Obtaining Multiple Separate Food Sources: Behavioural Intelligence in the Physarum Plasmodium

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Obtaining multiple separate food sources: behavioural intelligence in the *Physarum* plasmodium

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To evaluate performance in a complex survival task, we studied the morphology of the *Physarum* plasmodium transportation network when presented with multiple separate food sources. The plasmodium comprises a network of tubular elements through which chemical nutrient, intracellular signals and the viscous body are transported and circulated. When three separate food sources were presented, located at the vertices of a triangle, the tubular network connected them via a short pathway, which was often analogous to the mathematically shortest route known as Steiner's minimum tree (SMT). The other common network shape had high fault tolerance against accidental disconnection of the tubes and was known as cycle (CYC). Pattern selection appeared to be a bistable system involving SMT and CYC. When more than three food sources were presented, the network pattern tended to be a patchwork of SMT and CYC. We therefore concluded that the plasmodium tube network is a well designed and intelligent system.

Keywords: *Physarum*; small-world network; network dynamics; amoeboid behaviour; cell intelligence

1. INTRODUCTION

Living organisms are systems that effectively perform specific tasks that are necessary for survival. For example, the large unicellular amoeba-like true slime mould *Physarum polycephalum* can find the shortest route through a maze to locate food sources placed at the exits, and to absorb the maximum amount of nutrients in the shortest time possible (Nakagaki *et al.* 2000*a*). This suggests that the cell is capable of 'intelligent' behaviour, even in complicated situations in which it is difficult to optimize survival tasks. Two important questions are raised by this hypothesis: how smart is this behaviour and how is a smart solution reached?

The transport network in plasmodial slime moulds is a useful system in which to address these issues. The *P. polycephalum* plasmodium is an aggregate of protoplasm with a network of tubular elements through which nutrients and chemical signals circulate, the geometry of which is related to internal communication. Moreover, the tubes act as 'legs', allowing the organism to navigate around its environment, and can be disassembled and reassembled within a few hours in response to changes in external conditions. This system is therefore well suited to the study of behavioural and morphological cell 'smartness'.

When many small food sources—agar blocks containing oat flakes—are presented at various positions to a starved plasmodium, it endeavours to reach them all; as a consequence, only a few tubes are in contact with each individual food source. The organism attempts to optimize the shape of the network to facilitate effective absorption of the available nutrients. However, this might be difficult to achieve when multiple food sources are presented because of the limited body mass of the organism.

The effects of two separate food sources have previously been studied, and minimizing the total length (TL) of the network was found to have an important role (Nakagaki *et al.* 2000*a*, 2001). This network shape of the body enables certain physiological requirements to be met: (i) absorption of nutrients from food souces as efficiently as possible because almost all the body mass stays at the food sources to enable absorption; (ii) maintenance of the connectivity and intracellular communication throughout the organism; and (iii) meeting the constraint of limited resource of body mass. The network shape is regarded as a solution for the organism's survival problems.

Contrary to this, if food is plentiful, the organism finally splits into two pieces on two food sources. Even this case, however, just before the splitting, the organism traces the shortest connection only (Nakagaki *et al.* 2001). Therefore, the shortest connection appears once as a transient shape and is lastly disconnected, then the disconnection results in separation of the organism.

In addition, a model with multiple food sources has been investigated (Nakagaki *et al.* 2004) in which additional factors such as fault tolerance (FT) and the average degree of separation were examined. The shortest path connecting three points on a plane is not always a combination of the straight lines that connect any two of these points. In the case of less than 120° in the widest angle of a triangle, the position where these paths intersect, known as the Steiner point, must be found. For example, the minimum route between the three vertices of an equilateral triangle consists of three straight lines between each vertex and the mass centre of the triangle; the Steiner point corresponds to the mass centre in this case. The biological problem becomes more complex as the number of food sources increases to more than three. Here, we mainly investigate the effects of three food sources and discuss the results in the context of previous studies.

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2305

2. METHODS

(a) *Organism and presentation of food sources*

The frontal parts of a *P. polycephalum* plasmodium (15 cm × 30 cm) that had been regenerated from the sclerotia and starved for 12 h before the experiment, were cut into small pieces (1 cm × 1 cm) and placed on agar surfaces of the required shape. The small plasmodia extended and fused spontaneously to form a single large plasmodium. Three equal-sized agar blocks, which contained concentrated nutrients in the form of powdered oat flakes (100 mg ml^{-1}), were then presented at the required positions; for example, the vertices of an equilateral or isosceles triangle. The macroscopic morphology of the resulting tube network was observed using the methodology described by Nakagaki *et al.* (2004).

(b) *Two measures for evaluating the network shape*

Two measures were used to evaluate the network pattern: the TL of the tube network and FT against accidental disconnection of the tubes (Strogatz 2001; Nakagaki *et al.* 2004). TL is expressed as a dimensionless value, which is the TL divided by that of Steiner's minimum tree (SMT). FT_N is defined as the probability that the organism (strictly speaking, every part of the body staying at the food source) is not fragmented if N accidental disconnections occur at random points along the tubes. FT_1 is calculated as follows: let us assume that one disconnection occurs and that the probability of a disconnection is proportional to the ratio of tube length to total network length. We must test whether or not the organism is fragmented. For instance in figure 1a(i), because there are three straight lines with an equal length, each has the same probability of disconnection leading to inevitable fragmentation of the organism, hence $FT_1 = 0$. For figure 1a(iv), there are also three lines with an equal probability of disconnection, but the organism is never fragmented, hence $FT_1 = 1$. Because figure 1a(iii) consists of a triangle and an additional line, the organism is not fragmented if and only if a disconnection occurs on the triangle, which has 79% of the TL of all tubes; so $FT_1 = 0.79$.

Consider now the case for two disconnections on the network as illustrated in figure 1a(iv). If an identical line is disconnected twice, then the organism is not fragmented, but disconnection of two different lines leads to fragmentation. Hence $FT_2 = 0.33$ because all three lines have equal length and the probability of choosing the same line twice is 0.33. In figure 1a(iii), fragmentation does not occur if the same line of the triangle is disconnected twice, and there are two different lengths of line in the triangle which have probability of choice, 0.21 and 0.37, so that $FT_2 = (0.21)^2 \times 2 + (0.37)^2 = 0.23$. A longer tube has a higher risk of disconnection. Therefore, smaller values of TL but higher FT values are advantageous. These trends result from the physiological requirements of the organism. Generally, FT becomes higher as the TL increases, which reflects the relative cost of redundant connections to food sources. Therefore, we also calculated the combined FT/TL index, which expresses the cost–benefit ratio. Examples of networks with good scores are shown in figure 1a.

3. RESULTS

(a) *Network shapes with short total length and high fault tolerance*

Figure 2 shows the variation in macroscopic cell structure over time, starting from sheet-like initial conditions, in a system with three food sources located at the corners of an equilateral triangle. The thicker tubes persisted and the thinner tubes gradually diminished (figure 2b–e) until a simple tube network had formed (figure 2f). The final pat-
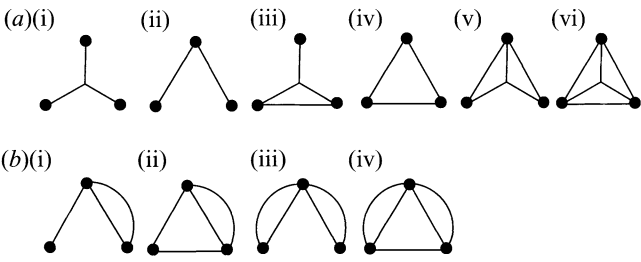

Figure 1. Possible connection paths between three vertices of an equilateral triangle. (a) Examples of efficient network shapes: (i) SMT; (ii) minimum spanning tree; (iii) an analogue of SMT; (iv) cycle (CYC); (v) an analogue of SMT; and (vi) a combination of SMT and (CYC) (COM). (b) Examples of alternative network shapes produced in the experiment; (ii) and (iv) are both analogues of CYC. The connectivity between food nodes was the focus of this investigation, rather than other factors, such as the zigzag and the wave of the tube paths.

tern of the tube network showed connections to all three food sources through a few thick tubes. This was analogous to the shortest connection route: that is SMT, according to graph theory (figure 1a(i)). A range of final shapes of the network patterns was observed 30 h after food presentation, as shown in figure 3. However, in all cases the three food sources were connected through only a few thick tubes.

Figure 4 illustrates the TL, FT_1 and FT_2 values of these experimental network patterns. Reference values from the high-scoring network patterns shown in figure 1a are included for comparison. Despite large deviations in TL, the FT values were similar at a fixed TL and a master curve of the relationship was plotted (indicated by the dotted lines in figure 4). All of the network patterns (indicated by circles in figure 4) had similar FT_1 and FT_2 values to the reference values (indicated by crosses); that is, for all of the TL values, the FT observed represented the optimal value under the constraints of that specific TL. This shows that the organisms consistently maximized FT at different TLs.

We also calculated the combined index $\alpha = FT/TL$ as a cost–benefit ratio. The α-values were graphically obtained from figure 4 and represent the intersection points of the FT/TL relationship curve and the straight line of $FT = \alpha TL$. As α is a slope of the line, we can visualize the scale of α as shown by the grey dashed lines in figure 4. One-half of all data points were over 70% and 80% maximum level in FT_1 and FT_2, respectively. This implies that the organism made a trade-off between FT and TL. Shapes from reference networks with high scores were superimposed close to the corresponding crosses in figure 4.

(b) *Bistable selection of Steiner's minimum tree and cycle*

The most frequent network shape was a combination of SMT (figure 1a(i)) and CYC (figure 1a(iv)), defined here as COM (figure 1a(vi)); this occurred in 22% of the samples (total number of samples was 50) and was sometimes an intermediate state during network formation, which subsequently transformed into SMT-analogous (figure 1a(i)(iii)(v)) or CYC-analogous shapes (figure 1a(iv),b(ii)(iv)). The percentage occurrence of SMT and its analogues was 26%, and that of CYC and its analogues
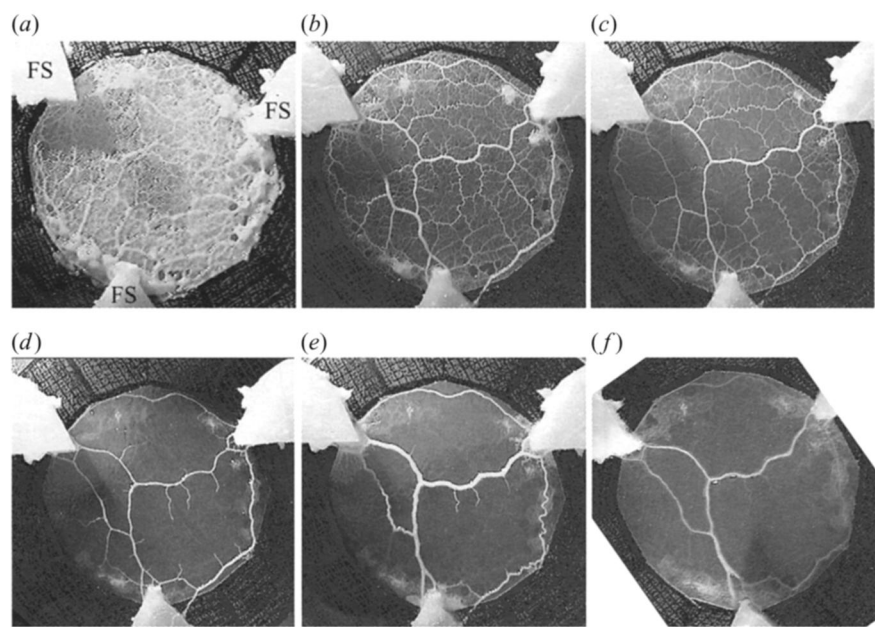
Figure 2. Time series of changes in network shape (top view) after the presentation of three food sources located at the vertices of an equilateral triangle. The diameter of the initial circular organism was 3 cm. The outer black section of each panel is the plastic film covering the agar surface. The network pattern is illustrated: (*a*) 0, (*b*) 6, (*c*) 9, (*d*) 13, (*e*) 26 and (*f*) 33 hours after food source presentation. FS, food source.
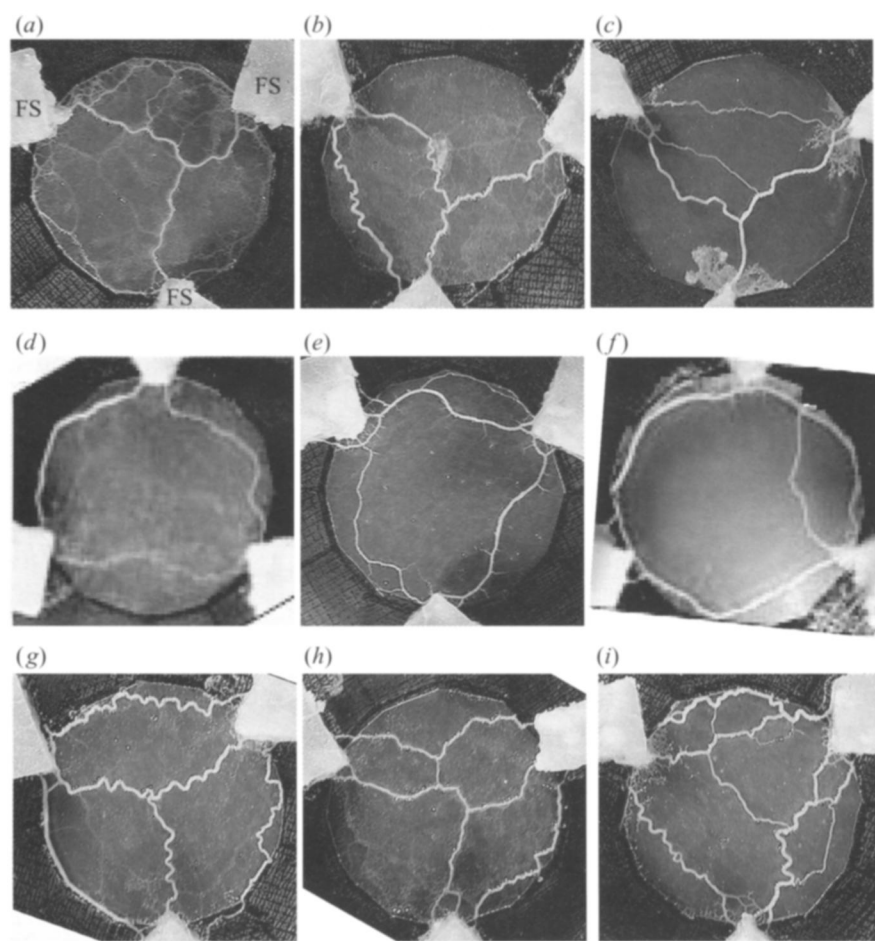

Figure 3. Different tube-network patterns 30 h after food presentation. FS, food source.

was 20%. These three types (SMT, CYC and COM) together accounted for 68% of all those seen. The remainder comprised miscellaneous shapes, such as those shown in figure 1*a*(ii),*b*(i)(iii) and 3*h,i*. It therefore seems that CYC and SMT represent two stable states, with COM as a 'separatrix' between them. Separatrix is a technical term
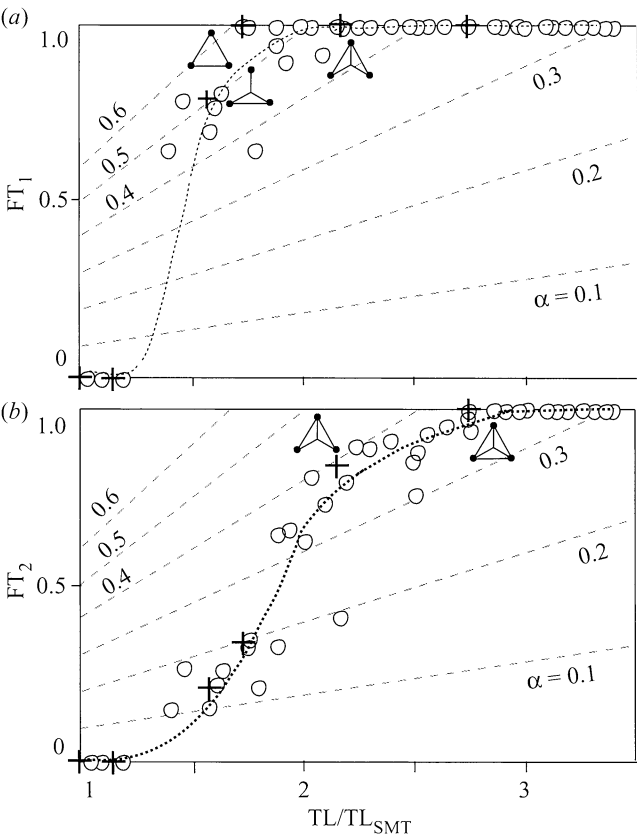
Figure 4. FT scores at different TLs. The abscissa indicates the TL normalized to that of the shortest path (SMT). The ordinate indicates FT against one (FT_1 (a)) or two (FT_2 (b)) accidental disconnections. The circles correspond to the different network shapes and the bold crosses correspond to the reference networks shown in figure 1a. The dotted lines indicate the master curve of the FT/TL relationship. The grey dashed lines show the contours of the ratio of $\alpha = FT/TL$. A few reference networks with high α are superimposed near to the corresponding crosses.

from nonlinear dynamics, indicating a dividing ridge of two flow directions towards each valley. The flow is slower in the vicinity of the divide than on the steep slope of mountain and the flow speed corresponds to speed of change in state. Therefore, the state just on the divide can be observable as a long-time transient.

To further investigate this theory, we examined network evolution starting from an initial COM shape, as shown in figure 5a. The final shape was SMT (64%), CYC (21%) and COM (15%); however, the intermediate shape shown in figure 5b was not observed. The duration of shape change was longer than that observed when starting from conventional sheet-like initial conditions (figure 2a). However, when the initial shape was artificially disconnected at specific points in the CYC- and SMT-components of COM (shown by the arrows in figure 5c,d), the final shapes were SMT and CYC, respectively. In addition, these disconnections shortened the period of shape change compared with an intact initial COM shape. This confirmed that pattern selection appeared to be a bistable system of SMT and CYC.
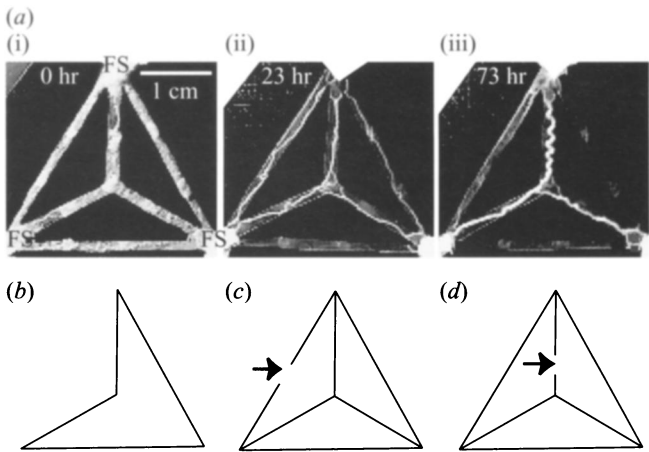
Figure 5. Final network shapes produced from an initial COM shape, which was prepared by using COM-shaped space as the required shape (see § 2). (a) The SMT analogue (similar to figure 1a(v)) and SMT shapes produced after (i) 0 h, (ii) 23 h and (iii) 73 h, respectively. (b) The intermediate shape between SMT and CYC, which was not observed here. (c) An initial shape with an artificial disconnection of the CYC component. (d) An initial shape with an artificial disconnection of the SMT component. FS, food source.

(c) Short tube network with large deviations of the junction location

The position of the junction in the SMT and COM networks deviated around the Steiner point in the equilateral triangle (figure 6a). TL was not sensitive to this deviation, which is shown in the figure by white boxes and black boxes corresponding to paths that are 5% and 10% longer, respectively, than the SMT. Most junctions were found inside the 5% boundary.

To determine whether or not the organism seeks the exact Steiner point, a different food-location pattern was presented. Figure 6b shows the results obtained with an isosceles triangle. The junctions were not distributed around the centre of the Steiner point. Therefore, the organism did not seem to seek the Steiner point, although TL was kept short. This implies that the organism is able to make a good approximation of SMT through finding the shortest connection path. The bio-computational algorithm involved here is not known and represents a challenging problem to be solved in the future.

(d) Local selection of Steiner's minimum tree and cycle

Figure 7a(ii),b(i) shows tube networks similar to SMT with four and six food sources, respectively, in which the positions of the junction differed only slightly from the Steiner point. However, this SMT-like pattern was rare and most of the networks appeared as a patchwork of COM-, SMT- and CYC-like elements. Figure 7b(ii) shows an example with SMT-like connections in the upper half and CYC-like connections in the lower half. It is therefore predicted that the basic process of pattern selection between these two types underlies the morphogenesis of the tube network in complicated situations with multiple food sources.
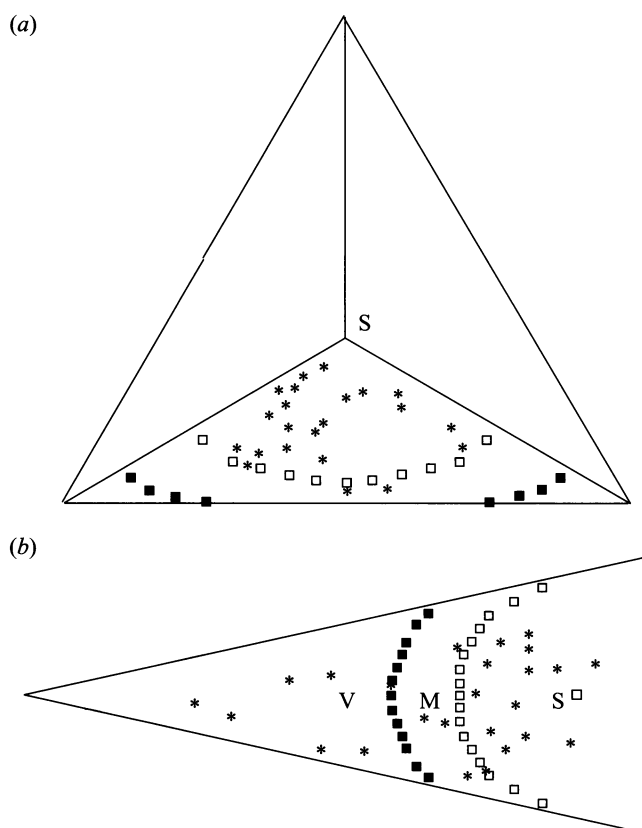
(a)

(b)

Figure 6. Positions of the junction of SMT-like patterns in (a) equilateral and (b) isosceles triangles. The asterisks correspond to those patterns obtained from the experiments. The positions of the Steiner junction (S), mass centre (M) and intersection of the Voronoi diagram (V) are shown. The white and black boxes show junctions corresponding to paths that are 5% and 10% longer, respectively, than the SMT. In the case of the equilateral triangle, all data were transferred to one of three equal sub-triangles because of its symmetry.

4. DISCUSSION

We found that the bulk of the *P. polycephalum* plasmodium accumulated at and covered the food sources presented, leaving only a few thick tubes connecting the quasi-separated components of the organism. The driving force for transportation is the difference in hydrostatic pressure along the tubes (Kamiya 1959); hydrodynamic theory suggests that thick short tubes are, in principle, the most effective for transportation. According to the approximation of Poiseuille flow, increasing thickness allows the flux to increase to the fourth power, which is a particularly effective strategy. The flux also increases in inverse proportion to the length. Therefore, by forming thick short connections, *P. polycephalum* achieves the most efficient exchange of nutrients and chemical signals. This is an effective strategy that allows the plasmodium to solve a complex problem and maximize its performance of a survival task.

How does the organism achieve this optimized solution? Two empirical rules describe changes in the tubular structure of the plasmodium: first, open-ended tubes are likely to disappear; second, when two or more tubes connect the same two food sources, the longer tubes tend to disappear (Nakagaki *et al.* 2001). These changes are closely related to the spatio-temporal dynamics of cellular rhythms as follows.
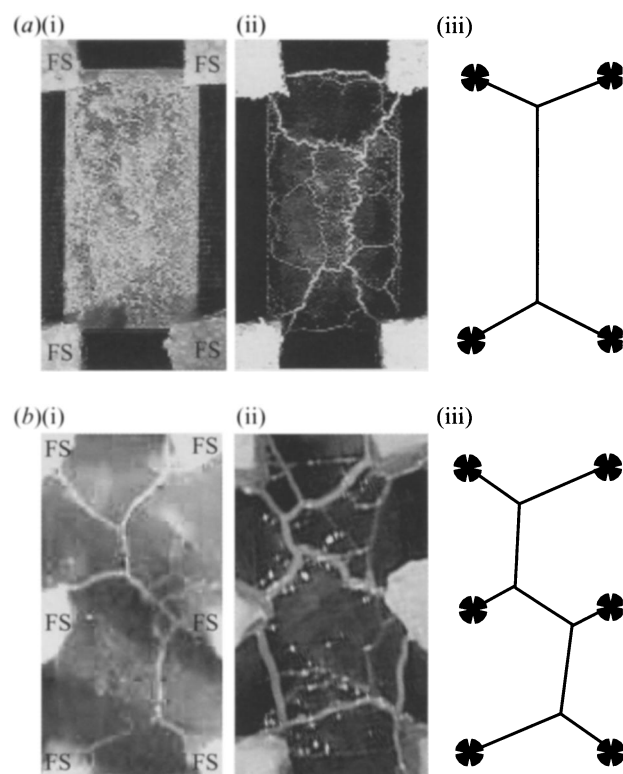
Figure 7. Tube networks with four or six food sources. (a)(i) The initial sheet-like structure just after food source presentation at four vertices of a rectangle. (a)(ii) The final shape 30 h after food source presentation. Thick tubes connect every food source through a connection path analogous to SMT. (a)(iii) The SMT structure. (b)(i) An SMT-like network connecting six food sources, starting from the initial sheet-like structure. (b)(ii) An example of a patchwork pattern with an SMT-like pattern in the upper half and a CYC-like pattern in the lower half. (b)(iii) The SMT structure. FS, food source.

Tubular structures are formed in a specific direction when shuttle streaming of the protoplasm, which is driven by hydrostatic pressure induced by rhythmic contraction, persists in that direction for a certain period (Nakagaki *et al.* 2000b). This experimental result could be explained at a molecular level. In the cortex of the tube, actomyosin fibres are arranged along the tube length, forming a basic architecture of the tube (Stockem & Brix 1994). A similar kind of fibre orientation is induced by artificial stretching of a plasmodial tissue, well-known as the stretch activation effect (Kamiya 1959). This is natural property for a fibrous molecule: for instance, when a sheet made by vinyl chloride is stretched, randomly oriented molecules tend to orient in a uniform direction along the stretching force. This implies that if there is a stretching force inside the organism, it is possible to for it to organize tubular structures by itself. A candidate is the shear stress of fast flow ($1\,\text{mm}\,\text{s}^{-1}$) of a protoplasm. In fact, the estimated magnitude of the shear stress is strong enough to produce the stretch activation. In summary, shear stress of protoplasmic flow induces the stretching effect, which in turn leads to regular orientation of actomyosin fibre as a basic framework of tubes. So the flow pattern of protoplasm, driven by the pattern of contraction rhythms, plays a key role for network formation. In general, we can actually observe various patterns of spatio-temporal variations in phase and amplitude of contraction

oscillation in relation to amoeboid movement of the plasmodium. These pattern dynamics have been modelled by a coupled oscillator system (Nakagaki *et al.* 1999).

In the case of food application, frequency and amplitude of the contraction force are changed just locally at each plasmodial part which contacts with the food source (Matsumoto *et al.* 1986, 1988). Responding to the number and location of food stimulation, contraction oscillators are modulated, otherwise oscillators are almost the same. Under these conditions the process of pattern formation may undergo. Notice that the tube network not only determines the shape of the system but also evolves over time depending on the state of the system. As above, the feedback regulation between the tube network and contraction pattern is a key process.

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