Cell population dynamics in *Coprinus cinereus*: co-ordination of cell inflation throughout the maturing basidiome

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Cell sizes were measured in microscope sections of fruit bodies of *Coprinus cinereus* whose temporal development was defined by the stage they had reached in meiosis and sporulation. Comparison of length/width ratios suggested that in pre-meiotic stages of primordium development, stipe elongation was more due to cell proliferation than inflation. Major inflation was a post-meiotic event and could account for all the basidiome expansion involved in maturation. Inflation of cells in the pileus was closely correlated with inflation of cells in the stipe. These studies provide new data about the spatiotemporal co-ordination of basidiome tissue development and indicate that some form of long range signalling system exists.

Most of the changes in shape during basidiome development in basidiomycetes depend on two types of cell inflation: a slow process typical of young primordial stages and a more rapid one characteristic of maturation. (Reijnders & Moore, 1985). Local cell inflation during basidiome development has attracted much attention [e.g. Gooday (1974, 1982, 1985), Moore, Elhiti & Butler (1979) and Rosin, Horner & Moore (1985) with *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray; Bret (1977) on *C. congregatus* (Bull.) Fr.; Wong & Gruen (1977), Gruen (1982), Williams, Beckett & Read (1985) and Gruen (1991) on *Flammulina velutipes* (Curt.: Fr.) Karsten; Bonner, Kane & Levey (1956) and Craig, Gull & Wood (1977) on *Agaricus bisporus* (Lange) Imbach]. However, what is lacking is an holistic account of inflation over the whole basidiome and an assessment of the correlation between cell behaviour in widely separated locations. This is what we have attempted in the research with *Coprinus cinereus* reported here. The hyphal compartment of most fungi does not conform to the traditional concept of the cell, but we will use the word 'cell' to describe all of the hyphal compartments considered here. Some of the arguments in favour of this opinion are discussed by Moore (1988).

Reijnders (1963) showed that the different zones of fruit bodies enlarge proportionally, so that different tissues mature without being impeded, compressed or distorted by the growth of other parts. Such co-ordination of differentiation of cells in relation to their location is one of the most important general principles of morphogenesis. It is thought to be based upon the migration through the developing tissues of pattern-forming morphogenetic factor(s) or signals, the nature of which is obscure. In basidiome primordia this mechanism would operate over distances of many millimetres. However, apparent 'co-ordination' could also result if ontogenetic events are arranged in a consequential series such that one (secondary) event is only instigated by the initiation or completion of an earlier (primary) event. For any conclusion to be reached on this issue it is necessary that the events can be accurately timed.

Many earlier studies have relied on morphological events to define the temporal stages in development of whole fruit bodies (Madelin, 1956; Takemaru & Kamada, 1972; Matthews & Niederpruem, 1973; Morimoto & Oda, 1973; Moore *et al.*, 1979). Pukkila *et al.* (1984) defined these stages in terms of progress through meiosis and spore formation in the hymenium, dividing the 48 h encompassing these processes into four 12 h periods (Table 1), but this lacks the resolution necessary for assessment of co-ordination. In our study we have expanded on this approach by examining a sufficiently

Table 1. Developmental stages of basidiome development in *Coprinus cinereus* designated by Pukkila *et al.* (1984)

Stage	Time	Predominant feature	Other designations in the literature* a	b	c	d
1	0–12 h	Basidial differentiation	Stages 1–2	Stages 1–3	Day 8	—
2	13–24 h	Karyogamy and meiotic prophase	Stage 2	Stage 2	—	-2 Days
3	25–36 h	Meiotic divisions and spore formation	Stages 3–4	Stage 4	Day 9	-1 Day
4	37–48 h	Stipe elongation and spore release	Stage 5	—	—	Day 0

*References: (a) Moore, Elhiti & Butler, 1979; (b) Morimoto & Oda, 1973, 1974; (c) Kamada, Kurita & Takemaru, 1978; (d) McLaughlin, 1982.

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large sample of fruit bodies to establish the exact timing of major meiotic and sporulation events, so providing a baseline to which any other process can be referenced simply by microscopic examination of a sliver of pileus tissue. This baseline is objective in the sense that it depends upon processes which are endogenously controlled. It is reliable because these processes are central to basidiome function and it is versatile since by examining slivers at known time intervals the effects of any change in cultivation conditions or culture genotype become apparent.

MATERIALS AND METHODS

Cultivation

In all experiments a *Coprinus cinereus* dikaryon called 'Meathop' was used, originally isolated from a dung heap in Lower Meathop Hill farm, Cumbria. The dikaryon was grown in darkness at 37 °C on Complete Medium (Moore & Pukkila, 1985) in 9 cm Petri dishes and maintained by serial transfer. Fruit bodies were produced on sterilised horse dung. The dung was inoculated with pieces of dikaryon from Petri dish cultures, incubated in darkness for 3 d at 37° to allow the mycelium to establish and then transferred to a 27° incubator with a 16 h light/8 h dark photoperiod which induces the production of fruit bodies. Illumination was provided by white fluorescent lights with an average illuminance of 800 lx.

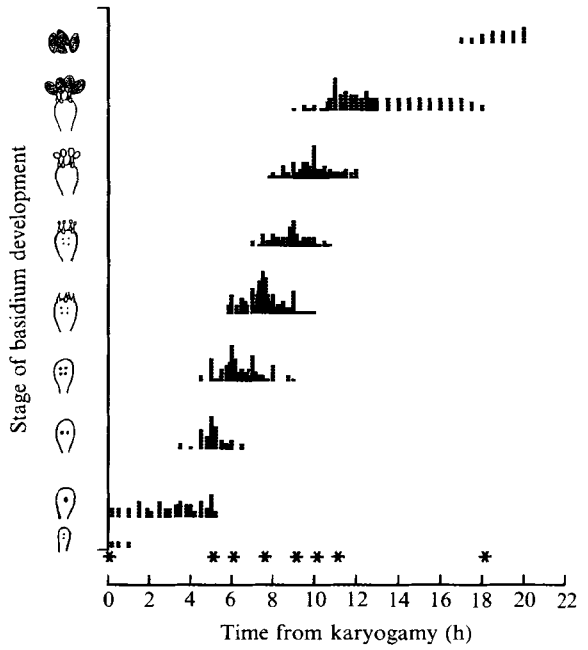

Fig. 1. The rate of progress through meiosis and sporulation in *C. cinereus*. The abscissa shows elapsed time. Samples were removed from basidiomes at regular time intervals and sample populations of hymenial cells observed in squashes were quantitatively categorised into the developmental stages represented in the cartoons on the ordinate. Time 0 h is arbitrarily chosen to be when nuclear fusion (karyogamy) starts. Other timings emerged from the observations: meiosis I occurred at 5 h, meiosis II at 6 h, sterigmata appeared at 7·5 h, basidiospores started to appear at 9 h, began pigmentation at 11 h and mature basidiospores were being discharged from 18 h. The asterisks on the abscissa emphasize these stages. Developmental stages of the basidia are illustrated as cartoons in the ordinate.

Determination of the time course of meiosis and sporulation

Meiotic division stages were examined using the silver staining technique described by Pukkila & Lu (1985). Sporulation stages, such as appearance of sterigmata, spore formation and pigmentation, were visible without staining. Samples of a few gills were removed from the pileus at 0·5 to 1 h intervals during meiosis and every 2 h before and after meiosis. The tissue was fixed in 4% (w/v) formaldehyde for 10 min at room temperature. A small piece (*ca* 1 mm^2) of a single layer of basidia was removed using a pair of fine tweezers and transferred to a drop of 45% (v/v) acetic acid on a coverslip. The gill segment was torn into several pieces, squashed and silver stained. Using phase contrast optics, over 100 basidia were counted in each sample and the stage of development of each recorded. The proportions of the different developmental stages in each specimen were calculated and plotted against sampling time; 35 fruit bodies were investigated (Fig. 1).

Video 'photography'

VHS video recordings of developing fruit bodies were made using a domestic-quality video recorder. Measurements of stipe height (= basidiome overall height) were made directly on the video screen. The progress of pileus expansion was determined by measuring the angle to the vertical subtended by the pileus at the apex of the stipe.

Stipe structure in longitudinal sections

Techniques used for preparing microscope sections for image analysis were as described by Hammad, Watling & Moore (1993). Specimens from fruit bodies at a range of different developmental stages were fixed and embedded in glycol-methacrylate resin. Longitudinal sections of the stipe were cut 5 μm thick and stained with Mayer's haemalum or using the periodic acid-Schiff reagent. Microscope images were analysed using an image analysis program (Skye Instruments) to measure cell sectional areas. A total of 50 measurements were made for each position within each basidiome. In addition to the areas, the maximum and minimum diameters of the profile of each stipe cell measured were also noted. From this information the length/width ratio of the cells was calculated. Only inflated hyphae were measured in these studies. The narrow hyphae (diameters < 5 μm) which are so evident in transverse sections of the stipe (Hammad *et al.*, 1993) only rarely remained in the plane of longitudinal sections sufficiently for successive cross-walls to be identified.

Hymenium structure

Basidium length and width were measured in squashes using the Measure Mouse graphics system (Analytical Measuring Systems, Pampisford, Cambridge, England). All other measurements of cell sizes were made on microscope images of 5 μm glycolmethacrylate sections using the Skye Instruments image analysis system (Hammad *et al.*, 1993).

RESULTS AND DISCUSSION

Time course of basidial development

Using the culture conditions described here, basidiome primordia were formed 5 d after inoculation of the horse dung and developed into mature fruit bodies within 2–3 days, during which the basidia underwent a sequence of morphologically and physiologically distinctive stages. The dikaryotic basidioles underwent nuclear fusion (karyogamy) and then entered meiosis I, followed immediately by meiosis II. After completion of meiosis, four sterigmata emerged followed by formation of four basidiospore initials, nucleus migration, maturation and discharge of the spores. All these events were completed within 18 h. However, basidial development was not totally synchronous. The overlap between different stages varied through development. In a 'meiosis I specimen' about 60% of the basidia had two nuclei; at meiosis II about 70% had four nuclei; and when sterigmata first appeared about 90% of the basidia were at the same stage. Defining karyogamy as time zero, basidia took 5 h to reach meiosis I (Fig. 1) and meiosis II was completed after a further hour. From meiosis II to the emergence of sterigmata required 1·5 h, spores emerging 1·5 h after that. Spore formation continued for 1 h and then nuclear migration started. Spore pigmentation commenced 1 h after spore formation and spores matured and were discharged 7 h later.

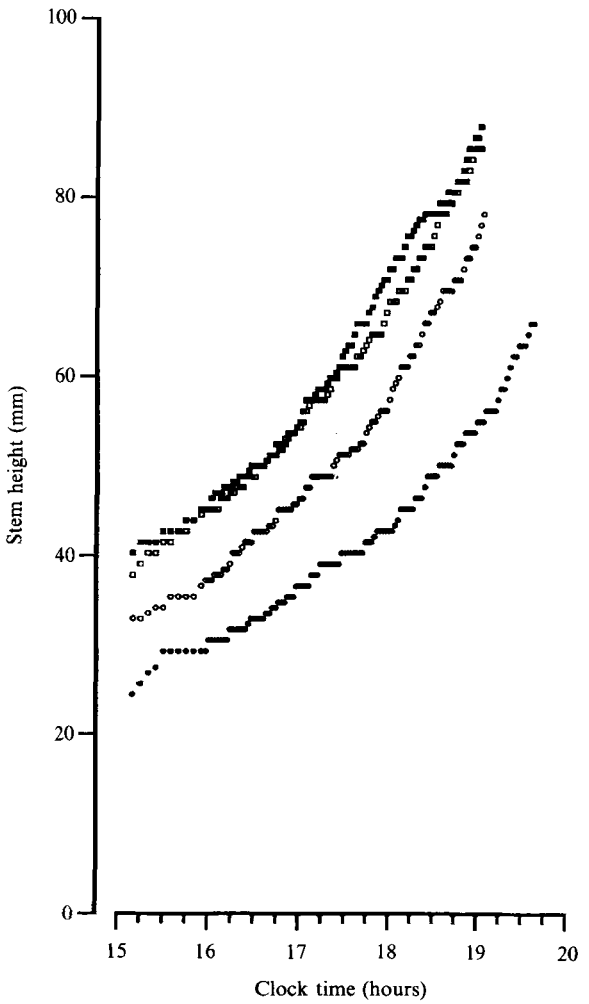

Fig. 2. Stipe extension time courses for four basidiomes of *Coprinus cinereus* in the same culture.

Stipe elongation and pileus expansion

The phase of most rapid stipe elongation occupied the 5 h (approx.) prior to spore discharge and pileus autolysis (Fig. 2) and started 8 h after karyogamy (Fig. 3). Pileus expansion started as spores matured, about 14 h after karyogamy (Fig. 3). The rate of stipe elongation more than doubled with 10° increases in temperature (Fig. 4). Stipe elongation was significantly greater ($P = 0{\cdot}005 - 0{\cdot}05$) when the pileus was left attached than when it was removed (Table 2) and this was true whether the pilei were removed before (10 h after karyogamy) or after (12 h after karyogamy) onset of the phase of rapid elongation. Leaving a half pileus in place was sufficient to ensure normal elongation (Table 2). These stipes curved away from the side with the half-pileus on it during the 8 h immediately following treatment (height range 80–85 mm), but in the next 4 h, as they grew to 104–137 mm in height, growth was strictly vertical.

Stipe structure in longitudinal sections

There was little increase in cell sectional area between a 3 mm basidiome and an 8 mm tall basidiome (Table 3) both of which were at pre-meiotic developmental stages. Presumably any stipe elongation occurring at these stages is due primarily to cell proliferation rather than cell elongation. By contrast, there was a large increase in cell sectional area between the stipes of the 8 mm basidiome (pre-meiotic) and that of a 25 mm basidiome undergoing meiosis. Initially the cells in the basal and middle regions of the stipe inflated. The apical cells did not expand to the same extent, and even in a fully elongated basidiome apical cells were considerably shorter than other cells. Cells in the extreme basal and apical regions were always shorter than those in other regions of elongated stipes. For example, cells near the pileus/stipe junction at the extreme apex of an 83 mm basidiome (fully elongated) had a typical longitudinal sectional area of 3000 μm^2 compared with an average for the whole of the apical section examined (about 10 mm long) of 6258 μm^2. The most elongated cells were found in the upper mid-region of the stipe.

The length/width ratios of about 2 in pre-meiotic stipes (3 mm and 8 mm basidiomes) increased greatly after meiosis, particularly in the upper middle regions, to 10, 20 and approximately 35 in 48 mm, 55 mm and 83 mm tall basidiomes.

Co-ordination of cell expansion

Sectional areas of veil cells, basidia, spores, paraphyses, cystidia and cystesia in longitudinal sections of the same specimens are given in Table 4. Veil cells were very heterogeneous in size, but showed a gradual increase in sectional area in basidiomes 3 mm, 6 mm and 8 mm tall, then tended to be sloughed off. Those closest to the pileus surface were smallest and least liable to slough off. It is not clear whether the representativeness of the population sampled was affected by the preparative procedures.

Table 2. Effect of the pileus on stipe elongation in *Coprinus cinereus*

Treatment	No.	Stipe size: Initial length (mm)	Final length (mm)	Elongation (mm)
(a) pilei removed 10 h after karyogamy (just before onset of rapid elongation phase)	6	35·8 ± 4·9	109·2 ± 12·2	73·3 ± 8·8
(a) control	5	36·0 ± 5·5	130·4 ± 2·9	94·4 ± 6·3
(b) pilei removed 12 h after karyogamy (just after onset of rapid elongation phase)	8	35·5 ± 5·1	123·0 ± 22·9	87·5 ± 19·3
(b) control	5	37·6 ± 5·4	155·6 ± 16·0	118·0 ± 13·7
(c) half of pileus removed 12 h after karyogamy	4	47·0 ± 9·4	121·5 ± 14·2	74·5 ± 9·2
(c) control	4	48·0 ± 10·8	124·0 ± 12·8	76·3 ± 3·9

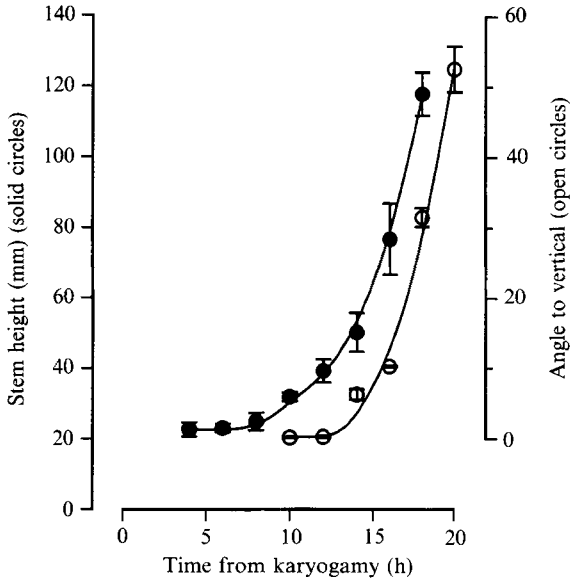

Fig. 3. Correlation of stipe elongation and pileus expansion with meiosis.

Table 3. Sectional area and length/width ratios of cells in longitudinal sections of stipes of *Coprinus cinereus*

Basidiome height (mm)	Zone	Mean stipe cell area (μm²)	Length/Width ratio
3	Middle	148	1·9
8	Middle	211	2·0
25	Apex	292	1·8
	Middle	3857	11·5
	Base	2705	6·2
48	Apex	3184	9·0
	Upper middle	6813	12·7
	Lower middle	5735	10·3
	Base	3449	10·6
55	Apex	9243	11·5
	Upper middle	9496	18·1
	Lower middle	10522	19·9
	Base	6533	13·8
83	Upper apex	6258	12·6
	Lower apex	11960	22·0
	Middle region 4	12894	26·0
	Middle region 3	11672	30·2
	Middle region 2	10448	35·1
	Middle region 1	5538	20·4
	Upper base	4785	14·1
	Lower base	2681	8·8

Each entry represents the mean of 50 measurements.

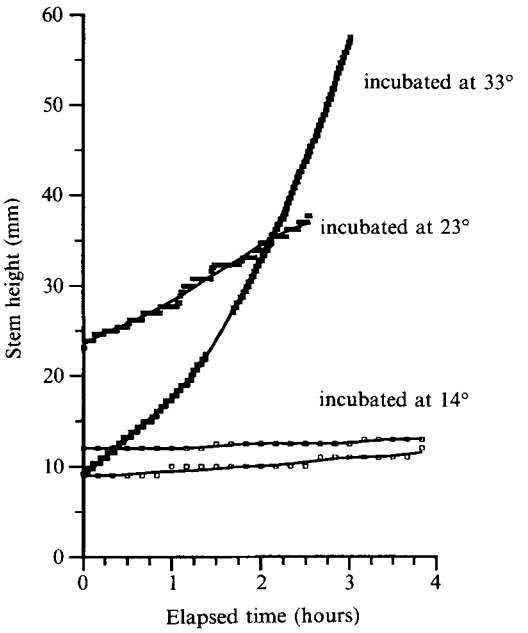

Fig. 4. Effect of incubation temperature on stipe elongation.

Table 4. Sectional areas (μm²) of hymenial cells measured in longitudinal sections together with pooled data for cells of the stipes of the same specimens

Cell type	Basidiome height (mm): 3	6	8	25	27	48	55	83
Stipe apex	—	—	—	292 ± 25	—	3184 ± 174	9243 ± 548	9109 ± 390
Stipe middle	148 ± 7	—	211 ± 13	3857 ± 194	—	6274 ± 214	10009 ± 434	10138 ± 308
Stipe base	—	—	—	2705 ± 181	—	3449 ± 200	6533 ± 474	3733 ± 182
Veil	238 ± 14	242 ± 11	276 ± 16	—	—	—	—	—
Basidia	—	—	—	—	151 ± 3	181 ± 3	177 ± 3	138 ± 3
Paraphyses	—	—	—	—	193 ± 7	244 ± 9	253 ± 7	215 ± 7
Spores	—	—	—	—	39 ± 1	48 ± 1	43 ± 1	45 ± 1
Cystidia	—	—	—	—	1194 ± 28	1423 ± 44	2495 ± 93	1391 ± 42
Cystesia	—	—	—	—	305 ± 7	303 ± 11	387 ± 17	260 ± 8

Each entry for the hymenial cell types is a mean of 50 measurements. The data for the cells of the stipes derive from Table 2 and are means of 50 to 400 measurements.

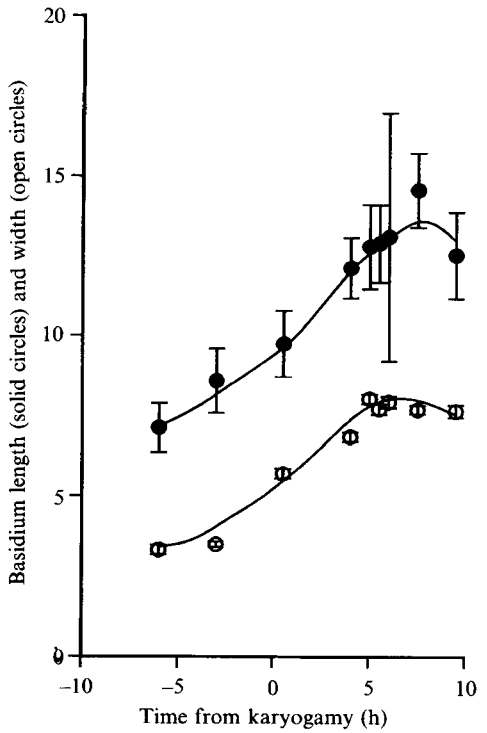

Fig. 5. Basidial length and width measurements in squashes of hymenial tissues during basidiome development. Soon after nuclear fusion and when basidia were undergoing meiosis, they rapidly increased in size but reduced in size when basidiospores appeared. Each point is the mean ± S.E.M. of between 12 and 37 replicates.

It was difficult to measure hymenial cells in sections of basidiomes just after meiosis (25 mm tall) because the cells were extremely closely packed, and so basidium sizes were measured in squashes (Fig. 5). Although these data were obtained in such a different manner they are comparable with the other measurements (Table 4), particularly in showing the basidium sectional area increased in basidiomes up to 48 mm tall but then decreased as the spores matured, possibly due to vacuolation and transfer of material to the spores. The other cell types increased in sectional area up to the 83 mm basidiome; in this specimen the pileus had already begun to autolyse and consequential cell collapse may be reflected in the generally lesser mean sectional area of a randomly chosen sample of cells.

The basidiome is a device whose function is to produce and liberate spores as economically as possible. Its development involves the integrated growth and differentiation of its components. The rate of stipe elongation increases from in the region of 10 μm min^{-1} to 110 μm min^{-1} (averaged between 1600 and 1900 h of Fig. 2). During the most rapid phase this means that an average stipe may elongate 80 mm in less than 12 h (Moore & Ewaze, 1976). The kinetics of this elongation have attracted a great deal of attention since Buller (1924) examined *C. sterquilinus* (Fr.) Fr. *C. radiatus* (Bull.: Fr.) S. F. Gray has been studied by Borriss (1934, using the name *C. lagopus* (Fr.) Fr.), Hafner & Thielke (1970), and Eilers (1974); *C. congregatus* by Bret (1977); and *C. cinereus* by Gooday (1974), Cox & Niederpruem (1975, using the name *C. lagopus*) and Kamada & Takemaru (1977, using the name *C. macrorhizus* (Pers.: Fr.) Rea). In all these species the upper half, and generally the upper mid-region, was the most active zone of elongation and this was reflected in our data showing the most dramatic increases in cell sizes in these regions (Table 3). However, there are species differences with respect to the degree of stipe autonomy and relative roles of cell proliferation and cell elongation in the overall process. In *C. congregatus* stipe elongation was dependent on both the parental mycelium and the pileus during the whole period of basidiome expansion (Bret, 1977). In *C. radiatus* elongation occurred after separation from the parent mycelium, but was dependent on the pileus only until the final phase of rapid elongation was reached: once the stipe had reached 25% of its final size decapitation did not impair elongation (Borriss, 1934; Eilers, 1974). In contrast, Gooday (1974) found that stipe elongation in *C. cinereus* had no requirement for connection either with the pileus or the parental mycelium. Although the fruit bodies which he used were at fairly late stages in development, Cox & Niederpruem (1975) confirmed and extended the observation by showing that primordia about 5 mm in height (equivalent to 5–10% of final size) were able to elongate after excision and decapitation. We have demonstrated another aspect of this pileus/stipe interplay. Stipe elongation is not dependent, but is markedly enhanced by the presence of the pileus (Table 2).

Eilers (1974) found that stipe elongation in *C. radiatus* was accompanied by a 6- to 8-fold increase in cell length and a doubling of the cell number. Although the DNA content of the stipe of *C. cinereus* has been found to increase abruptly just before the most rapid phase of elongation (Kamada, Miyazaki & Takemaru, 1976) and stipe cells become multinucleate (Lu, 1974; Moore *et al.*, 1979; Stephenson & Gooday, 1984), stipe elongation has been attributed solely to cell elongation (Gooday, 1975; Kamada & Takemaru, 1977) and our data supports this view. Only between the 3 and 8 mm tall primordia was the increase in overall size greater than the increase in mean cell length. The increase in size of stipe throughout the rest of the size range was easily accounted for by the increase in cell size (Table 3).

The most remarkable feature of our data was that rapid stipe elongation is correlated with the ending of meiosis (Fig. 3). Indeed, expansion of the different cell types in the pileus as well as inflation of cells of the stipe began immediately post-meiotically (Table 4). Expansion of the stipe is necessary to elevate the pileus into the air for more effective spore dispersal; expansion of the different cell types in the gill tissue is also necessary for effective spore dispersal and co-ordination with stipe expansion is clearly advantageous.

Other than coincidence, two general interpretations of these data impact on understanding basidiome development. Firstly, the different tissue types may be co-ordinated only by being independently synchronized at some early stage of development to the same external cue, their shared time scale then being maintained throughout differentiation. This is presumably the explanation for the co-ordination which is seen amongst groups of fruit bodies in the same culture (Fig. 2). Secondly, co-ordination may be achieved by some sort of signalling system that 'reports' the end of meiosis to spatially distant parts of the basidiome. The information we have about hormonal effects in agarics is sparse and unsatisfactory

(Moore, 1991). Further, the route such a signal might take is not clear, but since primary gills are attached to the stipe, with their tramal regions in full hyphal contact with its tissues (Reijnders, 1963, 1979; Moore, 1987), the connection between tissues undergoing meiosis and the upper (most reactive) regions of the stipe may be fairly direct.

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REFERENCES

Bonner, J. T., Kane, K. K. & Levey, R. H. (1956). Studies on the mechanics of growth in the common mushroom, *Agaricus campestris*. *Mycologia* **48**, 13–19.

Borriss, H. (1934). Beitrage zur Wachstums und Entwicklungsphysiologie der Fruchtkörper von *Coprinus lagopus*. *Planta* **22**, 28–69.

Bret, J. P. (1977). Respective role of cap and mycelium on stipe elongation of *Coprinus congregatus*. *Transactions of the British Mycological Society* **68**, 262–269.

Buller, A. H. R. (1924). *Researches on Fungi* **vol. 3**. Longman Green & Co.: London.

Cox, R. J. & Niederpruem, D. J. (1975). Differentiation in *Coprinus lagopus*. III. Expansion of excised fruit bodies. *Archives of Microbiology* **105**, 257–260.

Craig, G. D., Gull, K. & Wood, D. A. (1977). Stipe elongation in *Agaricus bisporus*. *Journal of General Microbiology* **102**, 337–347.

Eilers, F. I. (1974). Growth regulation in *Coprinus radiatus*. *Archives of Microbiology* **96** 353–364.

Gooday, G. W. (1974). Control of development of excised fruit bodies and stipes of *Coprinus cinereus*. *Transactions of the British Mycological Society* **62**, 391–399.

Gooday, G. W. (1975). The control of differentiation in fruit bodies of *Coprinus cinereus*. *Reports of the Tottori Mycological Institute, Japan* **12**, 151–160.

Gooday, G. W. (1982). Metabolic control of fruit body morphogenesis in *Coprinus cinereus*. In *Basidium and Basidiocarp: Evolution, Cytology, Function and Development* (ed. K. Wells & E. K. Wells), pp. 157–173. Springer-Verlag: New York.

Gooday, G. W. (1985). Elongation of the stipe of *Coprinus cinereus*. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 311–331. Cambridge University Press: Cambridge, U.K.

Gruen, H. (1982). Control of stipe elongation by the pileus and mycelium in fruitbodies of *Flammulina velutipes* and other Agaricales. In *Basidium and Basidiocarp: Evolution, Cytology, Function and Development* (ed. K. Wells & E. K. Wells), pp. 125–155. Springer-Verlag: New York.

Gruen, H. (1991). Effects of grafting on stipe elongation and pileus expansion in the mushroom *Flammulina velutipes*. *Mycologia* **83**, 480–491.

Hafner, L. & Thielke, C. (1970). Kernzahl und Zellgrösse im Fruchtkorperstiel von *Coprinus radiatus* (Solt.) Fr. *Berichte Deutsche Botanische Gesellschaft* **83**, 27–31.

Hammad, F., Watling, R. & Moore, D. (1993). Cell population dynamics in *Coprinus cinereus*: narrow and inflated hyphae in the basidiome stipe. *Mycological Research* **97**, 275–282.

Kamada, T. & Takemaru, T. (1977). Stipe elongation during basidiocarp maturation in *Coprinus macrorhizus*: mechanical properties of stipe cell wall. *Plant & Cell Physiology* **18**, 831–840.

Kamada, T., Kurita, R. & Takemaru, T. (1978). Effects of light on basidiocarp maturation in *Coprinus macrorhizus*. *Plant & Cell Physiology* **19**, 263–275.

Kamada, T., Miyazaki, S. & Takemaru, T. (1976). Quantitative changes of DNA, RNA and protein during basidiocarp maturation in *Coprinus macrorhizus*. *Transactions of the Mycological Society of Japan* **17**, 451–460.

Lu, B. C. (1974). Meiosis in *Coprinus*. V. The role of light on basidiocarp initiation, mitosis and hymenium differentiation in *Coprinus lagopus*. *Canadian Journal of Botany* **52**, 299–306.

McLaughlin, D. J. (1982). Ultrastructure and cytochemistry of basidial and basidiospore development. In *Basidium and Basidiocarp: Evolution, Cytology, Function and Development* (ed. K. Wells & E. K. Wells), pp. 37–74. Springer-Verlag: New York.

Madelin, M. F. (1956). Studies on the nutrition of *Coprinus lagopus* Fr. Especially as affecting fruiting. *Annals of Botany* **20**, 307–330.

Matthews, T. R. & Niederpruem, D. J. (1973). Differentiation in *Coprinus lagopus*. II. Histology and ultrastructural aspects of developing primordia. *Archives für Mikrobiologie* **88**, 169–180.

Moore, D. (1987). The formation of agaric gills. *Transactions of the British Mycological Society* **89**, 105–108.

Moore, D. (1988). Recent developments in morphogenetic studies of higher fungi. *Mushroom Journal for the Tropics* **8**, 109–128.

Moore, D. (1991). Perception and response to gravity in higher fungi – a critical appraisal. *New Phytologist* **117**, 3–23.

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.

Morimoto, N. & Oda, Y. (1973). Effects of light on fruit-body formation in a basidiomycete, *Coprinus macrorhizus*. *Plant & Cell Physiology* **14**, 217–225.

Morimoto, N. & Oda, Y. (1974). Photo-induced karyogamy in a basidiomycete, *Coprinus macrorhizus*. *Plant & Cell Physiology* **15**, 183–186.

Pukkila, P. J. & Lu, B. C. (1985). Silver staining of meiotic chromosomes in the fungus, *Coprinus cinereus*. *Chromosoma* **91**, 108–112.

Pukkila, P. J., Yashar, B. M. & Binninger, D. M. (1984). Analysis of meiotic development in *Coprinus cinereus*. In *Controlling Events in Meiosis* (ed. C. W. Evans & H. G. Dickinson), Symposia of the Society for Experimental Biology **38**, pp. 177–194. Cambridge University Press: Cambridge, U.K.

Reijnders, A. F. M. (1963). *Les problèmes du développement des carpophores des Agaricales et de quelques groupes voisins*. Dr W. Junk: The Hague.

Reijnders, A. F. M. (1979). Developmental anatomy of *Coprinus*. *Persoonia* **10**, 383–424.

Reijnders, A. F. M. & Moore, D. (1985). Developmental biology of agarics – an overview. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 333–351. Cambridge University Press: Cambridge, U.K.

Rosin, I. V., Horner, J. & Moore, D. (1985). Differentiation and pattern formation in the fruit body cap of *Coprinus cinereus*. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 333–351. Cambridge University Press: Cambridge, U.K.

Stephenson, N. A. & Gooday, G. W. (1984). Nuclear numbers in the stipe cells of *Coprinus cinereus*. *Transactions of the British Mycological Society* **82**, 531–534.

Takemaru, E. M. & Kamada, T. (1972). Basidiocarp development in *Coprinus macrorhizus*. I. Induction of developmental variations. *Botanical Magazine (Tokyo)* **85**, 51–57.

Williams, M. A. J., Beckett, A. & Read, N. D. (1985). Ultrastructural aspects of fruit body differentiation in *Flammulina velutipes*. In *Developmental Biology of Higher Fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 429–450. Cambridge University Press: Cambridge, U.K.

Wong, W. M. & Gruen, H. E. (1977). Changes in cell size and nuclear number during elongation of *Flammulina velutipes* fruitbodies. *Mycologia* **69**, 899–913.

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