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ORIGIN OF THE HYMENOPHORE AND ESTABLISHMENT OF MAJOR TISSUE DOMAINS DURING FRUIT BODY DEVELOPMENT IN *COPRINUS CINEREUS*

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In *Coprinus cinereus* gill differentiation from the protenchyma is initiated before formation of an annular cavity in a region corresponding to the boundary between stipe and pileal 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 organised plates of columnar cells which constitute the primordial hymenia of adjacent gills separated by a developing gill cavity. The annular cavity arises only after gills are well formed. Tramal tissues of primary gills remain intimately connected to the periphery of the stipe for a considerable time, becoming secondarily freed only in well developed primordia. The morphogenetic polarities established during differentiation are maintained throughout fruit body development. From the earliest stage right through to maturation and autolysis, developmental changes proceed along two major vectors: from inner edge of the gill, i.e. the edge closest to the stipe, towards the outer edge (that closest to the pileipellis) and from the cap margin towards the cap apex.

Maturation of the fruit body of *Coprinus cinereus* (Schaeff.: Fr.) S.F. Gray is accompanied by a specific pattern of changes in enzyme activity and metabolite levels (Ewaze, Moore & Stewart, 1978; Moore, 1981, 1984), especially in the cap. The net result in the cap is an accumulation of urea, and probably other nitrogenous metabolites, as osmotic solutes which drive water into the cells of the hymenium. This leads to the inflation of these cells and their expansion can account for the changes in form through which the cap progresses as it matures (Moore, Elhiti & Butler, 1979).

The biochemical data identify specific enzyme regulatory mechanisms that can be associated with morphogenetic changes in the fruit body. Understanding how this regulation is integrated endogenously during development requires detailed knowledge of the processes leading to establishment of tissue domains. A variety of developmental patterns have been recognised in agarics. Reijnders (1963, 1979) showed that different *Coprinus* species could exhibit different ontogenies but he did not include *C. cinereus* in his survey. The particular isolates used here have been frequently misidentified in the literature (Pinto-Lopes & Almeida, 1970; Moore *et al.*, 1979) so it was important to determine the mode of development (of the hymenophore especially) in this species in preparation for more detailed study of the biochemical aspects of morphogenesis.

MATERIALS AND METHODS

Organism and culture conditions

A dikaryon of *Coprinus cinereus* (Schaeff.: Fr.) S.F. Gray sensu Konr. was used throughout; it was originally isolated in Birmingham in 1973 by Dr G. M. Butler and given the isolation number 177/1. It is deposited in ATCC under no. 42721. Cultures were grown as described by Moore & Ewaze (1976), using sterilised horse dung to produce fruit bodies. Fruit bodies at two stages of development were used. Moore *et al.* (1979) have described the six stages into which fruit body development has been divided; the material used here was categorised as Stage 1 primordia (2–6 mm in height, prekaryogamy) and Stage 2 primordia (6–9 mm in height, meiosis occurs during this stage).

Microscopic preparation

Fruit bodies were excised from the parental mycelium and cut into segments vertically. These were fixed in 5·75% glutaraldehyde dissolved in Sorenson phosphate buffer (52·5 mM, pH 6·9) containing 0·01 mM magnesium sulphate and 1 mM sucrose. Penetration was assured by fixation under reduced pressure and the usual fixation period was 4 h although overnight fixation at 2 °C had no adverse effect.

The samples were post-fixed by adding an equal

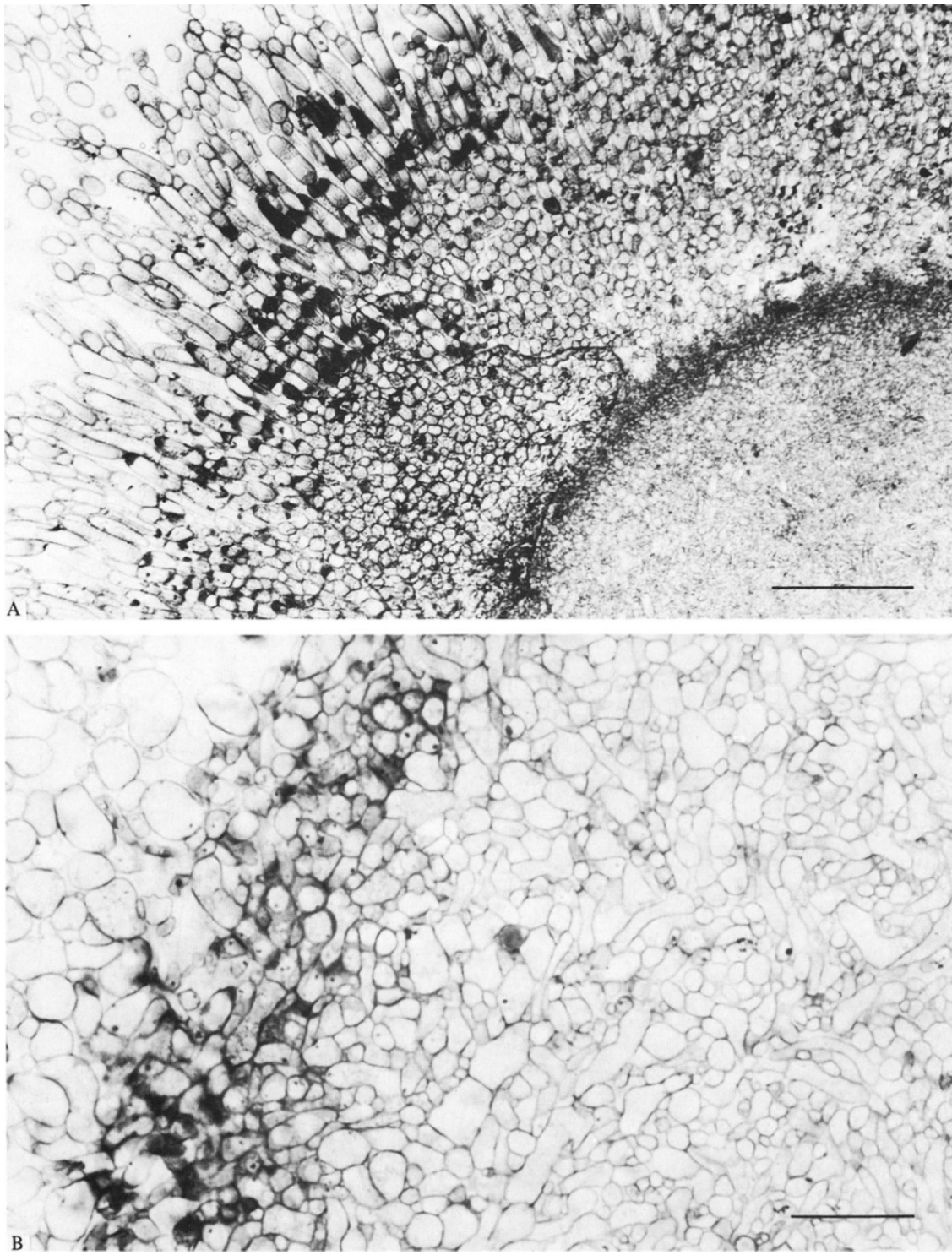


Fig. 1. Transverse sections of the apex of the Stage 1 primordial cap of *C. cinereus*. In this region cap and stipe tissues are not differentiated. The fruit body protenchyma (lower right) is separated from the veil by a band of presumptive pileipellis, and still shows (B) considerable evidence of its hyphal origin. Scale bar: A = 50 μm, B = 10 μm.

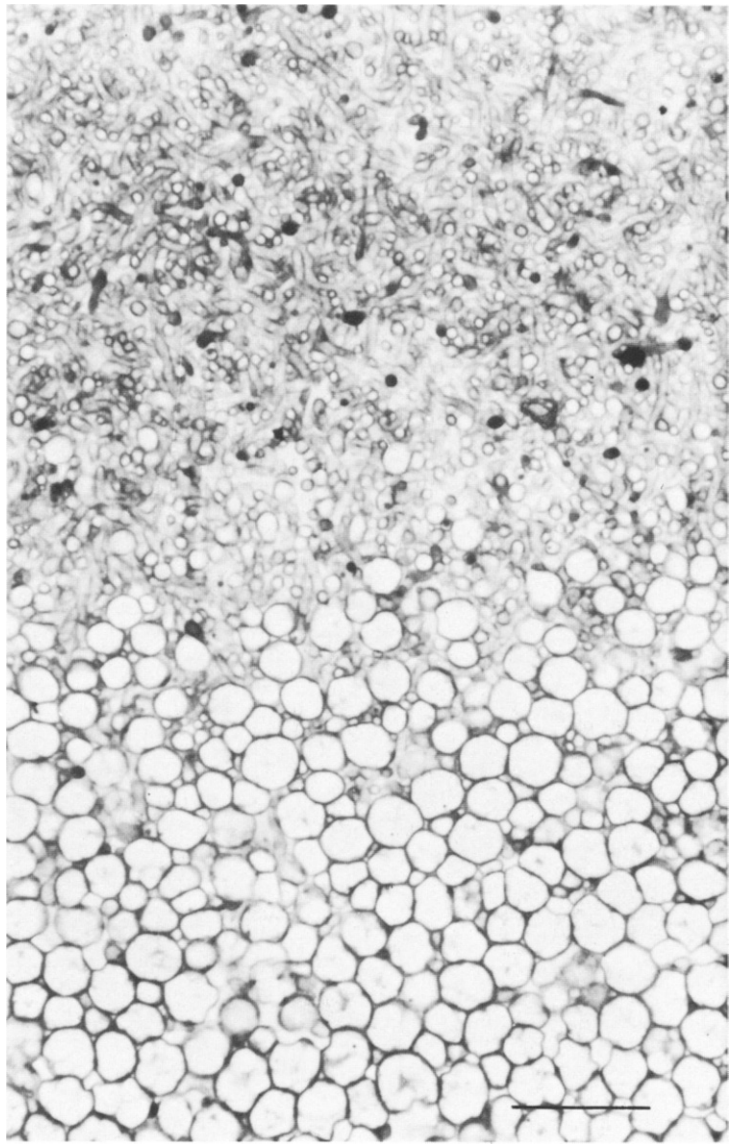


Fig. 2. Section of a stage of development of *C. cinereus* which shows differentiation of stipe tissues (bottom) while the 'cap' (top) is still protenchymal. Scale bar = 10 μm.

volume of the above buffer solution containing 1 % osmium tetroxide. After 90 min the tissues were dehydrated through an ethanol series and embedded in low-viscosity resin (Spurr, 1969).

Serial sections of 2 μm thickness were cut in a plane at right angles to the long axis of the stipe. Sections were transferred from the glass knife onto water droplets on 'Multispot' PTFE-coated glass slides (C. A. Hendley (Essex) Ltd, Oakwood Hill Industrial Estate, Loughton, Essex) and then dried. Sections were stained in 1 % toluidine blue in 1 % boric acid for 5 min at 60° for examination by light microscopy.

RESULTS AND DISCUSSION

Observations are derived from serial sections cut transversely from the apex to the margin of the cap. The apex of the cap consisted of a central core of multidirectional and interlaced hyphae referred to

generally as the context (Fig. 1 A). It was surrounded by a deep layer of radially oriented veil cells. The deposition of stain at the periphery of the context (Fig. 1 B) is attributed to polysaccharide accumulations and this zone of cells, which differentiates to form a distinctive layer in older stages, is identified as the presumptive pileipellis.

Sections descending the cap reached a point where the central area of the context was occupied by parallel and vertically oriented stipe cells. The outer layer of the stipe was poorly defined, pileal and stipe hyphae intermingling (Fig. 2). In subsequent sections a wave-like contour was observed around the stipe, the crests of the waves consisting of cells with a cross-sectional area intermediate between that of the majority of stipe and pileal hyphal cells. Hyphae radiated from the pileus in the troughs of the waves and appeared to diverge into the surrounding areas (Fig. 3 A). As successively more differentiated tissues were examined this pattern became more pronounced and regular groups of densely packed cells were seen to surround the stipe (Fig. 4 C, right). These groups of cells became chromophilic, elongated and formed two radially arranged rows of oblong cells (Fig. 3 B, C). A cavity appeared near the stipe and began to separate the paired rows of oblong cells (Fig. 3 B). The cavity elongated centrifugally; this is clearly the gill cavity which now separated two poorly differentiated hymenial layers. The trough area of the original wave-like contour consisted of the poorly developed gill trama and hyphae extended from it into the hymenium and the stipe tissues (Figs 3 B, 4). No annular cavity was observed at this stage.

The differentiation described, namely development from ill-defined groups of cells to small but recognisable gills which had their trama continuous with the stipe, took place over a very small fraction of the length of the cap; on average, as few as fifteen 2 μm sections were required to encompass the entire sequence. In sections taken lower down the pileus the gills were wider and the formation of secondary gills was well advanced (Fig. 4). The gills continued to increase in width until the outer end of the gill cavity was about 15 μm from the pileipellis.

The area enclosing the outer end of the gill cavity consisted of a group of narrow, tightly appressed cells in all sections throughout the cap of the Stage 1 fruit body. Similarly in all sections at this stage, the hymenium was discontinuous over the inner edge of primary gills, their tramal layers remaining in intimate connexion with the stipe tissues. In contrast, secondary gills were characterized by an enclosed trama, the hymenium being continuous over the inner edge of the gill (Fig. 4).

In Stage 2 fruit bodies a similar series of images was observed, though in these larger fruits the region between the stipe and the differentiating pileus had a rather more open structure which implied initiation by this stage of the development of an annular cavity (Fig. 5).

The origin of secondary gills was especially studied in sections of Stage 2 material. These were initiated by an enlargement of the outer end of the developing gill cavity (Fig. 6 A) which then bifurcated as the overlying group of appressed, small cells divided into two such groups, each at the head of what now corresponded to two developing gill cavities (Fig. 6 B, C). The secondary gill widened centrifugally but the distance between the inner edge and the stipe remained constant while the distance between the outer edge of the gill and the pileipellis decreased. In the lower portions of the Stage 2 pileus, where all primary gills were fully extended, tertiary gills were observed. These differed in their geometrical structure from the secondaries in that in successive sections towards the pileus margin the distance between the stipe and the inner edge of the tertiary gill diminished.

From the time of their formation, secondary and tertiary gills had a presumptive hymenium extending completely over their inner edge. On the other hand, primary gills, when first formed, had the trama at the inner edge in continuity with the lipsanenchyma and stipe tissue. However, towards the cap margin in Stage 2 primordia, sections showed that the hymenium extended over the inner edge of most primary gills, separating the trama from the annular cavity (Fig. 7).

Schmitz (1842) was one of the first to observe the presence of a general annular cavity around the stipe, the roof of which was lined with a continuous palisade layer; this latter being the young hymenophore. These observations were confirmed in Brefeld's study on *C. lagopus* (1877), Hoffman's work on *C. fimetarius* (1860), and Atkinson's descriptions of *Agaricus* spp. (1906, 1914). A second mode of development was originally thought to be of limited applicability (Atkinson, 1914) until Levine (1914) reported it in *C. micaceus* and then in other species and concluded that this course of development prevailed in most Agaricaceae. According to Levine (1914) no annular prelamellar cavity is found. Instead, the palisade layer develops a series of groups or ridges which then elongate, split, and halves of adjacent ridges unite to form the lamellae. Levine (1914) maintained that the protenchyme tissue between the ridged groups of palisade cells is continuous with the underlying stipe tissue from the earliest stage.

Atkinson (1916) refuted Levine's work in his treatise on *C. comatus*, *C. atramentarius* and

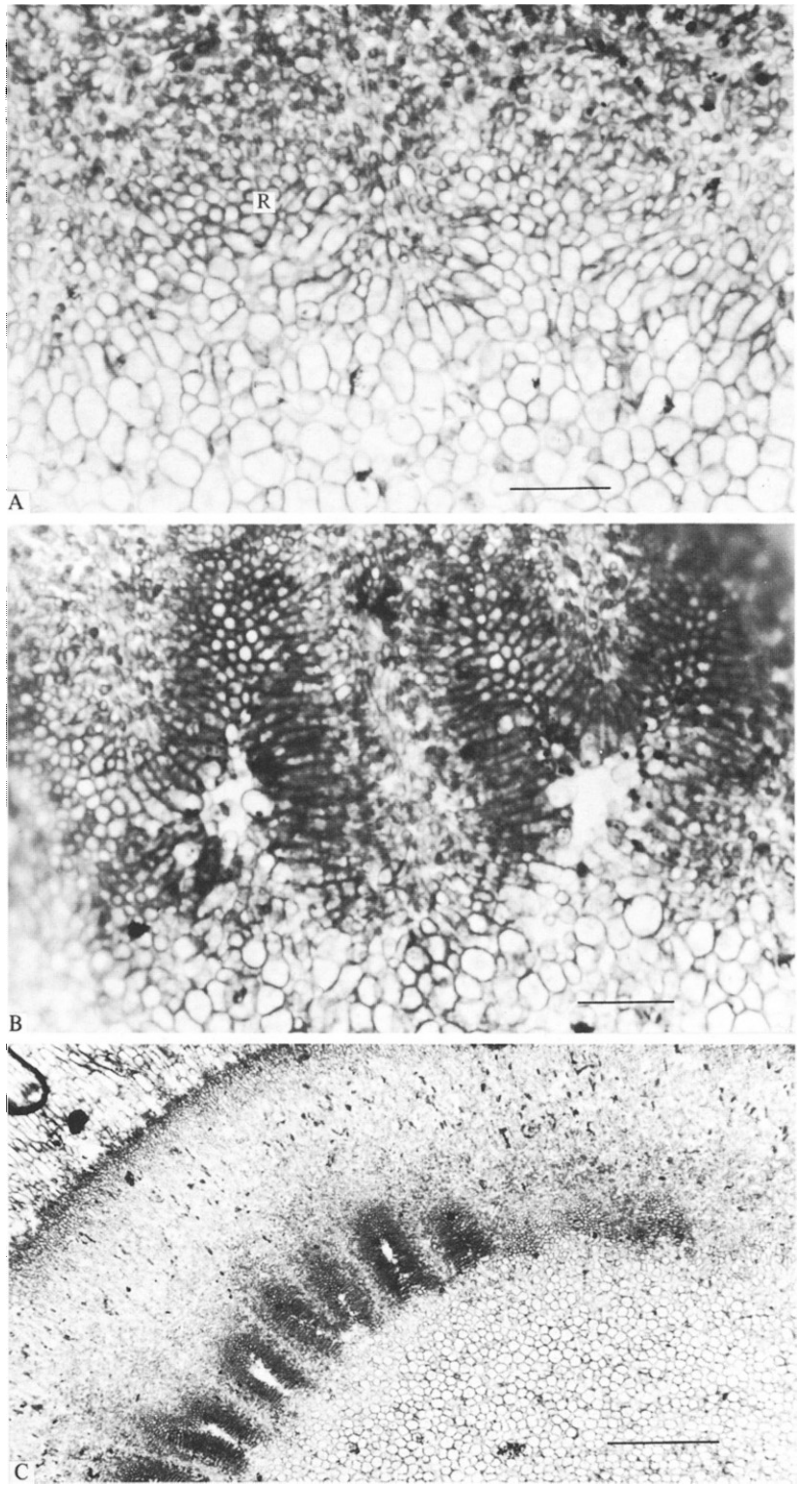


Fig. 3. Development of palisade ridges (R) of closely packed cells which act as organizing centres for gill differentiation. (3C) An oblique section showing a variety of successive stages in the process. Note that tramal tissues of primary gills are in intimate connexion with the stipe periphery, and that in B (right) a secondary gill has already been initiated. In all cases the stipe is at the bottom of the picture. Scale bars: A = 10 μm, B = 10 μm, C = 40 μm.

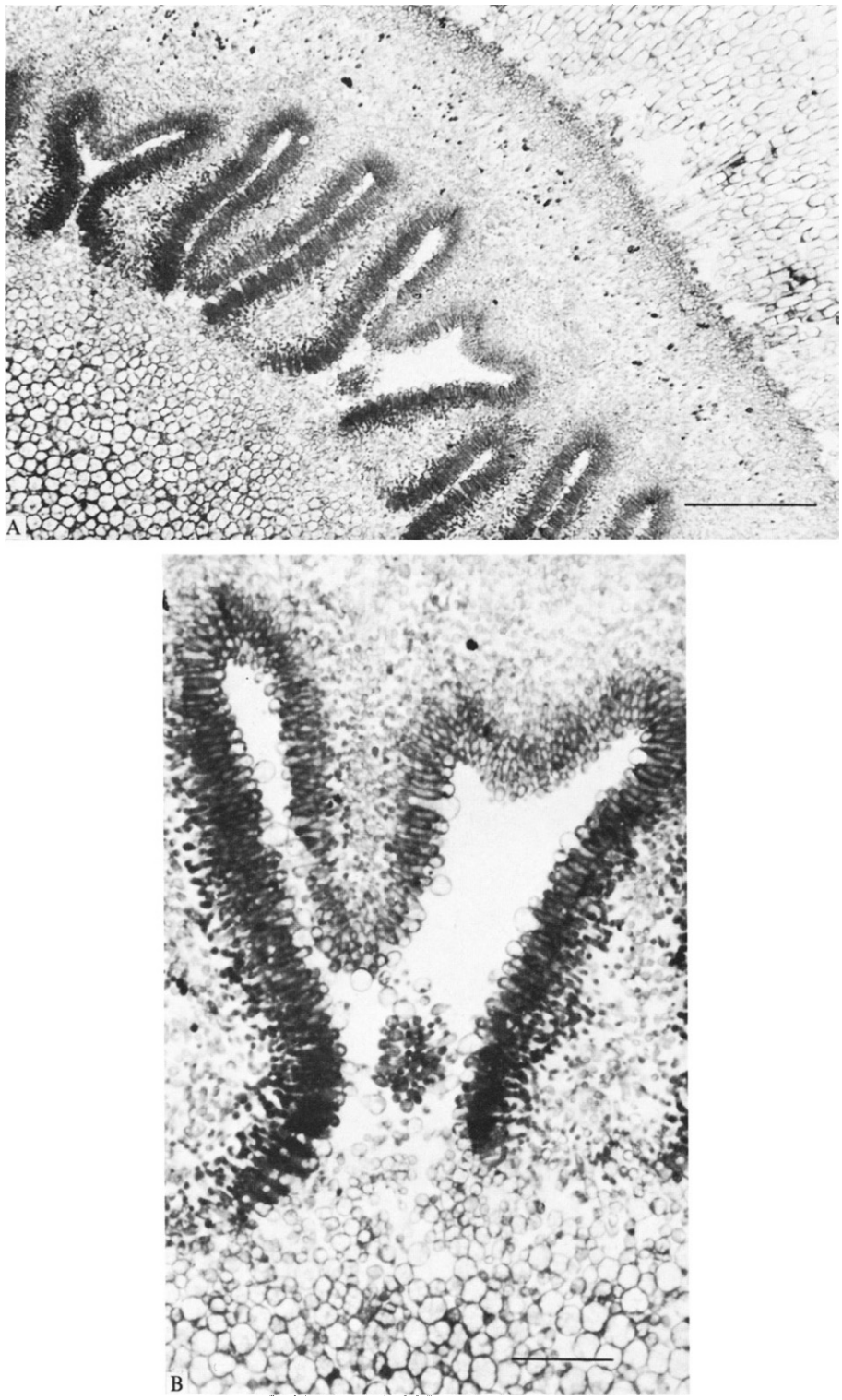


Fig. 4. The most highly developed gills of a Stage 1 primordium of *C. cinereus*. Note the continued connexion between trama of primary gills and the stipe periphery. Scale bars: A = 40 μm, B = 10 μm.

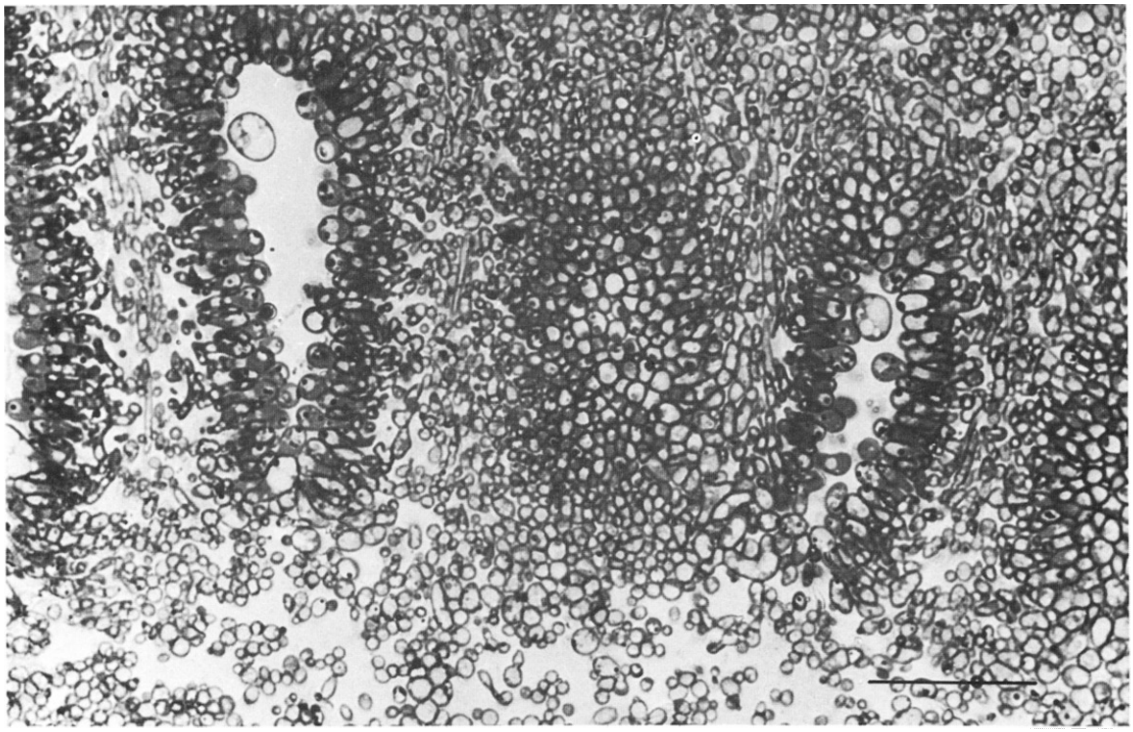


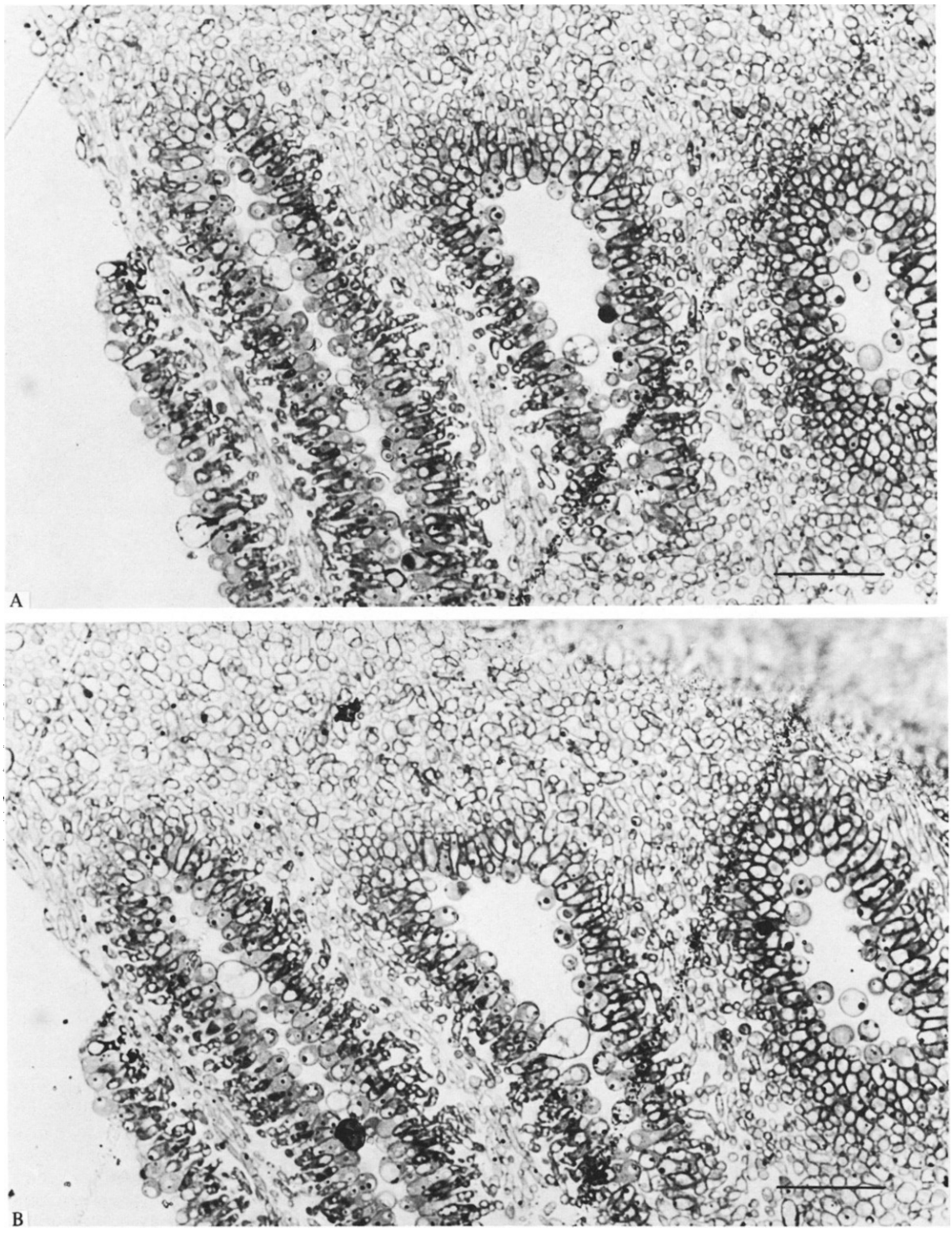
Fig. 5. Gills of a stage 2 primordium at a roughly equivalent grade of development to that shown in Fig. 4, but note the much looser organization of the boundary between cap and stipe tissues which denotes initiation of the annular cavity. Scale bar = 20 μm.

C. micaceus. He maintained that, in Agaricaceae, there is first a general annular cavity with a continuous palisade layer which grows 'outward in a centrifugal direction over the under-surface of the pileus, following the centrifugal growth of the latter'. According to Atkinson (1916) the unequal growth of areas of the palisade layer gives rise to folds which are the fundaments of the lamellae: as these widen in the annular cavity, they reach the underlying stipe or the fundamental plectenchyma surrounding the stipe. The stipe tissues and the gill trama therefore come to appear continuous. Atkinson (1916) argued that it is this secondary attachment of the gill trama to the stipe which led Levine to his 'erroneous' conclusions. Similarly, Chow (1934) reported palisade pockets with the interlying tissues continuous with the stipe, but, again, attributed this feature to the growth of the gills into the stipe.

Observations made since have shown that both modes of development do occur, but in different species (Reijnders, 1963, 1979). These observations indicate that certain species do not have a continuous palisade layer or a general annular cavity and that pileus and stipe hyphae are intimately intermingled in the regions between the palisade ridges. Reijnders (1979) examined a number of *Coprinus* spp. and found some to be rupthymenial (gill differentiation proceeding away from the stipe) while others were levhymenial (gills differentiate towards the stipe).

The observations reported here show quite clearly that *Coprinus cinereus* exhibits the rupthymenial mode of development. The groups of small, tightly-packed cells seen at the crests of a wave-like contour surrounding the stipe in the youngest tissues correspond to Levine's palisade ridges and their subsequent development parallels Levine's description exactly. The important points are that there is no initial annular cavity, this arises later when the gills are well-formed, and the gills differentiate from a plectenchyma along axes which are polarised such that the 'wave' of differentiation migrates from the stipe towards the pileipellis across the width of the gill, and from the cap margin towards the cap apex along the length of the gill.

This developmental polarity is the same as that seen during the later stages of maturation. In


A
B

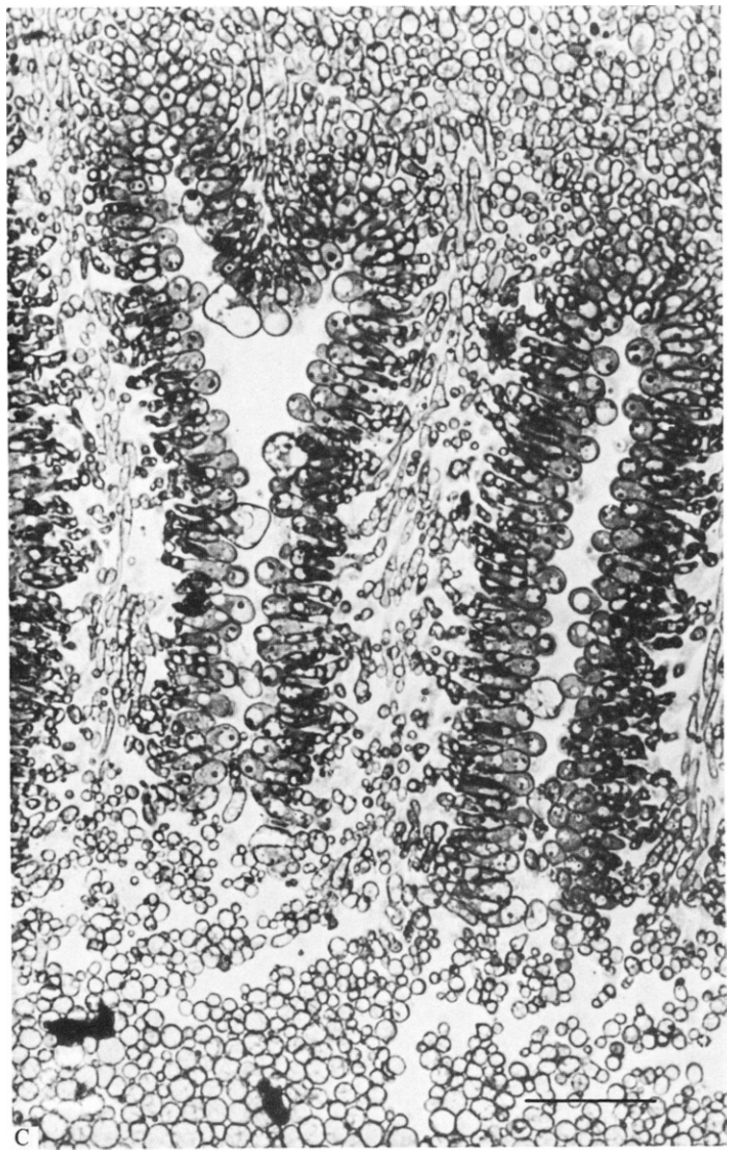


Fig. 6. Successive serial sections of a Stage 2 primordium showing origin of secondary gills by bifurcation of the advancing end of the differentiating gill cavity. Scale bars = 20 μm.

C. cinereus it is the tissue at the cap margin which first reaches the stage of development at which meiosis is initiated (Raju & Lu, 1970). Chow (1934) noted that the maturation of the basidia follows the same general order in *Coprinus* spp. and begins at the interior-inferior margin of each lamella, and it is a matter of simple observation that the production and pigmentation of spores, as well as autolysis of the gill, are initiated at the edge of the gill closest to the stipe and that pigmentation and autolysis proceed from that edge towards the outer edge of the cap and from the cap margin towards the apex. These observations indicate that events associated with maturation progress in an upward direction, from the cap margin to the apex, and across the gill, from the inner edge (adjacent to the stipe) to the

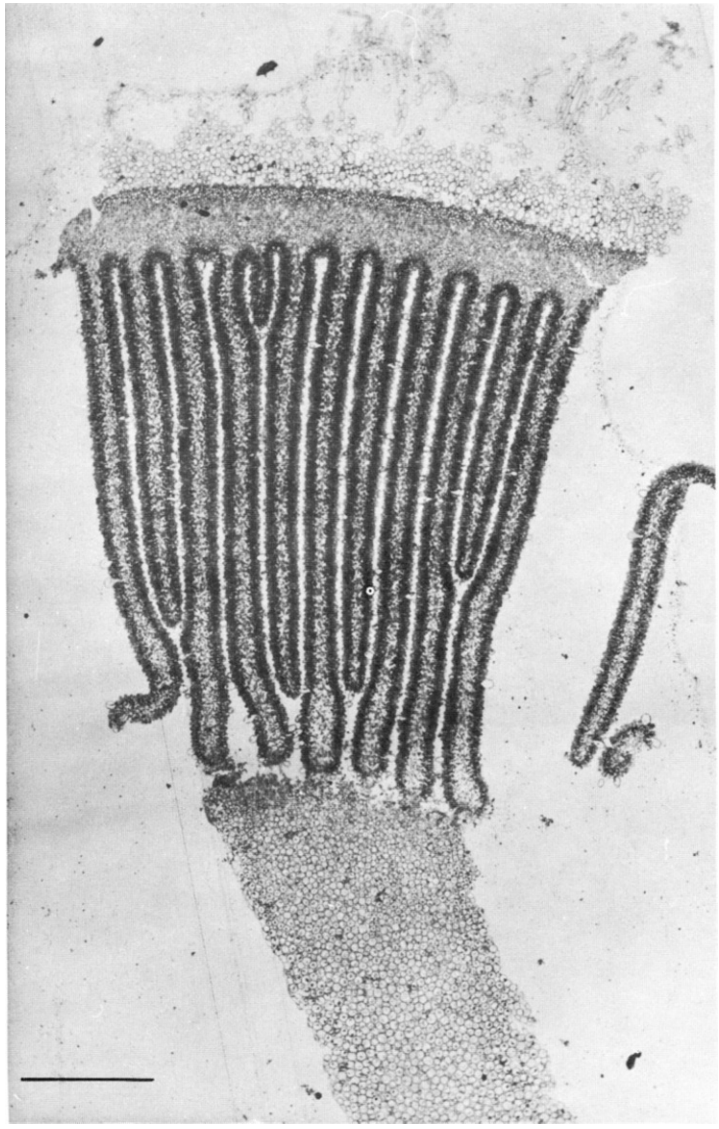


Fig. 7. Section (near the cap margin) of a segment of a Stage 2 primordium showing union of hymenium over the inner edge of the primary gills which are now free of the stipe periphery. Scale bar = 100 μm.

outer. Thus the developmental polarity established when the gill tissues are first delimited is maintained throughout the fruit body maturation process.

Only the formation of tertiary gills in *C. cinereus* could possibly be interpreted as being levhymenial. Even in this case, though, we feel that the better interpretation is that they, too, are rupthymenial but that the points at which their successive sectors are initiated vary in distance from the stipe along their length as a function of whatever morphogenetic signal is perceived that controls gill spacing in the essentially conical fruit body cap.

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