A mechanism for gill pattern formation in *Coprinus cinereus*

SIU WAI CHIU* AND DAVID MOORE

Microbiology Research Group, Department of Cell and Structural Biology, Stopford Building, The University, Manchester M13 9PT

A mechanism for gill pattern formation in *Coprinus cinereus*. *Mycological Research* **94** (3): 320–326 (1990).

Light and scanning electron microscope observations comparing basidiome morphogenesis of the wild type of *Coprinus cinereus* with that in two strains carrying developmental mutations are reported. In the wild type and in meiotic mutants producing basidiomes of normal morphology, gills are formed as convoluted, folded plates. It is envisaged that these must be tensioned into the regular parallel arrangement of the mature basidiome as the cap expands. The process is accomplished through the anchorages provided by the connexion of primary gills to the stem and by cystidium–cystesium pairs acting as tension elements, rather than buttresses, interconnecting gill plates into a fully tensioned structure around the stem as cap expansion pulls the gills into shape. The mutant *revoluta* lacks the primary gill connexion to the stem. This allows premature rolling of the cap margin and hinders formation of cystidium–cystesium pairs and the distorted hymenophore (an 'embryonic' condition) persists.

Keys words: Basidiome, Development, Morphogenesis, Cystidium, Gill pattern, Developmental mutant, *Coprinus cinereus*.

Examination of abnormal development in order to infer morphogenetic patterns and controls is a classic experimental strategy in developmental biology. Recently, we have made a study of spontaneous development polymorphism in *Volvariella bombycina* (Schaeff.: Fr.) Sing. and suggested that normal basidiome developmental 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). 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. Abnormal basidiomes 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.

A more specific technique for studying morphogenesis is the use of developmental mutants. Swamy, Uno & Ishikawa (1984) described a strain of *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray *sensu* Konr. carrying mutations in both of its incompatibility factors, and Kanda & Ishikawa (1986) showed how such strains could be used for the isolation of developmental mutants. We have adopted this approach, and in this paper report scanning electron microscope (SEM) observations comparing basidiome development of the wild type with that in some strains carrying developmental mutations.

* Present address: Department of Biology, Hong Kong Baptist College, Kowloon, Hong Kong.

MATERIALS AND METHODS

Cultures used

The Meathop wild-type dikaryon of *C. cinereus* was collected at Lower Meathop Hill in Cumbria, U.K., by R. A. Johnson. Strain 240 is a homokaryon carrying mutated mating-type factors ($A_{mut}B_{mut}$) and is derived from the original Japanese strains (Swamy *et al.*, 1984; Kanda & Ishikawa, 1986). The $A_{mut}B_{mut}$ genotype causes the homokaryon to behave as a dikaryon phenocopy and to produce basidiomes of normal morphology. Strain 240 is the outcome of further mutagenesis treatment; it has the genotype $A_{mut}B_{mut}$ *rad-9 paba-1* (P. J. Pukkila, pers. comm.; Zolan, Tremel & Pukkila, 1988). The *revoluta* strain was isolated by Dr L. A. Casselton from a parental strain of genotype $A_{mut}B_{mut}$ *paba-1* after ultraviolet irradiation; *revoluta* is a morphological mutant producing abnormal basidiomes. We have demonstrated that heterokaryotic dikaryons resulting from mating with strain 240 or with a range of other monokaryons from our collection all produced basidiomes with normal morphology, so *revoluta* is recessive; preliminary results suggest it segregates in a simple Mendelian pattern.

Cultivation

All the strains were kept as stock cultures on the mushroom complete medium (CM) of Raper & Miles (1958) consisting of (gl^{-1}): glucose (20), yeast extract (2), peptone (2), monopotassium phosphate (0·46), dipotassium phosphate (1), magnesium sulphate (0·5) and agar (15).

Fruiting cultures were grown on CM or horse-dung compost in darkness for 3 d at 37 °C and then transferred to a 28° incubator with 12 h light/12 h dark photoperiod at 554 lx.

Light microscopy

Nuclei were stained using the silver staining technique of Pukkila & Lu (1985) and examined by light microscopy, or by ethidium bromide (50 μg ml^{-1}) in squash preparations examined by fluorescence microscopy. Calcofluor white M_2R (50 μg ml^{-1} in water, freshly prepared before use) was used to visualize newly synthesized chitin in cell walls by fluorescence (Maeda & Ishida, 1967; Yoon & McLaughlin, 1980; Rico, Miragall & Sentandreu, 1985; Paris *et al.*, 1986).

Scanning electron microscopy (SEM)

Specimens were fixed with 6% glutaraldehyde in 0·2 M sodium cacodylate buffer (pH 7·2) for 1 h under reduced pressure to aid penetration. This step was repeated once. Then the specimen was rinsed with the same buffer, and post-fixed in 2% osmium tetroxide in 0·2 M sodium cacodylate buffer (pH 7·2) for 1 h. After a further rinse in buffer the specimen was dehydrated through an alcohol series with two changes of absolute ethanol in the final step before critical-point drying in CO_2. Before SEM examination, the specimen was coated with gold. Hitachi, Cambridge 200 or Camridge 360 scanning electron microscopes were used.

RESULTS AND DISCUSSION

Hymenium differentiation

An unusual feature of both strains 240 and *revoluta* was that the basal cells surrounding a facial cystidium were raised above the general hymenium surface (Fig. 1). Buller (1924) called such cells 'clasping cells'. They have not previously been observed in *C. cinereus*. They do not occur in British dikaryotic strains we have examined, but we have found them in dikaryotic strains of Japanese origin. This appears to be a population polymorphism between geographically isolated races. A similar structural polymorphism in tissue patterning during sclerotium formation has already been reported in *C. cinereus* (Hereward & Moore, 1979).

Hymenophore development in normal basidiomes

Basidiomes of the wild type dikaryon (Meathop) and strain 240 developed similarly. Their morphogenesis generally followed the descriptions given earlier (Moore, Elhiti & Butler, 1979; Rosin & Moore, 1985*a*, *b*; Rosin, Horner & Moore, 1985; Horner & Moore, 1987). Although strain 240 was unable to complete meiosis, it was able to complete the normal sequence of cap maturation, as were other *rad* mutants (Zolan *et al.*, 1988) and the meiotic mutants investigated by Miyake, Takemaru & Ishikawa (1980).

Normal development of the basidiome is hemi-angiocarpous; the hymenophore arises internally, but during further growth of the basidiome the hymenium is exposed by rupture of the outermost tissue layers (the veil). Development of the different parts of the basidiome is isocarpous – stem, cap and hymenophore being initiated at more or less the same time (Reijnders, 1963, 1979; Reijnders in Singer, 1986). Developmental changes proceed along two major vectors (Rosin & Moore, 1985*a*, *b*; Rosin *et al.*, 1985): from the inner edge of the gill, i.e. the edge closest to the stem, towards the outer edge (that closest to the context of the cap) and from the cap margin towards the cap apex. The morphogenetic polarities established at the very earliest stages of differentiation are maintained throughout basidiome development, to spore maturation and autolysis.

Gill differentiation from the protenchyma (cap context) is initiated before formation of an annular cavity in a region corresponding to the boundary between stem and cap tissues. Vertical ridges of small, closely packed cells arise, and as this wave of differentiation moves towards the outer surface of the cap it leaves behind two organized plates of columnar cells,

Fig. 1. View of a cystidium and the immediate surrounding hymenium of the *revoluta* strain of *Coprinus cinereus* showing the clasping cells around the base of the cystidium. Scale bar = 20 μm.

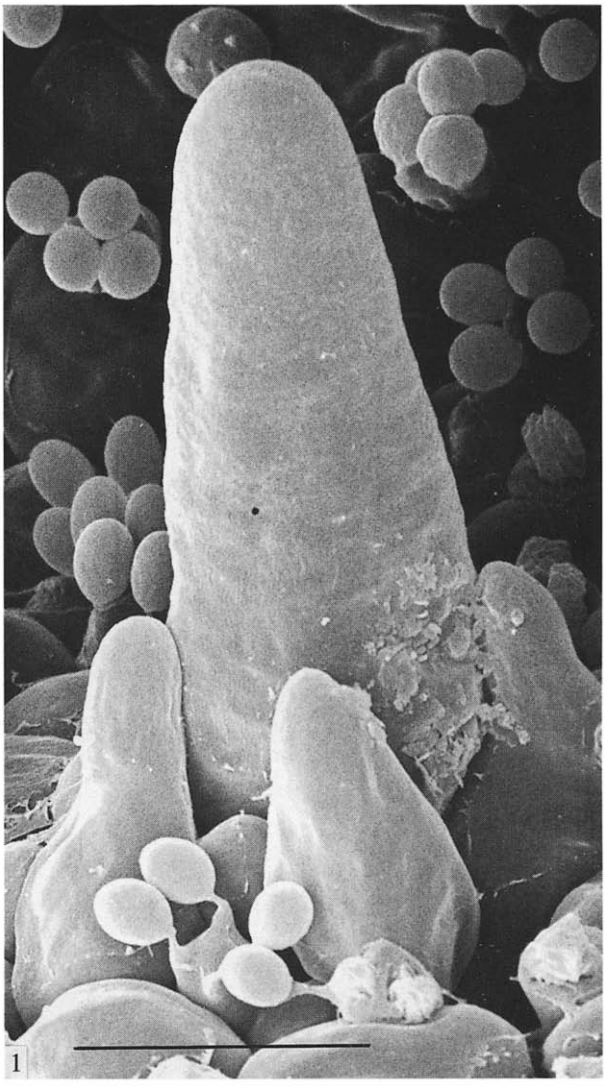
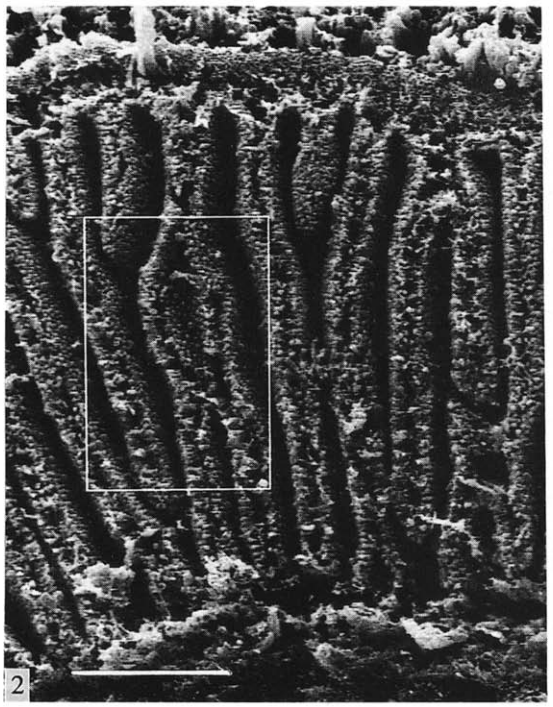
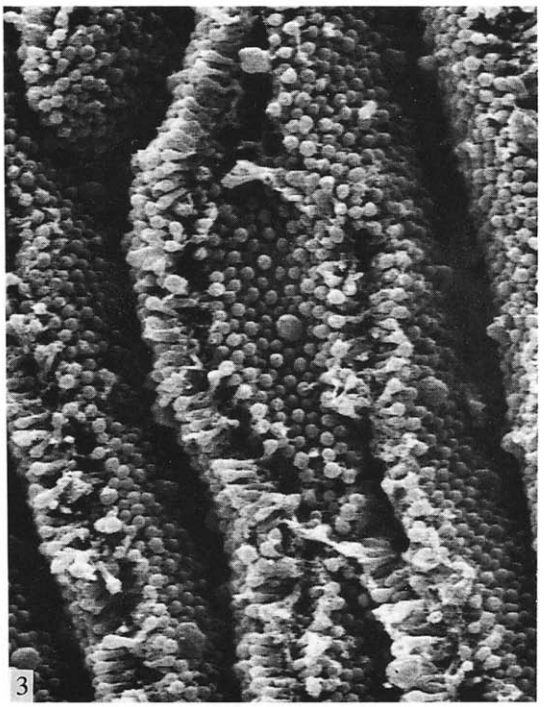


Fig. 2. SEM micrograph of a transverse 'section' of a basidiome of the Meathop strain at the meiotic stage of basidium development. The specimen is mounted so that we are looking 'upwards' towards the apex of the cap, and the stem is at the bottom of the illustration. Secondary gills and gills of lesser ranks arise as folds of the cap context between the roots of existing gills and by bifurcation of primary gills. Scale bar = 200 μm. **Fig. 3.** Threefold magnification of the highlighted region of Fig. 2 showing the protohymenial roof of a bifurcating region as it invades the primary gill trama.





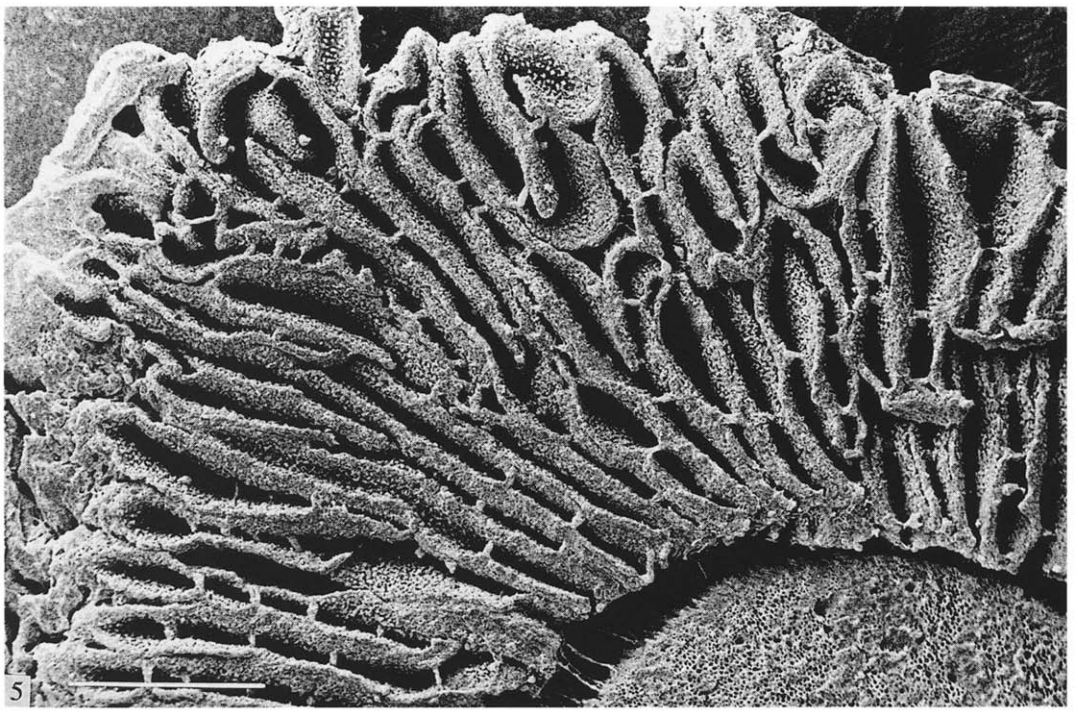
which constitute the primordial hymenia of adjacent gills separated by a developing gill cavity. Tramal tissues of primary gills remain intimately connected to the periphery of the stem for a considerable time, becoming secondarily freed only in well-developed primordia. The annular cavity arises only when these gills detach from the stem (Rosin & Moore, 1985*a*; Moore, 1987).

Gills of lesser ranks form by bifurcation of the gill in the region attached to the central stem (Moore, 1987) or by formation of new palisade ridges on the cap context between the roots of two existing gills (Figs 2–5).

Figures 2 and 3 show that the wave of development which gives rise to these changes in the gills does not penetrate into new areas over a uniform front, rather its border has a sinuate outline. This is the explanation for those gills in Figs 2 and 3 which appear to have a closed 'bubble' of hymenium within their tramal regions. In this specimen we are looking 'upwards' into a broken cap and see a gill in process of bifurcation, and can observe that the developing front at the time of fixation was lobed. In Fig. 2 the plane of sectioning passed just below the 'roof' of one of the lobes so that the continuous protohymenial palisade which is invading the trama above it is revealed (Fig. 3). Repeated bifurcation produced a pattern of complex folds in the cap apex which was evident even in sporulating specimens (Fig. 5) as well as in younger material (fixed at or before meiotic stages; Fig. 4). These folded, convoluted gills in the most apical regions of the cap have not been reported before. Their occurrence suggests that new gills can form where space is available. Clearly, there must be a

Fig. 4. SEM micrograph of a sector of a transverse 'section' of a basidiome of the Meathop strain excised during meiosis. The specimen was taken from near the apex of the cap and is mounted with the stem at the bottom of the picture. Note the frequent (and eccentrically placed) bifurcation forks, contorted gills and cystidium–cystesium pairs spanning gill cavities. Scale bar = 500 μm.

Fig. 5. SEM micrograph of a sector of a transverse 'section' of a basidiome of the Meathop strain taken from near the apex of the cap at the sporulation, i.e. post-meiotic, stage of basidium development. The specimen is mounted with the stem at the bottom of the picture. Note convoluted gill plates. Scale bar = 500 μm.



mechanism which ensures that these folded gills are resolved into the regularly radial pattern characteristic of the mature cap.

If the gills are formed as convoluted plates they may be tensioned into the regular parallel arrangement of the mature basidiome as the cap expands – like a folded cloth being straightened by stretching. Such a mechanism requires that the folded elements (in this case the gills) are anchored. The connexion of primary gills to the stem (note traces of this in Fig. 4) provides the initial anchorage; subsequently, cystidia could interconnect gill plates into a fully tensioned structure completely surrounding the stem.

Although the conventional view is that cystidia act as buttresses to keep adjacent hymenia apart (Buller, 1924), these observations of highly folded gills in the formative region of the apex of the cap lead us to suggest the opposite. A buttress requires a firm foundation, yet *Coprinus* gills are characteristically much thinner than those of other agarics and have a loose tramal structure which can have very little strength. In the immature stages when the gill pattern is being established, the hymenium lacks the close packing of paraphyses which may provide some stability in later stages (illustrations in Moore *et al.*, 1979 and Rosin & Moore, 1985*b*). Thus, at the early, critical stages in morphogenesis, the gill tissues are too weak to withstand the compression forces which would be imposed by buttresses.

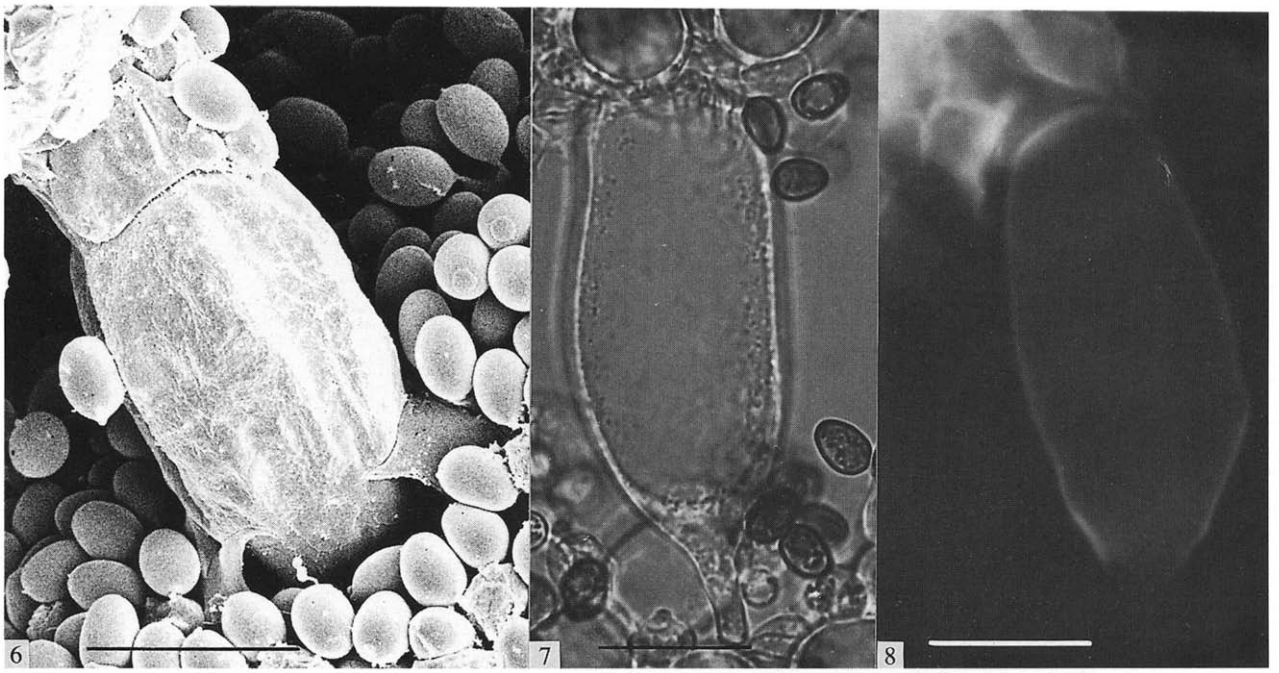
The strength of the adhesion between cystidia and the opposing hymenium has often been remarked upon (see discussion on p. 486 in Horner & Moore, 1987). Facial cystidia adhere to cystesia in the opposing hymenium (Horner & Moore, 1987). These attachments were sufficiently robust to survive procedures involved in SEM and squash preparations (Figs 6–8). Where the latter were treated with Calcofluor white the most intense staining occurred at the junction between the two cells (Fig. 8). This might suggest the presence of nascent chitin chains in the region of contact between the cells and, taken together with the vacuolation of cystesia induced by contact with the cystidium (Horner & Moore, 1987), implies considerable redifferentiation of these cells, which may include synthesis of wall polymers specifically in the region of contact of the two cells.

In structural terms, firm adhesion is not required of buttressing elements but it is essential for tensioning members. The centripetal stretching forces generated by expansion-growth of the cap could then be communicated and balanced throughout the structure. Eventually, of course, these same forces pull the primary gills away from the stem, open out the cap and split the gills. But in the early stages, if cystidium–cystesium pairs act as tension elements holding adjacent hymenia together (rather than as buttresses keeping hymenia apart) cap expansion will pull the convoluted gills into shape.

Basidiome development in the revoluta *mutant*

The *revoluta* basidiome developed hemi-angiocarpously and pileocarpously – the cap appearing before the hymenophore and stem (Reijnders in Singer, 1986). Its basidiome featured a convoluted hymenophore with revolute cap margin (Fig. 9) and short solid stem. There was a distinct annular cavity, in which the hymenophore palisade layer differentiated to form

Fig. 6. SEM view of a cystidium–cystesium pair spanning the gill cavity of two adjacent hymenia of the Meathop (wild-type) strain of *Coprinus cinereus*. Scale bar = 20 μm. **Fig. 7.** Optical microscope view of a squash preparation showing a cystidium–cystesium pair spanning the gill cavity of two adjacent hymenia of the Meathop (wild-type) strain of *Coprinus cinereus*. Scale bar = 20 μm. **Fig. 8.** Fluorescent optical micrograph of a squash preparation of hymenial tissues of the Meathop strain. The preparation is stained with Calcofluor white M_2R to reveal sites of fresh chitin deposition. Note the enhanced fluorescence in the region of contact between the cystidium and the cystesium on the opposing hymenium. Scale bar = 20 μm.



the hymenium. Gills arose as ridges on this continuous palisade, a mode of development described as levhymenial (Reijnders in Singer, 1986) (Fig. 10). The first-formed gills were radial (Fig. 10), but as the hymenophore grew the gills became characteristically convoluted (with hollow gill trama; Figs. 11, 12) and the margin of the cap became revolute. In most cases, basidia sporulated on this convoluted hymenophore. Wild-type basidiomes are characterized by a high level of synchrony in the development of basidia. This synchrony was lost in the *revoluta* mutant in the sense that adjacent gills could be at different stages of sporulation.

The *revoluta* mutation is clearly pleiotropic in that stem development, cap morphology, hymenophore initiation and developmental synchrony all differ from normal. The relative normality of strain 240 shows that neither the mutations in mating-type factors (Swamy *et al.*, 1984) nor the further auxotrophic and radiation-sensitivity mutations affect basidiome morphogenesis. Thus the complex phenotype of *revoluta* must be a consequence of the particular additional mutation it carries rather than being due to its unusual background genotype. This additional mutation arrests the development of the hymenophore such that gills bearing mature spores were still broad and bifurcated like those of an immature normal basidiome. Comparing Fig. 11 with Fig. 4, it is significant that cystidium–cystesium pairs are clearly evident in the latter (the wild-type strain), but are not so in *revoluta*. It would seem that in *revoluta*, stresses generated during cap

Fig. 9. SEM view of a bisected cap of the *revoluta* strain of *C. cinereus*. It bears sinuous hymenophores on the lower surface which are revealed by the revolute margin of the cap. Scale bar = 1 mm.

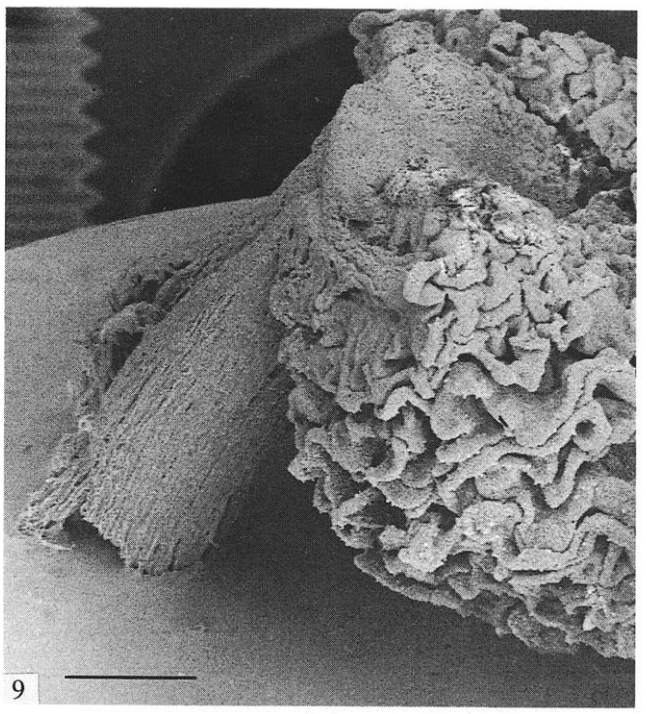


Fig. 10. SEM of the cap of a bisected basidiome of the *revoluta* strain of *C. cinereus*. The stem is in the bottom left corner and the image shows that gill ridges arise in a preformed annular cavity, connexions of primary gills to the stem being absent. Gill inception is consequently levhymenial. Scale bar = 100 μm.

growth cannot be properly directed because the lack of gill anchorage and inequalities in distribution of cystidium–cystesium pairs on the sides of the gill; consequently the 'embryonic' hymenophore persists. The fundamental fault is possibly the lack of primary gills, especially their characteristic connexion to the stem. This allows initial stresses to be dissipated by premature rolling of the cap margin and consequently prevents the close alignment of gills necessary for formation of cystidium–cystesium pairs. The lack (or even paucity) of these secondary anchorages will then amplify the disorder. In terms of the ideas referred to in the introduction, the interpretation would be that *revoluta* fails to invoke the primary gill subroutine, but whether through a direct or indirect consequence of the mutation remains to be established.

We thank Drs P. J. Pukkila and L. A. Casselton for the provision of strains. S.W.C. thanks the Committee of Vice-Chancellors and Principals for an Overseas Research Students Award, the University of Manchester for a Bursary, and her family, especially parents, for their care and support.

REFERENCES

BULLER, A. H. R. (1924). *Researches on Fungi* **3**, London: Longmans, Green & Co.

CHIU, S. W., MOORE, D. & CHANG, S. T. (1989). Basidiome polymorphism in *Volvariella bombycina*. *Mycological Research* **92**, 69–77.

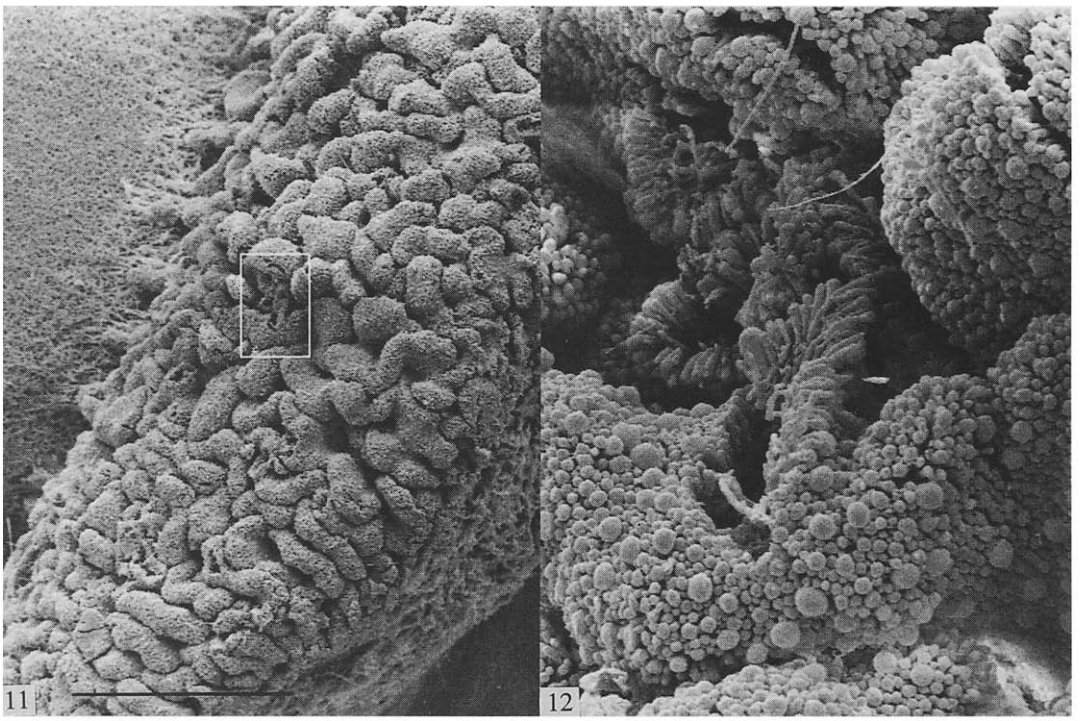
HEREWARD, F. V. & MOORE, D. (1979). Polymorphic variation in the structure of aerial sclerotia of *Coprinus cinereus*. *Journal of General Microbiology* **113**, 13–18.

HORNER, J. & MOORE, D. (1987). Cystidial morphogenetic field in the hymenium of *Coprinus cinereus*. *Transactions of the British Mycological Society* **88**, 479–488.

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

MAEDA, H. & ISHIDA, N. (1967). Specificity of binding of hexopyranosyl polysaccharides with fluorescent brightener. *Journal of Biochemistry* **62**, 276–278.

Fig. 11. Hymenophore of a young primordium of *revoluta*. Note the contorted, folded gills. Scale bar = 500 μm. **Fig. 12.** Eight-fold magnification of the damaged (highlighted) part of the hymenophore of Fig. 11 shows that the gills are apparently hollow.



MIYAKE, H., TAKEMARU, T. & ISHIKAWA, I. (1980). Sequential production of enzymes and basidiospore formation in fruiting bodies of *Coprinus macrorhizus*. *Archives of Microbiology* **126**, 201–203.

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.

PARIS, S., PREVOST, M. C., LATGE, J. P. & GARRISON, R. G. (1986). Cytochemical study of the yeast and mycelial cell walls of *Paracoccidioides brasiliensis*. *Experimental Mycology* **10**, 228–242.

PUKKILA, P. J. & LU, B. C. (1985). Silver staining of meiotic chromosomes in the fungus *Coprinus cinereus*. *Chromosoma* **91**, 108–112.

RAPER, J. R. & MILES, P. G. (1958). The genetics of *Schizophyllum commune*. *Genetics* **43**, 530–546.

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

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

RICO, H., MIRAGALL, F. & SENTANDREU, R. (1985). Abnormal formation of *Candida albicans* walls produced by Calcofluor white: an ultrastructural and stereologic study. *Experimental Mycology* **9**, 241–253.

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* (British Mycological Society Symposium vol. 10) (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 333–351. Cambridge: Cambridge University Press.

ROSIN, I. V. & MOORE, D. (1985*a*). 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. (1985*b*). Differentiation of the hymenium in *Coprinus cinereus*. *Transactions of the British Mycological Society* **84**, 621–628.

SINGER, R. (1986). *The Agaricales in Modern Taxonomy*, 4th edn. Königstein: Koeltz Scientific Books.

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.

YOON, K. S. & McLAUGHLIN, D. J. (1980). Fluorescence microscopic examination of basidiosporogenesis in *Boletus rubinellus* and *Coprinus cinereus*. *Mycologia* **72**, 1150–1158.

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.

(*Received for publication 21 February 1989 and in revised form 8 July 1989*)