstances which require large scale movement of nutrients (including water) to and from particular sites. They are formed in mushroom cultures to channel nutrients towards developing fruit bodies; Mathew (1961) described their development in the cultivated mushroom *Agaricus bisporus*. For a similar function, they are also formed by mycorrhizal fungi to radiate into the soil, greatly supplementing the host plant's root system and gathering nutrients for the host (Read, Leake & Langdale, 1989; Read, 1991). Although mycelial strands extend into the soil, the mycelium does not aggregate into the pseudoparenchymatous tissue of the mycorrhizal sheath either in the soil or *in vitro*, this tissue development requires a surface, oxygen and a supply of nutrients (Read & Armstrong, 1972).

In saprotrophic phases, strands are also migratory organs, extending from an existing food base to explore nutrient-poor surroundings for new nutrient sources. Strands of *Serpula lacrimans*, the dry-rot fungus, are able to penetrate several metres of brick-work from a food base in decaying wood (Butler, 1957, 1958; Watkinson, 1971; Jennings & Watkinson, 1982) and to overgrow Perspex and many building materials (Jennings, 1991). The strands hasten capture of new substrate by increasing the inoculum potential of the fungus at the point of contact with it (Garrett, 1954,1956, 1960,1970) but they also facilitate concentration of mycelial resources on capture and consolidation of the new food base by providing translocation routes in both directions. The distribution of strands around a food base changes with time (Thompson, 1984). By resorption of hyphae and redistribution of the nutrients so recovered the strands enable migration of the colony from place to place (Rayner, Watling & Frankland, 1985; Rayner *et al*., 1985; Boddy, 1993; Rayner, 1993).

Although mycelial strands contain morphologically differentiated hyphae (see below), their constituent hyphae are relatively loosely aggregated. Certain fungi produce highly differentiated aggregations of hyphae with well developed tissues (Fig. 3). These structures are very root-like in appearance and are called **rhizomorphs.** A prime example is *Armillaria mellea*, a pathogen of trees and shrubs, which spreads from one root system to another by means of its rhizomorphs (Rishbeth, 1985). Here, again, the structure serves translocatory and migratory functions and, as with strands, translocation is bidirectional, glucose being translocated towards and away from the apex simultaneously (Granlund, Jennings & Thompson, 1985). In moist tropical forests aerial rhizomorphs, mainly of *Marasmius* spp., form a network which intercepts and traps freshly fallen leaves, forming a suspended litter layer (Hedger, 1985; Hedger, Lewis & Gitay, 1993).

Mycelial strands originate when young branches adhere to, and grow over, an older leading hypha (Fig. 2). Further localised growth and incorporation of other hyphae it may meet leads to increase in size of the strand (Nuss, Jennings & Veltkamp, 1991). Anastomosis between the hyphae of the strands consolidates them and narrow hyphal branches ('tendril' hyphae) from the older regions of the main hyphae intertwine around the other hyphae (Fig. 2). From the beginning, some of the central hyphae may be wide-diameter, thin walled so-called vessel hyphae and in older strands narrow, but thick-walled, `fibre' hyphae appear, running longitudinally through the mature strands. Strand formation occurs in ageing mycelium on an exhausted substrate when the hyphae are likely to be the main repositories of nutrients (especially nitrogen) and it has been argued that stranding results from the limitation of new growth to the immediate vicinity of the remaining nutrient (Watkinson, 1975; 1979). As long as the strand is the main supplier of nutrient the integrity of the strand will be reinforced, but when the strand encounters an external source greater than its own endogenous supply the stimulus to cohesive growth will be lost and spreading, invasive, hyphal growth will envelop the new substrate.

Rhizomorphs differ from strands fundamentally by having a highly organised apical growing point and extreme apical dominance. The apical region of the rhizomorph contains a compact growing point of tightly packed cells, protected by a cap of intertwined hyphae in (and producing) a mucilaginous matrix. Behind is a medullary zone containing vessel-hyphae composed of swollen, vacuolated and often multinucleate cells surrounded by copious air- or mucilage-filled

spaces. The medullary region forms a central channel through the rhizomorph and, in mature tissues, is traversed by narrow-diameter, thick-walled fibre hyphae (Fig. 3)(Townsend, 1954; Motta, 1969, 1971; Botton & Dexheimer, 1977; Motta & Peabody, 1982; Powell & Rayner, 1983; Cairney, Jennings & Veltkamp, 1989; Cairney & Clipson, 1991).

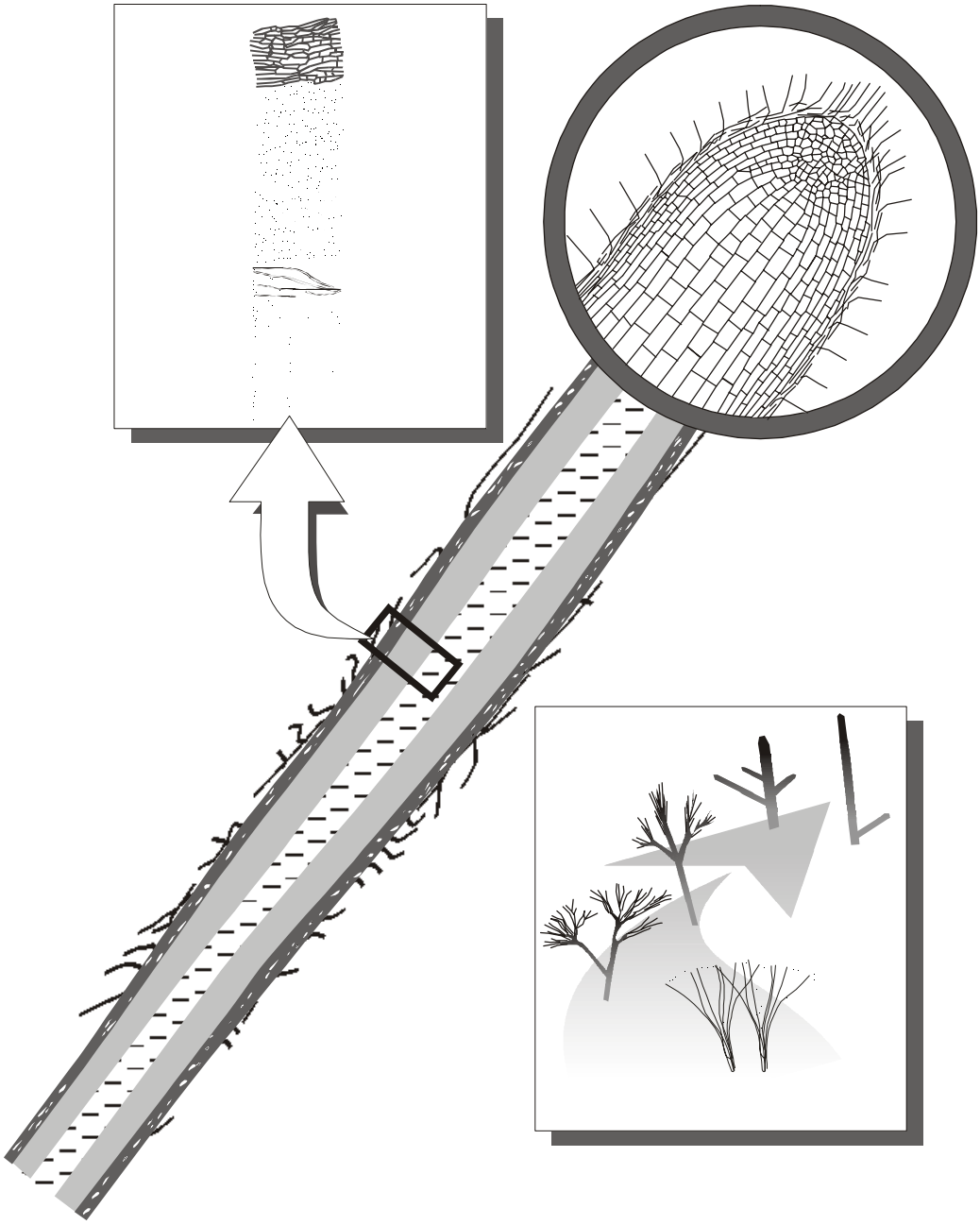

Fig. 3. Rhizomorph structure. The diagonal diagram is a sectional drawing showing general structure, with the apical region magnified to show the appearance of a growing point of tightly packed cells (redrawn after de Bary, 1887). Behind the tip is a medullary zone containing swollen, vacuolated and often multinucleate cells surrounded by copious air- or mucilage-filled spaces. The medullary region forms a central channel through the rhizomorph and, in mature tissues, is traversed by narrow fibre hyphae and wide-diameter vessel hyphae, the microscopic appearance being indicated in the drawing in the top left panel (redrawn after Webster, 1980). The panel at bottom right depicts mycelial fans, strands, cords and rhizomorphs as a series showing increasing apical dominance (redrawn after Rayner *et al*., 1985).

Towards the periphery of the rhizomorph, the cells are smaller, darker, and thicker-walled, and there is a fringing mycelium extending outwards between the outer layers of the rhizomorph, resembling the root-hair zone in a plant root. The similarity, at least in microscope sections, with the plant root has prompted the suggestion that rhizomorph extension results from meristematic

activity (Motta, 1967; 1969, 1971; Motta & Peabody, 1982). However, ultrastructural (especially scanning electron microscopical) observations reveal the hyphal structure of the rhizomorph tip (Botton & Dexheimer, 1977; Powell & Rayner, 1983; Rayner *et al*., 1985). A meristem-like structure would be totally alien to the growth strategy of the fungal hypha (Fig. 1) and, as suggested by Rayner *et al*. (1985) the impression of central apical initials giving rise to axially arranged tissues is undoubtedly an artefact caused by sectioning compact aggregations of parallel hyphae.

The rhizomorph apex is not unique in this: tissue layers involved in rapid cell formation in which the hyphae run parallel to one another have been recognised in agaric fruit bodies. They frequently demarcate the major tissue layers of the fruit body and were called **meristemoids** by Reijnders (1977). However, Reijnders was careful to emphasise how they differed from true meristems: `These meristemoids closely resemble the meristems of the phanerogams; sometimes they are referred to as such. This is not correct because the meristemoids initiate from hyphae which together form a simple tissue; cell division, therefore, can only take place in one direction, and the cell walls between the cells of different hyphae are double' (Reijnders, 1977). Some of the Deuteromycotina are said to have 'meristem arthrospores' or 'meristem blastospores' (Hughes, 1953), but this is, again, an unfortunate misuse of the word. Hughes (1971) compared cell proliferation in fungi with some lower plants and concluded that '...septation of fungal conidia results, not from the activity of a single [i.e. meristematic] cell, but by division of any or all of the cells. An apparent dictyoseptate condition of some conidia or other reproductive units in fungi may arise from the compacting of a coiled septate hypha or of hyphae which may branch repeatedly to form a more or less solid mass of cells.' It is quite clear that meristems do not occur in fungi.

The development of rhizomorphs *in vitro* was described by Garrett (1953, 1970) and Snider (1959) but details of their inception are sparse. They have arisen as protuberances on mounded mycelial aggregates of strains of *Stereum hirsutum*, a fungus which does not normally produce rhizomorphs (Rayner *et al*., 1985). Usually, rhizomorphs are initiated as compact masses of aggregated cells the ultimate origin being ascribed to locally enhanced acute-angled branching of some marginal hyphae in a mycelium; a phenomenon described as 'point-growth' (Coggins *et al*., 1980). Thus it seems likely that these linear organs originate from originally unpolarised hyphal aggregations that somehow become apically polarised.

Mycelial strands and rhizomorphs are extremes in a range of hyphal linear aggregations. Many intergrading forms can be recognised and have been given particular names (Townsend, 1954; Butler, 1966; Garrett, 1970) which have value in a descriptive sense, enabling distinctions to be made between species and their life style strategies, but different names should not obscure the close developmental relationships which exist between the structures. By all means call a hand a hand and a flipper a flipper; but recognise that a human hand and a dolphin flipper are variations on the same theme. Rayner *et al*. (1985) suggested that all linear hyphal aggregations could be related together in a hierarchy depending on apical dominance (Fig. 3).

Fruit bodies should also be included in this arrangement. In describing the structure of litter trapping rhizomorph networks in moist tropical forests, Hedger *et al* (1993) showed that the rhizomorphs have a reduced fruit body pileus at their tips that may protect the apex against desiccation. Overall, these linear organs are functionally analogous to soil rhizomorphs, but developmentally analogous to indefinitely extending fruit body stipes (Jacques-Félix, 1967). Many fruit bodies are served by radiating strands that convey nutrients towards the fruiting structure. In cases where the fruit body is stipitate (i.e. it has a stipe (=stem)) these can be so highly developed that the junction between strand and stipe is obscure. The term 'radicating' is used to describe fruit bodies whose stipes are elongated into root-like **pseudorhizas** which extend to the surface from some buried substrate (Fig. 4). Even in species that do not normally produce pseudorhizas, they can be induced by keeping fruiting cultures in darkness (Buller, 1924) whereupon the stipe base can extend for many cm, driving the fruit body primordium on its tip towards any source of light. Rhizomorphs? Pseudorhizas? Extending stipes? What they are called is less important than the implication that a close morphogenetic relationship underlies all fungal linear hyphal aggregations.

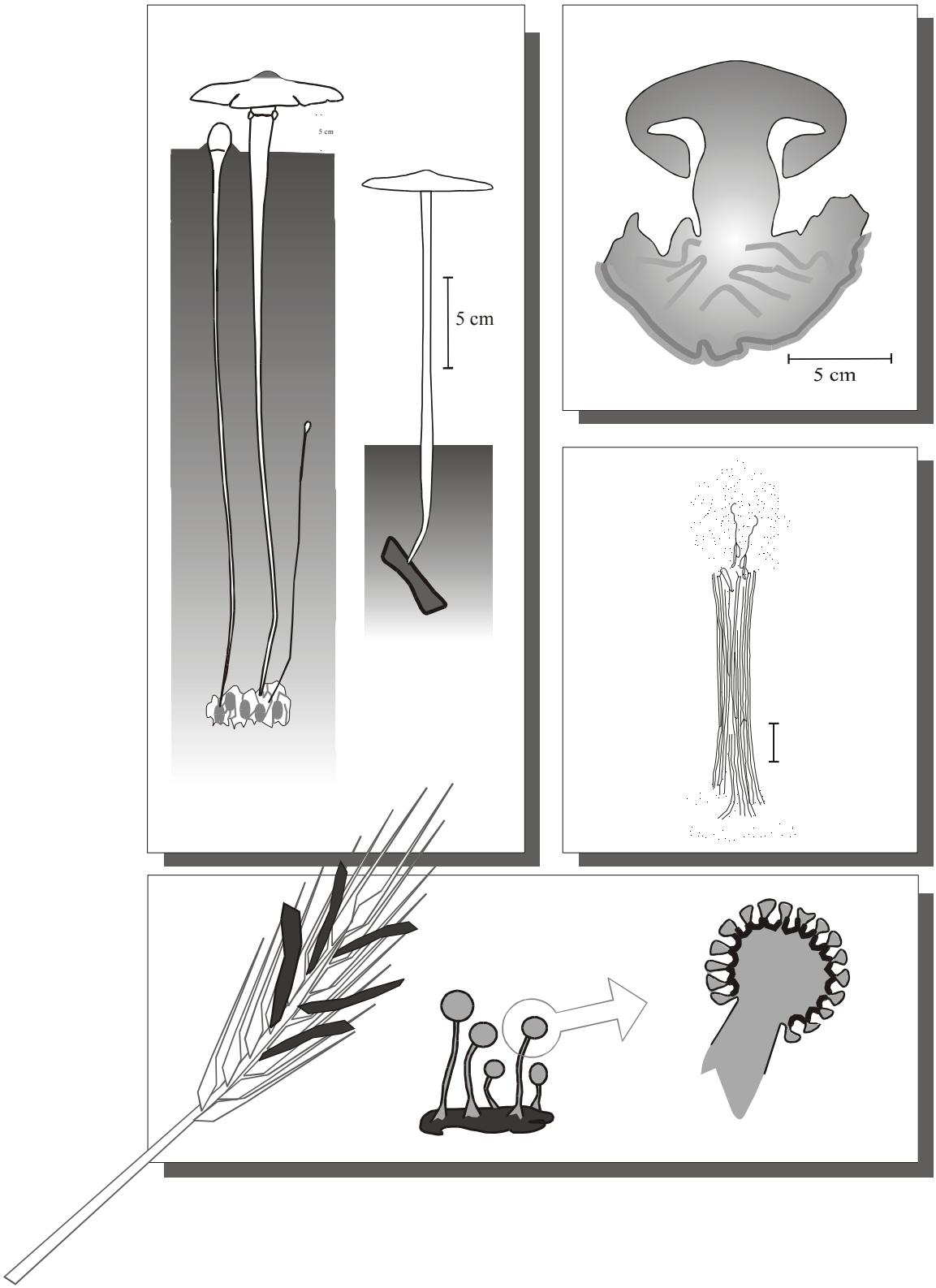

Fig. 4. Relationships between fungal multicellular structures. The panel at top left shows the radicating stipes of fruit bodies of *Termitomyces* spp. (left), connected by their pseudorhizas to the fungal galleries of an abandoned termitarium many feet below, and of *Oudemansiella (= Xerula) radicata*, where the pseudorhiza extends from tree roots or buried wood (scale bar = 50 mm; redrawn from Ingold, 1979). The panel at top right diagrammatically depicts the giant sclerotium of *Polyporus mylittae* germinating to form the fruit body (scale bar = 50 mm; drawn from photographs in Macfarlane *et al*., 1978). The panel at centre right shows the synnemata (bunched conidiophores) of *Podosporium elongatum* (scale bar = 50 ∶m; redrawn after Chen & Tzean, 1993). The bottom panel shows *Claviceps purpurea* which transforms the ovaries of its host (grasses and cereals, especially rye) into a hard, blackish, banana-shaped sclerotium (the Ergot) which overwinters on the ground and the following year gives rise to pinkish, drumstick-like **perithecial stromata** which contain the flask-shaped **perithecia**, within which the asci are formed (redrawn after Burnett, 1968).

2.3 Globose structures -- sclerotia, stromata, and fruit bodies

Sclerotia are pseudoparenchymatous hyphal aggregations in which concentric zones of tissue form an outer rind and inner medulla, with a cortex sometimes distinguishable between them. Sclerotia are tuber-like and detach from their parental mycelium at maturity. Some sclerotia consist of very few cells and are therefore of microscopic dimensions. At the other extreme, the sclerotium of *Polyporus mylittae*, found in the deserts of Australia, can reach 20 -- 35 cm in diameter and is known as native or black fella's bread.

Sclerotia are resistant survival structures which pass through a period of dormancy before utilizing accumulated reserves to 'germinate', often by producing fruiting structures (Fig. 4). Dormant sclerotia may survive for several years (Sussman, 1968; Coley-Smith & Cooke, 1971; Willetts, 1971), owing their resistance to the rind being composed of tightly-packed hyphal tips which become thick-walled and pigmented (melanized) to form an impervious surface layer. The medulla forms the bulk of the sclerotium, and its cells (and those of the cortex where present) may accumulate reserves of glycogen, polyphosphate, protein and lipid.

Sclerotium ontogeny comprises **initiation**, when the hyphae begin to aggregate to form small, distinct **initials**; **development**, when the initials expand and grow to full size, accumulating nutritional reserves from the parent mycelium; and **maturation**, which is most obviously characterised by clear demarcation of the surface and pigmentation of its constituent cell walls, but which also involves conversion of the reserve nutrients to forms suitable for long-term storage (Chet & Henis, 1975). Townsend & Willetts (1954) and Willetts & Wong (1971) distinguished several kinds of development in sclerotia. In the **loose type** (as in *Rhizoctonia solani*) sclerotial initials arise by branching and septation of hyphae; the cells become inflated and fill with dense contents and numerous vacuoles. The mature sclerotium is pseudoparenchymatous, but the tissue has an open structure and its hyphal nature is readily seen. At the periphery of this type of sclerotium the hyphae are more loosely arranged and generally lack thickened walls (Willetts, 1969). The **terminal type** of development is characterised by repeated dichotomous branching and cross-wall formation. It is exemplified by *Botrytis cinerea* and *B. allii* and an illustration of it is used in Fig. 1 as an example of the fungal space-filling strategy. Eventually the hyphal branches cohere to give the appearance of a solid tissue. In *Botrytis*, a (usually flattened) mature sclerotium may be about 10 mm long, 3--5 mm wide and 1--3 mm thick. It is differentiated into a rind composed of several layers of round cells with thickened, pigmented walls, a narrow cortex of thin-walled pseudoparenchymatous cells with dense contents, and a medulla of loosely arranged filaments. The **lateral type** is illustrated by *Sclerotinia gladioli* (which causes dry rot of corms of *Gladiolus*, *Crocus* and other plants). Sclerotial initials arise by formation of numerous side branches from one or more main hyphae or strands of several parallel hyphae. The mature sclerotium, about 0.1 -- 0.3 mm in diameter, is differentiated into a rind of small thick-walled cells and a medulla of large thin-walled cells.

More complex sclerotia and other types of sclerotial development have been found (Butler, 1966; Chet, Henis & Kislev, 1969). Many fungi enclose portions of the substrate and/or substratum (which may include host cells if the fungus is a pathogen) within a layer of pigmented, thick-walled cells; the whole structure may be regarded as a kind of sclerotium. Such a layer of impervious tissue, which is especially protective against desiccation, may be developed in other circumstances to form what has been called a **pseudosclerotial plate**, for example, over the surface of hyphal structures of the fungus *Hymenochaete corrugata* binding hazel branches together (Ainsworth & Rayner, 1990) and in similar adhesions between rhizomorphs and leaf litter in tropical forests (Hedger *et al*., 1993). When such plates completely enclose the mass on which they are formed, the structure which results is called a sclerotium. The term sclerotium is, therefore, a functional one. The structure is defined as a multihyphal aggregate which can remain dormant or quiescent when the environment is adverse and then, when conditions improve, germinate to reproduce the fungus (Willetts & Bullock, 1992). Since a number of dissimilar structures are encompassed by this definition it is thought that the different forms arose by convergent evolution, most probably evolving, in Ascomycotina, from aborted spore forming organs like perithecia, cleistothecia and conidial masses (Willetts, 1972; Cooke, 1983; Willetts & Bullock, 1992).

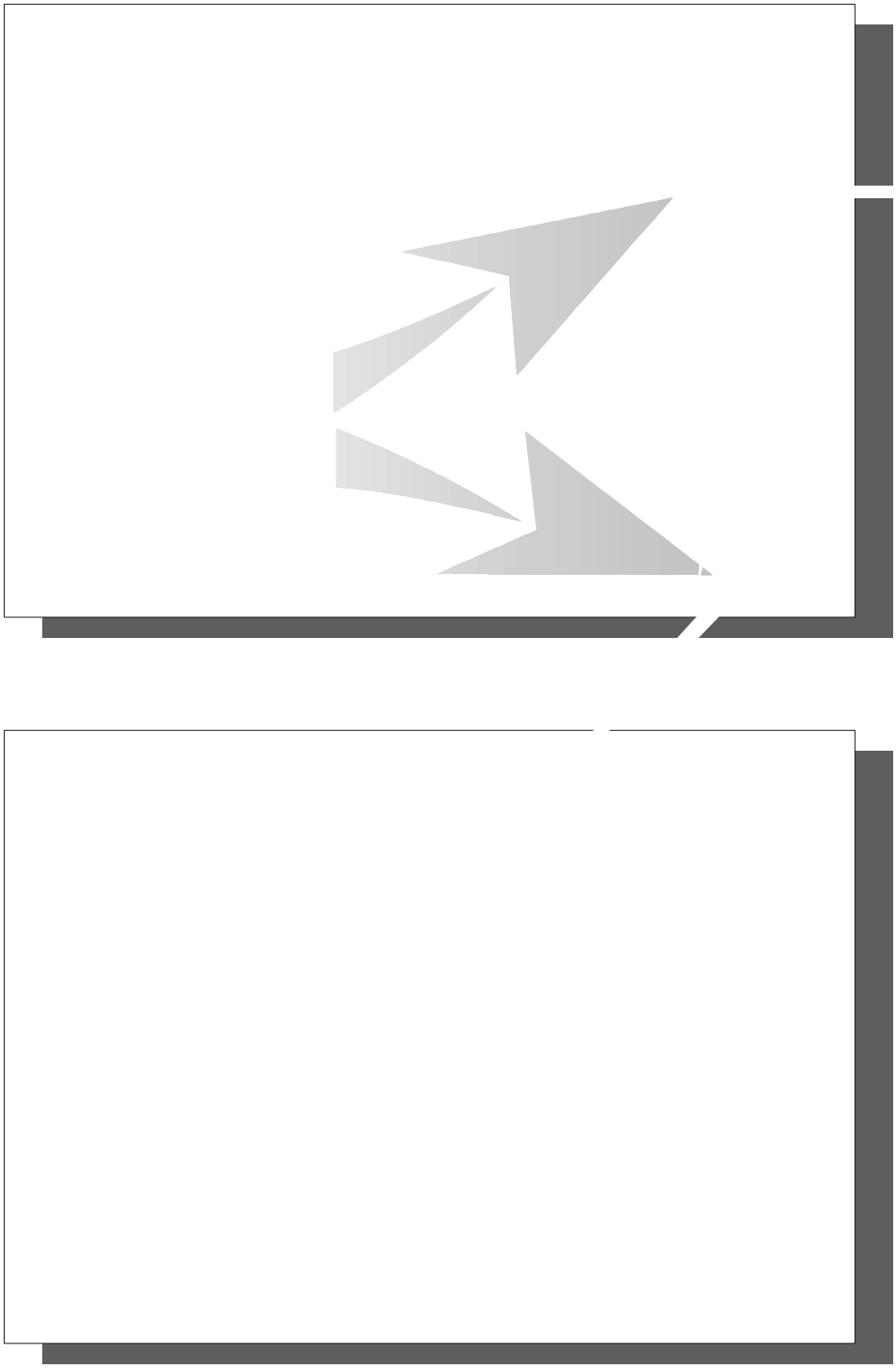

Fig. 5. The variety of multicellular fruiting bodies of fungi in the form of simplified diagrammatic sectional drawings (redrawn after Burnett, 1968); in each case the hymenial tissue is represented by the black line. The upper panel shows a range of ascomata with, at top left a basic flask-like perithecium (e.g. *Sordaria*) alongside the perithecial stroma of *Daldinia*. The rest of the drawings are arranged to show how the simple cup-like ascome of *Peziza* might, hypothetically, have given rise, on the one hand, to the morel (*Morchella*) via the fruit body forms of *Sarcoscypha*, *Helvella*, and *Mitrula*, and on the other hand to the subterranean fruit body of *Tuber* via a form like *Genea*. The lower panel shows a number of basidiomata, including the agaric (mushroom) form of fruit body, the poroid bracket and toadstool polypore, and toothed (hydnoid) form. At bottom left is an encrusting fruit body (as in *Stereum* and *Phellinus*) and at bottom right the mature stink-horn (*Phallus*).

Their close relationship with sporulating organs is shown in many ways. Inhibition of conidial differentiation in *Monilinia fructicola* by high humidity occasionally leads to formation of sclerotium-like stromata (Willetts, 1968; Willetts & Calonge, 1969). This is an example of the environment directly influencing the pathway of development, but another example provides

evidence for the same genes being involved in both sclerotium and fruit body initiation in the basidiomycete *Coprinus cinereus* (Moore, 1981; and see section 4.1 and Fig. 15). Sclerotia of *Coprinus cinereus* are polymorphic (Hereward & Moore, 1979) and strains which were either unable to make sclerotia or made abnormal sclerotia were shown to result from single gene recessive defects. In crosses with wild type strains the 'heterozygous' dikaryons were able to make both sclerotia and fruit bodies, but dikaryons 'homozygous' for sclerotium-defective genes were unable to make fruit bodies. The conclusion was that a common initiation pathway gave rise to hyphal aggregations which, under permissive environmental conditions (22--26EC plus illumination), developed axial symmetry and became fruit body initials, but under non-permissive conditions (30--37EC plus continuous darkness) developed radial symmetry and became sclerotia.

Sclerotia may 'germinate' to form mycelium, conidia, ascomata or basidiomata. Mode of germination seems in some cases to be a matter of size. The small sclerotia of *Coprinus cinereus* germinate to produce a mycelium but many other species produce spores and fruit bodies (Fig. 4). The giant sclerotium of *Polyporus mylittae* can form a basidiome (Fig. 4) without being supplied with water as the flesh is honeycombed with blocks of translucent tissue where the hyphae form copious amounts of an extrahyphal gel which is thought to serve as both nutrient and water store (Macfarlane *et al*., 1978).

In many Ascomycotina, Basidiomycotina and Deuteromycotina hyphae may aggregate to form fruiting structures which are responsible for producing and, just as important, distributing spores of various kinds. In Ascomycotina, the sexually produced **ascospores** are contained in **asci** (singular: **ascus**) enclosed in an aggregation of hyphae termed an **ascoma**. Ascomata are formed from non-dikaryotic sterile hyphae surrounding the ascogonial hyphae of the centrum; a number of distinct types can be recognised (Booth, 1966; Turian, 1978; Reynolds, 1981; Chadefaud, 1982*a, b, c*; Read, 1993)(Fig. 5). The fruit-bodies of Basidiomycotina, the mushrooms, toadstools, bracket fungi, puff-balls, stinkhorns, bird's nest fungi, etc., are all examples of **basidiomata** which bear the sexually produced **basidiospores** on **basidia**. Simplified diagrammatic drawings of some of the different types of ascomata and basidiomata are shown in Figs 5 & 6 so that the rest of this chapter can concentrate on the establishment of the patterns which result in formation of defined tissues in multicellular fungal structures. Many more details could be added but, in most cases, it is impossible to give much further ***information*** about possible mechanisms involved in defining morphogenesis of the structures, because of the lack of appropriately structured research. The great bulk of the published research has been done with taxonomic intentions. It has great value for its descriptive and comparative content, but precise developmental accounts are extremely rare and ***experimental*** approaches rarer still. Most work has been done on development of mushroom fruit bodies, most particularly with *Coprinus cinereus* and other *Coprinus* spp., so for the rest of this chapter the main theme will be the morphogenesis of the basidiome of *C. cinereus* with emphasis on how tissue patterns may arise, how the patterns contribute to morphogenesis and how they are expressed in morphological and biochemical differentiation.

3 Morphogenetic patterns

Development of any multicellular structure in fungi requires modification of the normal invasive growth of vegetative mycelium so that hyphae no longer characteristically diverge, but grow towards one another to co-operate in forming the differentiating organ. Although we are beginning to understand the kinetics of growth and branching patterns (Prosser, 1993; and see Chapters 13 & 14) we remain ignorant of the control processes responsible for changing the fundamental growth pattern of the hyphae. The sex-hormones of lower aquatic and terrestrial fungi (Chapter 16) and molecules determining mating-type specific agglutination in yeasts (Beavan *et al*., 1979; Rahary *et al*., 1985; Kihn, Masy & Mestdagh, 1988) are about the limit of current knowledge about the control of hyphal interactions (Moore, 1984a). As pointed out before (Reijnders & Moore, 1985), fungi offer a unique system for study of cell-to-cell tropisms and specific cell-to-cell adhesion since the change from one state to another is part of their normal development. Once the prosenchymal mycelial tuft (constituting the initial of the developing structure) is established, major tissue domains are demarcated very quickly. In *Coprinus cinereus*, fruit body initials only 800 :m

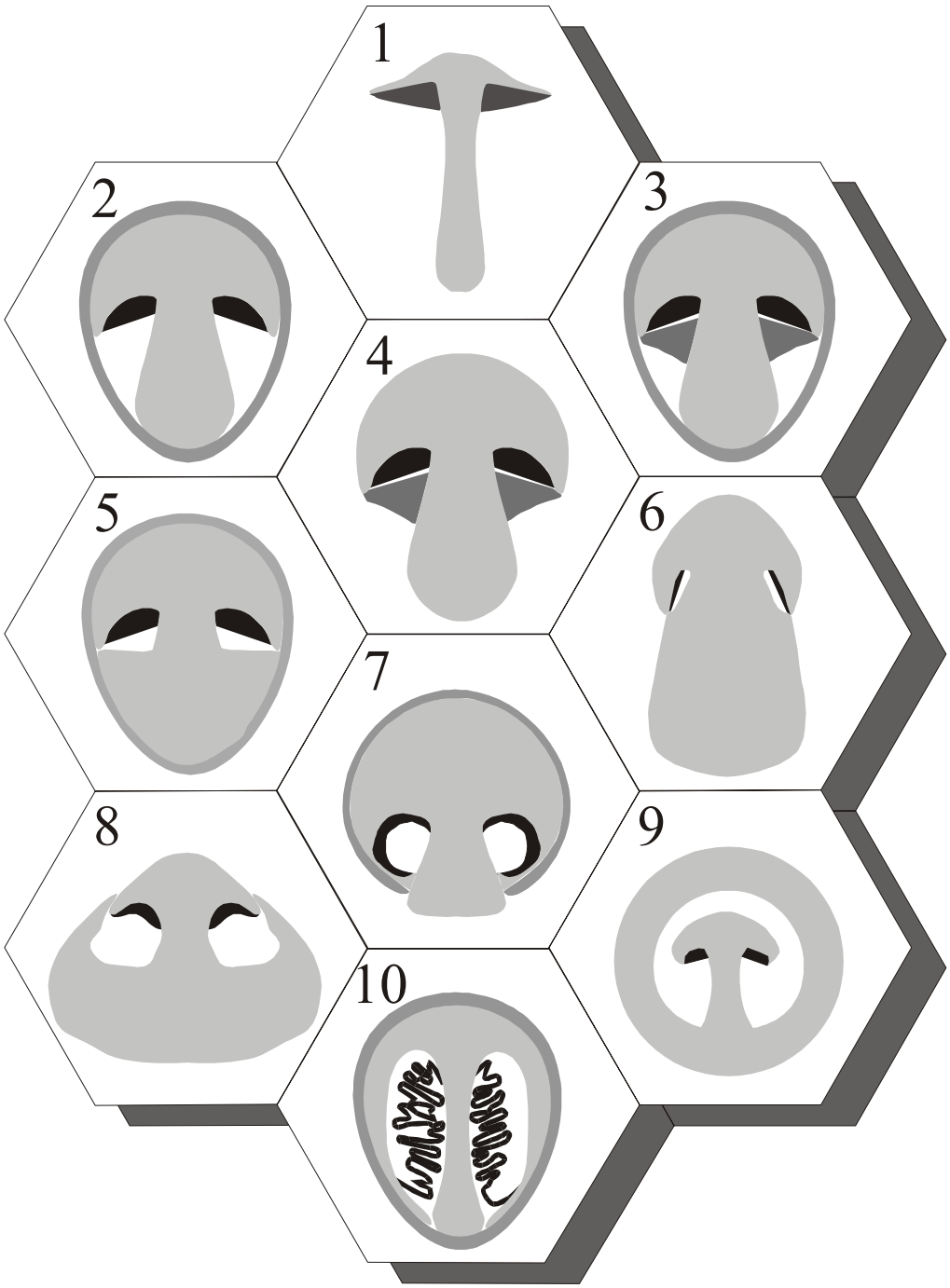

Fig. 6. Ten ways to make a mushroom. A montage of diagrammatic sections illustrating the various primordial tissue patterns which eventually mature to form mushroom-like basidiomata; hymenial tissues are shown in black (redrawn after Watling & Moore, 1993). 1 = gymnocarpic, where the hymenium is naked at first appearance and develops to maturity on the fruit body surface; 2 = monovelangiocarpic, with a single (universal) veil enveloping the whole primordium; 3 = bivelangiocarpic, in which an inner (partial) veil provides additional protection to the hymenium; 4 = paravelangiocarpic, where the veil is reduced and often lost at maturity; 5 = metavelangiocarpic, where a union of secondary tissues emerging from the pileus and/or stipe forms an analogue of the universal veil; 6 = gymnovelangiocarpic, in which the hymenium is protected by a very reduced veil, seen only at adolescence, formed between the stipe and the closely applied pileus; 7 = pilangiocarpic, the hymenium is protected by tissue extending downwards from the margin of the pileus; 8 = stipitoangiocarpic, the hymenium is protected by tissue extending upwards from the stipe base, but this does not enclose the primordium; 9 = bulbangiocarpic, where the tissue protecting the hymenium is largely derived from the basal bulb of the stipe and initially completely encloses the primordium; 10 = endocarpic, where the mature hymenium is enclosed or covered over, just one (the pileate type) of a number of patterns of this gasteromycetous form of fruit body is shown.

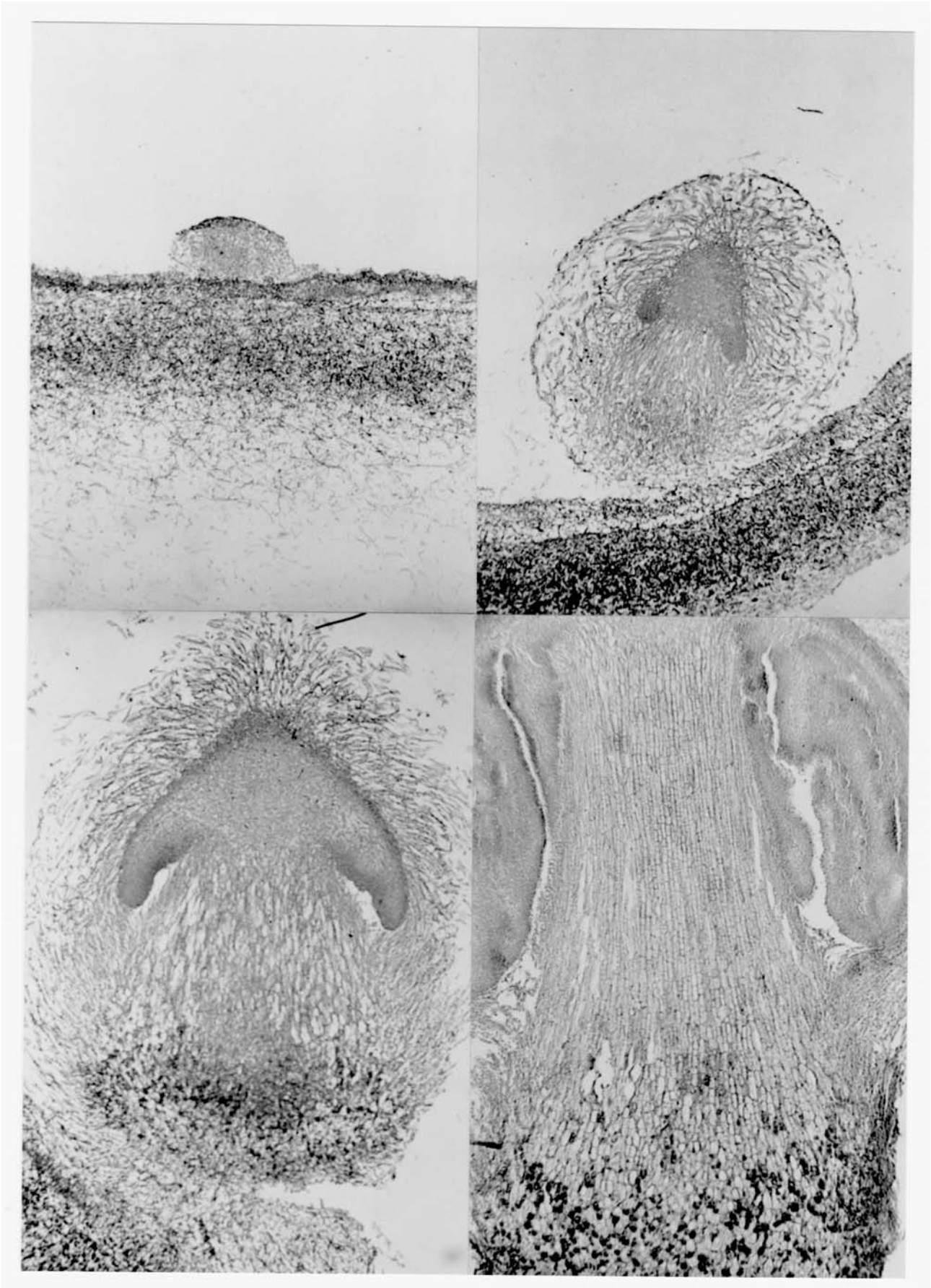

Fig. 7. Micrographs of median vertical sections of basidiome initials and primordia of *Coprinus cinereus*. At top left the initial is approx. 0.2 mm tall, showing hyphal aggregation but no internal pattern. Top right is an 0.8 mm tall initial which is clearly demarcated into veil, pileus and stipe tissues. Bottom left shows a 1.2 mm basidiome initial and a 3 mm tall primordium is shown at bottom right.

tall are clearly differentiated into pileus and stipe (Moore, Elhiti & Butler, 1979) though this is only 1% of the size of a mature fruit body (Fig. 7). This sort of example brings to mind establishment of the basic body plan very early in development of the animal embryo and this is worth exploration. The components in the overall process of animal embryo development have been characterised as: formation of inhomogeneous cell populations from homogeneous ones; regional specification of tissues (pattern formation) directed by organizers producing morphogens; specification and commitment of particular cells to particular fates; cell differentiation; and regulation of gene activity in ways specifically geared to morphogenesis (Slack, 1983). These statements highlight the major events contributing to morphogenesis in both animals and plants. The challenge is whether evidence exists for such mechanisms in the development of fungal structures.

3.1 Regional specification of tissue domains

Initials of multihyphal structures are composed of a mass of prosenchymal tissue which originates from communities of hyphae and their branches which grow together. This is clearly the case for strand formation (Butler, 1958; Nuss *et al*., 1991) and initiation of fruit bodies and sclerotia involves aggregation by hyphal congregation (Matthews & Niederpruem, 1972; Waters, Moore & Butler, 1975a; Van der Valk & Marchant, 1978). A range of observations show that fungal fruit bodies consist of a population of cells assembled from contributions of a number of co-operating hyphal systems. Hyphal congregation is so fundamental that it can lead to the formation of chimeric fruit bodies. Kemp (1977) described fruit bodies of *Coprinus* consisting of two different species, *C. miser* and *C. pellucidus*. The hymenium comprised a mixed population of basidia bearing the distinctive spores of the two species but the chimera extended throughout the fruit body as both species could be recovered by outgrowth from stipe segments incubated on nutrient medium.

Development of the basidiome has been a rich seam for open-cast mining of terminology. Some of this can be traced back to the last century (Brefeld, 1877; de Bary, 1884), but in recent years Reijnders (1948, 1963, 1979) has been instrumental in formalising the descriptive terminology on the basis of extremely extensive observation. He stresses the importance of three sets of features: (a) development and nature of the veil and **pileipellis** (the `epidermis' of the pileus) in relation to covering the developing hymenophore (the **hymenophore** carries the **hymenium**, a cell layer responsible for eventually producing the basidiospores); (b) the sequence of development of the stipe, pileus and hymenophore, which are the major functional zones of the basidiome; (c) the mode of development of the hymenophore. The terminology is discussed by Watling (1985) and illustrated in Fig. 6.

Microscope sections of even extremely small fruit body initials can be resolved into regions of recognisable pileus and stipe (Fig. 7), and provide *prima facie* evidence for regional specification, since the creation of such histologically distinct regions requires that some organisation is imposed upon the homogeneous prosenchyma. The most highly differentiated cellular elements seem to occur at the boundaries of tissue regions (Williams, 1986). In the youngest specimens, before this cell differentiation has occurred, the boundaries are frequently denoted by closely appressed parallel hyphae (called meristemoids by Reijnders, 1977; see section 2.2) which seem to be involved in rapid cell formation in the sense that the distance between successive hyphal cross walls is minimised.

3.2 Mushrooms make gills

The gills of agaric fungi are plates suspended from the fruit body pileus tissue. Intuitively one might expect such plates to develop and extend by 'downward' growth of the distal edge of the gill (that is, the edge which is eventually exposed) but this is not the case. The direction of gill development has been a matter of controversy for many years but experimental proof has recently become available from work with both *Coprinus cinereus* and *Volvariella bombycina*. A crucial aspect of understanding how the final structure of the fruit body is attained is appreciation of the geometrical consequences of the ***differential growth*** of the primordium (Fig. 7). As a typical fruit body of *C. cinereus* grows from 1 to 34 mm in height, the circumference of the stipe increases 9-fold and the circumference of the outer surface of the pileus increases 15-fold; this latter corresponds to more

than a 3000-fold increase in volume (Fig. 7). The implications of primordium enlargement for tissue relationships must be kept in mind as we turn to the first question, which is the origin of the space between gills.

3.2.1 Cavitation

The terminology describing hymenophore development was originally defined by Locquin (1953). Fruit body primordia may be categorised as being **levhymenial** (an initially continuous hymenium becomes folded) or **rupthymenial** (the hymenium originates in fragmented form). To these categories was later added **schizohymenial** (where gills and gill cavities differentiate together from the background tissue). Since originally coined, the definitions of these terms have become vectorised: rupthymenial gills are assumed to develop away from the stipe whilst levhymenial gills are alleged to push down into a preformed gill cavity (see definitions in Watling, 1985). Unfortunately, this latter definition is wrong, all gills grow at their roots -- the developmental vector ***moves away from the stipe*** (see below). However, as defined in the sense of contrasting preformed or simultaneously-formed gill cavities, the terms levhymenial and schizohymenial are useful alternatives.

The internal structure of the *Coprinus* primordium is uniformly solid at the time that gills begin to arise (Fig. 7) so gills and gill space arise together. Lu (1991) has claimed that the gill cavities arise as a result of 'programmed cell death' which plays a part in development in many plants and animals. However, no such suggestions have arisen in previous work on gill formation (e.g. Reijnders, 1963, 1979) and Lu's identification of cell degeneration depends on observation of precipitates in cell vacuoles and in gill cavities which have been dismissed previously as artefacts produced by precipitation of vacuolar contents during fixation (Waters, 1972; Waters, Butler & Moore, 1975b). Because of the enormous increase in size of the fruit body primordium, however, programmed cell death is not necessary to form a gill cavity. When two groups of hyphal tips 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 which can be opened out into a cavity when the expansion of the underlying tissue puts tension across the 'fracture' and pulls the palisades apart (Fig. 8). If the 'fracture planes' form an annulus around the top of the stipe (one palisade might be the stipe apical meristemoid, the other might be the hymenophore meristemoid), then an annular cavity may arise before gill formation (a mode of development which would be described as levhymenial). On the other hand, where the fracture planes are defined by the hymenia of neighbouring gills, cavities would be isolated, no annular cavity would be apparent, and the process would be described as schizohymenial. Though applied here to agaric primordia, this argument applies to cavitation in all differentially expanding cellular structures. It is a mechanical consequence of developmental change in their geometry like the faceting of cells in compressed tissues discussed by Dormer (1980).

3.2.2 *Coprinus* gills

In *Coprinus*, the pileus of the fruit body primordium encloses the top of the stipe and gills are formed as essentially vertical plates arranged radially around the stipe. Transverse sections show the pileus as an annulus concentric with the stipe, the inner circumference of the annulus being the surface of the stipe and the outer the surface of the pileus tissue (Fig. 9). There are two types of gill: primary gills which, from formation, have their inner, tramal tissue in continuity with the outer layers of the stipe (Fig. 9), and secondary (and lesser ranked) gills in which the hymenium is continuous over the gill edge (Fig. 10; and see Reijnders, 1979; Rosin & Moore, 1985a; Rosin, Horner & Moore, 1985; Moore, 1987). Tramal tissues of primary gills remain connected to the stipe until being freed when expansion of the pileus detaches them from the stipe (Rosin & Moore, 1985a; Moore, 1987).

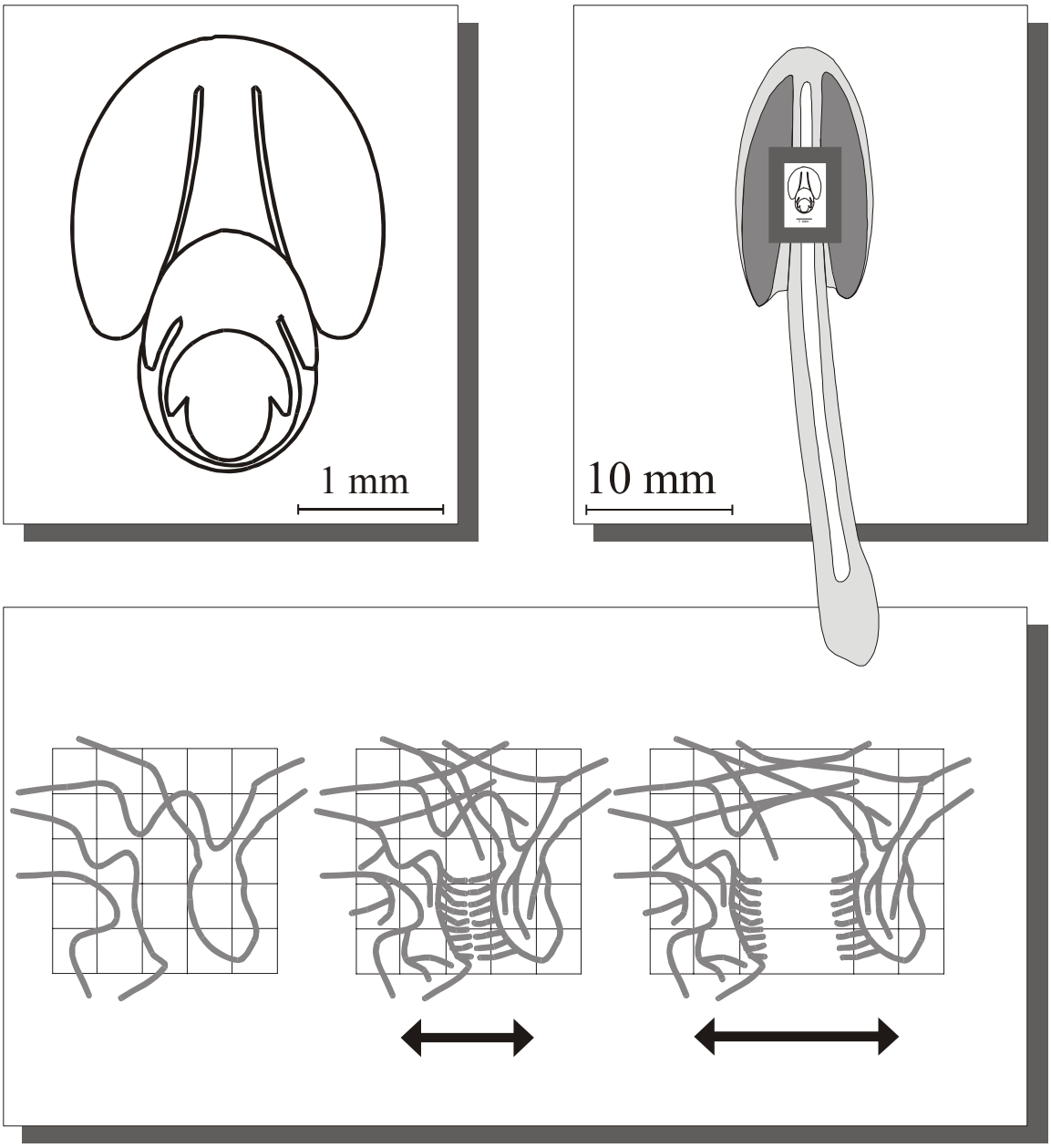

Fig. 8. Dynamics of basidiome expansion in *Coprinus cinereus*. In the panel at top left outline diagrams traced from the basidiome initials shown in Fig. 7 are nested together to illustrate the steady outward expansion of the tissue layers. These diagrams are superimposed (to scale) onto a median diagrammatic section of a mature basidiome in the top right panel to demonstrate the extent of the outward movement of tissue boundaries. The bottom panel shows how this expansion can generate cavities by putting tension stress across an incipient fracture plane. In these diagrams, a small region of prosenchyma is shown first against a reference grid. In the central diagram a round of branching is assumed to have taken place forming branches of determinate growth arranged in two opposing palisades. This constitutes the incipient fracture plane and when tension is applied the palisades will be pulled apart (right hand figure).

Primary gills are connected with pileus tissue at their outer edge and with the stipe at their inner edge; since the circumference of the stipe increases so much during maturation why doesn't the gill thickness increase by the same extent? The answer is that the tendency to widen as the stipe 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. This forms a Y-shaped structure (Fig. 10). Observation of fruit body sections shows that these Y-shaped gill structures are oriented exclusively as though the new gill organizer originates at the stipe circumference (Moore, 1987) so that the crotch of the Y-shape moves outwards towards the pileus (Fig. 10). This clearly sets the direction of development as outwards ***from the stipe***.

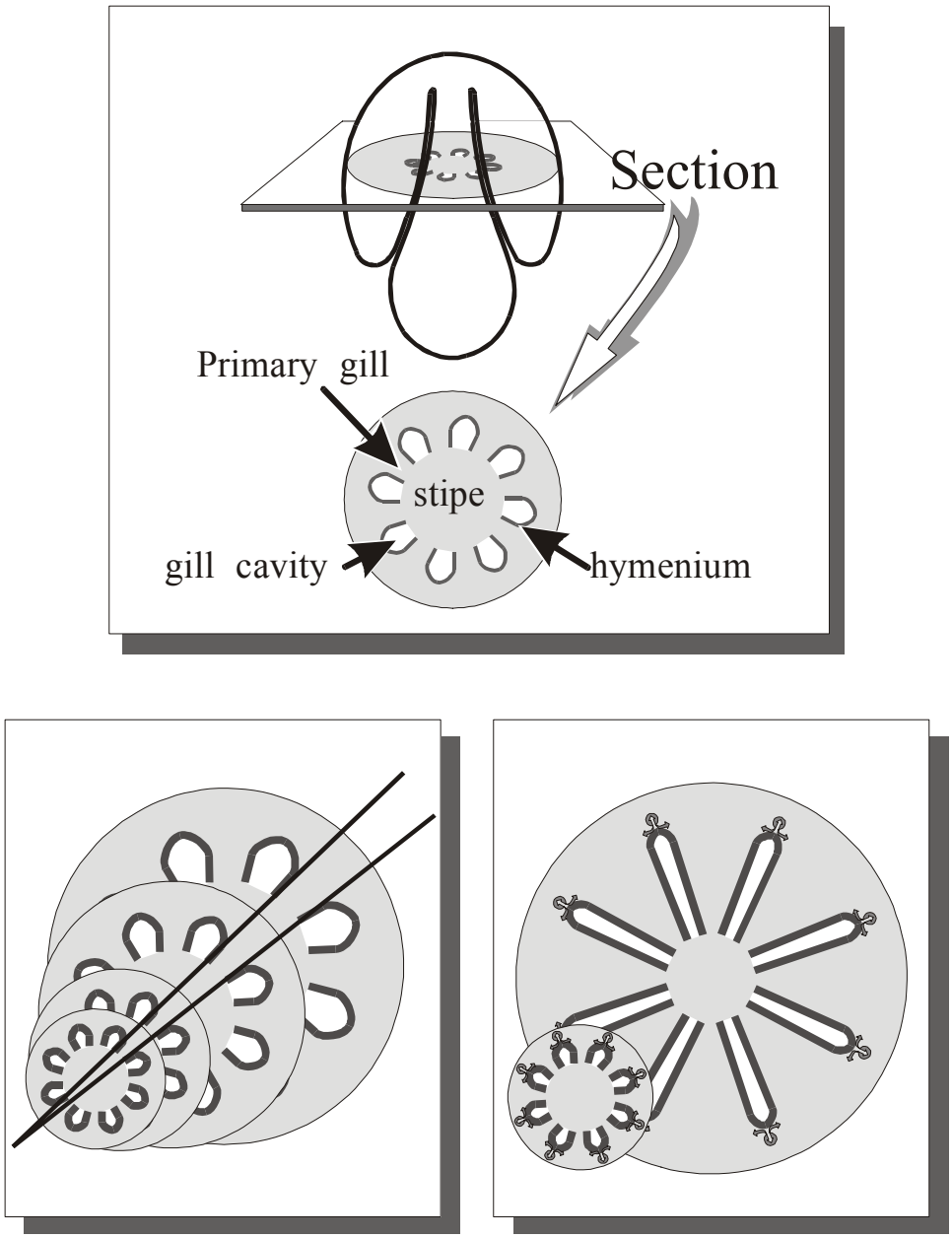

Fig. 9. Primary gills of *Coprinus* are connected to the stipe. The top panel is intended to orient the reader and to illustrate the basic layout of a transverse section of the primordial pileus. If these geometrical relationships remained unchanged during expansion, gill thickness would increase greatly for two reasons: (a) because the primary gills are connected to the stipe circumference, increase in stipe circumference would be accompanied by increase in gill thickness (bottom left panel); (b) because gill organisers migrate *radially* outwards (generating the branching pattern which forms the protohymenia as palisades bounding an incipient fracture plane as illustrated in Fig. 8) the gills would inevitably become thicker as their radial paths diverge (bottom right panel). The solutions to these problems are illustrated in Fig. 10.

These observations indicate that gills in the *C. cinereus* fruit body grow radially outwards, their roots extending into the undifferentiated tissue of the pileus context. The formative element appears to be an **organiser** in the tissue at the extreme end of the gill cavity where the change in structure occurs from the randomly intertwined prosenchymatous tissue of the pileus context to the highly compacted hymenial plates separated by the gill cavity. The gill organiser is responsible for the progression of the gill cavity radially outwards, away from the stipe. The prosenchyma/protohymenium transition need be no more complex than an increase in branch frequency to produce branches of determinate growth that are mutually ‘attracted’ so that they form the opposing palisades of a fracture plane allowing pileus expansion to separate the two

protohymenia (Fig. 8). The organiser responsible for this is moving radially outwards penetrating, as the primordium grows, successive generations of undifferentiated prosenchymatous tissue in the context, which lies between the gills and the 'epidermis' (pileipellis) of the pileus (Rosin & Moore, 1985b). Since it is a ***radial*** progression, neighbouring organisers become further and further separated from one another as development proceeds (Fig. 9). As the distance between neighbouring organisers increases a new one can arise between them (Rosin & Moore, 1985a; Fig. 10); 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 gill organisers which bracket it into the undifferentiated prosenchyma of the pileus context.

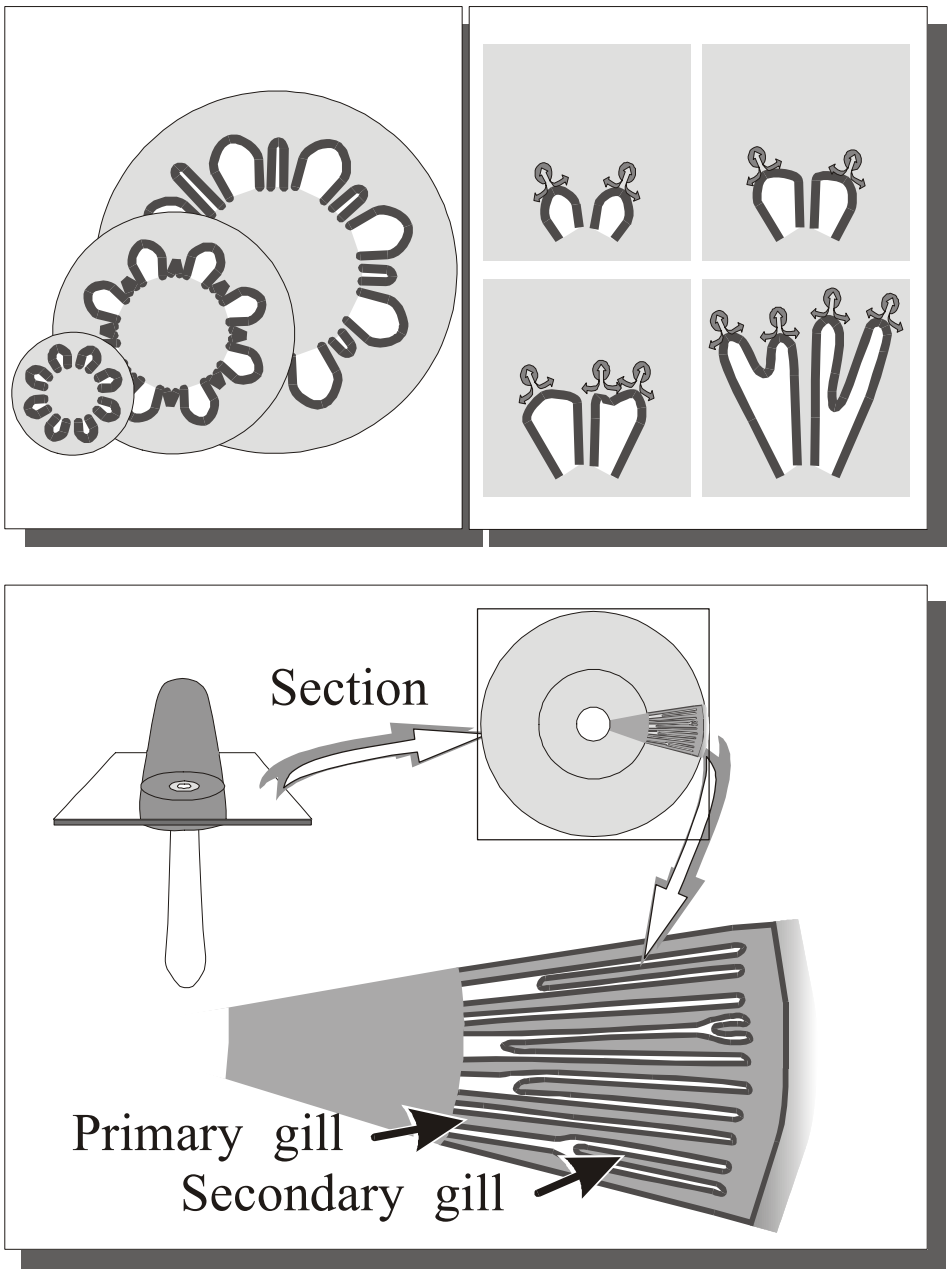

Fig. 10. Generation of new primary and secondary gills in *Coprinus*. Top left: increase in the thickness of the primary gill at its junction with the expanding stipe is compensated by the appearance of new gill cavities *within* the trama of the original primary gills. Top right: the formative element in outward extension of the gill is the gill organiser located in the tissue which borders the outermost part of the gill cavity. As their radial paths diverge, this part of the gill cavity expands tangentially until sufficient space exists between neighbouring gill organisers for a new organiser to appear between them. Continued outward migration of parent and daughter gill organisers creates a secondary gill between them. These two mechanisms are *not* alternatives; they occur together to generate the community of radial, narrow gills which characterise the mature *Coprinus* basidiome (bottom panel).

The observations summarised in Fig. 10 and the explanation given above provide *prima facie* evidence for two classic components of theoretical morphogenesis -- activation and inhibition by diffusing morphogens. First, we can suggest that diffusion of an activating signal along the fruit body radius assures progression of the gill organiser along its radial path. Second, each organiser can be assumed to produce an inhibitor that prevents formation of a new organizer within its diffusion range (i.e. the gill organiser uses this inhibitor to control its morphogenetic field). As radial progression into the extending pileus context 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. Interaction between the diffusion characteristics of the activator and the inhibitor is all that is necessary to control gill spacing, gill number, gill thickness, and the radial orientation of the gill field.

3.2.3 *Volvariella* gills

In fruit body development of *Volvariella bombycina*, primary gills arise as ridges on the lower surface of the pileus, projecting into a preformed annular cavity - a levhymenial mode of development. The gills clearly ***project*** into the annular cavity, but the question is, do they grow ***into*** it? I believe that the answer to this question is **no**, and see gill development as being exactly homologous with the process in *Coprinus*; i.e. growth of any one gill occurs by outward progression into outwardly expanding pileus context, of gill organisers either side of the foot of the gill. Effectively, therefore, the gill margins (the edges which arise as projections into the cavity when the under-surface of the pileus first becomes folded) remain positionally fixed in space while the gill cavities enfold and extend around them. Research on *Coprinus* development has been aided by the geometrical structure of the primordial fruit body, which provides spatial reference points (e.g. the initial attachment of primary gills to the stipe). The *Volvariella* primordium is not so 'user-friendly'.

Volvariella bombycina is bulbangiocarpic (Fig. 6) but occasionally primordia with exposed gills arise. Young fruit bodies of this sort were used to trace the relative growth rates of the different parts of the hymenophore by painting black ink marks on the tissues (Chiu & Moore, 1990a). During further fruit body development, ink marks placed on the pileus margin and those placed on the edges of the gills ***remained at the margin or the gill edges respectively***. The growth increment here is quite considerable, the radius of the pileus increasing from 0.5 to 2.5 cm and the depth of the gills from 1.5 to 5 mm. If growth of the pileus and gill margins resulted from apical growth of the hyphal tips that occupied the margin, then ink particles placed on those hyphal tips would be left behind as the hyphal apices extended (Fig. 11).

Indeed, this approach has been used to study growth of sporangiophores of *Phycomyces* (Castle, 1942) and *Aspergillus giganteus* (Trinci & Banbury, 1967); both papers present time-lapse photographic sequences showing the sporangiophore tip growing beyond externally applied markers (*Lycopodium* spores in Castle (1942), starch grains in Trinci & Banbury (1967)). In the *Volvariella* experiment, extension at the margin would consequently have resulted in the ink marks being left at their original absolute positions, being buried beneath 4 to 20 mm of newly formed tissue by the end of the experiment (Fig. 11). It follows, therefore, that gills of *V. bombycina* extend in depth by growth of their roots into the pileus context and by insertion of hymenial elements into their central and root regions. The developmental vector is directed ***away from the stipe***, as it is in *Coprinus*, and in both the schizohymenial and levhymenial modes of gill development the gill grows ***at its root and not at its margin*** (Moore, 1987). Similarly, the hyphal tips that form the pileus margin when it is established at the very earliest stage of development remain ***at*** the margin. They do not continue to grow apically to extend the margin radially, nor are they overtaken by other 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.

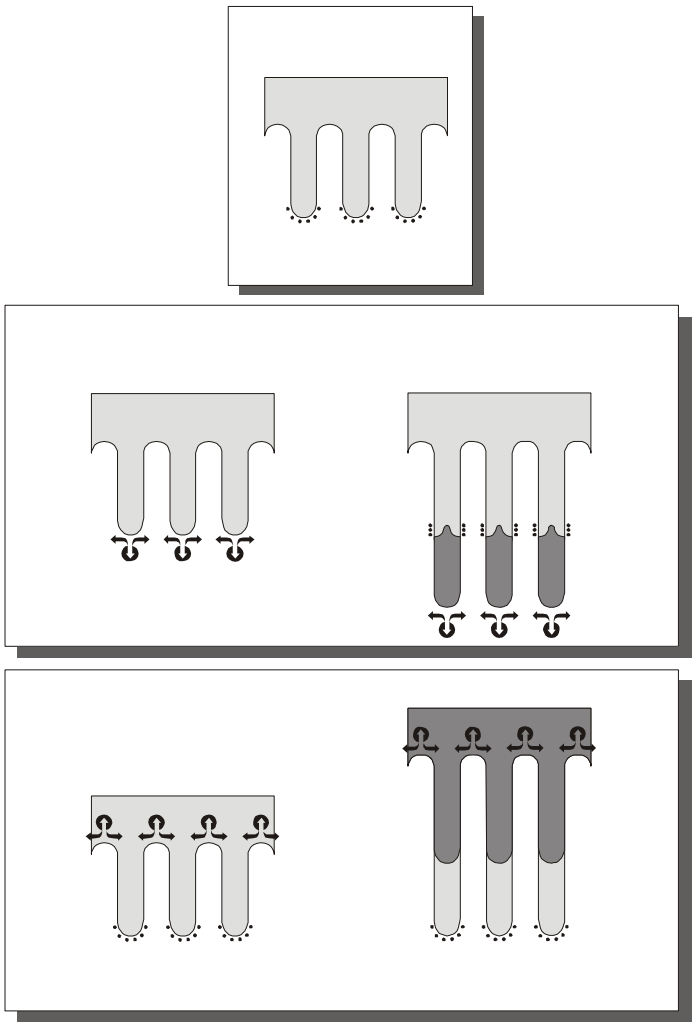

Fig. 11. Gill formation in *Volvariella bombycina*. Line drawings illustrating the outcome of marking experiments (Chiu & Moore, 1990a) and their implications for development of gills in this organism. The top panel diagrams ink particles on the primordial gills, shown in diagrammatic section. The other two panels illustrate *alternative* strategies for gill growth and their predicted outcome for this simple experiment. If the gill organiser is located at the gill margin (centre panel) growth at the margin will extend beyond the initial ink marks burying the ink deep within the gill cavities. If the gill organiser is located at the foot of the gill (bottom panel), originating the gill cavity as illustrated in Fig. 10, then growth of the gill will simply push the already-formed, ink-marked, gill margins further from the cap context but will leave the ink marks in full view. In the experiments reported by Chiu & Moore (1990a), ink marks were painted on the gill margins of primordial fruit bodies and were still clearly visible on the gill margins of mature fruit bodies. It is concluded that agaric gills grow by extension at their roots, and *not* by extension from the free margin.

Because so much stress is placed on apical wall growth, there is a popular misconception that growth occurs only at the hyphal tip, so many people judge the above interpretations as radical. Yet the hyphal tips at pileus and gill margins are growth-limited, differentiated cells, so it should come as no surprise that growth of the structure they comprise is concentrated ***behind*** them. This fact has been appreciated for over 30 years, as the *Dictionary of the Fungi* defines 'inflated hypha' as 'one in which cells behind the growing apex enlarge and cause the apparent rapid rate of growth characteristic of most agaric and gasteromycete fruit bodies' (Ainsworth, 1961). If a parallel to the paradigm that the agaric gill grows at its base is required, then only consider the lilies of the field, how they grow; they toil not, neither do they spin, but their leaves grow at the base.

3.2.4 Mushroom mechanics

In both *C. cinereus* and *V. bombycina* the first-formed gills were radially arranged. As the pileus expanded more gills were formed. In *V. bombycina*, new gills were formed in two ways (Chiu & Moore, 1990a). First, by bifurcation of an existing gill near its free edge. Initiation of the folding which produced bifurcations on existing gills was a localized and irregular event, resulting in

sinuous, contorted gills (Fig. 11). The formation of two daughter gills depended on completion of the bifurcation along the entire edge of the parental gill. Second, new generations of gills appeared as ridges in the region between existing gill roots, creating new folds on the pileus context representing the free edges of new secondary or tertiary gills, the gill spaces on either side extending into the pileus context as the gill grew by its root differentiating from the context. In *C. cinereus*, more gills are added as the basidiome enlarges by bifurcation of existing gills either on one side or at the stipe-gill junction, and by division of gill organisers at the roots of existing gills (section 3.22 and Fig. 10). Consequently, *Coprinus* gills are also formed as convoluted plates (Chiu & Moore, 1990b). Thus, the summary description is the same in each case and a sinuous, labyrinthiform hymenophore is a normal 'embryonic' stage in basidiome development in agarics, yet a regular radial arrangement of the gills is characteristic of the mature basidiome. How this is achieved seems, again, to be a function of the expansion of the maturing primordium but in this case the tensions generated by differential growth between tissue layers stretch the convoluted gills into strict radii.

In *C. cinereus*, tension stresses generated by growth of other parts of the basidiome place geometrical stress on the 'embryonic' gills -- like a folded cloth being straightened by stretching. Such a mechanism requires that the folded elements (in this case the gills) are anchored. The connection of primary gills to the stipe provides the initial anchorage; subsequently cystidium-cystesium pairs interconnect gill plates around the stipe. Tensions generated by expansion of the pileus will then be communicated and balanced throughout the structure. Cystidium-cystesium pairs act, therefore, not as buttresses to keep hymenia apart (the conventional view; Buller, 1924) but as tension elements whose function is to hold adjacent hymenia together as pileus expansion pulls the gills into shape.

For *Volvariella bombycina* an alternative mechanism operates because of the lack of cystidium-cystesium pairs. The hymenium of *V. bombycina* is a layer of tightly appressed cells, and the trama of the gill becomes filled with greatly inflated cells as maturation proceeds. These features suggest that expansion of tramal cells in gills enclosed by the hymenial 'epidermis' will generate compression forces, which will effectively inflate, and so stretch, the embryonic gills to form the regularly radial pattern of the mature pileus.

3.3 Cell distribution patterns in hymenia and fruit body stipes

3.3.1 Cellular elements in the hymenium of *Coprinus*

What the gill organizer leaves behind in the developing hymenophore of *Coprinus* is a protohymenium. A hyphal tip in this protohymenium has a probability of about 40% of becoming a cystidium, but when a cystidium does arise, it inhibits formation of further cystidia in the same hymenium within a radius of about 30 :m (see section 3.3.3, below). As a result, only about 8% of these tramal hyphal branches become cystidia; the rest become probasidia (Horner & Moore, 1987), which proceed to karyogamy and initiate the meiotic cycle ending with sporulation. Paraphyses arise as branches of sub-basidial cells and insert into the hymenium. About 75% of the paraphysis population is inserted before the end of meiosis, the rest insert at later stages of development (Rosin & Moore, 1985b). There is, therefore, a defined temporal sequence: probasidia appear first and then paraphyses arise as branches from sub-basidial cells. Another cell type in the hymenium of *C. cinereus* (called the **cystesium**; Horner & Moore, 1987) illustrates how a contact stimulus can set in train a pathway of differentiation leading to an adhesive cell type. At early stages in growth of the cystidium across the gill cavity the cell(s) with which it will collide in the opposing hymenium are indistinguishable from their fellow probasidia. However, when the cystidium contacts the opposing hymenium, the cells with which it collides develop a granular, vacuolated cytoplasm, more similar to that of the cystidium than to their neighbouring probasidia. The contact triggers the differentiation -- a phenomenon that is met in other organisms.

3.3.2 Cellular elements in the stipe of *Coprinus*

Until recently there was surprisingly little information concerning the structure of the stipe of *Coprinus* basidiomata. Recently, though, Hammad, Watling & Moore (1993) have demonstrated

that the stipe contains both narrow and inflated hyphae. Narrow hyphae (cross-sectional area <20 $:m^2$) always comprise a significant numerical proportion (23% to 54%) of the cells seen in microscope sections of stipe tissue, although they only contribute 1% to 4% to the overall cross-sectional area of the stipe (Fig. 12). Narrow hyphae tend to be concentrated on the outside of the stipe and fringe the central lumen (they fill the lumen at the extreme bases and apices of elongating stipes). Elsewhere, narrow hyphae were interspersed between the inflated hyphae (see section 3.3.3, below). Narrow hyphae stain differentially as well as having varied spatial arrangements, suggesting that although morphologically alike, they may serve distinct functions. The narrow hyphae form interconnections independent of inflated hyphae; being branched and fused laterally with other narrow hyphae, whilst there is no evidence that inflated hyphae are either branched or associated in networks of this sort. Since nutrients can be translocated through the stipe, avoiding barriers inserted into the tissue (Ji & Moore, 1993), it is likely that the network of narrow hyphae is important in nutrient translocation.

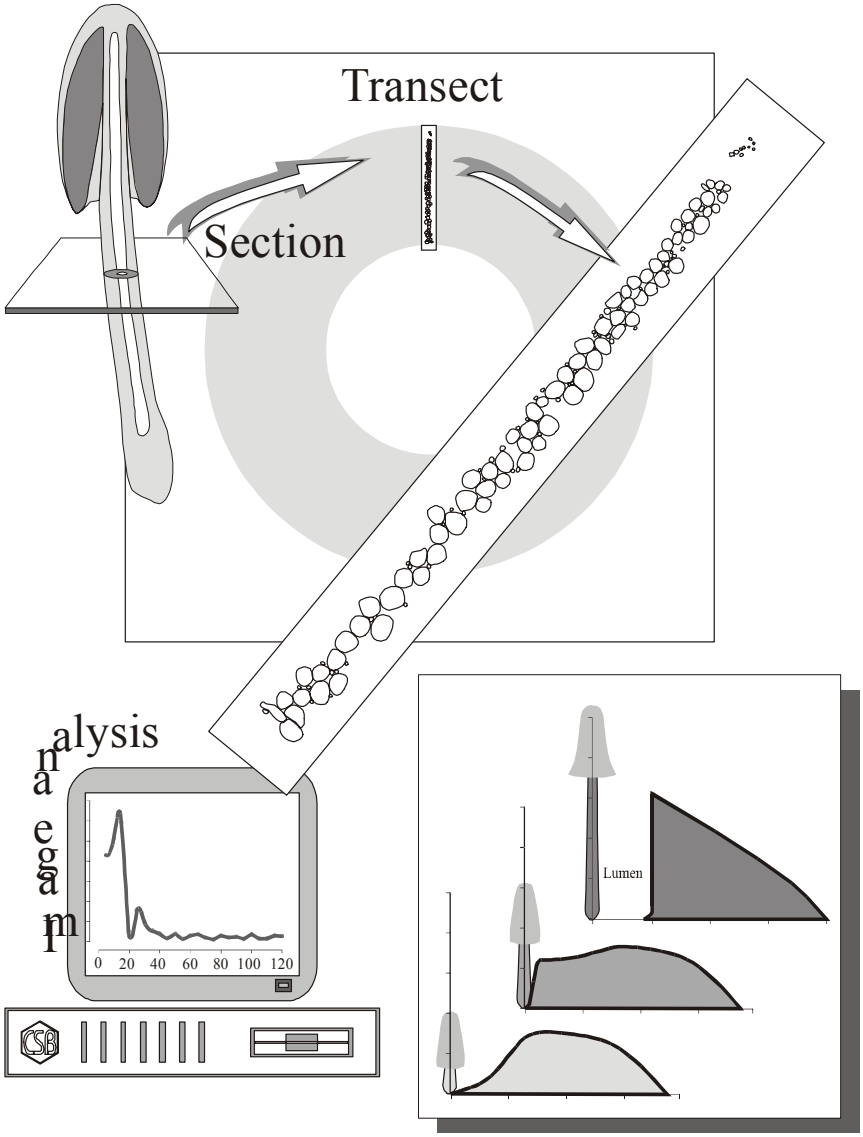

Fig. 12. Stipe structure and development in *Coprinus cinereus*. The main diagram shows how cell-size distribution data were obtained from microscope sections of basidiome stipes. The cell size distribution plot on the computer monitor illustrates the very distinct population of narrow hyphae and the very disperse population of inflated hyphae. The panel at lower right shows average cell area along a radius extending outwards from the centre of the stipe for basidiomes which were 27, 45 and 70 mm tall. The changing distribution of cell size shows that stipe growth is accompanied by inflation of cells in an annulus deep within the original stipe tissue. The outcome of this is that the inner region is torn apart (forming the central lumen) and the outer region is stretched.

During normal stipe growth the greatest cell expansion is seen in the inflated cells situated between the mid-cortex and the lumen rather than at the periphery of the stipe (Fig. 12). Such a distribution of expansion would magnify the tensions (especially compression forces) generated by the expansion process and would obviously contribute greatly to the stretching mechanisms (remember that the barrel-like pileus surrounds the stipe) discussed in section 3.2.4.

3.3.3 Patterns of distribution

Nearest neighbour analysis of cell distributions in *Coprinus* stipes shows that inflated hyphae are evenly rather than randomly distributed regardless of the age of the basidiome or position within the stipe (Hammad *et al.*, 1993a).The proportion of narrow hyphae decreases with time, presumably due to some becoming inflated, and the even distribution of inflated hyphae could be due to some sort of control over the pattern of inflation.

Other evidence for local control of morphogenesis has been obtained from a comparison of the distributions of cystidia on adjacent hymenia in the *Coprinus* fruit body (Horner & Moore, 1987). Cystidia are large, inflated cells which are readily seen in microscope sections so their relationships are open to numerical analysis. Thus, 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*) (Fig. 13). If the distribution of cystidia is entirely randomised there should be an equal number of *cis* and *trans* in both the distant and adjacent categories. However, quantitative data showed a distinct shortage of adjacent-*cis* cystidia (Fig. 13), suggesting that formation of a cystidium lowers the probability of another being formed in the immediate vicinity (Horner & Moore, 1987).

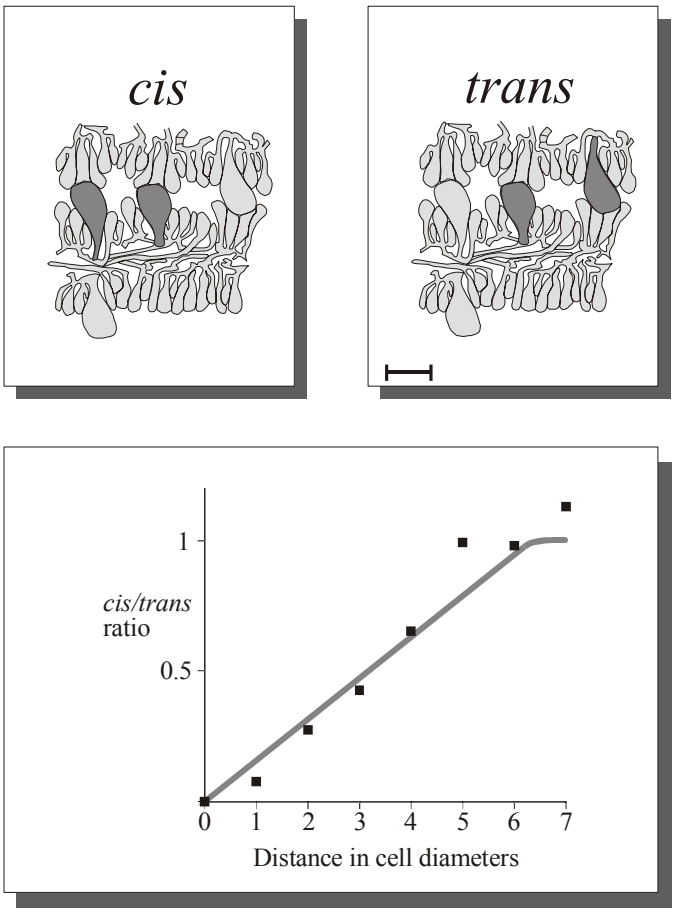

Fig. 13. Cystidium distribution in the *Coprinus cinereus* hymenium. The upper drawings show the categorisation of neighbouring pairs of cystidia in micrographs as either *cis* (both emerge from the same hymenium) or *trans* (emerging from opposite hymenia). The plot in the lower panel compares the frequencies 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.

The inhibition extends over a radius of about 30 : m and is limited to the hymenium of origin. The region around a cystidium is a morphogenetic field controlled by the cystidium at its centre.

The distribution pattern of cystidia might, therefore, be dependent on interplay between activating and inhibiting factors. In this instance (as with the determination of gill development mentioned in section 3.2.2) the patterning process is open to interpretation using the activator-inhibitor model developed by Meinhardt & Gierer (1974; Meinhardt, 1984) which suggests that morphogenetic pattern results from interaction with an activator which autocatalyses its own synthesis, and an inhibitor which inhibits synthesis of the activator. Both diffuse from the region where they are synthesised, the inhibitor diffusing more rapidly and consequently preventing activator production in the surrounding cells. A wide variety of patterns can be generated in computer simulations by varying diffusion coefficients, decay rates and other parameters (Meinhardt, 1984). The model readily accounts for stomatal, cilial, hair and bristle distributions, and has been applied successfully to simulate leaf venation and phylotaxy (Meinhardt, 1984). For animal systems, Green & Smith (1991) conclude that the answer is 'probably yes' to the question 'do gradients and thresholds of growth factors acting as morphogens establish body plan?' A belief that the same may be true for such fungal phenomena as have been outlined in this chapter is implicit in the way in which they are described here. Unfortunately, there are no clues to the nature of the **morphogens** that might be the activating and/or inhibiting growth factors in these phenomena. Another problem is that ***lateral*** contacts between fungal hyphae are extremely rare, being represented only by lateral hyphal fusions. The constituent cells of plant and animal tissues are interconnected laterally by frequent plasmodesmata, gap junctions and cell processes. The absence of similar structures connecting adjacent hyphae suggests that any morphogens that do exist are likely to be communicated exclusively through the extracellular environment (Reijnders & Moore, 1985; and see section 2.1). Although it is abundantly clear that co-ordination of developmental processes is successfully achieved in fungal multicellular structures, the evidence for chemicals able to perform the signal communication involved is sparse and disappointingly unconvincing (discussed in Moore, 1991).

3.4 Reijnders' hyphal knots - a basic building block?

Although the work of Hammad *et al*. (1993a, b) is the first ***quantitative*** hyphal analysis, hyphal analysis of basidiomata was introduced into taxonomy by Corner (1932), coining the terms monomitic, dimitic and trimitic to describe tissues consisting of one, two or three kinds of hyphae. Later, sarcodimitic and sarcotrimitic were used to describe basidiomata where there are two or three types of hyphae of which one is inflated and thickened (Corner, 1966), and Redhead (1987) recognised a group of closely related agarics with such structures. Some specialized gill trama structures have been highly rated as taxonomic criteria: the heteromerous trama of the Russulaceae, acrophysalidic trama of the Amanitaceae and Pluteaceae, and the sarcodimitic trama of *Trogia* (Corner, 1991) and the Xerulaceae (Redhead, 1987). The heteromerous trama in Russulaceae (see below) was described by Fayod (1889) and he was also aware of the presence of narrow hyphae amongst the more easily seen cells of the basidiome tissues he examined, but although differentiated hyphae and cells have been recognized as taxonomically important (Lentz, 1971), they have not been used in identification of agarics to the extent that the mitic system has in polypores.

These have all been examined in detail by Reijnders (1993) who came to the general conclusion that cell structures which are considered peculiar to each of the specialized trama types can be found in some form (either less well developed or restricted to a particular developmental stage) in many other, unrelated taxa. Of particular interest is the heteromerous trama, to which has been attached particular taxonomic importance. The heteromerous trama is characterized by sphaerocysts, first depicted by Corda (1839), which are inflated cells situated in a ring surrounding a central ('induction') hypha (Reijnders, 1976; Watling & Nicoll, 1980). Very similar aggregations of hyphae, termed hyphal knots (Reijnders, 1977) have been observed 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. Some hyphal knots do not show conspicuous differentiation, remaining as systems of tightly interwoven hyphae of uniform structure, but at the extreme, swollen cells in a ring or cylinder around a central hypha may be formed in species taxonomically far removed from the Russulaceae.

Hyphal knots are found particularly frequently in plectenchymatous tissue and occur in bulb, stipe, veil and pileus as well as tramal tissues. Reijnders (1993) discusses the impact of their widespread occurrence on taxonomic and phylogenetic arguments about the Agaricales. I wish to point out that their analogues (perhaps, even, homologues) might occur much more widely than that because elements of Reijnders' description of hyphal knots are detectable in descriptions of many fungal multihyphal structures, from strand formation (Butler, 1958) through development of sclerotia (Townsend & Willetts, 1954), and on to the descriptions given above of paraphysis distributions around basidia as well as the influence exerted by cystidia on their surroundings in the *Coprinus* hymenium, and the possible relationships between narrow and inflated hyphae in the *Coprinus* stipe.

Perhaps, in all multihyphal fungal structures, the ultimate morphogenetic regulatory structure 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 -- mycological macramé?

4 Controlling cell differentiation

It is important to appreciate that all the cell types so far mentioned arise as branches from some other hypha-like elements. At some point sister branches of the same hyphal system, cellular elements separated only by a dolipore septum in Basidiomycotina or a much simpler septum in Ascomycotina, will follow totally different pathways of differentiation (Fig. 14). Although evidence for different dolipore ultrastructures has been found in the basidiomycete hymenium (Gull, 1976, 1978; Moore, 1985), even in this extreme example there is no indication that the septa are physically sealed, so it is not at all clear how alternate pathways of differentiation are regulated on the two sides of a septum which appear to be in physiological contact.

4.1 Genes and things

Despite a generally smaller genome size, fungi share with other organisms the fact that only a small proportion of the genome is associated with any particular morphogenetic process. This has been demonstrated in *Schizophyllum* (Zantinge, Dons & Wessels, 1979; de Vries, Hoge & Wessels, 1980; de Vries & Wessels, 1984; Wessels, Mulder & Springer, 1987), *Coprinus* (Yashar & Pukkila, 1985; Pukkila & Casselton, 1991), *Neurospora* (Nasrallah & Srb, 1973, 1977, 1978), *Sclerotinia* (Russo, Dahlberg & Van Etten, 1982), *Sordaria* (Broxholme, Read & Bond, 1991), and *Saccharomyces*. The yeast example is especially interesting because of its small genome (haploid genome = about 1.4×10^4 kilobase pairs, which is less than four times the size of the genome of the bacterium *Escherichia coli*), yet only 21 to 75 of the estimated 12,000 genes in yeast are specific to sporulation (i.e. meiosis and ascospore formation)(Esposito *et al*., 1972). Thus, the emphasis in morphogenetic gene regulation is on differential integration of activity rather than on large-scale replacement of one set of gene products by another. This could be seen as conflicting with Timberlake's estimates that 11 -- 18% of poly(A) RNA sequences accumulated in conidiating cultures of *Aspergillus nidulans* are not detectable in vegetative hyphae (Timberlake, 1980) and that 6% of the unique sequences are expressed during this aspect of development (Timberlake & Marshall, 1988). But Timberlake's estimates are for the entire portfolio of ***differentiation*** events rather than morphogenetic events *per se*: his vegetative material comprised spores germinated for only 16 h, conidiating cultures were grown for 40 h (Timberlake, 1980). The other comparisons between fruiting and non-fruiting cultures referred to above involve cultures of similar age which, for environmental or genetic reasons, differ in their ability to fruit. As mycelia undergo vegetative morphogenesis, many aspects of cell differentiation expected of the fruit body (cell inflation, wall thickening, accumulation of metabolites, etc.) will be found in non-fruiting mycelia, so comparisons

made in these cases emphasise differences ascribable to the ***morphogenetic*** events contributing to fruit body formation rather than the differentiation of its component cells. It is these morphogenetic processes that we might hope to identify by studying the genetic control of fruiting.

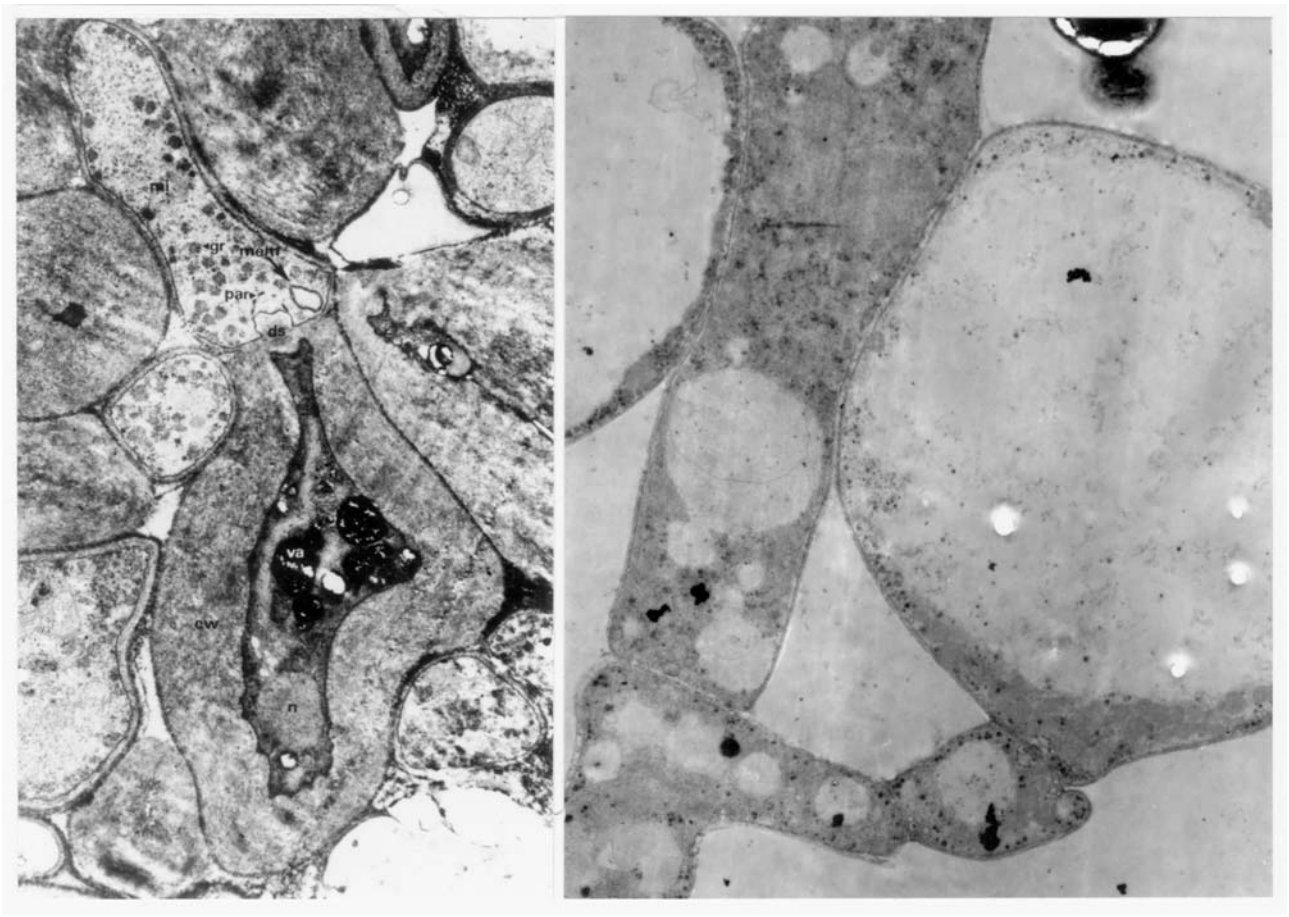

Fig. 14. Differentiation across the dolipore septum in *Coprinus cinereus*. The transmission electronmicrographs illustrate neighbouring cells showing extremes of differentiation in the hymenium and sclerotium. In the hymenium (left hand image) a greatly-inflated paraphysis is developing at the apex of a branch which emerged from the cell immediately beneath a basidium. The left hand image shows adjacent compartments of a hypha in a section of the medulla of an aerial sclerotium. The upper compartment is thin-walled and has an electron-translucent cytoplasm with scattered glycogen `rosettes' whilst the lower compartment is thick-walled, has a more electron-dense cytoplasm and vacuoles with extremely electron-dense contents. Scale bars = 5 :m.

Classical genetic studies, namely identification of variant strains, application of complementation tests to establish functional cistrons, construction of heterokaryons to determine dominance/recessive and epistatic relationships (to indicate the sequence of gene expression) have revealed a 'developmental pathway' for perithecium formation in *Sordaria* (Esser & Straub, 1958; Fig. 15), and 29 complementation groups involved in perithecium development have been identified in *Neurospora* (Johnson, 1978). Johnson (1976) used genetic mosaics (heterokaryons in which one nucleus carried a recessive colour mutant) to show that perithecia of *Neurospora* arise from an initiating population of 100 to 300 nuclei, and that the perithecium wall is composed of three developmentally distinct layers. Recently, Ashby & Johnstone (1993) have used the *Escherichia coli* $-glucuronidase gene as a reporter gene to study development of ascomata in *Pyrenopeziza brassicae* and have also revealed three tissue layers. One to which both mating types contribute and two to which the two mating types contribute separately. The significance of extensive tissue layers in which only one mating type is expressed is unknown.

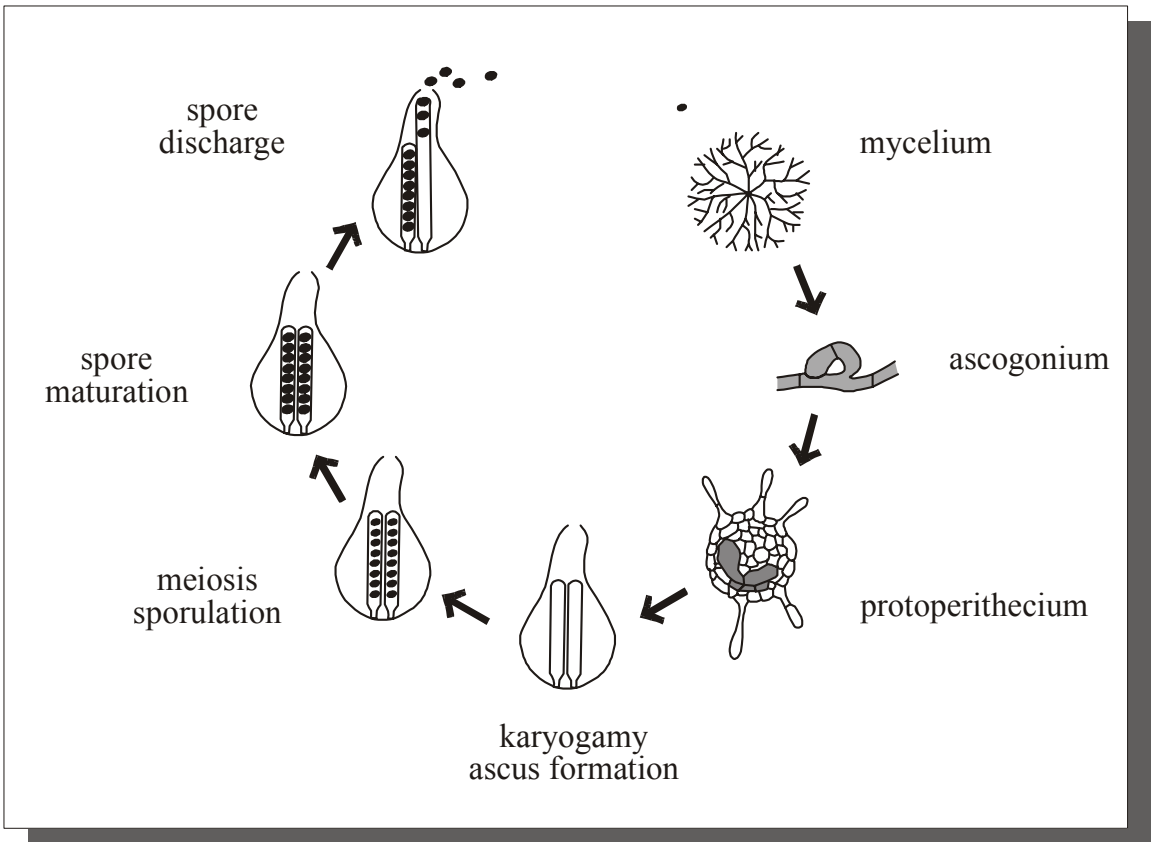

Fig. 15. The life cycle diagram and perithecium developmental pathway of *Sordaria macrospora* (after Esser & Straub, 1958). A variety of mutants are known which block the pathway at each of the stages represented by arrows, so the whole pathway is interpreted as being essentially a single sequence. Contrast this with the multiple parallel 'subroutines' which seem to characterise basidiome development (Figs 16 and 17).

In basidiomycetes, the picture revealed by classical genetic approaches is less clear. One reason is that fruit bodies in basidiomycetes are normally formed by heterokaryotic mycelia and the presence of two (or more) nuclei (and, hence, two or more genotypes) makes study of the genetics of development by conventional means very difficult. However, fruiting on monokaryotic mycelia has been reported for a diverse selection of agarics and polypores (Stahl & Esser, 1976; Elliott, 1985) and this allowed a start to be made on the genetic control of fruit body development.

4.1.1 Monokaryotic fruiting

The frequency of monokaryons able to fruit differs drastically between genera: 27% of *Sistotrema* isolates formed monokaryotic fruit bodies (Ullrich, 1973), 7% of *Schizophyllum* strains did so (Raper & Krongelb, 1958), but only one of 16 monokaryons of *Coprinus cinereus* tested by Uno & Ishikawa (1971). Horton & Raper (1991) identified a DNA sequence which induced monokaryotic fruiting in strains of *Schizophyllum commune* into which it was introduced by transformation, but there is no indication yet as to how it operates.

Stahl & Esser (1976) carried out genetic crosses between various monokaryotic fruiting strains of *Polyporus ciliatus* and identified three unlinked genes involved in monokaryotic fruiting. The way in which the genes function is unknown but fi^+ is interpreted as initiating monokaryotic fruiting while fb^+ is seen as being responsible for 'moulding' the structure of the fruit initiated by fi^+ into a fruit body, while mod^+ directs development into a futile pathway which leads to formation of compact mycelial masses called stromata. In the dikaryon mod^+ inhibited fruiting, but neither fi^+ nor fb^+ showed any expression even when homozygous.

A broadly similar genetic system was revealed in analogous experiments with the agaric *Agrocybe aegerita* (Esser & Meinhardt, 1977). Here, again, one gene, fi^+, was identified as being responsible for initiation of monokaryotic fruiting, while a second, fb^+, was thought to be

responsible for modelling the initiated structures into fruit bodies. Unlike *Polyporus*, these genes were found to be concerned with fruiting in the dikaryon of *A. aegerita*. Only dikaryons carrying at least one allele of both *fi*$^+$ and *fb*$^+$ were able to produce fertile fruit bodies.

In *Schizophyllum commune*, Raper & Krongelb (1958) examined some monokaryotic fruiting strains (called *hap*) and were able to show that there was no correlation between monokaryotic and dikaryotic fruiting, and the former was probably under polygenic control. The polygene complex involved may have been identified by Esser, Saleh & Meinhardt (1979), who found monokaryotic fruiting to be controlled by at least four genes in *S. commune*. Two 'fruiting initiation genes' (*fi*-1$^+$ and *fi*-2$^+$, either of which alone allowed differentiation into 2 -- 3 mm 'initials', whereas when both were present stipes 6 -- 8 mm long were formed), a third gene (*fb*$^+$) was required for formation of complete monokaryotic fruit bodies. The fourth gene *(st*$^+$) prevented expression of the others; any mycelium carrying *st*$^+$ produced only stromata. Although *st*$^+$ also blocked dikaryotic fruiting when homozygous, the other three genes had no affect on differentiation of fruit bodies in the dikaryon though they did influence the time of fruiting. The most rapid fruiting occurred on dikaryons which were homozygous for all three monokaryotic fruiter genes. The slowest fruiting occurred when the dikaryon did not carry any of the monokaryotic fruiter alleles, but the fact that fruiting did eventually occur in this latter case implies a major difference between the genetic control of monokaryotic and dikaryotic fruiting. Barnett & Lilly (1949) also reported an increased frequency of fruiting in dikaryons made from monokaryotic fruiters in *Lenzites trabea*, but they concluded that the same factors might be involved in the genetic control of monokaryotic and dikaryotic fruiting.

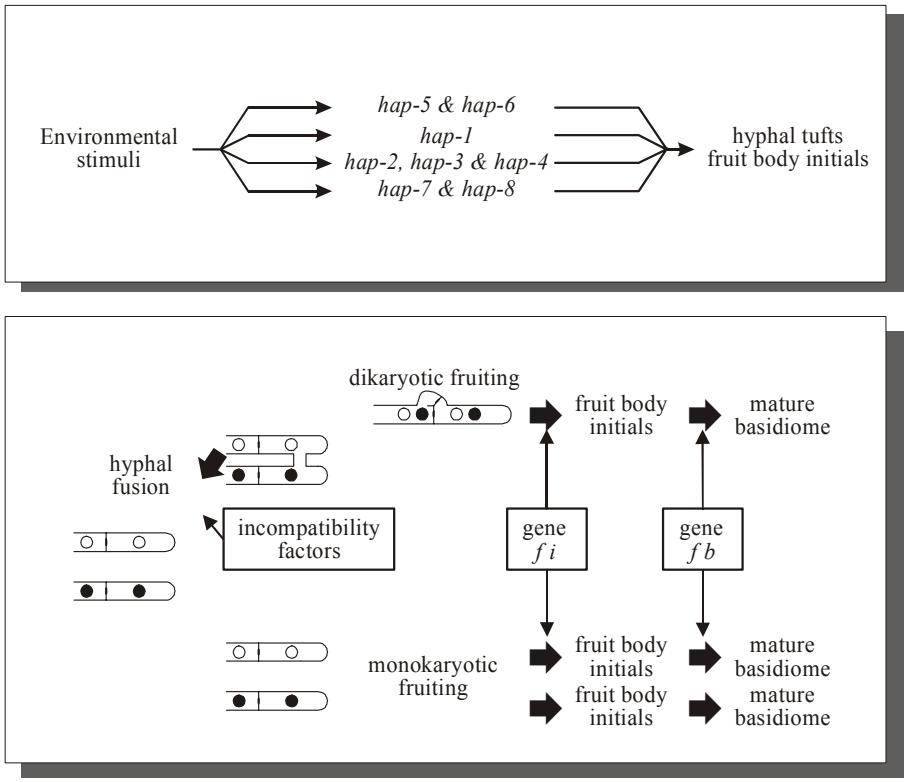

Fig. 16. Models for the genetic control of basidiome development. The top panel shows the genes involved in monokaryotic fruiting in *Schizophyllum commune* (after Leslie & Leonard, 1979b) in which *hap*-5 and *hap*-6 control the spontaneous initiation of basidiomes, *hap*-1 alone or *hap*-2, -3 and -4 acting together control fruiting as a response to injury, and *hap*-7 and *hap*-8 determine fruiting in response to applied chemicals. The bottom panel is a proposed model for the action of major genes controlling basidiome formation in *Agrocybe aegerita* (after Esser & Meinhardt, 1977).

Leslie & Leonard (1979a, b) identified 8 genes in *Schizophyllum commune* involved in four distinct pathways enabling monokaryons to initiate fruiting bodies in response to mechanical and chemical treatments (Fig. 16). As Leslie & Leonard (1979b) place the operation of these genes prior to fruiting stage I, which corresponds to masses of aggregated cells which have no defined

shape (Leonard & Dick, 1968), these systems are probably distinct from and perhaps operate at stages prior to those governed by the genes identified by Esser *et al*. (1979), which produce stipe-like structures. However, descriptive comparisons are the only basis for speculation about relationships between these systems.

The multiplicity of genetic factors involved in monokaryotic fruiting mirrors the multiplicity of physiological conditions which are able to promote such fruiting. In the overwhelming majority of cases 'monokaryotic fruits' are abnormal structures, being incomplete, sterile or both. An essential question, then, is whether genes which influence fruiting in monokaryons are actually relevant to the normal process of dikaryotic fruiting. Stahl & Esser (1976) report that neither fi^+ nor fb^+ showed expression in the dikaryon of *Polyporus ciliatus* even when homozygous, while Esser *et al*. (1979) came to a similar conclusion for the genes to which they gave the same names in *Schizophyllum commune*, but in both species there are examples of genes with some expression in both mycelial states: the gene mod^+ in the former and st^+ in the latter each blocked fruiting in the dikaryon. In *Agrocybe aegerita*, both fi^+ and fb^+ were essential for the dikaryon to produce fertile basidiomata (Esser & Meinhardt, 1977; Esser *et al.*, 1979; Fig. 16).

4.1.2 Dikaryotic fruiting

Uno & Ishikawa (1971) concluded that more than one gene is involved in controlling monokaryotic fruiting in *Coprinus cinereus* (= *macrorhizus*) but *C. cinereus* is the only organism in which any attempt has been made to study the genetic control of fruit body formation by the dikaryon. Dikaryons of *C. cinereus* can form sclerotia and basidiomata, monokaryons can also form sclerotia but normally do not form basidiomata. The initial stages in the development of both structures have been described separately and the descriptions are remarkably similar (Matthews & Niederpruem, 1972; Waters, Moore & Butler, 1975a). For both structures, development from the mycelium must involve equivalent patterns of hyphal aggregation so the likeness observed may indicate a shared initial pathway of development or coincidentally analogous separate, but parallel, pathways. The opportunity to distinguish between these possibilities arose with the identification of monokaryons unable to form sclerotia.

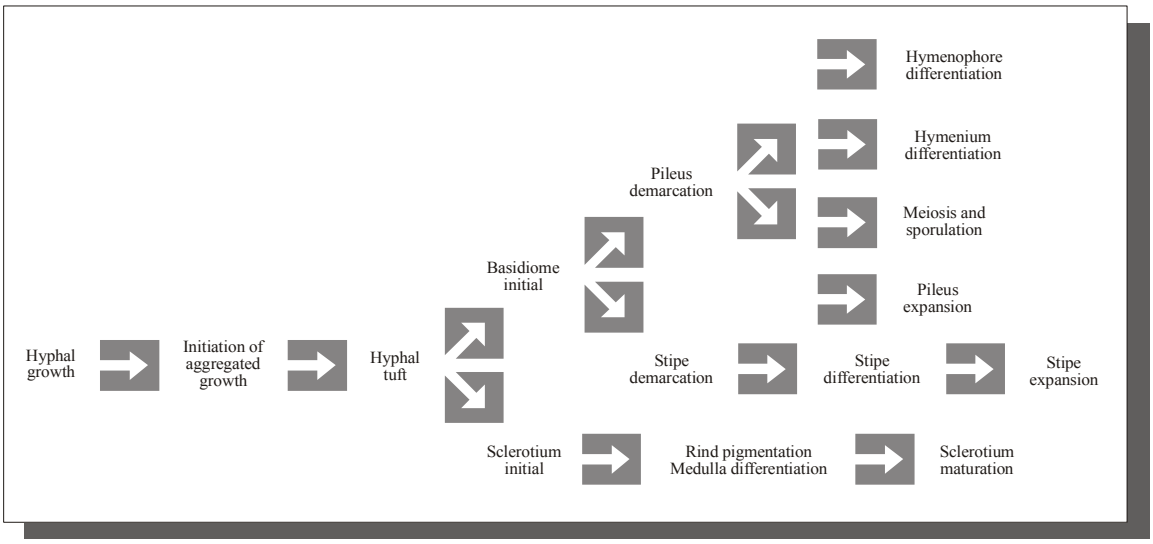

Fig. 17. Genetically distinct pathways involved in sclerotium and basidiome development in *Coprinus cinereus* (revised after Moore, 1981).

This monokaryon phenotype segregated in crosses as though controlled by a single major gene. Four such *scl* (sclerotium-negative) genes were characterised; one, *scl*-4, caused abortion of developing fruit body primordia even when paired in the dikaryon with a wild type nucleus but the other three behaved as recessive genes in such heteroallelic dikaryons and were mapped to existing linkage groups (Waters, Moore & Butler, 1975a). Subsequently, Moore (1981) showed that

homoallelelic dikaryons (i.e. dikaryons in which both nuclei carried the same *scl* allele) were unable to form either sclerotia or fruit bodies. Since these single genetic defects blocked development of both dikaryon structures it was concluded that in the initial stages sclerotia and basidiomata share a common developmental pathway which is governed by the *scl* genes (Fig. 17).

When they mutate they are usually recessive so the pathway can proceed only in the heteroallelic dikaryon where the missing *scl* function is provided by the wild type nucleus from the other parent. The *scl* mutations remove the ability to make sclerotia -- a normal aspect of the monokaryon phenotype. In this respect they seem to be the antithesis of the *fis* mutants, some of which cause monokaryotic fruiting (Uno & Ishikawa, 1971), and the *roc* gene, which causes stromatic proliferations (Nyunoya & Ishikawa, 1979) of *C. cinereus*, and the *hap*, *fi* and *fb* genes in *Schizophyllum* (discussed above) which confer on the monokaryon the ability to form a fruit body -- a phenotype which is normally a character of the dikaryon. Thus, recessive mutations can lead both to loss and gain of the ability to form multicellular structures. Attempts have been made to simplify many of these observations into a single developmental pathway (Esser & Hoffman, 1977; Esser, Stahl & Meinhardt, 1977; Meinhardt & Esser, 1983; Fig. 16), yet much of the evidence points to their being a number of discrete partial pathways which can run in parallel.

Basic characterisation of the genetic control of dikaryon fruit body development has only been attempted in *Coprinus cinereus* (under the name *C. macrorhizus*) by Takemaru & Kamada (1971, 1972). These workers treated macerated dikaryon fragments with mutagens and then searched for developmental abnormalities among the survivors. Including spontaneous mutations, a total of 1594 were identified out of 10641 dikaryotic survivors tested. They were classified into categories on the basis of the phenotype of the fruit body produced: (i) 'knotless' - no hyphal aggregations are formed; (ii) 'primordiumless' - aggregations are formed but they do not develop further; (iii) 'maturationless' - primordia are produced which fail to mature; (iv) 'elongationless' - stipe fails to elongate but pileus development is normal (v) 'expansionless' - stipe elongation normal but pileus fails to open; (vi) 'sporeless' - few or no spores are formed in what may otherwise be a normal fruit body. Since dikaryotic mutagen survivors were isolated, the genetic defects identified are all dominant. Elongationless mutants have been exploited to study stipe elongation (Kamada & Takemaru, 1977a & b, 1983), and sporeless mutants have been used to study sporulation (Miyake, Takemaru & Ishikawa, 1980; Miyake, Tanaka & Ishikawa, 1980).

These mutants suggest that different aspects of basidiome development are genetically separate. Prevention of meiosis still permits the fruit body to develop normally, demonstrating, as do monokaryotic fruit bodies, that meiosis and spore formation are entirely separable from construction of the spore-bearing structure. Perhaps more interesting is the fact that mutants were obtained with defects in either pileus expansion or stipe elongation. Both processes depend on enormous cell inflation, and the fact that they can be separated by mutation indicates that the same result (increase in cell volume) is achieved by different means (Moore, Elhiti & Butler, 1979).

However, there is a profound problem in accounting for the induction of dominant mutations at the high frequency observed by Takemaru & Kamada (1972). Among the mutagens used, ultraviolet light and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine both increased the mutation frequency, together producing a total of 1582 mutants among 8547 survivors examined. This 18.5% mutation rate is extraordinarily high. Another peculiarity is that over 72% of the mutants belong to just two phenotypes; there being 595 maturationless and 582 sporeless isolates. Takemaru & Kamada (1972) account for these frequencies with the suggestion that genes involved in development may be easy to mutate. The absence of reports of such mutations in other populations of *C. cinereus* dikaryons argues against this proposition. An alternative interpretation (Moore, 1981) is that the genes which were being caused to mutate were not those involved directly in development, but rather genes which modify the dominance of pre-existing developmental variants; i.e. the dikaryon subjected to mutagenesis carried genetic defects affecting maturation or spore formation, but that their recessiveness depended on modifying genes. Exposure to mutagen caused mutations in one or other of these modifiers and consequent change in the balance dominance modification resulted in the recessive phenotype becoming a dominant one. That dominance (or penetrance) is dependent on the modifying action of other genes is a well established and perfectly respectable idea in genetical

theory (Fisher, 1928, 1931; Sheppard, 1967; Manning, 1976, 1977), indeed one can imagine considerable selective advantage in a system which imposed recessiveness on variants in genes concerned with development. This interpretation was arrived at following work showing that the penetrance of *scl* genes in heteroallelic dikaryons depended on the segregation of modifiers (Moore, 1981). Nyunoya & Ishikawa (1979) also showed that *roc* and fis^{C} segregated in ratios suggestive of multiple gene control and dominance modification has also been invoked to explain segregation patterns of a gene conferring resistance to *p*-fluorophenylalanine in *C. cinereus* (Senathirajah & Lewis, 1975; Lewis & Vakeria, 1977). As differentiation in basidiomycetes involves extensive protein processing (Zantinge *et al*. 1979; de Vries *et al*. 1980; Moore & Jirjis, 1981), modifiers might be involved in processing signal sequences of structural proteins. In the presence of particular modifier alleles (those which cause the change in penetrance), signal processing might lead to normal structural proteins failing to reach their correct destination, or abnormal proteins being partially corrected so that they do reach the target site, despite being defective.

Isolation of strains of *C. cinereus* that have mutations in both mating type factors (*Amut Bmut* strains) has opened up new possibilities for genetic analysis of morphogenesis in this organism. These strains are homokaryotic dikaryon phenocopies; i.e. they emulate the dikaryon in that their hyphae have binucleate compartments and extend by conjugate nuclear division with formation of clamp connections, and the cultures can produce apparently normal basidiomata. On the other hand they are homokaryons, being able to produce asexual spores (usually called oidia) and, most importantly, containing only one (haploid) genetic complement (Swamy, Uno & Ishikawa, 1984). This last feature allows expression of recessive developmental mutants and these strains have been used to study a number of developmental mutants (Kanda & Ishikawa, 1986) especially in meiosis and spore formation (Zolan, Tremel & Pukkila, 1988; Kanda *et al*., 1989a, 1990; Kamada *et al*., 1989) and in the formation of basidiome primordia (Kanda *et al*., 1989b), but no overall basidiome developmental pathway has yet emerged.

4.1.3 Expression of fruiting genes

The work discussed so far gives no guidance about the way in which genes causing developmental variants exercise their effects. As stated at the beginning of this section, only a small fraction of the genome is specific to morphogenesis, and correspondingly few morphogenesis-specific polypeptides have been identified. A development specific protein has been identified in sclerotia of *Sclerotinia sclerotiorum* (Russo, Dahlberg & Van Etten, 1982) and a polypeptide specific to ascomatal development has been detected in *Neurospora tetrasperma* (Nasrallah & Srb, 1973, 1977) and localized to the mucilaginous matrix surrounding the asci and paraphyses (Nasrallah & Srb, 1978). In *Sordaria brevicollis*, 17 out of over 200 polypeptides detected after pulse labelling were found in perithecia after crossing (Broxholme, Read & Bond, 1991). De Vries & Wessels (1984) found only 15 polypeptides specifically expressed in fruit body primordia of *Schizophyllum commune*. Other techniques also suggest that expression of only a small proportion of the genome is devoted to morphogenesis in both *S. commune* (Zantinge, Dons & Wessels, 1979; de Vries, Hoge & Wessels, 1980) and *Coprinus cinereus* (Yashar & Pukkila, 1985; Pukkila & Casselton, 1991).

Reallocation of ribosomal-RNA between fruit bodies and their parental vegetative mycelium was demonstrated by *in situ* hybridisation in *S. commune* (Ruiters & Wessels, 1989a) and concentration of fruiting-specific RNAs in the basidiomata has also been demonstrated (Mulder & Wessels, 1986; Ruiters & Wessels, 1989b). From among the fruiting-specific sequences, Dons *et al*. (1984) cloned a gene belonging to a family of sequences encoding **hydrophobins**, cysteine-rich polypeptides which are excreted into the culture medium but polymerise on the wall of hyphae which emerge into the air (to form fruit body initials, for example) and invest them with a hydrophobic coating (Wessels, 1992; and see Fig. 18).

In *Coprinus cinereus* some enzymes are specifically derepressed in the fruit body pileus, being absent from its stipe; among these is the NADP-linked glutamate dehydrogenase (NADP-GDH) and glutamine synthetase (GS)(Moore, 1984b). Cytochemical examination shows that the NADP-GDH appears first in isolated islands of cells in the very young primordium (Elhiti, Butler & Moore, 1979) where the enzyme is localized to cytoplasmic microvesicles in the peripheral regions

of specific cells (Elhiti, Moore & Butler, 1987). Other analyses associated initial derepression of the enzyme with karyogamy and the progress of meiosis (Moore, Liu & Kuhad, 1987). It was suggested that the enzymes functioned in ammonium detoxification, rather than being primarily ammonium assimilators, to protect meiosis and sporulation (Moore, Horner & Liu, 1987). Subsequently, it was demonstrated that supplementation of the transplantation medium with ammonium salts abolished the commitment to sporulation that basidia otherwise show *in vitro*, causing them to form vegetative hyphae (Chiu & Moore, 1988b). Another remarkable feature of *C. cinereus* is that the helical arrangement of chitin microfibrils, which has been related to stipe extension growth in both *C. cinereus* (Kamada *et al*., 1991) and *Agaricus bisporus* (Mol, Vermeulen & Wessels, 1990), is established in the hyphal tufts which represent the earliest discernable fruit body initials, the walls of the latter being distinguishable from vegetative hyphal walls both in having less chitin and in having the helical microfibril arrangement (Kamada & Tsuru, 1993).

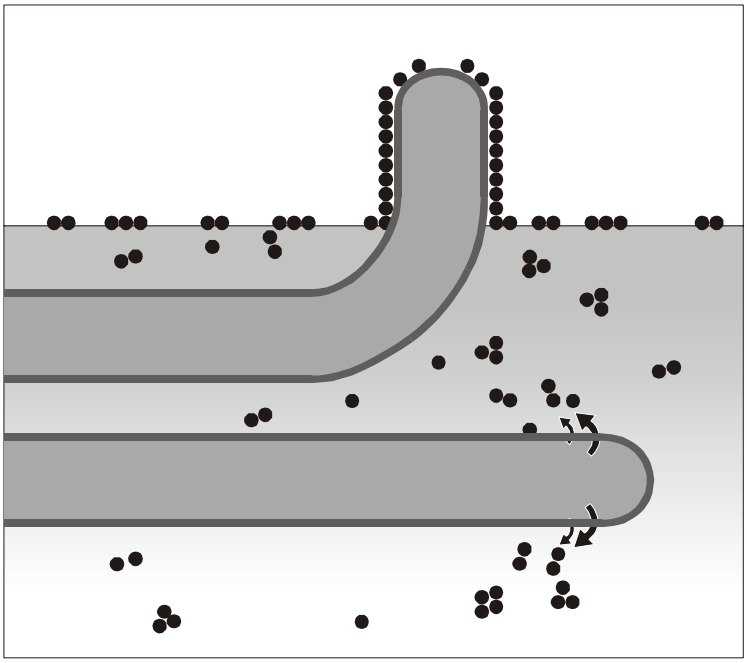

Fig. 18. The excretion of hydrophobins into the culture medium by submerged hyphae. The hydrophobins polymerise onto the wall of aerial hyphae to form a hydrophobic coating. If the molecules are hydrophobic when excreted they would be expected to congregate together and at the liquid surface as shown here (modified after Wessels, 1992).

Schizophyllum hydrophobins, and *Coprinus* NADP-GDH and wall structure are disparate examples of two generalizations. Firstly, the roles of morphogenesis-specific proteins provide surprises; they do not promote the sorts of function which might be postulated in speculative models. Secondly, they operate at, or in the vicinity of, the cell surface ***so as to modify the immediate environment of the differentiating hyphae***. Hydrophobicity is presumably essential for aerial hyphae (and perhaps aggregating hyphae?); removing ammonium ions from the vicinity is certainly crucial for even a committed basidium to remain committed. Could this be the biochemical/molecular basis of the 'tenuous grasp on differentiation' and 'need for continual reinforcement' discussed in section 4.2? Perhaps fungal morphogenesis depends on the differentiating hypha controlling its immediate environment. The degree and complexity of differentiation which can be achieved may be a function of the extent and potency of that control so that when control is diluted (by experimental explantation, for example) the state of differentiation can no longer be maintained.

Whatever genes are directly involved in morphogenesis, they are presumably ultimately controlled by the mating type factors (Kües & Casselton, 1992; and see Chapter 18) and most seem to be transcriptionally regulated (Schuren, van der Lende & Wessels, 1993). Since variation in fruit body morphology is common in higher fungi (Watling, 1971; Chiu, Moore & Chang, 1988) and can span generic (Bougher, Tommerup & Malajczuk, 1993) and even wider taxonomic boundaries

(Watling & Moore, 1993), it has been suggested that normal morphogenesis may be an assemblage of distinct developmental subroutines (Chiu *et al*., 1988); in other words that the genetic control of overall morphogenesis is compartmentalised into distinct segments which can be put into operation independently of one another. Thus, this model postulates subroutines for hymenophore, hymenium, stipe, pileus, etc., which in normal development appear to be under separate genetic control (Fig 17). In any one species they are thought to be invoked in a specific sequence which generates the particular ontogeny and morphology of that species but the same subroutines may be invoked in a different sequence as an abnormality in that same species or as the norm in a morphologically different species. The model provides a unifying theme for categorising fruit body ontogeny and for clarifying phylogenetic and taxonomic relationships (Watling & Moore, 1993).

4.2 Concepts of commitment

There is, then, a wealth of evidence for highly specific differentiation of individual cells in fungi, but very little direct evidence for the developmentally-important concept of commitment. This is the process whereby a cell becomes firmly committed to one of the developmental pathways open to it ***before expressing the phenotype of the differentiated cell type***. The classic demonstration of embryological commitment involves transplantation of the cell into a new environment; if the transplanted cell continues along the developmental pathway characteristic of its origin then it is said to have been **committed** prior to transplant. On the other hand, if the transplanted cell embarks upon the pathway appropriate to its new environment then it was clearly not committed at the time of transplant. Most fungal structures produce vegetative hyphae very readily when disturbed and `transplanted' to a new `environment' or medium. This is an essential feature of collecting fungi `from the wild'. Mycologists would expect to be able to recover cultures from the stipes of many fruit bodies collected from the field, incubate them with the minimum of fuss and obtain a fresh generation of fruit bodies *in vitro* within a matter of weeks. Try doing that with tissue pulled off a cow from the same field! The readiness with which fungi regenerate creates the impression that fungal cells express little commitment to their state of differentiation.

Unfortunately, very little transplantation experimentation has been reported with fungal multicellular structures. The clearest examples of commitment to a developmental pathway have been provided by Bastouill-Descollonges & Manachère (1984) and Chiu & Moore (1988a) who, respectively, demonstrated that basidia of isolated gills of *Coprinus congregatus* and *C. cinereus* continued development to spore production if removed to agar medium after initiation of meiosis, but immediately regressed to form vegetative mycelium if removed 12 hours earlier. In *C. cinereus*, basidia in the prekaryogamy (dikaryotic) stage became arrested on transplantation, neither developing further nor reverting to hyphal growth. Cystidia, paraphyses and tramal hyphae readily reverted to hyphal growth at all times. Evidently, probasidia become committed to complete the sporulation programme during meiotic prophase I. Once initiated, the maturation of basidia is able to proceed *in vitro*, though it can be inhibited by application of ammonium salts and some other compounds (Chiu & Moore, 1988b, 1990c). Clearly, then, even if only to a limited extent (only cells entering meiosis?), commitment to a pathway of differentiation some time before realisation of the differentiated phenotype can evidently occur in fungi. Butler (1988, 1992a) described development of the pore field in cultures of the resupinate polypore *Phellinus contiguus*, and has demonstrated that hyphal differentiation is autonomous in explants (Butler, 1992b). Differentiation in explants was similar to that which occurred in intact basidiomata at positions equivalent to those from which the explants were removed. This implies that the mode of differentiation of the explant was somehow specified, i.e. the cells were developmentally committed, prior to its excision.

4.3 Absolutes or probabilities?

The above discussion (section 4.2) of the explantation experiments of Chiu & Moore (1988b) concentrated on the commitment shown by basidia to the sporulation pathway. It also important to appreciate that the other cells of the hymenium and hymenophore, which immediately reverted to hyphal growth on explantation, do not default to hyphal growth *in situ*. While the fruit body is intact these cells exhibit extremes of differentiation, yet as soon as they are separated from

their fellows they produce hyphal outgrowths. It is as though they have an extremely tenuous grasp on their state of differentiation, and that, *in situ*, their state of differentiation is somehow continually reinforced by something in the environment of the tissue that they comprise.

A cell described as a basidium is quite clearly characterized by karyogamy, meiosis and the formation of basidiospores. In other words, the nomenclature encompasses a portfolio of features which cells of that sort are expected to express. However, on the one hand, the full portfolio of features may not always be expressed, and on the other hand, the portfolio of features that are expressed may not all be appropriate to the cell expressing them. Facial cystidia (those in the hymenium on the surface of the gill) in *C. cinereus* are established as components of the very first population of dikaryotic hyphal tips which form hymenial tissue (Rosin & Moore, 1985b; Horner & Moore, 1987) and are generally binucleate, reflecting their origin and the fact that they are sterile cells. Yet, occasional examples can be found of cystidia in which karyogamy has occurred (Chiu & Moore, 1993). This suggests that entry to the cystidial pathway of differentiation does not totally preclude expression of at least the start of the nuclear differentiation pathway characteristic of a different cell type.

Other aspects of developmental uncertainty are the facts that cells of different sorts can serve the same function, and cells of similar form can serve different functions and arise in different ways. To illustrate the first of these propositions: the 'epidermal pavement'-like structure of the *Coprinus* hymenium is constructed of highly differentiated cells -- paraphyses -- which arise as branches from beneath the basidia (Rosin & Moore, 1985b). At maturity, individual basidia are surrounded by about 5 paraphyses, so that about 80% of the hymenial cells serve a structural function. In the hymenium of *Agaricus bisporus*, the 'epidermal pavement' which provides the structural support for basidia is made up of immature basidia in an arrested meiotic state (Allen *et al*., 1992). Even after many days existence, when the fruit body is close to senescence, 30 to 70% of the immature basidia are in meiotic prophase. Rather than this being wastage of reproductive potential, it is constructive use of one differentiation pathway to serve two distinct but essential functions. *Agaricus* and *Coprinus* hymenophore tissues reach essentially the same structural composition by radically different routes.

Marginal and facial cystidia of *C. cinereus* are very similar in shape and size, but whilst facial cystidia (those in the hymenium on the surface of the gill) arise from the hymenial population of hyphal tips, cystidia on the edges of primary gills (marginal cystidia) are the apical cells of branches from the multinucleate gill trama, which become swollen to repair the injury caused when primary gills are pulled away from the stipe. Marginal cystidia retain the multinucleate character of their parental tramal hyphae (Chiu & Moore, 1993), serve to heal an injury rather than act as tension ties, and yet, apart from position, are indistinguishable from their analogues, the facial cystidia. Basidia provide more examples of 'convergent development'. A hymenial cell initial being binucleate in *C. cinereus* but uninucleate in *V. bombycina* lead to inevitable differences in the course of meiotic division, which are magnified by the fact that DNA synthesis takes place before karyogamy in *C. cinereus* but after karyogamy in *V. bombycina*. Yet the two basidia exhibit remarkably similar morphologies and clearly serve the same function.

Discussion of fungal cell differentiation often involves use of the word 'switch' in phrases which imply wholesale diversion between alternative developmental pathways at some point -- like the 'mode switches' in vegetative mycelial development discussed by Gregory (1984). But the examples given above suggest that fungal cells can assume a phenotype even when all conditions for it have not been met. Rather than rigidly following a prescribed sequence of steps, such differentiation pathways seem to be based on allowing latitude in interpreting the rules on which the pathways are based. From his work with genetic mosaics, Johnson (1976) concluded: 'My observations suggest a very different mechanism of pattern formation for *Neurospora* perithecia than is observed in *Drosophila* development. In contrast to *Drosophila*, no rigid determination of the number of nuclear divisions and the pattern of cellular growth can be detected. Rather, there seems to be control on the average amount of growth which an entire tissue or perithecium undergoes.' Perhaps choice between, and progress of, fungal differentiation pathways has more to do with the balance of probabilities than with switching between absolute alternatives.

5 Conclusions

The existing information about developmental mycology is fragmentary and relies too much on inelegant, long-superseded techniques which inevitably give rise to inelegant interpretations. Even at the basic observational level, inappropriate techniques are still employed. Observations made with the light-microscope using sections 60, 40 or even 20 μm thick are useless. The ready availability of resins which can be sectioned routinely at 5 to 10 μm thick leaves no excuse for continued use of such material. Similarly, the accessibility of electron microscopy, especially cryo-SEM, should abolish all attempts to interpret, often with more imagination than accuracy, light microscope images at, and even beyond, the limits of resolution and magnification of the equipment. The value of recent technical innovations should also be more rapidly appreciated. The personal computer can be applied easily to gain new insight into the dynamic communities of hyphae in developing fruit bodies. Capture of video images for computerised image analysis takes some of the pain out of the sort of painstaking analysis which is essential to obtain the precise and detailed ***quantitative*** descriptions of temporal and spatial relationships between hyphae in developing structures.

These types of investigation can be done at relatively low levels of funding. Going up a step we need to know more about the physiology and biochemistry of multihyphal structures. In many cases even the most simple questions cannot be answered at the moment. For example, how is oxygen supplied to the centre of a 20 cm diameter sclerotium of *Polyporus mylittae*? Oxygen tension has been shown to fall to zero within 150 μm of the surface of pellets of *Penicillium chrysogenum* formed in liquid cultures (Wittler *et al*., 1986) but no similar information is available for more normal hyphal aggregates. It goes without saying that the study of fungal morphogenesis would be greatly advanced by deeper understanding of the molecular events involved. Involved in what? Involved in defining hyphal branch initiation, in directional growth of branches, in the nature of the hyphal surface, in the nature of the extracellular matrix, in hypha-to-hypha and hypha-to-extracellular matrix interactions, in the signalling processes and molecules which discriminate between tissues, in the expression of developmental specific genes and the nature of their polypeptides. Projects in these areas require high levels of funding and consequently depend upon reaching beyond the gulf of ignorance and prejudice evident in those who populate, and review for, the committees of granting agencies to persuade them that research on this unique group of organisms is pertinent and important.

Acknowledgements

Thanks are due to Lily Novak Frazer, Tony Trinci and Roy Watling for helpful comments on an early version of this manuscript. I extend my sincere thanks to all my collaborators and postgraduate students, past and present, but wish to record my particular gratitude to Isabelle Rosin, for opening the Pandora's Box of mushroom development for me, and Siu-wai Chiu, for raking out its contents.

References

Ainsworth, A. M. & Rayner, A. D. M. (1990). Aerial mycelial transfer by *Hymenochaete corrugata* between stems of hazel and other trees. *Mycological Research* **92**, 263-266.

Ainsworth, G. C. (1961). *Ainsworth & Bisby's Dictionary of the Fungi*, 5th edition. Commonwealth Mycological Institute: Kew, Surrey, U.K.

Allen, J. J., Moore, D. & Elliott, T. J. (1992). Persistent meiotic arrest in basidia of *Agaricus bisporus*. *Mycological Research* **96**, 125-127.

Ashby, A. M. & Johnstone, K. (1993). Expression of the *E. coli* -glucuronidase gene in the light leaf spot pathogen *Pyrenopeziza brassicae* and its use as a reporter gene to study developmental interactions in fungi. *Mycological Research* **97**, in press.

Barnett, H. L. & Lilly, V. G. (1949). Production of haploid and diploid fruit bodies of *Lenzites trabea* in culture. *Proceedings of the West Virginia Academy of Science* **19**, 34-39.

Bartnicki-Garcia, S. (1990). Role of vesicles in apical growth and a new mathematical model of hyphal morphogenesis. In *Tip Growth in Plant and Fungal Cells*, (ed. I. B. Heath), pp.211-232. Academic Press, Inc.: San Diego & London.

Bastouill-Descollonges, Y. & Manachère, G. (1984). Photosporogenesis of *Coprinus congregatus*: correlations between the physiological age of lamellae and the development of their potential for renewed fruiting. *Physiologia Plantarum* **61**, 607 - 610.

Beavan, M. J., Belk, D. M., Stewart, G. G. & Rose, A. H. (1979). Changes in electrophoretic mobility and lytic enzyme activity associated with the development of flocculating ability in *Saccharomyces cerevisiae. Canadian Journal of Microbiology* **25**, 888-895.

Boddy, L. (1993). Saprotrophic cord-forming fungi: warfare strategies and other ecological aspects. *Mycological Research* **97**, in press.

Boddy, L. & Rayner, A. D. M. (1983a). Mycelial interactions, morphogenesis and ecology of *Phlebia radiata* and *P. rufa* from oak. *Transactions of the British Mycological Society* **80**, 437-448.

Boddy, L. & Rayner, A. D. M. (1983b). Ecological roles of basidiomycetes forming decay columns in attached oak branches. *New Phytologist* **93**, 77-88.

Booth, C. (1966). Fruit bodies in Ascomycetes. In *The Fungi: An Advanced Treatise*, **vol. II**, (ed. G. C. Ainsworth & A. S. Sussman), pp. 133-150. Academic Press: New York & London.

Botton, B. & Dexheimer, J. (1977). The ultrastructure of the rhizomorphs of *Sphaerostilbe repens* B. & B. *Zeitschrift für Pflanzenphysiologie* **85**, 429-443.

Bougher, N. L., Tommerup, I. C. & Malajczuk, N. (1993). Broad variation in developmental and mature basidiome morphology of the ectomycorrhizal fungus *Hydnangium sublamellatum* sp. nov. bridges morphologically-based generic concepts of *Hydnangium*, *Podohydnangium* and *Laccaria*. *Mycological Research* **97**, in press.

Brefeld, O. (1877). *Botanische Untersuchungen über Schimmepilze*. III Heft. *Basidiomyceten I.* Arthur Felix: Leipzig.

Broxholme, S. J., Read, N. D. & Bond, D. J. (1991). Developmental regulation of proteins during fruit-body morphogenesis in *Sordaria brevicollis*. *Mycological Research* **95**, 958-969.

Buller, A. H. R. (1924). *Researches on Fungi*, vol. 3. Longman Green & Co.: London.

Burnett, J. H. (1968). *Fundamentals of Mycology*. Edward Arnold: London.

Butler, G. M. (1957). The development and behaviour of mycelial strands in *Merulius lacrymans* (Wulf.) Fr. I. Strand development during growth from a food-base through a non-nutrient medium. *Annals of Botany* **21**, 523-537.

Butler, G. M. (1958). The development and behaviour of mycelial strands in *Merulius lacrymans* (Wulf.) Fr. II. Hyphal behaviour during strand formation. *Annals of Botany* **22**, 219-236.

Butler, G. M. (1966). Vegetative structure. In *The Fungi: An Advanced Treatise*, **vol. II**, (ed. G. C. Ainsworth & A. S. Sussman), pp. 83-112. Academic Press: New York & London.

Butler, G. M. (1988). Pattern of pore morphogenesis in the resupinate basidiome of *Phellinus contiguus*. *Transactions of the British Mycological Society* **91**, 677-686.

Butler, G. M. (1992a). Location of hyphal differentiation in the agar pore field of the basidiome of *Phellinus contiguus*. *Mycological Research* **96**, 313-317.

Butler, G. M. (1992b). Capacity for differentiation of setae and other hyphal types of the basidiome in explants from cultures of the polypore *Phellinus contiguus*. *Mycological Research* **96**, 949-955.

Cairney, J. W. G. & Clipson, N. J. W. (1991). Internal structure of rhizomorphs of *Trechispora vaga*. **95**, 764-767.

Cairney, J. W. G., Jennings, D. H. & Veltkamp, C. J. (1989). A scanning electron microscope study of the internal structure of mature linear mycelial organs of four basidiomycete species. *Canadian Journal of Botany* **67**, 2266-2271.

Castle, E. S. (1942). Spiral growth and reversal of spiralling in *Phycomyces*, and their bearing on primary wall structure. *American Journal of Botany* **29**, 664-672.

Cavalier-Smith, T. (1981). Eukaryote Kingdoms: seven or nine? *BioSystems* **14**, 461-481.

Chadefaud, M. (1982a). Les principaux types d'ascocarpes: leur organisation et leur évolution. *Cryptogamie Mycologie* **3**, 1-9.

Chadefaud, M. (1982b). Les principaux types d'ascocarpes: leur organisation et leur évolution. Deuxième partie: les discocarpes. *Cryptogamie Mycologie* **3**, 103-144.

Chadefaud, M. (1982a). Les principaux types d'ascocarpes: leur organisation et leur évolution. Troisième partie: les pyrénocarpes. *Cryptogamie Mycologie* **3**, 199-235.

Chen, J. L. & Tzean, S. S. (1993). *Podosporium elongatum*, a new synnematous hyphomycete from Taiwan. *Mycological Research* **97**, in press.

Chet, I. & Henis, Y. (1975). Sclerotial morphogenesis in fungi. *Annual Review of Phytopathology* **13**, 169-192.

Chet, I., Henis, Y. & Kislev, N. (1969). Ultrastructure of sclerotia and hyphae of *Sclerotium rolfsii* Sacc. *Journal of General Microbiology* **57**, 143-147.

Chiu, S. W. & Moore, D. (1988a). Evidence for developmental commitment in the differentiating fruit body of *Coprinus cinereus*. *Transactions of the British Mycological Society* **90**, 247-253.

Chiu, S. W. & Moore, D. (1988b). Ammonium ions and glutamine inhibit sporulation of *Coprinus cinereus* basidia assayed *in vitro*. *Cell Biology International Reports* **12**, 519-526.

Chiu, S. W. & Moore, D. (1990a). Development of the basidiome of *Volvariella bombycina*. *Mycological Research* **94**, 327-337.

Chiu, S. W. & Moore, D. (1990b). A mechanism for gill pattern formation in *Coprinus cinereus*. *Mycological Research* **94**, 320-326.

Chiu, S. W. & Moore, D. (1990c). Sporulation in *Coprinus cinereus*: use of an *in vitro* assay to establish the major landmarks in differentiation. *Mycological Research* **94**, 249-253.

Chiu, S. W. & Moore, D. (1993). Cell form, function and lineage in the hymenia of *Coprinus cinereus* and *Volvariella bombycina*. *Mycological Research* **97**, 221-226.

Chiu, S. W., Moore, D. & Chang, S. T. (1989). Basidiome polymorphism in *Volvariella bombycina*. *Mycological Research* **92,** 69-77.

Coggins, C. R., Hornung, U., Jennings, D. H. & Veltkamp, C. J. (1980). The phenomenon of `point-growth' and its relation to flushing and strand formation in mycelium of *Serpula lacrimans*. *Transactions of the British Mycological Society* **75**, 69-76.

Coley-Smith, J. R. & Cooke, R. C. (1971). Survival and germination of fungal sclerotia. *Annual Review of Phytopathology* **9**, 65-92.

Colson, B. (1935). The cytology of the mushroom *Psalliota campestris* Quél. *Annals of Botany* **49**, 1-17.

Cooke, R. C. (1983). Morphogenesis of sclerotia. In *Fungal Differentiation, a Contemporary Synthesis*, (ed. J. E. Smith), pp. 397-418. Marcel Dekker: New York.

Corda, A. C. J. (1839). *Icones Fungorum Hucusque Cognitorum III*. Prague.

Corner, E. J. H. (1932). A *Fomes* with two systems of hyphae. *Transactions of the British Mycological Society* **17**, 51-81.

Corner, E. J. H. (1966). *A monograph of cantharelloid fungi.* Annals of Botany Memoirs no. 2. Oxford University Press, London.

Corner, E. J. H. (1991). *Trogia* (Basidiomycetes). *The Garden's Bulletin, Singapore*, supplement **2**, 1-100.

Damsky, C. H. & Werb, Z. (1992). Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. *Current Opinions in Cell Biology* **4**, 772-781.

de Bary, A. (1884). *Vergleichende Morphologie und Biologie der Pilze*. U.S.W.: Leipzig.

de Bary, A. (1887). *Comparative Morphology and Biology of the Fungi*, *Mycetozoa and Bacteria*. Oxford University (Clarendon) Press: London & New York.

de Silva, L. R., Youatt, J., Gooday, G. W. & Gow, N. A. R. (1992). Inwardly directed ionic currents of *Allomyces macrogynus* and other water moulds indicate sites of proton-driven nutrient transport but are incidental to tip growth. *Mycological Research* **96**, 925-931.

de Vries, O. M. H., Hoge, J. H. C. & Wessels, J. G. H. (1980). Translation of RNA from *Schizophyllum commune* in a wheat germ and rabbit reticulocyte cell-free system: comparison of *in vitro* and *in vivo* products after two-dimensional gel electrophoresis. *Biochimica et Biophysica Acta* **607**, 373-378.

de Vries, O. M. H. & Wessels, J. G. H. (1984). Patterns of polypeptide synthesis in non-fruiting monokaryons and a fruiting dikaryon of *Schizophyllum commune*. *Journal of General Microbiology* **133**, 145-154.

Dons, J. J. M., Springer, J., de Vries, S. C. & Wessels, J. G. H. (1984). Molecular cloning of a gene abundantly expressed during fruiting body initiation in *Schizophyllum commune*. *Journal of Bacteriology* **157**, 802-808.

Dormer, K. J. (1980). *Fundamental Tissue Geometry for Biologists*. Cambridge University Press: Cambridge, U.K.

Elhiti, M. M. Y., Butler, R. D. & Moore, D. (1979). Cytochemical localization of glutamate dehydrogenase during carpophore development in *Coprinus cinereus*. *New Phytologist* **82**, 153-157.

Elhiti, M. M. Y., Moore, D. & Butler, R. D. (1987). Ultrastructural distribution of glutamate dehydrogenases during fruit body development in *Coprinus cinereus*. *New Phytologist* **107**, 531-539.

Elliott, T. J. (1985). Developmental genetics - from spore to sporophore. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 451-465. Cambridge University Press: Cambridge, U.K.

Esposito, R. E., Frink, N., Bernstein, P. & Esposito, M. S. (1972). The genetic control of sporulation in *Saccharomyces*. II. Dominance and complementation of mutants of meiosis and spore formation. *Molecular and General Genetics* **114**, 241-248.

Esser, K. & Hoffman, P. (1977). Genetic basis for speciation in higher basidiomycetes with special reference to the genus *Polyporus*. In *The Species Concept in Hymenomycetes* (ed. H. Clémençon), pp.189-214. J. Cramer: Vaduz.

Esser, K. & Meinhardt, F. (1977). A common genetic control of dikaryotic and monokaryotic fruiting in the basidiomycete *Agrocybe aegerita*. *Molecular and General Genetics* **155**, 113-115.

Esser, K. & Straub, J. (1958). Genetische Untersuchungen an *Sordaria macrospora* Auersw., Kompensation und induktion bei genbedingten Entwicklungsdefekten. *Zeitschrift für Vererbungslehre* **89**, 729-746.

Esser, K., Saleh, F. & Meinhardt, F. (1979). Genetics of fruit body production in higher basidiomycetes. II. Monokaryotic and dikaryotic fruiting in *Schizophyllum commune*. *Current Genetics* **1**, 85-88.

Esser, K., Stahl, U. & Meinhardt, F. (1977). Genetic aspects of differentiation in fungi. In *Biotechnology and Fungal Differentiation* (ed. J. Meyrath & J. D. Bu'Lock), pp. 67-75. Academic Press: London.

Evans, H. J. (1959). Nuclear behaviour in the cultivated mushroom. *Chromosoma* **10**, 115-135.

Fayod, V. (1889). Prodrome d'une histoire naturelle des Agaricinés. *Annales des Sciences Naturelles, Botanique Série*, **7-9**, 179-411.

Fisher, R. A. (1928). The possible modifications of the wild type to recurrent mutations. *American Naturalist* **62**, 115-126.

Fisher, R. A. (1931). The evolution of dominance. *Biological Reviews* **6**, 345-368.

Garrett, S.D. (1953). Rhizomorph behaviour in *Armillaria mellea* (Vahl) Quél. I. Factors controlling rhizomorph initiation by *Armillaria mellea* in pure culture. *Annals of Botany* **17**, 63-79.

Garrett, S.D. (1954). Function of the mycelial strands in substrate colonization by the cultivated mushroom *Psalliota hortensis*. *Transactions of the British Mycological Society* **37**, 51-57.
Garrett, S.D. (1956). *Biology of Root-Infecting Fungi*. Cambridge University Press: Cambridge, U.K.
Garrett, S.D. (1960). Inoculum potential. In *Plant Pathology: an Advanced Treatise*, **vol. 3**, (ed. J. G. Horsfall & A. E. Dimond), pp. 23-56. Academic Press: New York & London.
Garrett, S.D. (1970). *Pathogenic Root-Infecting Fungi*. Cambridge University Press: Cambridge, U.K.
Girbardt, M. (1979). A microfilamentous septal belt (FSB) during induction of cytokinesis in *Trametes versicolor* (L. ex Fr.). *Experimental Mycology* **3**, 215-228.
Gooday, G. W. (1985). Elongation of the stipe of *Coprinus cinereus*. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 311-331. Cambridge University Press: Cambridge, U.K.
Granlund, H. I., Jennings, D. H. & Thompson, W. (1985). Translocation of solute along rhizomorphs of *Armillaria mellea*. *Transactions of the British Mycological Society* **84**, 111-119.
Green, J. B. A. & Smith, J. C. (1991). Growth factors as morphogens: do gradients and thresholds establish body plan? *Trends in Genetics* **7**, 245-250.
Gregory, P. H. (1984). The fungal mycelium -- an historical perspective. In *The Ecology and Physiology of the Fungal Mycelium*, (ed. D. H. Jennings & A. D. M. Rayner), pp. 383-417. Cambridge University Press: Cambridge, U.K.
Griffin, D. H., Timberlake, W. E. & Cheney, J. C. (1974). Regulation of macromolecular synthesis, colony development and specific growth rate of *Achlya bisexualis* during balanced growth. *Journal of General Microbiology* **80**, 381-388.
Grove, S. N. (1978). The cytology of hyphal tip growth. In *The Filamentous Fungi*, **vol. 3**, *Developmental Mycology*, (ed. J. E. Smith & D. R. Berry), pp. 28-50. Edward Arnold: London.
Gull, K. (1976). Differentiation of septal ultrastructure according to cell type in the basidiomycete *Agrocybe praecox*. *Journal of Ultrastructure Research* **54**, 89-94.
Gull, K. (1978). Form and function of septa in filamentous fungi. In *The Filamentous Fungi*, **vol. 3**, *Developmental Mycology*, (ed. J. E. Smith & D. R. Berry), pp. 78-93. Edward Arnold: London.
Hammad, F., Watling, R. & Moore, D. (1993a). Cell population dynamics in *Coprinus cinereus*: narrow and inflated hyphae in the basidiome stipe. *Mycological Research* **97**, 269-274.
Hammad, F., Ji, J., Watling, R. & Moore, D. (1993b). Cell population dynamics in *Coprinus cinereus*: co-ordination of cell inflation throughout the maturing basidiome. *Mycological Research* **97**, 275-282.
Harold, F. M. & Caldwell, J. H. (1990). Tips and currents: electrobiology of apical growth. In *Tip Growth in Plant and Fungal Cells*, (ed. I. B. Heath), pp.59-90. Academic Press, Inc.: San Diego & London.
Hedger, J. N. (1985). Tropical agarics: resource relations and fruiting periodicity. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 41-86. Cambridge University Press: Cambridge, U.K.
Hedger, J. N., Lewis, P. & Gitay, H. (1993). Litter-trapping by fungi in moist tropical forest. In *Aspects of Tropical Mycology*, (ed. S. Isaac, R. Watling, A. J. S. Whalley & J. C. Frankland), pp. in press. Cambridge University Press: Cambridge, U.K.
Hereward, F. V. & Moore, D. (1979). Polymorphic variation in the structure of aerial sclerotia of *Coprinus cinereus*. *Journal of General Microbiology* **113**, 13-18.
Horner, J. & Moore, D. (1987). Cystidial morphogenetic field in the hymenium of *Coprinus cinereus*. *Transactions of the British Mycological Society* **88**, 479-488.
Horton, J. S. & Raper, C. A. (1991). A mushroom-inducing DNA sequence isolated from the basidiomycete, *Schizophyllum commune*. *Genetics* **129**, 707-716.
Hughes, S. J. (1953). Conidiophores, conidia, and classification. *Canadian Journal of Botany* **31**, 577-659.
Hughes, S. J. (1971). On conidia of fungi, and gemmae of algae, bryophytes, and pteridophytes. *Canadian Journal of Botany* **49**, 1319-1339.
Hynes, R. O. (1992). Integrins: versatility, modulation, and signalling in cell adhesion.*Cell* **69**, 11-25.
Ingold, C. T. (1979). *The Nature of Toadstools*. Studies in Biology Series, no. 113. Edward Arnold: London.
Jacques-Félix, M. (1967). Recherches morphologiques, anatomiques, morphogénétiques et physiologiques sur des rhizomorphes de champignons supérieurs et sur le déterminisme de leur formation. I. Observations sur les formations 'synnémiques' des champignons supérieurs dans le milieu naturel. *Bulletin Trimestrial de la Societé Mycologique de France* **83**, 5-103.
Jennings, D. H. (1991). The physiology and biochemistry of the vegetative mycelium. In *Serpula lacrymans: Fundamental Biology and Control Strategies*, (ed. D. H. Jennings & A. F. Bravery), pp. 55-79. John Wiley & Sons: Chichester.
Jennings, D. H. & Bravery, A. F. (1991). *Serpula lacrymans: Fundamental Biology and Control Strategies*. John Wiley & Sons: Chichester.
Jennings, D. H. & Watkinson, S. C. (1982). Structure and development of mycelial strands in *Serpula lacrimans*. *Transactions of the British Mycological Society* **78**, 465-474.
Ji, J. & Moore, D. (1993). Glycogen metabolism in relation to fruit body maturation in *Coprinus cinereus*. *Mycological Research* **97**, 283-289.

Johnson, T. E. (1976). Analysis of pattern formation in Neurospora perithecial development using genetic mosaics. *Developmental Biology* **54**, 23-36.
Johnson, T. E. (1978). Isolation and characterisation of perithecial development mutants in *Neurospora*. *Genetics* **88**, 27-47.
Kamada, T. & Takemaru, T. (1977a). Stipe elongation during basidiocarp maturation in *Coprinus macrorhizus*: mechanical properties of stipe cell wall. *Plant & Cell Physiology* **18**, 831-840.
Kamada, T. & Takemaru, T. (1977b). Stipe elongation during basidiocarp maturation in *Coprinus macrorhizus*:changes in polysaccharide composition of stipe cell wall during elongation. *Plant & Cell Physiology* **18**, 1291-1300.
Kamada, T. & Takemaru, T. (1983). Modifications of cell wall polysaccharides during stipe elongation in the basidiomycete *Coprinus cinereus*. *Journal of General Microbiology* **129**, 703-709.
Kamada, T., Sumiyoshi, T., Shindo, Y. & Takemaru, T. (1989). Isolation and genetic analysis of resistant mutants to the benzimidazole fungicide benomyl in *Coprinus cinereus*. *Current Microbiology* **18**, 215-218.
Kamada, T., Takemaru, T., Prosser, J. I. & Gooday, G. W. (1991). Right and left handed helicity of chitin microfibrils in stipe cells in *Coprinus cinereus*. *Protoplasma* **165**, 64-70.
Kamada, T. & Tsuru, M. (1993). The onset of the helical arrangement of chitin microfibrils in fruit body development of *Coprinus cinereus*. *Mycological Research* **97**, in press.
Kanda, T. & Ishikawa, T. (1986). Isolation of recessive developmental mutants in *Coprinus cinereus*. *Journal of General and Applied Microbiology* **32**, 541-543.
Kanda, T., Goto, A., Sawa, K., Arakawa, H., Yasuda, Y. & Takemaru, T. (1989a). Isolation and characterization of recessive sporeless mutants in the basidiomycete *Coprinus cinereus*. *Molecular and General Genetics* **216**, 526-529.
Kanda, T., Ishihara, H. & Takemaru, T. (1989b). Genetic analysis of recessive primordiumless mutants in the basidiomycete *Coprinus cinereus*. *Botanical Magazine, Tokyo* **102**, 561-564.
Kanda, T., Arakawa, H., Yasuda, Y. & Takemaru, T. (1990). Basidiospore formation in a mutant of the incompatibility factors and mutants that arrest at meta-anaphase I in *Coprinus cinereus*. *Experimental Mycology* **14**, 218-226.
Kemp, R. F. O. (1977). Oidial homing and the taxonomy and speciation of basidiomycetes with special reference to the genus *Coprinus*. In *The Species Concept in Hymenomycetes*, (ed. H. Clémençon), pp. 259-273. Cramer: Vaduz.
Kihn, J. C., Masy, C. L. & Mestdagh, M. M. (1988). Yeast flocculation: competition between nonspecific repulsion and specific bonding in cell adhesion. *Canadian Journal of Microbiology* **34**, 773-778.
Kligman, A. M. (1943). Some cultural and genetic problems in the cultivation of the mushroom *Agaricus campestris*. *American Journal of Botany* **30**, 745-763.
Kropf, D. L., Caldwell, J. C., Gow, N. A. R. & Harold, F. M. (1984). Transcellular ion currents in the water mould *Achlya*. Amino acid proton symport as a mechanism of current entry. *Journal of Cell Biology* **99**, 486-496.
Kropf, D. L., Lupa, M. D. A., Caldwell, J. C. & Harold, F. M. (1983). Cell polarity: endogenous ion currents precede and predict branching in the water mould *Achlya*. *Science* **220**, 1385-1387.
Kües, U. & Casselton, L. A. (1992). Fungal mating type genes -- regulators of sexual development. *Mycological Research* **96**, 993-1006.
Lentz, P. L. (1971). Analysis of modified hyphae as a tool in taxonomic research in the higher Basidiomycetes. In *Evolution in the Higher Basidiomycetes* (ed. R. H. Petersen), pp. 99-127. University of Tennessee Press: Knoxville.
Leonard, T. J. & Dick, S. (1968). Chemical induction of haploid fruiting bodies in *Schizophyllum commune*. *Proceedings of the National Academy of Sciences, U.S.A*. **59**, 745-751.
Leslie, J. F. & Leonard, T. J. (1979a). Three independent genetic systems that control initiation of a fungal fruiting body. *Molecular and General Genetics* **171**, 257-260.
Leslie, J. F. & Leonard, T. J. (1979b). Monokaryotic fruiting in *Schizophyllum commune*: genetic control of the response to mechanical injury. *Molecular and General Genetics* **175**, 5-12.
Lewis, D. & Vakeria, D. (1977). Resistance to *p*-fluorophenylalanine in diploid/haploid dikaryons: dominance modifier gene explained as a controller of hybrid multimer formation. *Genetical Research* **30**, 31-43.
Locquin, M. (1953). Recherches sur l'organisation et le développement des Agarics, des Bolets et des Clavulaires. *Bulletin de la Société Mycologique de France* **69**, 389-402.
Lu, B. C. (1991). Cell degeneration and gill remodelling during basidiocarp development in the fungus *Coprinus cinereus*. *Canadian Journal of Botany* **69**, 1161-1169.
Macfarlane, T. D., Kuo, J. & Hilton, R. N. (1978). Structure of the giant sclerotium of *Polyporus mylittae*. *Transactions of the British Mycological Society* **71**, 359-365.
Manning, J. T. (1976). Is sex maintained to facilitate or minimise mutational advance? *Heredity* **36**, 351-357.
Manning, J. T. (1977). The evolution of dominance: Haldane v Fisher revisited. *Heredity* **38**, 117-119.
Margulis, L. (1974). Five-Kingdom classification and the origin and evolution of cells. *Evolutionary Biology* **7**, 45-78.
Mathew, K. T. (1961). Morphogenesis of mycelial strands in the cultivated mushroom *Agaricus bisporus*. *Transactions of the British Mycological Society* **44**, 285-290.
Matthews, T. R. & Niederpruem, D. J. (1972). Differentiation in *Coprinus lagopus*. I. Control of fruiting and cytology of initial events. *Archives of Microbiology* **87**, 257-268.
McGillivray, A. M. & Gow, N. A. R. (1986). Applied electrical fields polarize the growth of mycelial fungi. *Journal of General Microbiology* **132**, 2515-2525.

Meinhardt, F. & Esser, K. (1983). Genetic aspects of sexual differentiation in fungi. In *Fungal Differentiation* (ed. J. E. Smith), pp. 537-557.Marcel Dekker: New York.

Meinhardt, H. (1984). Models of pattern formation and their application to plant development. In *Positional Controls in Plant Development*, (ed. P. W. Barlow & D. J. Carr), pp. 1-32. Cambridge University Press: Cambridge, U.K.

Meinhardt, H. & Gierer, A. (1974). Applications of a theory of biological pattern formation based on lateral inhibition. *Journal of Cell Science* **15**, 321-346.

Miller, J. H. (1980). Orientation of the plane of cell division in fern gametophytes: the roles of cell shape and stress. *American Journal of Botany* **67**, 534-542.

Miyake, H., Takemaru, T. & Ishikawa, T. (1980). Sequential production of enzymes and basidiospore formation in fruiting bodies of *Coprinus macrorhizus*. *Archives of Microbiology* **126**, 201-205.

Miyake, H., Tanaka, K. & Ishikawa, T. (1980). Basidiospore formation in monokaryotic fruiting bodies of a mutant strain of *Coprinus macrorhizus*. *Archives of Microbiology* **126**, 207-212.

Miyata, M., Miyata H. & Johnson, B. F. (1986). Establishment of septum orientation in a morphologically altered fission yeast, *Schizosaccharomyces pombe*. *Journal of General Microbiology* **132**, 2535-2540.

Mol, P. C., Vermeulen, C. A. & Wessels, J. G. H. (1990). Diffuse extension of hyphae in stipes of *Agaricus bisporus* may be based on a unique wall structure. *Mycological Research* **94**, 480-488.

Moore, D. (1981). Developmental genetics of *Coprinus cinereus*: genetic evidence that carpophores and sclerotia share a common pathway of initiation. *Current Genetics* **3**, 145-150.

Moore, D. (1984a). Positional control of development in fungi. In *Positional Controls in Plant Development*, (ed. P. W. Barlow & D. J. Carr), pp. 107-135. Cambridge University Press: Cambridge, U.K.

Moore, D. (1984b). Developmental biology of the *Coprinus cinereus* carpophore: metabolic regulation in relation to cap morphogenesis. *Experimental Mycology* **8**, 283-297.

Moore, D. (1987). The formation of agaric gills. *Transactions of the British Mycological Society* **89**, 105-108.

Moore, D. (1991). Perception and response to gravity in higher fungi - a critical appraisal. *New Phytologist* **117**, 3-23.

Moore, D., Elhiti, M. M. Y. & Butler, R. D. (1979). Morphogenesis of the carpophore of *Coprinus cinereus*. *New Phytologist* **83**, 695-722.

Moore, D., Horner, J. & Liu, M. (1987). Co-ordinate control of ammonium-scavenging enzymes in the fruit body cap of *Coprinus cinereus* avoids inhibition of sporulation by ammonium. *FEMS Microbiology Letters* **44**, 239-242.

Moore, D. & Jirjis, R. I. (1981). Electrophoretic studies of carpophore development in the basidiomycete *Coprinus cinereus*. *New Phytologist* **87**, 101-113.

Moore, D., Liu, M. & Kuhad, R. C. (1987). Karyogamy-dependent enzyme derepression in the basidiomycete *Coprinus*. *Cell Biology International Reports* **11**, 335-341.

Moore, R. T. (1985). The challenge of the dolipore/parenthesome septum. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 175-212. Cambridge University Press: Cambridge, U.K.

Motta, J. J. (1967). A note on the mitotic apparatus in the rhizomorph meristem of *Armillaria mellea*. *Mycologia* **59**, 370-375.

Motta, J. J. (1969). Cytology and morphogenesis in the rhizomorph of *Armillaria mellea*. *American Journal of Botany* **56**, 610-619.

Motta, J. J. (1971). Histochemistry of the rhizomorph meristem of *Armillaria mellea*. *American Journal of Botany* **58**, 80-87.

Motta, J. J. & Peabody, D. C. (1982). Rhizomorph cytology and morphogenesis in *Armillaria tabescens*. *Mycologia* **74**, 671-674.

Mulder, G. H. & Wessels, J. G. H. (1986). Molecular cloning of RNAs differentially expressed in monokaryons and dikaryons of *Schizophyllum commune* in relation to fruiting. *Experimental Mycology* **10**, 214-227.

Nasrallah, J. B., & Srb, A. M. (1973). Genetically related protein variants specifically associated with fruiting body maturation in *Neurospora*. *Proceedings of the National Academy of Sciences, U.S.A.* 70, 1891-1893.

Nasrallah, J. B. & Srb, A. M. (1977). Occurrence of a major protein associated with fruiting body development in *Neurospora* and related Ascomycetes. *Proceedings of the National Academy of Sciences, U.S.A*. 74, 3831-3834.

Nasrallah, J. B. & Srb, A. M. (1978). Immunofluorescent localization of a phase-specific protein in *Neurospora tetrasperma* perithecia. *Experimental Mycology* **2**, 211-215.

Nathan, C. & Sporn, M. (1991). Cytokines in context. *Journal of Cell Biology* **113**, 981-986.

Nuss, I., Jennings, D. H. & Veltkamp, C. J. (1991). Morphology of *Serpula lacrymans*. In *Serpula lacrymans: Fundamental Biology and Control Strategies*, (ed. D. H. Jennings & A. F. Bravery), pp. 9-38. John Wiley & Sons: Chichester.

Nyunoya, H. & Ishikawa, T. (1979). Control of unusual hyphal morphology in a mutant of *Coprinus macrorhizus*. *Japanese Journal of Genetics* **54**, 11-20.

Powell, K. A. & Rayner, A. D. M. (1983). Ultrastructure of the rhizomorph apex in *Armillaria bulbosa* in relation to mucilage production. *Transactions of the British Mycological Society* **81**, 529-534.

Prosser, J. I. (1993). Growth kinetics of mycelial colonies and hyphal aggregates of ascomycetes. *Mycological Research* **97**, in press.

Pukkila, P. J. & Casselton, L. A. (1991). Molecular genetics of the agaric *Coprinus cinereus*. *More Gene Manipulations in Fungi*, (ed. J. W. Bennett & L. A. Lasure), pp. 126-150. Academic Press: New York.

Rahary, L., Bonaly, R., Lematre, J. & Poulain, D. (1985). Aggregation and disaggregation of *Candida albicans* germ tubes. *FEMS Microbiology Letters* **30**, 383-387.

Raper, J. R. & Krongelb, G. S. (1958). Genetic and environmental aspects of fruiting in *Schizophyllum commune* Fr. *Mycologia* **50**, 707-740.

Rayner, A. D. M. (1993). Differential insulation and the generation of mycelial patterns. In *Shape and Form in Plants and Fungi*, (ed. D. S. Ingram), pp. *in press*. Academic Press: London.

Rayner, A. D. M., Powell, K. A., Thompson, W. & Jennings, D. H. (1985). Morphogenesis of vegetative organs. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 249-279. Cambridge University Press: Cambridge, U.K.

Rayner, A. D. M., Watling, R. & Frankland, J. C. (1985). Resource relations -- an overview. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 1-40. Cambridge University Press: Cambridge, U.K.

Rayner, A. D. M. & Webber, J. F. (1984). Interspecific mycelial interactions -- an overview. In *The Ecology and Physiology of the Fungal Mycelium*, (ed. D. H. Jennings & A. D. M. Rayner), pp. 383-417. Cambridge University Press: Cambridge, U.K.

Read, D. J. (1991). Mycorrhizas in ecosystems -- Nature's response to the "Law of the Minimum". In *Frontiers of Mycology*, (ed. D. L. Hawksworth), pp. 101-130. CAB International: Wallingford, U.K.

Read, D. J. & Armstrong, W. (1972). A relationship between oxygen transport and the formation of the ectotrophic mycorrhizal sheath in conifer seedlings. *New Phytologist* **71**, 49-53.

Read, D. J., Leake, J. R. & Langdale, A. R. (1989). The nitrogen nutrition of mycorrhizal fungi and their host plants. In *Nitrogen, Phosphorus and Sulphur Utilization by Fungi*, (ed. L. Boddy, R. Marchant & D. J. Read), pp. 181-204. Cambridge University Press: Cambridge, U.K.

Read, N. D. (1983). A scanning electron microscopic study of the external features of perithecium development in *Sordaria humana*. *Canadian Journal of Botany* **61**, 3217-3229.

Read, N. D. (1993). Multicellular development in fungi. In *Shape and Form in Plants and Fungi*, (ed. D. S. Ingram), pp. *in press*. Academic Press: London.

Read, N. D. & Beckett, A. (1985). The anatomy of the mature perithecium in *Sordaria humana*; and its significance for fungal multicellular development. *Canadian Journal of Botany* **63**, 281-296.

Redhead, S. A. (1987). The Xerulaceae (Basidiomycetes), a family with sarcodimitic tissues. *Canadian Journal of Botany* **65**, 1551-1562.

Reijnders, A. F. M. (1948). Études sur le développement et l'organisation histologique des carpophores dans les Agaricales. *Recuil des Travaux Botaniques Néerlandais* **41**, 213-396.

Reijnders, A. F. M. (1963). *Les problèmes du développement des carpophores des Agaricales et de quelques groupes voisins.* Dr W. Junk: The Hague.

Reijnders, A. F. M. (1976). Recherches sur le développement et l'histogénèse dans les Asterosporales. *Persoonia* **9**, 65-83.

Reijnders, A. F. M. (1977). The histogenesis of bulb and trama tissue of the higher Basidiomycetes and its phylogenetic implications. *Persoonia* **9**, 329-362.

Reijnders, A. F. M. (1979). Developmental anatomy of *Coprinus*. *Persoonia* **10**, 383-424.

Reijnders, A. F. M. (1993). On the origin of specialised trama types in the Agaricales. *Mycological Research* **97**, 257-268.

Reijnders, A. F. M. & Moore, D. (1985). Developmental biology of agarics - an overview. In *Developmental Biology of Higher Fungi*, (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 333-351. Cambridge University Press: Cambridge, U.K.

Reynolds, D. R. (1981). *Ascomycete Systematics: The Luttrellian Concept*. Springer-Verlag: New York.

Rishbeth, J. (1985). *Armillaria*: resources and hosts. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 87-101. Cambridge University Press: Cambridge, U.K.

Rosin, I. V., Horner, J. & Moore, D. (1985). Differentiation and pattern formation in the fruit body cap of *Coprinus cinereus*. In: *Developmental Biology of Higher Fungi*, (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 333-351. Cambridge University Press: Cambridge, U.K.

Rosin, I. V. & Moore, D. (1985a). Origin of the hymenophore and establishment of major tissue domains during fruit body development in *Coprinus cinereus*. *Transactions of the British Mycological Society* **84**, 609-619.

Rosin, I. V. & Moore, D. (1985b). Differentiation of the hymenium in *Coprinus cinereus*. *Transactions of the British Mycological Society* **84**, 621-628.

Ruiters, M. H. J. & Wessels, J. G. H. (1989a). *In situ* localization of specific RNAs in whole fruiting colonies of *Schizophyllum commune*. *Journal of General Microbiology* **135**, 1747-1754.

Ruiters, M. H. J. & Wessels, J. G. H. (1989b). *In situ* localization of specific RNAs in developing fruit bodies of the basidiomycete *Schizophyllum commune*. *Experimental Mycology* **13**, 212-222.

Russo, G. M., Dahlberg, K. R. & Van Etten, J. L. (1982). Identification of a development-specific protein in sclerotia of *Sclerotinia sclerotiorum*. *Experimental Mycology* **6**, 259-267.

Schuren, F. H. J., van der Lende, T. R. & Wessels, J. G. H. (1993). Fruiting genes of *Schizophyllum commune* are transcriptionally regulated. *Mycological Research* **97**, in press.

Senathirajah, S. & Lewis, D. (1975). Resistance to amino acid analogues in *Coprinus*: dominance modifier genes and dominance reversal in dikaryons and diploids. *Genetical Research* **25**, 95-107.

Sheppard, P. M. (1967). *Natural Selection and Heredity*. Hutchinson: London.
Slack, J. M. W. (1983). *From Egg to Embryo: Determinative Events in Early Development*. Cambridge University Press: Cambridge, U.K.
Smith, A. H. (1966). The hyphal structure of the basidiocarp. In *The Fungi: An Advanced Treatise*, **vol. II**, (ed. G. C. Ainsworth & A. S. Sussman), pp. 151-177. Academic Press: New York & London.
Snider, P. J. (1959). Stages of development in rhizomorphic thalli of *Armillaria mellea*. *Mycologia* **51**, 693-707.
Stahl, U. & Esser, K. (1976). Genetics of fruit body production in higher basidiomycetes. I. Monokaryotic fruiting and its correlation with dikaryotic fruiting in *Polyporus ciliatus*. *Molecular and General Genetics* **148**, 183-197.
Stephenson, N. A. & Gooday, G. W. (1984). Nuclear numbers in the stipe cells of *Coprinus cinereus*. *Transactions of the British Mycological Society* **82**, 531-534.
Streuli, C. H. (1993). Extracellular matrix and gene expression in mammary epithelium. *Seminars in Cell Biology*, in press
Sussman, A. S. (1968). Longevity and survivability of fungi. In *The Fungi: An Advanced Treatise*, **vol. III**, (ed. G. C. Ainsworth & A. S. Sussman), pp. 447-486. Academic Press: New York & London.
Swamy, S., Uno, I & Ishikawa, T. (1984). Morphogenetic effects of mutations at the *A* and *B* incompatibility factors in *Coprinus cinereus*. *Journal of General Microbiology* **130**, 3219-3224.
Takemaru, T. & Kamada, T. (1971). Gene control of basidiocarp development in *Coprinus macrorhizus*. *Reports of the Tottori Mycological Institute, Japan* **9**, 21-35.
Takemaru, T. & Kamada, T. (1972). Basidiocarp development in *Coprinus macrorhizus*. I. Induction of developmental variations. *Botanical Magazine (Tokyo)* **85**, 51-57.
Talbot, P. H. B. (1968). Fossilized pre-Patouillardian taxonomy? *Taxon* **17**, 622-628.
Thompson, W. (1984). Distribution, development and functioning of mycelial cord systems of decomposer basidiomycetes of the deciduous woodland floor. In *The Ecology and Physiology of the Fungal Mycelium*, (ed. D. H. Jennings & A. D. M. Rayner), pp. 185-214. Cambridge University Press: Cambridge, U.K.
Timberlake, W. E. (1980).Developmental gene regulation in *Aspergillus nidulans*. *Developmental Biology* **78**, 497-510.
Timberlake, W. E. & Marshall, M. A. (1988). Genetic regulation of development in *Aspergillus nidulans*. *Trends in Genetics* **4**, 162-169.
Todd, N. K. & Aylmore, R. C. (1985). Cytology of hyphal interactions and reactions in *Schizophyllum commune*. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 231-248. Cambridge University Press: Cambridge, U.K.
Townsend, B. B. (1954). Morphology and development of fungal rhizomorphs. *Transactions of the British Mycological Society* **37**, 222-233.
Townsend, B. B. & Willetts, H. J. (1954). The development of sclerotia of certain fungi. *Transactions of the British Mycological Society* **37**, 213-221.
Trinci, A. P. J. (1974). A study of the kinetics of hyphal extension and branch initiation of fungal mycelia. *Journal of General Microbiology* **81**, 225-236.
Trinci, A. P. J. (1978). The duplication cycle and vegetative development in moulds. In *The Filamentous Fungi*, **vol. 3**, *Developmental Mycology*, (ed. J. E. Smith & D. R. Berry), pp. 133-163. Edward Arnold: London.
Trinci, A. P. J. (1984). Regulation of hyphal branching and hyphal orientation.In *The Ecology and Physiology of the Fungal Mycelium*, (ed. D. H. Jennings & A. D. M. Rayner), pp. 23-52. Cambridge University Press: Cambridge, U.K.
Trinci, A. P. J. & Banbury, G. H. (1967). A study of the tall conidiophores of *Aspergillus giganteus*. *Transactions of the British Mycological Society* **50**, 525-538.
Tubaki, K. (1966). Sporulating structures in Fungi Imperfecti. In *The Fungi: An Advanced Treatise*, **vol. II**, (ed. G. C. Ainsworth & A. S. Sussman), pp. 113-131. Academic Press: New York & London.
Turian, G. (1978). Sexual morphogenesis in the Ascomycetes. In *The Filamentous Fungi*, **vol. 3**, *Developmental Mycology*, (ed. J. E. Smith & D. R. Berry), pp. 315-333. Edward Arnold: London.
Ullrich, R. C. (1973). Sexuality, incompatibility, and intersterility in the biology of the *Sistotrema brinkmannii* aggregate. *Mycologia* **65**, 1234-1249.
Uno, I., & Ishikawa, T. (1971). Chemical and genetical control of induction of monokaryotic fruiting bodies in *Coprinus macrorhizus*. *Molecular and General Genetics* **113**, 228-239.
Van der Valk, P. & Marchant, R. (1978). Hyphal ultrastructure in fruit body primordia of the basidiomycetes *Schizophyllum commune* and *Coprinus cinereus*. *Protoplasma* **95**, 57-72.
Waters, H. (1972). *Aspects of sclerotium morphogenesis in Coprinus lagopus (sensu) Bull.* Ph.D. Thesis, University of Manchester.
Waters, H., Moore, D. & Butler, R. D. (1975a). Morphogenesis of aerial sclerotia of *Coprinus lagopus*. *New Phytologist*, **74**, 207-213.
Waters, H., Butler, R. D. & Moore, D. (1975b). Structure of aerial and submerged sclerotia of *Coprinus lagopus*. *New Phytologist* **74**, 199-205.
Watkinson, S. C. (1971). The mechanism of mycelial strand induction in *Serpula lacrimans*: a possible effect of nutrient distribution. *New Phytologist* **70**, 1079-1088.
Watkinson, S. C. (1975). The relation between nitrogen nutrition and the formation of mycelial strands in *Serpula lacrimans*. *Transactions of the British Mycological Society* **64**, 195-200.

Watkinson, S. C. (1979). Growth of rhizomorphs, mycelial strands coremia and sclerotia. In *Fungal Walls and Hyphal Growth*, (ed. J. H. Burnett & A. P. J. Trinci), pp. 93-113. Cambridge University Press: Cambridge, U.K.

Watling, R. (1971). Polymorphism in *Psilocybe merdaria*. *New Phytologist* **70**, 307-326.

Watling, R. (1985). Developmental characters of agarics. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 281-310. Cambridge University Press: Cambridge, U.K.

Watling, R. & Moore, D. (1993). Moulding moulds into mushrooms: shape and form in the higher fungi. In *Shape and Form in Plants and Fungi*, (ed. D. S. Ingram), pp. *in press*. Academic Press: London.

Watling, R. & Nicoll, H. (1980). Sphaerocysts in *Lactarius rufus*. *Transactions of the British Mycological Society* **75**, 331-333.

Webster, J. (1980). *Introduction to Fungi*, 2nd ed. Cambridge University Press: Cambridge, U.K.

Wessels, J. G. H. (1992). Gene expression during fruiting in *Schizophyllum commune*. *Mycological Research* **96**, 609-620.

Wessels, J. G. H., Mulder, G. H. & Springer, J. (1987). Expression of dikaryon-specific and non-specific mRNAs of *Schizophyllum commune* in relation to environmental conditions and fruiting. *Journal of General Microbiology* **133**, 2557-2561.

Whittaker, R. H. (1969). New concepts of kingdoms of organisms. *Science* **163**, 150-160.

Willetts, H. J. (1968). The development of stromata of *Sclerotinia fructicola* and related species. II. In fruits. *Transactions of the British Mycological Society* **51**, 633-642.

Willetts, H. J. (1969). Structure of the outer surfaces of sclerotia of certain fungi. *Archiv für Mikrobiologie* **69**, 48-53.

Willetts, H. J. (1971). The survival of fungal sclerotia under adverse environmental conditions. *Biological Reviews* **46**, 387-407.

Willetts, H. J. (1972). The morphogenesis and possible evolutionary origins of fungal sclerotia. *Biological Reviews* **47**, 515-536.

Willetts, H. J. & Bullock, S. (1992). Developmental biology of sclerotia. *Mycological Research*. **96**, 801-816.

Willetts, H. J. & Calonge, F. D. (1969). Spore development in the brown rot fungi (*Sclerotinia* spp.). *New Phytologist* **68**, 123-131.

Willetts, H. J. & Wong, A. L. (1971). Ontogenetic diversity of sclerotia of *Sclerotinia sclerotiorum* and related species. *Transactions of the British Mycological Society* **57**, 515-524.

Williams, M. A. J. (1986). *Studies on the structure and development of Flammulina velutipes (Curtis: Fries) Singer*. Ph.D. Thesis, University of Bristol.

Williams, M. A. J., Beckett, A. & Read, N. D. (1985). Ultrastructural aspects of fruit body differentiation in *Flammulina velutipes*. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 429-450. Cambridge University Press: Cambridge, U.K.

Wittler, R., Baumgartl, H., Lubbers, D. W. & Shugerl, K. (1986). Investigations of oxygen transfer into *Penicilliun chrysogenum* pellets by microprobe measurements. *Biotechnology and Bioengineering* **28**, 1024-1036.

Yashar, B. M. & Pukkila, P. J. (1985). Changes in polyadenylated RNA sequences associated with fruiting body morphogenesis in *Coprinus cinereus*. *Transactions of the British Mycological Society* **84**, 215-226.

Zantinge, B., Dons, H. & Wessels, J. G. H. (1979). Comparison of poly(A)-containing RNAs in different cell types of the lower eukaryote *Schizophyllum commune*. *European Journal of Biochemistry* **101**, 251-260.

Zolan, M. E., Tremel, C. J. & Pukkila, P. J. (1988). Production and characterization of radiation-sensitive meiotic mutants of *Coprinus cinereus*. *Genetics* **120**, 379-387.