Published as: Moore, D., Reeve, M. A., Meškauskas, A., Sánchez, C. & Chiu, S W. (2000). Mushroom mechanics and mathematical models of mushroom morphogenesis. In *Science and Cultivation of Edible Fungi, Mushroom Science XV*, **vol. 1** (L. J. L. D. Van Griensven, ed.), pp. 103-107. Balkema: Rotterdam.

Conference paper: Chairman's Introduction
Mushroom Mechanics and Mathematical Models of Mushroom Morphogenesis

David Moore
School of Biological Sciences, The University of Manchester, UK

Audrius Meškauskas
Department of Chemistry and Bioengineering, Gediminas Technical University, Vilnius, Lithuania

Mark A. Reeve
School of Biological Sciences, The University of Manchester, UK

Carmen Sánchez
Research Centre for Biological Sciences, Universidad Autónoma de Tlaxcala, México

Siu Wai Chiu
Department of Biology, The Chinese University of Hong Kong, Shatin, Hong Kong S.A.R., China.

There is no reason why the "rules" which govern morphogenesis should not be established. From the rules and a few dimensions, times and rate values a mathematical expression to describe the entire morphogenetic process could emerge. Nice idea! But where do you start? Start simple. Making a stem bend in response to a tropic stimulus is a suitably simple ***experimental*** approach. The experimenter can choose when to apply the stimulus, it is easily replicated and reaction and response times can be measured readily. Also, the response itself can be ***measured*** so the quantitative demands of mathematical modelling can be satisfied. We have used the gravitropic reactions of mushroom fruit bodies to study control of morphogenesis because being the right way up is crucial to a mushroom. Changing orientation is a ***non-invasive*** stimulus. We've coupled video observation and image analysis to get detailed descriptions of the kinetics, and made and used clinostats to vary exposure to gravity, all combined with a variety of microscopic observation techniques to make quantitative observations. Bending rate is determined by the balance between signals from gravity (a function of the ***angle*** of the stem) and curvature compensation detectors (a function of the local amount of ***curvature***). This model is ***predictive*** and successfully describes the gravitropic reaction of stems treated with metabolic inhibitors, confirming the credibility of the model and indicating possible links between the functions of the equations and actual physiological processes. To take the model into three spatial dimensions we are developing the use of laser confocal microscopy to establish an accurate data set describing the geometrical arrangement of the hyphal components of fungal tissues. This cannot be done using conventional microscopy because z-axis (vertical) dimensions and internal branch angles cannot be measured. The confocal images are readily converted to red/green anaglyphs (using Confocal Assistant) which provide an easily realised three-dimensional visual sensation. However, the intention is to produce three-dimensional visualisations (using

AVS/Express). These are fairly primitive at the moment (though they can be rotated for viewing from various angles), but they hold the promise of development to full 3-D visualisations which can be inspected 'from within' and used to extract geometrical measurements.

Introduction

The mushroom industry is totally dependent on the development of mushroom fruit bodies yet there is very little research done on the morphogenesis of mushrooms. Fungal cells behave as though they have considerable latitude in expression; decisions between developmental pathways seem to be able to cope with a degree of uncertainty (Moore, 1998a) and study of fungal morphogenesis involves more than morphology. Measuring and counting cells in different regions of fruit bodies at different stages of development reveals specific patterns of cell differentiation which mechanically generate the final form of the fruit body. The patterns revealed must be organised by signalling molecules, so these studies establish the very foundation for detailed analysis of the control of fungal morphogenesis. By revealing that positional information is regulated in time and space, investigations of this sort raise crucial questions about the nature of the signalling molecule(s) involved, their transduction pathways and the responses they elicit.

The evolutionary separation between the major Kingdoms must have occurred at a stage when the most highly evolved things were single cells. Consequently, each Kingdom has independently evolved ways to organise populations of cells to make the multicellular organisms we now know as mushrooms, mice or marigolds. The three major Kingdoms we see today are very different from one another in ways that determine shape and form. A key feature during the embryology of even lower animals is the movement of cells and cell populations, so cell migration (and everything that controls it) plays a central role in animal development. Being encased in walls, plant cells have little scope for movement and their changes in shape and form are achieved by regulating the orientation and position of the wall which forms when a plant cell divides.

Fungi are also encased in walls; but their basic structural unit is a tubular cell, called a hypha. It has two peculiarities which result in fungal development being totally different from that in plants. These are that the hypha grows only at its tip and that new walls form only at right angles to the growth axis of the hypha. Studying ***how*** a mushroom ***makes*** a mushroom is an investigation every bit as deep and meaningful (and difficult!) as studying how a human embryo develops or how a tree is shaped and sculptured in the forest. Sadly, in the popular imagination mushrooms don’t have the same status as human animals or forest trees and, anyway, mycologists have never been very good at thinking in four dimensions.

We need an ***objective*** view of tissue structure, yet the best descriptions available are ***subjective interpretations***. We are only at the brink of this research, but it is going forward. Attempts to determine the nature of the signals used in fungi have been initiated (Novak Frazer, 1996) and attempts are under way to exploit further the numerical approach and computer-aided image analysis. The aim of the latter is to establish mathematical models describing mushroom morphogenesis. Too much reliance is currently placed on drawings of hyphal distributions in fruit body tissues. Such drawings are often a delight to behold from an artistic point of view, but suffer from subjective interpretation and lack of a quantitative dimension and have no experimental basis.

If we are to understand ***how*** fungal morphogenesis works we need first to find out ***what*** it consists of. We need to catalogue the patterns of hyphal growth and hyphal branching that produce particular shapes and morphologies. We can't do that from 2-dimensional renditions - even when they are combined with educated guesses about how they might be related. Guesses are still only guesses no matter how erudite the guesser!

Mathematical modelling

Truly ***experimental*** approaches to study of fungal morphogenesis are difficult because the experimental intervention alters the progress of morphogenesis. We have used the gravitropic reactions of mushroom fruit bodies to study control of morphogenesis in fungi because altered orientation to the gravity vector provides a non-invasive initiator of a defined morphogenetic change which is easily replicated and open to precise quantification. Video image analysis defines the kinetics and micrographic image analysis the cellular morphometrics of the tropic bending response. These quantitative observations provide the basis for development of mathematical models aimed at producing computer simulations of the tropic response.

The attraction (and potential power) of this approach is that the mathematics does not rely on specific events. Rather, the value(s) and unit(s) of the parameters in the equations specify events. The model itself is assembled first from parameters chosen on the basis of some sort of theoretical understanding (provided by observation *in vivo).* The simulations that the model provides are compared statistically with the reality of a specific situation and to make the simulations objectively realistic it may be necessary to introduce parameters and/or parameter-interactions that are unanticipated on the basis of prior expectation. Nevertheless, once a satisfactory model has emerged from reiteration of the cycle "experiment - compare model - adjust model if necessary - experiment" it should be applicable to a range, if not all morphogenetic events (subject to suitable change in parameter values), and should be able to predict morphogenetic reactions which are outside the envelope of events used for its construction.

There is no *a priori* reason why the "rules" which govern morphogenesis should not be established. When the rules are known and the dimensional and temporal constraints are discovered then a mathematical expression could be written to describe the morphogenetic process. Computer models could be derived from that to simulate the whole process. Nice idea! But morphogenesis is rather complicated, so where do you start?

Well, start simple. Making a stem bend in response to a tropic stimulus looks like a nice simple ***experimental*** approach. ***YOU*** can choose when to apply the stimulus so it is easily replicated and reaction and response times can be measured easily. Also, the response itself can be ***measured*** so the quantitative demands of mathematical modelling can be satisfied.

We have used the gravitropic reactions of mushroom fruit bodies as an experimental system to study control of morphogenesis in fungi because being the right way up is crucial to a mushroom. More importantly, changing the orientation to the gravity vector provides a ***non-invasive*** initiator of the defined morphogenetic (bending) reaction (no chemicals, no change in light or temperature = minimum disturbance). We've coupled video observation and image analysis to get detailed descriptions of the kinetics. We've made and used clinostats to vary exposure to gravity, and we've combined a variety of microscopic observation techniques to make quantitative observations. All these provide the basis for mathematical models which simulate the tropic response. Now we've got the simulations we can get rid of the mushrooms

and all that smelly compost ... ..!

The first step in the process is to invest about 15 years of effort into observations and experiments which can then be summarized into a flow chart. Creating a flow chart can be a real contribution to understanding because you have to minimize and simplify everything. The approach concentrates attention on critical features and, in a non-mathematical way, produces a formalized description which is a good starting point for mathematical analysis Moore, D. Greening, J. P., Hatton, J. P. & Novak Frazer, L. (1994). Gravitational biology of mushrooms: a flow-chart approach to characterising processes and mechanisms. *Microgravity Quarterly* **4**, 21-24.

Next, an explanatory "mechanism" or "scheme" needs to be constructed in a way which lends itself to mathematical expression whilst still keeping a firm footing in cell physiology. Fortunately, people have been worrying about gravitropism (mostly in plants, of course) for most of the twentieth century and a fair chunk of the nineteenth so there is general agreement on "a scheme". The basic assumptions of this are that change in the angle of the apex (of stem, root, coleoptile or other organ) occurred as a result of four consecutive stages:

- the physical change which occurs when the subject is disoriented (this is called susception)
- conversion of the physical change into a physiological change (this is called perception)
- transmission of the physiological signal to the competent tissue (called transduction)
- the growth response in which differential regulation of growth generates the change in apex angle (called response).

We used this scheme to estimate and calculate numerical values for the various parameters. Then developed a combined equation that could generate apex angle kinetics which imitated the reaction of mushroom stems quite well (Stočkus & Moore, 1996).

This imitational model dealt with change in apex angle only, no attention was given to the shape of the stem as it reacted. Observations of real stems show a complex distribution of bending rates in mushroom stems during their tropic reactions. There are regions, which, after reaching a particular angle, start to straighten. Almost 90% of the initial curvature is reversed by this process (we call it 'curvature compensation') so it must be incorporated into any model. Overall, a more realistic model of the mushroom stem gravitropic reaction would describe the bending process in space as well as in time. This is where 'imitation' ends and 'simulation' begins.

The model we have now describes the spatial organization of the gravitropic reaction in *Coprinus cinereus* stems. This is called the local curvature distribution model and it is able to simulate accurately the ***shapes*** assumed by real stems. The first version was a descriptive mathematical model which successfully simulated the morphology of experimentally manipulated tissue, and so approached the desired aim of a mathematical description of fungal morphogenesis (Meškauskas, Moore & Novak Frazer, 1998).

From this we have developed the **revised local curvature distribution model** by including curvature compensation by supposing that the actual bending rate is determined by the balance between the gravitropic and curvature compensation signals. It is argued that the

gravitropic signal is a function of the local ***angle*** in the signal perception site. The curvature compensation signal can be assumed to be a function of the local ***curvature*** in the signal perception site. Hence, the actual bending rate in any subsection can depend on the ***difference*** between the gravitropic and curvature compensation signals in this subsection. In the displaced stem, the gravitropic signal makes the subsection bend. In a straight stem displaced to the horizontal the gravitropic signal is maximal and curvature compensation signal is zero. As the stem bends the gravitropic signal weakens (as the angle of displacement of the perception system lessens) but the bending enhances the curvature compensation signal. In the final LOCAL CURVATURE DISTRIBUTION MODEL, therefore, straightening is determined by local curvature, independently of the spatial orientation of that part of the stem (Meškauskas, Novak Frazer & Moore, 1999).

This model is ***predictive***, once fitted into the gravitropic reaction of a specimen for a certain angle it can simulate the bending process from any other angle. Furthermore, the simple wave equation is sufficient to describe transmission of the hypothetical gravitropic signal so the final form of this new model is simpler, making the biological interpretation of its parameters much easier.

The model successfully describes the gravitropic reaction of stems treated with metabolic inhibitors, confirming the credibility of the model and indicating possible links between the functions of the equations and actual physiological processes..The model describes gravitropic stem bending in the standard assay with great accuracy and has the virtue of operating well outside the experimental data set used in its derivation. The same model also produces accurate simulations of the gravitropic bending of wheat coleoptiles (Meškauskas, Jurkoniene & Moore, 1999). Thus, depending on the values given to its parameters, our model is able to simulate the spatial organisation (= morphogenesis) of plant and fungal axial organs. The model promises to be a valuable predictive tool for future research.

Three-dimensional reconstruction

Further refinement needs the mathematics to be developed into three spatial dimensions and to take into account the behaviour of hyphal communities. We know virtually nothing about these factors.

We need to ‘get inside’ a 3-dimensional chunk of tissue to ***measure*** growth directions, branching frequencies, branch directions and branch growth kinetics. And not just one chunk of tissue, but hundreds, thousands even, so that we have a library of such descriptions large enough to reveal underlying developmental strategies and show similarities and differences.

Conventional sections for the light microscope (20, 30, 40 μm thick) are not good enough to study hyphal distributions. They are so thick that hyphae disappear within them and even with serial sections you find a totally different population of hyphal profiles exposed at each successive cut surface. Good for tissue morphologies and distribution, maybe, but not for individual hyphae.

Embedding in resins like glycol methacrylate and even Spurr’s resin enables very thin serial sections to be cut. But we are left with three problems:

- specimen preparation (fixation, dehydration, embedding) raises the usual question of how much of what is left is ***real***?
- section orientation (on the slide or grid) becomes a major issue; if you remember that

you (or your computer) will be trying to marry up the position of the same hyphal wall over a (hopefully large) number of successive sections you will appreciate that those successive sections need to be oriented in *x* and *y* directions to an accuracy ***less than the thickness of the hyphal wall***.

- the wall might be 100 to 200 nm thick; if you are skilled enough to produce sections which are only half a micrometre in thickness, the wall you (or your computer) are trying to follow can extend ***within the plane of one section*** so that hyphal profiles in serial sections lose their spatial relationships.

We have found greatest promise with the confocal microscope. Fresh specimens can be examined, and the observation technique produces digitised optical sections without mechanical disturbance.

It's not free of problems. Specimens can move during observation so an embedding agent to restrict this is necessary - glycerol or low-gelling temperature agarose work well. The microscope depends on laser-excited fluorescence so we need a fluorescence "stain" for chitin. FITC-conjugated wheat-germ agglutinin and calcofluor are effective, but penetration of the "stain" into the block of tissue, uniformity of fluorescence and fading are issues which remain.

Nevertheless data sets have been obtained and three-dimensional reconstruction was achieved utilising a standard desktop personal computer, with AVS/Express and Confocal Assistant software. The confocal images are readily converted to red/green anaglyphs that give a good ***visual*** impressions of depth and parallax and consequently go one step further than conventional 2-D images. But these are not suitable for quantitative measurements.

Fortunately, the digitised images which contribute to the anaglyphs can be extracted and prototype ***3D visualisations*** have been produced using AVS, an industry-standard work-station/mainframe software suite that generates 3-dimensional models within the computer. These are fairly primitive at the moment (though they can be rotated for viewing from various angles), but they hold the promise of development to full 3-D visualisations which can be inspected 'from within' and used to extract geometrical measurements.

A number of factors still need to be addressed to make this a routinely useful procedure. Such visualisations will be the source of the accurate 3-D observational data needed to enable tissue structure to be mathematically described. Parameters extracted from the visualisations will enable computer modelling to be extended into 3 spatial dimensions. The aim is a computer model able to simulate the hyphal architecture of mushroom tissues that can be explored to catalogue the structural effects of changes in its parameters. Study of these simulations will reveal morphogenetically important parameters and define experiments to improve knowledge of *in vivo* morphogenetic control very considerably. The virtual mushroom is attainable!

References

Meškauskas, A., Jurkoniene, S. & Moore, D. 1999. Spatial organisation of the gravitropic response in plants: applicability of the revised local curvature distribution model to *Triticum aestivum* coleoptiles. New Phytol. 143: 401-407.

Meškauskas, A., Moore, D. & Novak Frazer, L. 1998. Mathematical modelling of morphogenesis in fungi: spatial organization of the gravitropic response in the mushroom stem of *Coprinus cinereus*. New Phytol. 140: 111-123.

Meškauskas, A., Novak Frazer, L. & Moore, D. 1999. Mathematical modelling of morphogenesis in fungi: a key role for curvature compensation (‘autotropism’) in the local curvature distribution model. New Phytol. 143:387-399.
Moore, D. Greening, J. P., Hatton, J. P. & Novak Frazer, L. 1994. Gravitational biology of mushrooms: a flow-chart approach to characterising processes and mechanisms. Micrograv. Quart. 4: 21-24.
Stočkus, A. & Moore, D. 1996. Comparison of plant and fungal gravitropic responses using imitational modelling. Plant, Cell & Environ. 19: 787-800.