Trans. Br. mycol. Soc. **84** (4), 621–628 (1985) *Printed in Great Britain*

DIFFERENTIATION OF THE HYMENIUM IN *COPRINUS CINEREUS*

BY ISABELLE V. ROSIN AND DAVID MOORE
Department of Botany, The University, Manchester M13 9PL

When first formed the hymenium of *Coprinus cinereus* consists of protobasidia and a scattering of cystidia. The latter arise as the terminal compartments of little-branched tramal hyphae. Paraphyses originate as branches of sub-basidial cells and insert into the basidial layer. About 75% of the paraphyseal population inserted as meiosis was completed, the rest at later stages of development. Basidial numbers did not increase once paraphyseal insertion commenced.

A fully differentiated fruit body cap of *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray sensu Konr. consists of a thin layer of pileal flesh (the pileipellis) bounded exteriorly by the veil cells and with the gills suspended on the inner side. The gills develop parallel to one another and retain this arrangement throughout development. The gill surface is the hymenium; it is composed of three highly differentiated cell types, basidia, cystidia and paraphyses, all of which arise from the central layer of the gill, the trama, the constituent cells of which maintain a basically hyphal structure.

Although considerable interest has centred on basidial and cystidial structure for taxonomic and phylogenetic purposes (Smith, 1966; Oberwinkler, 1982) and in studies of basidiosporogenesis (McLaughlin, 1982; Thielke, 1982), there has been no extensive treatment of the hymenium as a differentiating tissue. It is, nevertheless, a highly organized morphogenetic system which presents many of the development problems more usually associated with higher organisms (Wolpert, 1969; Barlow & Carr, 1984; Moore, 1984*a*). These problems relate to the ways in which cells, of the same genetic constitution, differentiate in a developing tissue in particular patterns that imply some sort of positional control extending over the tissue as a whole. Understanding of such regulation of pattern formation will depend on determination of biochemical events governing differentiation and subsequently the orchestration of control of the biochemistry over large populations of spatially-scattered cells. Description of some biochemical events associated with hymenium development in *C. cinereus* has reached an advanced stage (Ewaze, Moore & Stewart, 1978; Moore, Elhiti & Butler, 1979; Moore, 1981), and some attempt has been made to relate findings derived from work with tissue homogenates to cytochemically-observable processes in individual cells (Elhiti, Butler & Moore, 1979) and to extend the analysis to the polynucleotide level (Yashar & Pukkila, 1985). An important point, though , is that hymenial tissue is a fungal tissue and is a derivative of the hyphal grade of organization, the characteristics of which may well impose restrictions on the applicability of theoretical models derived from other organisms.

An essential prerequisite for study of any tissue is a knowledge of its exact structure and development. Although fungal fruiting body structure has been studied for many years, and often from a developmental point of view, the published information is inadequate to establish the nature of cell-to-cell relationships in the detail which is required in morphogenetic analysis. In this paper we describe the structure of the developing hymenium of *C. cinereus*, with emphasis on structural, developmental and geometrical cell relationships.

MATERIALS AND METHODS

The dikaryon, culture conditions and preparative techniques described by Rosin & Moore (1985) were employed.

RESULTS AND DISCUSSION

The young hymenium consisted of a palisade of tightly packed club-shaped cells with a scattering of much larger, highly inflated cells (Fig. 1). The latter are presumed to be developing cystidia though on the basis of these images there is no way of categorising the former either as basidia or paraphyses. However, two lines of evidence indicate that in these young primordia (representing Stage 1, 2–6 mm in height, and Stage 2, 6–9 mm in height) the hymenium is largely composed of differentiating basidia. The first is that, as we shall show below, paraphyses were observed to appear later and were inserted into the basidial layer; the second is that in studies of meiosis (which occurs

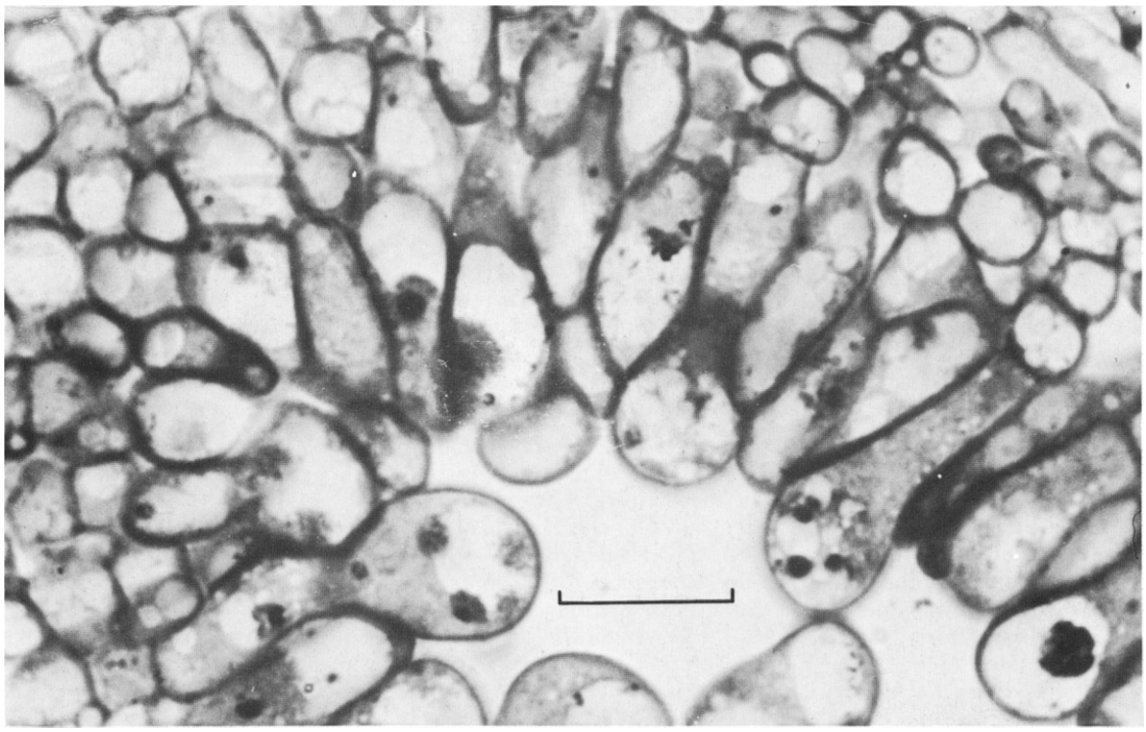

Fig. 1. Protohymenium which has just differentiated from the protenchymal tissue of the cap of the *C. cinereus* fruit body. Scale bar = 5 μm.

during Stage 2) Raju & Lu (1970) showed that meiotic figures could be demonstrated in virtually all of the hymenial cells, an observation which has recently been confirmed (Pukkila, pers. comm.). We will therefore refer to these cells as basidia since only this cell type is expected to undergo karyogamy and meiosis.

Basidia originated as slightly swollen chromophilic cells at the apex of protenchyme (tramal) hyphae. This apical differentiation follows the pattern of most basidiomycetes (Sundberg, 1978; Oberwinkler, 1982) but McLaughlin (1982) emphasised the ultrastructural differences between the developing basidium and the growing vegetative hyphal apex, especially the lack of apical vesicles in the basidium which he suggested may indicate a slow growth rate. In *C. cinereus* basidia were the terminal cells of branches which curved out from the generally parallel tramal hyphae. During the early stages of hymenium development it was noticeable that adjacent basidia arose at the apex of sister branches of parental tramal hyphae. This is the normal mode of basidium proliferation in agarics (Oberwinkler, 1982) and contrasts with proliferation at sub-basidial clamp connexions described in *Schizophyllum commune* (Niederpruem, Jersild & Lane, 1971; Niederpruem & Jersild, 1972).

Cystidia arose from tramal hyphae which branched relatively much less than those subtending basidia (Fig. 2). From the earliest stage cystidia inserted into the opposite hymenium. Cystidia stretched considerably when two gills were pulled apart, indicating a firm attachment. Reijnders (1963) gives an account of the nomenclature and structure of these cells.

Towards Stage 2, cystidia and the apices of basidia enlarged. The expansion of basidial apices was often associated with basidiocarps in which the hyphae of the trama, subhymenium and hymenium, showed similar expansion (Oberwinkler, 1982). Paraphyses developed as outgrowths of sub-basidial cells during Stage 2. During Stage 1 basidia were the most chromophilic cells of the gill but at Stage 2 the most chromophilic region was in the subhymenium corresponding in position to the emerging paraphyses. This distribution appears to reflect the actively growing zone of the lamellae. In *C. micaceus* and *C. lagopus*, Chow (1934) observed a similar chromophilic pattern but concluded that basidia grew from the subhymenium and into a layer of paraphyses. We do not agree with this

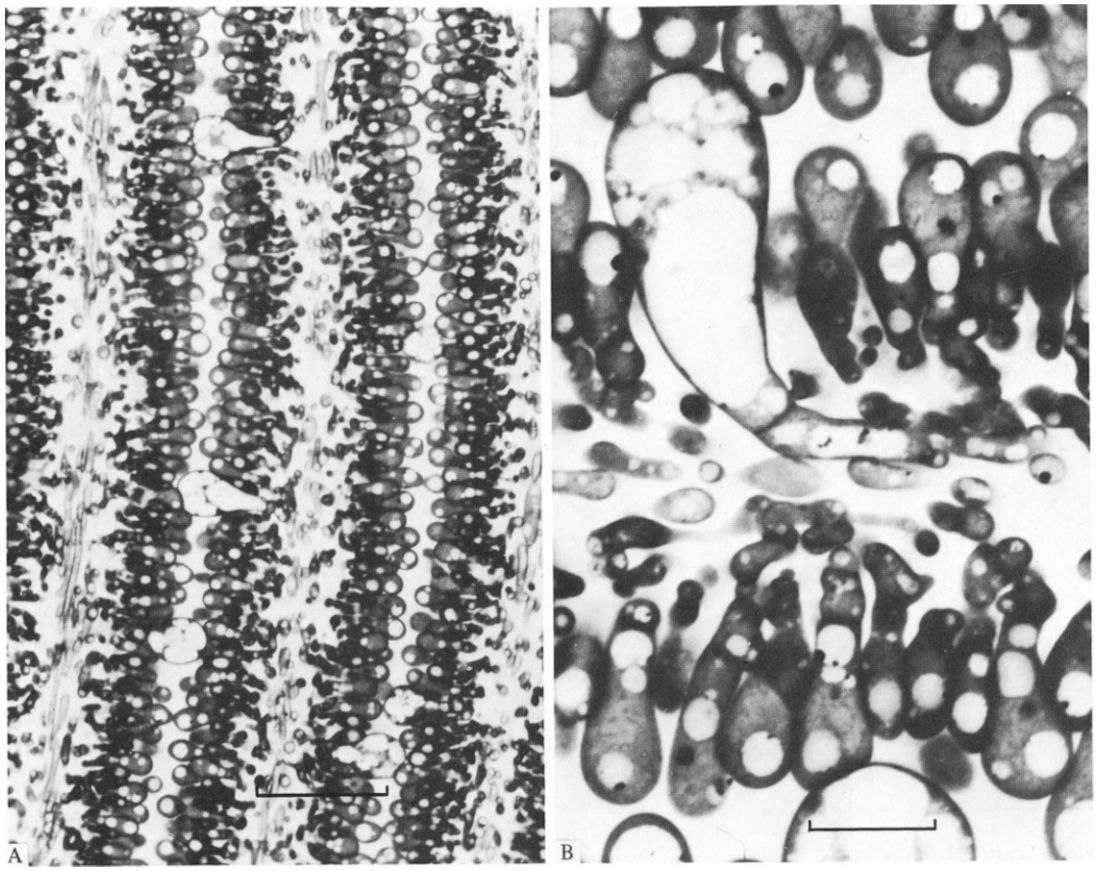

Fig. 2. Differentiating hymenium of *C. cinereus*. Note that the large cystidial cells are terminal compartments of tramal hyphae. The hymenium at this stage consists of a closely packed layer of basidia, though the highly chromophilic paraphyseal branches from the sub-basidial cells are becoming evident. Scale bars: A = 20 μm, B = 5 μm.

interpretation. The strongly chromophilic region in the *C. cinereus* subhymenium was composed of branches communicating between the trama and hymenium and of small, rather oblong, cells (Fig. 2). These latter cells (which are young paraphyses) arose as branches from the sub-basidial cells and then inserted between the basidia (Fig. 3). By Stage 3 (immature fruit body over 10 mm in height, postmeiotic) these cells, still highly chromophilic, had expanded and taken on the characteristic rectangular profile of paraphyses (Fig. 4). By this stage the number of paraphyses had increased to the point where no two basidia were adjacent.

The most important points arising from these observations are that when first formed the hymenium consists of cells destined to become basidia or cystidia. The latter are fewer in number and are the apical cells of tramal hyphae which branch much less frequently than the majority. Only more detailed analysis of serial sections at the EM level will show whether 'cystidial tramal hyphae' constitute a hyphal population distinct from 'basidial tramal hyphae', but on the basis of present observations that possibility certainly exists. The final point is that paraphyses arise as branches from sub-basidial cells and insert into the basidial cell layer. Formation of paraphyseal branches appears to occur at about the time that meiosis is taking place.

In primordia of Stages 1–2 the gill hymenium consisted essentially of a cell layer of basidia and was about 100 μm wide at the cap margin. At maturity, Stage 5, the hymenium was composed of basidia embedded in a pavement of paraphyses and the gill was about 1·5–2 mm wide at the cap margin. Observations described above showed that paraphyses were inserted into the hymenium layer at about Stage 2/3. At this time about 100 cells

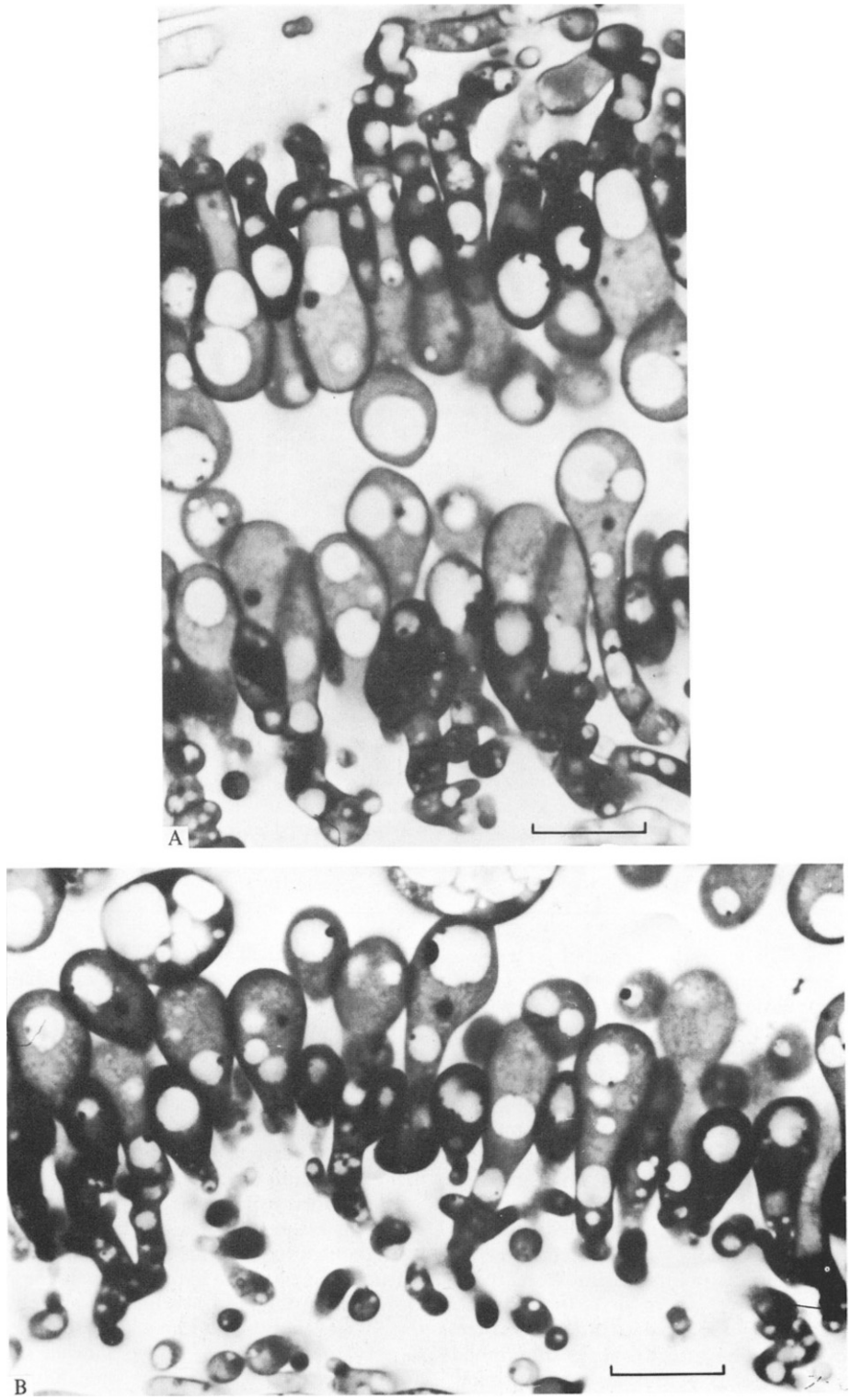

Fig. 3. Insertion of paraphyses (the most chromophilic cells) into the basidial layer of the *Coprinus* hymenium. Scale bars = 5 μm.

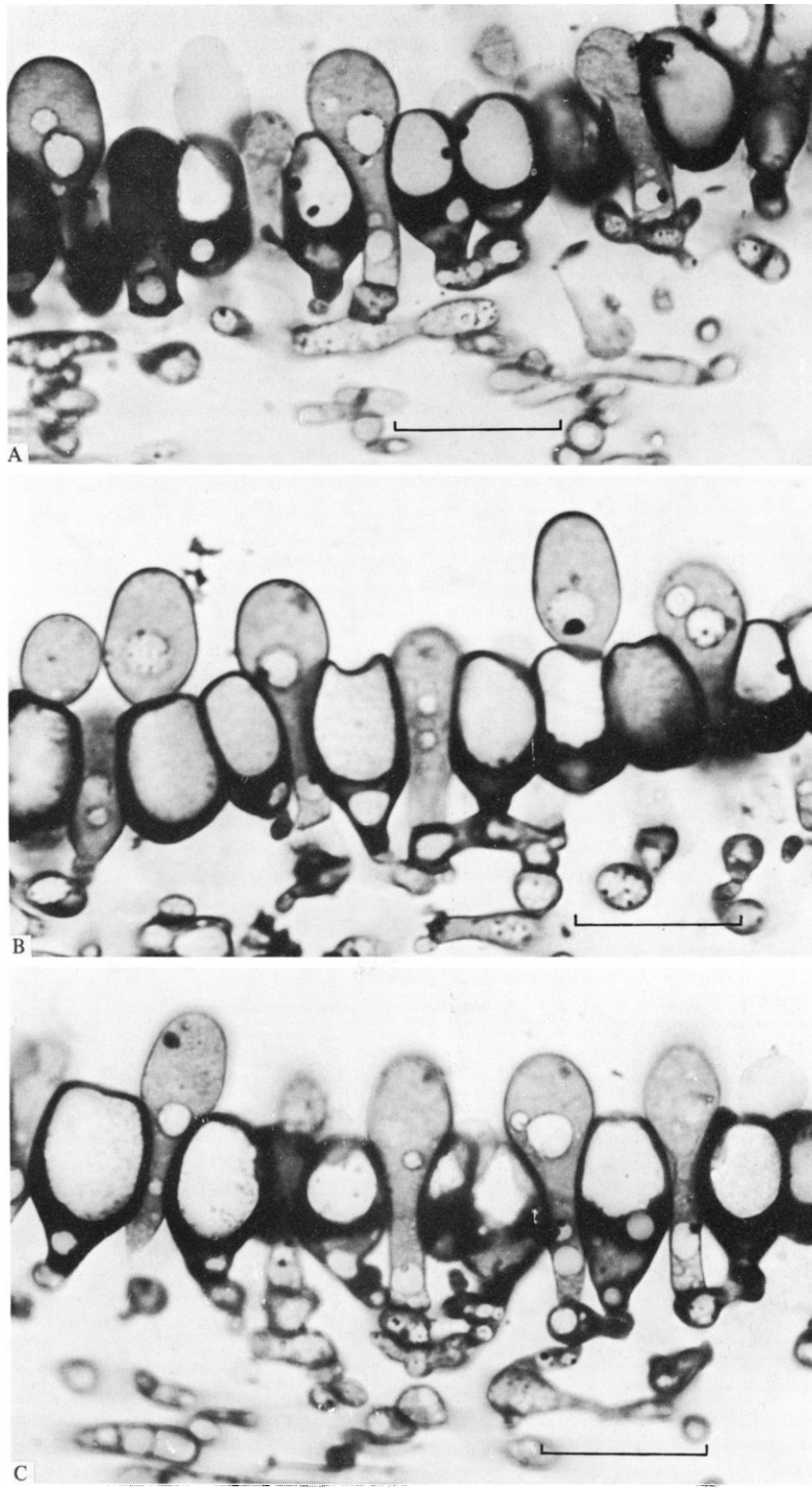

Fig. 4. Expansion of the inserted paraphyses to form the characteristic paraphyseal pavement of the *Coprinus* hymenium. Scale bars = 10 μm.

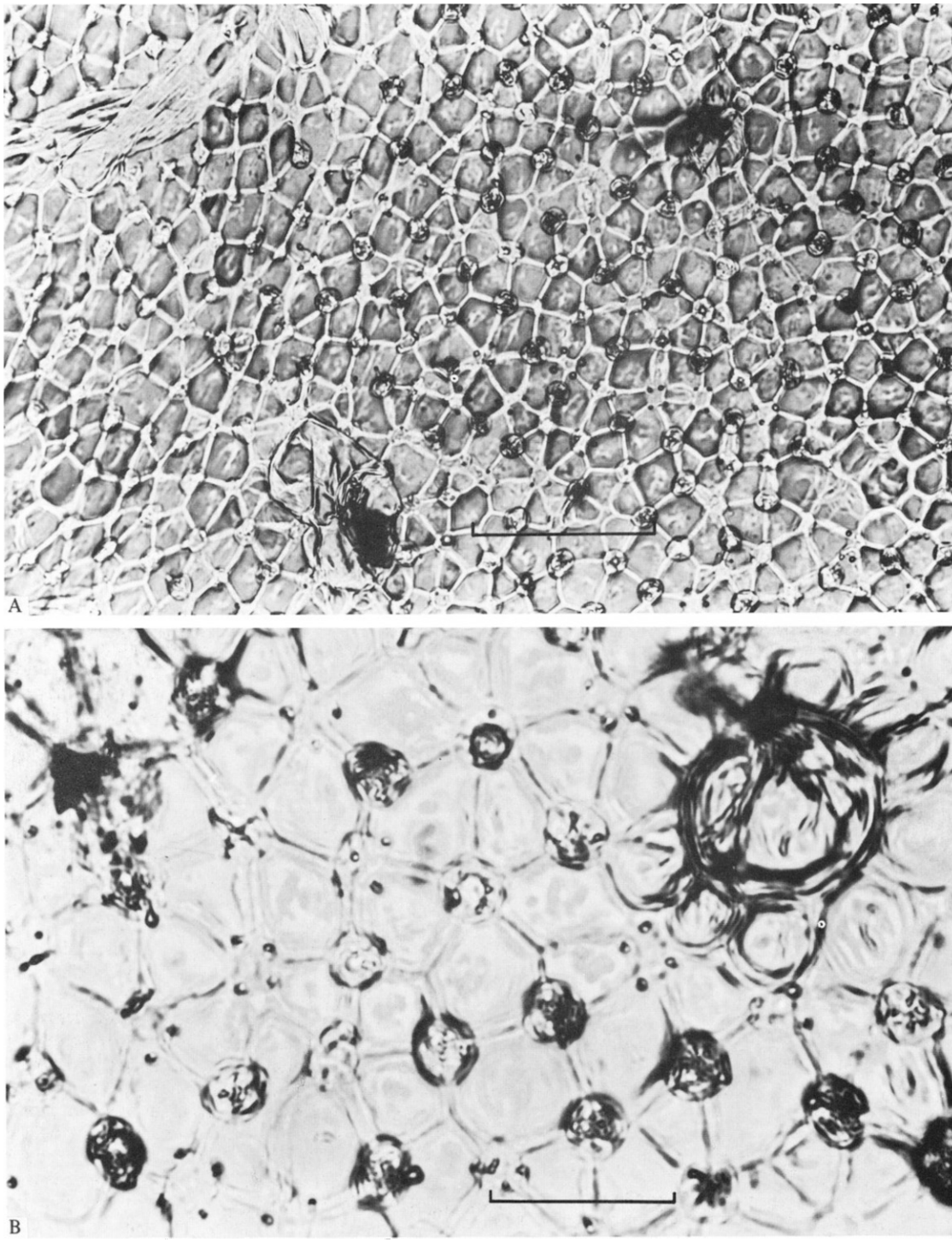

Fig. 5. Surface view of the almost mature hymenium of a sporeless mutant of *C. cinereus* to show the patterns of paraphyseal boundaries and entrapped basidia (ba). Nomarski interference optics. Scale bars: A = 75 μm, B = 25 μm.

inserted into the gill transect and the observed gill width was 300–400 μm. The average width of the inserted paraphyses was 3–4 μm. At Stage 4, approx. 140 paraphyses were counted along gill transects and they had an average width of 8 μm. Thus, although the main production of paraphyses occurred at the end of Stage 2, about 25% of the population was formed and inserted during the later stages of maturation. Insertion and expansion of paraphyses was sufficient to account for the entire increase in gill width. Increase in gill length will, of course, depend on the same factors but is further dependent on continued differentiation of gill tissues from the protenchyma at the apex of the cap (Rosin & Moore, 1985). Paraphyseal enlargement proceeded continuously during maturation. Increase in gill area is clearly dependent on an enormous increase in the constituent cell volumes. Paraphyses expand as much as two and a half times their original volume. The ingress of water into the hymenium elements leads to considerable vacuolation as the cells expand (Moore *et al.*, 1979). The exact identity of an osmoticum has not been established, but amplification of urea cycle activity, which occurs specifically in the cap tissue, probably contributes to the osmotic influx of water (Ewaze *et al.*, 1978; Moore, 1984*b*).

The distribution of paraphyses and basidia has been analysed geometrically using a method based on that of Lewis (1931, 1943). The principles for geometrical analysis of tissues have been derived for layers of dividing cells, in applying them here we are assuming that the insertion of cells (specifically paraphyses) into a cell layer is formally equivalent to cell division within the layer. Geometrically this seems to be valid providing the argument does not depend on constraints on the plane of division. It is clear that theoretical aspects of fungal tissue construction (and especially the unique considerations arising from the hyphal origin of the cells and their characteristic transverse dividing septum) have so far been ignored. We intend to return to this theme in a later paper. The inherent property of a mosaic of cells is that, in a system of linked polygons, the average number of cell sides is exactly six, and that the area of a cell is proportional to the number of sides of the cell (Lewis, 1943). The nature of this proportionality can identify different cell populations. Measurements were made of maximum cell diameter (replacing area) and the number of sides for 50 randomly chosen basidia and paraphyses in photographs of the hymenium surface (Fig. 5) of fruit bodies at Stages 3 and 5. A computer-generated unweighted regression of the plot (Fig. 6) shows that basidia and paraphyses fall into two distinct populations. Basidial diameter remained approximately constant irrespective of the number of sides, while for paraphyses the number of sides increased with size.

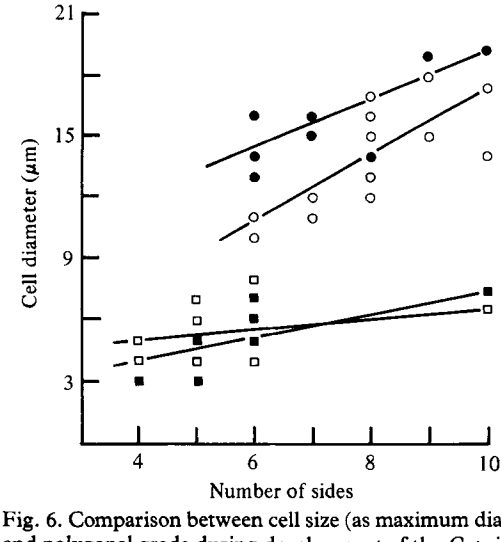

Fig. 6. Comparison between cell size (as maximum diam) and polygonal grade during development of the *Coprinus* hymenium. Plot shows a regression analysis for basidia of Stage 3 primordia (□) and Stage 4 immature fruit bodies (■), and paraphyses of Stage 3 (○) and Stage 4 (●).

The failure of many-sided cells to attain the size required to comply with the expected progression has been explained in terms of the division of cells around a non-dividing cell (Lewis, 1943). In the context of our present knowledge of the ontogeny of the hymenium in *C. cinereus*, the analogous statement for this tissue would be that additional paraphyses insert around the basidia. Thus the geometrical analysis of basidia and paraphyses reinforces the view that the basidial population is numerically static. Only paraphyses insert into the hymenial layer after its initial differentiation from the protenchyma (Rosin & Moore, 1985).

The key features which appear to determine cell patterning in the *Coprinus* hymenium are therefore the establishment of a cell layer comprised of basidia with a scattering of cystidia, and cessation of basidial growth which seems to remove a constraint on branching and lateral proliferation of the sub-basidial cells so that paraphyseal branch formation can take place. This latter is accompanied by an accumulation of carbohydrate in the sub-basidial cells as these become the active growth zone of the hymenium. These processes seem to require three major controls: specification of the distribution of cystidia; determination of the apical differentiation of tramal hyphae into basidia and, as a consequence of basidial differentiation, the third control sequence dictates differentiation of sub-

basidial branches into paraphyses. Further analysis of these processes and their correlation with specific metabolic events is underway.

REFERENCES

BARLOW, P. W. & CARR, D. J. (1984). *Positional Controls in Plant Development*. Cambridge University Press.

CHOW, C. H. (1934). Contribution a l'etude du developpement de coprins. *Le Botaniste* **26**, 89–233.

ELHITI, M. M. Y., BUTLER, R. D. & MOORE, D. (1979). Cytochemical localization of glutamate dehydrogenases during carpophore development in *Coprinus cinereus*. *New Phytologist* **82**, 153–157.

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**, 343–375.

LEWIS, F. T. (1931). A comparison between the mosaic of polygons in a film of artificial emulsion and the pattern of simple epithelium in surface view (cucumber epidermis and human amnion). *Anatomical Record* **50**, 235–265.

LEWIS, F. T. (1943). The geometry of growth and cell division in epithelial mosaics. *American Journal of Botany* **30**, 766–776.

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

MOORE, D. (1981). Evidence that the NADP-linked glutamate dehydrogenase of *Coprinus cinereus* is regulated by acetyl-CoA and ammonium levels. *Biochimica et Biophysica Acta* **661**, 247–254.

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

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

MOORE, D., ELHITI, M. M. Y. & BUTLER, R. D. (1979). Morphogenesis of the carpophore of *Coprinus cinereus*. *New Phytologist* **83**, 695–722.

NIEDERPRUEM, D. J. & JERSILD, R. A. (1972). Cellular aspects of morphogenesis in the mushroom *Schizophyllum commune*. *CRC Critical Reviews in Microbiology* **1**, 545–576.

NIEDERPRUEM, D. J., JERSILD, R. A. & LANE, P. L. (1971). Direct microscopic studies of clamp connection formation in growing hyphae of *Schizophyllum commune*. I. The dikaryon. *Archives of Microbiology* **78**, 268–280.

OBERWINKLER, F. (1982). The significance of the morphology of the basidium in the phylogeny of basidiomycetes. In *Basidium and Basidiocarp* (ed. K. Wells & E. K. Wells), pp. 9–35. New York: Springer-Verlag.

RAJU, N. B. & LU, B. C. (1970). Meiosis in *Coprinus*. III. Timing of meiotic events in *Coprinus lagopus* (*sensu* Buller). *Canadian Journal of Botany* **48**, 2183–2186.

REIJNDERS, A. F. M. (1963). *Les Problemes du Developpement des Carpophores dans les Agaricales et de Quelques Groupes Voisins*. The Hague: Junk.

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.

SMITH, A. H. (1966). The hyphal structure of the basidiocarp. In *The Fungi*, vol. II (ed. G. C. Ainsworth & A. S. Sussman), pp. 151–177. New York: Academic Press.

SUNDBERG, W. J. (1978). Hymenial cytodifferentiation in basidiomycetes. In *The Filamentous Fungi*, vol. III (ed. J. E. Smith & D. R. Berry), pp. 209–314. London: Arnold.

THIELKE, C. (1982). Meiotic divisions in the basidium. In *Basidium and Basidiocarp* (ed. K. Wells & E. K. Wells), pp. 75–91. New York: Springer-Verlag.

WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *Journal of Theoretical Biology* **25**, 1–47.

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

(Received for publication 31 August 1984)