Aethalium cortex formation in the myxomycete *Lycogala terrestre*

Roland McHugh [1]
Colin Reid [2]

[1] *Dublin Institute of Technology, Cathal Brugha Street, Dublin, Ireland*
[2] *Centre for Microscopy and Analysis, Trinity College, Dublin, Ireland*

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ORIGINAL

Formación de la corteza en el etalio del myxomycete *Lycogala terrestre*

Resumen. En su fase inicial, el etalio de *Lycogala terrestre* es un glóbulo simple del cual se proyectan unas papilas, en tanto el material cortical se dirige hacia el interior del glóbulo, separándolo para formar el pseudocapilicio lleno de aire. Las mitocondrias que se alinean a lo largo del pseudocapilicio en formación parecen ser las responsables del bombeo de agua hacia su exterior, dando como resultado la separación que le dará origen.
Palabras clave: Mixomycetes, esporulación, mitocondria.

Abstract. The aethalium of *Lycogala terrestre* is initially a simple blob from which papillae are temporarily extruded whilst partitions of cortical material running into it split to form the airfilled pseudocapillitium. Mitochondria aligned alongside these partitions appear responsible for the pumping of water out of them, resulting in this splitting.
Keywords: Myxomycetes, sporulation, mitochondria.

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Introduction

Lycogala terrestre Fries is a common and conspicuous myxomycete. Indeed, T.H.Macbride [4] claimed that it was 'no doubt the most common slime-mould in the world' (this of course was prior to the discovery of bark culture species). By this date several accounts of fruiting in *Lycogala* had appeared. Rostafinski's brief treatment in Cooke [1] had been expanded by Massee [5], who based his taxonomy on the 'capillitium', as both authors termed the mass of branching tubes in the cortex. Eventually Lister [2] discovered that these tubes were filled with air, and therefore quite unlike the capillitium of other myxomycetes. He referred to them as a 'pseudocapillitium'. For a view of the pseudocapillitium under SEM see the paper by Schoknecht & Small [6].

Lister's description of the fruiting process appears very painstaking, although sometimes a little confusing. The plasmodium, he informs us 'first rises from the wood as a group of small coral-red papillae which soon extend to form a cushion-like mass of closely convoluted veins or sporangia; these are more or less separated from each other by narrow tubular air-passages'. The airpassages give rise to the pseudocapillitium. Lister's account is somewhat curtailed in his third edition [3] but substantially identical, despite his restriction there of the term 'pseudocapillitium' to the innermost airpassages.

The following is an attempt to improve on Lister's account.

Autor para correspondencia: Roland McHugh
roland.mchugh@dit.ie

Materials and Methods

Developing aethalia were obtained by first locating recently matured ones on *Picea* logs and slicing them away together with the underlying bark. This was kept wet and scanned at intervals of 2-3 hours with a lowpower stereoscope. As more aethalia emerged from the wood and developed samples were removed and fixed in 2.5% v/v glutaraldehyde in 0.1 M sodium cacodylate buffer prior to postfixation in 1% osmic acid and subsequent examination with SEM and TEM.

Results

The stages in aethalium formation characterized by Lister as 'papillae' form bunches of bladders, resembling diminutive pink blackberries. Still earlier stages are not at all papillate but rather form simple blobs of protoplasm bounded by a thin peridium. Figure 1a shows an example after critical point drying. If such a specimen is split with a razor blade, recoated with gold-palladium alloy and replaced in the SEM, the peridium is seen to have curled back, revealing a smooth inside surface as no trace of pseudocapillitium has yet developed. The outer surface however exhibits a scattering of shallow punctures. In aethalia a few hours older these will deepen and an arrangement of sulci appears, sometimes connecting and sometimes surrounding them, and giving the fructification a lobate appearance (Figure 1b). Views into the punctures at this stage show some entrances to deeper passages (Figure 1c) but the inside surface of the peridium still appears smooth when separated.

Figure 1d shows a section across a sulcus with TEM. Nuclei and storage material in the protoplast may be identified. A surface membrane covers a superficial water layer but folds in to meet the peridium along the floor of the sulcus. From this line of contact a narrow partition of medium electron-density projects vertically into the protoplast. More of this material appears to the right of the sulcus: it will become the principal substance of the cortex, which term replaces 'peridium' from this point.

Figure 1e corresponds to Lister's 'group of papillae' and occurs some hours later. Essentially, papillae are extruded from the regions between sulci. Lister's characterization of them as 'sporangia' appears dubious. Figure 1e also shows the beginning of the subsequent process of collapse of the papillae, giving rise to folds. At the same stage, sections across sulci exhibit a proliferation and extension of the partitions running into them, as in Figure 1f. Lister observes that 'tubular air-passages are enclosed between the folds, which together with the deeper air-passages and the surface of the aethalium are bounded by a delicate membrane'. The membrane, enclosing water, is visible, and the partition running into the protoplast is now bisected by a central electron-dense lamina. The same feature appears in another partition from a slightly later preparation, in Figure 2a. Note here the alignment of numerous mitochondria at the sides of the lamina, and the many water vacuoles in the entire region. Such alignments have been seen in several other sections.

After perhaps 12 hours the aethalium resembles Figure 2b. The inner surface of the cortex is no longer smooth but instead incorporates a tangle of airtubes (Figure 2c). Collapsed matter around the mouths of some tubes may form a pleated rim (Figure 2d). Such material is the remains of what were folds. Protoplasm trapped within it becomes separated from the main protoplast and forms the multinucleate warts found on the surfaces of mature aethalia. Rostafinski and Massee referred to these as 'cells', the latter author considering that the pseudocapillitium originated in them. Lister called them 'vesicles' and noted that their nuclei remained sharply defined until spore cleavage was completed.

As the aethalium matures, the pseudocapillitial tubes continue to thicken, and in fractured SEM preparations a concentrically layered structure is visible (Figure 2e).

The layers appear more closely adpressed when viewed with TEM (Figure 2f).

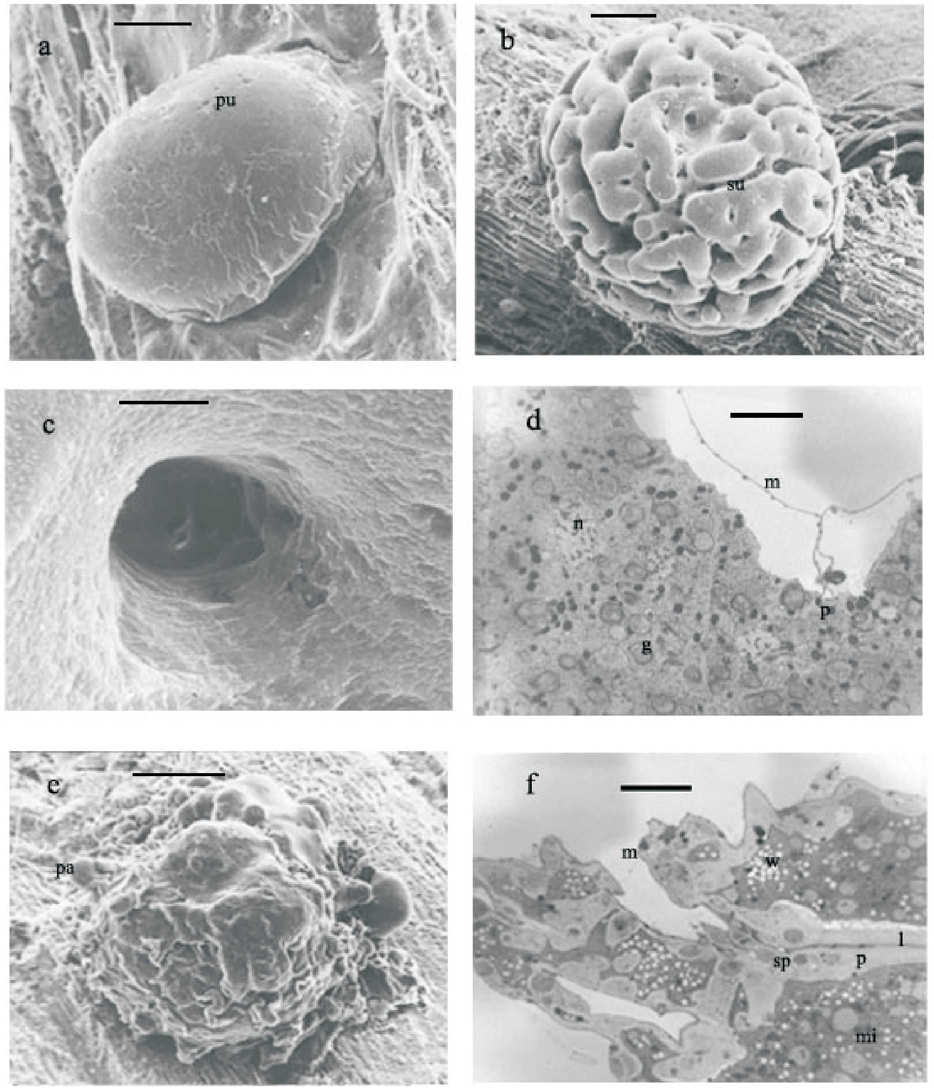

Figure 1. a. Very early aethalium showing punctures (pu), SEM. Scale bar = 100 µm . **b.** Early aethalium showing sulci (su), SEM. Scale bar = 250 µm. **c.** View into puncture in early aethalium, SEM. Scale bar =10 µm . **d.** VS sulcus showing surface membrane (m), storage granule (g), nucleus (n) and partition of cortical material in early stage (p), TEM. Scale bar = 10µm . **e.** Aethalium at papillate stage showing papilla (pa), SEM. Scale bar = 250 µm. **f.** VS aethalium at papillate stage showing surface membrane (m), mitochondria (mi), water vacuoles (w) and partition (p) with central lamina (l) containing split (sp), TEM. Scale bar = 10µm .

Discussion

Lister's concept of collapsing folds which trap air could easily explain large bubbles of it but can hardly account for the reticulum of minute air tubes. Somehow air would need to be forced or sucked into the tubes. It seems likely that ATP from the mitochondria along the insides of the partitions could provide the energy needed to achieve this. A similar alignment appears at the edges of epithelial cells in the mammalian kidney and is responsible for water translocation. In *Lycogala* we may have an active withdrawal of water from the central laminae within the partitions, splitting them at intervals to produce branching passages (obliquely aligned to the plane of our sections) into which air is sucked by the splitting process. Such splits in the central laminae are seen in Figures 1f and 2a. At their edges occur a number of electron-dense nodules which may have been produced by compaction resulting from water removal. The water would have subsequently left the

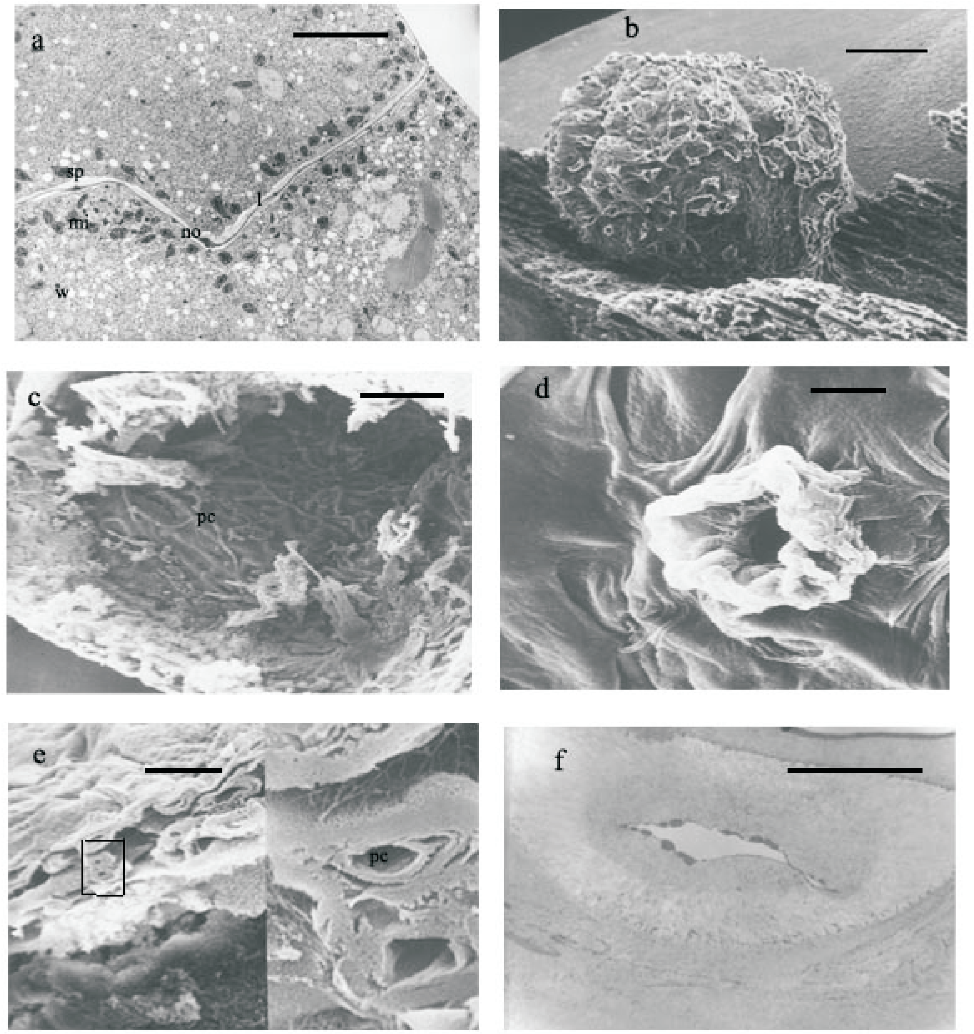

Figure 2. a. VS aethalium at papillate stage showing mitochondria (mi), central lamina (l) with splits (sp) and nodules (no), and water vacuoles (w), TEM. Scale bar = 10 µm. **b.** Aethalium appx 12 hours after emergence, SEM. Scale bar = 100 µm. **c.** Fractured aethalium at same stage as **b**, showing pseudocapillitium (pc) on inner cortical surface, SEM. Scale bar = 100 µm. **d.** Portion of exterior surface of same preparation, SEM. Scale bar = 25 µm . **e.** Detail of **c**, SEM. Scale bar = 25 µm. Righthand portion is enlargement of window, showing fractured pseudocapillitium (pc). **f.** TS pseudocapillitium, TEM. Scale bar = 10µm .

partitions as vacuoles which would have emptied at the edge of the cortex causing the bulging-out of the papillae as in Figure 1e. In living material some of these papillae appear almost colourless and seem to be little more than water-sacs. Once the pseudocapillitium has formed they gradually collapse, the water being presumably returned to the center of the aethalium.

Processes similar to these probably occur in the other *Lycogala* species. The remaining Reticulariaceae, *Reticularia, Tubulifera* and *Dictydiaethalium* all produce a sporocarp in which distinct sporangia are compacted. The extrusion of papillae in *Lycogala* is an element of pseudocapillitium formation and there is no certainty that it evolved from a partial cleavage of sporangia. It may however have done so, and the distinction would not thereby justify the removal of *Lycogala* to a separate family.

Finally, what function has this web of airpassages? *Lycogala* often appears on the exposed upper surfaces of logs and stumps, and the insulating cortex may help to reduce overheating in direct sunlight, which would impair spore survival. The silvery reflective peridium of the aethalia of *Reticularia lycoperdon* Bull., to be seen, for example, on sunlit fenceposts, may serve a similar function. Other species such as *Stemonitis fusca* Roth which also occur in exposed situations but disperse their spores rapidly would suffer less from longterm baking in the hot sun and would not require such devices.

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