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PATTERN OF PORE MORPHOGENESIS IN THE RESUPINATE BASIDIOME OF *PHELLINUS CONTIGUUS*

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On malt agar in Petri dishes in the light, basidiome development of the resupinate polypore *Phellinus contiguus* proceeded centrifugally after a minimum but variable colony diameter had been attained. Dissepiments were initiated as more or less separate islands of aerial hyphae approximately 3 mm behind the mostly submerged mycelial margin. Localized aerial fascicles of apically-extending hyphae grew from these island initials both horizontally, where they coalesced with fascicles from adjacent initials and delimited pores, and vertically, resulting in lengthening of dissepiments. On dilute (0·2 %) malt agar and towards the edge of dishes containing more concentrated malt agar, initials tended to fail to unite laterally and grow as vertical spines. Horizontal growth of branching and interconnecting aerial fascicles also occurred at the margins of more mature poroid basidiome patches, increasing the poroid area. Time lapse studies of development from widely spaced initials transferred to fresh medium weekly showed that on 0·2 % malt agar, areas with basidia and lacking extending aerial hyphae remained as the bases of developing primary tubes. On 2 % malt agar secondary tubes were formed at a later stage by differential vertical growth within dissepiment areas. Where groups of initials were removed poroid basidiome tissue was regenerated mainly by lateral fascicle development from the surrounding basidiome tissue.

The development of pattern in multihyphal structures of fungi is a challenging problem (Moore, 1984). In basidiomes, pattern in differentiation is exhibited at the levels of the distribution of differentiated hyphal masses, e.g. in determining hymenophore configuration, and of distribution of differentiated hyphae, e.g. in the organization of the hymenium (Horner & Moore, 1987) as well as intrahyphally. The absence of a separate inflation phase during basidiome development in many of the Aphyllophorales could be an advantage for studies of the development of hymenophore configuration. In his pioneer developmental studies of non-inflating growth in the stipitate polypore *Polystictus xanthopus* Fr., Corner (1932*a*) described the initiation of pore patches and dissepiments in the 'pore field', an area on the underside of the young part of the pileus. Since that time few studies on the development in culture of polypore basidiomes have paid attention to hymenophore differentiation (Plunkett, 1956; Kitamoto *et al.*, 1974). A strain of *Phellinus contiguus* has been found to provide good material for studies of pore morphogenesis since the basidiome is resupinate and develops readily in culture under certain environmental conditions (Butler & Wood, 1988). The poroid hymenophore is formed directly on the mycelium at the agar surface and pore initials can be recognized with the naked eye close to the colony margin. The objectives of this work were first to establish the temporal and spatial sequence of development of the two major domains of the hymenophore, the pore areas and the dissepiments, and second to investigate the effect of removal of initials on the pattern of pore development.

MATERIALS AND METHODS

The same strain of *Phellinus contiguus* (Pers.: Fr.) Pat. was used as in Butler & Wood (1988). Cultures were grown in inverted 9 cm plastic Petri dishes each containing approximately 25 ml malt extract agar (0·02 %–2 % (w/v) Oxoid malt extract) and incubated at 25 °C in light from fluorescent tubes providing a total photon flux density between 400 and 700 nm of 26 μmol m^{-2} s^{-1} for 18 h d^{-1}. Conditions in the remaining 6 h d^{-1} varied between experiments, being either darkness or a low light intensity of 1·2 μmol m^{-2} s^{-1} (depending upon availability of growth facilities).

The surface view of living basidiome tissue, supplemented in some cases by a lateral view of vertical slices, was recorded photomicrographically using a Leitz ×1 objective with oblique reflected light illumination by means of fibre optics. In some instances a combination of reflected and transmitted light was used. Each dish was briefly inverted and the lid removed during recording. Time lapse studies were carried out on 10 mm agar disks excised from parts of colonies with basidiome

initials, notched at one side for ease of orientation, and placed on malt agar in Petri dishes. At weekly intervals the disks were photographed before transfer to fresh agar medium. Where appropriate, initials were removed with fine forceps under a stereoscopic microscope, in still air in order to minimize drying out. There were four replicate disks of each treatment.

Material for SEM observations was grown on coverslips coated with a thin film of malt agar which were placed in the predicted fruiting region on the surface of similar agar in Petri dishes before inoculation. Approximately 5 mm squares of agar were excised, fixed in 3% glutaraldehyde in 0·025 M phosphate buffer at pH 6·8 and dehydrated in a water/acetone series before critical point drying.

RESULTS

Profile of basidiome development in the intact colony

In the light at 25° basidiome initiation from a vegetative inoculum usually began when colonies were 30 mm or more in diameter, a centrifugally expanding annulus of pores developing as the colony extended in radius at a rate of 6·5 mm wk^{-1} (Butler & Wood, 1988). The first macroscopic sign of dissepiment development was the formation approximately 3 mm inside the colony margin of more or less discrete irregularly-shaped islands of rosy buff aerial hyphae. Under optimum conditions for pore formation, i.e. in colonies on 2% malt agar soon after the onset of fruiting, complete pores were distinguishable 2–3 mm inside the initials (Fig. 1).

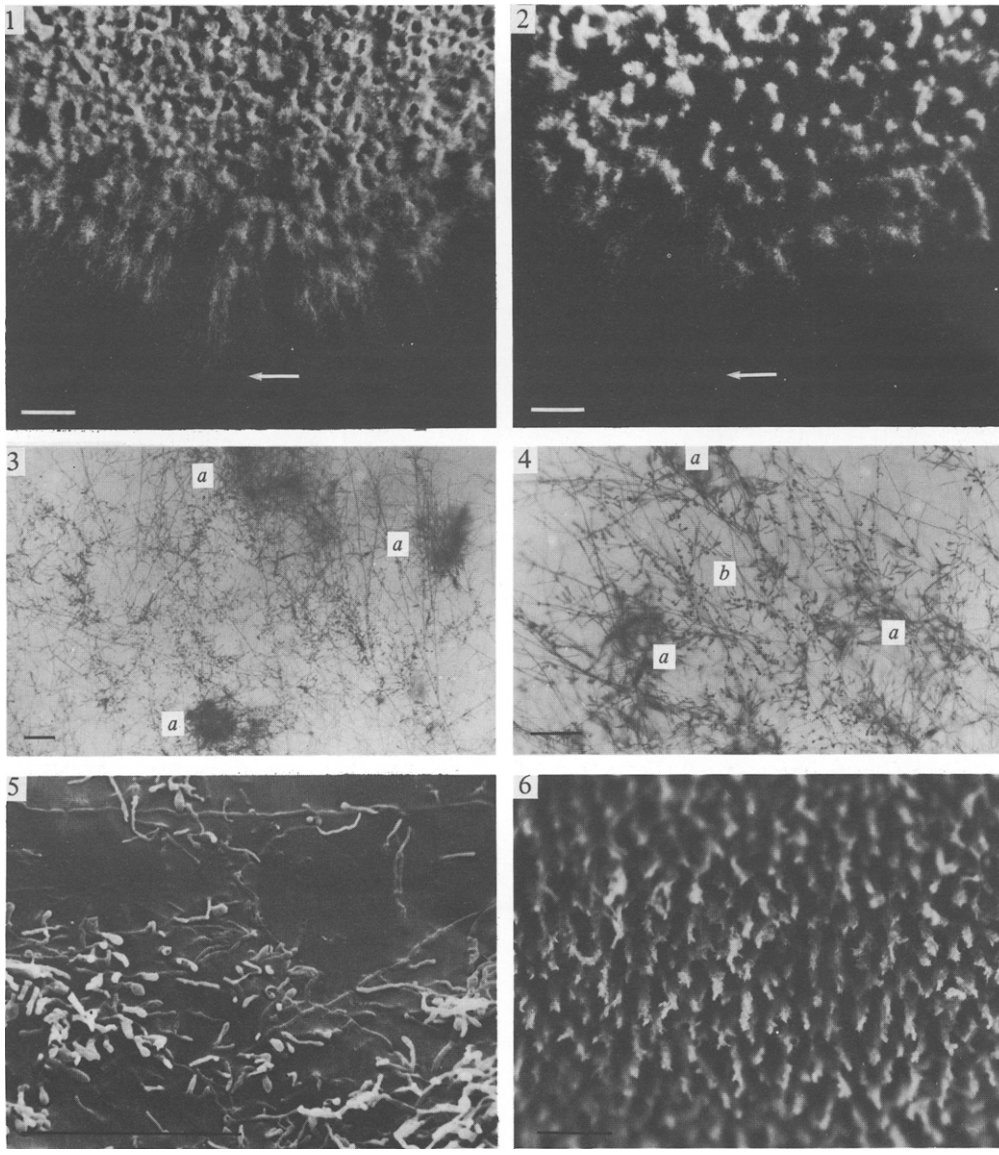
When first discernible the initials were round to elongate in shape and variable in size, approximately 100–300 μm in shortest diameter and 100–400 μm or occasionally up to 750 μm from their nearest neighbour. The density of these initials varied within as well as between dishes and their pattern appeared to be relatively uniform. Although sometimes radial or circumferential alignments of initials could be discerned, neither occurred consistently. On 0·5% and lower concentrations of malt there was virtually no vegetative aerial mycelium at the margin. SEM observations showed that the first basidia and the elongating hyphae of the dissepiment initials both arose as branches from submerged or closely adpressed vegetative hyphae (Fig. 5). Light microscope observations of parallel regions stained in aniline blue in lactic acid showed that different areas along the same hyphal branching system formed either basidial initials or dissepiment initials (Figs 3, 4).

In slightly older parts of colonies these island initials were both vertically longer and laterally unevenly wider. The amount and major direction of this extension varied with nutrient supply and with the age of the whole culture. On 2% malt the initials coalesced laterally to form pore dissepiments close to the margin and in favourable moisture and aeration conditions further development consisted of vertical extension at the outer, i.e. lower, surface of the developing basidiome. In section the dimitic flesh of the dissepiments contained hyphae with thick, brown and presumably inextensible walls. At the apex of growing dissepiments thick-walled hyphae occurred within 125 μm of the tips of the leading thin-walled hyphae. On lower malt concentrations lateral coalescence occurred further from the margin and was sometimes incomplete (Fig. 2). On 0·5% malt agar lateral extension was by localized fascicles in which presumed extending regions of loosely-arranged unbranched and more or less horizontally-growing hyphal tips (Fig. 7*e*) could be distinguished from more densely-packed non-extending lateral regions (Fig. 7*n*). In Fig. 7 pioneer hyphal tips of two fascicles can be seen approaching one another. The stage shown in Fig. 8 was frequently observed. Initials were connected by a few aerial hyphae often bearing liquid droplets. The taut appearance of these bridging hyphae in opened dishes suggested firm attachment at both ends of the hyphae. These localized fascicles were aerial structures, above the sparse hymenial elements at the agar surface (Fig. 9). By this stage the characteristic setae were visible in the dissepiment initials (Fig. 8). On 0·2% and lower concentrations of malt, lateral spread of the island initials was much reduced compared with vertical extension so that individual spines often occurred. Such isolated spines also occurred near the dish edge on more concentrated malt agar (Fig. 6), at locations where initials had developed 7 or more weeks after inoculation.

The younger edge of the initiation zone consisted of vegetative hyphae and a defined basidiome margin could not be distinguished. In older parts of cultures lateral extension of established poroid areas quite often occurred. This varied in form from a more or less laterally united 'shelf' to isolated fascicles (Figs 10, 11). However even the 'shelf' form showed some evidence of fasciculation. A series of cavities under such horizontal shelves could be seen in vertical sections of older basidiome tissue (Fig. 13). This contrasted with the primary pores developed directly from island initials (Fig. 12).

Time course studies

When agar disks bearing fruiting initials were transferred to fresh medium they developed to



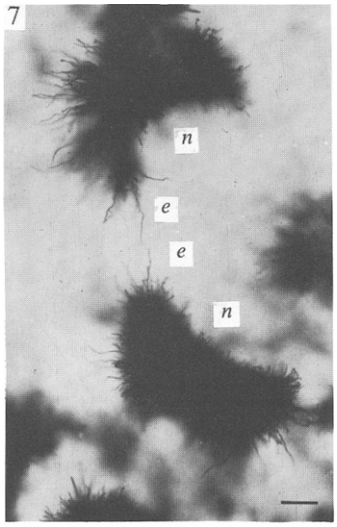
Figs 1–6. Basidiome initials in *Phellinus contiguus*.

Figs 1, 2. Initiation zones on 2 % and 0·2 % malt agar respectively (arrows mark the positions of the mycelial margins, bar = 1 mm).

Figs 3, 4. Surface views of initials stained in aniline blue in lactic acid (bar = 100 μm). Fig. 3. Three islands of aerial hyphae (*a*) in relation to the submerged parental hyphae; Fig. 4. Focussed on basidia (*b*) between islands of aerial hyphae which are out of focus.

Fig. 5. SEM of initiation zone with basidia and elongating aerial hyphae arising from submerged hyphae (bar = 100 μm).

Fig. 6. Oblique view of basidiome tissue from near the edge of a Petri dish culture where the initials have continued to elongate separately forming spines (bar = 1 mm).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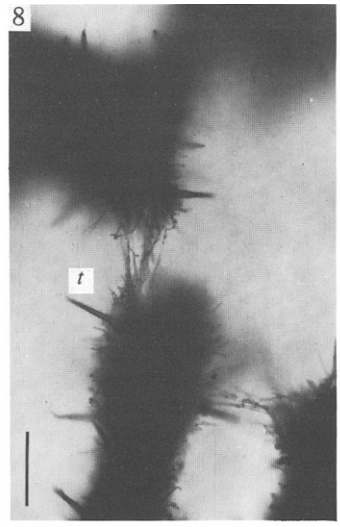
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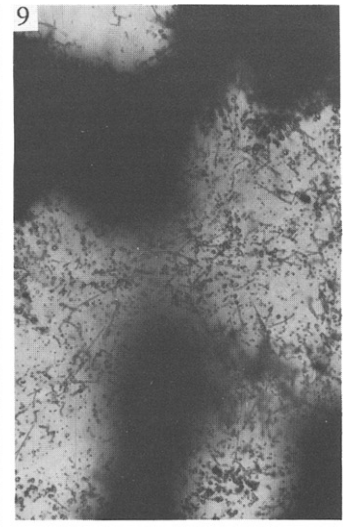
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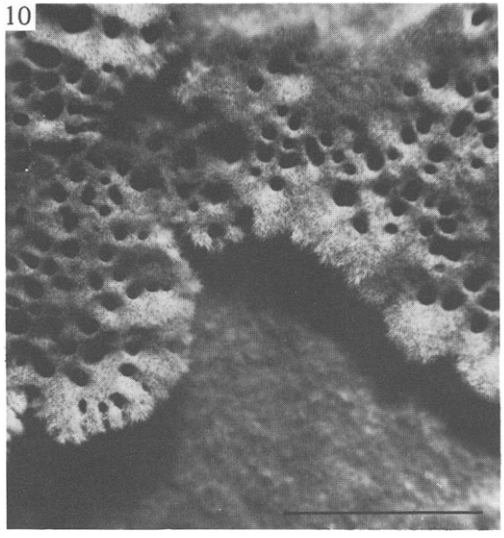
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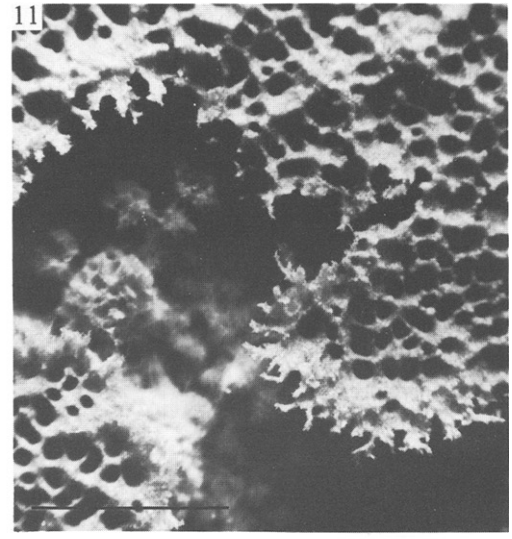
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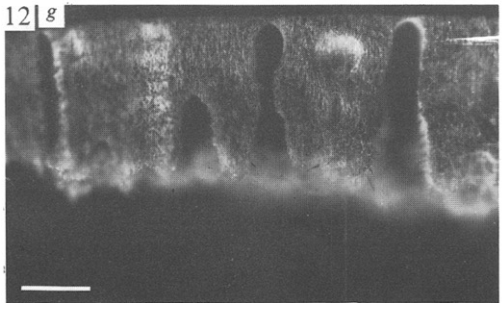
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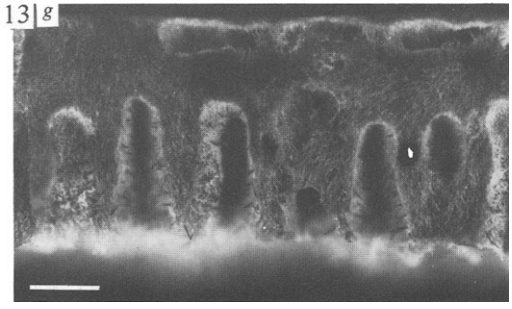
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form pore dissepiments which continued to extend for more than one year during successive disk transfers to fresh medium. Pore development also continued after photographic recording.

The development of dissepiments on different concentrations of malt from standard initials was investigated using disks from the initiation zone on 0·2% malt agar transferred to 0·02, 0·2 and 2% malt agar and transferred weekly to similar medium. On 0·02% malt agar virtually no further development took place. On 0·2% malt agar progressive enlargement of the first initials can be followed (Figs 14–19). A comparison of the positions of the initials after 1 week with those of subsequent pores shows that the two areas were complementary. Areas of non-growth, e.g. at *p*, remained as the bases of the pores whose walls were vertical and lateral extensions of the initials. Lateral infilling of a larger space by more or less horizontal fascicles of dissepiment growth can be seen at *i*. On 2% malt agar (Figs 20–25) the initials extended rapidly in all directions to form a lumpy carpet. Small 'primary' pores with thick dissepiments were soon discernible. Subsequently additional 'secondary' pores developed within the dissepiments, e.g. at *s*, by different amounts of vertical growth in different areas. Two weeks after the final transfer the outer surfaces of the dissepiments were dentate on both media, as if growth had taken place at localized points on the edges of the dissepiments (Figs 19, 25). At this stage the basidiome on 0·2% malt also showed rather irregular secondary pores. Droplets occurred on the basidiome tissue in later stages of development.

Effect of removal of initials on pore pattern

The central areas, approx. 1–2 mm in diameter, of similar disks of initials on 0·2% malt agar were denuded of initials in two ways. Either individual island initials were picked off or a central sub-disk of agar and hyphae was excised. Poroid tissue covered both types of gap over a period of 5 weeks (Figs 26–31). In all replicates which were denuded of initials direct regeneration from the hyphae at the agar surface was slow and infrequent. In both types of removal the infilling poroid tissue seemed to develop largely from the surrounding poroid areas by lateral outgrowths of branching fascicles from the dissepiment tissue. Droplets (*d*) associated with development of basidiome tissue are evident in Figs 28 and 31.

DISCUSSION

The poroid hymenophore characteristic of this species developed well in these cultures. The basidiome tissue is robust since pore development continued after disk transfers, other surgical operations and photography, all of which involved temporary gravity reversal and removal of the dish lids. Caution is necessary in interpreting development in such disturbed cultures as representing the normal sequence, especially since aeration has been shown to have a marked effect on pore density (Butler & Wood, 1988). However, structures consistent with all the developmental stages distinguished in time course studies have been observed in undisturbed cultures. These observations of living unmounted material indicate the importance of aerial spatial relationships between adjacent structures, which would be lost easily during fixation and embedding.

Two developmental processes can be distinguished, island initiation and aerial fascicle growth. Basidiome tissue arose as islands in a more or less narrow initiation zone close behind the mycelial margin. The island initials are formed in regions where the mycelium is mainly submerged, especially on dilute nutrients, so that pattern formation could be mediated through the agar medium. The pattern of initials seems to be more related to position than to hyphal lineage, a common feature of basidiome development (Reijnders & Moore, 1985).

Later development involves aerial fascicle growth which is characterized by the distinction between localized regions of loose relatively rapidly extending hyphae associated with lateral regions which either do not extend or extend more slowly.

Figs 7–13. Lateral extension of basidiome tissue.

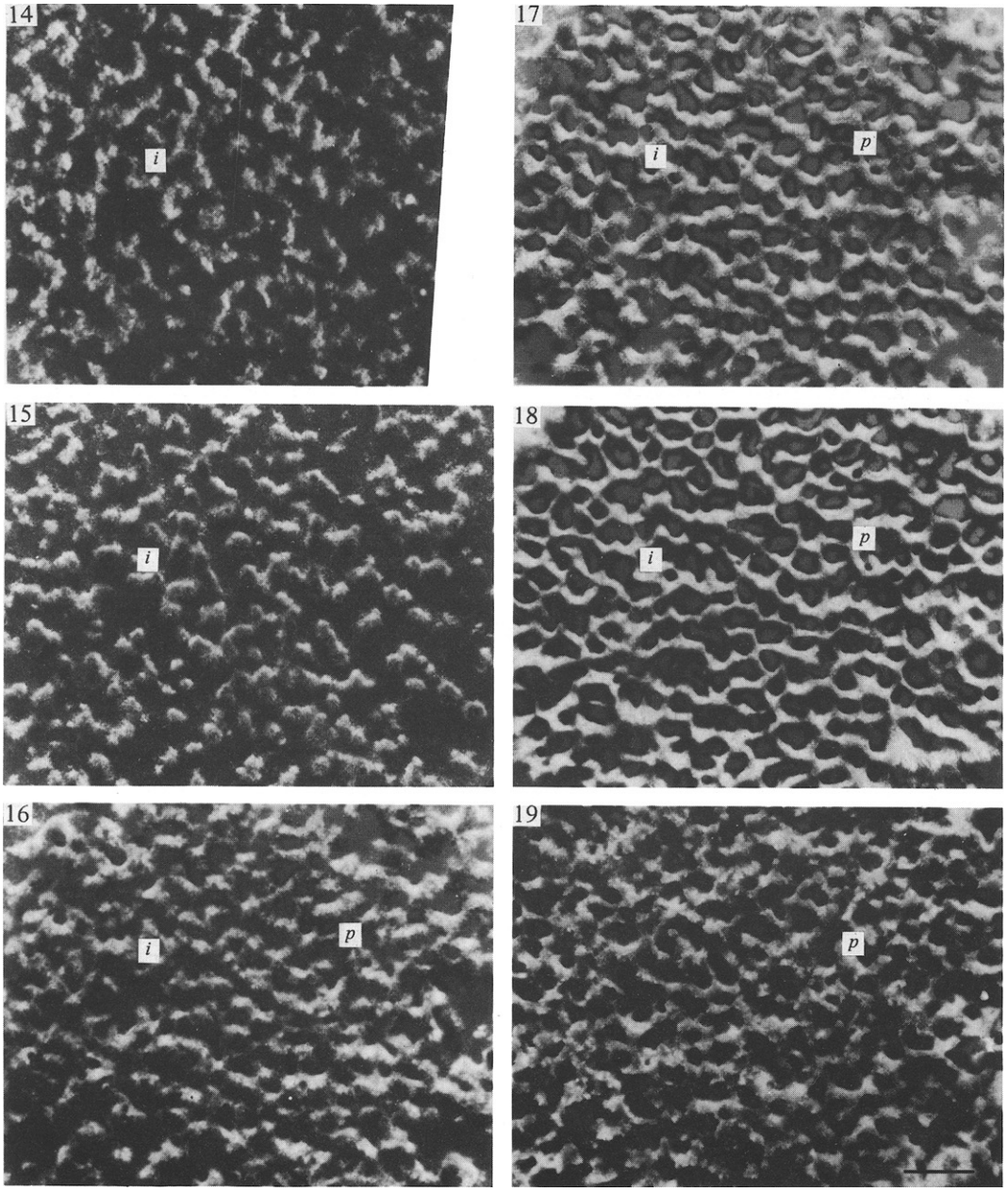
Fig. 7. Surface view of aerial fascicle outgrowths from island initials (*e*, extending region; *n*, non-extending region; bar = 100 μm).

Figs 8, 9. Surface views of the same area focussed respectively on fascicles connected by aerial hyphae and on the underlying agar surface (*t*, seta; bar = 100 μm).

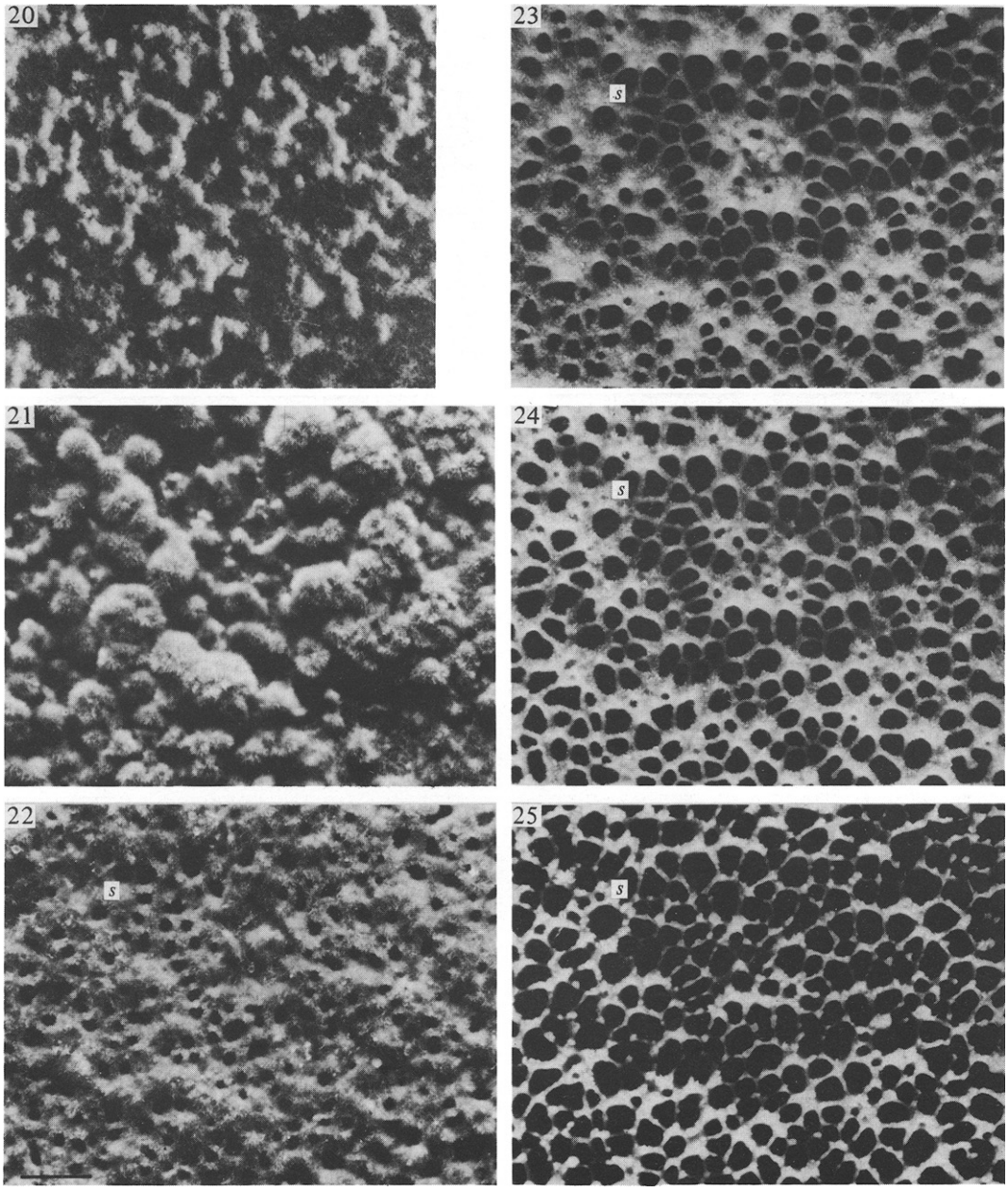
Figs 10, 11. Surface views of two examples of extending edges of older poroid areas (bar = 1 mm).

Fig. 12. Vertical section of basidiome tissue formed by primary pore development from island initials (bar = 1 mm).

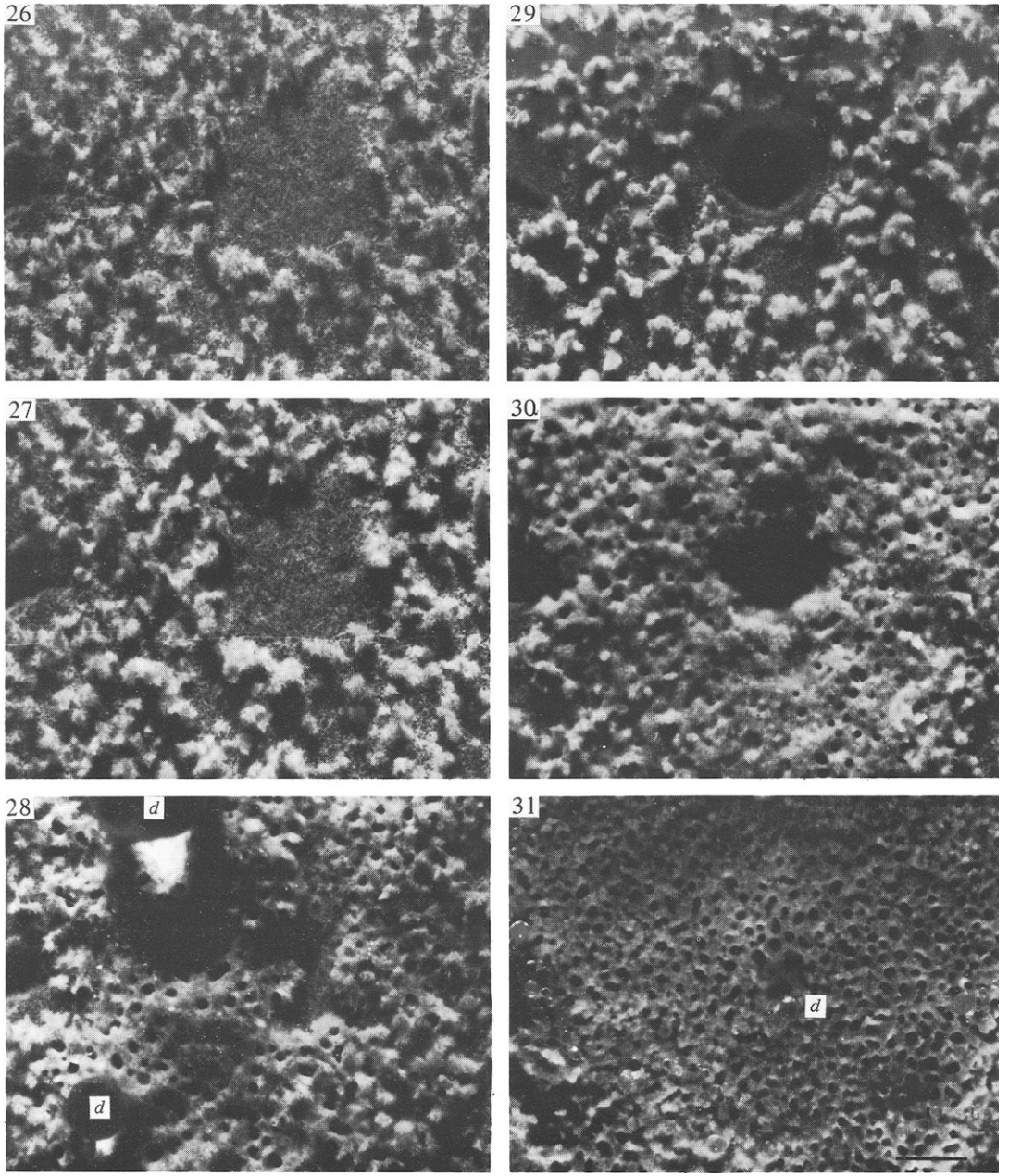
Fig. 13. Vertical section of basidiome tissue formed by lateral outgrowth of older poroid tissue with cavities beneath the shelf of basidiome tissue (*g*, agar; bar = 1 mm).



Figs 14–19. Time lapse sequence of basidiome development on a disk of initials formed on 0·2 % malt agar and transferred weekly to fresh 0·2 % malt agar.
Figs 14–18. Appearance two days and one, two, three and four weeks after first transfer respectively.
Fig. 19. Appearance six weeks after first transfer and two weeks after final transfer. (*p*, one example of a persistent primary pore; *i*, position of progressive infilling of a larger gap by lateral fascicle growth; bar = 1 mm).



Figs. 20–25. Time lapse sequence of basidiome development on a disk of initials formed on 0·2 % malt agar and transferred weekly to fresh 2 % malt agar (*s*, one example of development of secondary pores; other details as in Figs 14–19).



Figs 26–31. Infilling by poroid basidiome tissue two, three and five weeks after denuding a central area by removal of initials (Figs 26–28) and of an agar disk (Figs 29–31) (*d*, droplet; bar = 1 mm).

This localized extension is essentially an aerial process so that co-ordination to form pores raises questions of mode of communication. It is possible to interpret all the features of later development, i.e. primary and secondary pore delimitation, vertical dissepiment elongation and lateral extension of the basidiome area, in terms of localized aerial fascicle growth. This interpretation would place the nature of the fascicle and its mode of growth in a central position in determining pore configuration. A striking feature of this later development is that there is not a clear phase separation between a period of lateral diageotropic growth, resulting in pore delimitation, and a period of vertical positively geotropic growth, resulting in dissepiment elongation. It seems unlikely that lateral outgrowth at a late stage is an artifact of tip damage as a result of opening dishes since it occurred in unopened dishes and moreover the nature and amount of lateral outgrowth varied with nutrition as well as aeration.

Corner (1932*a*) defined the 'pore field' in *Polystictus xanthopus* as an annulus near the margin on the underside of the pileus in which localized development of different modes of hyphal growth resulted in initiation of pore dissepiments and delimitation of pore areas. He described the origin of dissepiments in the 'pore field' as excrescent ridges which branched and united round non-extending pore patches. This strain of *P. contiguus* is similar in that initiation occurred in a distinct 'field' but there are several differences. In *P. contiguus* the 'field' was formed on the mycelium rather than on a differentiated pileus, the initials were more or less separate islands whose growth only later in development coalesced to form pores and the final pore density was not necessarily defined in the 'pore field'. There is evidence that secondary pore formation can occur both by differential growth in thick dissepiment areas and by subdivision of primary pores. The observation that the bases of pores on 2% malt were either light transparent or light opaque (Butler & Wood, 1988) could be interpreted as due to the occurrence respectively of primary pores and secondary pores formed in dissepiment areas.

Phellinus contiguus is a member of the Hymenochaetaceae (Corner's xanthochroic series), a group closely united by microscopic characters but exhibiting a wide diversity of basidiome and hymenophore form (Donk, 1964). Corner (1932*b*) reported that the spatial distribution of dissepiment initials in *Fomes levigatus* Corner, a member of the Hymenochaetaceae with a polyporoid bracket, was similar to that of *P. xanthopus*. However initiation in *P. contiguus* is more similar to his description (Corner, 1948) of that in *Asterodon ferruginosus*, a member of the Hymenochaetaceae with a resupinate hydnoid basidiome. He described a 'field' corresponding to the 'pore field' of polypores in which spines arose as localized areas of downgrowth 0·2–0·3 mm in diameter in which the hyphal tips were positively geotropic. The main difference in *P. contiguus* is that not all hyphae contributing to dissepiment extension grew downwards, i.e. were positively geotropic, so that pores were formed by lateral extension from the island initials. The relation of these observations on a resupinate member of the Hymenochaetaceae to the development of other resupinate polypores remains to be seen.

These experiments demonstrate that this system provides good material for the study of pattern in basidiomycetes (Moore, 1984). The development of the dissepiments from separate islands of growth has a striking superficial similarity to one of the illustrated models of pattern morphogenesis described by Meinhardt (1984). Further study at more fundamental levels is required to understand the basis of pattern in this fungus.

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