The fungal colony

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Introduction

For so long neglected in the development and promulgation of evolutionary theory, there are increasing signs that mycelial fungi can bring new insights into the origins of phenotypic diversity and change. They challenge some of our most fundamental assumptions about natural selection and its significance relative to other processes in determining the direction of evolutionary pathways. This is because of the way mycelia are physically organized as versatile systems of interconnected tubes that can span heterogeneous environments in which energy is often in very variable supply (Rayner 1994; Rayner, Griffith & Ainsworth, 1995*a*).

Current models of evolutionary change effectively treat the boundaries of living systems and their components as fixed (that is, determinate). Consequently, the dynamic processes underlying change are assumed to be driven by purely external forces acting on discrete objects – genes and individuals (see Dawkins, 1995). However, such discretist models of evolutionary and ecological dynamics are potentially very misleading because all known life forms, from single cells to communities, are dynamic systems which assimilate supplies of free energy from their surroundings and distribute this energy into growth, development, reproduction and movement. They achieve this by possessing boundaries through which they regulate energy exchange with their surroundings and other life forms (Rayner, 1997*a*). For life forms to thrive and survive as energy supplies wax and wane, these boundaries have to be capable of enhancing gains through the proliferation of assimilative free surface in energy-rich environments whilst minimizing losses by various means of containment in inhospitable environments. This requires the configuration and properties of boundaries to change according to circumstances.

Living system boundaries cannot therefore be absolutely fixed any more than they can be absolutely sealed: rather they are dynamic, reactive interfaces, forever in some degree of flux. Their properties both define and are defined by the properties of the interactive arenas – the 'dynamic contexts' – incorporated by living systems. (Please note that in this sense 'context' does not equate with external environment as such, but is rather the 'domain', 'territory' or 'field' occupied by and including a life form as it develops through space and time.) Many of these properties derive from materials or energy sources – for example, water, air, minerals and light – that are not encoded in DNA, and are overlooked in much evolutionary theory, but are nevertheless salient in moulding the dynamic interplay between genetic information and environment into diverse phenotypic forms and behaviours. The dynamic boundaries of living systems therefore define both the sites and mode of action of natural selection as an interactive, channelling process rather than a mechanical sifting of particle-like units. They enable living systems to respond to the environmental heterogeneity that these systems both interact with and help to generate.

This chapter aims to show how the interconnectedness and versatility of mycelial organization uphold a 'systemic' evolutionary approach which explains phenotypic diversity in terms of how the properties of dynamic contextual boundaries regulate processes of input, throughput and output of energy. Special emphasis will be given to the way mycelia epitomize the integrational processes of boundary-sealing, boundary-redistribution and boundary-fusion. These much neglected processes counteract the tendency for living systems to subdivide into discrete, competitive, energy-dissipating units.

Order, organization and chaos – the mycelial example

Recent decades have witnessed significant developments in the way that pattern-generating processes in dynamic physical systems can be understood and modelled mathematically. These developments are encompassed within an array of interrelated concepts, variously described as non-linearity, chaos, complexity, fractal geometry and self-organization. Using fungal mycelia for cross-reference, we will try here to clarify the biological relevance of these developments. We hope to show how the concepts extend beyond rather than negate discretist paradigms, and so open up new prospects for future understanding.

'Self-differentiation' – the route to 'incoherence'

Many of the developments just referred to arise from consideration of the effects of two kinds of feedback and their counteraction. Positive feedback – autocatalysis – arises from the ability of a system to amplify itself using energy input from its local environment. This ability generates an expansive drive which, if unconstrained, causes the system to increase exponentially. Negative feedback damps down expansive drive by directly or indirectly increasing resistance or dissipation as input increases.

The counteraction between positive and negative feedback causes systems to be non-linear (non-additive) and to become unstable if the rate of input exceeds a critical threshold or 'throughput capacity'. Below this threshold, the counteraction causes a smooth build-up to a dynamic equilibrium at which there is no net increase in the system's expansion: the system then remains, in effect, self-contained. Above this threshold, the system becomes 'forced' and hence prone to subdivide, by means of a series of bifurcations, into increasing numbers of subdomains or states. These subdomains may be manifest as increasingly complex, but nonetheless recurrent and predictable, oscillations, countercurrents or branches. Above a yet higher threshold, the subdivisions cease to occur recurrently. Instead, the system traverses what approaches an infinite variety of states in a manner which is apparently erratic and extremely sensitive to initial conditions, and therefore unpredictable in the long term. This is deterministic chaos.

An implicit feature of physical systems that exhibit non-linear dynamics is the presence of one or more dynamic boundaries. The very term 'feedback' implies a reactive interface that mediates this influence. Without a boundary, whether of attraction or constraint, that allows assimilation but prevents instantaneous dispersion, there can be no autocatalysis and no containment. The fact that the importance of dynamic boundaries is often overlooked, has led consciously or unconsciously to discretist interpretations of non-linear systems. These interpretations arise because attention is focused on the behaviour of individual components of the systems rather than the boundaries which shape and are shaped by these behaviours. An example occurs in what has been termed 'self-organization theory' and its attendant metaphor of 'order out of chaos' (Prigogine & Stengers, 1984). The most generally accepted idea of self-organization is that it involves the production of potentially complex patterns or structures through the operation of simple calculational procedures (algorithms) in a many-bodied system. Since the algorithms do not themselves directly encode the patterns or structures, generation of the latter is described as an 'emergent property' of the system (see Bonabeau *et al.*, 1997).

For self-organization to occur, it has been considered necessary for the systems to be thermodynamically open and far from equilibrium, so that they can be sustained by high rates of input and dissipation of energy. Consequently, the emergent structures or patterns they produce are described as 'dissipative', maximizing the conversion of free energy input to entropy (Prigogine & Stengers, 1984). Since emergence of dissipative structures occurs in what appears to be a previously patternless or structureless domain, it is assumed to originate from chaos or even randomness. Examples commonly used to illustrate this idea include 'random' mixtures of autocatalytic ('activator') and constraining ('inhibitor') chemicals, and 'random' arrays of social organisms (for example, slime mould amoebae, ants). These systems generate annular and spiral patterns if suitably prompted by local perturbations (e.g. Goodwin, 1994).

The assumption of a chaotic or random origin for self-organizing patterns may, however, be inappropriate. In fact, it is thought more apt to describe chaos as an extreme form of order which emerges as a consequence of high rates of input of free energy into an initially coherent (self-contained) system (Rayner, 1997*b*). Here, it is important to understand what is implied systemically by coherence, randomness, homogeneity and heterogeneity, and how these terms relate to concepts of order, organization, chaos and entropy. The systemic application of all these terms and concepts depends on the way that systems both define and are defined by their dynamic boundaries, and so differs in some important respects from conventional analytical usage. To begin with, it is vital to realize that randomness is the converse of homogeneity. This fact is often overlooked because for purposes of calculation, random assemblies are assumed analytically to be sets of independent (discrete) data points whose density can be treated on average as homogeneous – the same in different samples – provided that sufficient numbers are accounted for. When sample sizes are small, however, random distributions exhibit extreme heterogeneity. Furthermore, although the distribution of data between set intervals does not imply that these data are interdependent and so non-random from an analytical perspective, in which boundaries are absolute, the same cannot be said from a systemic viewpoint. What randomness, that is, total 'incoherence', implies systemically is the lack of a containing boundary, so the components of a system can be anywhere, anytime and incapable of concerted action. Such absolute disorder is equatable with entropy. By contrast, homogeneity implies being the same everywhere, at all scales, that is, absolute order. Whilst a fully random system is incapable of concerted action, a fully coherent system is incapable of change. Dynamic systems therefore operate between these extremes – that is, with increasing degrees of freedom from relative coherence to relative incoherence. The boundaries of these systems represent sites of relative order which, when in disordered surroundings, tend to lose coherence.

These considerations focus attention, at any particular scale of reference, on the boundary of a system as the expression of its relative order and dynamic state. The effect of introducing free energy into a dynamically bounded system is, directly or indirectly, to cause an expansion of the system's boundary. If the rate of input to the system is below the 'throughput capacity' defined by the resistances imposed by the system's boundary (see above), the boundary expands smoothly, retaining its symmetry and minimizing its surface area and consequent dissipation to its surroundings. A germinating fungal spore exhibiting initial spherical growth exemplifies this. However, if the rate of input exceeds the throughput capacity, the system begins to lose coherence by 'breaking its symmetry' and generating emergent structure. It first polarizes and then subdivides to produce more and more dissipative (and assimilative) free surface – as epitomized by the emergence and subsequent branching of a germ tube (see Fig. 1.1). All this emergent, increasingly complex structure, the most extreme form of which is chaotically distributed, represents proliferated boundary – and hence as presently defined, increased order. However, the origin of this order is not disorder, but a highly integrated, coherent initial state. We view this initial state as more highly organized.

Like the packaging that is used to enclose all kinds of commodities, the order invested in boundaries is energetically costly, for two reasons. Firstly, a high rate of energy input is required to cause systems to become unstable and break symmetry. Secondly, proliferating boundaries present an increased dissipative free surface which renders the system more susceptible to random environmental influences and counteracts the input of free energy, so that more erratic but less labile structures emerge.

Given that boundary-proliferation can only be sustained by continuing energy input, an important question is what happens to dissipative struc-

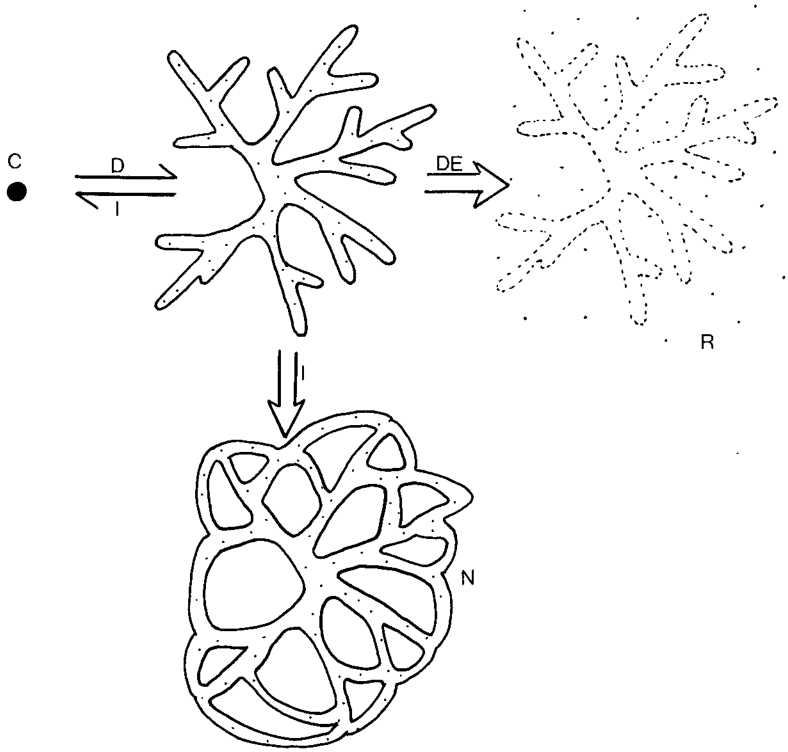

Fig. 1.1. The role of dynamic boundaries in the production of dissipative structure ('order') and coherent 'organization'. Assimilation of free energy into a coherent initial state (C) results in the proliferation and subdivision of boundary (dissipative structure) by 'self-differentiation' (D). Irreversible decay or degeneration (DE) of this structure in the absence of energy replenishment leads to random disorder (R). 'Self-integration' of this structure by boundary-fusion, boundary-sealing and boundary-redistribution minimizes its dissipative free surface, enabling it to reconfigure into coherent initial states or persistent networks (N). (From Rayner, 1997*b*.)

tures when external supplies of free energy are restricted? A related, fundamentally important, question is what is the origin of the initial coherent state from which dissipative structures emerge in energetically unrestricted environments? Essentially, if external energy supplies are withdrawn from a dissipatively structured system, the long-term survival of the system (or part of it) rests on a stark alternative (Fig. 1.1). The system may continue to dissipate, or it undergoes processes that minimize exposure of free surface. The first option leads to dissolution, an irreversible decay into an entropic state. The second option results in a reduction of order and an increase in organization. The sustainability and persistence of life forms in energetically variable environments depends on this second option, which involves three dissipation-minimizing processes: boundary-fusion, boundary-sealing and boundary-redistribution. We term this second option 'self-integration', as distinct from the emergence of dissipative structures, which we term 'self-differentiation'.

'Self-integration' – retaining and regaining coherence

Since dissipative free surface is energetically costly, any process that minimizes this surface is energy-saving and even energy-yielding. The three self-integrational processes depicted in Figs. 1.1 and 1.2 all have these effects. Operating separately or in concert, these processes enable living systems to conserve, explore for and recycle resources by means of fundamentally simple adjustments in their boundary properties that accord with local circumstances. They are well illustrated by fungal mycelia.

Boundary-fusion both lessens the amount of dissipative surface and releases energy that was previously contained in this surface through its dissolution. It is most obvious amongst mycelial fungi in the process of anastomosis. Anastomosis can occur both between individual hyphae, and between hyphal aggregates such as mycelial cords (Thompson & Rayner, 1983; Dowson, Rayner & Boddy, 1988*a*). It converts a dendritic branching system with resistances in series, to a more coherent network with resistances at least partially in parallel. It thereby makes the system more retentive and less prone to proliferate branches. At the same time it enables the system to amplify its organizational scale, through enhanced delivery to sites of emergence of distributive or reproductive structures, for example rhizomorphs and fruit bodies, on its boundary.

Boundary-sealing involves various ways of reducing permeability and hence increasing the 'insulation' of a system. Sealing a fixed boundary results in the production of survival structures, as in various kinds of constitutively dormant spores, sclerotia and pseudosclerotia by fungal mycelia. Sealing a deformable boundary results in the emergence of distributive structures that serve reproductive or explorative/migratory functions. Since the sites of input to these structures are distal to their sites of proliferation, their branching pattern will be distributary- or fountain-like, contrasting with the tributary-like branching pattern of

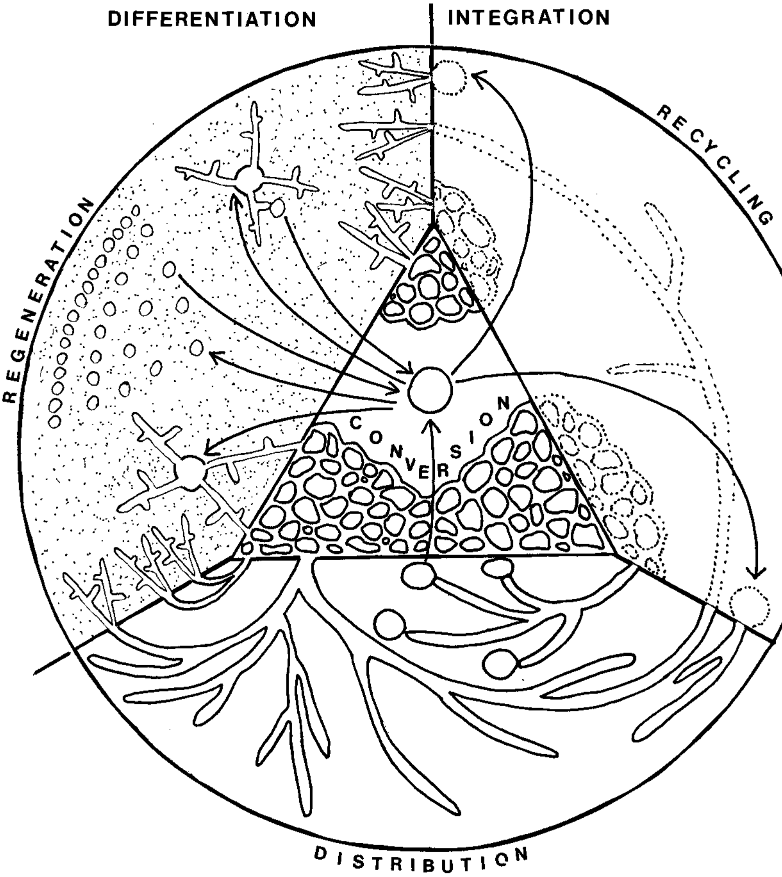

Fig. 1.2. The interplay between self-integration and self-differentiation to produce distinctive organizational states in resource-rich (stippled) and resource-restricted environments. The interplay enables energy to be assimilated (allowing regeneration of boundaries), conserved (by conversion of boundaries into impermeable form), explored for (through internal distribution of energy sources) or recycled (via redistribution of boundaries) according to circumstances. Fine lines indicate permeable contextual boundaries, bold lines impermeable boundaries and dotted lines degenerating boundaries. (From Rayner, 1997*a*.)

assimilative structures. Moreover, sealing the lateral boundary of a tube or channel whilst maintaining its apical boundary in a deformable, responsive state enables a much more focused response to a directional stimulus – much like blinkering a horse! This correlates with the observation that migratory structures in fungal mycelia are more prone to exhibit tropic responses than assimilative structures (Rayner & Boddy, 1988).

Such responses greatly enhance the energetic efficiency of any distributive system.

Boundary-redistribution involves the transfer of resources from degenerative to generative sites. In mycelia, it is evident in various examples of ‘autolysis’ and ‘self-parasitism’ (e.g. Rayner, 1977*a*; Rayner & Boddy, 1988).

Boundary properties and life history patterns

From a discretist standpoint, life cycles are commonly thought of as reproductive cycles – the means by which genes make more of themselves. The ability of a particular genotype, given a particular set of niche parameters, to make use of these cycles in delivering as many as possible of its own offspring into succeeding generations defines its adaptive fitness.

Even from this standpoint, however, it has long been appreciated that reproduction is subject to biotic or abiotic environmental constraints. Recognition of the effects of these constraints on population dynamics has given rise to classical theories of *r*- and K-selection, based on the reproductive rate (r) and equilibrium or carrying capacity ($K = 1 - 1/r$) terms of the non-linear logistic equation (see, for example, Andrews, 1992). Correspondingly, high rates of reproduction are associated with *r*-selection in unrestrictive environments, whilst lower rates of reproduction occur as a result of K-selection in restrictive environments.

Whilst *r*–K-selection theories explain why, in an adaptational sense, reproductive rates in unrestrictive and restrictive environments differ, how, in an organizational sense, life forms and life cycle stages are attuned physically to their surroundings has attracted less attention. Consequently, important insights into the origins and versatility of phenotypic form in response to inconstant environmental circumstances may have been missed.

From the systemic perspective illustrated in Figs. 1.1 and 1.2, far from defining the beginnings and endings of discontinuous generations of discrete individuals within finite niches, life cycles represent a means of generating and maintaining a continuous dynamic context in changeable surroundings. This is the answer to the riddle of the chicken and the egg (or the spore and the sporophore): neither came first – rather they represent distinctive boundary configurations of the same dynamic system!

The continuity of context that is ensured by life cycles is an expression of the fundamental indeterminacy of living systems, that is, their capacity for ongoing production and reconfiguration of boundaries, and obscures the discretist distinction between growth and reproduction. Examples of this indeterminacy can be found from molecular to social scales of biological organization, with boundaries being defined anywhere from intracellular to extra-organism locations (Rayner, 1997*a*). Correspondingly, the dynamic contexts of motile organisms – such as many animals – are not defined by where the body boundaries of these organisms are at a particular instant. Rather, they are defined topographically by the trajectories that these organisms map out as they use their powers of locomotion to follow and create paths of least resistance, and regionally by the territories within which the trajectories are confined. By contrast, the topographical and regional contexts of organisms, such as many plants and mycelial fungi, that grow rather than move bodily from place to place, coincide directly with the proliferation and overall extent of their body boundaries.

The concept that life forms inhabit and generate indeterminate contexts introduces the need to develop a more dynamic view of niches not as fixed but as fluid and variably interconnected space–time–energy domains. This in turn has important implications for the way *r*–K-selection theory can be used to understand the relationship between life form and life cycle in the generation of exploitative and/or persistent organizations.

When supplies of readily accessible resources are temporarily plentiful – that is, under *r*-selective conditions – following destructive or enrichment disturbance of natural habitats, the self-differentiation or regenerative processes depicted in Figs. 1.1 and 1.2 are promoted. These processes result in rapid proliferation, associated with high metabolic rates, but produce highly dissipative structures that are only sustainable as long as there is continual enrichment. In the absence of replenishment, conditions in any habitat are prone to become more restrictive – that is, to change from *r*- to *K*-selective – due to increasing competition or abiotic stress (including resource depletion). This necessitates self-integration into a more coherent organization if total dissipation is to be avoided.

Systems in which boundary-redistribution into relatively discrete dispersal and survival units predominates are characteristically strongly exploitative and somatically non-persistent – for example, many mitosporic (asexual) fungi. By contrast, boundary-sealing, accompanied by fusion and redistribution, produces a more retentive, coherent organization that allows resources to be conserved or distributed within a protective or explorative context, for example within sclerotia or rhizomorphs.

These processes therefore enable either ‘stress management’ through the production of survival and emigratory structures, or the development of overtly territorial, invasive and resistive organizations.

These propositions are supported by the general (but not invariable) differences evident between life styles typical amongst basidiomycetes and those of other fungi. Basidiomycetes are generally regarded as having more K-selected properties than other fungi in that they become dominant at late stages of fungal succession, associated with greater powers of persistence, invasiveness and reproduction via meiotic pathways (Frankland, 1992). Persistence is due to the production of resilient, often sclerotized and anastomosed boundaries (for example, pseudosclerotial crusts) which sequester rather than release hydrophobic metabolites. Invasiveness is associated with the formation of cable-like mycelial cords and rhizomorphs, and meiospores are produced in macroscopic basidiomes.

Dynamic networks – varying scale and pattern

The way that self-differentiation and self-integration processes are coordinated in a system capable of changing its scale and mode of operation according to circumstances can be demonstrated by growing mycelia, especially those of basidiomycetes, in heterogeneous culture systems. For example, experiments in which certain wood-decay fungi are grown between colonized inocula and uncolonized ‘baits’ in trays of soil have revealed a variety of long-range and short-range ‘foraging strategies’ that produce patterns extraordinarily similar to, for example, the raid swarms of army ants and the roots and stoloniferous systems of plants (e.g. Boddy, 1993; Dowson, Rayner & Boddy, 1986, 1988*b*).

Especially revealing are patterns produced by mycelia grown in matrix systems of the kind illustrated in Fig. 1.3. Here it is possible to see how, purely by responding to local circumstances and without any central administration, a mycelium can generate a persistent network which is reinforced along avenues of successful exploration and capable of producing coherent, scaled-up outgrowths with reproductive and migratory functions.

These patterns can be explained by the organization of mycelia as nonlinear hydrodynamic systems (Rayner, Ramsdale & Watkins, 1995*b*; Rayner, 1994, 1996*a*, *b*, *c*). The uptake of water and nutrients generates an expansive drive that results in the hydraulic displacement of deformable components of hyphal boundaries. Whenever its ‘through-

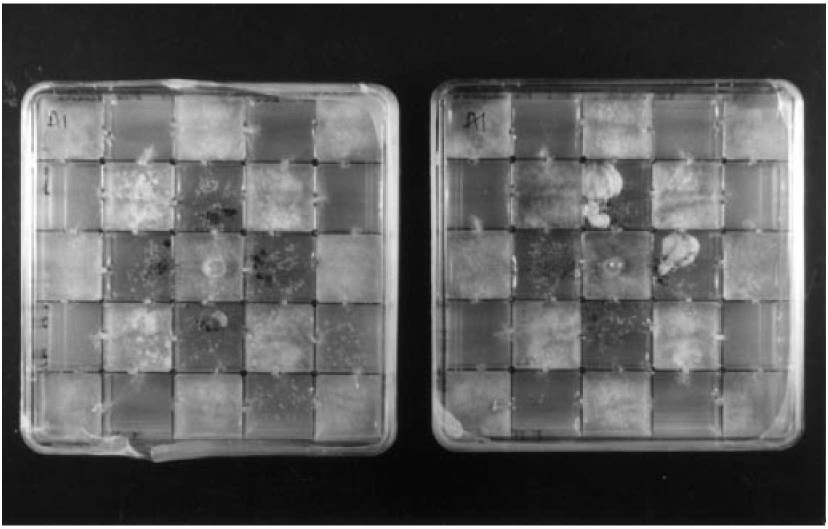

Fig. 1.3. Two examples of development of mycelium of the basidiomycete, *Coprinus radians*, when grown through a matrix of 25×4 cm^2 chambers. The chambers alternately contain 2% (w/v) malt agar and distilled water agar and are interconnected by narrow channels cut in the plastic partitions just above the level of the medium. Notice the diffuse proliferation in the high nutrient chambers and production of fruit bodies and fruit body initials in the low nutrient chambers. (Photograph by Timothy Jones.)

put capacity', due to the resistance to displacement to existing sites of boundary deformation, becomes exceeded by the rate of uptake, the non-linear system becomes prone to branch. In purely assimilative hyphae, generated by self-differentiation, the branches will form in a tributary-like pattern – due to the fact that the sites of uptake and proliferation coincide. In hyphae where uptake is distal to the site of proliferation, the branches form in a distributary-like pattern.

The feasibility of the theory that the versatility of mycelial systems can be explained in terms of varied hydraulic resistances to uptake, throughput and discharge of resources, can be tested using non-linear mathematical models. A simple reaction-diffusion model of this kind has been developed by Davidson *et al.* (1996, 1997). This model has four fundamental components: (1) a diffusible substrate, which is the energy source of the system; (2) replenishment of this substrate at a constant specific rate; (3) an autocatalytic activator, which facilitates conversion of the substrate into energy, drives the proliferation of biomass and decays at a constant specific rate; (4) diffusion of the activator at a rate which is inversely related to the system's resistance to throughput.

As shown in Fig. 1.4, the model used by Davidson *et al.* (1996,1997) was capable of reproducing many of the macroscopic patterns of biomass distribution actually observed in fungal mycelia. These included the production of smooth, annular and irregular biomass density profiles depending on the resistance to throughput. Smooth profiles were produced by low resistance (that is, highly networked) systems that ceased to expand radially unless replenishment was prevented – simulating the effect of internal degeneration – whence a 'fairy-ring'-like travelling wave front was propagated.

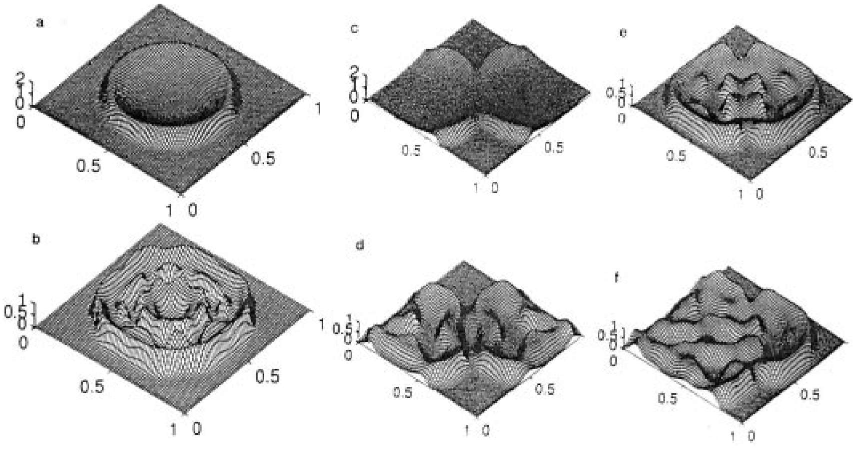

Fig. 1.4. Use of a reaction-diffusion model to predict energy assimilation patterns in growing and interacting mycelial networks with varied resistance to throughput and replenishment of substrate. Numbers on the vertical and horizontal axes respectively represent units of activator concentration (and hence, biomass-generating capacity) and spatial intervals. (a) Fairy-ring-like travelling-wave solution produced when replenishment is low or absent. (b) Irregularly lobed pattern produced in a relatively high resistance system when replenishment is sufficient to offset decay. In low resistance systems, the heterogeneity is reduced and expansion ceases (but can be resumed as a travelling wave if replenishment is prevented). (c) Mutual extinction of the interface between colliding travelling waves, as is observed in natural fairy rings. (d) Formation of a demarcation zone between established, replenished systems – as commonly exhibited by self-inhibiting cultures. (e) Coalescence of immature, replenished systems – as commonly seen in self-pairings between mycelia. (f) Formation of ridges protruding from an established into an immature replenished system – simulating the commonly observed penetration of mycelial cords from one colony into another (from Davidson *et al.*, 1996).

It should be noted, however, that the model could not sustain either an evenly growing margin without a decline in the capacity for substrate utilization in the interior, or the extension and expansion of activator peaks. The latter would correspond with the emergence of distributive structures such as mycelial cords and fruit bodies, due to enhanced delivery to local sites on the boundary of an integrated system. These inabilities may be related to the fact that as a first step, the model used was unable to seal its boundaries and/or alter its resistance as a consequence of throughput and so was incapable of effective conservation or distribution.

A more recent model based on circuitry has, however, succeeded in generating emergent structures (D. Marles, S.C. Harris, D. Williams & A.D.M. Rayner, unpublished). This model consists of a set of distributive channels emerging from a resource base and travelling along a gradient towards a resource source. The channels were allowed to branch and to anastomose at random. Their extension rate was dependent on their conductivity, which increased as a consequence of throughput. Under appropriate circumstances, anastomosis and consequent increased conductivity enabled the rapid proliferation of fan-like branching systems in a manner strongly reminiscent of the slow/dense – fast/effuse transitions in fungal mycelia that have been implicated in switches from predominantly assimilative to explorative growth patterns (e.g. Rayner & Coates, 1987).

Feedback mechanisms – the primary role of oxidative stress?

Having proposed roles for self-differentiation and self-integration in the dynamics of mycelia, the next step is to identify the specific feedback mechanisms responsible for bringing these processes into operation. Given that their physical properties of hyphal boundaries depend on their chemistry, the key questions are how does this chemistry affect, and how is it influenced by the external and internal environments of hyphae?

Since the energy which drives proliferation derives most fundamentally from external sources of reducing and oxidizing power, a primary consideration is how the availability of these sources affects boundary chemistry. Here, the ability of oxygen to serve constructive and destructive as well as energy-releasing roles may be important. These contrasting roles arise from the affinity of oxygen for electrons, which it accepts one at a time in the course of its reduction to water or other compounds. Such reduction releases chemical energy but also generates reactive oxygen species (ROS) capable of bringing about oxidative cross-linking as well as destroying the chemical order of living protoplasm through the generation of free radicals (species with one or more unpaired electrons; Halliwell & Gutteridge, 1989).

Here, an important possibility has been suggested by Hansberg & Aguirre (1990), who found that the development of a 'hyperoxidant state' is a necessary prelude to aerial mycelium and spore formation in the ascomycete, *Neurospora crassa*. This is a state in which the capacity of protoplasm to neutralize ROS is exceeded. Unless mitigated in some way, it can lead to protoplasmic degeneration. It is promoted by any factors that diminish availability of reducing power, enhance exposure to oxygen (especially in the gaseous phase, as in terrestrial habitats) or impede oxidative phosphorylation. On the other hand it is attenuated by any mechanisms that maximize resource uptake whilst minimizing intracellular oxygen concentrations.

Mycelia have four main ways of responding to this threat and promise of oxygen, each with contrasting effects on boundary chemistry. Firstly, they can assimilate nutrients in solution from plentiful external supplies through hydrophilic, permeable boundaries: in so doing they acquire respirable substrate and the ability to proliferate as dissipative systems – but only as long as nutrients are replenished. Secondly, they can neutralize intracellular ROS and molecular oxygen by means of antioxidant enzymes, pathways and metabolites, many of which are currently classified under the general heading of 'secondary metabolism'. Thirdly, they can produce a relatively oxygen- (and thereby also solute- and water-) impermeable boundary. This can be achieved by anastomosis (so restricting proliferation of branches), aggregation and the generation and oxidative cross-linking of hydrophobic phenolic, proteinaceous and lipid compounds in the presence of phenol oxidase and peroxidase enzymes. Fourthly, they can actively enable or passively allow a hyperoxidant state to arise and lead to protoplasmic disorder and cell degeneration (Rayner, 1996*b*, 1997*a*).

Onset of the hyperoxidant state, due to an inability to reduce intracellular oxygen fully to water via the respiratory chain, may therefore be an important, and possibly the most fundamental cue for self-integration. Moreover, degenerative processes would be initiated above a high oxidative stress threshold, whereas protective mechanisms would come into play above a lower threshold (Rayner, 1996*b*). A recent study, which indicated that initiation of sclerotia in the Basidiomycete, *Sclerotium rolfsii*, is characterized by a high degree of peroxidation in its total lipids, accords with this interpretation. Lipid peroxidation is characteristic of oxidative stress, and factors that in the past have been shown to inhibit or promote sclerotium initiation respectively act as free radical scavengers or pro-oxidants (Georgiou, 1997).

A general hypothesis that arises from these considerations is that when the ratio of external to internal supplies of resources providing reducing power exceeds a threshold, mycelia differentiate as assimilative, dissipative structures. However, when the ratio falls below this threshold, self-integration is induced – accompanied to varying degrees by the production, sequestration and release of extracellular compounds. Where these compounds are sequestered, they reduce the permeability of hyphal boundaries. This hypothesis accords both with the changes in mycelial organization associated with resource enrichment or attenuation (for example, in matrix plates), and with the onset of 'secondary metabolism' (e.g. Bushell, 1989*a*,*b*). Moreover, it suggests why organization and metabolite production are interrelated. These propositions are also consistent with data obtained from three phenotypically distinctive strains of the basidiomycete, *Hypholoma fasciculare* (Crowe, 1997). One of these strains was a typical dikaryon, which produced silky, cord-forming mycelium with distributary-like branching. Several major hydrophobic components were present in extracts from the mycelium as opposed to the growth medium of this dikaryon. These hydrophobic components were also consistently produced by other dikaryotic genotypes. By contrast, monokaryons of *H. fasciculare*, which have predominantly tributary-like branching, as well as 'flat' dikaryons, which did not produce mycelial cords and aerial mycelium, lacked these components. The importance of hydrophobic compounds in the formation of emergent mycelium has been demonstrated more explicitly for an increasingly wide range of fungi that produce the cysteine-rich proteins known as 'hydrophobins'. These proteins coat the walls of emergent hyphae, but are released into the medium from submerged hyphae (Wessels, 1994).

An important adjunct of the general hypothesis is that the nature of boundary-sealing compounds may be expected to vary with habitat – notably with respect to the degree of aeration and availability of nitrogen sources. Correspondingly, whereas relatively aquatic systems often produce abundant mucilage at their external boundaries, terrestrial systems undergo sclerotization. Sclerotization characteristically involves the oxidative cross-linking of reducing compounds, notably phenolics, lipids and cysteine-rich proteins. It not only makes outer boundaries impermeable to water, but also consumes and reduces permeability to oxygen.

That basidiomycetes generally display the most overtly terrestrial life styles amongst fungi is consistent with this proposition. Moreover, a striking example of the transition from mucilaginous to sclerotized boundaries within a single invasive structure is found in the rhizomorphs of honey fungus (*Armillaria* spp.), which both create and grow down oxygen gradients. The apex of a growing rhizomorph is coated by a thick layer of mucilage, which both lubricates the structure and makes it deformable (Rayner *et al.*, 1985). Behind the apex, the outermost layer progressively darkens and rigidifies due to the oxidative cross-linking of phenolic compounds in the presence of the extracellular phenoloxidase, laccase (Worrall, Chet & Hüttermann, 1986; Rehmann & Thurston, 1992). Within the core of the structure is a medulla, which probably acts as an air channel (Griffin, 1972). Surrounding the medulla is a cortex, the innermost layer of which, forming a dome over the medulla, contains hyphae rich in mitochondria and bundles of microfilaments (Rayner *et al.*, 1985). The rhizomorph is remarkable for its ability to invade anoxic environments and to penetrate the intact bark and vascular cambium of woody roots, with increased aeration in its wake. If it emerges into air, it ceases extension and its tip sclerotizes. The mitochondria-rich hyphae presumably serve both to generate the energy necessary to drive extension and to use up the oxygen supplied through the medulla that would otherwise sclerotize the tip. The same basic principle of progressive sclerotization is demonstrated by many other examples of the 'hardening off' of maturing boundaries, ranging from insect cuticles to tree bark, and also links in with the general principle, described earlier, of lateral boundary-sealing in distributive structures.

Interactive boundaries – mycelia in conflict and partnership

Given their proposed significance and sensitivity, boundary properties will both influence and be influenced by the outcome of encounters between fungi and between fungi and other organisms. Here we will signpost the ways we think self-integration theory may help to clarify how these influences are brought to bear.

Competition

Competition occurs when the boundaries of systems with similar resource requirements occupy the same contextual domain but fail to integrate: the outcome may then be mutual dissolution, mutual restriction or the overtaking of one system by another (Rayner, 1996*c*, 1997*a*). An indication of how these distinctive outcomes are embedded in the physical organization of mycelia as dynamic, assimilative systems is provided by observations of collisions between the mathematical model systems of Davidson *et al.* (1996, 1997; Fig. 1.4). Collisions between travelling waves resulted in mutual extinction of their interactive interface, as with natural fairy rings (Dowson, Rayner & Boddy, 1989). Collisions between irregular systems resulted in coalescence, formation of demarcation zones and reflective waves or incursion from one system into the other, depending on the distance apart and relative timing. All these patterns occur in real mycelial interactions.

In understanding the mechanisms and consequences of competition, it is important to appreciate the distinction between 'primary resource capture', primacy in locating and assimilating suitable external supplies of free energy, and combat, the gaining or denial of access to resources already incorporated by a system (Cooke & Rayner, 1984). Whereas primary resource capture is maximized through assimilative free surface, combative prowess is enhanced by increased coherence and a boundary that is both resilient to and capable of serving as a secretory surface for allelopathic (damaging/inhibitory) substances.

Numerous observations point to the importance of combat in fungal successions (Frankland, 1992). Here, relatively more *r*-selected and/or aquatic forms with permeable boundaries might be expected to lack combative properties altogether, or release diffusible inhibitors/antibiotics. By contrast, forms with oxidatively polymerized boundaries would be more capable of producing resistive or invasive structures. These structures would not only be relatively immune to, but could benefit territorially from, the secretion of reactive molecules, such as hydrogen peroxide (which in the presence of peroxidase could also provide a mechanism for eliminating excess oxygen and boundary-sealing).

The dominance of basidiomycetes late in succession, and the characteristic way in which they produce emergent mycelial phases and induce cell death following contact with one another and other organisms accords with these expectations. So too does evidence that the release of hydrophobic metabolites by these fungi is suppressed when they interact with one another or metabolic inhibitors such as 2,4-dichlorophenol, associated with enhanced phenol oxidase and peroxidase activity (Griffith, Rayner & Wildman, 1994*a*,*b*,*c*). The production of hydrogen peroxide, accompanied by the formation of invasive mycelial cords and lysis of opposing mycelium, has been demonstrated in *Phanerochaete velutina* (Z.R. Watkins & A.D.M. Rayner, unpublished). Also, of the three phenotypes of *H. fasciculare* described earlier, the typical dikaryon was more invasive than the monokaryon and flat dikaryon when paired with another fungus (Crowe, 1997).

Mating

In those fungi where plasmogamy involves integration of systems that are genetically non-identical (i.e. 'non-self'), a context is thereby produced for the encounter of disparate nuclear and mitochondrial genomic organelles. The boundaries and activities of these organelles may be 'compatible' – capable of integration, or 'incompatible' – incapable of integration.

Since any mechanism that compromises a cell's ability to maintain reactive oxygen species below a critical level will induce oxidative stress, incompatibility and consequent interference between gene products that directly or indirectly influence electron flows could be critical to survival. For example, disparity between nuclei with regard to the specification of key components of mitochondria could induce dysfunction of respiratory pathways and protoplasmic degeneration (e.g. Rayner & Ross, 1991). This could explain the *dynamic origin* of post-fusion somatic incompatibility reactions amongst higher fungi (Rayner, 1991, 1996*c*) and the general difference between ascomycetes and basidiomycetes with respect to their ability to form stable heterokaryons in strongly aerated conditions (Rayner, 1996*c*). Ascomycetes may generally lack the latter ability because they do not, except in their fruit bodies, produce distributive hyphae with strongly insulated boundaries. The relatively terrestrial, independently growing heterokaryotic states of many Basidiomycetes, do, however, form such boundaries.

In Basidiomycetes, the smoothness of the transition between homokaryotic and heterokaryotic stages may depend on the rapidity with which an insulated boundary is formed by heterokaryotic states resulting from hyphal fusion, *before* cellular degeneration. The sensitivity of this transition is evident in matings of *Stereum* from different geographical regions (that is, 'allopatric' matings), where takeover, degeneracy and subdivision into conflicting local domains have all been observed (Ainsworth & Rayner, 1989; Ainsworth *et al.*, 1990, 1992) accompanied by the release of secondary metabolites. The latter include the sesquiterpene, (+)-torreyol, and can be suppressed by the antioxidant, N-acetyl-L-cysteine (Z.R. Watkins & A.D.M. Rayner, unpublished).

Parasitic symbiosis

Parasitism occurs when the boundary of a host entity provides a context for, but does not coalesce with, the boundary of an invasive entity, so establishing an interface across which the invader can unilaterally assimilate resources. The nature and sustainability of the parasitism then depends on the integrity and responsiveness of host and parasite system boundaries. Necrotrophic parasitism requires the destruction of host boundaries before they can be sealed, so that their contents are released and made available to the parasite. Biotrophic parasitism depends on the host boundary remaining intact, but permeable, so that the parasite can assimilate resources from living host cells or tissues. Any mechanism that directly or indirectly disrupts the boundary of the parasite or seals the boundary of the host obviates the parasitism. This fact probably underlies the varied mechanisms and processes that underlie resistance and susceptibility to infection, including the production of reactive oxygen species during hypersensitive, cell death, responses (Baker & Orlandi, 1995).

Mutualistic symbiosis

Mutualistic symbiosis depends on the boundaries of disparate entities associating in such ways as to produce an interactive interface for reciprocal exchange of resources. In mycorrhiza-forming fungi, the provision of a linking mycelium between an array of such interfaces can serve to interconnect separate host plants in a manner analogous to computer networks (Read, 1997; Simard *et al.*, 1997). However, degenerative incompatible responses can be induced and need to be suppressed by specific host genes if successful integration is to occur (Gianinazzi-Pearson *et al.*, 1995).

Conclusions – the wider context

We have sought to show how the behaviour of life forms in general and fungi in particular as dynamic systems within a changeable context