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Tolerance of Imprecision in Fungal Morphogenesis

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Summary
A variety of experiments and observations contribute to the conclusion that differentiated cells in fungal fruit bodies, except the meiocyte, have an extremely tenuous grasp on their state of differentiation. So tenuous that when removed from their normal tissue environment they revert immediately to the vegetative hyphal growth mode. The possibility has been discussed that fungal differentiation pathways exhibit what would be described as 'fuzzy logic' in cybernetic programming terms. Instead of viewing fungal cell differentiation as involving individual major 'decisions' which switch progress between alternative developmental pathways which lead inevitably to specific combinations of features, this idea suggests that the end point in fungal differentiation depends on the ***balance*** of a network of minor 'decisions'. Observation shows that developmental decisions between pathways of differentiation are able to cope with a degree of uncertainty, allowing fungal cells to assume a differentiation state even when all conditions of that state have not been met. So, rather than rigidly following a prescribed sequence of steps, fungal differentiation pathways must be based on application of rules that allow considerable latitude in expression - fuzzy constraints - which in the ultimate can lead to polymorphic fruit bodies.

Among the three major Kingdoms of eukaryotes (Mycota, 'true' fungi; Plantae, bryophytes and tracheophytes; and Animalia) the fungi could well be the oldest. Yet through most of the history of science fungi have been confused with plants. This particular imprecision has had an adverse effect on the development of knowledge and understanding of fungi and its echoes are still with us. Margulis (1992) put it this way: "...This classification scheme...requires changes in social organization of biologists, many of whom as botanists and zoologists, still behave as if there were only two important kingdoms (plants and animals)...". Ironically, the most recent molecular phylogenies are indicating that far from being some peculiar kind of plant, "... animals and fungi are sister groups while plants constitute an independent evolutionary lineage..." (Baldauf & Palmer, 1993). Furthermore, the fungal kingdom has been separate from animals and plants for almost one-third of the time that life has existed on planet Earth. Although there is still uncertainty in the exact sequence of divergence of the major kingdoms, probably because of the effect of variable rates of evolution between the different groups (and, indeed, between the different molecules analysed), it is clear that:

- there is evidence for the activities of living organisms in terrestrial rocks which are 3.5×10^9 years old
- eukaryotes and eubacteria last shared a common ancestor about 2×10^9 years ago
- eukaryotic kingdoms diverged about 1×10^9 years ago (Doolittle *et al*., 1996).

The relevance of this to the argument I want to develop is that fungi have been evolving independently of animals and plants for a thousand million years. Since the three main eukaryotic Kingdoms probably separated from each other at some unicellular grade of organization, aspects of cell biology contributing to multicellular grades of organisation must have evolved separately in the three Kingdoms. Equally, if the eukaryotic grade of organisation arose first in a fungal organism,

from which first plants, and then animals diverged (Cavalier-Smith, 1981, 1987), then the fungi must have originated the common features of eukaryotes which plants and animals presumably then adapted to their own uses.

Remains of two mushrooms have been found in amber which is 90 to 94 million years old (Hibbett, Grimaldi & Donoghue, 1995). They bear a strong resemblance to the existing genera *Marasmius* and *Marasmiellus* yet when they were preserved the dinosaurs still ruled the Earth. Form and structure in fungi is maintained for enormous periods of evolutionary time. In Pirozynski's view (1976): '...evidence accumulates to support the long-held view that the history of fungi is not marked by change and extinctions but by conservatism and continuity...'

In other words, the fungal 'rule of thumb' is this: if it works,… don't fix it! In terms of evolution theory this must mean that selection pressure is minimised in some way, and my argument is simply that it is the in-built flexibility of fungal structures which minimises selection pressure against abnormality of form.

Modular organisms - plastic organisms

Fungi are 'modular organisms', like clonal corals and vegetatively-propagated plants, in which growth is repetitive and a single individual will have localized regions at very different stages of development (Harper, Rosen & White, 1986; Andrews, 1995). Though the definition of 'individual' is open to debate, it must be remembered also that mushrooms and similar fruit bodies are appendages of their mycelium, not individual organisms. Yet, in many ways such fruit bodies can be treated as individuals. The early development of a mushroom has many of the characteristics of an embryonic development (Moore, 1984, 1998a & b) and like the larval and embryonic forms of animals, study of the different patterns and modes of that development can reveal taxonomic and phylogenetic relationships (Watling, 1985, 1996; Watling & Moore, 1994). Surveys of mushrooms, again treating them effectively as individuals, are used to reveal changing distributions for conservational and ecological analyses (Watling, 1996). Fungi have attributes which are unique to them which must affect their developmental mechanisms. Most significant among these is probably that their constituent cells are generally considered to be totipotent (able to follow any pathway of differentiation), because a mycologist would *expect* to be able to produce a mycelial culture in a culture dish from a fragment removed from a mature, fully differentiated structure, like a mushroom fruit body, collected from the field. This cannot be done routinely with animals, and most plants demand far more stringent *in vitro* growth media and conditions than do most fungi. This behaviour reflects the nutrient-absorptive fungal lifestyle, but it also says something about the control of fungal development because even highly differentiated fungal cells will revert readily to vegetative growth if they are explanted to a (relatively simple) nutrient medium (see below).

This is not to say that fungal cell differentiation is any less sophisticated or complex than is found in animals and plants, but fungi can vary the timing, extent, and mode of differentiation in response to external signals, interconverting growth forms and reproductive phases of their life cycle in ways which make them supremely able to adapt to challenging conditions. This results in a morphological plasticity which surpasses that of other organisms and provides an intellectual challenge in terms of developmental biology, taxonomy and genetics (Watling & Moore, 1994).

Features of fruit body form

We all know what mushrooms look like, and any fruiting culture will reveal the truism that the basic shape, form and structure of an organism (whether fungal, plant or animal) does not arise all at once. Rather, the shape and form emerge as a result of a sequence of developmental adjustments. Each of these is usually irreversible within its morphogenetic sequence although often reversible by some gross disturbance; e.g. differentiated cells being put into tissue culture, nuclear and cell transplants, regeneration after injury, and so on. The whole process in which the final organization

and pattern of the organism is established is termed ‘morphogenesis’.

The most extensive research has been done with animals and from this a vocabulary has been established which describes morphogenetic events without prejudging the mechanisms which may be involved (Slack, 1991). As an embryonic organism develops towards maturity, each intermediate state represents a reduction in developmental potential compared with the previous. Each adjustment (or developmental ‘decision’) is made by cells already ***specified*** by earlier adjustments to belong to a particular developmental pathway. Consequently, developmental decisions are made from among progressively smaller numbers of alternatives until the particular structure to which the cell will contribute is finally ***determined***. Classic embryological transplantation experiments revealed these states. Where the explant differentiated to a state representative of its old position then it was said to have been determined prior to transplantation. If it developed in accord with its new position, then it had not been determined, but may have been specified. Within the developing tissues, cells embark on particular routes of differentiation in response to the playing out of their intrinsic genetic programme, in response to external physical signals (light, temperature, gravity, humidity), or in response to chemical signals from other regions of the developing structure. These chemicals may be termed organisers, inducers or morphogens, and seem to inhibit or stimulate entry to particular states of determination. Chemical signals may contribute to a ***morphogenetic field*** around a structure (cell or organ) which permits continued development of that structure but inhibits formation of another structure of the same type within the field. All of these phenomena contribute to the ***pattern formation*** which characterises the ‘body plan’ which is created by the particular distribution of differentiated tissues in the structure (organ or individual). Pattern formation depends on ***positional information***, which prompts or allows the cell to differentiate in a way appropriate to its position in the structure. Positional information is usually thought to be conveyed by concentration gradients of one or more morphogens emitted from one or more spatially distinct organisers. The responding cell senses the concentration of the morphogen and initiates a differentiation programme appropriate to the physical position at which that morphogen concentration is normally found.

The basic rules of pattern formation seem to be that regional specification (directed by organisers producing morphogens) occurs first, regulating gene activity in ways specifically geared to morphogenesis so that particular cells are first specified (a state which is still flexible) and then determined (a state which is inflexible) to their differentiated fates. Cell differentiation is a consequence of these events - cells which are either specified or determined are not necessarily morphologically different from their neighbours or predecessors (the morphological change may occur much later, or the differentiation may involve change only in molecular or metabolic attributes).

Control of fungal morphogenesis

Unfortunately, fungal development has been somewhat ignored as a topic in its own right. The great majority of the published research on fungal morphogenesis has been done with taxonomic intentions. It has great value for its descriptive and comparative content, but precise developmental accounts are extremely rare and ***experimental*** approaches rarer still. Nevertheless, homologues and analogues of all of the developmental mechanisms known in animals and plants can be found in fungi:

- mechanical effects
- temporal sequencing
- pattern formation and morphogenetic fields
- reaction with extracellular matrix
- a fungal kind of programmed cell death.

A major key to form and structure in fungi which has emerged from the experimental work which

has been done is that the total environment (the 'network' or 'context') within which new gene products must work includes chemical, electrical and structural or mechanical tensions as well as cell and organelle structures. Consequently, expression of the developmental programme is:

- place-dependent
- time-dependent
- epigenetic,

and variation in any of these will expose the plasticity of the developmental process.

***In vitro* tissue transplantations.** Removal of pieces of tissue of the young mushroom fruit body of *Coprinus cinereus* from its normal location to a simple medium results in most cell types of the gill reverting to vegetative hyphal growth, but basidia are an exception. If transplanted after the start of meiosis, then meiosis and sporulation continue, and, by analogy with classic embryological experimentation, this experiment implies that basidia are committed to their developmental pathway (Chiu & Moore, 1988a). The transplantation medium is required to provide physical support and humidity, its chemical constitution is irrelevant *unless* ammonium ions are present. Ammonium ions break basidial commitment to sporulation (Chiu & Moore, 1988b) and cause the sporulation process to be aborted, with immediate return to vegetative growth whatever stage had been reached at the time of the ammonium exposure. Significantly, the normal developmental programme of the *C. cinereus* fruit body includes specific derepression of ammonium scavenging enzymes in the basidia and hymenium which detoxify ammonium (by conversion to glutamate and glutamine) and therefore protect the sporulation process (Moore, Liu & Kuhad, 1987; Moore, Horner & Liu, 1987).

Time dependence. A specific temporal sequence of events is often obscured by the lack of synchrony in most developing fruiting structures which results in there being many stages of development present at any one time. The synchronised development of *Coprinus*, however, reveals such sequences, particularly during hymenium assembly. When first formed, the hymenium of *Coprinus cinereus* consists of a population of hyphal tips which are mostly destined to be basidia (the minority cell type represented are cystidia). Only after this basidial layer is formed do the paraphyses arise as sub-basidial branches which insert themselves into the hymenial palisade. Eventually, of course, the paraphyses expand to become the main structural elements of the hymenium, but they are not present when the hymenial layer is first defined.

Epigenetic influences. Morphogenesis is not simply a matter of playing out a predefined genetic programme. Gene expression is so much influenced by the events which surround it that it is doubtful whether true patterning genes exist. At the moment, it seems more likely that a specific gill pattern in an agaric, say, may result because the application of general rules within the context of its development 'invariably' results in that pattern (and *not* because the pattern is predefined as such genetically). Embryonic gills of both *Coprinus* and *Volvariella* are contorted, with little evidence of the regular 'parallel' (actually radial) pattern of the mature mushroom. The contorted embryonic gills are stretched (*Coprinus*) or inflated (*Volvariella*) into their mature pattern as a result of the mechanical changes which occur as the fruit body matures (Chiu & Moore, 1990a, b).

Other important epigenetic influences are the extracellular matrix and a fungal kind of programmed cell death. Both are crucially important in fungi but scandalously under-researched. Fungal tissues are sculptured and trimmed to shape by extensive sacrifice of hyphal cells. Although it is very similar to the apoptosis of animal cells there are many differences. This is not surprising, however, since many features of apoptosis are geared to the internalisation of the products of cell death to avoid autoimmune response in the animal. This is not a consideration in fungi and, indeed, fungal programmed cell death seems to be the consequence of some product which is externalised by lysis of the cell. In the most widely known example, the autolysis of the ink-cap Coprini, it is hydrolytic enzymes which are overproduced. But there are more recent examples in which the programme leading to cell death involves the sacrificed cells over-producing extracellular matrix and then

lysing to release it (Umar & Van Griensven, 1997, 1998). Removal of differentiating cells from their normal location leads to their regression to vegetative hyphae (see above). Consequently, hyphal cells require reinforcement of their differentiation 'instructions'. This reinforcement is part of the context within which they normally develop and the extracellular matrix is of prime importance in establishing and maintaining that context.

Developmental subroutines. Another common feature is that fungal morphogenesis is compartmentalized into a collection of distinct developmental processes, called 'subroutines' (Chiu, Moore & Chang, 1989). These are recognizable at all levels; for example, increase in cell length seems to be distinct from increase in cell girth, and at the other end of the spectrum, the structure of the hymenium seems to be a complete subroutine which can be invoked in different places. Developmental subroutines are distinct genetically and physiologically, and may run in parallel or in sequence. When played out in their correct arrangement, 'normal' morphology results, but if some subroutines are disabled (genetically or through physiological stress), the rest may still proceed. Such partial execution produces an abnormal morphology, ***but spores are still produced***.

Tolerance of imprecision

This flexibility in expression of developmental subroutines allows the fruit body to react to adverse conditions and still produce a crop of spores. Tolerance of imprecision is an important attribute of fungal morphogenesis. Evidently, fungal development uses fuzzy logic rather than crisp logic. Fuzzy logic is a respectable branch of cybernetic theory which has been described as computing with words (Zadeh, 1996). It is, fact, the sort of logic with which we are most familiar on a personal level. As scientists we might well demand precise numerical descriptions (= crisp functions) which can be graphed in *x*, *y* (and maybe *z*) coordinate systems, but on a personal level we are much more likely to describe something as being "in front of you" rather than "*x* metres forward and *y* metres left of your current position". The fuzzy statement "in front of you" depends for its exact meaning on the context within which it is made. If the conversation so far has been centred on the landscape, then "in front of you" may refer to the mountain 20 km away on the horizon. On the other hand, if the previous words had been an offer of a drink, then "in front of you" may refer to a glass within arm's reach on a table on a balcony overlooking that same mountain scenery. As in this example, so in the fungi, function and performance depends on context and on what has gone before. Basidia can arrest meiosis and become structural cells (Allen, Moore & Elliott, 1992), but they remain basidia; sterile cells, like cystidia, can enter the meiotic cycle (Chiu & Moore, 1993), but remain as functional cystidia; and similar sterile cells may assume basidial morphologies by producing sterigmata (Watling, 1971), but without jeopardising their function. As already pointed out, developmental subroutines permit circumstances in which hymenia may arise on the outer (rather than under-) surface of an agaric cap. This can often be ascribed to physiological stress. The outcome, as already stressed, is that the abnormally-placed hymenium can still produce a large crop of spores, some of which at least may be successfully dispersed. This will consequently lessen the selection pressure against occurrence of abnormality.

Ability to cope with uncertainty in development is what makes the fungi so successful. It is the outcome of two main features:

- the modular way in which these modular organisms have organised their developmental genetic architecture (i.e. into distinct subroutines);
- the relatively weak grasp which fungal cells have on their level of differentiation and their ensuing need for continuous reinforcement of their differentiation instructions.

Tolerance of imprecision has enabled many fungi to exist almost unchanged over enormous reaches of geological time. The agaric forms which were caught in amber 90 million years ago had already seen the dinosaurs come to rule the Earth. The same agarics survived whatever catastrophe saw an end to the terrible lizards, and then witnessed, in their own quiet way, the rise of mammals and

appearance of the thinking primates. There is no reason to doubt that they will still be here when all that is left of those primates are their buildings and artifacts. And most of them suitable substrates for mushrooms!

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