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EVIDENCE FOR DEVELOPMENTAL COMMITMENT IN THE DIFFERENTIATING FRUIT BODY OF *COPRINUS CINEREUS*

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Commitment of hymenial tissues of *Coprinus cinereus* to continued development after being explanted to agar medium was determined as a function of the stage of development reached immediately prior to explantation. Probasidia at the dikaryotic (prekaryogamy) stage at excision were arrested at that stage; explants made at later physiological ages did complete meiosis and/or sporulation, though at a slower rate than in vivo. Reversion to hyphal growth was observed to occur from hymenial and tramal cells but not often from probasidia. It is concluded that dikaryotic (prekaryogamy) probasidia are specified irreversibly as meiocytes and that they become determined to complete the sporulation programme during meiotic prophase I. Once initiated, maturation of basidia is an autonomous, endotrophic process.

The potential of different fruit-body tissues of *C. cinereus* to form fruit-body primordia when explanted to agar medium was tested. Stipe bases and pseudorhizal (dark-grown) stipe bases always gave rise directly to primordia; most lamellae explanted at the dikaryotic stage showed such direct fruiting, but gills explanted after meiosis did not. This behaviour correlated with the disposition of accumulated glycogen and it is suggested that competence to fruit in these circumstances depends on the glycogen content of the tissue rather than representing commitment to a morphogenetic pathway.

Fungal development, whilst presenting particular problems of its own, may nevertheless have parallels with morphogenesis in higher organisms. Such parallels are worth pursuing because of the conceptual framework which is already established. Recently, we have attempted to draw comparisons with development in other eukaryotes by seeking examples of the major phenomena identified therein. For example, cell differentiation and regulation of gene activity in ways geared to morphogenesis (Moore, Elhiti & Butler, 1979; Moore, 1984*a*, *b*; Yashar & Pukkila, 1985); formation of inhomogeneous cell populations from homogeneous ones (Rosin, Horner & Moore, 1985; Rosin & Moore, 1985*a*, *b*); and regional specification (pattern formation) directed by organisers producing morphogens (Rosin & Moore, 1985*a*; Horner & Moore, 1987). In this paper we address the problem of commitment of particular cells to particular fates.

It is a common expectation among mycologists that vegetative cultures should be recoverable readily from tissues of fruiting (and other multicellular) structures collected in the field. This expectation is more often fulfilled than not, usually with quite simple media and frequently with the ability to re-form the fruiting organ given appropriate environmental and nutritional conditions. Neither botanists nor zoologists can contemplate such routine preparation of cell cultures from fully differentiated tissues, still less the regeneration of the whole organism. The question therefore arises as to whether fungal multicellular structures consist of cells as fully committed to a differentiated state as are their plant or animal counterparts.

Animal embryologists distinguish a number of different types of commitment (see discussion in Slack, 1983) but the two which seem applicable here are the successive steps, specification and determination. A tissue explant is said to be specified to become a particular structure if it will develop autonomously into that structure after isolation from the embryo, subject to its being provided with appropriate conditions. An explant is determined to become a particular structure if it develops autonomously into that structure irrespective of the conditions into which it is explanted.

Commitment in the *Coprinus* hymenium has been demonstrated in *C. cinereus* (Schaeff.: Fr.) S. F. Gray (sensu Konr.) by McLaughlin (1982), and in *C. congregatus* Bull.: Fr. by Bastouill-Descollonges & Manachère (1984). However, these authors did not discuss their experiments from this viewpoint, placing more stress in the former case on sterigma formation, and in the latter on the potential for renewed fruiting from excised lamellae – a regeneration phenomenon related to, but

distinct from, developmental commitment. We have, therefore, repeated and extended these observations using *C. cinereus*. The two species are similar in certain respects: both have synchronized meiotic divisions in their basidia, so that progress through meiosis can be employed as an objective-marker for the physiological age of a fruit body. Also, both have similar response to light induction of primordium formation (Morimoto & Oda, 1973; Ross, 1985), have dark-inhibitory and dark-recovery processes in the maturation of caps (Kamada, Kurita & Takemaru, 1978; Durand, 1983), and show tissue-specific accumulation of glycogen (Moore *et al.*, 1979; Ross, 1985). They differ in at least one aspect: stipe elongation in *C. congregatus* is regulated by the cap (Manachère *et al.*, 1983) while excised and decapitated stipes of *C. cinereus* continue to elongate (Gooday, 1974). In the present study we have established the stage of commitment to sporulation and have demonstrated the differing abilities of fruit-body tissues to revert to hyphal growth on the one hand, or give rise to fruit-body primordia on the other.

MATERIALS AND METHODS

Organism and culture conditions

The Meathop dikaryon of *C. cinereus* was grown on a nutrient agar (10 g malt extract, 4 g yeast extract, 4 g glucose, 10 g agar per l) (Rao & Neiderpruem, 1969). Petri dish cultures were incubated first at 37 °C for 3 d in complete darkness then transferred to 27° under a photoperiod of 16 h light–8 h dark to induce fruit bodies. For dark-grown fruit bodies, plates were wrapped with aluminium foil prior to transfer to the 27° incubator.

Determination of commitment to hymenium development

Gill lamellae were removed from caps at various stages of development and placed on the surface of the above nutrient-agar explantation medium or 1% (w/v) water-agar. Their development was then observed at daily intervals during incubation at 27° in an illuminated incubator operating with the photoperiod described above.

Potential for renewed fruiting

In addition to lamellae, 1 cm lengths of dark-grown 'stipes' (= pseudorhizal stipe bases; Buller, 1931, pp. 112–117) and of normally-grown stipes were used as inocula; one per 9 cm plate, placed on the surface of nutrient-agar medium and incubated at 27° under the illumination conditions described above. The ability to form new fruit-body primordia was scored. In doing so, only first-flush primordia were taken into consideration. Direct fruiting was used to refer to formation of primordia directly on the inoculum; indirect fruiting was used for primordia that were formed on the outgrowing mycelium away from the inoculum. 'Mixed fruiting' was a combination of both of these patterns (Bastouill-Descollonges & Manachère, 1984).

Cytological examination

The physiological age of all fruit-body tissues was determined by examining the stage attained in meiotic division using fluorescent staining of tissue squashes with ethidium bromide or acridine orange (Chiu, 1986). Development of explanted lamellae was examined using fluorescence microscopy, and scanning electron microscopy of glutaraldehyde-osmium tetroxide-fixed specimens which were dehydrated in ethanol prior to critical point drying (Chiu & Chang, 1987).

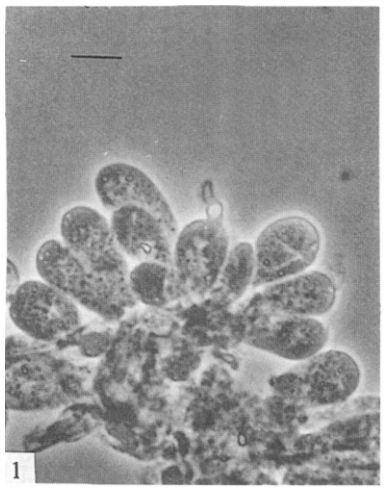
RESULTS AND DISCUSSION

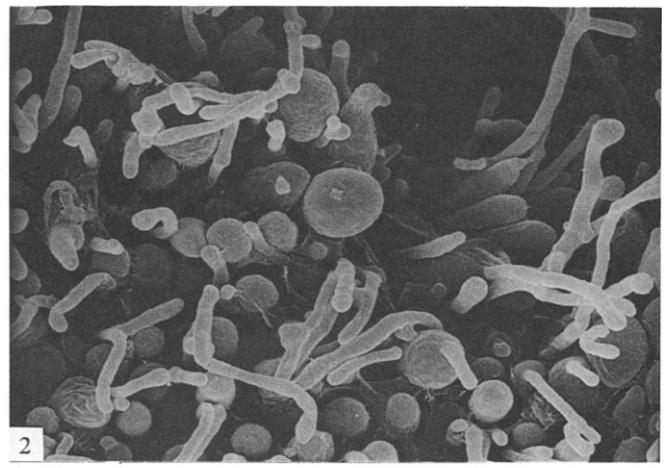
Commitment to hymenium development

Cytological examination of 16 specimens of explanted lamellae taken at the dikaryotic stage (prior to meiosis) showed that very few probasidia in some samples proceeded to prophase I, even after 2 d incubation on explantation medium. The majority of probasidia in such samples were arrested at the stage which they had reached at the time of explantation, even though the incubation period was sufficient to permit hyphal outgrowths to be formed, largely from tramal tissues (Figs 1, 2). Probasidia of samples taken at or after prophase I all completed meiosis and sporulation after explantation (25 specimens). In contrast, paraphyses (and cystidia) in the same samples reverted to hyphal growth by unipolar or multipolar apex formation (Figs 3–6).

Lu (1972) suggested that the 10 h period before prophase I was the stage programmed for initiation of karyogamy and chromosome pairing, and Raudaskoski & Lu (1980), using hydroxyurea to arrest DNA synthesis in the dikaryotic stage, showed that this treatment stopped further development of fruit bodies. Lu (1982) suggested that duplication of DNA at the dikaryotic stage immediately prior to meiosis commits the basidia to genetic recombination. Our explantation experiments, however, demonstrate that such tissues are specified but not determined for sporulation. Determination to sporulation is demonstrable, but only in material explanted at prophase I or later.

This situation is similar to that in *Saccharomyces cerevisiae* Hansen, where commitment to re-

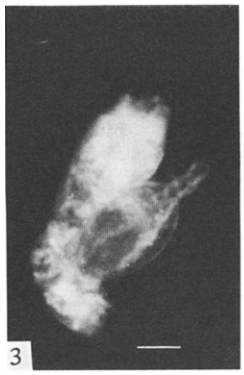




Figs 1, 2. Behaviour of hymenia of *Coprinus cinereus* lamellae explanted to agar medium at the dikaryotic (prekaryogamy) stage.
Fig. 1. Light-micrograph (LM, scale bar = 10 μm) of a squash preparation of a small piece of hymenium with few probasidia (forming the bulk of hymenium at this stage) regenerating to form hyphal outgrowths, the majority are arrested in development.
Fig. 2. SEM showing that reversion to hyphal growth occurs in few of the hymenial cells; most regenerated hyphae penetrate through the hymenium from the trama (×975).

combination does not inevitably lead to commitment to meiotic division, the latter requiring duplication of the spindle pole body which occurs early in the first meiotic division (Berry, 1983; Dawes, 1983). Raju & Lu (1973) found that the spindle pole body duplicated at diplotene in *C. cinereus*. Thus, *S. cerevisiae* and *C. cinereus* share similar requirements for the attainment of competence and commitment to recombination and meiotic division. There is a difference, in that *S. cerevisiae* cells removed from sporulation medium after commitment to recombination, but before commitment to sporulation, can return to mitotic vegetative growth (Berry, 1983). Here, though, all the isolated lamellae explanted at the dikaryotic stage maintained their hymenial structure even after 2 d incubation; the majority of hyphal outgrowths penetrating through the hymenium from below (Fig. 2). Thus, although such young probasidia are unable to continue development on explantation, they are somehow inhibited from reversion to the vegetative state. That is they are specified as meiocytes but not yet determined for sporulation. Paraphyses, although highly differentiated by being much swollen, retain the ability to revert immediately to (dikaryotic) vegetative growth on explantation (Figs 3–6).

Whilst basidia appear to be specified for meiosis and sporulation, these processes are slowed in explants. Some lamellae isolated at prophase I had formed only sterigmata after 1 d, producing spores after 2 d. Raju & Lu (1970) claimed that basidia required 11·5 h to complete meiosis and 8–10 h for sporulation. In the Meathop dikaryon, karyogamy to spore maturation occupies 10 h (Moore, Liu & Kuhad, 1987). Therefore, the isolation procedure employed here slows the rate of maturation quite considerably. A similar effect was noted in McLaughlin's (1982) experiments on the effects of applied electrical fields on sterigma formation by isolated gills floating on a liquid nutrient solution. In *C. congregatus*, Bastouill-Descollonges & Manachère (1984) also demonstrated that isolated lamellae carried out meiosis and sporulation at a retarded rate. All the evidence suggests that prophase I is the critical stage at which basidia become determined for the division programme. In our experiments similar results were obtained whether water-agar or nutrient-agar was used as the explantation medium. Thus, meiosis in basidia, once initiated, is endogeneously regulated and proceeds autonomously. Only gross interference with events such as DNA or protein synthesis arrests nuclear division and sporulation (Lu, 1982). This autonomous, endotrophic phenomenon and the synchrony of nuclear division in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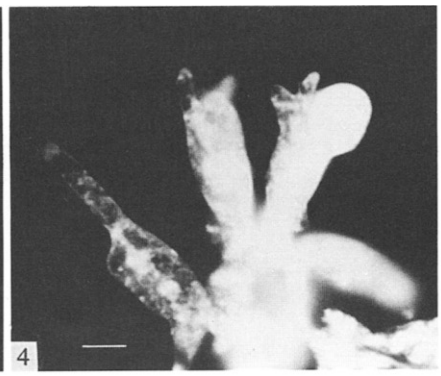
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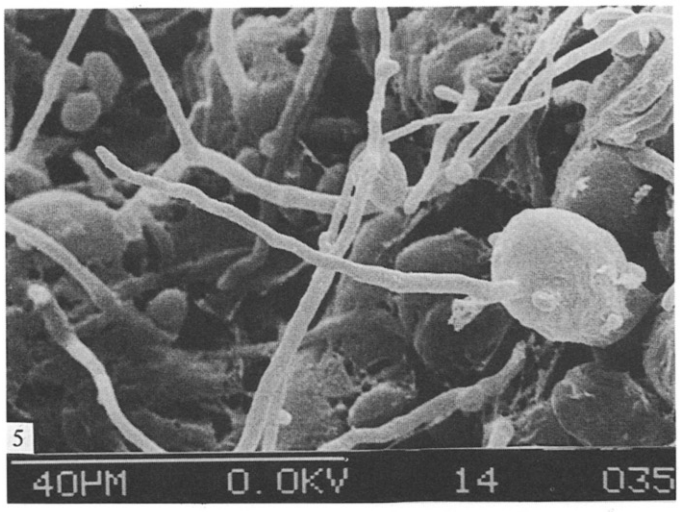

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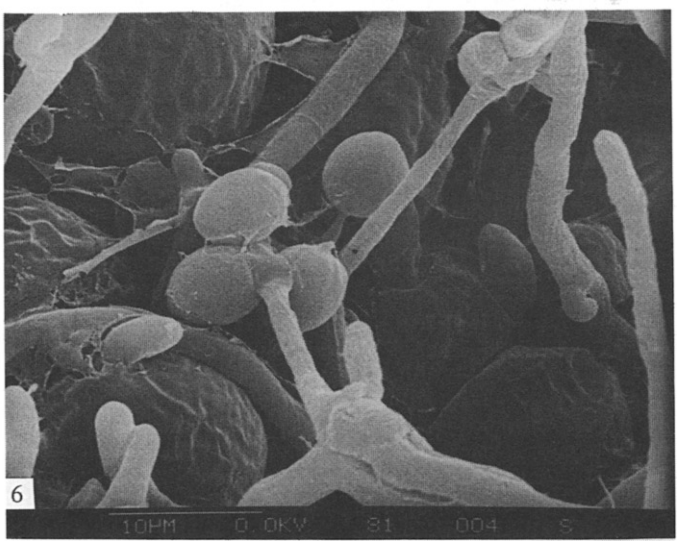

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Table 1. *Fruiting pattern of cultures inoculated with lamellae of* Coprinus cinereus

Physiological age at explantation	Total number	Fruiting pattern observed* (% of cultures)		
		Direct	Mixed	Indirect
Dikaryotic (pre-karyogamy)	19	63	5	32
Prophase I	38	24	39	37
Meiotic division	25	8	60	32
Sporulation	39	18	56	26

* Direct fruiting = new primordia formed only on the original inoculum; indirect = primordia formed only on the outgrowing mycelium; mixed = a combination of both.

C. cinereus make isolated lamellae an ideal material to study cell differentiation.

Potential for renewed fruiting

All basal stipe portions of normally-grown fruit bodies (16 samples) and all parts of the pseudorhizas of dark-grown fruit bodies (10 samples) made direct fruiting within 4 d. This compares with cultures inoculated with vegetative dikaryon which, under the same conditions, formed fruit bodies in 10–14 d. The other portions of stipes of normally-grown fruit bodies (16 samples) produced (in 9–12 d) all types of fruiting pattern, these were unpredictable from their physiological age or physical size at the time of inoculation. The types of fruiting patterns observed on cultures inoculated with isolated lamellae are summarized in Table 1. This shows a clear dependence of the type of fruiting pattern on physiological age; tissues explanted prior to karyogamy showing a preponderance of direct fruiting, those explanted during or after meiosis showing a minimum of direct fruiting. Identical results were obtained for *C. congregatus* by Bastouill-Descollonges & Manachère (1984).

Unusually rapid formation of fruit-body primordia upon segments of fruit bodies used as inocula for cultures (direct fruiting) is not uncommon, but the high degree of developmental synchrony characteristic of the smaller *Coprinus* species permits assessment of its dependence on the physiological developmental state of the tissue. Such tissues are clearly rather more competent to initiate fruiting than the average vegetative dikaryon inoculum, but it is unlikely that this indicates a morphogenetic phenomenon akin to commitment to a developmental pathway. The only physiological aspect of normal fruit body development with which a correlation is evident is the disposition of accumulated glycogen. The developing fruit body of *C. cinereus* accumulates large quantities of glycogen, which appear first in the stipe base and later in the subhymenial regions of the gills (Moore *et al.*, 1979; Gooday, 1985). Further, the greatly reduced frequency of direct fruiting in cultures initiated with lamellae explanted during or after meiosis correlates with the rapid, immediately post-meiotic, utilization of glycogen (Moore, Liu & Kuhad, 1987). The implication, therefore, is that direct fruiting occurs on a tissue with a high glycogen content. Whether attainment of fruiting competence is a simple response to the ready availability of carbohydrate or results from some consequential circumstance (such as cAMP content of glycogen-rich cells) is not known.

Apart from scattered cystidia and cystesia (Horner & Moore, 1987), the mature hymenium of *Coprinus* comprises the two highly differentiated cell types, basidia and paraphyses. The more

Figs 3–6. Behaviour of hymenia of *Coprinus cinereus* lamellae explanted to agar medium at or after prophase I. At this stage of development the majority of cells in the hymenium are young paraphyses formed as branches from immediately beneath the probasidia. Most of these regenerate hyphal outgrowths on explantation.

Figs 3, 4 (scale bars = 10 μm). LM of squash preparations; Fig. 3 shows a paraphysis (forming a hyphal outgrowth) connecting with a basidium which is forming only sterigmata; Fig. 4 shows a clutch of paraphyses (parental basidia are out of the plane of focus), all of which show evidence of hyphal outgrowth (contrast with Fig. 1).

Fig. 5. SEM of an enlarged cell, probably a developing cystidium, forming numerous outgrowths.

Fig. 6. SEM of sterile hymenial elements reverting to vegetative hyphal growth with an adjacent basidium continuing to form spores.

numerous paraphyses provide the structural foundation for the gill, and their inflation produces fruit body expansion. Yet it seems likely that only the basidial (meiocyte) morphogenetic pathway is one to which a true developmental commitment is made. An important implication of the ready ability of paraphyses and trama to revert to hyphal growth on explantation is that this growth mode must be actively and continually inhibited in vivo to ensure the orderly formation and development of the fruit body.

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