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Artifacts in video measurements cause growth curves to advance in steps

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Summary

Measurement of video recordings of mushroom growth seemed to reveal a stepwise mode of stipe elongation in fruit bodies of *Coprinus cinereus* and *Agaricus bisporus* in which brief episodes of explosive growth were followed by quiescent periods which could last many minutes. Similar stepped growth curves were obtained for hyphal extension growth in agar cultures. The stepped nature of these growth curves proved to be an artifact, probably caused by the fact that the video image is composed of a defined number of lines, phosphor dots and/or pixels. An easy test to verify the reality of any discontinuities in such observations is to make video records at a range of magnifications. If the steps (or other discontinuities) are real their absolute magnitude will be unchanged by the altered magnification (though the precision of the measurement will increase). If the steps are artifacts, their apparent absolute magnitude will decline at higher magnifications because the line interval in the image corresponds to a smaller absolute distance on the subject.

Key words: Mushroom; Fruit body; Growth

Introduction

Use of video observation of microbial growth and behaviour is increasing because of the ready availability of cheap video cameras and recorders, digitiser cards for converting video images into computer graphics and software for simple image analysis of those graphics. Even equipment designed (and priced) for the domestic market can be used successfully to record, and measure, morphogenetic events in revealing detail. We have used video records to study the kinetics of gravitropism [1,2] and morphogenesis [3,4] in fruit bodies of the basidiomycete *Coprinus cinereus* (Schaeff.: Fr.) S.F. Gray *sensu* Konr. Early in these studies, though, measurement of video images of growing fruit bodies of both *C. cinereus* and *Agaricus bisporus*

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(Lange) Imbach seemed to show a stepwise mode of stipe elongation where episodes of explosive growth lasting a few seconds were followed by quiescent periods of many minutes before the next episode of explosive growth. In *C. cinereus*, the 'growth rate' of a single step could be as high as 0.8 mm min^{-1} – much greater than the average extension rate of the stipe. Similar observations were made of hyphal extension in Petri dish cultures. In this note we show that these observations are artifactual and describe a simple test for this.

Materials and Methods

Strains and cultivation

In all experiments with *C. cinereus* a dikaryon called 'Meathop' was used; this was originally isolated from a dung heap in Lower Meathop Hill farm in Cumbria. Cultivation techniques have been described previously [5]. Fruit bodies of *Agaricus bisporus* were produced using a commercial mushroom growing kit (B&Q Superstores) treated according to the supplier's instructions.

Video recording

Video images of the development of *C. cinereus* and *A. bisporus* fruit bodies were recorded at either normal speed or in time-lapse mode at 1 frame s^{-1}. The video images were recorded using a Panasonic time-lapse video recorder (model AG-6010) in conjunction with either a black & white Hitachi CCTV camera or a JVC colour camera. During recording, cultures were enclosed within a humidity chamber. Illumination was provided by a white fluorescent light.

Measurements were made directly on a television screen, being converted into absolute values by measuring against a scale incorporated into the image. Initial measurements were made every 5 min during periods of slow growth and every 2 min during rapid growth. Subsequently more frequent measurements (at 30 s and 1 s intervals) of stipe length were made of the same videos to expand the data set over particularly interesting phases. The data was presented as graphs with growth plotted against time.

Hyphal growth of the Meathop dikaryon across a film of water agar was also recorded. The culture was covered by a glass coverslip and video recorded using the × 40 objective of a light microscope.

Results and Discussion

In the first videos the fruiting culture was recorded so that the whole growth of the fruit body was recorded in a single take. In these cases the resultant screen image used for measurement was 0.82 × natural size. Later cultures were recorded in a range of 1.3–4.7 × natural size.

Fig. 1A shows measurements of the heights of three fruit bodies in a single culture of *C. cinereus* at 30 s intervals: measurement was begun just before the phase of most rapid stipe elongation. The growth curves take the form of a series of steps with short bursts of rapid growth (over a few sec) being followed by quiescent periods (which can last many minutes). As the rate of stipe elongation accelerated, the quiescent periods

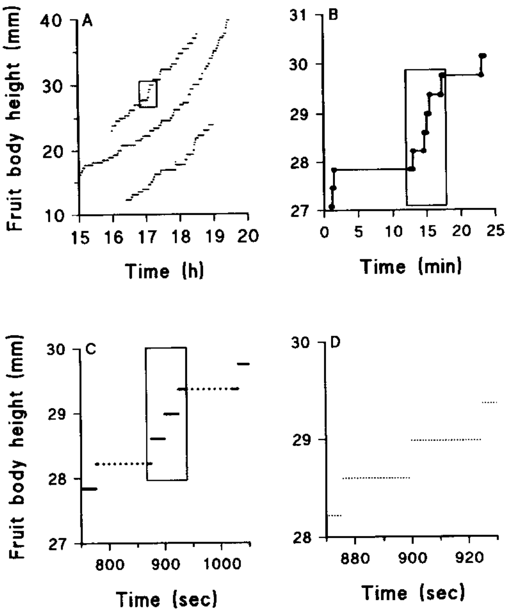

Fig. 1. Growth curves of fruit bodies of *Coprinus cinereus*. (A) Stipe elongation time courses for three fruit bodies of *C. cinereus* in the same culture. Measurements were taken every 30 s. The boxed region is expanded in 1B. (B) Expanded plot of the boxed data shown in the previous plot, relatively long periods of quiescence are punctuated by rapid growth episodes. (C) Expanded plot of the rapid growth episode represented by the boxed data shown in Fig. 1B; measurements were made at 1 s intervals. (D) Part of the same data set (boxed in 1C) with the scale expanded to show individual measurements.

between the episodes of rapid extension became much shorter; but throughout, and in all fruit bodies, stipe extension growth plots were stepped. Where (as in Fig. 1A) a number of fruit bodies were observed in the same culture, the active growth steps and quiescent episodes occurred at different times; this lack of synchrony implying that the phenomenon was intrinsic to the fruit bodies and did not result from cyclical environmental variation.

Further analysis was carried out at 1 s intervals for selected steps (Fig. 1B–D). This

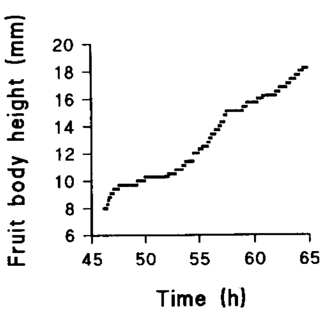

Fig. 2. Growth curve of a fruit body of *Agaricus bisporus*. Measurements were made at 5 min intervals.

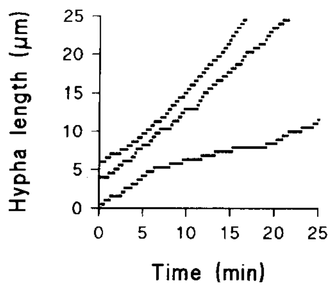

Fig. 3. Pattern of extension of three hyphae of a *C. cinereus* dikaryon growing across a water agar surface. Incubation was at 20–21°C.

suggested that the main risers in a series of steps were themselves made up of lesser discrete stepwise increments. Even at this higher resolution, the quiescent periods occupied the great majority of the time. Similar observations of fruit bodies of *Agaricus bisporus* (Fig. 2) also indicated a stepped mode of growth.

The observations presented so far were all made at temperatures in the range of 20–27°C, which is the normal range for laboratory culture of *C. cinereus* (Fig. 3). In cultures recorded at other temperatures [3], the length of the quiescent periods was extended at lower temperature (14°C) and very much reduced at higher temperature (33°C). A stepped growth curve was also obtained from observations of individual hyphae growing across a film of agar (Fig. 4), though the quiescent periods were not as pronounced as they were in stipe growth curves.

As we shall show later, these stepped growth curves proved to be artifactual. However, Kretschmer [6,7] has used a different method of analysis to reveal a stepwise mode of growth for hyphae emerging from germinating spores of *Streptomyces granaticolor*. In these studies photographs were taken every 3 or 5 min and the length of developing hyphae was determined from the photographic film using a stereomicroscope. Thus, a stepwise growth curve in basidiomycetes would not be unique, nor would it be difficult to explain, either in terms of discontinuities in nutrient supply, dependence of stipe extension on cycles of nuclear division, or the purely mechanical effect of friction between adjacent hyphae causing 'inertia' which

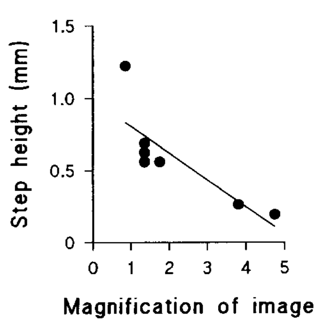

Fig. 4. Relationship between step height and the magnification of the recorded images.

was overcome periodically as accumulated growth potential was expressed as a sudden extension of the stipe – like slippage along a tectonic fault.

The first doubts about the validity of our data were raised by Dr Karl McKnight (St. Lawrence University, New York) who analysed the numerical data and found that they fell into distinct groups with a deficit of intermediate values. He interpreted this to mean that the steps were artifacts caused by the smoothly growing subject being displayed (and measured) as images comprised of discrete pixel rows. We found that the easiest test of the stepped mode of extension was to determine step height at a number of different magnifications (Fig. 4). This showed that as the magnification of the image was increased, so the step height decreased. If the steps were 'real' then the height of the step should not have been affected by the magnification, i.e. real steps would be characteristic of the material examined, not the mode of examination. Thus it appears that the stepped growth pattern was, indeed, an artifact of the video-image based data collection technique. Other users of video measurements should beware of this limitation.

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