Leucoagaricus basidiomata from a live nest of the leaf-cutting ant *Atta cephalotes*

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The formation of basidiomata of *Leucoagaricus gongylophorus* in a live nest of *Atta cephalotes* is described and illustrated.

Möller (1893) was the first to provide detailed descriptions of fungi isolated from ant fungus gardens. He isolated the fungi from the nests of various attine species, including members of the genera *Acromyrmex* Mayer, *Apterostigma* Mayer and *Cyphomyrmex* Mayer. He also described and drew the development of agaricaceous basidiomata, which he obtained from the surfaces of leaf-cutting ant nests in the field. He accurately drew gongylidia, the typical swollen hyphal tips of the vegetative mycelium in ant nests, which developed after he had inoculated nutrient agar with fragments of fruit body taken from inside sterile surfaces of basidiomata. He also successfully grew vegetative mycelium from basidiospores which later formed gongylidia. He named the species *Rozites gongylophorus* A. Møller. Weber (1957, 1966), using the fungi isolated from nests of the primitive attines *Cyphomyermex costatus* Mann and *Myrmicocrypta buenzli* Borgmeier, and Hervey, Rogerson & Leong (1977), using the fungus from *Apterostigma auriculatum* Wheeler, obtained basidiomata in culture but in no case did they report a gongylidia-bearing mycelium obtained from these basidiomata. According to Heim (1957) and Singer (1975), both Weber's and Hervey's agarics are specifically identical and congeneric with *Leucoagaricus* (Locq.) Singer and should be known as *Leucoagaricus gongylophorus* (A. Møller) Singer. The combination *Leucoagaricus gongylophorus* was later formally proposed by Singer (1986). More recently Muchovej, Della Lucia & Muchovej (1991) have described *Leucoagaricus weberi* as a new species and the first agaric found growing in a living nest of *Atta sexdens* L.

This work describes the successive formation of four separate basidioma aggregates over a period of 9 wk in a living nest of *Atta cephalotes* L. Cultures of attine ants have been maintained in the Department of Biological Sciences at the University of Exeter since 1980. Colonies with queens of *Atta cephalotes*, *Acromyrmex octospinosus* Reich, *Sericomyrmex urichi* Forel and *Trachymyrmex urichi* Forel were collected in Trinidad during July and August in 1980, 1986 and 1991. Those of *Atta cephalotes* were about 1 yr old, with a single fungus garden *ca* 10 cm diam. In addition to the above, five young *A. cephalotes* colonies were collected by Mr R. Jackman at Gilpin Trace, Roxborough, Tobago on 20 Nov. 1989 and delivered to the laboratory at Exeter three days later. Their fungus gardens were destroyed during transit. To regenerate the colonies each surviving queen with her workers and brood was placed on a moist sponge cloth in a plastic container 10 × 7 × 3·5 cm and supplied with approximately 20 g of mature fungus garden taken from a laboratory colony (Exeter) of *A. cephalotes* collected in the Caura Valley, Trinidad in July 1986. All five of these colonies survived, successfully adopting the new fungus gardens. Cultures were kept at 25–26 °C, relative humidity 70–80% in a 12:12 photoperiod. The nests were housed under Perspex containers isolated on separate boards supported by legs resting in paraffin moats to prevent ant escape. Each colony was provided with a dish of water and a daily supply of a variety of plant material, principally privet leaves (*Ligustrum ovalifolium* Hassk.), grapefruit skins (*Citrus paradisi* Macfad. = *Citrus aurantium* L.), and cultivated rose leaves and petals. Expansion of the colonies was normally restricted to approx. 15 × 15 × 20 cm by their covers, but one colony was eventually rehoused in a larger container 20 × 20 × 30 cm, where the ants ultimately expanded the fungus garden to occupy 75% of the available space with an estimated fresh weight of 1·75 kg. In June 1993 an irregular structure with the appearance of an incipient basidioma began to develop in this enlarged colony. Three subsequent aggregations of new basidiomata developed at 2–3 wk intervals, usually just above the central line of the fungus garden, until after about 9 wk the process terminated (Figs 1, 2). All specimens have been deposited in 'K' under J. Fisher (KM 23677, 23974). Addition of inert markers indicated that the turnover time for plant substrata was about 5–6 wk, so that the basidiomata were gradually moving downwards during their formation as new fungus garden was added at the top of the colony and old garden was removed from below. The ants appeared to feed their larvae preferentially on developing basidioma tissue, perhaps because the fungus is

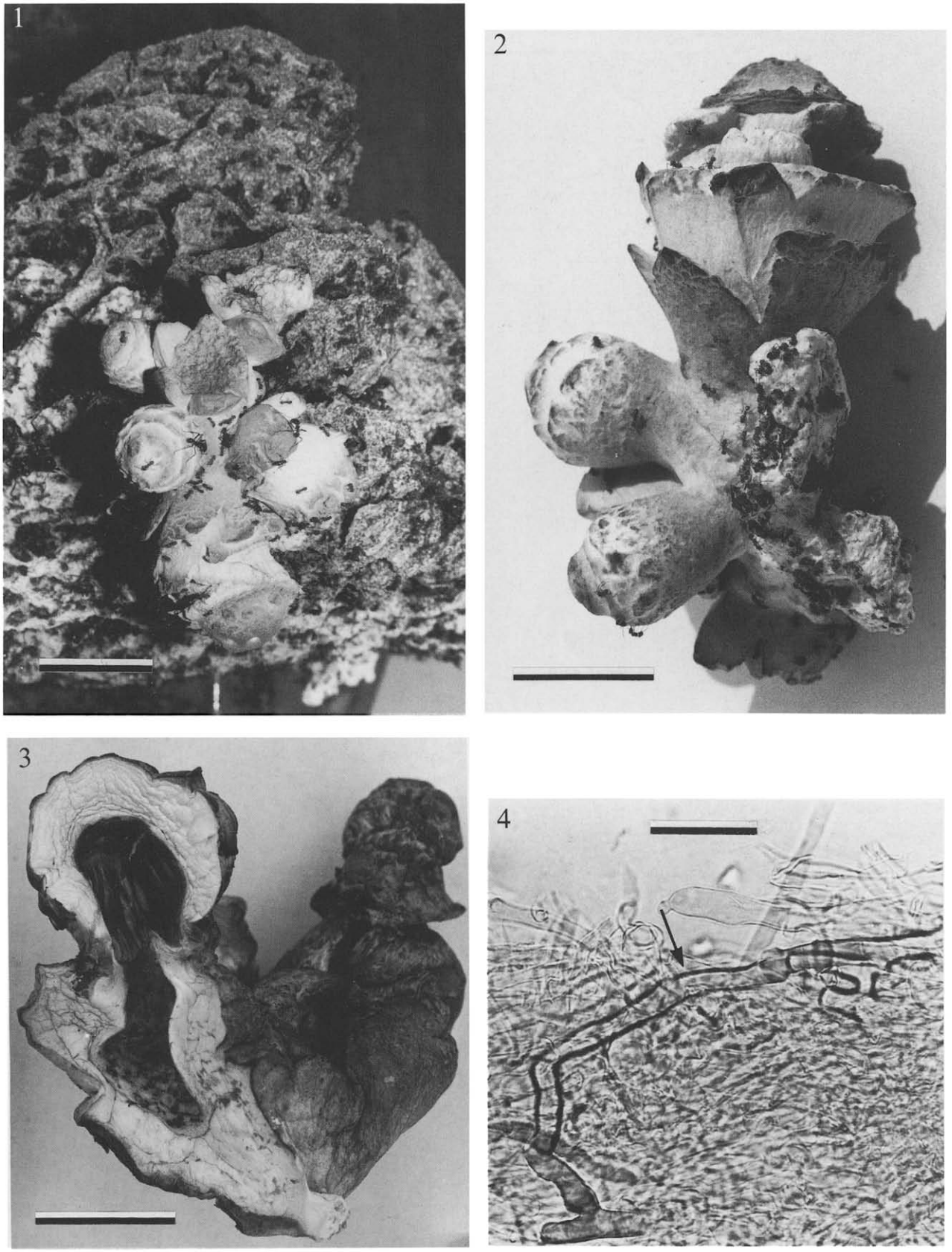
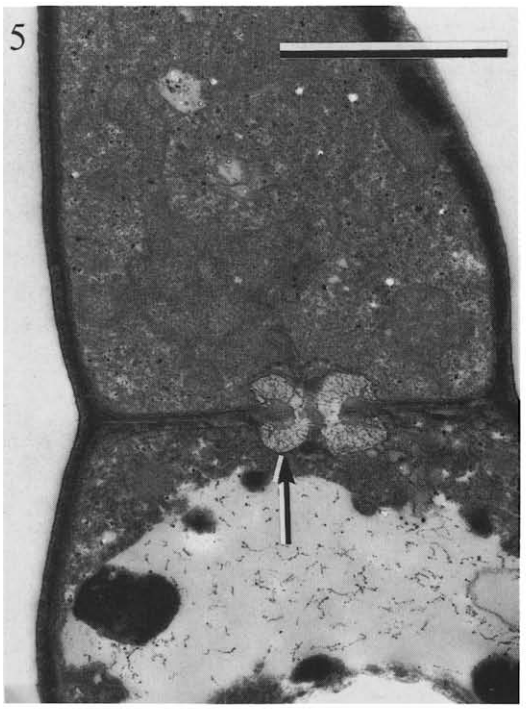
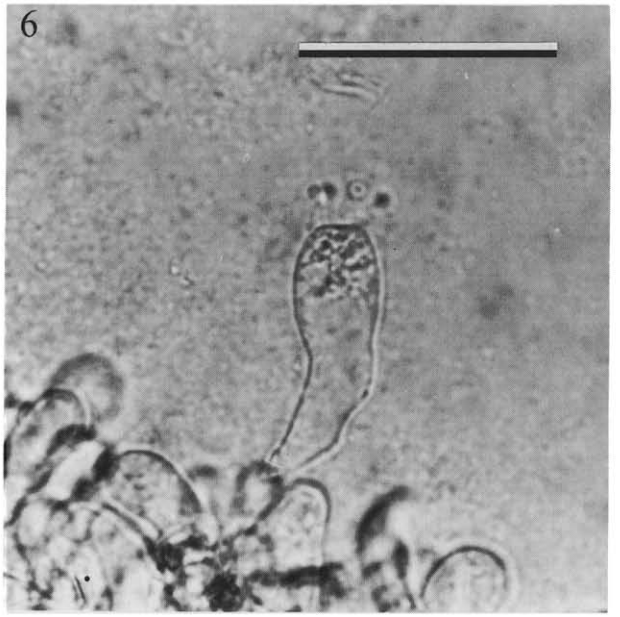


Fig. 1. Developing basidiomata of *Leucoagaricus gongylophorus* on a fungus garden in a live nest of *Atta cephalotes*. Bar, 4 cm. **Fig. 2.** Some stages of development of basidiomata removed from the ants' nest. Bar, 2 cm. **Fig. 3.** A basidioma which has been hollowed out by the ants. Bar, 2 cm. **Fig. 4.** Teased tissue taken from the inside of the stipe showing gloeoplerous hyphae (arrowed) . Bar, 30 μm.





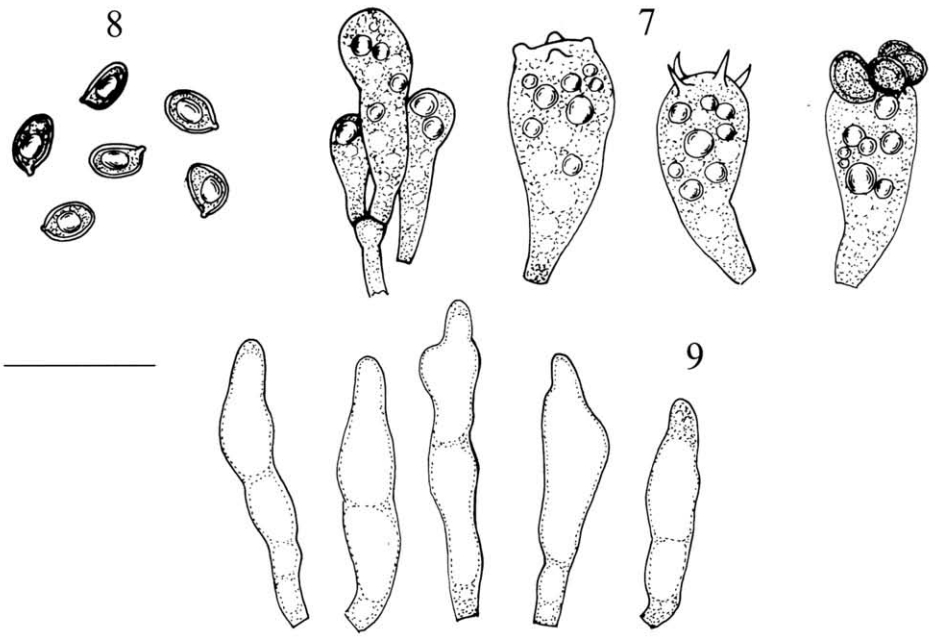
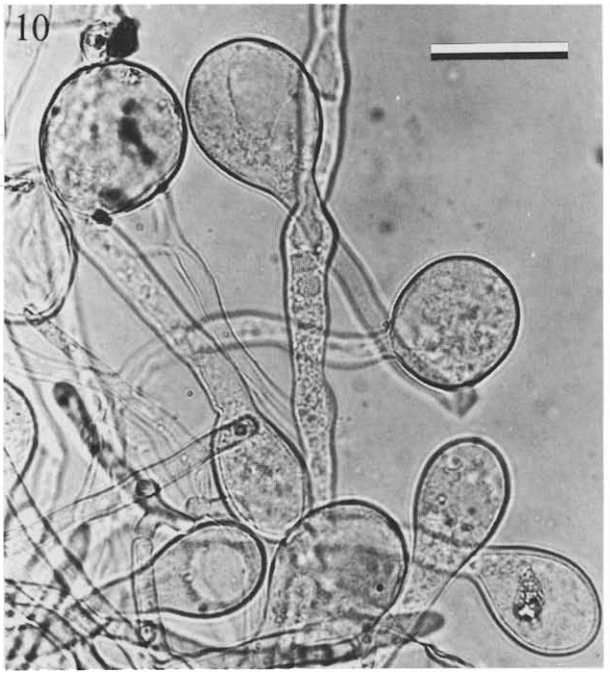
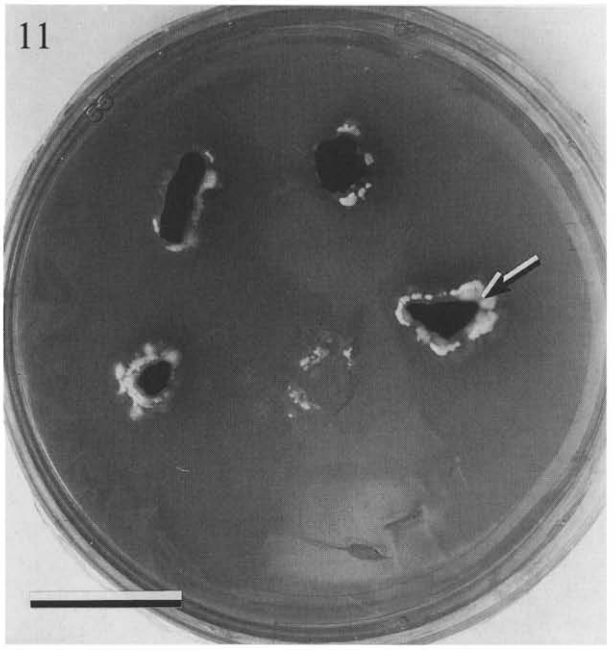


Fig. 5. E.M. longitudinal section of hypha from stipe tissue showing dolipore septum (arrowed). Bar, 2 μm. **Fig. 6.** Basidium with sterigmata and spores. Bar, 30 μm. **Fig. 7.** Some developmental stages of basidia. **Fig. 8.** Basidiospores. **Fig. 9.** Cheilocystidia. Bar, 20 μm.

present there in a concentrated form similar to that of the gongylidia in the fungus garden, which is the principal larval food. The basidiomata were hollowed out by the ants from the inside (Fig. 3), with the result that most of the hymenial tissues were destroyed before they could ripen to produce basidiospores. Some basidioma aggregates were therefore removed (40–50 g wet weight each) at an appropriate stage. Samples of teased tissue taken from the inside of the stipe showed a structure traversed by gloeoplerous hyphae (7–8 μm diam) with oleaginous contents which stained deeply with lacto-fuchsin (Fig. 4). E.M. longitudinal sections revealed dolipore septa similar to those in the mycelium found in the fungus garden (Fig. 5). One basidioma was sectioned (Fig. 13). Squash preparations of hymenial tissue yielded four-spored basidia, 22–32 × 8·5–14 μm (Figs 6–7) and individual basidiospores, 5·7–7·7 × 4·5–5 ($6{\cdot}7 \pm 0{\cdot}3 \times 4{\cdot}8 \pm 0{\cdot}2$) μm, $Q = 1{\cdot}4$ (Fig. 8); cheilocystidia, 24–44 × 5·5–8·5 μm, taken from a sterile gill edge were also present (Fig. 9). The strongly dextrinoid spore wall, the lack of apical differentiation into a germ-pore or similar structure and the absence of clamp-





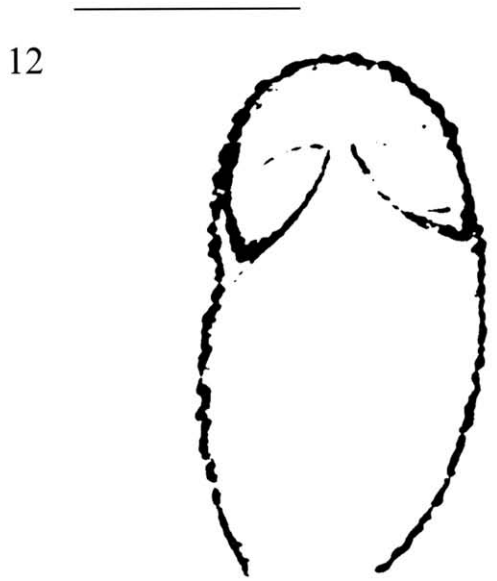


Fig. 10. Club-shaped gongylidia, typical to all attine fungus gardens and the principal food of ant larvae. Bar, 40 μm. **Fig. 11.** Fragments of stipe tissue plated on to PDA showing white gongylidia formations. Bar 2 cm. **Fig. 12.** A drawing by Möller (1893) showing developing basidioma. Bar, 2·5 cm. **Fig. 13.** Freshly sectioned developing basidioma. Bar, 2·5 cm.

connections are all features typical of *Leucoagaricus*. Attempts to germinate immature basidiospores failed, and although a number of basidiomata were placed on moist cotton wool in a damp chamber the basidiomata failed to expand to yield mature basidiospores. Ten aggregates of cheilocystidial elements, each aggregate measuring $\leqslant$ 1 mm, were then dissected from the hymenium under sterile conditions and plated on to potato dextrose agar. Thirty fragments of stipe tissue were similarly dissected and plated. Intubation was at 25°. After about 1 wk all the plated fragments began to grow new mycelial tissue, and approximately 10 d later typical aggregates of gongylidia formed (centre transverse measurement 35–40 μm) at the edges of the colonies (Figs 10–11). All colonies obtained from fragments of the basidiomata looked and behaved identically to those obtained from the fungus garden. Care was taken by using × 600 light microscopy to ensure as far as possible that no contaminant was present in the plated fragments. It is also noteworthy that ants secrete antibiotic substances which suppress the growth of many micro-organisms (Maschwitz, Koob & Schildknecht, 1970). One such substance, myrmicacin, $C_{10}H_{20}O_3$ (Schildknecht & Koob, 1971), inhibits the growth of a large number of fungi including typical soil organisms such as *Penicillium* spp. and plant epiphytes such as *Cladosporium* spp. and

Alternaria sp. (Stradling & Fisher, unpublished). This indicates that the total mass of basidioma tissue (approx. 200 g) must have grown from the cultivated fungus, since an invading fungus would have been suppressed.

This work therefore strongly suggests the teleomorph connection between the basidiomata and the mycelium of the fungus garden, although it would have been preferable to obtain further proof from germinating basidiospores. According to Muchovej *et al.* (1991), their description of *Leucoagaricus weberi* in a nest of *Atta sexdens* was the first record of any agaricoid fungus in an active nest in which ants were still cultivating the fungus garden. This work therefore describes a second such event, but this time in a nest of *Atta cephalotes*.

Our successful method for isolating mycelium from the basidioma tissue to obtain gongylidia reflects Möller's work, and his illustration of a developing basidioma (Fig. 12) is remarkably similar to our observations (Fig. 13). The original herbarium material from Möller's work appears to have been lost or destroyed. It is regrettable that except for Möller, none of the earlier workers reported on gongylidia formed by the vegetative mycelium obtained from basidiomata, a feature common to all fungi isolated from true attine fungus gardens. Chapela *et al.* (unpublished) have recently examined the cultural and micromorphological characters of 42 fungi cultured by ants in the tribe *Attini* to clarify their taxonomic and broad phylogenetic status. They found three consistent morphological groups with wide morphological and whole-genome differences, which suggests that they belong to at least different species within the Basidiomycotina. This may well explain in part why there is a descriptive divergence of the few reported cases of teleomorph states of this group.

What induces the formation of teleomorphs in a fungus garden remains unknown. In the absence of clamp-connections in the mycelium bearing the gongylidia, and observations on the cytological state of the mycelium, it is not known whether the mycelium in the nest is normally monokaryotic or dikaryotic. More information is required on the cytology of the mycelium in the nest, and whether cultures derived from single basidiospores are capable of fruiting.

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However, since the ant colonies with their fungus gardens have been maintained over several years under identical conditions but without fruiting in our laboratory, it is thought unlikely that the development of basidiocarps would have been preceded by a change in the cytological state, i.e. a change from a monokaryotic to a dikaryotic state. Instead it is thought more likely that some subtle change in the state of the nest, for example a failure of the ants to remove basidomata initials in their earliest stages, thus allowing them to grow into more mature structures, might be responsible. This is supported by the fact that the queen of this nest and consequently the whole colony died approximately 8 wk after the last fruit body had formed. Thus the colony had probably entered the final ageing process when the ants allowed the basidomata to form.

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