

Development of the basidiome of *Volvariella bombycina*

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Basidiome development of *Volvariella bombycina* was examined with optical and scanning electron microscopy. Primary gills arose as ridges on the lower surface of the cap, projecting into a preformed annular cavity. Secondary and tertiary gills were added whenever space became available by bifurcation of an existing gill either from one side or at the free edge, or by folding of the palisade layer near or at the roots of existing gills. These processes gave rise to sinuous, labyrinthiform hymenophores as a normal transitional stage to the mature regularly radial gill pattern. Although many cystidia spanned the gill space at the early stages, their tips causing depressions in the opposing hymenium, cryo-SEM examination showed that marginal and facial cystidia of mature gills bore droplets, suggesting that these cells are more likely to act as secretory elements than as structural members. Transformation of the convoluted gills into regularly radial ones is probably accomplished by cell inflation in the gill trama. Marking experiments and consideration of cystidial distribution suggest that *V. bombycina* gills grow at their root, not at their margin.

Key words: Hymenophore development, Morphogenesis, Gill pattern, Cystidium, *Volvariella bombycina*.

Volvariella bombycina (Schaeff.: Fr.) Sing., the silver-silk straw mushroom, is an edible fungus which fruits readily in culture. During fruiting trials of this fungus, two strains from different sources regularly produced a wide variation in basidiome morphology and this developmental plasticity has been described in detail recently (Chiu, Moore & Chang, 1989). In addition to the normal hemi-angiocarpous mode of development, basidiomes developed angiocarpously (with enclosed hymenium) or gymnocarpously (exposed hymenium) and in the latter the genus-specific basal volva might be absent or abruptly bulbous in contrast to the normal membranous form. Basidiomes could also be sterile carpophoroids, gasteromycetoid or morchelloid forms, or agaricoid forms with inverted caps or supernumerary hymenia. Hymenophore pattern ranged from the sinuous, labyrinthiform morchelloid hymenium to the normal radial gill of a typical agaric. These spontaneous variants were interpreted to imply that the basidiome ontogenetic programme is a sequence of subroutines which can be modulated independently. Co-ordinated activation of the subroutines specifies the ontogeny and the architecture of a fruiting structure. Spontaneous developmental plasticity may be a response to environmental stress causing expression of these morphogenetic subroutines in the wrong sequence and/or in the wrong place but nevertheless permitting spore dispersal under stress conditions.

This interpretation of basidiome morphogenesis as resulting

from expression of a series of essentially self-contained segments of a developmental programme implies that the sequence in which the segments are put into effect would determine the overall form of the fruit body. It may also be that its genetic control is organized into a similar logical hierarchy. With a view to establishing the morphogenetic mechanisms on which basidiome development is based we have examined the ontogeny of the *V. bombycina* basidiome in some detail and report the results here.

MATERIALS AND METHODS

Cultivation

Volvariella bombycina (Schaeff.: Fr.) Sing., wild-type strain Vo-1 was used. Cultures were maintained and fruiting was induced as described previously (Chiu *et al.*, 1989).

Ontogenetic studies

Development from the initiation of primordia to the completion of sporulation in each basidiome was followed during cultivation. Radial and transverse sections of caps or portions of caps at different developmental stages were excised and processed for conventional SEM examination. Hymenophore morphogenesis and hymenial cell differentiation were examined. Surgical experiments were performed on some basidiomes (normal forms and polymorphic variants) at different developmental stages. A small radial portion of the

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Fig. 1. Diagrammatic summary of basidiome development in *Volvariella bombycina*. The shaded area in the sequence of bisected 'half basidiomes' in the lower part of the figure shows the gill area. The diagrams are not drawn to scale but photographs on which they are based can be found in Chiu *et al.* (1989).

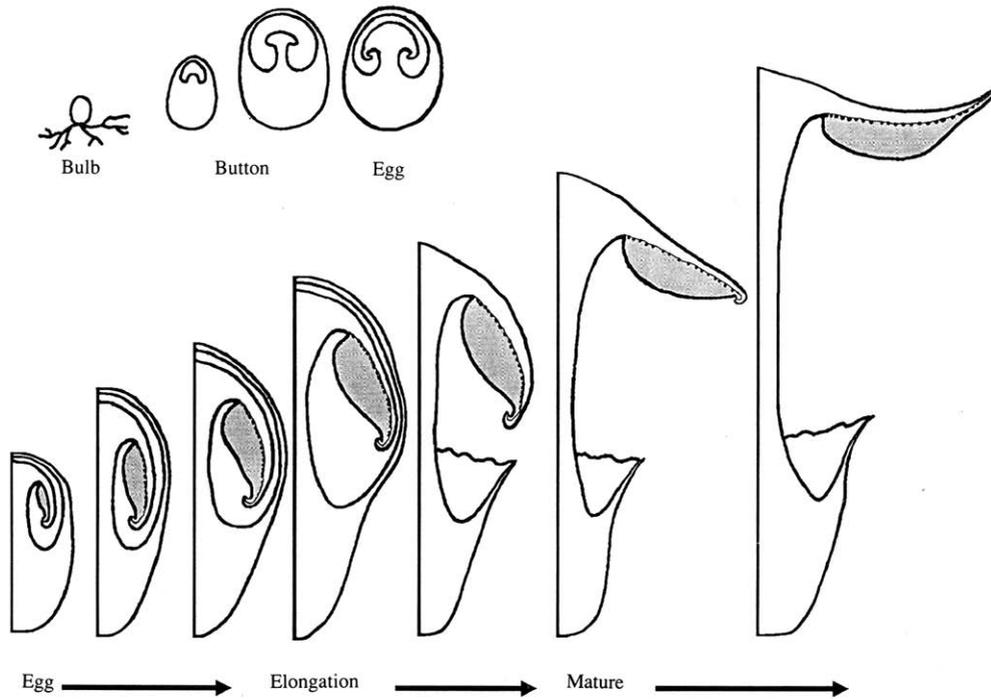
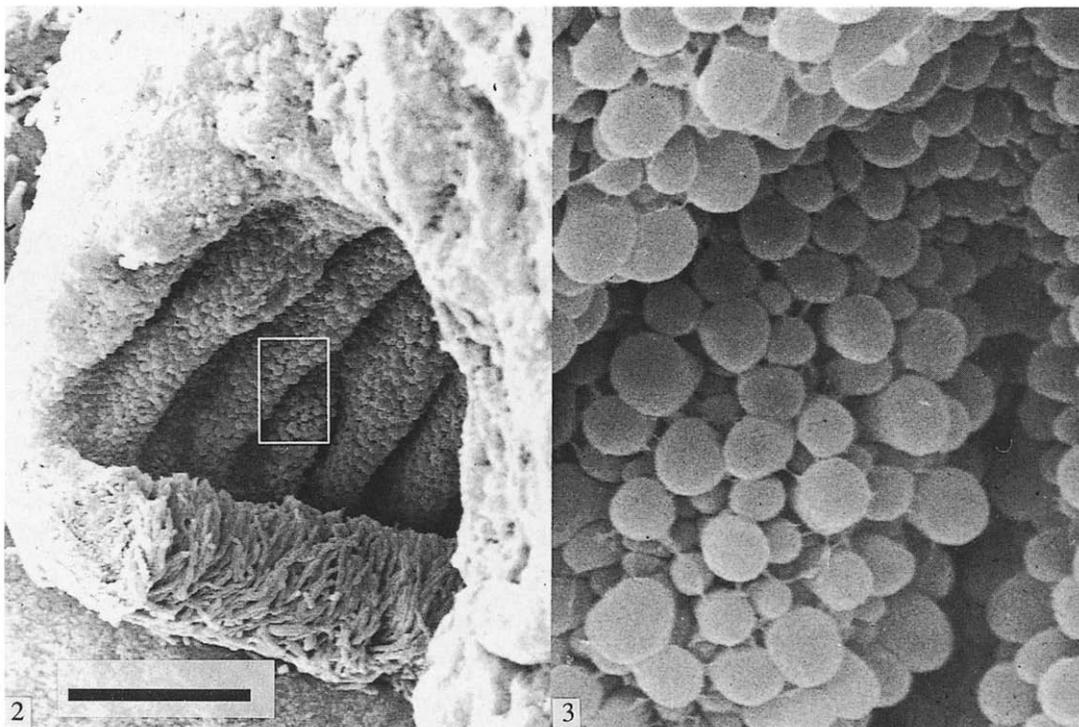


Fig. 2. SEM view of a bisected cap of *Volvariella bombycina* at the late button stage, looking at the roof of the annular cavity where it is thrown into the radial folds or ridges which represent the primary gills. Scale bar = 100 μ m. **Fig. 3.** View of the highlighted region of Fig. 2 (8-fold magnification) showing the cell distribution pattern of the first-formed hymenium.



cap tissue bearing 2–3 gills was excized daily from primordia of egg to maturation stages (see below) and fixed for SEM examination. Similar surgical experiments were performed on a gymnocarpous basidiome (6 cm in length) bearing multiple sites of supernumerary hymenia. Excision of tissues bearing the supernumerary hymenia attaching to the lower hymenophore was carried out, and growth of the largest supernumerary hymenia was recorded photographically. In all cases, gross morphological change of the hymenophore and hymenial cell distribution and differentiation were examined.

Marking experiments

Black ink marks were painted on the upper surface of the cap and/or along the free margins of the gills of young gymnocarpous fruit bodies with a Chinese calligraphic brush in order to trace the relative growth rates of the different parts of the hymenophore; successive stages were then photographed.

Scanning electron microscopy (SEM)

Chemically-fixed, dehydrated and critical-point dried specimens were prepared for conventional SEM as described previously (Chiu & Moore, 1989). Frozen-hydrated specimens were prepared for cryo-SEM as follows: fresh gills excized from basidiomes at different developmental stages were flash-frozen in nitrogen slush in the Hexland Cryotrans system CT1000. The specimen was transferred to the specimen chamber of the Cambridge 200 SEM and maintained at

–160 °C unless condensate was to be sublimed. The specimen was examined directly or after coating with gold.

RESULTS AND DISCUSSION

The life-history, especially nuclear behaviour, of strain Vo-1 has been reported before (Chiu, 1986; Chiu & Chang, 1987a, b). A homokaryon of *V. bombycina* is self-fertile (Elliott & Challen, 1985), simple-septate and multinucleate (Chiu, 1986) and will form heterokaryons when paired with other monosporous isolates (Elliott & Challen, 1985; Chiu & Chang, 1987a) but no incompatibility factors have been identified (Chiu & Chang, 1987a). Normal basidiome development (summarized in Fig. 1) has been illustrated by Chiu *et al.* (1989). Throughout development from button to mature stages the cap context increased in thickness. The cap margin and the gill curled inwards (involute margin); this involute margin was straightened only at the final mature stage at which the cap changed from campanulate to flat.

Basidiome development

Excision of tissues at the button stage caused abortion of fruit bodies; primordia at the egg stage (see Fig. 1) survived surgery, so successive removal of segments for examination was possible from that stage. Normal fruit bodies and fruit bodies undergoing daily surgical excision development through the same sequence of events. Depending on the size and stage of the developing primordium at the start of the experiment, daily excision could be continued for up to 7 d.

Fig. 4. Surface view of a young gill at the mid-egg stage of development in *Volvariella bombycina* to show the cell distribution pattern of the hymenium. Scale bar = 25 µm. **Fig. 5.** Edge view of three adjacent gills at the mid-egg stage of development. Note how inflated cells (presumed to be facial cystidia) span the gill spaces, impinging on the opposing hymenium. Scale bar = 25 µm.

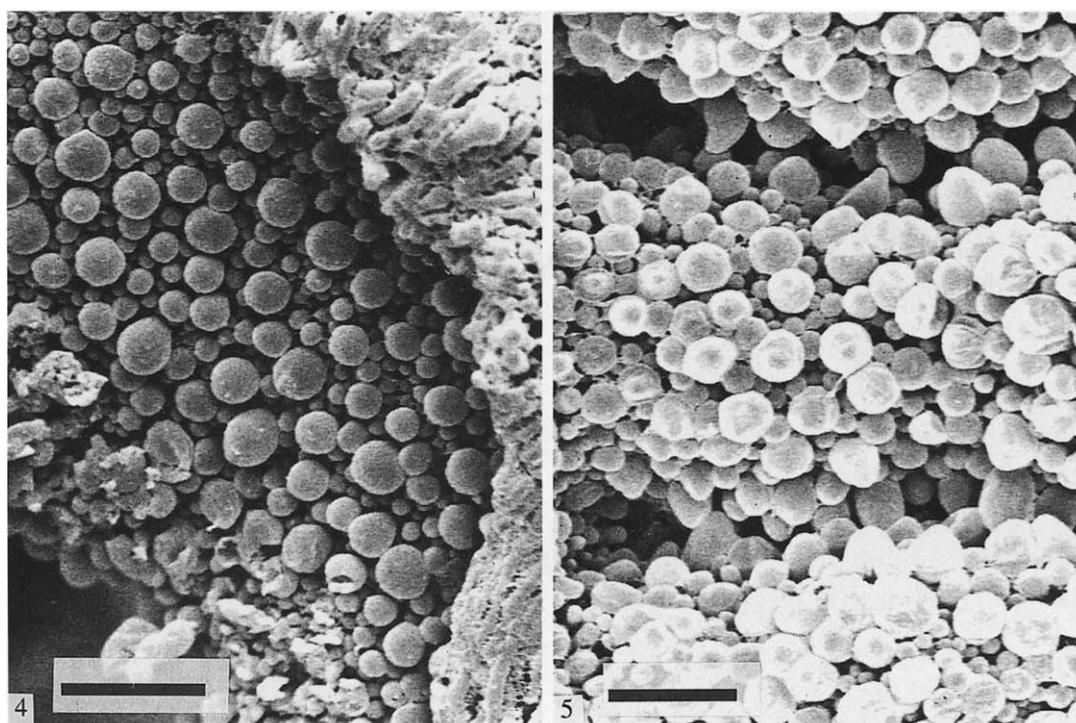
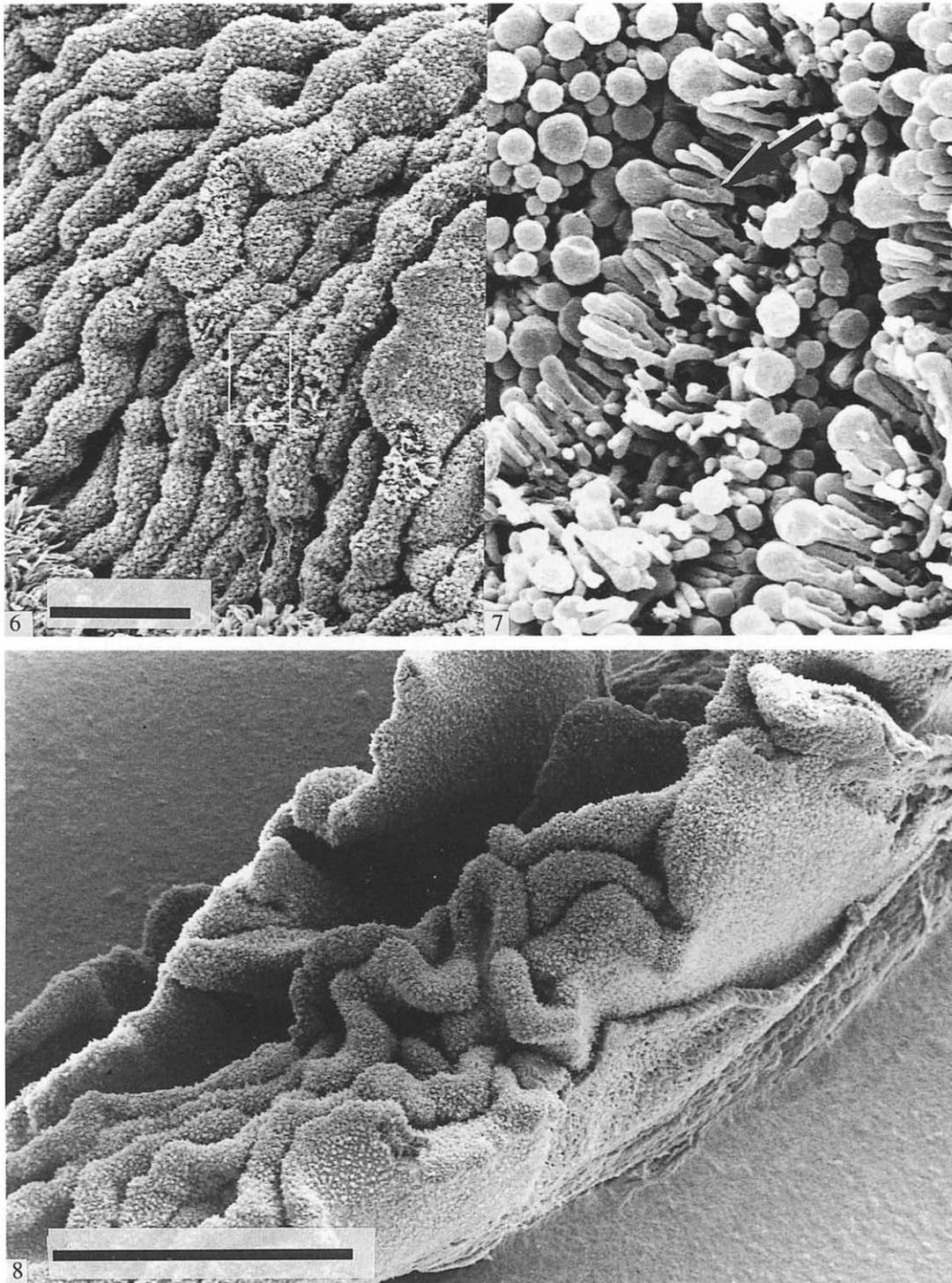


Fig. 6. Surface view of an upturned excized cap at the mid-egg stage of development in *Volvariella bombycina*. The young gills are broad, becoming regionally sinuous and labyrinthiform. Scale bar = 250 μ m. **Fig. 7.** Magnified view (8-fold magnification) of the highlighted region of damage in Fig. 6 reveals the pattern of branching of the young hymenial cells and particularly shows that hymenial cells of different size can arise from the same branching system (arrow). **Fig. 8.** Surface view of a segment of the cap of *Volvariella bombycina* excized at the late egg stage of development. Note the very sinuous and labyrinthiform gills. Scale bar = 1 mm.

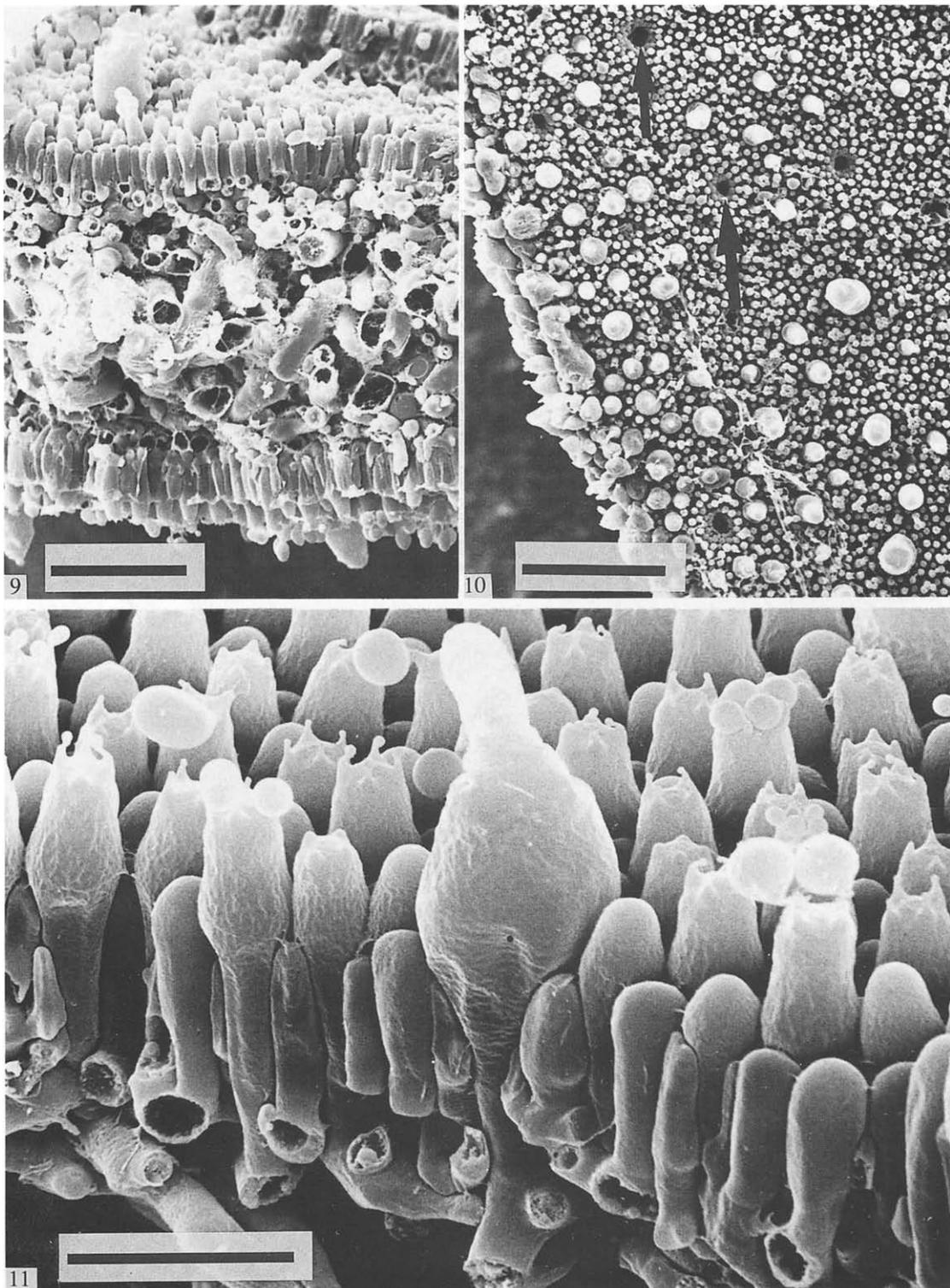


Fruit bodies undergoing surgical excision were still able to expand the remains of their pilei and complete sporulation.

Button stage. Cap differentiation was initiated by the hemispherical growth of the apical portion of the cap/stipe initial in the schizogenous cavity enclosed by the universal

veil and the basal bulb. The hymenophore developed more or less simultaneously with the annular cavity (Fig. 2). The continuous palisade layer lining the hymenophore started cell differentiation, the elongated cylindrical hyphae swelling at their apices to become clavate, forming the hymenium (Fig. 3). The hymenial cells were uniform in size. This very earliest

Fig. 9. SEM view of a sectioned gill excized from *Volvariella bombycina* at the elongation stage of development. Note the closely packed, greatly inflated cells of the trama. Scale bar = 40 μm . **Fig. 10.** View of the free (marginal) edge of a gill of *Volvariella bombycina* excized at the elongation stage. Note the depressions in the hymenium (arrowed) which are thought to be due to localized contact inhibition caused by buttressing facial cystidia from the opposing hymenium of the adjacent gill. Scale bar = 100 μm . **Fig. 11.** View of the mature hymenium of *Volvariella bombycina*. Scale bar = 10 μm .



hymenium then started growth into ridges, initiating formation of the gills.

Early to mid-egg stage. The distance between the central stipe and the hymenophore was increased to a fixed distance;

consequently, the future gills were free (Fig. 2), a genus-specific feature (Reijnders, 1963). Evenly intermingled large and small cells (presumed to be cystidia and basidia respectively) characterized the hymenium at this stage (Fig. 4). Intimate contact between large cells of the same gill (Fig. 4)

and of opposing gills (Fig. 5) was observed. Neighbouring hymenial cells of different size on the same hymenium could arise from the same gill tramal branch (fascicle; Fig. 7, arrowed).

Gills at the mid-egg stage were short, narrow and closely packed (Fig. 6). Marginal cystidia started to differentiate, locating the free edge of the gill. Because gills were crowded, marginal cystidia and other cells, especially facial cystidia, of opposing gills were in touch (Fig. 5). Contact inhibition by facial cystidia caused depressions to appear in the opposing hymenium (shown in Fig. 10).

As this stage progressed the distribution of facial cystidia began to change, from being uniformly crowded over young gills (Figs 3, 4) to being aggregated near the free edge of the gill but more scattered over the rest of the hymenium.

Late egg stage. Local proliferation of the gills was observed first in the upper region of the hymenophore, though samples excised at a later date had the region of proliferation shifted to the middle and lower regions (Fig. 8). At the site of proliferation the hymenophore appeared to be sinuous and labyrinthiform (Fig. 8) in comparison with the regular folding observed at earlier stages (Figs 2, 6).

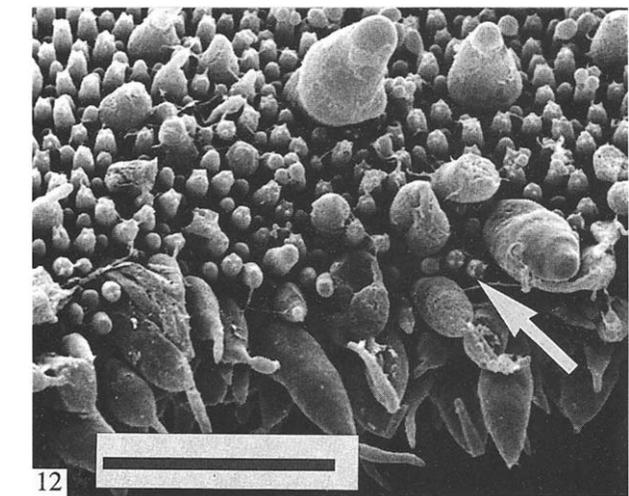
Mature marginal cystidia, each with a short apical papilla, marked the free edges of the gills. Contact between gills at the early stages of development accounts partly for the polymorphism of facial cystidia (Shaffer, 1957); small facial cystidia bore single apical or eccentric knobs or papillae whilst the large ones were cylindrical. Large facial cystidia were more numerous near the free edge of the gill while the small ones were scattered in the hymenium.

Elongation stage. Tramal hyphae of gills excised during the brief elongation stage (at which stipe elongation and cap expansion rupture the universal veil to expose the hymenophore) were greatly inflated (Fig. 9). Depressions in the hymenium were again evident (Fig. 10, arrows); their persistence through to the elongation stage suggests that for the major part of the development of the cap adjacent gills are buttressed apart by facial cystidia spanning the gill cavity. This arrangement does not persist beyond the elongation stage, however.

In addition to the gills which ran along the whole length of the radius of the cap (primary gills, Reijnders, 1979), there were gills of lesser ranks (secondary or tertiary gills) extending along only a portion of this length. Gills of lesser ranks did not arise at the same distance away from the central stipe. No regular pattern was observed. Yet the final phenotype of the hymenophore was of a radially well-spaced gill pattern with the central free stipe (shown in Fig. 19).

Mature stage. A narrow-bladed gill at maturity was generally ventricose with an entire, erose or wavy margin. Veins (raised lines) sometimes appeared on the surface of the gill. In the hymenium, basidia of various developmental stages were scattered, those of similar developmental stage being well-separated by differential maturation of hymenial cells between (Fig. 11) (Chiu *et al.*, 1989). Basidia were also intermingled with marginal cystidia at the free edge of the gill (Fig. 12);

thus, the free edge is not a totally sterile region. In contrast, the edge of the gill within the involute margin was devoid of marginal cystidia.



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Facial cystidia were scattered and solitary, but aggregated near the free edge and showing decreasing density of distribution towards the edge attaching to the cap context (Fig. 13, also evident in Fig. 10); a similar distribution of facial cystidia has been reported in *Flammulina velutipes* (Williams, 1986). The absence of adjacent facial cystidia suggests that as a hyphal tip differentiates into a cystidium it exerts an inhibitory influence over the surrounding zone as has been demonstrated in *Coprinus cinereus* (Horner & Moore, 1987).

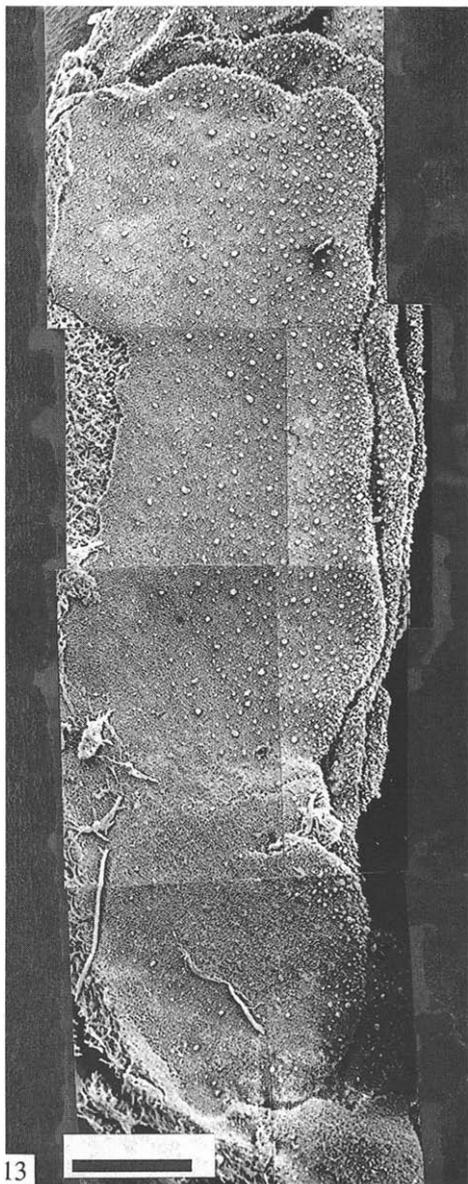
Cryo-SEM observation revealed droplets of different size on both facial and marginal cystidia (Figs 14, 15 respectively). Such observations support Thielke's (1983) suggestion that facial cystidia of *V. bombycina* have a secretory role. Similar secretory cystidia have been observed on the surface of basidiomes of *Flammulina velutipes* (Williams, Beckett & Read, 1985), in contrast to the non-secretory 'true' cystidia in the hymenium of *Coprinus cinereus* (Jones & McHardy, 1985).

Marking experiments

The greatest increase in the distance between two adjacent marks painted on the outer surface of the cap occurred in the middle and marginal regions of the cap. Marks in these regions also became more diffuse (Figs 16, 17). Marks painted along the margin of the gills of exposed (gymnocarpous) fruit bodies indicated that the free margin of the gill was fixed early in development and remained as the free edge throughout. Marks persisted at the free edge from the exposed egg stage to the mature stage (Figs 18, 19).

In this and earlier work we have made comparative studies on basidiome development in *Coprinus cinereus* and *Volvariella bombycina*. These two species provide an interesting comparison since earlier workers have placed them in opposing groups with regard to gill inception. This is described as ruphymental in *C. cinereus* (the hymenophore is formed from

Fig. 13. SEM montage showing the surface of an entire gill of *Volvariella bombycina* excized at the elongation stage. The image is oriented as in life, cap apex at the top, cap margin at the bottom and the stipe would be on the right of the figure. Note that cystidia (the scattered inflated cells) are distributed unevenly, being congregated near the free edge of the gill and more dispersed near the cap context and the margin of the cap. Scale bar = 2 mm.



two blocks of a discontinuous palisade layer, gill differentiation proceeding away from the stipe) and levhymenial in *V. bombycina* (a continuous palisade layer forms into ridges to form the young hymenophores which '... push down into a preformed gill cavity ...'; gill differentiation is consequently presumed to proceed towards the stipe) (Reijnders, 1963; Reijnders in Singer, 1986; the quotation comes from Watling, 1985). Our concern is exclusively with the developmental mechanics by which the regular radial pattern of gills can be established. We do not wish to draw any taxonomic, systemic or phylogenetic conclusions.

In *Coprinus cinereus*, gills initiate from the ground tissue

(context or protenchyma) of the cap (Reijnders, 1963, 1979; Rosin & Moore, 1985 *a, b*). As the basidiome enlarges more gills are added by bifurcation of an existing gill either from one side or at the stipe-gill junction, and by folding of the palisade layer near or at the roots of existing gills (Moore, 1987) and neighbouring gills are interconnected by cystidia spanning the gill cavity being firmly connected to cystidia in the opposing hymenium (Horner & Moore, 1987).

Extensive stipe-gill junctions and cystidium-cystidium 'ties' across gill spaces are absent from most mushrooms but despite fundamental structural differences, it seems to us that there are two common features between the ontogenetic pathways of *Volvariella bombycina* and *Coprinus cinereus* which are of mechanistic importance; one is that the directions of growth vectors between the gill edge and the cap context are identical, the other is that new gills are formed whenever space becomes available so that, because of the geometry of the formative regions, young gills may be tortuously folded.

Direction of growth vectors

In establishing the directions of growth vectors we attach much weight to the marking experiments illustrated in Figs 16 to 19. In these experiments, marks placed adjacent to each other at the cap apex remained close together whereas marks originally close together in the mid regions of the outer surface of the cap became widely separated (and were made more diffuse) by subsequent growth. Further, ink marks placed on the cap margin and those placed on the free edges of the gills remained at the margin or the free edge. These observations show (a) that the greatest contribution to cap expansion occurred in an annulus some way in from the margin and not extending to the apex; (b) that the free edges of the gills remained intact and were not replaced; and (c) that the margin of the cap was similarly not replaced though intussusception increased its circumference.

Growth of these tissues must be achieved through the growth of their component hyphae. Hyphal extension, of course, is exclusively apical, a mode of growth which leads to ink marks made on hyphal structures being left behind as the apex grows on (Burgeff, 1915). In our experiments the ink marks were carried forward along with, and in the direction of, the growing tissue. Thus, it follows from observation (b) that individual gills of *V. bombycina* do not grow by extension at their free edge, and from (c) that the cap margin is not a growth centre for radial extension. Rather, the gills extend in depth by growth at their roots and by insertion of hymenial elements into their central and root regions. The cap extends radially through intussusceptive growth in a broad region behind the margin. Thus, the hyphal tips which form the cap margin when it is established at the very earliest stage of development remain *at* the margin. They do not continue to grow apically to extend the margin radially, nor are they overtaken by other hyphae; instead they are 'pushed' radially outwards by the press of fresh growth behind, and they are joined by fresh branches appearing alongside as the circumference of the margin is increased.

An independent indicator of the direction of growth vectors in *V. bombycina* is the developing distribution of large

Fig. 14. Cryo-SEM image of facial cystidia of a mature hymenium showing the occurrence of droplets (arrowed). Scale bar = 20 μm .

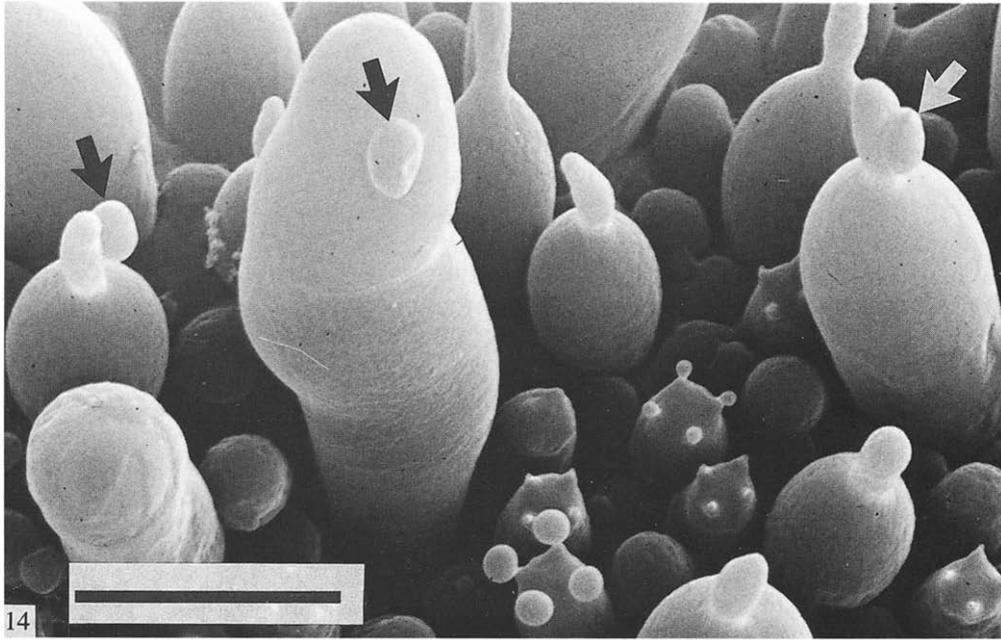
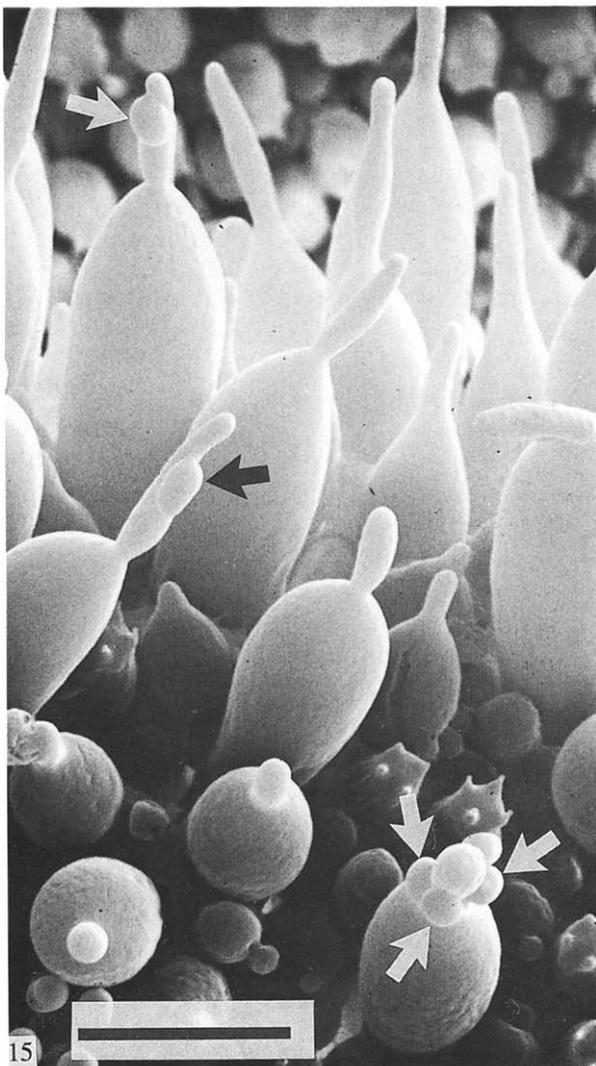


Fig. 15. Cryo-SEM image of marginal cystidia of a mature hymenium showing the occurrence of droplets (arrowed). Scale bar = 20 μm .



facial cystidia. At the button stage, broad and short gills were formed on which facial cystidia on the same hymenium were uniformly in close proximity (Figs 3, 4). Increase in the area of the hymenium was achieved mainly by cell insertion from the trama. The inserted cells could be either basidia or small facial cystidia, so from the egg stage onwards the large solitary facial cystidia became scattered over the hymenium. Since the large facial cystidia were tightly aggregated at the earliest stage their skewed distribution in later stages suggests that the rate of cell insertion from the trama into the hymenium was not equal in every region. At the later stages of development large facial cystidia were aggregated near the free edge but showed a decreasing density in the hymenium towards the gill root, i.e. towards the cap context (Fig. 13). If this distribution does reflect the extent of growth then the growth rate of the gill was greatest in these latter regions. On the same basis of cystidial distribution, the radial growth rate of the gill, i.e. along the length of the gill, was least near the apex of the cap.

Both the results of marking experiments and consideration of cystidial distribution lead to the same interpretations. These are illustrated in Fig. 20 and compared with conclusions previously drawn about the polarity of growth vectors in *Coprinus cinereus* (Rosin, Horner & Moore, 1985). The direction of the radial vector obviously differs between the two species, which might be a reflection of the difference between the synchronized maturation of basidia in *C. cinereus* compared with the mosaic pattern of maturation in *V. bombycina*; perhaps the requirement for synchrony imposes additional constraints on cap construction in *Coprinus*. However, it is most significant that the growth vector across the width of the gill is directed *away from the stipe* in both species. In other words, for both the rythymenial and levhymenial modes of gill development the gill grows *at its root and not at its margin* (Moore, 1987).

Fig. 16. Disposition of ink marks painted on the outer surface of a gymnocarpous basidiome of *Volvariella bombycina* at the start of the experiment. Scale bar = 5 mm. **Fig. 17.** The same basidiome as is shown in Fig. 16 after maturation. Scale bar = 5 mm.

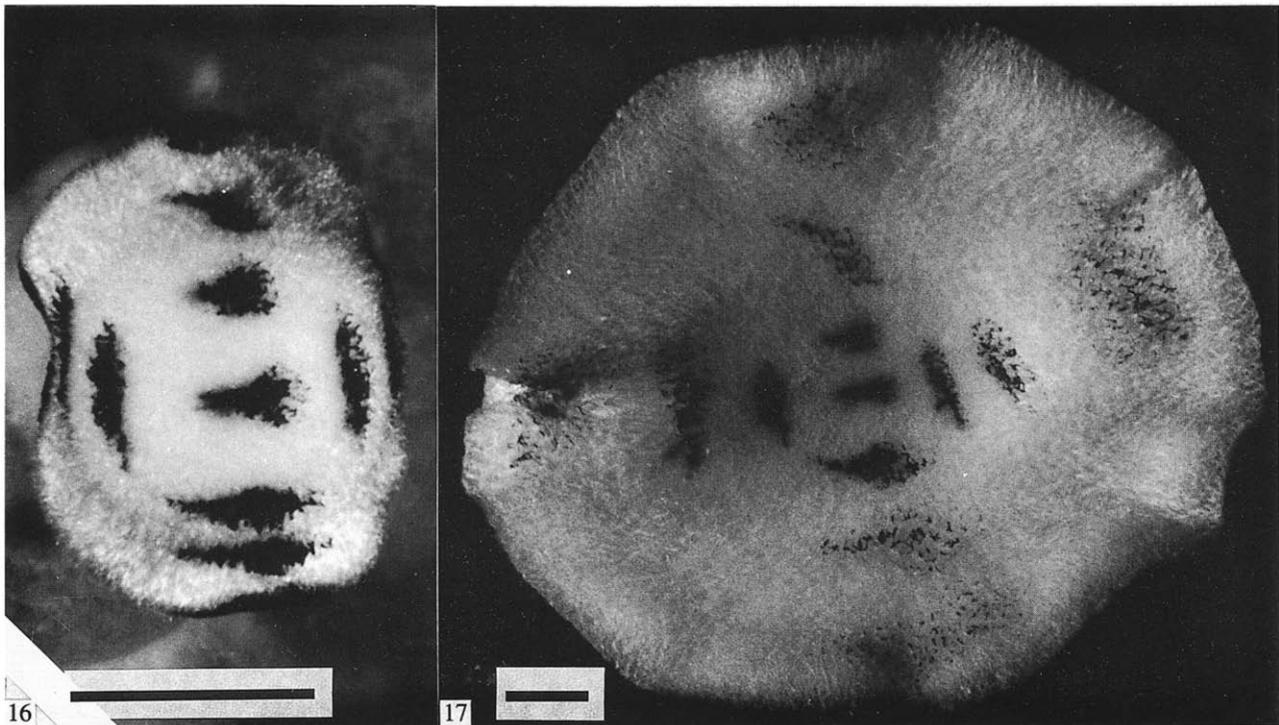
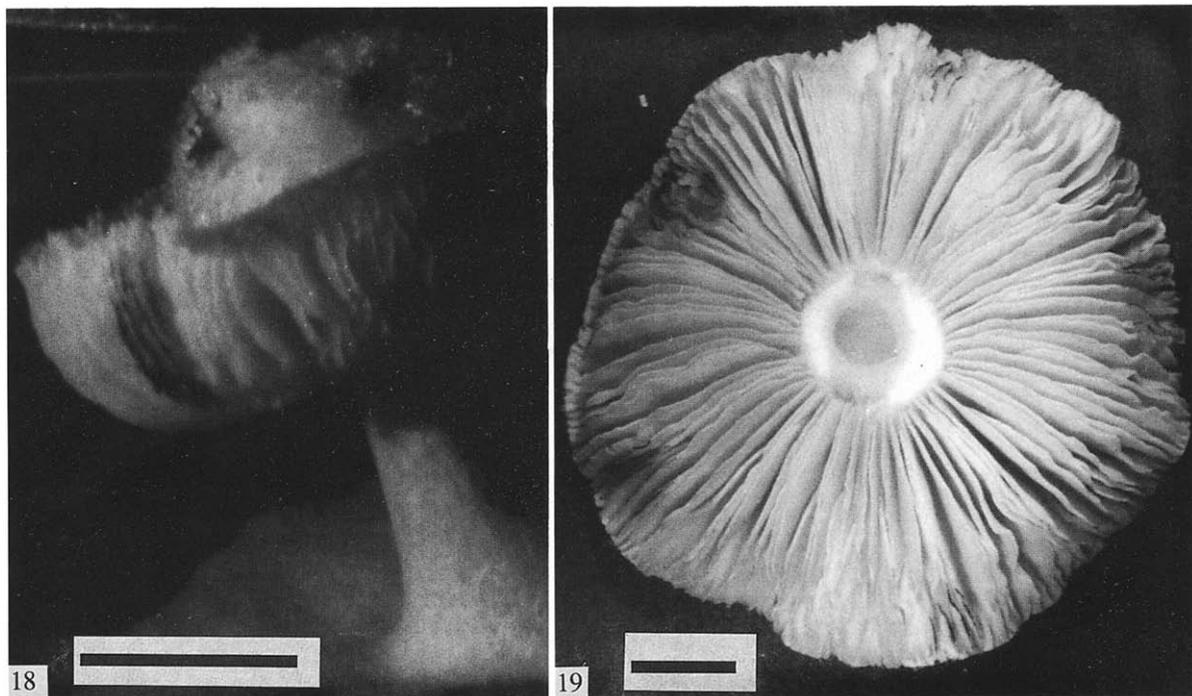


Fig. 18. Disposition of ink marks painted on the gill margins of a gymnocarpous basidiome of *Volvariella bombycina* at the start of the experiment. Scale bar = 5 mm. **Fig. 19.** The same basidiome as is shown in Fig. 18 after maturation. Scale bar = 5 mm.

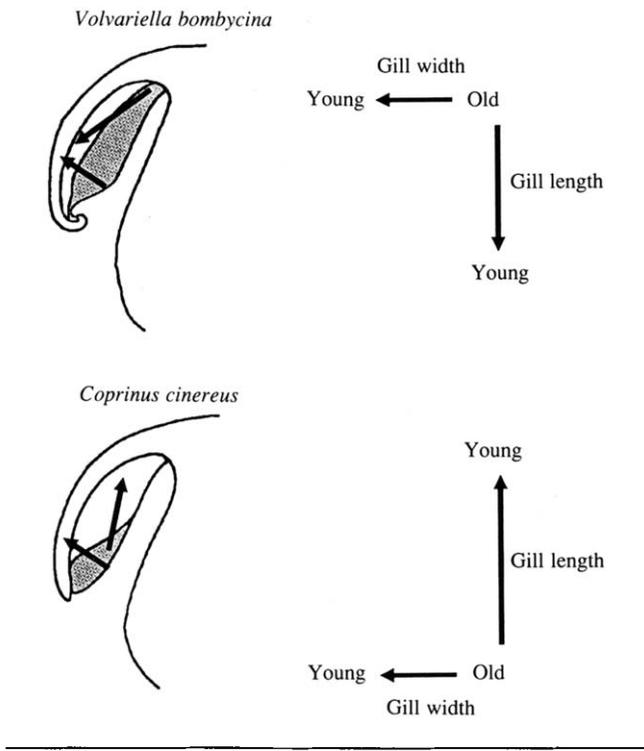


Formation of gills

In both *C. cinereus* and *V. bombycina* the first-formed gills were radially arranged. As the cap expanded more gills were formed. In *V. bombycina*, new gills were formed by successive

bifurcation of an old one near its free edge and growth of the continuous hymenial layer at or near the root of the old gill. In the former case a new 'gill cavity' or gill root formed close to the free edge and then invaded the trama of the existing gill as it extended radially outwards. Initiation of such folding on

Fig. 20. Diagrammatic interpretation of the major growth vectors in the gill of *Volvariella bombycina*, in comparison with those identified in *Coprinus cinereus* (Rosin *et al.*, 1985).



existing gills was a localized event, starting in the upper and then shifting to the middle and lower regions, formation of two daughter gills depending on completion of the bifurcation all along the free edge of the parental gill. This occurred as a wave from the upper (apical) towards the lower (marginal) region of the gill. However, as the mature gill is ventricose in plan view, the rate of its growth in width clearly differs along its length, so visible progress of this wave of completion was not entirely regular. If the wave of completion was arrested the fold was seen as a vein on the narrow-bladed gill at maturity.

In the alternative mode of formation of new generations of gills they appeared as folds in the region between existing gill roots, creating new ridges on the cap context which became the free edges of new secondary or tertiary gills, the gill spaces on either side extending as the root differentiated from the context.

The irregular placement of veins on the gills and the occurrence of ventricose and labyrinthiform regions (Figs 6, 8) implies that the rates of growth on the two sides of a gill were not fully co-ordinated. Indeed, at early stages what seems like a complete lack of co-ordination produced sinuous, contorted gills. Thus, a sinuous, labyrinthiform hymenophore is a normal transitional stage in basidiome development in *V. bombycina*.

Basidiome maturation

A significant event in basidiome morphogenesis is the transformation of the sinuous 'embryonic' hymenophores

into the linear, narrow-bladed gills characteristic of the mature basidiome. This must be achieved as growth of other parts of the cap places geometrical stress on the gills and constrains them along regular radii. The flattening and eventual eversion of the originally involute cap margin is indicative of the sorts of stresses which are established within the cap (Fig. 1).

Williams (1986) pointed out how, in the basidiome of *Flammulina velutipes*, the most highly differentiated cells occurred on the outside of the tissue blocks. Thus, major morphogenetic events in fungi, like those in animals and plants, are associated with tissue surfaces and their 'epidermal' layers of cells. Reijnders (1963) and Reijnders & Moore (1985) emphasized the role played by cell inflation in driving developmental events in fungal structures. We note that the hymenium of *V. bombycina* (Fig. 11) is a layer of tightly appressed cells, and the trama of the broken gill shown in Fig. 9 is full and crowded with greatly inflated cells. We suggest that expansion and growth of tramal cells in gills enclosed by the hymenial 'epidermis' will generate compression forces which will effectively inflate, and so stretch, the embryonic gills to form the regularly radial pattern of the mature cap.

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