This is a replica of a manuscript published as Chapter 6 in the book *Patterns in Fungal Development* the full reference being:

Bourne, A.N., Chiu, S.W. & Moore, D. (1996). Experimental approaches to the study of pattern formation in *Coprinus cinereus*. In *Patterns in Fungal Development*, (S.W. Chiu & D. Moore, eds), pp. 126-155. Cambridge, UK: Cambridge University Press. ISBN-10: 0521560470, ISBN-13: 978-0521560474.

Chapter 6
Experimental approaches to the study of pattern formation in *Coprinus cinereus*

Adrian N. Bourne, Siu-Wai Chiu & David Moore

Summary
The basidiomycete *Coprinus cinereus* has some special features which particularly suit it to experimental studies. In addition to ease of culture, which makes conventional approaches with various cytological techniques (including continuous video observation) practicable, we show in this chapter how a number of transplantation and bioassay experiments have been used to identify the sequence of commitment steps culminating in the final differentiated stage of sporulation in the meiocyte (basidium). Multicellular fruit bodies have the functions to produce, protect, support and help dispersal of the spores. We present here various case studies which have demonstrated the morphogens in control of the fruit body phenotype and identified the independent but co-ordinated morphogenetic subroutines which form a normal fruit body. These experimental approaches to agaric developmental biology enable formulation of various working models of fungal cell differentiation, tissue patterning and fruit body morphogenesis, thus providing a unifying theme for categorising fruit body ontogeny and for clarifying phylogenetic and taxonomic relationships.

Introduction

Although study of fungal developmental biology is not as flourishing as similar studies with plant and animal systems, a number of approaches have been pioneered in *Coprinus* which could be more widely applied. In addition to continuous monitoring and measurement on fruiting development (see Chapter 1), these approaches include surgical intervention in fruit body morphogenesis, explantation of fruit body tissues to defined media *in vitro*, use of metabolic inhibitors to interfere with development, study of mutants with defects in known developmental processes and use of tropic responses as models of morphogenesis (see Chapter 7).

Coprinus cinereus fruits readily on horse dung manure or semi-synthetic media within 14 to 21 days under controlled conditions (Anderson, 1971; Morimoto & Oda, 1973; Morimoto, Suda & Sagara, 1981; Moore & Pukkila, 1985). This fungus shows a typical haploid monokaryon - dikaryotic life cycle (Chiu & Moore, 1993; see Chapter 5). The genus *Coprinus* is unique because it carries out synchronous meiosis. This makes it a very convenient subject for study of the meiotic division both cytologically and biochemically (Lu, 1982; Montgomery & Lu, 1990; Moore & Pukkila, 1985; Pukkila, Shannon & Skrzynia, 1995; Zolan *et al*., 1995). Progress in the meiocyte pathway can be manipulated by environmental factors such as light and temperature (Lu & Chiu, 1978; Moore, Horner & Liu, 1978; Kamada, Kurita & Takemaru, 1978) or addition of chemicals such as hydroxyurea (Raudaskoski & Lu, 1980; Lu, 1982; Chiu & Moore, 1988b). This is also a good experimental tool for conventional genetic and cytogenetic as well as molecular analyses (Moore & Pukkila, 1985; Casselton *et al*., 1995; see Chapter 5) since it produces abundant oidia in monokaryons as well as sexual homokaryotic binucleate basidiospores. Both types of spores germinate readily on conventional agar media.

Surgery and explantation

Mycologists expect to be able to recover vegetative mycelial cultures from the tissues of fruit

bodies (and other multicellular structures) collected in the field. This expectation is more often fulfilled than not, and usually with quite simple media. In addition, it is frequently possible to find appropriate environmental and nutritional conditions in which to cultivate the resultant isolate to reform the fruit body *in vitro*. Neither plant nor animal scientists can contemplate such routine preparation of cell cultures from excised slivers of fully differentiated tissues, still less the regeneration of the whole organism. Such experience raises the question whether fungal multicellular structures consist of cells as fully committed to a differentiated state as are their plant or animal counterparts (see Section entitled *Fuzzy logic in fungal differentiation* in Chapter 1), but here we wish to emphasise the processes which do continue in explanted tissues rather than those which default to the vegetative state.

Renewed fruiting

Renewed fruiting is a phenomenon distinct from regeneration. In it, fragments of a fruit body explanted to a nutrient-rich or even water agar medium produce a new crop of fruit bodies with unusual rapidity instead of regenerating the missing tissues. Such rapid formation of fruit body primordia on excised fruit body tissues is not uncommon in agarics, but the high degree of developmental synchrony characteristic of the smaller *Coprinus* species permits assessment of its dependence on the physiological state of the tissue. The phenomenon was systematically investigated in *Coprinus congregatus* by Bastouill-Descollonges & Manachère (1984) who used the phrases '... this potential for direct regeneration ... remains "memorized" in the inocula' and '... the competence of hymenial lamellae to sporulate in an autonomous way ...', clearly implying their belief that fruit body tissues used as inocula in such experiments are in some way committed or channelled towards fruit body construction. Whether this represents some sort of cytological memory (and see Chapter 2) has not been clearly established. However, we have verified that explanted fruit body fragments of *C. cinereus* behave similarly (Chiu & Moore, 1988a).

Table 1. Fruiting pattern of cultures inoculated with gills of *Coprinus cinereus*

Physiological age at explantation	Number of cultures	Fruiting pattern observed (% of cultures)		
		Direct*	Indirect	Mixed
Dikaryotic (prekaryogamy)	19	63	32	5
Prophase I	38	24	37	39
Meiotic division	25	8	32	60
Sporulation	39	18	26	56

*, see text for definitions. Data from Chiu & Moore, 1988a.

Renewed fruiting is described as 'direct' when the new primordia are formed only on the original inoculum; as 'indirect' when primordia form only on the outgrowing mycelium; or as 'mixed' when primordia form on both inoculum and mycelium. All cultures inoculated with the basal portions of stems from normally-grown fruit bodies (16 samples) and all parts of the pseudorhizas (= extended stem bases) of dark-grown fruit bodies (10 samples) made direct fruit bodies within 4 d. This compares with cultures inoculated with vegetative dikaryon which, under the same conditions, formed fruit bodies in 10 - 14 d. The other portions of stems of normally-grown fruit bodies (16 samples) produced (in 9 - 12 d) all types of fruiting pattern, unpredictably from their physiological age or physical size at the time of inoculation. The types of fruiting pattern observed on cultures inoculated with isolated gills are summarised in Table 1. This shows a clear dependence of the type of fruiting pattern on physiological age; tissues explanted prior to karyogamy showing a preponderance of direct fruiting, those explanted during or after meiosis showing a minimum of direct fruiting. Exactly similar results were obtained for *C. congregatus* by Bastouill-Descollonges

& Manachère (1984).

Fruit body tissues used as inocula are clearly much more competent to initiate fruiting than the average vegetative dikaryon inoculum but the circumstances which confer this competence are completely obscure. The only physiological aspect of normal fruit body development with which a correlation might be evident is the disposition of accumulated glycogen. Intracellular glycogen features prominently in various aspects of growth and development in *Coprinus*, including the vegetative mycelium (Madelin, 1960; Jirjis & Moore, 1976), sclerotium maturation (Waters, Moore & Butler, 1975b) and fruit body development (Blayney & Marchant, 1977; Moore, Elhiti & Butler, 1979; Gooday, 1985; Moore, Liu & Kuhad, 1987). The developing fruit body of *C. cinereus* accumulates large quantities of glycogen, which appear first in the stem base and later in the subhymenial regions of the gills (Moore *et al*., 1979; Gooday, 1985). The greatly reduced frequency of direct fruiting in cultures initiated with lamellae explanted during or after meiosis (Table 1) seems to correlate with the rapid, immediately post-meiotic, utilization of glycogen (Moore *et al*., 1987). Whether intracellular glycogen serves as a nutrient or whether this carbohydrate accumulation represents a general nutritional/developmental commitment for fruiting is worth testing. Brunt & Moore (1989) claimed that the yield of fruit bodies arising directly on the initial inoculum showed a positive correlation with the glycogen content of the culture inoculum. The relationship between direct fruiting and glycogen content of the inoculum was complex and could only be fitted by third-degree polynomial regression. Meanwhile, supplementation of the medium with commercial rabbit liver glycogen had no effect. In *Pleurotus sajor-caju* (= *P. pulmonarius*), no correlation could be found between glycogen content and fruiting capacity in experiments featuring *in vivo* fruiting on media with different carbon sources and *in vitro* renewed fruiting of excised stems (Chiu & To, 1993). Subsequent detailed analysis of the relation between glycogen concentration and fruit body development in *C. cinereus* concluded that the carbohydrate cannot be linked exclusively, or even predominantly, with any one of the several processes during fruit body maturation pathway (Ji & Moore, 1993). Therefore, the endogenous glycogen level does not represent a fruiting signal.

In *C. cinereu*s, cAMP has been shown to activate glycogen phosphorylase and inhibit glycogen synthetase via the cAMP-dependent protein kinase *in vitro* (Uno & Ishikawa, 1981, 1982). Yet the addition of femtomolar concentrations of cAMP to vegetative cultures stimulated glycogen synthesis (Kuhad, Rosin & Moore, 1987). Fruiting ability was also correlated with high cAMP level in the data of Swamy, Uno & Ishikawa (1985). However, in these experiments fruit body tissues were not separated from the mycelium prior to analysis, and when this was done in *C. cinereus* it was shown that though cAMP concentration was elevated in the fruit body initial, it dropped to a level approaching that of the vegetative mycelium during meiosis and sporulation (Kuhad *et al*., 1987). Therefore, cAMP is an unlikely candidate as a fruiting signal molecule.

Fruit body surgery

Most surgical experiments on fruit bodies *in vivo* have been carried out on *Flammulina*, *Coprinus* and *Agaricus* with a view to studying the possible involvement of hormones in fruit body development (see Chapter 7). Here, we wish only to mention some experiments which demonstrate that nutrient translocation (as opposed to hormone flow) in *C. cinereus* occurred mainly, but not exclusively, in the ‘stem to cap’ direction (Ji & Moore, 1993; Chapter 1).

Surgical treatments were performed on both caps and stems to try to block potential translocation routes. Small pieces of aluminium foil were inserted into tangential incisions in caps of fruit bodies 4.5 h after karyogamy. The fruit bodies were then kept at 26^{o} in an illuminated incubator and glycogen content in the cap was measured every 2 h. After 2 h the glycogen concentrations in the surgically treated and untreated segments were not significantly different; after 4 h the surgically treated segment contained significantly less glycogen than the untreated segment; and 6 h after

surgery there was, again, no significant difference between the two segments. When the same type of surgery was performed on fruit bodies in which basidiospores were already pigmented (about 11 hours after karyogamy) there was no significant difference between the segments after 1.5 h but after 3 and 4.5 h further incubation the part of the cap beneath the inserted aluminium foil had a significantly lower glycogen content than the untreated part of the cap. In the young fruit bodies in which the margins of primary gills are connected to the stem (tramal hyphae interweaving with the stem hyphae), a transient reduction in glycogen content seemed to be compensated so that 6 h after surgery the glycogen content of the cap below the incision was not significantly different from the control sample. No such compensation occurred in older fruit bodies whose tramal connections were broken. The results of these surgical experiments consequently imply that materials could be translocated between the gills or between stem and gill in the young cap, so bypassing the incision and aluminium foil insert. Detached from the stem, the older cap was limited to radial translocation routes (i.e. from apex to margin) through the cap flesh and these were successfully blocked by the insert. In other words, net translocation of glycogen from stem to cap is indicated, continuing well beyond formation and pigmentation of basidiospores.

Thus, there must be an organised intracellular nutrient translocation circuit from mycelium to stem and to cap. However, there is reason to believe that the stem receives material from the cap, too. Hammad *et al*. (1993) demonstrated that stem elongation benefits considerably from the presence of the cap. Intact fruit bodies elongated about 25% more than decapitated ones, this amounting to 2 to 3 cm greater length (see Table 2 in Chapter 1). Furthermore, while the major supply route to the gills runs radially from the cap apex through the cap flesh, in young fruit bodies gills can be serviced by translocation of nutrients through the connections which exist between the stem and the 'edges' of primary gills. Taken together, these observations indicate that a fruit body is provided with sophisticated translocation flows in all directions throughout its structure. Translocation of growth factors through these translocation routes may help in defining the morphogenetic pattern of the developing structure (see Chapter 7).

Explantation experiments

The ultimate surgical intervention is complete removal of a segment of tissue from its place *in vivo* for transfer to a cultivation medium *in vitro* - this we describe as an explantation experiment. In classical (animal) embryology such a procedure is a test for the level of commitment of the explanted tissue (see discussion in Slack, 1983). 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. Most, but not all.

Commitment in the *Coprinus* hymenium was demonstrated in *C. cinereus* by McLaughlin (1982), and in *C. congregatus* by Bastouill-Descollonges & Manachère (1984). However, these authors did not discuss their experiments from this viewpoint, placing more stress in the former case on sterigma formation, and in the latter on the potential for renewed fruiting from excised lamellae (see above). Detailed analysis of commitment in *C. cinereus* using tissue explantation was done by Chiu & Moore (1988a). In this study commitment to hymenium development was determined by cytological examination of specimens of explanted gills.

In gills explanted at the dikaryotic stage (prior to meiosis) the majority of young basidia were arrested in differentiation at the stage which they had reached at the time of removal from the parent fruit body, even though the 2 day incubation period was sufficient to permit hyphal outgrowths to be formed, largely from tramal tissues of the gill. Young basidia of gill samples taken at or after prophase I all completed meiosis and sporulation after explantation (25 specimens). In contrast,

paraphyses and cystidia (Fig. 1) in the same samples reverted to hyphal growth by unipolar or multipolar hyphal apex formation and/or continued to swell into giant cells. Thus, determination to sporulation was demonstrable, but only in materials explanted at prophase I or later. This is similar to the situation in *Saccharomyces cerevisiae*, where commitment to recombination does not inevitably lead to commitment to meiotic division, the latter requiring duplication of the spindle pole body which occurs early in the first meiotic division (Berry, 1983; Dawes, 1983). Raju & Lu (1973) found that the spindle pole body duplicated at diplotene in *C. cinereus*. Thus, at least on this basis, *S. cerevisiae* and *C. cinereus* seem to share similar requirements for the attainment of competence and commitment to recombination and meiotic division.

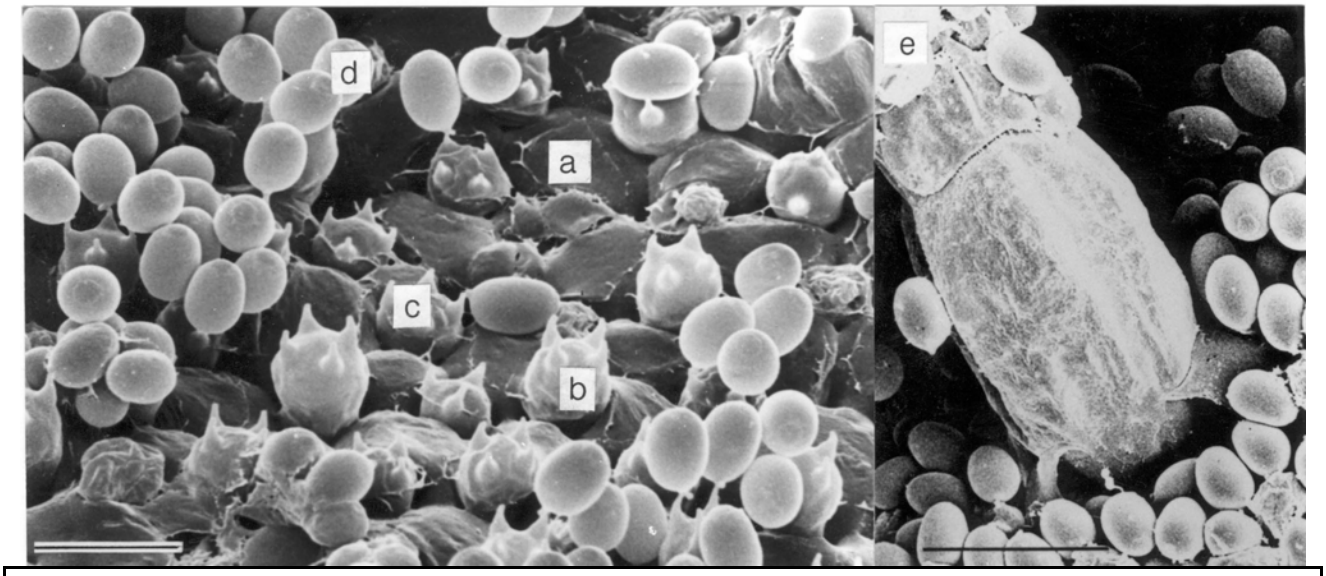

Fig. 1. Scanning electron micrographs showing the hymenial cell types of *Coprinus cinereus*. a, paraphysis; b, long basidium; c, short basidium; d, basidiospore; and e, the cystidium-cystesium pair spanning between two neighbouring gills. Bar = 20 μm.

A difference was evident, however, in that *S. cerevisiae* cells removed from sporulation medium after commitment to recombination but before commitment to sporulation were able to return to mitotic vegetative growth (Berry, 1983). In the experiments with *C. cinereus*, all the isolated gills which were explanted at the dikaryotic stage maintained their hymenial structure even after 2 days incubation; hyphal outgrowths which did occur penetrated through the hymenium from the tramal tissues below. Thus, although such young basidia were unable to continue development on explantation; they were somehow inhibited from reversion to the vegetative state; i.e. they were specified as meiocytes but not yet determined for sporulation.

All the evidence suggests that prophase I is the critical stage at which *C. cinereus* basidia become determined for the division programme. Similar results were obtained whether water agar, buffered agar or nutrient agar was used as explantation medium. Meiosis in basidia, once initiated, was endogenously regulated and proceeded autonomously. The autonomous, endotrophic phenomenon and the synchrony of nuclear division in *C. cinereus* make isolated gills and stems ideal subjects for *in vitro* bioassay to study stem morphogenesis and basidial differentiation (Chiu & Moore, 1988b, 1990b; Moore, Hammad & Ji, 1994; see Chapters 1 and 7).

Using metabolic inhibitors

Dissection of the basidial pathway

Coprinus cinereus is an ammonia fungus, favouring ammonia-rich substrata, and naturally fruits on animal manure or straw enriched with urea. Therefore, the first experiment using the explantation bioassay examined the effect of ammonium and found that ammonium ions halted meiocyte

differentiation; sporulation being terminated, with vegetative hyphae emerging from those parts of the basidium which were in active growth at the time of exposure (Chiu & Moore, 1988b). Tests at various pH values and ammonium (chloride and sulphate) concentrations showed that highly alkaline pH values inhibited gill development, but at permissive pH values (6-8) ammonium concentrations of 50 mM were inhibitory. Neither potassium chloride nor potassium sulphate had any effect. Two ammonium analogues, hydroxylamine and methylamine, were also effective in inhibiting sporulation *in vitro*. Other compounds, such as L-glutamine (but not D-glutamine) and L-methionine, were effective inhibitors, too (Chiu & Moore, 1988b). In contrast, two utilizable sugars (D-glucose, D-fructose), mannitol, and most metabolites of the tricarboxylic cycle, arginine cycle and urea cycle which are amplified during cap maturation (Ewaze, Moore & Stewart, 1978) were ineffective (Chiu & Moore, 1988b). Similarly, in *Saccharomyces cerevisiae*, L-methionine, ammonium and L-glutamine were also inhibitors of sporulation (Miller, 1963; Piñon, 1977; Delavier-Klutchko *et al*., 1980; Freese *et al*., 1984).

Ammonium salts injected into the caps of young fruit bodies with a microsyringe also terminated further meiocyte development. Very young primordia (prekaryogamy) were not able to withstand the damage caused by injection and in most cases aborted. Injections of 2.5 µl of 1 M ammonium salt solutions (buffered to pH 7) were effective in locally suppressing sporulation if injected in post-meiotic and early sporulation stages. White zones appeared around the point of injection as the rest of the cap matured and produced its crop of blackened spores. Similar injections of water or buffer had no visible effect on fruit body maturation. Ammonium ions inhibited the meiocyte development pathway *in vitro* when applied at any time during meiosis (stages prophase I through to the second meiotic division were tested). When applied at similar stages *in vivo*, ammonium retarded the rate of progress through meiosis but did not suppress sporulation. When applied at later sporulation stages (sterigma formation, spore formation, spore pigmentation), ammonium arrested sporulation completely both *in vivo* and *in vitro*.

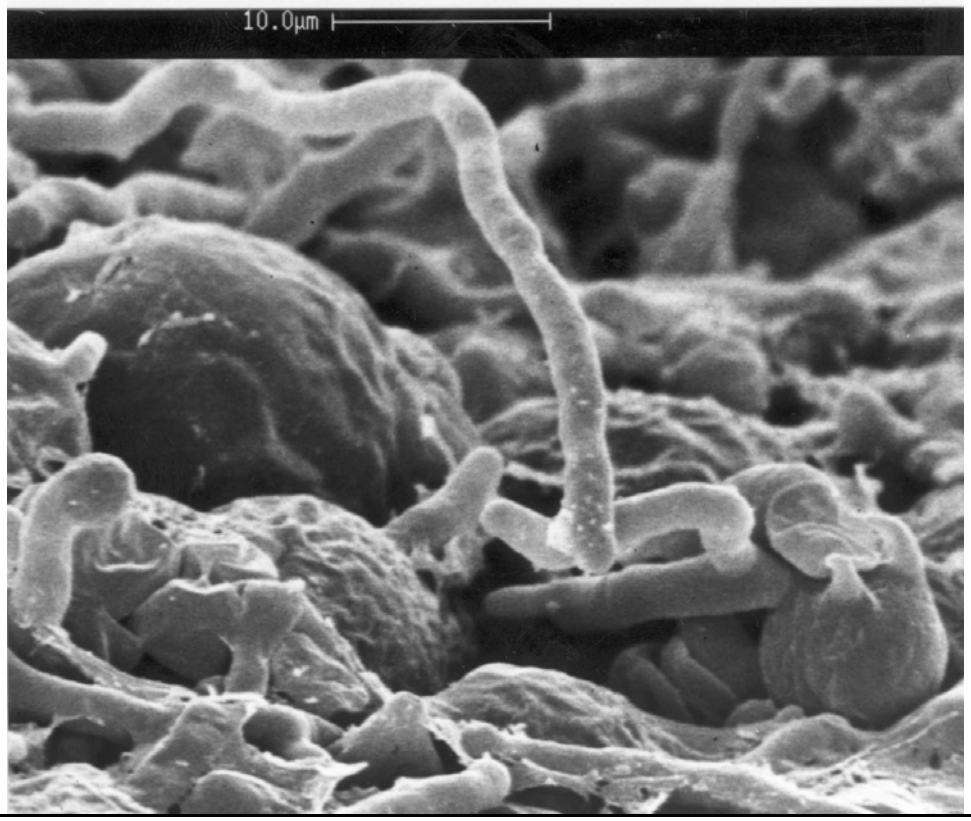

Fig. 2. Scanning electron micrograph showing a typical stress response from a basidium in comparison to a giant sterile hymenial cell and hyphae penetrating from the gill trama into the hymenium of an excised gill.

Cytological examination of gills excised at prophase I and explanted to ammonium-supplemented medium for 24 h showed a range of responses. Some were arrested at prophase I, others continued to metaphase I and some even completed the meiotic division, but no sporulation was observed. Although meiosis is well synchronised in *Coprinus*, synchrony is not perfect and the different stages at which development was arrested presumably reflected a combination of variation in exact time of exposure to ammonium and variation in stage reached by the time of exposure. Samples which were explanted at later stages suffered ammonium-arrest at correspondingly later stages. Tissue taken during meiosis (prophase I, meiotic divisions I and II) showed basidia arrested in later meiotic stages and in early sporulation stages. However, tissue explanted during those early sporulation stages seemed to become arrested immediately. Thus, exposure to ammonium caused termination of the normal developmental sequence of the basidium (Fig. 2).

Chiu & Moore (1988b) used a standard time of exposure in their bioassay. Moore *et al*. (1994) modified the technique to establish the time of exposure to 80 mM NH_4Cl required for inhibition of sporulation, and the most sensitive time period during the course of meiosis. The time of exposure required varied directly with the stage in meiosis in the tissue at the time of excision. Tissue excised as karyogamy was occurring required 7 to 8 h exposure to NH_4Cl for sporulation to be halted, tissue excised during meiotic division I required only 2 to 3 h exposure. Observations made with 16 different fruit bodies established that the stage just after completion of the second meiotic division but before the appearance of sterigmata (spanning 60 to 90 min) was most sensitive to inhibition by ammonium. In all of these bioassays (Chiu & Moore, 1988a; Moore *et al*., 1994), ammonium causes the rapid and regular promotion of reversion to hyphal tip growth from basidia, breaking down the commitment normally shown to their developmental pathway.

The demonstration that sterile elements of the hymenium immediately revert to hyphal growth on explantation to agar media (Chiu & Moore, 1988a) implies that such reversion must be actively inhibited during development of the normal hymenium. Since explantation to media containing ammonium ions caused basidia, the only committed cells of the hymenium, to abort sporulation and revert to hyphal growth, normal sporulation requires protection from the inhibitory effects of metabolic sources of ammonium and related metabolites. Significantly, the ammonium assimilating enzyme NADP-dependent glutamate dehydrogenase (NADP-GDH) is derepressed specifically in basidia (Moore, 1984), being localised in microvesicles associated with the cell periphery (Elhiti, Moore & Butler, 1987) where it could serve as a detoxifying ammonium scavenger. Such a function might also be ascribed to the glutamine synthetase which is derepressed co-ordinately with NADP-GDH (Moore, 1984; Moore *et al*., 1987).

Attempts to identify other specific inhibitory molecules on the basis of comparison of effects in other organisms have met with varied success. Ammonium depletion triggers a *Dictyostelium* slug to transform into a fruit body (Schindler & Sussman, 1977) and ammonium acts as an inhibitor of stalk-cell differentiation (Gross *et al*., 1983). Such inhibitory effect was antagonised by 1 to 2 μM diethylstilboestrol and zearalenone, which inhibit plasma membrane ATPase (Gross *et al*., 1983). Therefore, zearalenone and diethylstilboestrol were tested by transplanting gill segments of *Coprinu*s to medium containing ammonium plus diethylstilboestrol (concentration range 1 to 800 μM) or zearalenone (100 to 600 μM). No antagonism between the additives was observed, implying that *Coprinus* and *Dictyostelium* do not share the same mechanism of action (Moore *et al*., 1994).

The inhibition shown by ammonium may act through effects on the ionic balance of the basidium. The ammonium ion has the same dimensions as potassium, and its salts show many chemical similarities with salts of this metal. The effects of salts of potassium, sodium, rubidium, caesium, calcium and magnesium were assessed over the concentration range 25 to 150 mM (Chiu & Moore, 1990b). Exposure to alkali metal salts at any time from meiosis through to late sporulation stages resulted in premature termination of basidium development and outgrowth of vegetative hyphae.

These results would not support the contention that the ammonium effect is solely due to this ion acting as an analogue of potassium. If this were so, one would expect the same spectrum of effects to be caused by rubidium but this was not observed. Clearly though, the ionic environment, as affected by both cations and anions, does seem to exert some influence.

As uptake of ammonium and other ions will affect the electrochemical gradient, the effects of the membrane-depolarizing agents dinitrophenol (DNP) and sodium azide, the sodium channel gramicidin S and the potassium carrier valinomycin were examined. Tissues excised when in meiotic stages were inhibited by exposure to sublethal 0.75 mM DNP or NaN_3; and whilst many basidia completed sporulation and the explanted gills autolysed, some showed reversion with hyphae growing out in place of sterigmata. Much the same result was obtained with gramicidin-S and valinomycin. With all ionophores, gills excised at sporulation stages were not affected but those excised during meiosis were sensitive and in each case an increased concentration of the agent increased the proportion of basidia affected. Since dinitrophenol and other membrane-depolarizing agents can raise the cAMP level in *C. cinereus* (Uno & Ishikawa, 1981) and other fungi (Trevillyan & Pall, 1979; Thevelein *et al*., 1987), and cAMP levels during meiosis and sporulation were kept low in *C. cinereus* (Kuhad *et al*., 1987), the effect of exposure of explanted gills to cAMP was also tested, and experiments were also carried out with tunicamycin and nikkomycin, which are potent inhibitors of wall synthesis (in *S. cerevisiae*, tunicamycin inhibited epispore formation (Weinstock & Ballou, 1987)). Like the ionophores, cAMP and wall synthesis inhibitors were effective only if applied during meiosis.

In brief, the differential sensitivity of basidia between meiotic and sporulation stages towards these diverse groups of inhibitors implies that during the nuclear division the cell is prepared in advance for sporulation, rather like the egg cell of an animal or plant, so that by the end of the cytologically recognizable nuclear division sporulation can proceed despite treatment with ionophores and wall synthesis inhibitors. Basidial differentiation in *Coprinus cinereus*, therefore, can be seen as a sequence of integrated steps of commitment (Fig. 3) consisting of the following landmarks: (i) commitment to recombination (requires completion of DNA synthesis; Lu, 1982); (ii) commitment to meiosis (at prophase I; Lu & Chiu, 1978; Chiu & Moore, 1988a); (iii) commitment to sporulation (at or after meiotic II division; Raudaskoski & Lu, 1980; Chiu & Moore, 1988a & b); and (iv) commitment to maturation (Chiu & Moore, 1988b). It is not surprising to find a similar sequence of events in the meiocyte pathway of *S. cerevisiae* (Esposito & Klapholz, 1981).

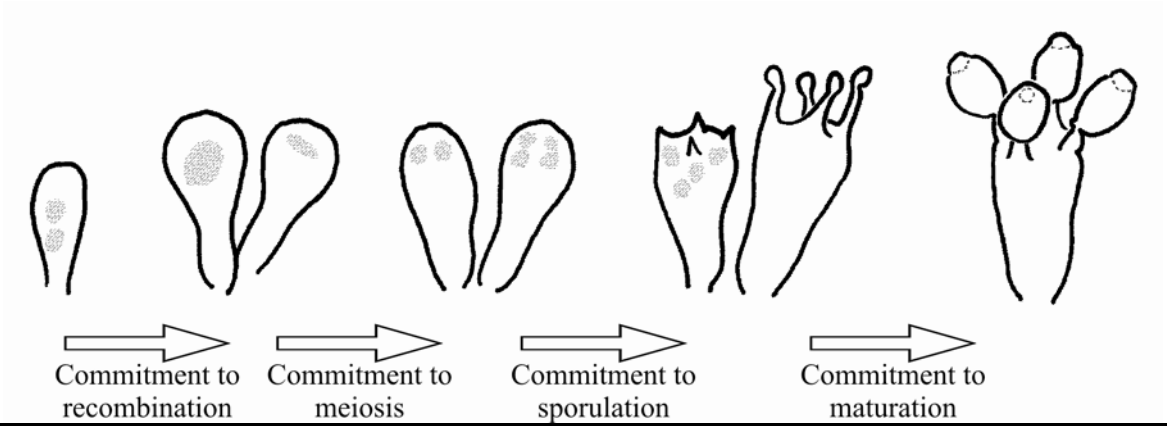

Fig. 3. The basidial differentiation pathway in *Coprinus cinereus*.

In view of the wide range of compounds which have been tested in these bioassays, it is remarkable that the pattern of reversion was highly similar. Most hyphal apices were formed at sites expected to be involved in active wall synthesis during the normal progress of development. The effect of ammonium is unique: inhibiting sporulation and spore maturation *in vitro*. When the tissue exposed

to ammonium treatment was in post-meiotic and early sporulation stages, the reversion hyphae grew out at the sites of sterigma formation; if the basidia had formed sterigmata, hyphae, instead of basidiospores, grew from their apices; if spores were in process of formation, exposure to ammonium caused termination of spore formation and direct outgrowth of hyphal tips from the immature spores still borne on basidia. It seems likely that ammonium ions interfere with cell wall metabolism and the cytoskeletal architecture which defines the number, position and nature of the outgrowths from the basidium.

Fungal walls and fruit body size

The fungal cell wall is a highly dynamic structure which is subject to modification during growth and development. Comparisons of wall composition between monokaryons and dikaryons of *C. cinereus* (Marchant, 1978; Blayney & Marchant, 1977) showed that whilst two compatible monokaryotic strains contained 33.4 and 26.8% (w/w) chitin, the chitin content of the walls of vegetative hyphae of their joint dikaryon was only 10.1%, although fruit body stems from the same dikaryon had a chitin content of 43.3%. Although there have been many studies of the architecture of fungal walls, particularly of the tip region (see Chapter 2), little is known of the dynamics, regulation and spatial organisation of wall synthesis and assembly.

The primary structural components of the fungal cell wall are polysaccharides which may be homo- or heteropolymers. Proteins are also significant components of the wall and are frequently covalently bonded to the polysaccharide constituents. Lipids and melanins are minor wall components in many fungi. Wall polysaccharides can be divided into two groups on the basis of their function and physical form. Structural (or skeletal) polysaccharides are water insoluble homopolymers and include chitin and β-linked glucans. The matrix polysaccharides are amorphous or slightly crystalline and are generally water soluble. Fungal walls are layered. The structural components (mainly chitin fibrils) are normally located on the inner side, frequently embedded in amorphous matrix material. The inner surface is characteristically fibrillar in appearance and is covered by protein and a glycoprotein reticulum with an outer layer of α- and β-glucans (Hunsley & Burnett, 1970; Cabib, Roberts & Bowers, 1982; Sietsma & Wessels, 1990). The cell wall of *C. cinereus* is composed of chitin, glucans with α-(1,4), β-(1,3) and β-(1,6) linkages, and glycans/glycoproteins containing xylomannans (Schaeffer, 1977; Kamada & Takemaru, 1977, 1983; Marchant, 1978; Bottom & Siehr, 1979, 1980).

Chitin microfibrils in stem cells of *C. cinereus* have been described as existing in right- or left-handed helices (Kamada *et al.,* 1991). About two-thirds are left handed, the sense of helicity is constant throughout a cell. The helicity was found to be the same before and after rapid elongation indicating that new microfibrils are inserted between existing ones. The onset of the helical arrangement of chitin microfibrils in the fruit body of *C. cinereus* was traced back to hyphal knots of 0.1 to 0.2 mm in diameter, prior to differentiation of recognisable fruit body tissues (Kamada & Tsuru, 1993). The walls of these hyphal knots contained lower amounts of chitin than hyphae in other developmental phases, so it appears that hyphal walls become differentiated in the fruit body before any obvious change in cell morphology. Mol, Vermeulen & Wessels (1990) found that in elongating hyphae of the mushroom stem of *Agaricus bisporus*, glucosaminoglycan chains were transversely oriented and not organised into distinct chitin microfibrils. Walls from the vegetative hyphae in the substrate showed randomly oriented microfibrils embedded in an amorphous matrix. A model was put forward explaining wall growth as occurring by diffuse extension. This was thought to be due to creeping of polymers in the walls caused by continuous breakdown and reformation of hydrogen bonds among the glucan chains and passive re-orientation of the glucosaminoglycan chains in a transverse manner.

Bartnicki-Garcia (1973) proposed a model for wall synthesis in which wall-bound lytic enzymes at the hyphal apex produce a controlled lysis to allow insertion of new polymers (see Chapter 2). It has

also been proposed that wall-bound enzymes have a role in activating new growth points during branch formation. Synthesis of chitin is catalysed by the enzyme chitin synthase which catalyses transfer of *N*-acetylglucosamine from uridine diphosphate-*N*-acetylglucosamine to a growing chitin chain. Chitin synthases are integral membrane bound proteins and require solubilisation with digitonin before purification. The intracellular distribution and location of chitin synthase still provokes controversy. Although Sentandreu, Mormeneo & Ruiz-Herrera (1994) indicated that chitin synthesis could only occur inside the cell, it remains to be proven whether chitin synthase is located on the inner or outer side of the membrane (Duran, Bowers & Cabib, 1975; Vermeulen & Wessels, 1983; Kang *et al.*, 1984).

Delivery of chitin synthetic machinery to the membrane seems to be the responsibility of chitosomes, which are microvesicles rich in zymogenic chitin synthase (Bartnicki-Garcia, Ruiz-Herrera & Bracker, 1979; Bartnicki-Garcia & Bracker, 1984). Chitosomes are intracellular microvesicles typically of 40-70 nm diameter. When activated *in vitro* by proteolytic enzymes and incubated in the presence of substrate UDP-GlcNAc, chitosomes generate chitin fibrils. One microfibril of chitin is produced from one vesicle. Although Cabib (1987) questioned whether chitosomes are real structures or whether they arise from disruption of other organelles it now seems generally accepted that at least '...some of the microvesicles observed at the apex of fungal hyphae may be chitosomes...' (Gooday, 1983). The current concept is that when vesicles meet the membrane at the hyphal tip they release their contents and merge with the wall.

In *Coprinus cinereus*, attempts have been made to isolate mutants resistant to calcofluor white. Calcofluor white is a fluor (commercially, a fabric whitening agent) which binds to nascent chitin microfibrils through hydrogen bonding with free hydroxyl groups and microfibril assembly by polymerisation is seriously disrupted. Calcofluor white-induced chitin products are profoundly different from the native microfibrillar one and abnormal walls are formed in yeasts exposed to the fluor (Elorza, Rico & Sentandreu, 1983; Roncero *et al.*, 1988).

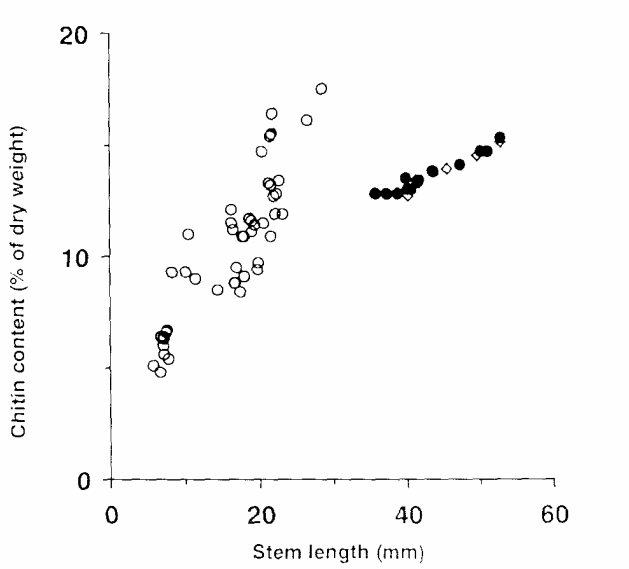

Fig. 4. Chitin content (% dry weight) compared with stem length (mm). The different symbols show data for micro-fruit body *cfw* resistant strains (open circles), macro-fruit body *cfw* resistant strains (closed circles) and the parental $A_{mut}B_{mut}$ strain (open diamonds). Means of 3 replicates.

The experiment used $A_{mut}B_{mut}$ strains of *C. cinereus* which are homokaryotic phenocopies of the dikaryon (see below). A total of 1.5×10^6 oidia was used to select mutants showing resistance to hyphal growth inhibition by calcofluor white. Of the 60 calcofluor white (*cfw*) resistant strains isolated, 46 produced micro-fruit bodies (mean height 18±6.4 mm) and 14 produced normal fruit

bodies (mean height 45.2±7.1 mm compared with the 48.2±4.6 mm tall fruit bodies formed by the parental $A_{mut}B_{mut}$ strain).

True dikaryons made between *cfw* resistant strains and the parental strain (verified by their failure to produce oidia) were sensitive to growth inhibition by calcofluor white. This indicates that resistance to calcofluor white was a recessive character. All the *cfw* × *cfw* dikaryons were resistant to concentrations inhibiting the growth of the parental strain, suggesting all were alleles of a single gene (*cfw*). Normal-sized *cfw* × micro-fruit body *cfw* dikaryons produced normal-sized fruit bodies, indicating that the micro-fruit body phenotype was also recessive, but micro-fruit body *cfw* × micro-fruit body *cfw* dikaryons only formed micro-fruit bodies. Thus, the apparently allelic *cfw* mutants were differentiated into two groups on the basis of fruit body size. Assay of chitin content in the cell walls showed that micro-fruit body *cfw* resistant strains contained significantly less chitin than normal (Fig. 4); mean values (± SEM) being 10.4±0.5% (of dry weight) for micro-fruit body *cfw* resistant strains, 13.6±0.2% for macro-fruit body *cfw* resistant strains and 13.9±0.3% for the parental $A_{mut}B_{mut}$ strain. Among the micro-fruit body strains, a positive correlation was observed between chitin content and stem length (Fig. 4). Interestingly, a negative correlation was observed between chitin content and gravitropic reaction time (Fig. 5). Horizontally placed stems of micro-fruit body *cfw* resistant strains responded to a change in the gravity vector within 57±3.8 minutes, macro-fruit body *cfw* resistant strains respond within 42.7±0.7 minutes compared with 40.2±0.4 minutes for the parental strain. At the moment the mechanism(s) which relate(s) wall structure to fruit body size remain unknown.

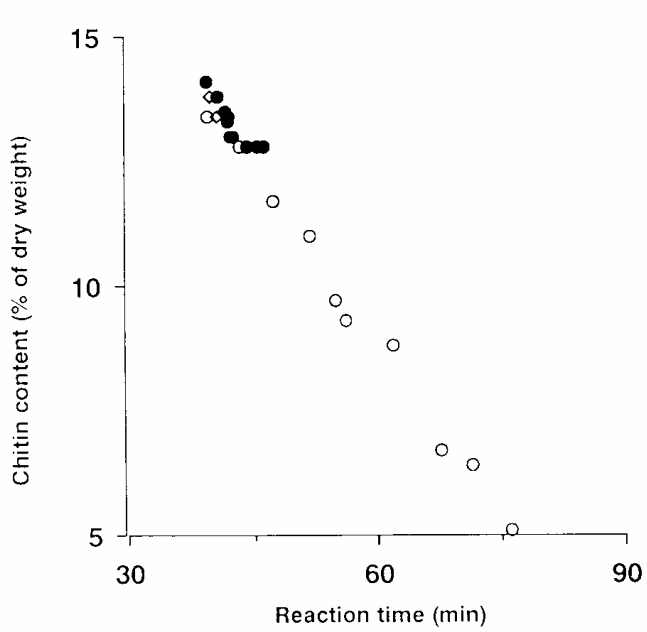

Fig. 5. Chitin content (% dry weight) compared with gravitropic reaction time. The different symbols show data for micro-fruit body *cfw* resistant strains (open circles), macro-fruit body *cfw* resistant strains (closed circles) and the parental $A_{mut}B_{mut}$ strain (open diamonds). Means of 3 replicates.

Using developmental mutants and spontaneous variants
Population polymorphisms and spontaneous mutants
Sclerotia of *Coprinus cinereus* are globose, small (*ca* 250 μm diam.) but multicellular, persistent resting structures. They are formed by vegetative mycelia, both monokaryotic and dikaryotic. Mushroom fruit bodies are borne more commonly on the dikaryon but monokaryotic fruiters are frequent. Both sclerotia and fruit bodies develop from undifferentiated mycelia through an organized process of hyphal growth and branching which forms an aggregate in which cellular

differentiation occurs. The first study of the internal structure of mature sclerotia (Volz & Niederpruem, 1970) revealed that mature sclerotia possessed an outer unicellular rind layer, composed of cells with thickened and pigmented walls, which enclosed a medulla composed of a compact mass of thin-walled bulbous cells and accompanying hyphae. A later study (Waters, Butler & Moore, 1972, 1975a) reported a very different structure; the sclerotia were found to have a multilayered rind enclosing a compact medulla composed predominantly of thick-walled cells. Further analysis of sclerotia produced by 47 monokaryotic strains of different geographical origins showed that this difference in structure was a genuine polymorphism within the wild population of the species (Hereward & Moore, 1979).

The genetic basis of the polymorphism was investigated by constructing dikaryons from selected monokaryotic strains, the sclerotia produced being scored as having either a multilayered or single layered rind by light microscopy of wax-embedded sections. The monokaryons used for dikaryon construction included a number known to be unable to produce sclerotia because of genetic defects. Three loci (*scl*-1, *scl*-2 and *scl*-3) at which alleles which prevent sclerotium formation have been identified were mapped genetically by Waters *et al*. (1975b) and representatives of these were combined in dikaryons together with strains able to produce either one of the two different sorts of sclerotium. The main conclusions were (i) the single layered rind was the dominant phenotype; (ii) the inability to form sclerotia was recessive; (iii) the different *scl*-negative genes complemented one another in doubly-heterozygous dikaryons (i.e. dikaryons made between sclerotium-negative strains were able to produce sclerotia).

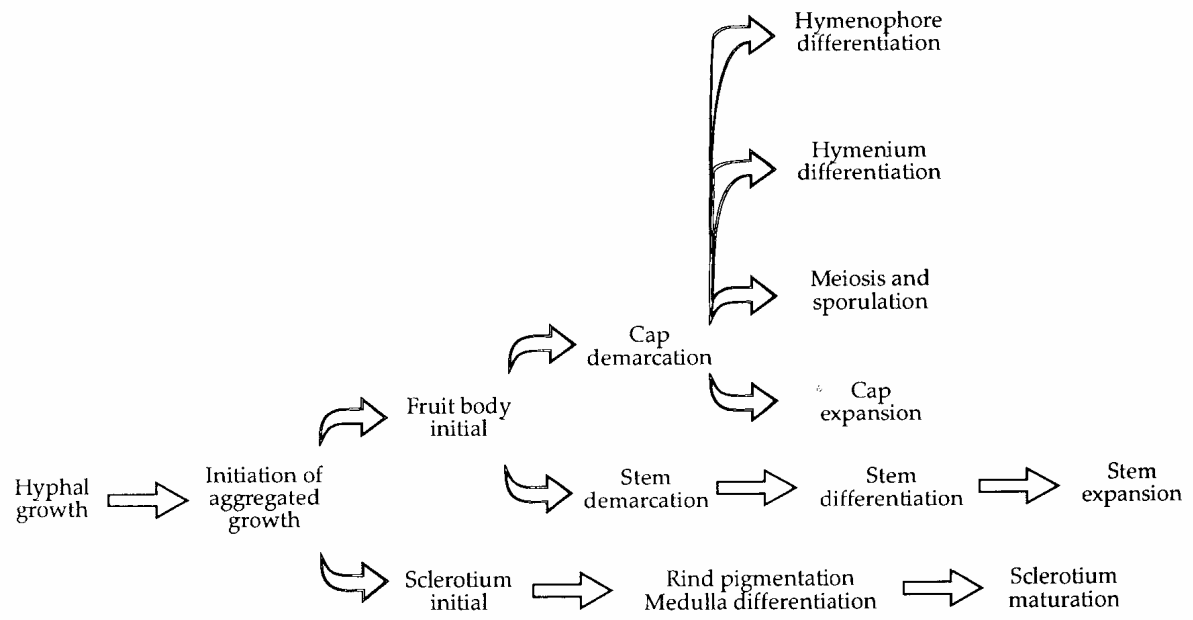

Fig. 6. The common initiation pathway to both fruit body and sclerotium development in *Coprinus cinereus*.

It was concluded that the type of sclerotium with a multi-layered rind structure was the phenotype resulting from a genetic defect which was an allele of the gene *scl*-1 (designated scl-1^H) which was recessive to wild-type but dominant to its silent allele (*scl*-1^0 strains exhibiting the sclerotium-negative phenotype). The *scl*-1^0 alleles segregated in crosses as expected of a single chromosomal gene (Waters *et al*., 1975b) and were assumed to represent a gene responsible for some factor involved in sclerotium morphogenesis. A change in gene being able either to completely block sclerotium production (the *scl*-1^0 phenotype) or to formation of sclerotia which were 'over endowed' with their component tissues (the scl-1^H phenotype). The scl-1 gene product may thus be

concerned with determining the extent and type of growth made during maturation of sclerotium initials.

Early stages of the pathway which leads to the formation of the fruit body (Matthews & Niederpruem, 1972) were strikingly similar to events described for the initiation of sclerotia (Waters *et al*., 1975b). For this and other circumstantial reasons it was suggested that the two structures were alternative outcomes of a single initiating pathway in the dikaryon (Moore & Jirjis, 1976). A test of this proposition was performed using the sclerotium-negative (*scl*) strains to make homoallelic dikaryons (i.e. dikaryons in which both nuclei carried the same *scl* allele)(Moore, 1981). Of the four *scl* genes 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 by Waters *et al*., 1975b). Dikaryons which were homoallelic for any of the four *scl* genes were unable to form either sclerotia or fruit bodies. These observations confirmed earlier suspicions that a common pathway of initiation is used by both fruit bodies and sclerotia (Fig. 6).

Selection of developmental mutants
'Dominant' dikaryotic strains.
The most extensive study of developmental mutants in mushrooms has been done with the Japanese strains of *C. cinereus*. It is worth emphasising that although these are called *C. macrorhizus* they have been demonstrated to be conspecific with the European isolates by mating tests (Moore *et al*., 1979). Takemaru & Kamada (1972) isolated over 1,500 developmental variants following mutagen treatment of macerated dikaryon fragments. Among the mutants isolated were some (designated 'knotless') which were unable to differentiate. This phenotype was similar to that of homoallelic *scl*-negative dikaryons. However, since Takemaru & Kamada (1972) dealt solely with dikaryotic isolates it seems likely that their knotless mutants were dominant and thus different in nature from the recessive *scl*-genes. Penetrance of *scl*-genes in heteroallelic dikaryons depended on segregation of modifiers (Moore, 1981). The effect of these modifiers may help in understanding the very high frequency of dominant mutations observed by Takemaru & Kamada (1972). The assumption made by these authors was that their developmental variants arose as the result of mutations in genes controlling development. However, mutations in modifiers could allow both the expression of previously recessive variants present in the original genome, and also make more likely the expression of newly-induced developmental gene mutations. About 15% of the survivors of mutagen treatment were found to carry dominant developmental variations (Takemaru & Kamada, 1972), but nearly 75% of the variants were assigned (in about equal number) to just two phenotypes. It is possible that recessive genes for these phenotypes occurred in the parental dikaryon and that the high frequency of their occurrence in dominant form in the mutagen survivors was due to increased penetrance because of mutations in members of what could be a large and heterogeneous population of modifying loci, rather than to mutations in genes involved in morphogenesis.

These mutants were classified into categories on the basis of the phenotype of the fruit body produced: (i) 'knotless' - no hyphal aggregations formed; (ii) 'primordiumless' - aggregations were formed but did not develop further; (iii) 'maturationless' - primordia failed to mature; (iv) 'elongationless' - stem failed to elongate but cap development was normal (v) 'expansionless' - stem elongation normal but cap failed to open; (vi) 'sporeless' - few or no spores formed in what were otherwise a normal fruit bodies (Takemaru & Kamada, 1972). These mutant phenotypes suggest that different aspects of fruit body development are genetically separate, and fruit body morphogenesis is under polygenic control. Prevention of meiosis still permits the fruit body to develop normally. However, the hymenia of both the 'elongationless' and 'expansionless' mutants showed frequent abnormal basidia (2- and 3-spored basidia and basidia with asynchronously maturing basidiospores) (Chiu, unpublished observations). Therefore, the defective development of

the fruit body may create different microhabitats in the hymenophore leading to abnormality in sporulation. Perhaps more interesting is the fact that mutants were obtained with defects in either cap expansion or stem elongation. Both processes depend on enormous cell inflation (Gooday, 1985; Moore *et al*., 1979), and the fact that they can be separated by mutation indicates that the same result (increase in cell volume) is achieved by different means.

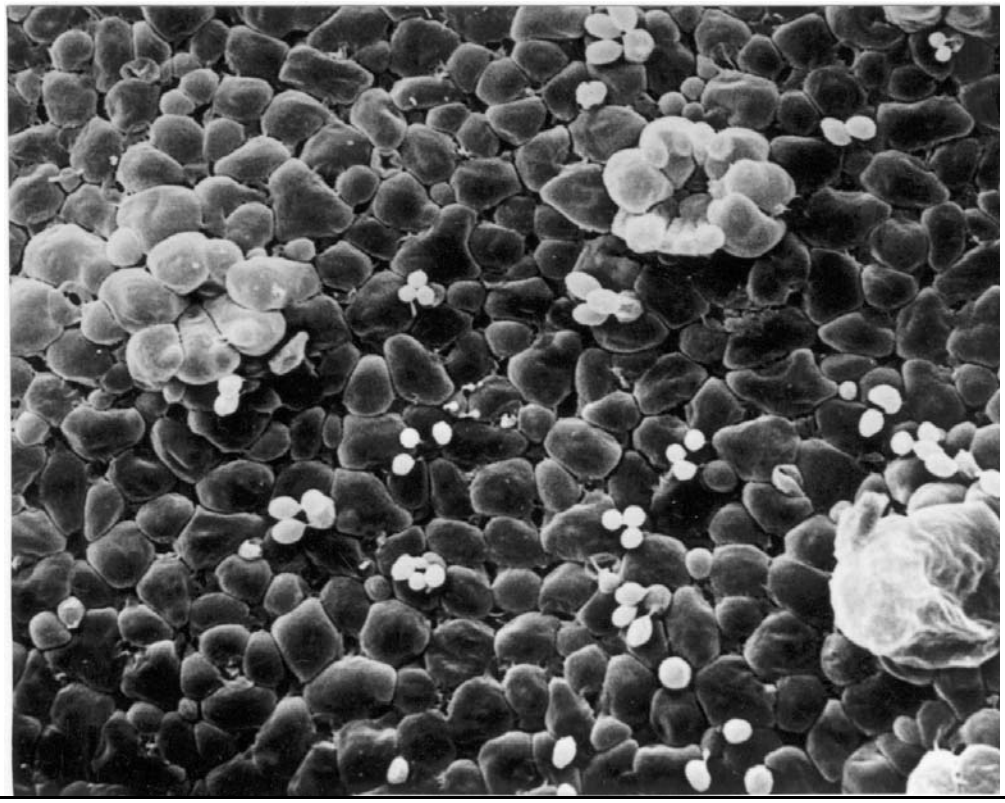

Fig. 7. Scanning electron micrograph showing the hymenial phenotype of a leaky radiation-sensitive mutant in *Coprinus cinereus*. Most of the basidia are arrested at prophase I and do not sporulate while some complete meiosis but, abnormally, bear 2 to 3 basidiospores.

Haploid fruiters especially with double mutations in the mating type factors.
Normally in *C. cinereus*, basidiospores germinate to form mycelium which has cells containing a single haploid nucleus. Mating between these monokaryons is controlled by an outbreeding mechanism dependent on the operation of two incompatibility (or mating type) factors termed A and B each of which harbours a series of linked genes of multiple alleles (Casselton *et al*., 1995). For an attempted mating to form a dikaryon, the two parental monokaryons must carry different alleles at both A and B loci, such a pairing is said to be compatible. The A and B genes are located on different chromosomes and segregate at random during meiosis. In a successful mating, hyphae from monokaryons of compatible mating types anastomose, forming a dikaryon which has two nuclei (one of each mating type) within each cell. This mycelium grows forming clamp connections at each newly formed septum, one of the two nuclei divides here while the other divides in the main part of the cell under the regulation of the mating type genes (Casselton *et al*., 1995). Upon suitable environmental trigger and the presence of functional fruit body developmental genes, a dikaryon gives rise to a fruit body and meiosis occurs in basidia in the hymenium of the gills of the fruit body cap, so completing the life cycle.

The fact that cells in the tissues of the fruit body contain two (parental) nuclei means that each cell is effectively a genetic diploid and presents the inevitable problem for genetic analysis of development that recessive mutations will not be expressed. Swamy *et al*. (1984) described a strain of C. *cinereus* carrying mutations in both of its mating type factors ($A_{mut}B_{mut}$). This strain is a

homokaryotic dikaryon phenocopy. It is similar to the dikaryon in that the hyphae have binucleate compartments and extend by conjugate nuclear division with formation of clamp connections, and the cultures can produce apparently normal fruit bodies. On the other hand, it is a homokaryon, being able to produce asexual spores (usually called oidia) and, most importantly, containing only one (haploid) genetic complement.

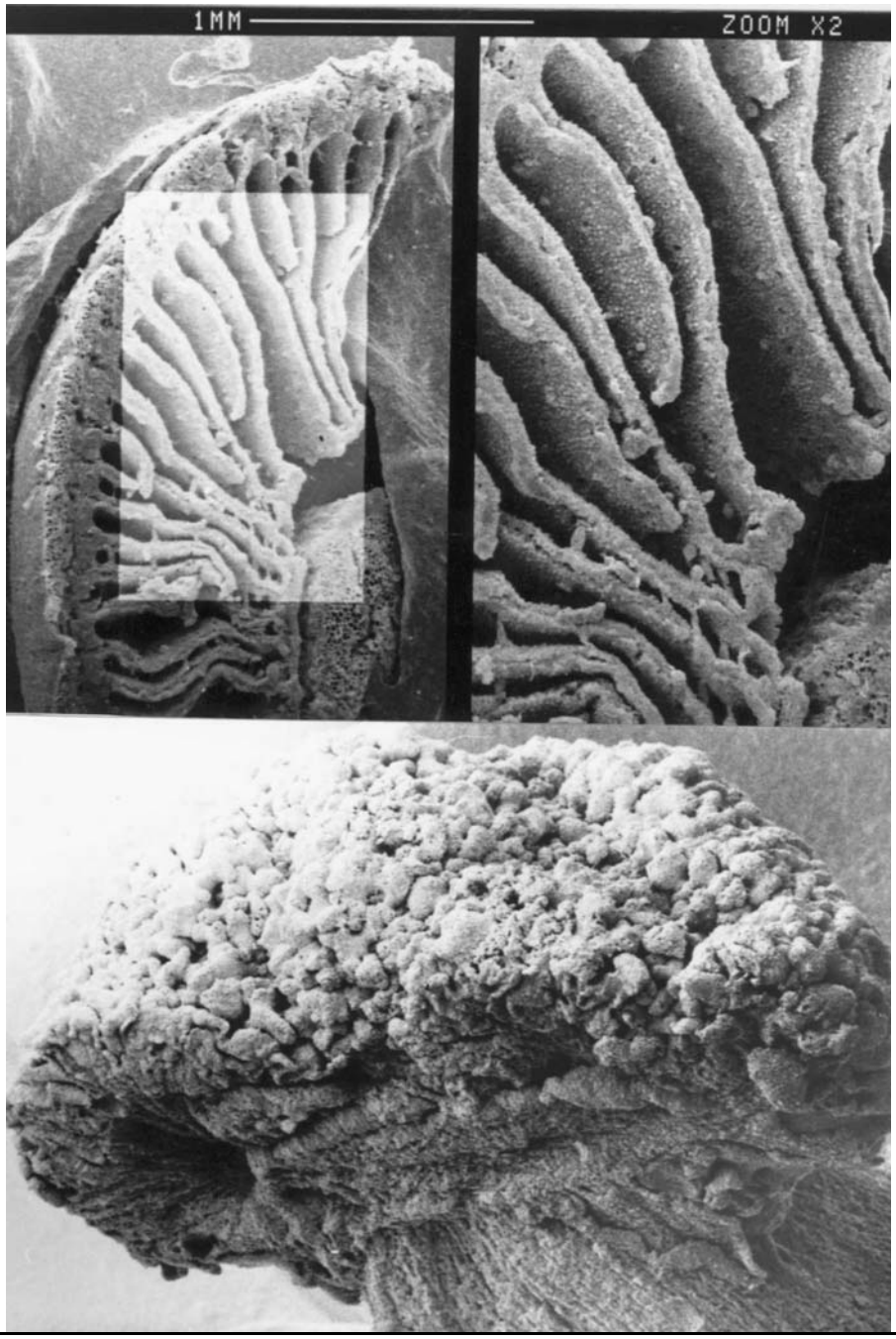

Fig. 8. Scanning electron micrographs showing the hymenophores of wild type (top) and strain *revoluta* (bottom) of *Coprinus cinereus*. Top, the horizontal section shows the radially arranged thin gills around the central stem; bottom, the longitudinal half fruit body shows the convoluted hymenophore. Bar = 200 μm.

Kanda & Ishikawa (1986) and later followed by Zolan, Tremel & Pukkila (1988) showed how such strains could be used for the isolation and characterization of developmental mutants (Kanda *et al*., 1989a & b; 1990). These strains have especially been used to study meiosis and spore formation (Zolan *et al*. 1988; Kamada *et al*., 1989; Kanda *et al*., 1989a, 1990; Pukkila *et al*., 1995; Zolan *et*

al., 1995; see Chapter 5). The sporeless (or, better, sporulation-deficiency; e.g. Fig. 7) mutants obtained so far showed defects in either one of the four steps of commitment identified in the meiocyte pathway (Fig. 2). They are grouped as: (i) failure to complete premeiotic DNA replication leading to arrestment at meta-anaphase I; (ii) meiotic mutants with defects in DNA repair machinery leading to formation of defective synaptonemal complex in meiosis and hypersensitivity towards radiation; (iii) failure to initiate sporulation; and (iv) failure to produce mature basidiospores (Miyake, Takemaru & Ishikawa, 1980; Miyake, Tanaka & Ishikawa, 1980; Pukkila *et al*., 1995; Zolan *et al*., 1995).

The mutant *revoluta*

The *revoluta* strain was isolated from a parental strain of genotype $A_{mut}B_{mut}$, *paba*-1 after ultraviolet irradiation; *revoluta* is a morphological mutant producing abnormal fruit bodies. Heterokaryotic dikaryons resulting from mating with other monokaryons all produced normal fruit bodies, so *revoluta* is recessive and it segregates in a simple Mendelian pattern. Characteristically, the *revoluta* fruit body had a convoluted hymenophore with revolute cap margin and short solid stem (Fig. 8). The first gills to be formed were radial but as the hymenophore grew, the gills became increasingly convoluted and the margin of the cap became revolute.

The *revoluta* mutation was clearly pleiotropic in that stem development, cap morphology, hymenophore initiation and developmental synchrony all differed from normal. But the crucial abnormality was that the mutant lacked the primary gill connection to the stem at its very early appearance of the hymenophore. As a result of the lack of gill anchorage and the uneven distribution of the cystidia-cystesia pairs between neighbouring gills, stresses generated during cap growth could not be properly directed. Consequently, the 'embryonic' convoluted hymenophore present in normal fruit bodies persisted instead of the normal radially symmetrical gills around the central stem at maturity (Chiu & Moore, 1990a). Basidia did sporulate on this convoluted hymenophore but the normally-observed synchrony was lost; adjacent gills could be at different stages of sporulation. Therefore, the enclosed cap with the primary gills connected to the central stem, a characteristic feature of genus *Coprinus*, is essential for maintaining synchrony of meiosis in basidia, another unique feature of the genus.

Conclusions

Experiments described here highlight a number of experimental approaches which could be more widely applied in other organisms to examine pattern formation in mushrooms, such as continuous video observation, explantation, use of inhibitors or metabolite analogues and genetic mutants or natural variants. In other chapters of this book, we show the use of chemical techniques, statistical analysis and video-imaging to give us a clear picture of fruiting morphogenesis in *C. cinereus*. The study of the *revoluta* strain and related investigations of spontaneous developmental polymorphisms in *Volvariella bombycina* have suggested that normal fruit body development comprises a sequence of independent but co-ordinated morphogenetic subroutines, each of which can be activated or repressed as a complete entity (Chiu, Moore & Chang, 1989; see Chapters 1 & 8). In this model the 'hymenium subroutine' (for example) in an agaric would be invoked normally to form the 'epidermal' layer of the gill; a 'hymenophore subroutine' producing the classic agaric gill plates. In any one species the subroutines are be invoked in a specific sequence which generates the particular ontogeny and morphology of that species. Abnormal fruit bodies which arise spontaneously are interpreted as being produced by correct execution of a morphogenetic subroutine which has been invoked in the wrong place or at the wrong time. In a morphologically different species the subroutines are invoked in yet a different sequence, or with different timing. The model provides a unifying theme for categorising fruit body ontogeny and for clarifying phylogenetic and taxonomic relationships (Watling & Moore, 1993; and see Chapters 1 & 8).

References

Anderson, G. E. (1971). *The Life History and Genetics of Coprinus lagopus*. Philip Harris Biological Supplies Ltd.: Weston-super-Mare, U.K.

Bartnicki-Garcia, S. (1973). Fundamental aspects of hyphal morphogenesis. In *Microbial Differentiation*, Symposium of the Society for General Microbiology, **vol. 23** (ed. J. O. Ashworth & J. E. Smith), pp. 245-267. Cambridge University Press: Cambridge, U.K.

Bartnicki-Garcia, S. & Bracker, C. E. (1984). Unique properties of chitosomes. In *Microbial Cell Wall Synthesis and Autolysis* (ed. C. Nombela), pp. 101-112. Elsevier: Amsterdam.

Bartnicki-Garcia, S., Ruiz-Herrera, J. & Bracker, C. E. (1979). Chitosomes, and chitin synthesis. In *Fungal Walls and Hyphal Growth* (ed. J. H. Burnett & A. P. J. Trinci), pp. 149-168. Cambridge University Press: Cambridge, U.K.

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.

Berry, D. R. (1983). Ascospore formation in yeast. In *Fungal Differentiation: a Contemporary Synthesis* (ed. J. E. Smith), pp. 147-173. Marcel Dekker: New York.

Blayney, G. P. & Marchant, R. (1977). Glycogen and protein inclusions in elongating stipes of *Coprinus cinereus*. *Journal of General Microbiology* **98**, 467-476.

Bottom, C. B. & Siehr, D. J. (1979). Structure of an alkali-soluble polysaccharide from the hyphal wall of the basidiomycete *Coprinus macrorhizus* var. *microsporus*. *Carbohydrate Research* **77**, 169-181.

Bottom, C. B. & Siehr, D. J. (1980). Structure and composition of the alkali-insoluble cell wall fraction of *Coprinus macrorhizus* var. *microsporus*. *Canadian Journal of Biochemistry* **58**, 147-153.

Brunt, I. C. & Moore, D. (1989). Intracellular glycogen stimulates fruiting in *Coprinus cinereus*. *Mycological Research* **93**, 543-546.

Cabib, E. (1987). The synthesis and degradation of chitin. *Advances in Enzymology* **59**, 59-101.

Cabib, E., Roberts, R. & Bowers, B. (1982). Synthesis of the yeast cell wall and its regulation. *Annals of Biochemistry* **52**, 763-793.

Casselton, L. A., Asante-Owusu, R. N., Banham, A. H., Kingsnorth, C. S., Kües, U., O'Shea, S. F. & Pardo, E. H. (1995). Mating type control of sexual development in *Coprinus cinereus*. *Canadian Journal of Botany* **73**, S266-S272.

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). A mechanism for gill pattern formation in *Coprinus cinereus*. *Mycological Research* **94**, 320-326.

Chiu, S. W. & Moore, D. (1990b). 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.

Chiu, S. W. & To, S. W. (1993). Endogenous glycogen is not a trigger for fruiting in *Pleurotus sajor-caju*. *Mycological Research* **97**, 363-366.

Dawes, I. W. (1983). Genetic control and gene expression during meiosis and sporulation in *Saccharomyces cerevisiae*. In *Yeast Genetics: Fundamental and Applied Aspects* (ed. J. F. T. Spencer, D. M. Spencer & A. R. W. Smith), pp. 29-64. Springer-Verlag: New York, Heidelberg, Berlin.

Delavier-Klutchko, C., Durian-Trautmann, O., Allemad, P. & Tarlitzki, J. (1980). Assimilation of

ammonia during sporogenesis of *Saccharomyces cerevisiae*. *Journal of General Microbiology* **116**, 143-148.
Duran, A., Bowers, B. & Cabib, E. (1975). Chitin synthase zymogen is attached to the yeast plasma membrane. *Proceedings of the National Academy of Sciences, U.S.A* **72**, 3952-3955.
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.
Elorza, M. V., Rico, H. & Sentandreu, R. (1983). Calcofluor white alters the assembly of chitin fibrils in *Saccharomyces cerevisiae* and *Candida albicans* cells. *Journal of General Microbiology* **131**, 2209-2216.
Esposito, R. E. & Klapholz, S. (1981). Meiosis and ascospore development. In *The Molecular Biology of the Yeast Saccharomyces cerevisiae,* **vol. 1**, *Life Cycle and Inheritance* (ed. J. N. Strathern, E. W. Jones & J. R. Brach), pp. 211-287. Cold Spring Harbor Laboratory: New York.
Ewaze, J. O., Moore, D. & Stewart, G. R. (1978). Co-ordinate regulation of enzymes involved in ornithine metabolism and its relation to sporophore morphogenesis in *Coprinus cinereus*. *Journal of General Microbiology* **107**, 343-357.
Freese, E. B., Olempske-Beer, Z., Hartig, A. & Freese, E. (1984). Initiation of meiosis and sporulation of *Saccharomyces cerevisiae*. *Developmental Biology* **102**, 438-451.
Gooday, G. W. (1983). The hyphal tip. In *Fungal Differentiation: A Contemporary Synthesis* (ed. J. E. Smith), pp. 315-356. Marcel Dekker: New York.
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.
Gross J. D., Bradbury J., Kay R. R. & Peacey M. J. (1983). Intracellular pH and the control of cell differentiation in *Dictyostelium discoideum*. *Nature* **303**, 244-245.
Hammad, F., Ji, J., Watling, R. & Moore, D. (1993). Cell population dynamics in *Coprinus cinereus*: co-ordination of cell inflation throughout the maturing basidiome. *Mycological Research* **97**, 269-274.
Hereward, F. V. & Moore, D. (1979). Polymorphic variation in the structure of aerial sclerotia of *Coprinus cinereus*. *Journal of General Microbiology* **113**, 13-18.
Hunsley, D. & Burnett, J. H. (1970). The ultrastructural architecture of the walls of some hyphal fungi. *Journal of General Microbiology* **62**, 203-216.
Ji, J. & Moore, D. (1993). Glycogen metabolism in relation to fruit body maturation in *Coprinus cinereus*. *Mycological Research* **97**, 283-289.
Jirjis, R. I. & Moore, D. (1976). Involvement of glycogen in morphogenesis in *Coprinus lagopus*. *Journal of General Microbiology* **95**, 348-352.
Kamada, T., Kurita, R. & Takemaru, T. (1978). Effects of light on basidiocarp maturation in *Coprinus macrorhizus*. *Plant & Cell Physiology* **19**, 263-275.
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. (1977). 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 Microbiol*ogy **129**, 703-709.
Kamada, T., Takemaru, T., Prosser, J. I. & Gooday, G. W. (1991). Right and left handed helicity of chitin microfibrils in stipe cells of *Coprinus cinereus*. *Protoplasma* **165**, 65-70.
Kamada, T. & Tsuru, M. (1993). The onset of the helical arrangement of chitin microfibrils in fruit body development of *Coprinus cinereus*. *Mycological Research* **97**, 884-888.

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.

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. & Ishikawa, T. (1986). Isolation of recessive developmental mutants in *Coprinus cinereus*. *Journal of General and Applied Microbiology* **32**, 541-543.

Kang, M. S., Elango, N., Mattia, E., Au-Young, J., Robbins, P. W. & Cabib, E. (1984). Isolation of chitin synthase from *Saccharomyces cerevisiae*. Purification of the enzyme by entrapment in the reaction products. *Journal of Biological Chemistry* **259**, 14966-14972.

Kuhad, R. C., Rosin, I. V. & Moore, D. (1987). A possible relation between cyclic-AMP levels and glycogen mobilisation in *Coprinus cinereus*. *Transactions of the British Mycological Society* **88**, 229-236.

Lu, B. C. (1982). Replication of deoxyribonucleic acid and crossing over in *Coprinus*. In *Basidium and Basidiocarp: Evolution, Cytology, Function and Development* (ed. K. Wells & E. K. Wells), pp. 93-112. Springer-Verlag: New York, Heidelberg & Berlin.

Lu, B. C. & Chiu, S. M. (1978). Meiosis in *Coprinus*. IX. The influence of premeiotic S-phase arrest and cold temperature on the meiotic cell cycle. *Journal of Cell Science* **32**, 21-30.

Madelin, M. F. (1960). Visible changes in the vegetative mycelium of *Coprinus lagopus* Fr. at the time of fruiting. *Transactions of the British Mycological Society* **43**, 105-110.

Marchant, R. (1978). Wall composition of monokaryons and dikaryons of *Coprinus cinereus*. *Journal of General Microbiology* **106**, 195-199.

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.

McLaughlin, D. J. (1982). Ultrastructure and cytochemistry of basidial and basidiospore development. In *Basidium and Basidiocarp: Evolution, Cytology, Function and Development* (ed. K. Wells & E. K. Wells), pp. 37-74. Springer-Verlag: New York, Heidelberg, Berlin.

Miller, J. J. (1963). Determination by ammonium of the manner of yeast nuclear division. Nature **198**, 214.

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.

Montgomery, G. P. & Lu, B. C. (1990). Involvement of *Coprinus* endonuclease in preparing substrate for *in vitro* recombination. *Genome* 33, 101-108.

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. (1984). 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., Elhiti, M. M. Y. & Butler, R. D. (1979). Morphogenesis of the carpophore of *Coprinus cinereus*. *New Phytologist* **83**, 695-722.

Moore, D., Hammad, F. & Ji, J. (1994). The stage in sporulation between the end of meiosis and

emergence of sterigmata is most sensitive to ammonium inhibition in *Coprinus cinereus*. *Microbios* **76**, 197-201.
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. (1976). Regulation of sclerotium production by primary metabolites in *Coprinus cinereus* (= *C. lagopus sensu* Lewis). *Transactions of the British Mycological Society* **66**, 377-382.
Moore, D., Liu, M. & Kuhad, R. C. (1987). Karyogamy-dependent enzyme derepression in the basidiomycete *Coprinus*. *Cell Biology International Reports* **11**, 119 - 124.
Moore, D. & Pukkila, P. J. (1985). *Coprinus cinereus*: an ideal organism for studies of genetics and developmental biology. *Journal of Biological Education* **19**, 31-40.
Morimoto, N. & Oda, Y. (1973). Effects of light on fruit-body formation in a basidiomycete, *Coprinus cinereus*. *Plant & Cell Physiology* 14, 217-225.
Morimoto, N., Suda, S. & Sagara, N. (1981). Effect of ammonia on fruit-body induction of *Coprinus cinereus* in darkness. *Plant & Cell Physiology* **22**, 247-254.
Piñon, R. (1977). Effects of ammonium ions on sporulation of *Saccharomyces cerevisiae*. *Experimental Cell Research* **105**, 367-378.
Pukkila, P. J., Shannon, K. B. & Skrzynia, C. (1995). Independent synaptic behavior of sister chromatids in *Coprinus cinereus*. *Canadian Journal of Botany* **73**, S215-S220.
Raju, N. B. & Lu, B. C. (1973). Meiosis in *Coprinus*. IV. Morphology and behavior of spindle pole bodies. *Journal of Cell Science* **12**, 131-141.
Raudaskoski, M. & Lu, B. C. (1980). The effect of hydroxyurea on meiosis and genetic recombination in the fungus *Coprinus lagopus*. *Canadian Journal of Genetics and Cytology* **22**, 41-50.
Roncero, C., Valdivieso, M. H., Ribas, J. C. & Duran, A. (1988). Isolation and characterisation of *S. cerevisiae* mutants resistant to calcofluor white. *Journal of Bacteriology* **170**, 1945-1949.
Rosin, I. V. & Moore, D. (1985). 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.
Schaeffer, H. P. (1977). An alkali-soluble polysaccharide from the cell walls of *Coprinus lagopus*. *Archives of Microbiology* **113**, 79-82.
Schindler J. & Sussman M. (1977). Ammonia determines the choice of morphogenetic pathways in *Dictyostelium discoideum*. *Journal of Molecular Biology* **116**, 161-169.
Sentandreu, R., Mormeneo, S. & Ruiz-Herrera, J. (1994). Biogenesis of the fungal cell wall. In *The Mycota,* **vol. I**, *Growth, Differentiation and Sexuality* (ed. J. G. H. Wessels & F. Meinhardt), pp. 111-124. Springer-Verlag: Berlin.
Sietsma, J. H. & Wessels, J. G. H. (1990). The occurrence of glucosaminoglycan in the wall of *Schizosaccharomyces pombe*. *Journal of General Microbiology* **136**, 2261-2265.
Slack, J. M. W. (1983). *From Egg to Embryo: Determinative Events in Early Development*. Cambridge University Press: Cambridge, U.K.
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.
Swamy, S., Uno, I. & Ishikawa, T. (1985). Regulation of cyclic AMP metabolism by the incompatibility factors in *Coprinus cinereus*. *Journal of General Microbiology* **131**, 3211-3217.
Takemaru, T. & Kamada, T. (1972). Basidiocarp development in *Coprinus macrorhizus*. I. Induction of developmental variations. *Botanical Magazine (Tokyo)* **85**, 51-57.
Thevelein, J. M., Beullens, M., Honshoven, F., Hoebeeck, G., Detremerie, K., Den Hollander, J. A. & Jans, A. W. H. (1987). Regulation of the cAMP level in the yeast *Saccharomyces cerevisiae*: intracellular pH and the effect of membrane depolarizing compounds. *Journal of*

General Microbiology **133**, 2191-2106.
Trevillyan, J. M. & Pall, M. L. (1979). Control of cAMP levels by depolarizing agents in fungi. *Journal of Bacteriology* **138**, 397-403.
Uno, I. & Ishikawa, T. (1981). Control of adenosine 3',5'-monophosphate level and protein phosphorylation by depolarizing agents in *Coprinus macrorhizus*. *Biochimica et Biophysica Acta* **672**, 108-113.
Uno, I. & Ishikawa, T. (1982). Biochemical and genetical studies on the initial events of fruitbody formation. In *Basidium and Basidiocarp: Evolution, Cytology, Function and Development* (ed. K. Wells & E. K. Wells), pp. 113-123. Springer-Verlag: New York, Heidelberg & Berlin.
Vermeulen, C. A. & Wessels, J. G. H. (1983). Evidence for a phospholipid requirement of chitin synthase in *Schizophyllum commune*. *Current Microbiology* **8**, 67-71.
Volz, P. A. & Niederpruem, D. J. (1970). The sclerotia of *Coprinus lagopus*. *Archiv für Mikrobiologie* **70**, 369-377.
Waters, H., Butler, R. D. & Moore, D. (1972). Thick-walled sclerotial medullary cells in *Coprinus lagopus*. *Transactions of the British Mycological Society* **59**, 167-169.
Waters, H., Butler, R. D. & Moore, D. (1975a). Structure of aerial and submerged sclerotia of *Coprinus lagopus*. *New Phytologist* **74**, 199-205.
Waters, H., Moore, D. & Butler, R. D. (1975b). Morphogenesis of aerial sclerotia of *Coprinus lagopus*. *New Phytologist* **74**, 207-213.
Watling, R. & Moore, D. (1994). Moulding moulds into mushrooms: shape and form in the higher fungi. In *Shape and Form in Plants and Fungi* (ed. D. S. Ingram & A. Hudson), pp. 270-290. Academic Press: London.
Weinstock, K. G. & Ballou, C. E. (1987). Tunicamycin inhibition of epispore formation in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **169**, 4384-4387.
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.
Zolan, M. E., Stassen, N. Y., Ramesh, M. A., Lu, B. C. & Valentino, G. (1995). Meiotic mutants and DNA repair genes of *Coprinus cinereus*. *Canadian Journal of Botany* **31**, S226-S233.