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CYSTIDIAL MORPHOGENETIC FIELD IN THE HYMENIUM OF *COPRINUS CINEREUS*

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Cystidia spanning the gill cavity may be 'distant', having other cells separating them, or 'adjacent', with no intervening cell; and, in either case, both cystidia may emerge from the same hymenium (described here as '*cis*') or from opposite hymenia ('*trans*'). If the distribution of cystidia is entirely randomized the frequency of adjacent pairs will depend on the population density and there will be an equal number of *cis* and *trans* in both the distant and adjacent categories. Quantitative data from serial sections of a primordium show that there is a positive inhibition of formation of neighbouring cystidia in the same hymenium such that formation of a cystidium actively lowers the probability of another being formed in the immediate vicinity. The extent of the inhibitory influence extends over a radius of about 30 μm and is strictly limited to the hymenium of origin. Cystidial density distribution on the face of the gill is fairly uniform, but at the gill edge the density of cystidia is locally increased. It is suggested that differentiation leading to cystidium formation is activated by the concentration of a component of the atmosphere, possibly water vapour, in the gill cavity immediately above the developing hymenium. The distribution pattern of cystidia is thus dependent on interplay between activating and inhibiting factors. At early stages in growth of the cystidium across the gill cavity the cell(s) with which the cystidium will come into contact in the opposing hymenium are indistinguishable from their fellow probasidia. However, when the cystidium comes firmly into contact with the opposing hymenium, the hymenial cells with which it collides develop a distinct granular and vacuolated cytoplasm, more akin to that of the cystidium itself than to the neighbouring probasidia. This suggests that a contact stimulus sets in train an alternative pathway of differentiation leading to an adhesive cell type called the cystesium.

The hymenium of *Coprinus cinereus* is commonly considered to comprise three cell types: basidia, paraphyses and cystidia. Although the paraphyses expand to become the major structural members of the gill lamellae, they arise secondarily as branches from sub-basidial compartments, and when first formed the hymenium consists of a carpet of probasidia with a scattering of cystidia (Rosin & Moore, 1985*a*). We prefer to use the term probasidia rather than basidiole to signify immature basidia, since the latter term has been defined as referring both to basidia which have not yet produced spores and to morphologically similar, but sterile cells which become coprinoid paraphyses (Smith, 1966). This is misleading, as we have shown (Rosin & Moore, 1985*a*) that the paraphyses arise as sub-basidial branches, so although they may pass through morphologically similar states, the two cell types are very different ontogenetically. The terminology used must reflect this.

Cystidia '· · · occur haphazardly in the hymenium, depending on the species, and vary from abundant to absent · · · ' (Smith, 1966). Much of the literature dealing with cystidia considers them from the taxonomic viewpoint (e.g. Lentz, 1954; Price, 1973), reflecting assiduous attention to fine distinctions of nomenclature at the expense of appreciation of the remarkable developmental plasticity revealed by their occurrence and form. Brefeld (1887, cited in Buller, 1910) concluded that cystidia are metamorphosed basidia, a view summarized by Corner (1947) in the phrase '· · · cystidia represent sterile basidia which become overgrown · · · '. Certainly, both probasidia and procystidia originate as the terminal compartments of branches from the hyphae of the sub-hymenium, but cystidia are not overgrown basidia. The mature cystidium is a cell that is highly differentiated for its particular (poorly understood) function. Thus, the development of a cystidium represents expression of a perfectly respectable alternative pathway of differentiation, and it follows from the observations of Rosin &

Moore (1985*a*, *b*) that commitment to the cystidial as opposed to the basidial pathway of differentiation must occur very early in development of the hymenium. Nothing is known about the controls which might determine formation of a cystidium, rather than a basidium, by a particular hyphal apex. Indeed, it is not even known whether the sorts of description commonly applied to the distribution of the former, e.g. scattering, haphazard (see above), 'fairly uniform' (Buller, 1910), are meaningful or whether there is some pattern in their distribution which might reflect those ontogenetic controls and/or the functions of these enormously inflated cells.

Cystidia are found in fair number in the hymenium of *C. cinereus*. As part of our attempt to define the fundamental characteristics which determine the pattern of hyphal differentiation in the hymenium of this organism we have investigated, and report in this paper, the origin and distribution of cystidia. We also identify a new cell type, the **cystesium**, which is derived from a probasidium that, rather than continuing development to a basidium, becomes specialized to adhere to a cystidium arising from the opposing hymenial face.

MATERIALS AND METHODS

Organism and culture conditions

A dikaryon of *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray sensu Konr. (ATCC 42721) was fruited on horse dung (Moore & Ewaze, 1976) and fruit bodies at two stages of development were used for microscopic analysis. Stage 1 primordia (2–6 mm in height, prekaryogamy) and Stage 2 primordia (6–9 mm in height, meiosis occurs during this stage) were harvested for microscopic analysis. Moore, Elhiti & Butler (1979) have described the six stages into which fruit body development has been divided.

Microscopic preparation

Sections used for light microscopy were 2 μm thick, being cut from resin blocks containing material prepared as described by Rosin & Moore (1985*b*). For scanning electron microscopy, tissue was dissected from fresh fruit bodies and either shock-frozen in nitrogen slush prior to freeze-drying, or fixed for 2 h in 3% glutaraldehyde in 0·05 M Sorenson's buffer (pH 6·8) and dehydrated through an ethanol series prior to critical-point drying. Mounted samples were coated with gold and viewed.

RESULTS AND DISCUSSION

Hyphal origin

Cystidia in *C. cinereus* are the terminal compartments of hyphal branches from the subhymenium, but they are not derived from any particular rank of branch (Corner, 1947), nor do they arise from a lower stratum in the hymenium (Boudier, 1886), and the idea that 'cystidial hyphae' are a separate population from 'basidial hyphae' (Rosin & Moore, 1985*a*) is not supportable. Careful examination of sections shows that cystidia belong to the same branching systems as their neighbouring probasidia (Fig. 1). The two cell types thus represent alternative fates for otherwise indistinguishable hyphal tips approaching the hymenium from the subhymenial hyphae. Lentz (1954) describes the branching pattern giving rise to the hymenium as cyme-like, corymbose or sympodial; a more vivid description might be 'crowded'. So crowded that it is doubtful whether the characteristic branching pattern, if there is one, could be distinguished with anything less than reconstruction from serial electron microscope sections. Incidentally, images such as those in Fig. 1 highlight dramatically the changed growth pattern exhibited by a dikaryon during fruit body formation. In vegetative growth on the surface of an agar medium, leading hyphae of this dikaryon produce branches at intervals of approx 73 μm.

Regulation of cystidium formation

Internal and/or external control signals must regulate branching and cystidium development and, since cystidia are dispersed, an analysis of their distribution may suggest the nature of those controls. There are a number of components to a description of cystidial distribution. Cystidia emerge from a particular hymenium and span the gill cavity. Thus two neighbouring cystidia may be (*a*) 'distant', having other cells (initially probasidia, later basidia and their daughter paraphyses) separating them, or (*b*) 'adjacent', with no intervening cell; and in either case, both cystidia may emerge from the same hymenium (described here as '*cis*'; e.g. Fig. 1C) or from opposite hymenia ('*trans*'; e.g. Fig. 6A). If the distribution of cystidia is entirely randomized the frequency of adjacent pairs will depend on the population density and there will be an equal number of *cis* and *trans* in both the distant and adjacent categories. Quantitative data were obtained from a series of 2 μm thick serial sections of a Stage 1 primordium (similar to Fig. 1). The first 169 cystidia observed in median section were scored for their direction of growth – either clockwise or anticlockwise as

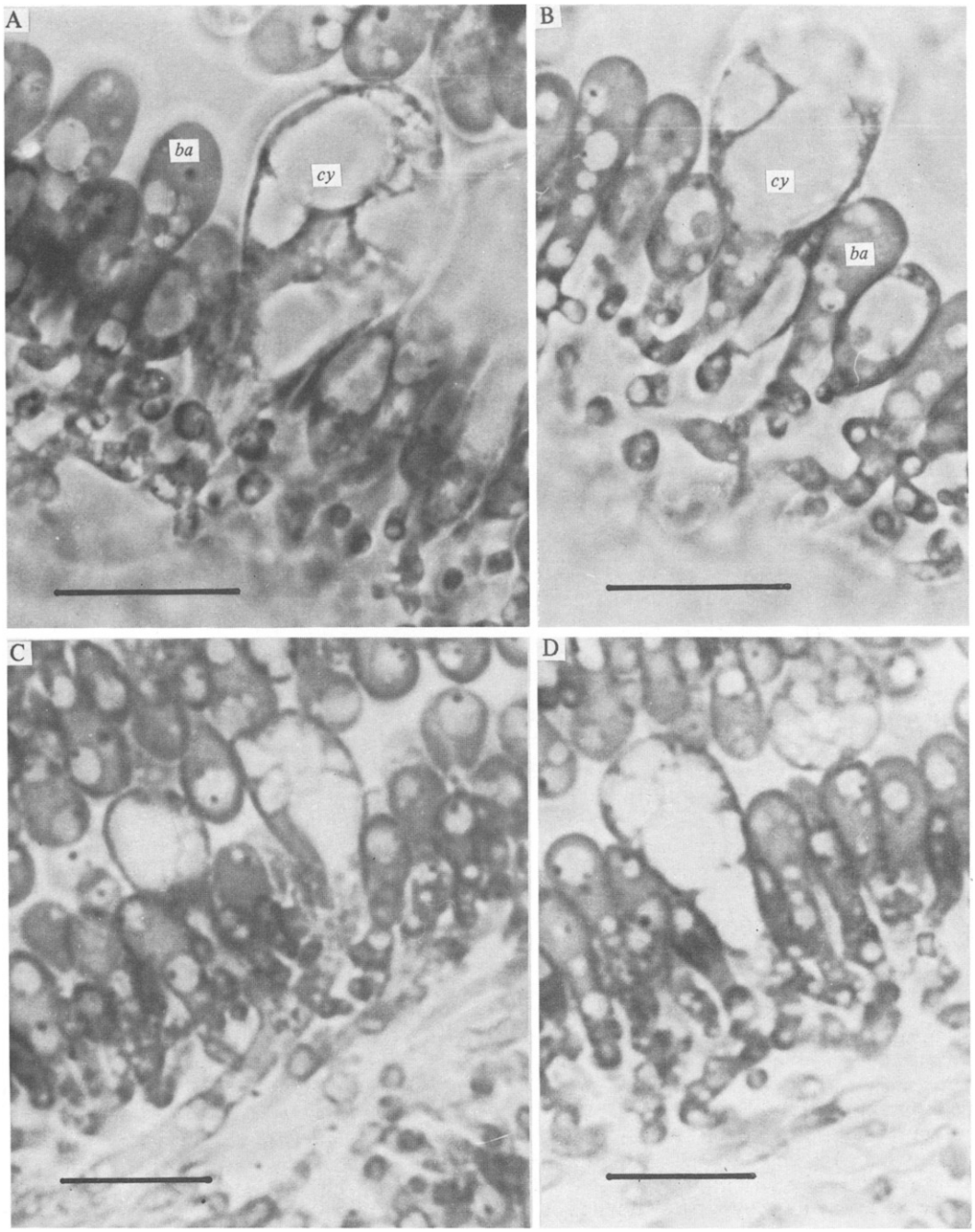

Fig. 1. Branching patterns at cystidium bases. Cystidia (*cy*) and probasidia (*ba*) arise from the same subhymenial branch systems with no indication of a particular arrangement of branches. Scale bars = 20 μm.

Table 1. *Distances between nearest-neighbour pairs of cystidia observed in gill sections, comparing pairs in which both members originated from the same hymenia* (cis) *with those in which the two cystidia arose from opposing hymenia* (trans)

	Number of pairs examined	Mean	S.D.	Units
Cystidia in *cis*	133	156·7	102·1	μm
		23	15	cell diameters (probasidia)
Cystidia in *trans*	129	113·4	96·3	μm
		17	14	cell diameters (probasidia)

viewed from the top of the fruit body. The outcome was a ratio of 82 : 87, not significantly different from equality, so we conclude that the two hymenia bordering a gill cavity are equally likely to give rise to cystidia spanning that cavity. Every fifth section was searched for pairs of nearest-neighbour cystidia, in which the hymenial origin of both was unambiguous; their separation distance was measured and they were scored as either *cis* or *trans*. A total of 262 cystidial pairs were examined, and results are shown in Table 1. Measurement of these distances is potentially contentious, as the hymenium is expanding by both cell enlargement and cell insertion throughout fruit-body development. However, the primordial stage 1 chosen for examination is that which precedes emergence of paraphyses (Rosin & Moore, 1985*a*), so the hymenium is structurally at its simplest and the measurements of physical distance can be translated easily into the potentially more meaningful unit of measurement of cell (probasidial) diameters. Both measures are given in Table 1, but whatever the units it is clear that the two categories of cystidial arrangement are differently distributed. Moreover, among the 262 pairs examined, only 20 were adjacent, and these were all in *trans*. The lack of *cis*-adjacent pairs of cystidia is not simply due to their slightly lower population density. This conclusion is based on comparison of the observed and expected numbers of *cis*-adjacent cystidia. If it is assumed that distances between neighbouring cystidia are normally distributed, the mean and S.D. (which mathematically describe the distribution) can be used to calculate the expected number of *cis*-adjacent pairs of cystidia at any given separation distance by calculating the appropriate area under the normal probability curve. The mean diameter of cystidia in these sections was 17 μm. If this value is used as the mean separation distance for adjacent cystidia, calculation shows that the expected number of cystidial pairs separated by this distance or less in this sample was 11·35; none was observed, and the probability of the null observation being due to chance is less than 0·0005. When the same calculation is applied to cystidia in *trans* it emerges that 20·5 *trans*-adjacent pairs are expected, whereas 20 were observed.

Thus the distances between neighbouring *trans*-disposed cystidia are approximately normally distributed (not strictly a normal distribution, of course, as there is a finite minimum distance corresponding to the mean cystidial diameter); but the distribution of the population of measured distances between the *cis*-disposed pairs does not approach a normal distribution. There is evidently a positive inhibition of formation of neighbouring cystidia in the same hymenium such that formation of a cystidium actively lowers the probability of another being formed in the immediate vicinity. The extent of the inhibitory influence can be judged by comparing observations with calculations of the expected number of *cis*-disposed pairs at successively greater separations. This is shown in Fig. 2, in which the distance between cystidia is expressed in terms of probasidial diameters (units of 7 μm). The inhibitory effect extends up to four probasidial diameters. This defines the radius of the cystidial morphogenetic field, which is strictly limited to the hymenium of origin.

Density distribution

Mature cystidia are usually essentially at right angles to their hymenia (see Fig. 2 in Moore & Pukkila (1985) and illustrations of various species in Buller (1924)), yet most of the increase in area of the hymenium takes place through insertion and expansion of paraphyses after cystidia are firmly connected to the two hymenia each side of the gill cavity which they span. For the cystidia not to be grossly distorted during maturation of the gills implies either that the two hymenia either side of a gill space are positively co-ordinated in terms of the number of paraphyses which insert and their subsequent extent of expansion, or that these features are so perfectly randomized over such a large cell population that, on balance, equality of

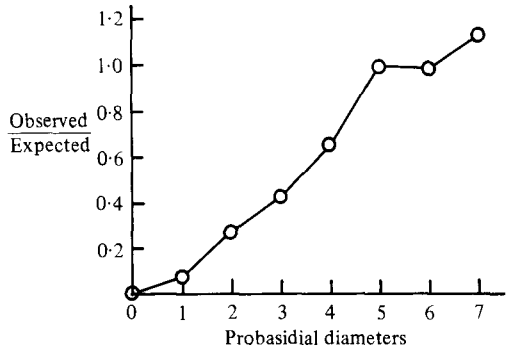

Fig. 2. Inhibition of cystidium differentiation within range of a preformed cystidium. The graph shows the observed number of *cis*-arranged pairs of cystidia (expressed as a fraction of the expected number) at successively greater distances of separation. The expected number was calculated for each distance as the area under the normal probability curve represented by the mean and S.D. shown in Table 1. The separation distance between cystidia is shown on the abscissa in cell (probasidial) diameters; in these specimens probasidia were about 7 μm diam. If the presence of a cystidium had no influence on occurrence of a second in the immediate vicinity the points would be stochastically scattered around an ordinate value of 1. In reality this is true only for cystidia separated by five or more probasidia. At lesser distances positive inhibition of cystidium differentiation in the vicinity is observed. We thank Dr J. M. Bond for suggesting the form of this graph to us.

extension occurs. Without experimental intervention (which is difficult, since the gill cavities are completely enclosed) it is probably impossible to distinguish between these two possibilities. Hence the opportunity to compare the distributions of *cis*- and *trans*-disposed cystidia permits a rare insight into the relationships between cells in a hymenium and between opposing hymenia. The indication that the cystidial morphogenetic field is limited to its own hymenium implies that opposing hymenia are integrated only in so far as similar events occur in both; those events are essentially randomized; and a large cell population is involved.

Randomness, of course, does not imply lack of order; nor would we wish to imply with the description we have just given that the gill is assembled in a mechanical, unresponsive way. Cystidial density distribution appears to be randomized and fairly uniform except at the gill edge (Fig. 3), where the density of cystidia is locally increased to the point where *cis*-adjacent pairs of cystidia can be found. This observation indicates that the rule for forming a cystidium must have some environmental cue. The distributions analysed above, which apply to the general gill surface, could well operate without reference to the environment external to the hyphal branching system on the basis of something like 'on average every fifth branch is a cystidium unless one of the previous four branches was a cystidium'. This would clearly not be responsive to position within the gill, nor can it be responsive unless the hymenial cells are capable of perceiving and responding to some sort of positional information from their environment which identifies the gill edge.

The gill cavity seems to be the appropriate environment to consider, and regulation based on sensing a gas or vapour concentration would enable explanation of the increased density at the gill edge. Wherever a gill edge is formed the atmospheric volume is expanded in the immediate vicinity. This is true both for the formation of secondary gills by division of the gill-organizing centre (Rosin & Moore, 1985*b*) and for primary gill edges which become exposed to the developing annular cavity. So if cystidium differentiation is activated by a locally decreased concentration of some vapour, activation would be amplified inevitably in the region of the gill edge, allowing for closure of the exposed trama as primary gills separate from the stipe (Fig. 4A) and additional buttressing of secondary gills (Fig. 4B). The nature of the controlling molecule is unknown. Carbon dioxide influences fruit-body growth differentially in *Agaricus* (Lambert, 1933; Turner, 1977); ammonia-scavenging enzymes are specifically derepressed in the *Coprinus* fruit-body cap (Moore, 1984), at least one of which has been localized to the cell surface in both vegetative mycelium (Elhiti, Moore & Butler, 1986) and the hymenium (M. M. Y. Elhiti, R. D. Butler & D. Moore, unpubl.), and ammonia is known to be a morphogenetic regulator in *Dictyostelium* (Schindler & Sussman, 1977). However, cystidia are thought of as functioning generally in maintenance of hymenial humidity (Smith, 1966) by 'transpiring' water vapour into the gill cavity. Thus it could be that the vapour pressure of water over the just-differentiating hymenium is the determining factor. Hyphal tips forming the hymenium may be sensitive to the water vapour pressures in the gill cavity, having a particular probability of differentiating into a cystidium when activated to do so by locally reduced humidity. It is significant that Gamow & Bottger (1982) concluded that water is a self-emitted, growth-stimulating avoidance gas in the sporangiophore of *Phycomyces*. If a similar situation obtains in the cystidium then, once activated, progress of cystidial differentiation would be essentially autocatalytic. Such a scheme would

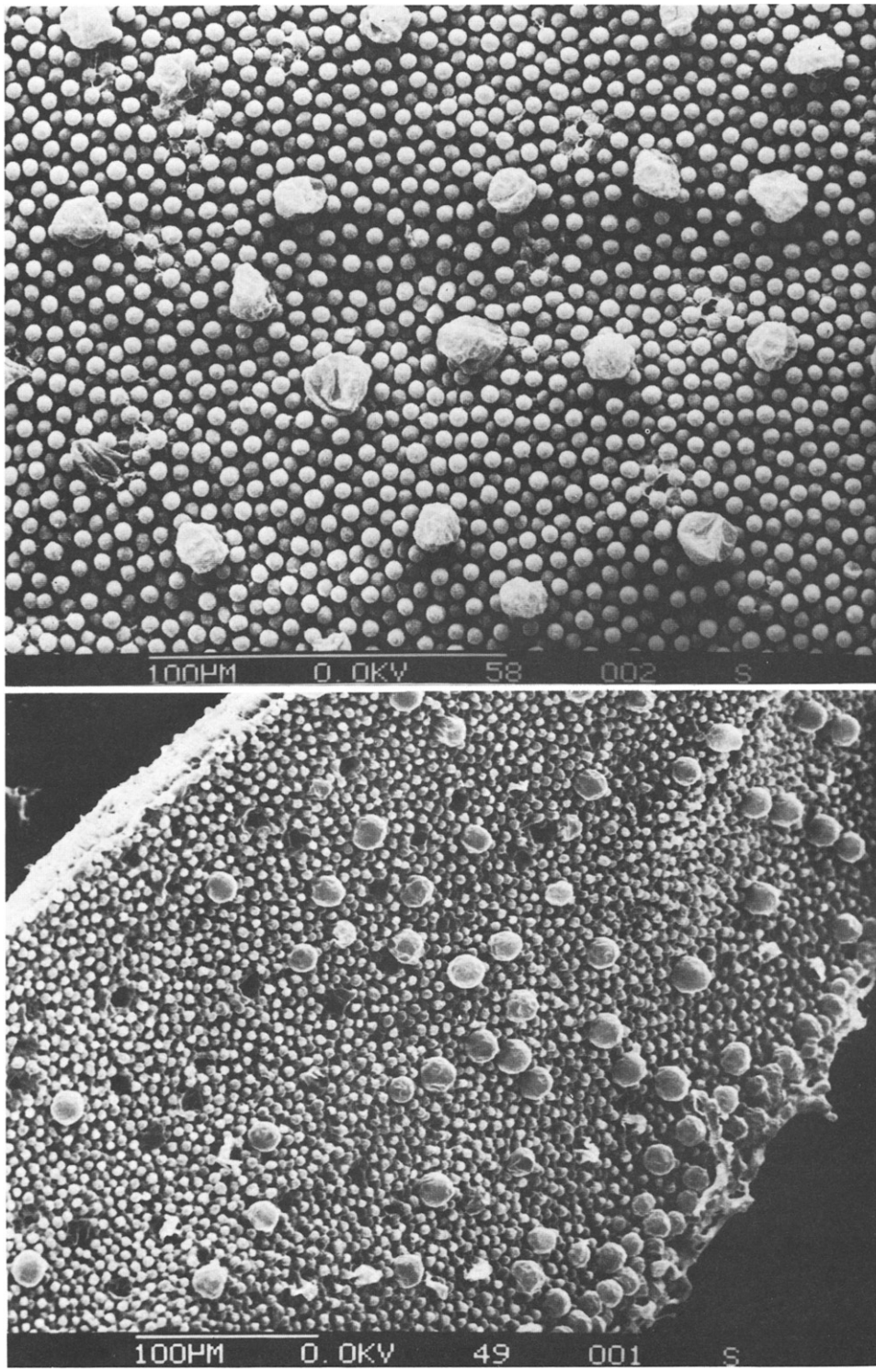

Fig. 3. Scanning electron micrographs of the hymenial surface. (A) Very regularly spaced distribution of cystidia arising from the hymenial surface on the open face of the gill. (B) Increased crowding as the gill edge – that closest to the stipe, at the right of the picture – is approached. The 'pits' in the hymenial surface are places where cystidia from the opposing gill face have been pulled away from their cystesia when the gills were separated. Scale bars = 100 μm.

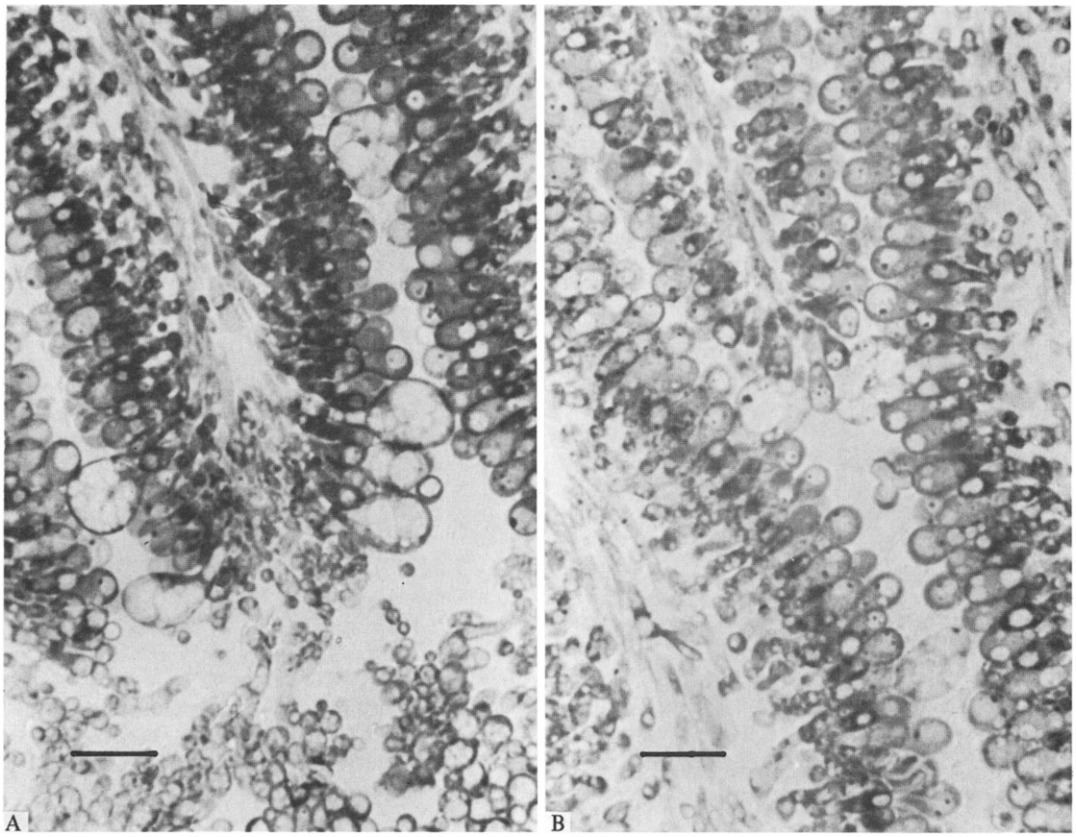

Fig. 4. The edge of a primary gill (A) showing increased frequency of cystidium formation in the expanded space where the gill has separated from the outer layers of the stipe (lipsanenchyma), compared with the edge of a secondary gill (B), where the increased space in the gill cavity has induced both neighbouring hymenia to produce cystidia to buttress the secondary gill edge symmetrically. Scale bars = 20 μm.

allow for coincident activation of cystidia in the equivalent positions in opposing hymenia, at a frequency equal to the square of the individual probability of formation of a cystidium. Using the figures given in Table 1 (20 adjacents in 129 *trans*-arranged pairs) the latter frequency is calculated to be 0·39; i.e. a hyphal tip in the hymenium has a probability of about 40% of forming a cystidium when exposed to activating conditions.

The differentiating cystidium inhibits further cystidial formation on its own hymenium over a radius of about 30 μm. This may be due to production of an active inhibitor or to local diversion of metabolites (particularly water?) to the already committed cystidium. In either case the patterning process is open to analysis and simulation using the activator–inhibitor model developed by Meinhardt (1982) to account for stomatal, cilial, hair and bristle distributions.

Hymenial adhesion

At the gill edge, expansion of the gill cavity presumably amplifies desiccation stress and activates cystidial formation to such an extent that the effect of the local inhibitor is masked and *cis*-adjacent neighbours can be formed (Fig. 4A). It is this circumstance which indicates most strongly that cystidium formation is responsive to cues received from outside the parental hymenium. Evidence that a cystidium exerts a formative influence on the opposing hymenium comes from examination of the cells which cystidia contact. Mature cystidia are embedded into the opposing gill face. The final structural result – firm con-

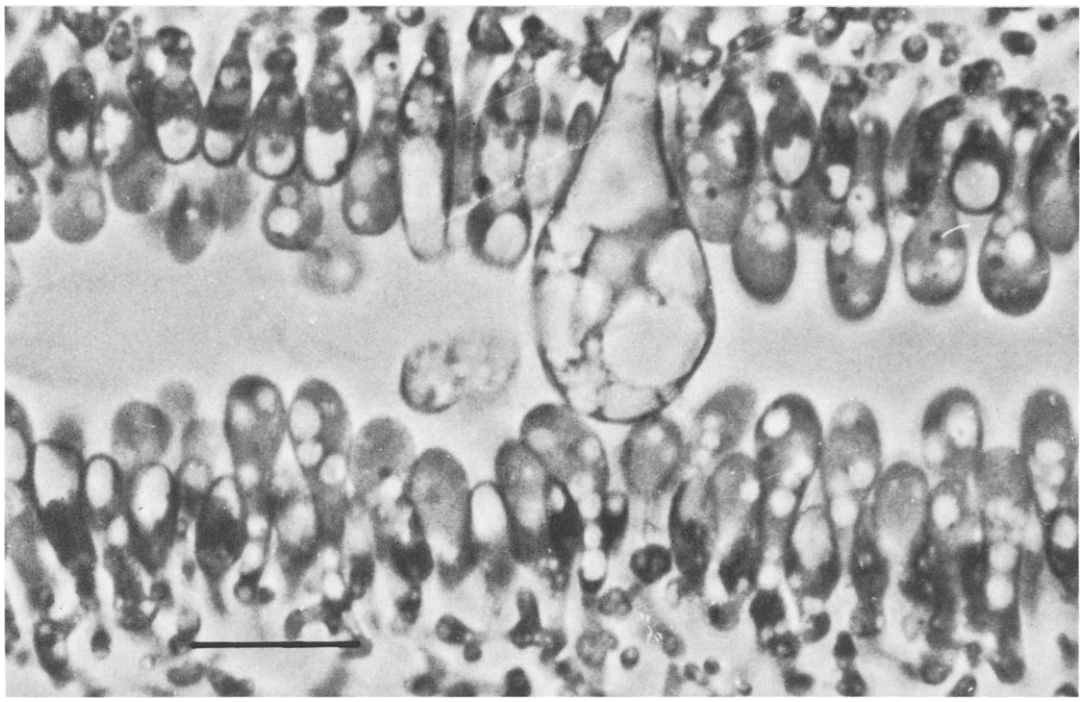

Fig. 5. An early stage in contact between a cystidium and its opposing hymenium showing that the cells with which the cystidium collides are not morphologically different from their neighbouring probasidia. Scale bar = 20 μm.

nexion between the cystidium and the opposing hymenium, has often been remarked upon and illustrated. Inevitably, the most relevant descriptions are found in Buller (1910, 1924). In *Coprinus atramentarius* Buller (1910) records that 'When one succeeds in forcibly separating parts of two neighbouring gills, one finds that although the cystidia have mostly separated from one of the gills at their apical ends, and have thus remained attached to the outer gill at their basal ends · · · not infrequently the reverse happens; the cystidia · · · have broken away at their basal ends and have remained attached at their apical ends.' Similarly, with reference to *C. lagopus*: 'If the gills are torn apart one end of each cystidium remains attached to one of the gills, whilst the other becomes free. Very often the free end has attached to it one of the clasping cells, thus proving how strongly these cells adhere to the cystidial wall' (Buller, 1924). Despite such graphic descriptions, with their clear indication that adhesion between the cystidium and the 'clasping cells' can be so strong as to exceed the breaking strain of the subhymenial hyphal branching system, causing cells to be torn out of their hymenium of origin, the fact that the cells that adhere to the tip of the cystidium show evidence of a specifically induced adjustment to their progress of differentiation has escaped description. At early stages in the growth of the cystidium across the gill cavity the sub-basidial branches which will become paraphyses have not yet formed and the cell(s) with which the cystidium will come into contact in the opposing hymenium are indistinguishable from their fellow probasidia, though they may show avoidance growth occasioned by the presence of the encroaching cystidium (Fig. 5, and see Fig. 2 in Rosin & Moore, 1985*b*). Yet when the cystidium comes firmly into contact with the opposing hymenium, the hymenial cells with which it collides develop a distinct granular and vacuolated cytoplasm, more akin to that of the cystidium itself than to the neighbouring probasidia (Fig. 6). This is suggestive of some contact stimulus aborting continuation of basidial differentiation in those cells and setting in train an alternative pathway of differentiation. The effect is highly localized, seemingly limited to cells which contact the tip of the cystidium, since more laterally placed neighbours remain unaffected. We believe that the differentiation of these cells warrants their recogni-

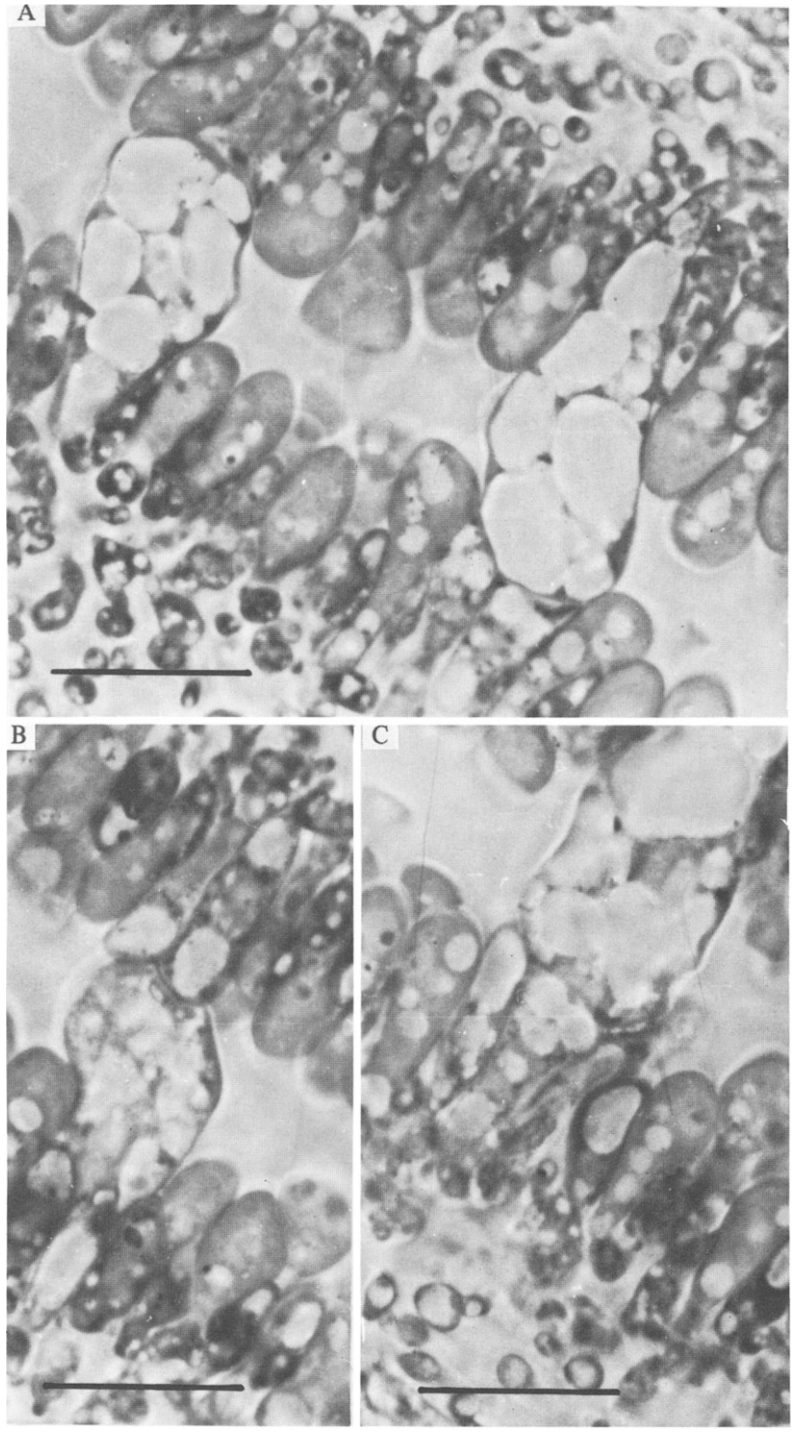

Fig. 6. Cystidia are firmly interlocked with cells in the opposing hymenia and have caused the latter drastically to change their cytoplasmic morphology – to become cystesia. Note that only the tip of the cystidium exerts this influence. Scale bars = 20 μm.

tion as a named cell type in the hymenium and propose the name **cystesium** (from cystidium + the Latin root of adhere) for a cell which differentiates to adhere to a cystidium arising from the opposing hymenium.

Differentiation of the hymenium in *Coprinus cinereus* therefore involves formation of the following cell types in the order indicated. Initially, the prohymenium consists of a palisade of morphologically similar hyphal tips, of which about 8% are induced to become cystidia while the rest remain as relatively undifferentiated probasidia. As the cystidia extend across the gill cavity they contact probasidia in the opposing hymenium and induce these cells to divert from their development as basidia and form cystesia. Subsequently, paraphyses arise as branches from sub-basidial cells which insert into the hymenium and expand greatly to form the characteristic coprinoid hymenium.

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REFERENCES

BOUDIER, J. L. E. (1886). Considérations générales et pratiques sur l'étude microscopique des champignons. *Bulletin de la Société Mycologique de France* **2**, 134–192.

BULLER, A. H. R. (1910). The function and fate of the cystidia of *Coprinus atramentarius*, together with some general remarks on *Coprinus* fruit bodies. *Annals of Botany* **24** (old series), 613–629.

BULLER, A. H. R. (1924). *Researches on Fungi*, vol. III. London: Longman, Green & Co.

CORNER, E. J. H. (1947). Variation in the size and shape of spores, basidia and cystidia in Basidiomycetes. *New Phytologist* **46**, 195–228.

ELHITI, M. M. Y., MOORE, D. & BUTLER, R. D. (1986). Cytochemical localisation of NADP-linked glutamate dehydrogenase newly induced in nitrogen starved mycelia of *Coprinus cinereus*. *FEMS Microbiology Letters* **33**, 121–124.

GAMOW, R. I. & BOTTGER, B. (1982). Avoidance and rheotropic responses in *Phycomyces*. Evidence for an 'avoidance gas' mechanism. *Journal of General Physiology* **79**, 835–848.

LAMBERT, E. B. (1933). Effect of excess CO_2 on growing mushrooms. *Journal of Agricultural Research* **47**, 599–608.

LENTZ, P. L. (1954). Modified hyphae of hymenomycetes. *The Botanical Review* **20**, 135–199.

MEINHARDT, H. (1982). *Models of Biological Pattern Formation*. London: Academic Press.

MOORE, D. (1984). 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.

MOORE, D. & EWAZE, J. O. (1976). Activities of some enzymes involved in metabolism of carbohydrate during sporophore development in *Coprinus cinereus*. *Journal of General Microbiology* **97**, 313–322.

MOORE, D. & PUKKILA, P. J. (1985). *Coprinus cinereus*: an ideal organism for studies of genetics and developmental biology. *Journal of Biological Education* **19**, 31–40.

PRICE, I. P. (1973). A study of cystidia in effused Aphyllophorales. *Nova Hedwigia* **24**, 515–618.

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

ROSIN, I. V. & MOORE, D. (1985*b*). 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.

SCHINDLER, J. & SUSSMAN, M. (1977). Ammonia determines the choice of morphogenetic pathways in *Dictyostelium discoideum*. *Journal of Molecular Biology* **116**, 161–169.

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.

TURNER, E. M. (1977). Development of excised sporocarps of *Agaricus bisporus* and its control by CO_2. *Transactions of the British Mycological Society* **69**, 183–186.

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