INSPIRATION FROM MICROBES: FROM PATTERNS TO NETWORKS

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Abstract

Many relatively simple organisms, such as bacteria, cellular and acellular slime moulds and fungi, can self-organise to form patterns or complex developmental networks with a rich variety of structure and behaviour. Many of these systems are intimately associated with nutrient acquisition or distribution, particularly under conditions where resources are limited and distributed patchily in time and/or space. It is postulated that emergent structures are likely to be efficient and resilient as they have been subject to many cycles of evolutionary selection pressure. In

comparison to many biological networks, such as neural networks, genetic and biochemical pathways or food webs, microbes are also extremely accessible, and provide tractable experimental systems. In this Chapter, we briefly review areas where emphasis has been given to morphological representation of microbial structures and discuss areas of potential overlap with current developments in network theory.

Introduction

The term microbe does not represent a conventional taxonomic group, but is used here as a convenient umbrella descriptor to cover an assemblage of relatively simple organisms, such as bacteria, cellular and plasmodial slime moulds and fungi, that have little or no multi-cellular organization in comparison to animals and plants. The population dynamics, colony morphology and organization are all strongly influenced by nutrient availability in the environment. This leads to overlap in their gross behaviour and growth habit and, as a consequence, the models that have been developed to describe them. They fit well into the current definitions of self-organising, complex systems provided by Camazine *et al.,* (2003) and Amaral and Ottino (2004).

"Self-organisation is a process in which pattern at the global level of a system emerges solely from numerous interactions among the lower-level components of the system. Moreover, the rules specifying interactions among the system's components are executed using only local information, without reference to the global pattern." (Camazine et al., 2003)

"A complex system is a system with a large number of elements, building blocks or agents, capable of interacting with each other and with their environment. The interaction between elements may occur only with immediate neighbours or with distant ones; the agents can all be identical or different; they may move in space or occupy fixed positions and can be in one of two states or multiple states. The common characteristic of all complex systems is that they display organization

without any external organizing principle being applied." (Amaral and Ottino, 2004).

Importantly, as most microbes can be readily grown under laboratory conditions they have proved a rich source of tractable experimental systems to investigate self-organisation and pattern formation, and are amenable to physical, chemical or genetic intervention (Ben-Jacob *et al.,* 1994,1995, 1998, 2000; Levine and Ben-Jacob, 2004). Populations of unicellular organisms, such as bacteria and cellular slime moulds are conceptually easy to identify with an agent-based modeling approach (Chowdhury *et al.,* 2004). The individual cells have limited morphological variation, but can generate exquisite patterns and dynamics at the population level. There has been considerable success in simulating emergent pattern formation as a consequence of iterative interactions between agents that follow relatively simple rule-sets. As the models are refined there is increasingly good correspondence between mathematical model and real-world behaviour (Ben-Jacob *et al.,* 1998, 2000; Dormann *et al.,* 2002; Levine and Ben-Jacob, 2004).

Plasmodial slime molds (myxomycetes) and mycelial fungi form more elaborate interconnected foraging networks that are highly responsive to local environmental conditions. At one level, study of these systems may enhance our understanding of the rules pertaining to pattern formation, in a similar manner to the bacterial systems described above, in addition, they may also provide an ideal opportunity to abstract the critical features that enable self-organisation of a network with de-centralised control, and may act as a future paradigm for robust network design. Inherent in this approach is the assumption that solutions adopted by biological networks will exemplify useful generic theoretical principles, such as persistence, robustness, error-handling or appropriate redundancy, as they have been honed by many cycles of evolutionary selection. The expectation is that the process of Darwinian natural selection based on variation, competition and survival has explored a significant range of possible network organizations and the resulting systems are likely to be well-adapted to survive and reproduce under particular biotic and abiotic conditions to solve certain ecological problems. A range of network architecture, development and dynamics

can be found within the fungi and myxomycetes, suggesting a comparative approach may also be instructive. A weakness is that the constraints imposed by the components used to construct the network (i.e. branching tubes) may have such a profound effect on the possible network organization and dynamics that any result can only be generalized to a very limited set of problems.

In other areas of biology, such as ecological food webs or metabolic and genetic networks, systems involving interactions between multiple agents have been successfully analysed using tools originally developed for graph theory (Strogatz, 2001; Albert and Barabási, 2001; Dorogovtsev and Mendes, 2002; Newman, 2003; Amaral and Ottino, 2004). To our knowledge, these approaches have so far only been applied to one microbial system, that of *Physarum polycephalum*, results of which will be described later. We therefore await with some anticipation to see whether similar approaches will yield useful insights into fungal behaviour and conversely whether analysis of fungal systems will provide an additional class of networks to develop and test universal ideas of network organization and dynamics.

Emergent pattern formation in bacteria

Bacteria produce circular colonies when grown under optimal conditions in a culture medium on a Petri dish. At the molecular level, although complete sequence information is available and a substantial fraction of the protein complement has been characterized, the behavior of bacteria is sufficiently complicated that it is still not completely understood. Indeed, recent approaches both highlight the complexity of the interactions in transcriptional and metabolic pathways but also suggest conceptual frameworks that may simplify the interpretation of such networks (Zaslaver *et al.,* 2004; Milo *et al.,* 2002, 2004). On the macroscopic level however, each bacterial cell can be considered as a single unit having behavior but no structure, allowing the development of colonies to be modeled effectively using stochastic cellular automata models (CA).

In a CA model a large group of simple computational units is collected in a regular array. Unfortunately, the conventional description

of these units as "cells" creates an immediate clash in terminology with the description required in a discussion of the growth of bacterial cells. Therefore, we shall in this paper refer to each position in the CA model as a node, in order to distinguish them from (bacterial) cells.

In most CA models, this array of nodes is two-dimensional, and is laid out in a hexagonal, triangular or, most commonly, rectangular pattern. Each node is defined as being in one of a finite number of states, and a closed and complete set of rules exists which defines the transitions which are possible between these states.

In one of the first applications of CA modelling, Eden (1960) simulated a 2-D biological growth process on a square grid by random addition (growth) of particles to the border of the structure formed in the previous steps from an initial seed. The resulting shape was roughly circular with a slightly irregular boundary. If conditions are made less favorable for growth, through the choice of an unsuitable temperature, the presence of a harmful chemical, or a reduction in the concentration of nutrient, a variety of complex growth patterns may appear, some of which show surprising evidence of co-operative behavior among bacterial cells (Ben-Jacob *et al.,* 1994, 1995, 1998, 2000). If diffusion of nutrients to the growing colony becomes limiting, a fractal branching structure develops in a process termed diffusion-limited aggregation (DLA; Witten and Sander, 1960) or diffusion limited growth (DLG; Meakin, 1986). Both DLA and DLG have found extensive application in biological and non-biological fields. In biological fields, DLG was initially used to simulate bacterial colony growth under conditions where expansion only involved cell-division but not movement (Matsushita *et al.,* 1990).

In such a simulation it is convenient to define the spacing of the grid as the size of a bacterial cell. Cells have a finite lifetime and do not move, but can grow and divide if environmental conditions, such as resource levels, are favourable. As the cell functions, it consumes that nutrient and, in the absence of any mechanism to replenish it, the bacterial cell will eventually starve, or place itself into a quiescent state. This leads to a variety of growth patterns (Fig. 1), ranging from very lush and rapid growth to broken or fractal growth, sparse growth, or the death of the colony depending on the interplay between the rate at which

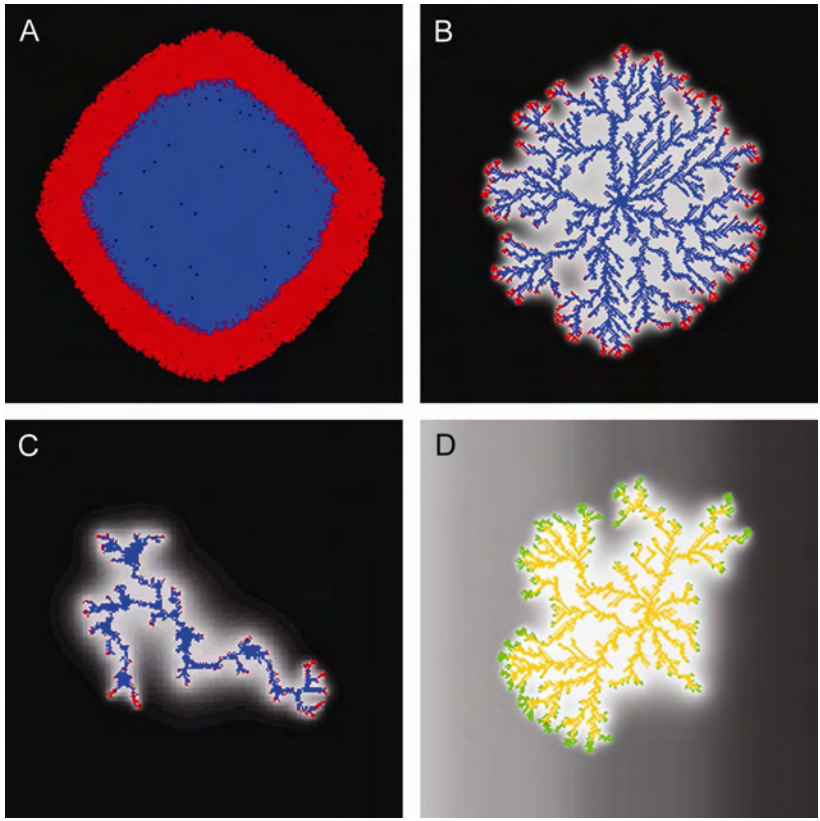

Fig. 1: Cellular Automaton models of bacterial colony development

A – Stochastic growth in the presence of large quantities of a non-diffusing nutrient. Cells are created if 2-5 living cells adjoin an empty node and live for 50 generations. Bacteria are coded red if alive and blue if dead.
B – Emergence of fractal growth under nutrient limitation. The depth of colour of the background indicates the level of available nutrient. 100 units of nutrient are present at each point initially, 10 are consumed when a cell is created and one is consumed every successive generation.
C – A colony with barely enough nutrient to survive has very few points of growth
D – Colony growth in the presence of limiting nutrients and a pollutant whose concentration increases to the right of the figure. The depth of colour of the background indicates the level of pollution.

nutrient at a target cell is consumed, competition for nutrient from

competing cells, the rate at which nutrient can be replenished by diffusion, the length of time a cell can survive without an adequate supply of nutrient, and the finite lifetime available to a cell. Each of these factors can be cast as one or more rules influencing the transitions that are possible between node states. The forms of growth predicted by the model closely follow the types of growth shown experimentally in the laboratory (e.g. Fujikawa, 1994).

Further influences on growth include temperature gradients, bulk liquid flow and the presence of chemicals. These may inhibit growth, cause the premature death of a cell, or reduce the chance of reproduction. Treatment of such factors is complicated by the likelihood that, for example, the chemicals may diffuse down concentration gradients, and the possibility that they may be partly or totally metabolized by the cells. The influence of such factors can be taken into account by appropriate rules. For example, Figure 1C reveals the characteristic way in which a colony shrinks away from a region of high chemical concentration, to the right hand side of the figure. It can be seen that the majority of growth from the inoculation point has occurred to the left hand side. The physical scale of these simulations is in the order of a mm.

Ben-Jacob and colleagues have extended the CA approach further to include bacterial motility and chemotaxis in the "communicating walkers model" and were able to simulate a wide range of tip-splitting (T), chiral (C) and vortex forming (V) colony morphotypes (reviewed in Ben-Jacob *et al.,* 1998, 2000). These patterns required intercellular communication mediated by external diffusion of signalling molecules and cooperative multicellular behaviour, in addition to control of growth through nutrient diffusion. To match the scale of pattern formation spanning tens of mm in Petri-dish cultures, the effective size of the agents was also increased to represent 10^2-10^4 bacterial cells.

Understanding of the structure and dynamics of bacterial growth under non-ideal conditions is becoming of increasing importance in diverse fields such as effective control of bacterial biofilms, utilization of bacteria in bioremediation, the use of biobarriers to contain or filter polluted groundwater plumes, and optimization of structural features of polymeric species to facilitate their degradation in the environment.

These CA models provide insights into the collective cooperative behaviour of agents in 2-D planar systems and the potentially complex, dynamic patterning that can arise with relatively simple rule-sets. The systems are driven by nutrient availability, and operate far from equilibrium. The most interesting patterns emerge when at least two processes combine with different length scales and each agent operates with less than total global information. One of the strengths of this field of research is the presence of both continuous and discrete models, capitalising on the speed and simplicity of CA models and the analytical and mechanistic power of continuum approaches. This is necessary to prevent the 'reminiscence trap' (Ben-Jacob *et al.,* 2000), in which the rules used to produce a visually appropriate CA output are automatically assumed to accurately describe the underlying process without a sound mechanistic explanation.

Fungal mycelial networks

Filamentous fungi grow out radially from an inoculum, by apical extension of slender (5-20 µm diameter) tubes termed hyphae that branch sub-apically to form fractal, tree-like structures (mycelium). In ascomycetes and basidiomycetes, as the colony develops tangential hyphal fusion, termed anastamosis, also occurs to form an interconnected mycelial network (Rayner *et al.,* 1994, Glass *et al.,* 2004), spanning millimeters to centimeters. The developing mycelial network functions to scavenge and sequester nutrients from the substratum in or on which it is growing (e.g. soil), concentrate nutrients from soil solution and decaying organic matter, relocate nutrients within the mycelium (which may be located in different organic resources), and ultimately make nutrients available to plants to maintain primary productivity (e.g. Boddy & Watkinson, 1995). Both saprotrophic and ectomycorrhizal basidiomycetes often develop specialised high-conductivity channels, termed cords, through aggregation and limited differentiation of hyphae. Cords are able to translocate nutrients between separate food resources, but their development, frequency, scale and distribution are all species specific in a way that suggests each cord forming species has evolved a different foraging strategy (or set of foraging strategies). The architecture

of the network is not static, but is continuously reconfigured in response to local nutritional or environmental cues, damage or predation, through a combination of growth, branching, fusion or regression (Boddy, 1999; Watkinson, 1999). Embedded within the physical structure is an equally complex set of physiological processes that contribute to uptake, storage and redistribution of nutrients throughout the network in a well co-ordinated manner. Local sensory perception and responses are coupled over different length scales leading to optimisation of long-term behaviour. The overall success of each foraging strategy arises from the iterative interaction between environmental sensing, physiological adaptation and developmental re-organisation. For example, long range foragers such as *Phanerochaete velutina* grow rapidly for long distances, with cords developing early in growth to form long supply lines from the large wood food bases this organism utilizes.

Advance only halts when the mycelium meets, and pauses to exploit, a substantial new wood resource (Boddy, 1999). By contrast, *Hypholoma fasciculare,* which lives on dead leaves as well as substantial pieces of wood, 'searches' intensively over a short range, spreading mainly as

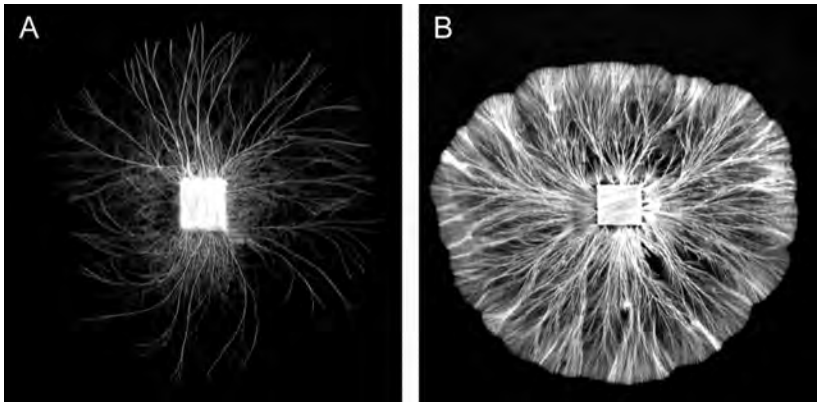

Fig 2 Mycelial networks in foraging basidiomycetes

Mycelial systems of *Phanerochaete velutina* (A) and *Hypholoma fasciculare* (B) growing from wood block of 2 cm side in 24 x 24 cm tray of compressed non-sterile soil. The mass fractal dimension for *P. velutina* is approxamtely 1.6 and *H. Fasciculare* > 1.9. Photos by George Tordoff.

fanned-out mycelium composed of a dense array of separate hyphae, with less obvious cords (Fig. 2); hyphae in older systems die-back leaving a much more open network than that which initially develops from an inoculum. The use of fractal dimension (Boddy *et al*., 1998) to describe the organisation of the mycelial network, and its responses to encounter with various newly supplied resources (baits) as it grows in soil microcosms (Figs 2 & 4), can highlight developmental shifts – some so subtle as to evade simple observation - between diffuse assimilative and corded distributive growth. Moreover, it provides a means to convey these patterns in quantitative form and to measure changes.

Whilst mycelial networks are typically grown in laboratory microcosms ranging from 0.1 – 1 m^2 with a few resource patches, in an undisturbed forest ecosystem almost all trees and fallen plant parts are interconnected by a diverse population of mycelial systems forming an extensive network. The mycorrhizal connection between trees, termed the "Wood Wide Web", allows carbon transfer between trees, including from a host tree to neighbouring seedlings, even of different species (Simard *et al.,* 1997; Read, 1997). Likewise, there is nutrient movement between dead resources via mycelial connections (Wells & Boddy, 1995). The true extent and degree of connectivity of such translocation networks is not known. However, it is worth noting that the largest organism on Earth are fungi (Smith *et al.,* 1992), the largest of which comprises genetically identical isolates of the fungus *Armillaria ostoyae* spanning 965 hectares with a maximum separation of 3810 m and an estimated age of 1900-8650 years (Ferguson *et al.,* 2003).

Unlike almost every other type of complex biological network, fungal mycelia have the major advantage that in some systems almost the entire network is visible and accessible. Thus, for example, saprotrophic cord forming fungi, though growing in the 3-D volume of wood and soil, are often naturally restricted to an approximate 3-D plane as they extend between resources at the soil litter interface. This greatly facilitates non-invasive imaging approaches to map and analyse dynamic changes in network architecture. In comparison with motile bacterial colonies, fungal colony development is constrained to a much greater degree by the previous history of hyphal growth and branching as this pattern, once established, only tends to be remodelled over a longer time-frame. There

is much greater potential for communication within the network as hyphae maintain continuity with both their immediate ancestors and with neighbouring branches through *de novo* formation of cross-links (anastomoses).

Although the network architecture is of considerable interest, it only defines which connections are possible at any given time, but not the

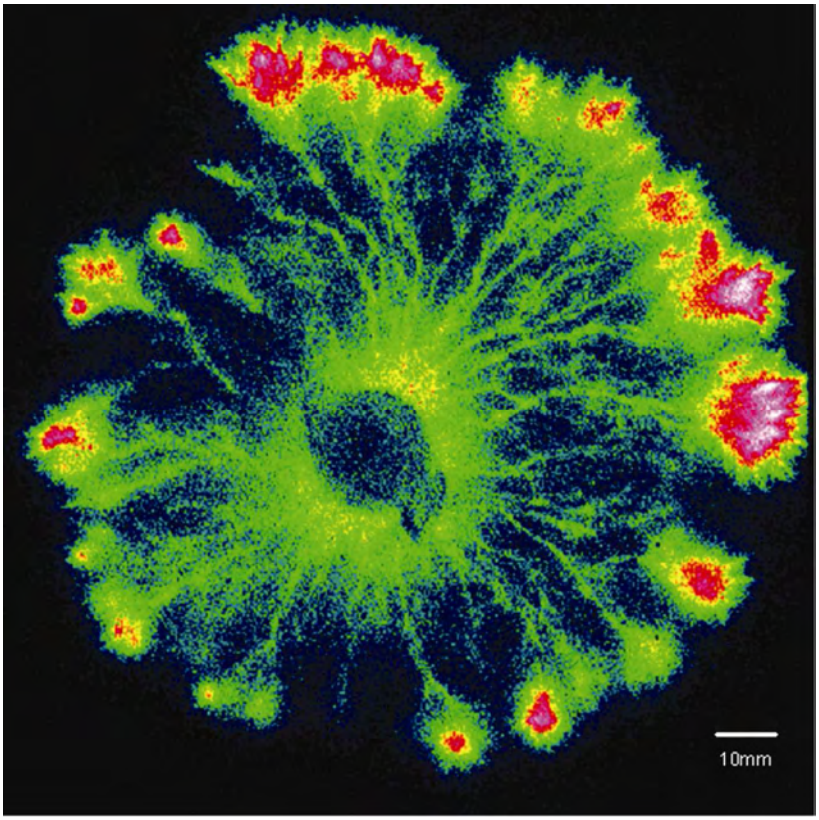

Fig. 3 Photon-counting scintillation imaging of nutrient transport transport in *Phanerochaete velutina*

The non-metabolisable amino-acid analogue, ^{14}C-aminoisobutyrate (AIB) was loaded in the center of the colony previously grown across a scintillation screen. Transport of the radiolabelled compound was imaged using a photon-counting camera. The resultant image is pseudo-colour coded blue for low intensities through green to red/white for high intensities

strength or direction of the transport or signaling fluxes. To begin to address this problem, we have developed a novel non-invasive technique to track movement of ^{14}C-labelled N-compounds in foraging mycelial networks in contact with an inert scintillation screen using photon-counting scintillation imaging (Tlalka *et al.*, 2002). This provides a highly sensitive, quantitative measure of N-distribution in near real time and gives us a unique opportunity to define the complex patterns of N-redistribution that occur in mycelial systems developing in patchy resource environments. In simple microcosms with mycelium growing out from a central inoculum, transport occurs towards the margin of the colony, concentrated in cords if these are present (Fig. 3). The signal from different regions of the mycelium can be quantified from these images and reveals that there is a strong pulsatile component associated with rapid transport, particularly through the corded system (Tlalka *et al.*, 2002; 2003).

We have now modified the approach to allow measurements from more realistic microcosms with wood-block inocula and sand or soil substrates overlaid with a more-sensitive translucent scintillation screen. With this system we can continuously image ^{14}C-AIB dynamics for extended periods in excess of 6 weeks and have observed rapid pulsatile fluxes operating both acropetally and basipetally between inoculum and baits consistent with a circulatory 'ring main' (Wells *et al.*, 1999; Lindahl *et al.,* 2001). A complex sequence of shifts in N-distribution and transport priority can also be observed throughout the network as it develops over time.

For example, Fig. 4 shows scintillation images of *Phanerochaete velutina* growing across sand from a wood inoculum as a foraging network of loosely aggregated cords. Contact with a second wood bait triggered an increase in local branching and proliferation after 2 months. The microcosm was overlaid with a translucent scintillation screen at this point and ^{14}C-AIB added to the initial inoculum. Within 1 h of loading the ^{14}C-AIB had travelled 250 mm along one of the major cords (Fig. 4A). After 4 h signal was present in most of the growing mycelium subtended by this cord (Fig. 4B). The signal from regions 1-3 along this cord (Fig. 4D) showed pronounced oscillations, superimposed on the longer term trend, that continued for around 5-7 days (Fig. 4E). The

overall level of signal decreases in the cords as the growing mycelial margin advanced out of this region. Not all the cords transported simultaneously. For example, the pre-existing cord in the area highlighted by the dotted circle (Fig. 1B) showed no ^{14}C-AIB movement until around 12h, then filled at a similar rate to the primary cord (region 4 in Fig. 1D&F), a process we term "route-switching". This cord appears

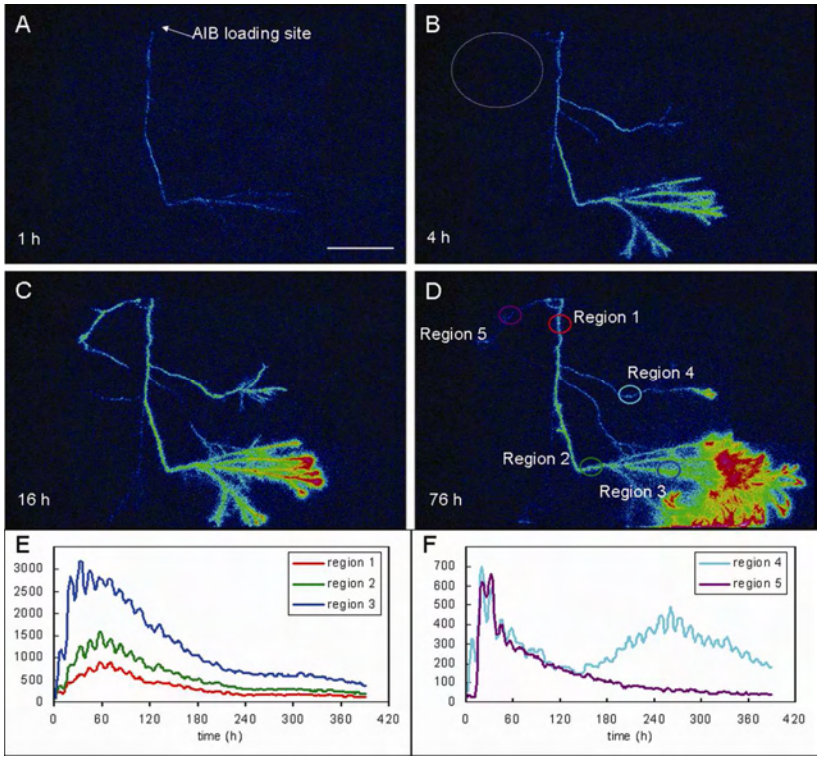

Fig. 4 Photon-counting scintillation-imaging of ^{14}C-AIB transport in *Phanerochaete velutina* growing from a wood-block inoculum across a 24 x 24 cm^2 sand microcosm

A-D: Pseudocolour coded scintillation images showing ^{14}C-AIB transport at the time points indicated.
E-F: Total signal intensity for the regions depicted in (D) illustrating a pulsatile component to transport superimposed on the longer term trends and the abrupt rout switching event in region 5.

to act as a transport route only transiently as the signal declined after around 30 h. Likewise, one of the other subsidiary cords showed two phases of transport, one initiated almost synchronously with the main cord and the second starting at around 150 h (region 5, Fig. 4D&F).

There is no agreed mechanism driving nutrient movement through corded mycelia, although the most likely explanations will involve a combination of diffusion, cytoplasmic streaming or vesicle transport and mass-flow. The observations of pulsatile fluxes and route switching are novel and have no explanation at present. In the context of this paper, however, they serve to highlight the need to consider the magnitude, dynamics and direction of fluxes in a biological network as well as just the topology.

Models of fungal development

A range of different modeling approaches have been applied to fungal growth and development (Bezzi and Ciliberto, 2004). There are several 'continuous models' that seek to model the collective attributes of the mycelium, rather than the growth of individual hyphae, but include morphological features such as tip growth, branching, anastomosis and cell death (Edelstein, 1982; Edelstein & Segal, 1983; Edelstein-Keshet & Ermentrout, 1989; Davidson *et al.*, 1996; Davidson, 1998; Davidson & Olsson, 2000; Boswell *et al.*, 2002, 2003). In such models growth is driven by nutrient concentration derived from uptake and internal passive or active transport. These models provide good descriptions of mass and substrate distributions for growth in both homogeneous and heterogeneous environments, but can only describe the topology architecture of the mycelium through its average properties and do not have an explicit morphological representation.

The first attempts to capture a direct representation of the morphology of the colony were based on cellular automata operating in discrete time, space and state. In a similar manner to the bacterial models, although the CA is discretized, growth is typically controlled through interaction with continuous fields of nutrients or signalling molecules (Regalado *et al.,* 1996; Ermentrout and Edelstein-Keshet, 1993, Liddell and Hansen, 1993; López and Jensen, 2002)

Most of the models above rely on external diffusion of nutrients, inhibitors and/or signalling molecules to supply information that is then interpreted locally to regulate growth and/or branching. Many foraging fungal systems are capable of growing over inert substrates that would preclude such interactions. Although growth is still exclusively confined to the hyphal tips and branch points, all the nutrient supply is derived from the initial inoculum source and has to be translocated through the network to the growing points through a combination of diffusion, mass flow and active transport systems.

To investigate whether pattern formation is possible in such asymmetric systems driven only by internal resource allocation, we have developed an agent based model in which nutrients required for growth are translocated through the structure created by the agents themselves, rather than diffusion through an external field.

The agents represent a physiological unit rather than just a morphological structure. They grow with random probability into adjacent, unoccupied nodes if sufficient nutrients are available, and die if not. A key feature of the model is that the agents pass on all, or a fraction, of their resources to 'daughter' agents at each time step. This captures active transport of nutrients through the network, without

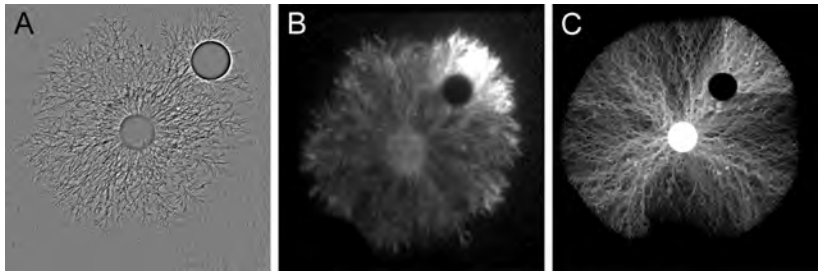

Fig. 5 Resource-driven agent-based modeling of mycelial development

A – Contrast enhanced bright field image showing the network topology for a growing fungal colony of *Phanerochaete velutina* with asymmetric food supply (bait - top right).
B – Photon-counting scintillation imaging of amino-acid translocation in a baited colony reveals preferential transport along mycelial strands towards the new food resource (dark circle, top right).
C – Emergence of canalised nutrient fluxes and anisotropic growth under asymmetric resource conditions using a resource-driven agent-based model.

specifying the mechanism. Cost functions can be assigned to each activity. The model generates an emergent branching, connected structure of living agents unlike the DLA type models, where the fractal structure is predominantly composed of dead agents incapable of further interactions. The model raises the intriguing possibility that the pattern of branching structure arises initially from stochastic variation in nutrient fluxes and the actual architecture is then defined by reinforcement of this pattern rather than through a sophisticated set of regulatory developmental processes defining the branching structure.

CA models can generate crude spatial representations of structure but are heavily constrained by the regular, often 2-D, lattice used in the simulation. The basic tubular structure and simple branching growth habit of fungi has led to an alternative approach to develop systems based on (empirical) rules governing growth rate and branching characteristics of 'vector-agents' to produce models with greater morphological realism. The rules may include stochastic sampling of experimentally determined parameter distributions of, for example, tip and branch angles, branching frequency or internode length (Hutchinson *et al.*,1980; Yang *et al.,* 1992a,b; Lejeune, Nielsen & Baron, 1995; Lejeune & Baron, 1997). In some cases (Liddell and Hanson, 1993; Soddell *et al.,* 1995; Tunbridge and Jones, 1995), the rules have been encapsulated using string re-writing 'Lindenmayer'- (or L-) systems (Lindenmayer, 1968; Prusinkiewicz & Lindenmayer, 1990; Prusinkiewicz, 2004).

Meškauskas *et al*. (2004a, b; Moore *et al*., 2004) developed the Neighbour-Sensing (NS) model of hyphal growth as an explicit 3-D mathematical model, and a Java™ computer program realization of it, that together generate realistic visualizations of filamentous hyphal growth. The model brings together the basic essentials of hyphal growth kinetics into a vector-based mathematical model in which the growth vector of each virtual hyphal tip is calculated at each iteration of the algorithm by reference to the surrounding virtual mycelium. In this model the branching frequency, position and orientation are determined directly by model components, rather than through a random stochastic process. Regulation of growth and branching occurs in response to evaluation of tropisms that are represented by local density-dependent

fields. The most important field is a negative autotropism, which is an abstract representation of growth regulation due to substrate depletion or accumulation of inhibitory metabolites. The model also includes a secondary long-range autotropism that represents the type of interaction that might arise from diffusible signalling molecules. Other tropisms reflect the impact of physical factors like gravity and electrical fields, and can also represent physical constraints. Among the latter is a field (a horizontal plane tropism) that limits growth to a layer similar to that which a mycelium will encounter in the surface layers of soil or in Petri-dish cultures on agar media.

Visualization of the output of the NS model show striking similarities to actual colony development. For example Fig 6A shows *in silico* growth of a spherical colony, similar to structures formed in liquid culture, whilst the effect a horizontal growth constraint, equivalent to growth of a colony on Agar, is shown in Fig. 6B. The model outputs various colony statistics, such as total mycelial length (which is proportional to total mycelial mass) and internode length, that assist in

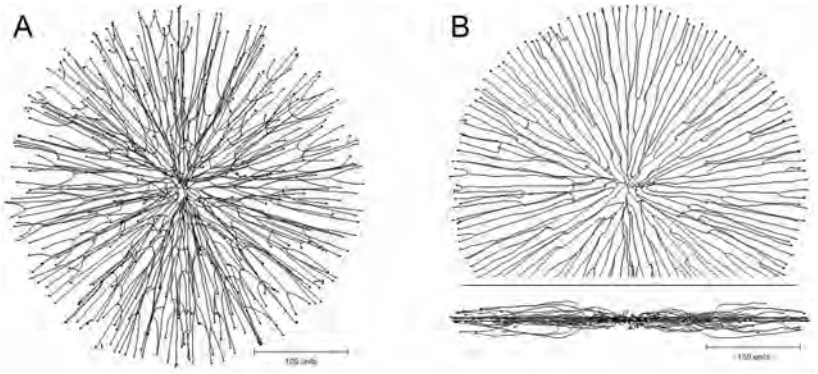

Fig 6 Visualizations produced by the Neighbour-Sensing model of hyphal growth, illustrating the effect of the horizontal plane tropism on the shape of the colony.

At extreme left is a view of a spherical colony grown in 220 iterations assuming a negative autotropic reaction and density-dependent branching (branching probability 40% per iteration), with the density field being generated by all of the mycelium. The spherical colony results when there is no physical constraint. Applying a horizontal plane tropism that restricts growth to a thin horizontal zone produces the morphology (shown at right) of a circular colony (viewed from above) with a narrow profile (side view).

comparison with real experimental data (Meškauskas *et al*. 2004a, b; Moore *et al*., 2004).

In the current context, a key limitation of most of the 'morphological' models presented (with the exception of the early 'branch' model developed by Ermentrout and Edelstein-Keshet, 1993) is the absence of anastamoses, that would create the loops and shortcuts needed to generate interesting behaviour from a network perspective. This is currently under development for the NS-model Once this is in place, the *in silico* approach will be able to rapidly generate biologically inspired networks, with tunable parameter sets for evaluation against their theoretical, physical and social counterparts.

Translation into network nomenclature

To explore whether it is possible to apply network analysis tools to fungal mycelia, it is necessary to translate the morphological structures observed or simulated into a form appropriate for network modeling. Our starting assumption is that the fungal mycelium forms a spatial network that can be represented as a graph comprising a set of nodes (or vertices) connected by links (or edges). The first decision is to fix the appropriate level of resolution needed to characterize the network structure. Even in a laboratory microcosm, the mycelium has a structure spanning several orders of magnitude from the branching of individual hyphae at around 0.1 mm length scale to the entire colony diameter around 24 cm and may comprise hundreds of thousands of tips and internodes.

As our first approximation we focus on cords as the most convenient spatial scale as these are readily identifiable discrete structures that represent the major transport pathways through the mycelium. Each branch point or junction is represented as a node and the persistant cords connecting them form the links. The degree of each node (k) is given by the number of links associated with that node. Thus tips will have a degree of 1 as they are only connected to the previous node, branch points will typically have a degree of three, because of the growth processes forming the network tends to give a single branch or a single fusion (anastomosis) at each point. It is unlikely that there will be any loops where a link curls back round on itself to re-join the same node,

although multiple parallel links between two nodes are possible. As the fine structure of the mycelium within a food resource (agar or wood block) cannot be resolved, each of these is represented as a node with many links, resembling a hub in other network systems.

A number of different quantities are typically measured for a network including:

(i) The minimum path length that must be traversed between two nodes;
(ii) The local clustering or transitivity that measures the probability that if a node is connected to two other nodes, they will also be connected to each other;
(iii) The degree distribution, which is given by the frequency of nodes of with different numbers of links
(iv) The network resilience, which estimates the extent that the network properties change as nodes or links are removed from the network.

To understand the behaviour of real-networks, it has proved instructive to generate model networks that are connected according to well-defined rules whose products have varying properties (Strogatz, 2001; Dorogovtsev and Mendes, 2002; Newman, 2003; Amaral and Ottino, 2004). For example, in the simplest random graph models, each node has a random probability of connection to any other node, giving a bell-shaped Poisson degree distribution for a network with a sufficiently large number of nodes and links. The average shortest-path length between two nodes scales with the logarithm of the total number of nodes. This means that, even in a large network it is possible to move between two nodes with relatively few steps, termed the 'Small World' effect. On the other hand, the transitivity or clustering coefficient is very low as the likelihood that adjacent nodes are all interconnected is low.

Many natural and artificial networks also have a mean path length that scales logarithmically or slower with network size, but also exhibit a very high clustering coefficient, unlike the random graph model. There are several ways that this behaviour could arise. One of the first models developed by Watts and Strogatz (1998) introduced a small number of 'shortcuts' between different parts of a regular network which had the

effect of producing low average shortest-path lengths typical of random graphs, but with the high clustering typical of regular lattices.

The degree distribution arising from the Watts and Strogatz small-world network construction was found to decay exponentially. In practice, the degree distribution for several networks, deviates substantially from either a Poisson or exponential form, with a much longer tail of highly connected nodes. These frequency distributions tend to follow an inverse power law relationship with increasing degree. Barabási and Albert (1999) suggested that a power law distribution (also termed 'scale-free' distribution) may arise in a growing network by preferential attachment of new vertices to older vertices that are already highly connected, giving rise to 'hubs'. These networks show short mean-path lengths, but with higher levels of local clustering than expected from a random graph model. Scale free networks are also resilient to random removal of nodes or links, but are highly sensitive to targeted removal of the most highly connected nodes (Albert, Jeong and Barabási, 2000).

Most network analysis has focused on the network topology rather than the spatial relationship of the nodes, even though this must impose constraints on the probability of their inter-connection (Gorman and Kulkarni, 2004; Artzy-Randrup *et al.,* 2004). In a spatial network, nodes are likely to have a much higher probability of connecting to their physical neighbours and, depending on the way the network is built, low or even zero (in planar networks) probability of links crossing-over each other without forming a new node. This makes it difficult or impossible to create topological equivalents to 'short cuts' between physically remote parts of the network. Equally, it is interesting to speculate that inclusion of weighting by transport speed and/or capacity may have the equivalent effect of long-range communication, bringing distant parts of the network into closer contact than expected from their spatial separation or unweighted path-length.

Although in theory it is possible to generate large 'hubs' characteristic of a scale-free network in a planar spatial network, there may be physical constraints on the number of links that can be accommodated, suppressing the emergence of nodes with very high degree (Amaral *et al.,* 2000).

In the case of the *Phanerochaete velutina* grown in a 24 cm square microcosm, the size of the corded experimental networks is around 300-500 nodes, depending on growth conditions. It might be appropriate to consider the links to be directed on the basis of their initial growth

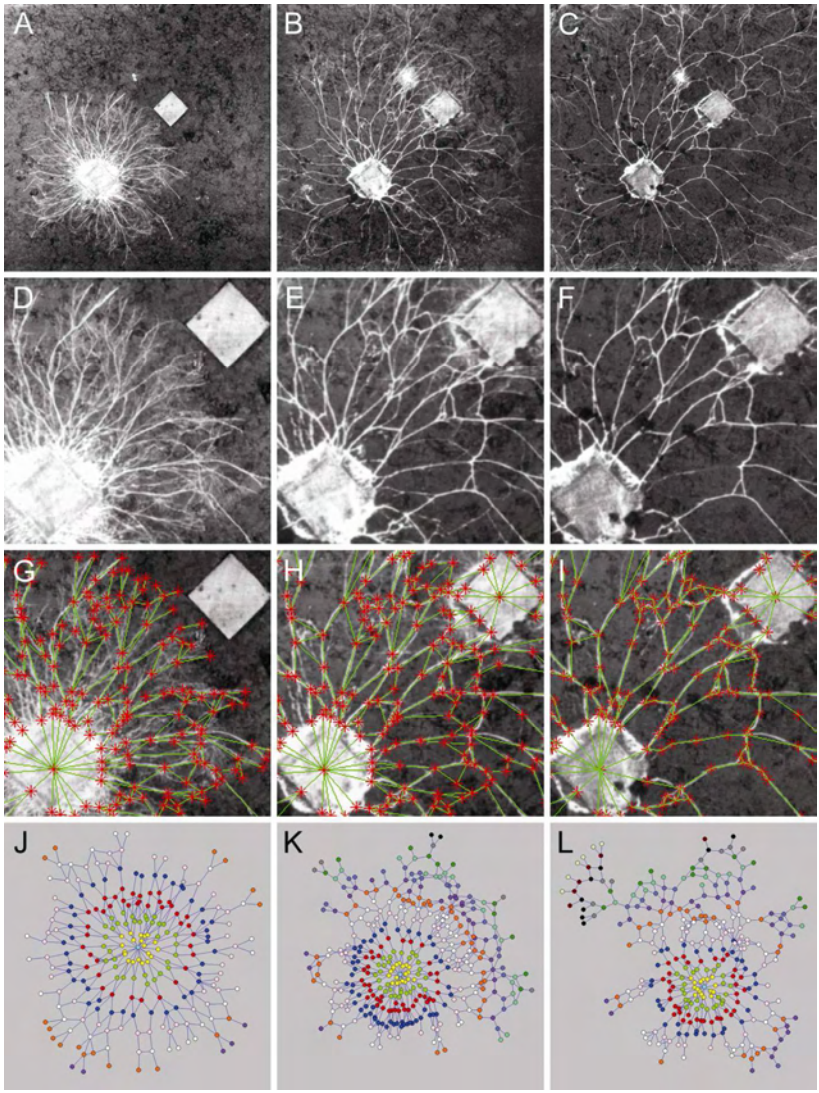

Fig. 7 Network analysis of mycelial development in *Phanerochaete velutina*

A-C: Digital images showing the development of *Phanerochaete velutina* mycelium extending from 2 cm^3 beech (*Fagus sylvatica*) inocula to 4 cm^3 beech wood resources in 24 x 24 cm trays of compressed non-sterile soil, after 9 d (A), 25 d (B), and 39 d (C). The prolific development of much branched, fine *P. velutina* mycelium in the 1 o'clock position, after 25 and 39d, corresponds with the location of a small piece of organic matter colonized by another fungus (visible in the 9 and 18 d images). Digital images were obtained from photographs taken by Rory Bolton.
D-F: Enlargement of the region between the initial inoculum and the bait showing the developing network structure in more detail
G-I: Result of manual superposition of nodes (red asterisks) and links (green) on the developing network image. By 39 d, several of the links have regressed leaving nodes of degree 2 on the remaining cords
J-L: Colour-coding of node distance from the central inoculum for each time point, following removal of degree 2 nodes. Images were generated using Pajak. ()

direction. In practice, the physiological direction of nutrient fluxes is more important and does not have to follow the developmental connection sequence. Unfortunately, we cannot predict *a priori* which direction the flux may move in and, indeed, we expect it to vary depending on the source-sink relationships within the network. We are currently developing techniques to map fluxes *in vivo* (Tlalka *et al.,* 2002; 2003; see Fig. 3&4), but at this stage it is simpler to assume that links are bi-directional.

The degree distribution of the fungal networks so far examined shows most nodes have three links as anticipated, a significant number have one connection (tips) and the inoculum and additional sources are more highly connected. The presence of 'weak hubs' means that the network may exhibit some of the properties associated with scale-free networks (Barabási and Albert, 1999), although at best the scaling can only operate over a few orders of magnitude. The construction algorithm is also initially somewhat different. Thus during growth of a conventional scale free networks new nodes are preferentially attached to older nodes with higher k. In the fungal case, 'hubs' initiate a large

number of new nodes (tips) and links, whilst subsequent new nodes arise almost exclusively from branching or anastamosis and therefore have a low degree. Moreover, new nodes are likely to form in the middle of existing edges rather than as connections to pre-existing nodes. The

network architecture is not static, but continuously evolves. For example, growth from the inoculum to connect to a new food source progress through an initial proliferation phase with many links forming (Fig. 7A&D), followed by selection and re-inforcement of a subset of paths to create a more limited number of strong links (Fig. 7B&E) eventually with regression of the remainder of the links to leave a sparser network (Fig. 7C&F). Nodes can be (manually) assigned to each tip, branch or fusion during colony development to try to understand the network topology that evolves (Fig. 7G-I). In the early stages of growth a substantial number of cords are developing. However, there are also a considerable number of fine foraging hyphae that cannot be resolved clearly (Fig. 7G). By 18 days, the mycelium has contacted the new food resource, established more obvious cords and much of the fine mycelium has receded. By 39 days, the number of interconnected cords has reduced further, although the history of the previous connections remains as a series of nodes with degree 2 left on the main connecting cords. This pattern of development is reflected in the frequency histogram of node degree (Fig. 8). Despite the massive growth of the colony, the number of cord tips stays around 50 through the period. However there is a dramatic rise in the number of degree 2 nodes emerging as a result of the history of link loss (Fig. 8). If these are excluded from the analysis, the average degree for each node stabilizes at around 3. The mean path length (L) increases slightly from 7 to 10, and the diameter (d) of the network increases from 16 to 24 (Fig. 8). The network forms a highly interconnected reticulate system with many 4, 5 or 6 node rings spreading away from the central inoculum (Fig. 7J-L). In this representation, nodes are colour-coded by their distance from the inoculum.

The fungal mycelium is a transport system so it is pertinent to ask how the structure described above fits into the recent discussions on topology of the fittest transportation networks (West *et al.,* 1997, Banavar *et al.,* 1999; 2000). The West *et al.* (1997) model seeks to explain allometric scaling laws in biology by minimization of the energy dissipated through space-filling fractal networks of branching tubes. At the moment the fungal network does not seem to sit well in the framework derived for non-pulsatile flow, as it is not clear that the size

of the transport tubes will fulfill the requirements for area-conservation and the transport velocity is not constant but varies considerably throughout the network. However, it is of considerable interest that the introduction of a pulsatile component can compensate for the lack of area-conservation and the variation in flow rate for the mammalian circulatory system (West *et al.,* 1997). One of the most striking features of radiolabelled amino acid transport so far is the presence of a pulsatile component (Tlalka *et al.,* 2002, 2003). The coincidence of these observations may prove a fruitful area for future research.

The approach taken by Banavar *et al.,* (1999, 2000) also seeks to explain allometric scaling laws, but focuses on the predicted network topology needed for efficient transport systems characterized by a minimum overall cost. A central result is that spanning trees are expected if the cost function for transport scales with a power of less than 1 with respect to the amount of material, whilst loops will emerge if the power

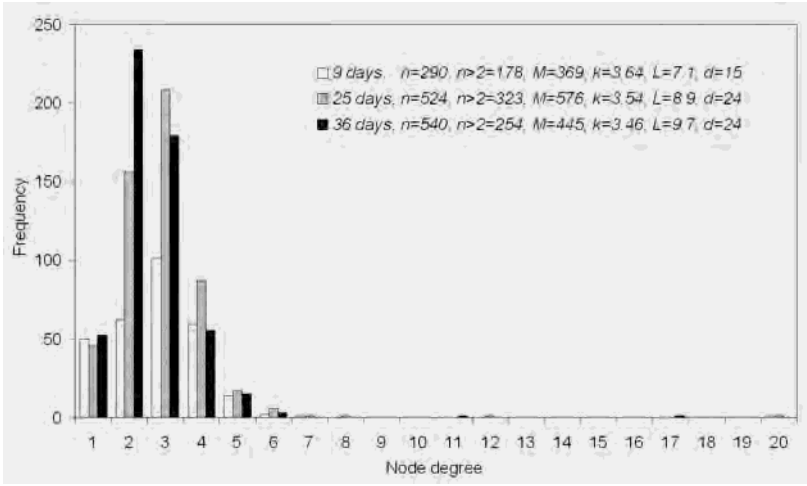

Fig. 8 Frequency histogram of node degree for mycelial networks of *Phanerochaete velutina* at different time points during development in a baited microcosm.

The legend gives the total number of nodes (*n*), the number of nodes with degree greater than 2, the number of links (*M*), the average degree of each node (*k*), the average minimum path length between nodes (*L*) and the diameter of the network (*d*).

relationship is greater than 1. The cost function for transport in the fungal system is not known. However it is interesting to note that the initial growth phase in the micron to mm range takes place as a branching tree when process such as diffusion may be sufficient to drive transport, whilst transport over distance longer than a few mm requires an active component (Davidson and Olsson, 2000; Boswell *et al.,* 2002). In the case of *Phanerochaete velutina* this length scale is also associated with anastomoses and the development of a network with many loops.

Whilst efficient transport is likely to shape fungal mycelial networks, resilience to accidental damage and predation is also likely to be important. In a spatial network, the probability of node or edge removal is unlikely to be random, and may also show a high degree of correlation between adjacent nodes. For example, grazing by soil invertebrates may be in specific locations in the network. This is because some regions are more palatable than others. Part of the resilience of such a biological network may not be just the architecture of the network prior to damage, but the ease and efficiency that the network can reconnect itself. In this respect, a self-organising spatial network may have considerable advantages over a random network in the cost, consistency and efficacy of the rewiring process needed to re-establishment of a functioning system.

Simple networks in the plasmodial slime mold Physarum

Whilst network analysis of mycelial fungi is in its infancy, considerable progress has already been made in the analysis of simple networks in the plasmodial slime mould *Physarum polycephalum* (Nakagaki, 2001). *Physarum polycephalum* forms a network of interconnected tubular elements that enable widespread foraging for resources whilst maintaining connectivity throughout the plasmodium. As isolated sections of the plasmodium will spread and coalesce back into a single organism, it is possible to establish an almost homogeneous sheet of tissue across a nutrient free agar substrate through experimental manipulation (Fig. 9A). If a number of localized food sources are placed on the sheet, the plasmodium preferentially colonises the resources, but also retains a network of tubes that interconnects the entire plasmodium

(Fig. 9A-C). By positioning the food sources in specific geometric patterns or mazes it is possible to assess the extent that the resulting networks balance the cost of maintaining a connection, encapsulated as the length of connecting tube, with the potential risk of the plasmodium becoming fragmented (Nakagaki *et al.,* 2000a; Nakagaki, 2001; Nakagaki *et al.,* 2004).

Slightly different criteria are needed to analyse spatial networks in comparison to the non-spatial, relative networks. Thus the degree of separation is defined as the number of transit food sources through along the shortest path between two food sources. The average separation (AS) is the degree of separation averaged over all pairs of food sources, and decreases as food sources are more closely coupled. To allow comparisons between different arrangements it is normalized to the average separation for the minimum spanning tree. The fault tolerance (FT) is the probability that the organism is not fragmented into separate pieces if an accidental breakage occurs at a random point along the tubes. Since the probability of disconnection of a tube is proportional to its length, a longer tube has a higher risk of disconnection. The combined index, FT/TL, can be regarded as a measure of the ratio of benefit to cost. By judicious positioning of food sources, the geometry of the network can be compared to possible theoretical solutions in terms of path length and fault tolerance, such as the minimal spanning tree (MST), the Steiner minimal tree (SMT) and a Delaunay triangulation network (DTN). Examples are given in Fig 9 for the predicted network with 3 food sources (Fig. 10 D) and experimental results for 3 food sources (Fig. 9E-G), 6 and 7 food sources (Fig. 9H&I) with the associated analysis of path length and fault tolerance (Fig. 9J), rings of 12 food sources (Fig. 9K&L) and grids of 64 food sources (Fig. 9M&N).

In all cases, the network that is established by the plasmodium has a relatively short total length of interconnecting tubes, but maintains close connections among all the food sources and exhibits a high tolerance to accidental fragmentation (e.g. Fig. 9J; Nakagaki *et al.,* 2004). The behaviour of *Physarum* is interesting not just because it suggests there is potential for analyzing biological systems in terms of their optimal network properties, but because the mechanisms that underpin its behaviour have parallels to dynamic phenomena observed in other

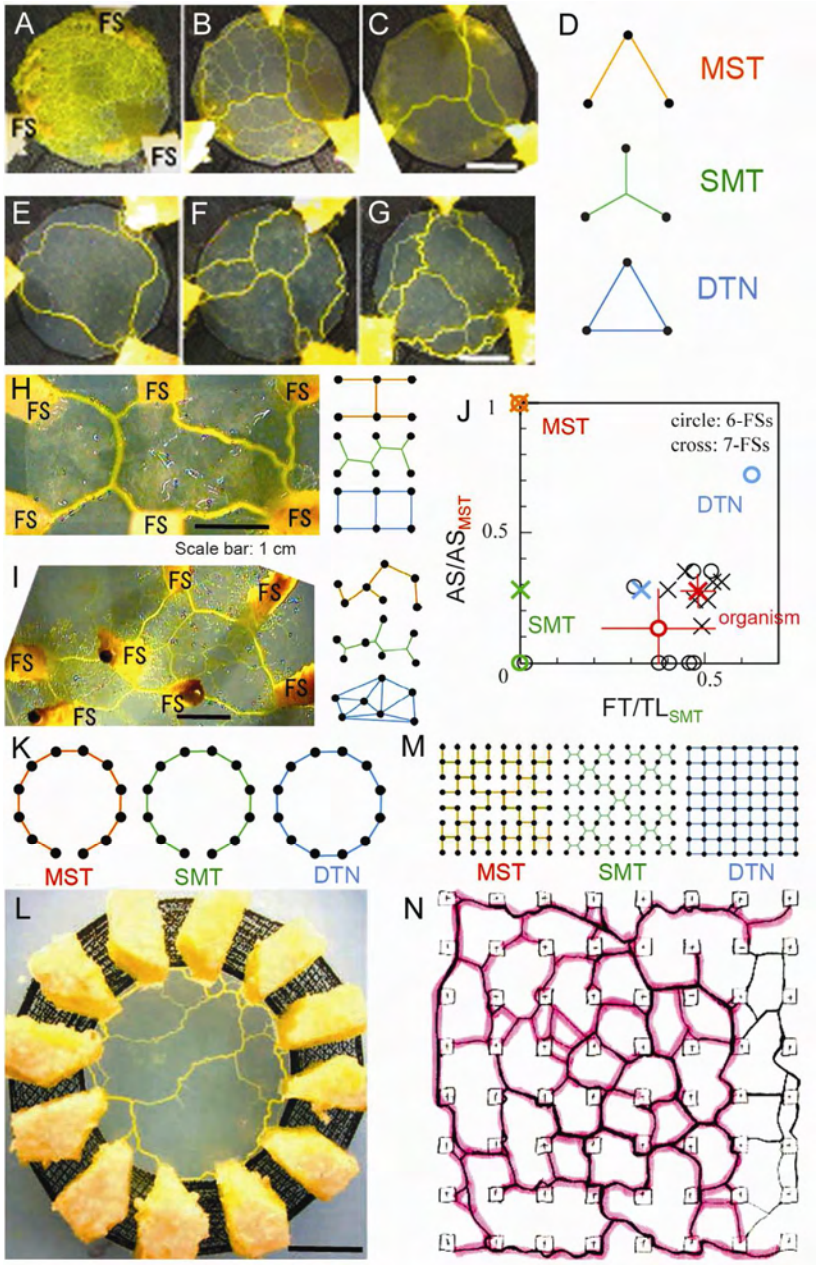
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Fig. 9: Self—organisation of robust network architecture in *Physarum polycephalum*

A-C: Development of a network between three food sources, starting from a continuous 'sheet' of tissue. Network structure at 0h (A), 6h (B) and 36h (C). Scale bar = 1 cm.
D: Schematic illustration of the arrangement of food sources (black dots). The orange, green and blue lines represent the network of minimum spanning tree (MST), Steiner's minimal tree (SMT) and Delauney triangulation network (DTN), respectively.
E-G: Three typical networks in ascending order of total length (TL) after 35 h. Scale bar = 1 cm..
H&I: Typical emergent network structure with six (H) and seven (I) food sources and schematic representation of the corresponding MST (orange), SMT (green) and DTN (blue).
J: Properties of the plasmodial networks, defined by average separation of food sources (AS), normalised to the value for the minimal spanning tree, and benefit to cost ratio, defined as the fault tolerance over the total length (FT/TL). Black symbols give the value for each specimen, and red, the value of the mean with associated S.D. Orange, green and blue symbols give the values of MST, SMT and DTN respectively. The organism maintains a short total length of tubes with close connections between food sources yet high tolerance of accidental disconnection.
K-N: Network organisation with ring (K&L) and grid (M&N) arrangement of food sources. These systems also show robust network architecture, with short path length but high fault tolerance.

network systems. A natural part of the growth and development of the plasmodium involves contractile pulsing and the changes in the tubular structure of the plasmodium observed are closely related to the spatio-temporal dynamics of cellular rhythms (Nakagaki, *et al.,* 2000b). When coupled in appropriate ring systems, these oscillations synchronise in accordance with predictions for coupled oscillators in other systems (Takamatsu *et al.,* 2001) and it is possible that such oscillatory coupling is a key mechanism underlying network formation across a wide range of network types (Strogatz, 2001).

Future perspectives

Bacterial systems provide good experimental systems to understand self-organisation and pattern formation where the underlying processes have strong parallels with self-organising systems in the physical sciences. They are perhaps less useful in providing insight into network design, as the physical structure of the colony (motile or non-motile

individuals) and the flow of information (local physical influences and coupling by diffusion fields) do not necessarily map well onto man-made networks. On the other hand, foraging systems in cellular slime moulds and mycelial fungi form more persistent transport networks that appear to exhibit interesting properties, in terms of self—organisation, adaptability, cost/benefit ratio and fault tolerance. Although at a very early stage, there appear to be potential benefits on both sides from using network analysis tools to better understand the biology and also to use the biology to highlight potential development of novel strategies in artificial network design. From our experience so far, it is clear that widespread expansion of this approach will need much more efficient ways of translating images of both the network architecture and its physiological function into network topologies suitable for analysis.

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