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Emergence of morphological order in the network formation of *Physarum polycephalum*

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Abstract

Emergence in a system appears through the interaction of its components, giving rise to higher order or complexity in the system. We tested for the presence of emergent properties in a biological system using the simplest biological entity of a unicellular organism; the plasmodium of *Physarum polycephalum*, a giant unicellular amoeboid organism that forms a network-like tubular structure connecting its food sources. We let two plasmodium networks within a single cell interact with each other, and observed how the intracellular interaction affected the morphologenesis of the plasmodium networks. We found that the two networks developed homologous morphology. We further discuss the presence of autonomous and emergent properties in homologous network formation.

Keywords: Physarum polycephalum; Emergence; Pattern formation; Network morphology; Rhythmic contraction; Cytoplasmic streaming

1. Introduction

Emergence is a process of generation of complexity or higher levels of organization in a system from basic constituent parts. Emergence occurs through interaction of the system's components and has come to be widely regarded as a key concept in various scientific fields including nonlinear physics, complex systems science, systems theory and the study of artificial life. Emergence appears in any system showing orderly behavior, and in a broad sense involves generation of higher order that is irreducible to lower level description, though the meaning and usage of "emergence" depends on the context of each field (J. Fromm, Types and forms of emergence, electronic preprint, http://arxiv.org/abs/nlin.AO/0506028). The simplest example of emergence is the evolution of sand ripples. Under certain conditions, interactions between sand and water, sand and wind, or between sand grains generate patterns that were not previously present in the sandy surface or among the sand grains [1,2]. Another example presents itself in the behaviors of cellular automata. Given some simple rules, automata produce complex patterns that would not be expected to emerge from a system governed by those rules [3]. Among such emergent systems, biological entities are sometimes considered as the most striking example [4].

The notion of emergence can also be applied within a biological context. For example, swarm intelligence is frequently quoted as an emergent phenomenon. Both ants and bees show orderly behaviors in optimal path finding and nest construction [5,6]. In a similar fashion, cellular function is realized through a collection of biomolecules, and cerebral function through a collection of neurons. These could be considered to constitute emergent systems because higher order appears through the collection and interaction of their components. However, the biomolecules and cells that comprise an organism themselves have a complex structure, so it is not always possible to define all of the rules that control their behavior. The hierarchical structure of a biological system has complexities in each layer and apparent emergent behavior of a biological system can be directly derived from the functions of its components. When we deal with a system in terms of

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emergence, we must therefore clarify the types of interaction that occur between the system's elements and the kind of system structure or organization that gives rise to emergent phenomena. Thus, to investigate the emergent properties of a biological system, we began with experimental observation of emergent phenomena in the simplest biological entity, a unicellular organism. In this study, we established an experimental system using the plasmodium of *Physarum polycephalum*.

The plasmodium of *P. polycephalum* is a large unicellular, multinuclear amoeboid organism. This giant amoeba crawls on plane surfaces and stretches itself to reach multiple food sources; however, all parts of the cell body remain connected by cellular tubes so as to maintain integrated unity as a single cell. In this process, the plasmodium forms a tubular network in an attempt to keep the cell volume required for the connecting tubes as small as possible. This process effectively requires the plasmodium to solve mathematical problems. If two food sources are placed at two points in a maze, the plasmodium connects them by the shortest path, effectively providing the solution for the maze [7–9]. Furthermore, the plasmodium makes connections between multiple food sources by forming efficient networks such as Delaunay triangulation network (DTN), minimum spanning tree (MST), or Steiner minimum tree (SMT) [10,11]. In such path finding and network formation processes, it is possible to clearly observe and estimate the generation of morphological order by mathematically analyzing the resulting tubular structure of the plasmodium.

In the present study, we attempted to establish an experimental system to observe emergent phenomena that appeared as a result of interactions in the cellular system. We let two plasmodium networks interact with each other and tested the effect of intracellular communication on network formation. We demonstrated that two connected networks form homologous networks, thus implying an underlying emergent mechanism in the morphogenesis of *Physarum* plasmodium.

2. Materials and methods

2.1. Physarum plasmodia culture

We cultivated *Physarum* plasmodia according to the method of Camp [12]. Briefly, plasmodia were cultured at 20 °C on wet paper towels laid out on glass Petri dishes in a plastic box. The space below the towels was filled with tap water to supply moisture. Oatmeal was fed daily.

2.2. Experimental system

After at least 24 h of starvation, the frontal parts of *Physarum* plasmodium were cut into small pieces (approximately 0.5 × 0.5 cm) for experimental use. Two types of experimental fields were prepared for the plasmodia (Fig. 1): a single circular field (the single network system; SNS) and a pair of circular fields with a connecting path (the dual network system; DNS). Briefly, circles 8 cm in diameter were cut out from plastic film. Then from each of these larger circles, 4 circles of 2 cm in diameter were cut out and removed. For the SNS, the experimental field consisted of a single circle, and for the DNS, a short path (0.5 × 2 cm) was cut to connect two circles so as to provide an experimental field consisting of a pair of circles. This 4-holed circular film was then placed on 1.5% agar gel in a 9 cm Petri dish.

A piece of plasmodium was placed on the edge of each circle and left undisturbed in the dark for 6–8 h at 20 °C until the

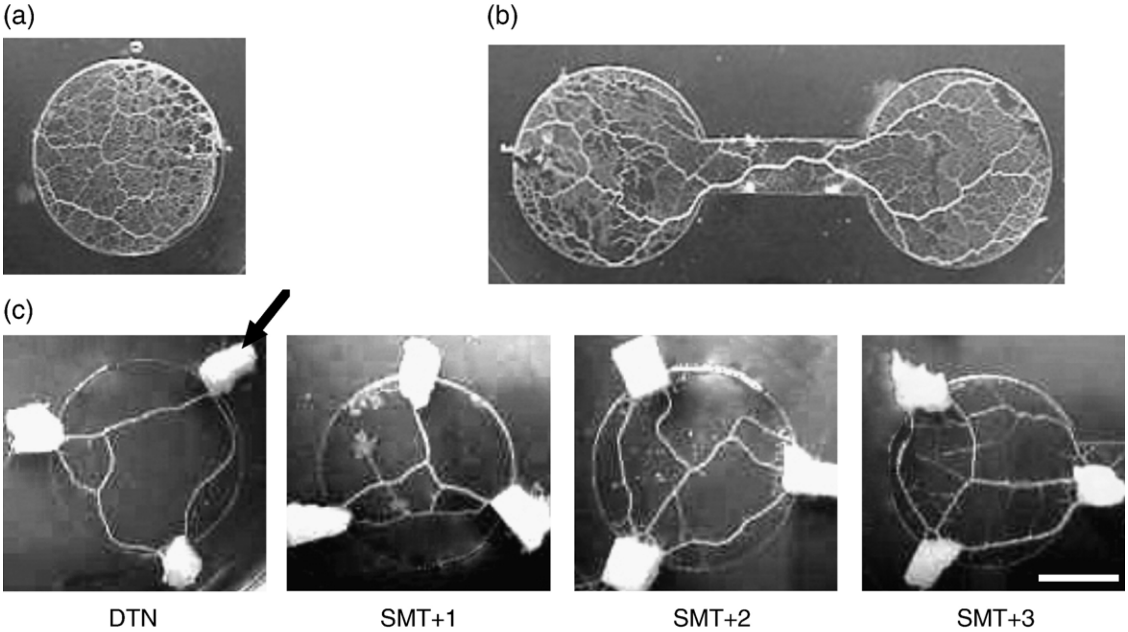


Fig. 1. Experimental set up for plasmodium network formation. (a), (b) Plasmodia spreading in a single network system (SNS) and dual network system (DNS). Plasmodia spread uniformly in the circular fields. Food sources were supplied. (c) Networks formed in the experimental fields; from the left, DTN, SMT+1, SMT+2 and SMT+3 formed. Arrow indicates food source. (Bar: 1 cm).

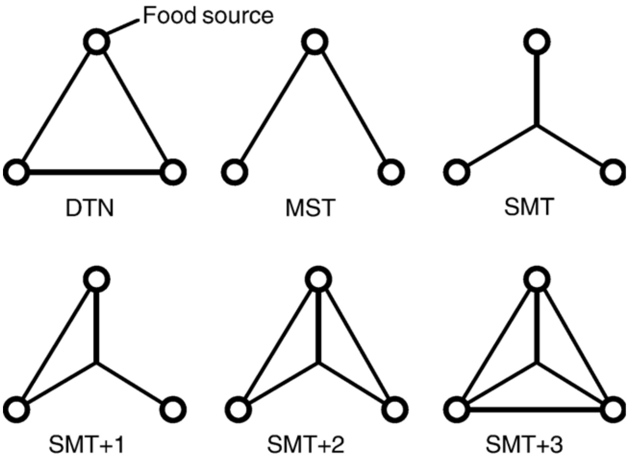


Fig. 2. Plasmodium network categorization. Six types of network connect the three triangularly arranged food sources.

plasmodium had spread homogeneously in the circle (Fig. 1(a), (b)). Plasmodium tends to avoid the dry plastic sheet and, as expected, it remained within the circle in all cases. After the plasmodium had completely spread into the circle, 3 food sources of cubic 1.5% agar gel blocks (approximately 0.5 × 0.5 × 0.5 cm) containing 0.1 g/ml crushed oatmeal were placed on the edge of the circle at the vertices of an equilateral triangle, the points of which touched the edge of the circle internally (Fig. 1(c)). After an additional 20 h of incubation, the plasmodia formed tubular networks. Images of plasmodium networks were captured with a USB camera (SONY, Japan) or video camera (SONY, Japan), and enhanced using Adobe Photoshop 7.0 (Adobe, CA, USA). We confined our observation and analysis here to only those networks that connected all 3 food sources.

2.3. Evaluation of plasmodium network

Analytical criteria for the plasmodium networks were established according to recent studies of self-organizing adaptive networks and plasmodium networks [7,8,13–17]. Neglecting the minor branches of the plasmodium networks, we classified network shape into the following three types based on network topology: DTN, MST and SMT. In addition, because SMT can coexist with the triangular edge formations observable in the DTN and MST, three intermediate types of network shape form between various configurations of SMT, MST, and DTN. These shapes were denoted SMT + 1, SMT + 2 and SMT + 3 (Fig. 2). Also, to measure centripetal intensity (CI), total length (TL) and fault tolerance (FT) of the plasmodium networks, we analyzed images of the networks using Adobe Photoshop 7.0 (Adobe, CA, USA) and Canvas 6™ package (Deneba Software, FL, USA). CI is defined as the area covered by the outermost tube of the plasmodium network, normalized to the area of DTN (Fig. 3). TL is defined in the literature as total length of the network. Note that minor branch length (neglected in shape determination) is included in this definition. The observed value of total length was then normalized to total SMT length, and this value was adopted as TL for each network. FT_N is defined by the probability that any part of the network is not fragmented when the network is dissected N times at random. We assumed that the probability of dissection at a network edge is proportional to edge length. For example, in DTN in Fig. 2, there are three edges of the same length, so the probability of dissection for each edge is calculated as 0.33. In DTN, if edge dissection occurs only once, it does not lead to network fragmentation, so FT_1 of DTN gives 1. On the other hand, SMT in Fig. 2 is always fragmented if it is dissected no less than once, so its FT_1 is 0. SMT + 1 has two types of edge, with different lengths, and a probability of dissection at each edge of 0.21 and 0.37, respectively. In SMT + 1 in Fig. 2, when dissection occurs twice, there are two cases that lead to network fragmentation: dissection at two different edges and dissection in the lower right edge. Therefore, for SMT + 1, $FT_2 = (0.21)^2 \times 2 + (0.37)^2 = 0.23$.

2.4. Evaluation of homology between two networks

Homology between two networks was evaluated using all of the criteria described above: shape, CI, TL, FT_1 and FT_2. Two networks were regarded as homologous when two conditions were satisfied: shape correspondence was more than 75% and differences in CI, TL, FT_1 and FT_2 were all less than 25% of the maximum value. Correspondence of the network shape was evaluated as follows. The six types of network have four types of edges that connect the food sources. When two networks have identical shape, their correspondence was assigned to be 100%; when three corresponding pathways exist in both networks, similarity was defined to be 75%. The combinations of two networks that have 75% similarity are as follows: DTN and MST, DTN and SMT + 3, MST and SMT + 2, SMT and SMT + 1, SMT + 1 and SMT + 2, and SMT + 2 and SMT + 3. The significance of increase in the number of homologous pairs in connected networks was tested statistically using the Chi squared test ($P < 0.01$).

2.5. Induction of DTN-type networks

The two connected circles shown in Fig. 1(b) were used as the field for the plasmodium. Plasmodia were inoculated in the same manner as described in Section 2.2, and incubated for 6–8 h at 20 °C. After incubation, food sources were placed at the same points (Fig. 1(c)), but this time, 1.5% agar cubes containing 100 mM of the repellent potassium chloride were

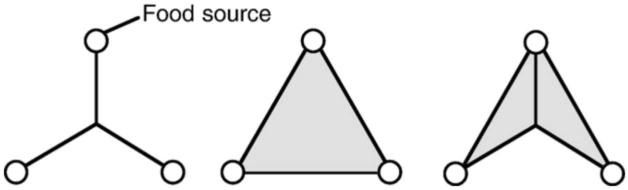


Fig. 3. Measurement of CI. The area covered by the outermost plasmodium tube was measured and normalized to the area of DTN. CI of SMT was counted as 0. As MST has only two edges, its CI was calculated supposing the presence of SMT edges. Shaded areas indicate the area measured.

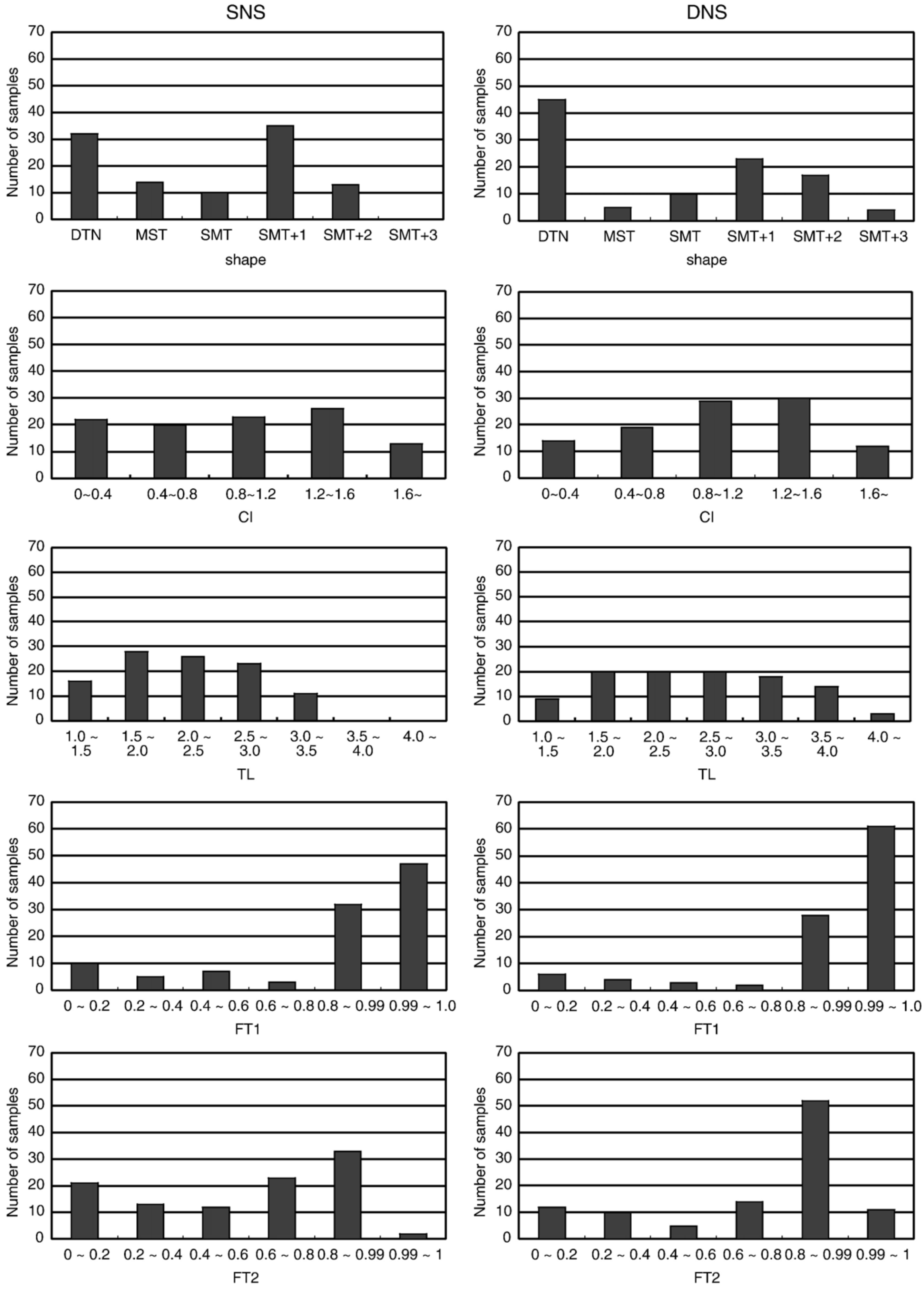


Fig. 4. Properties of 104 samples each in the SNS and DNS. Compared with the SNS networks, DNS networks showed an increased number of DTN, SMT+2 and SMT+3 networks, and increased values of TL and FT_2.

also placed between the food sources so as to prevent the formation of SMT-type edges. After 20 h of incubation, the plasmodium formed networks. Network images were captured using a video camera.

3. Results

The SNS and DNS provide the same experimental conditions locally within each circle; both have a locally identical field and

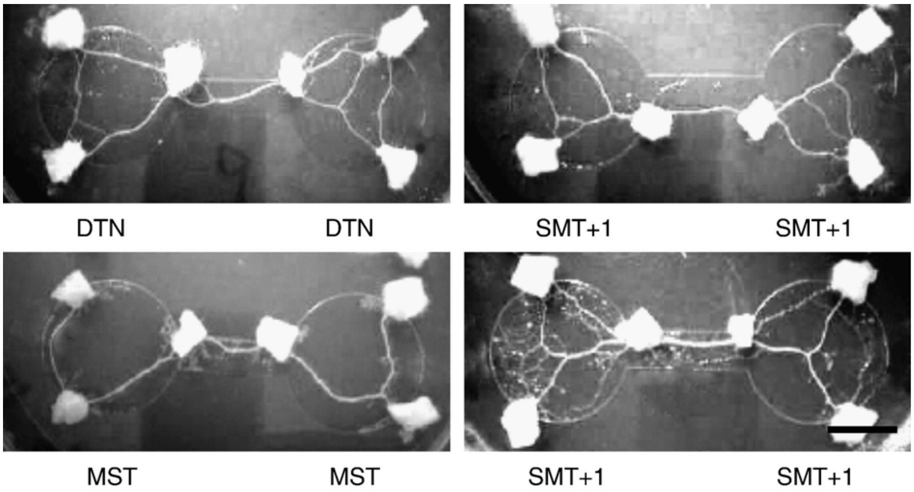


Fig. 5. Examples of DNS networks showing high levels of homology. (Bar: 1 cm).

food source arrangement for the plasmodium network. They differ only in that the DNS has a connecting path between two circular fields, thus allowing interaction between the two networks. It is therefore possible to test the effect of inter-network interaction on network formation in one field, by comparing the plasmodium networks that formed in the SNS and DNS. We analyzed each of 104 networks in the SNS and DNS, and determined their shape, TL, CI, FT_1 and FT_2 (Fig. 4). The SNS and DNS networks showed different characteristics, especially with regards to shape, TL and FT_2. The DNS showed an increased number of DTN and SMT+3 formations, that is to say, the networks were assigned more complex shapes. As expected, the DNS showed increased TL and FT_2, indicating that the networks became longer and denser. Such differences were observed even though total plasmodium volume per circle area remained constant.

Furthermore, we found that some DNS networks showed strong similarity in their properties (Fig. 5). To check whether the similarities resulted from the connection of two networks, we measured the degree of homology between two neighboring SNS networks (Fig. 6(a), group A), between two neighboring networks belonging to different DNS pairs (Fig. 6(a), group B), and between pairs which belong to the same DNS (Fig. 6(a), group C). Homology was seen in 13% of network pairs in group A and in 15% of network pairs in group B, but in a significantly higher percentage of 37% in group C ($P=0.0067$). This result indicates that the connection of two networks does indeed enhance network homology in the DNS.

To further demonstrate the cooperative aspect of network formation that results in homology generation, we confined network formation in one side of the DNS pair to a specific type of shape and tested the effect on the other side. In this experiment, we placed an agar cube containing potassium chloride repellent in one side of the DNS, so as to prevent the formation of SMT-type network edges (Fig. 7). This arrangement of repellent-containing cubes successfully inhibited the formation of SMT-type edges and induced the formation of DTN-type edges in the other side. We then statistically analyzed network shape in the side opposite the induced network. Induction of DTN promoted the same type of network formation in the opposite side (Figs. 7 and 8). Compared with each of the 104 SNS and DNS samples in the previous experiment, the DNS with DTN induction showed an increase in the number of DTN SMT+3 formations, which included DTN-type edges. This result strongly indicates that DNS networks mutually promote formation of the same type of network, implying that the process of morphogenesis is cooperative and mutually referential.

4. Discussion

To test the presence of emergent properties in the morphogenesis of *Physarum* plasmodium, we established two experimental systems–the SNS and DNS–to investigate the effect of intracellular interaction on *Physarum* plasmodium network formation. The comparison of the networks in SNS and DNS successfully indicated the presence of interactive, mutually referencing and cooperative morphogenesis in the plasmodium.

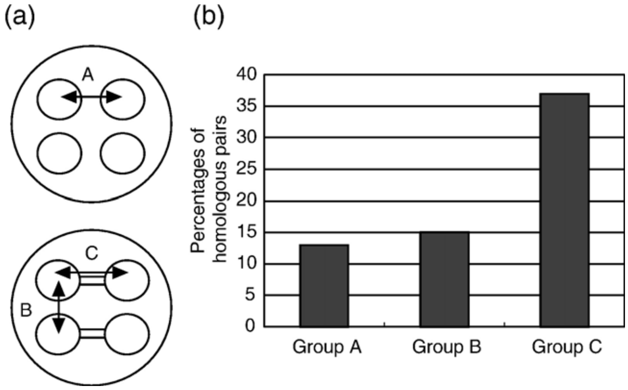


Fig. 6. Evaluation of homology between two networks. (a) Pairs studied for Groups A, B and C. (b) Percentages of homologous pairs in each group. Group C, a pair of neighboring DNS networks showed significantly higher homology when compared with A and B. (c) Images of 19 samples out of 52 pairs of networks in Group C, that were evaluated as homologous.

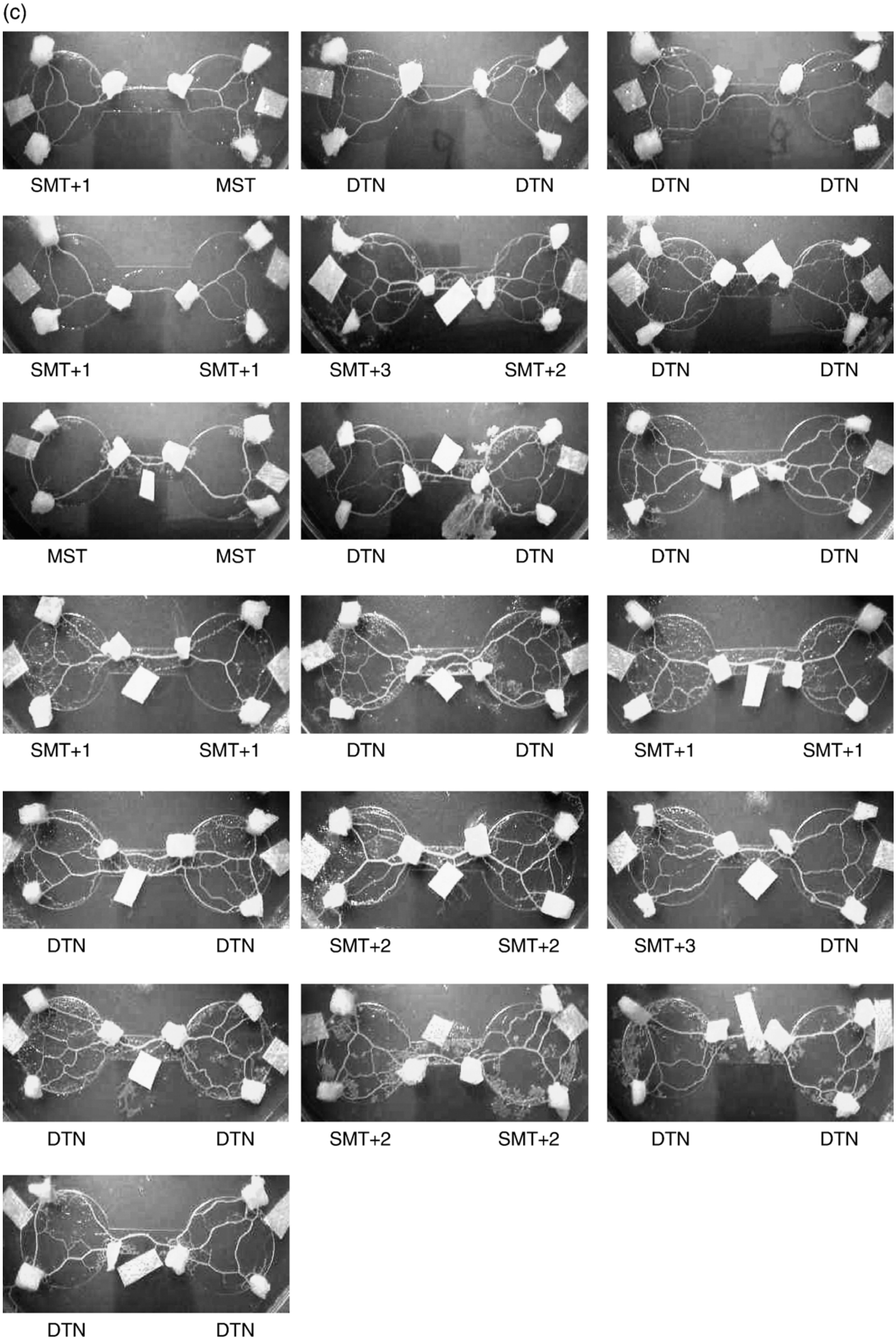


Fig. 6 (*continued*).

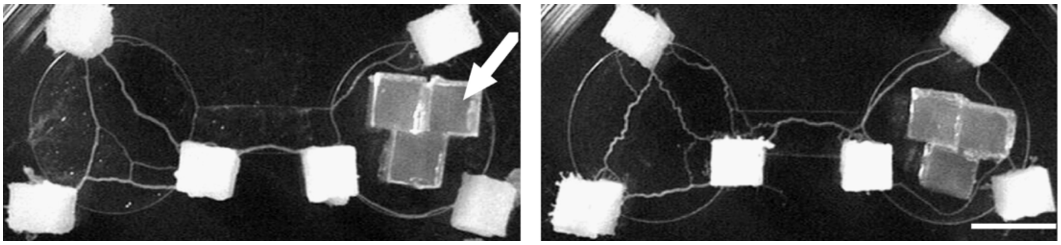


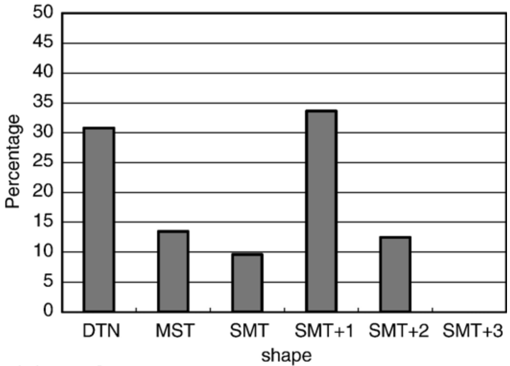
Fig. 7. Induction of DTN-type network edges using agar cubes containing repellent potassium chloride. Presence of the repellent successfully induced the desired formation, and promoted the same type of network formation as in the other side of the DNS. Arrow indicates repellent containing cube. (Bar: 1 cm).

The computational ability of the plasmodium has already been demonstrated; it is able to solve a maze [7–9] and to find a solution for an efficient network [10,11]. In such maze-solving or network formation, problem establishment is definite and the plasmodium gives relatively constant solutions to the problem. However, plasmodia in our experimental system not only responded to a given situation but also spontaneously generated morphological order. Such behavior is beyond computation in the conventional sense, and strongly implies the presence of emergent property in the plasmodium. The homology observed also implies a more direct physiological significance. The plasmodium under natural conditions crawls on plane surfaces that have relatively uniform pattern and texture, such as tree bark or soil. The plasmodium adapts to its field, trying to form optimal shape. In a sequence of search behavior, reference to the morphology in one place may help adaptation in the next place. Thus, homologous network formation would enable the plasmodium to search its field effectively, helping in its search for food.

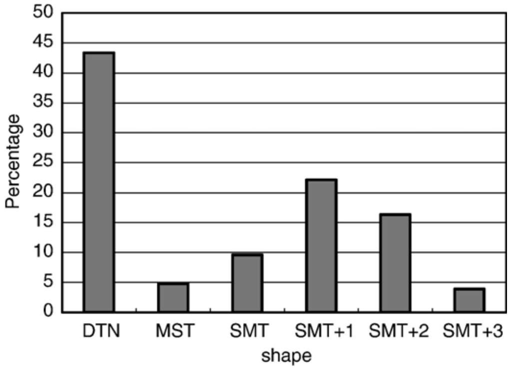
One of the most hopeful candidates as a contributor to homology generation is the rhythmic contraction in the plasmodium cell body and entailing cytoplasmic streaming. In the DNS, the two networks are separated by a 2-cm path. It does not therefore seem reasonable to suppose that the morphology of a network in one side is transferred to the other side via intracellular mechanical force or diffusive tactic molecules. On the other hand, the wave motions of contractile activity are possibly able to carry directional information. In fact, the direction of tubulogenesis in the plasmodium corresponds to the direction of the contractile wave [18]. We can therefore assume that cooperation between contractile activity in the cell body and/or share of cytoplasmic streaming in both sides of the DNS give rise to the generation of homology. The contraction of plasmodium is synchronous through its cell body, and the direction of contractile wave alternates periodically [18,19]. We assume that each plasmodium network has an eigen frequency-like property that is dependent on the properties of network, such as type of shape and values of TL, CI and FT. In our assumption, if two networks were to have similar network properties and similar eigen frequency, synchronization between the networks would be stable and the morphology of the networks in this state would also be stabilized.

Homologous network formation is emergent in that the plasmodia autonomously generate morphological order through intracellular interaction between two networks. However, to fully demonstrate the emergent aspect of the phenomenon, we

(a) SNS



(b) DNS



(c) DNS with DTN induction

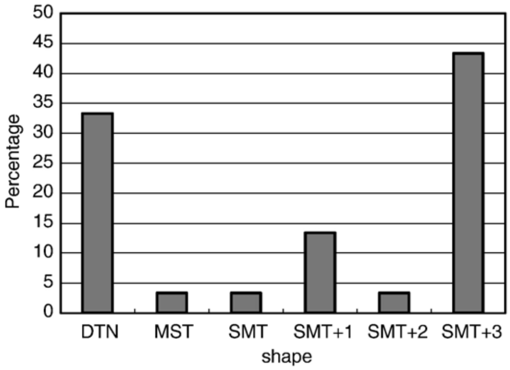


Fig. 8. Results of the induction experiment. (a), (b) Networks formed in the SNS and DNS without induction (same data as in Fig. 4, with percentile presentation). (c) Networks formed in DTN-induced DNS. DNS with DTN induction in one side showed a significantly increased number of networks that included a DTN-type edge (DTN and SMT+3), compared with SNS networks (a) and DNS networks without induction (b).

must study the mechanism of homologous network formation in more detail. Future study should focus on the time series development of the plasmodium network and cytoplasmic streaming dynamics. While there is room for further study of our results, the phenomenon observed in the present study is clearly of significance and our methods provide an opportunity to quantitatively analyze the autonomous and emergent behavior of a unicellular system.

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