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Paper

What do amoebae look before they leap? — an efficient mechanism before sporulation in the true slime mold *Physarum polycephalum* —

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Abstract: In this study, we investigate a moving behavior of the network which is observed before an amoeba turns into 'fruiting bodies', namely it sporulates. More specifically, we observe how *Physarum polycephalum* behaves after a severe environmental change; exposure to certain strong light. Through systematic, controlled experiments (in constant dark condition, 26°C) we obtained four evidences, suggesting an efficient mechanism of the network's moving by which *Physarum polycephalum* makes the sporulation more effective, which is considered to be important for its survival. Our finding adds a new knowledge to the biological aspect of network science.

Key Words: spatio-temporal pattern, true slime mold, sporulation, network science

1. Introduction

A network formation from cellular level has been recently investigated in an amoeboid organism; the true slime mold (*Physarum polycephalum*). To elucidate a hidden mechanism and functioning of its network formation, studies have been successively conducted in 'maze solving' [1], minimumrisk path finding [2], anticipation of periodic events [3], and searching for a solution to the traveling salesman problem [4]. All these studies use an aggregate of plasmodium for *Physarum polycephalum* (*Physarum*, in short); an amoeboid organism stably maintained in a dynamic fan-like network structure (Fig. 1(a)) when the environment is suitable for its survival. In contrast to these studies, here we focus on another facet of *Physarum*, *i.e.*, a moving behavior of the network in the process when the amoeba is going to turn into 'fruiting bodies' (Fig. 1(b)), namely it sporulates. More precisely, we observe how *Physarum* behaves in certain controlled environments before it sporulates after a severe environmental change; exposure to certain strong light. To the authors' knowledge, this sort of study has never been reported, although we empirically know fruiting bodies are often formed in a relatively dry place, for example on rotten trees or on the trunks of trees.

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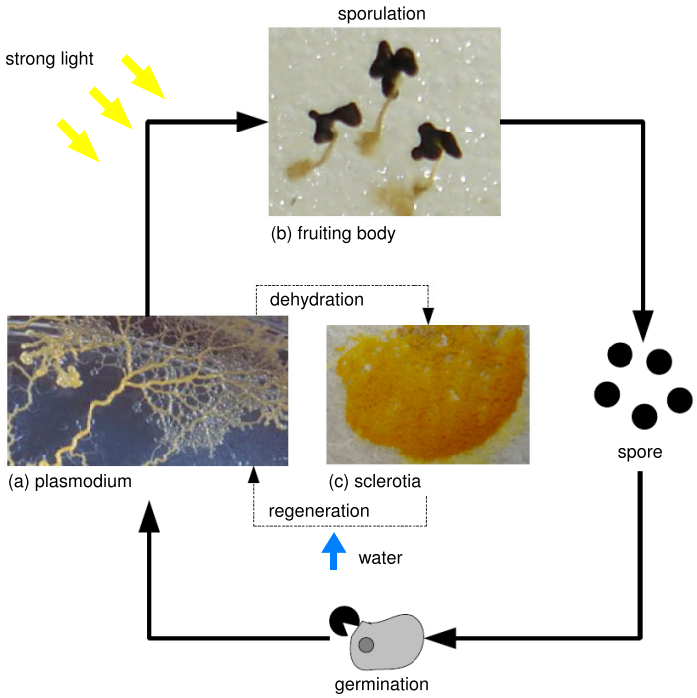

Fig. 1. Life cycle of *Physarum*. (a) Plasmodium; a single cell giant amoeba. (b) Fruiting body containing spores. (c) Sclerotia obtained from plasmodium after dehydration.

This paper is organized as follows. In Section 2 we describe systematic, controlled experiments to investigate how the environment (*i.e.*, humidity) affects a spatial pattern of fruiting bodies formations. Then, in Section 3 we show resulting four observations on the moving behavior of the network which *Physarum* exhibits before sporulation. Implications and insights from these observations are discussed in Section 4.

2. Methods

In all experiments, we used the plasmodium of the true slime mold *Physarum polycephalum* (sporulated and cloned in Ueda-Nakagaki Lab, in Hokkaido Univ.), which is regenerated from the sclerotia (Fig. 1(c)). It was cultured with oat flakes for at least about a few days after regeneration from sclerotia in the dark. Before experiments, the plasmodium was allowed to move and extend on a plain agar plate (1%) in a trough at 26°C for 12 hours.

2.1 Experiment A: climbing and gathering behavior

Two kinds of materials (*i.e.*, the amoebae) were prepared before experiments. One is the amoeba which has been exposed to the strong light; three fluorescent lights (27 W, FPL27EX-N, Panasonic) from distance of ten centimeters for 6 hours. The other one was kept in a constant dark condition at 26°C as described above. It is noted that this fluorescent light has enough power at around 360 nm wavelength which is known to be one of effective action spectra for sporulation [5], and the temperature of the amoeba was carefully maintained at around 26°C during this exposure.

Then, certain amount of amoeba was cut out and put on the center of a glass plate with a wet paper filter on it, and this glass plate was put steady in the cylindrical glass container (height 12 cm, diameter 9 cm) as shown in Fig. 2. This container has a glass lid on the top, and there is an invisibly small gap between the container and its lid. The average humidity in the container is around 90% RH. All experiments were carried out under a static condition; 26°C, 50% RH, constant dark in an incubator (Type KCL2000A, Eyela Co.). The behaviour of amoebae was continuously monitored by a CCD camera (Fig. 2) under infrared lights with 945 nm wavelength which is known not to cause any effect on *Physarum*.

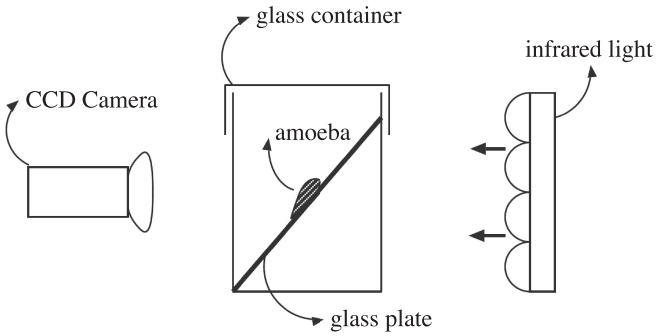

Fig. 2. Experiment A setup.

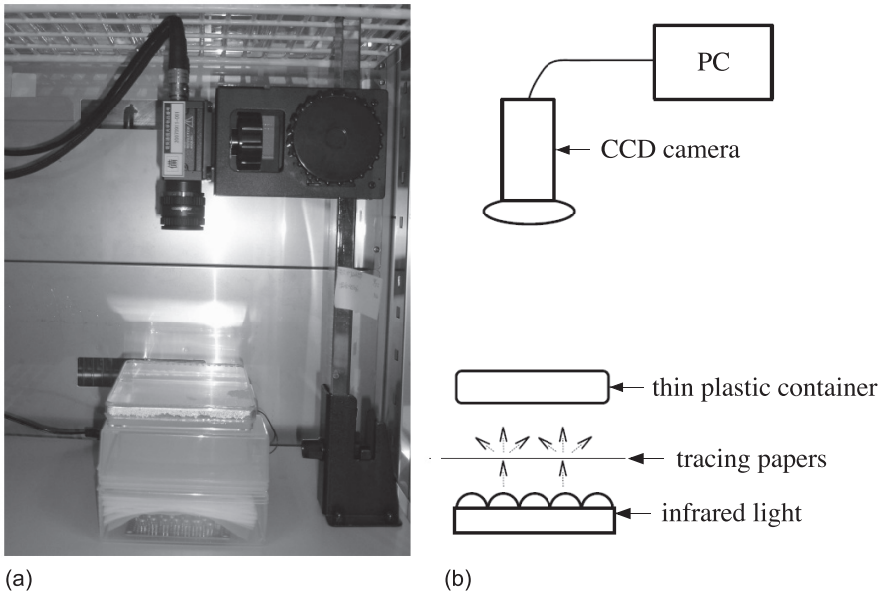

Fig. 3. Experiment B setup. (a) Setup in the incubator. (b) Detail of the setup.

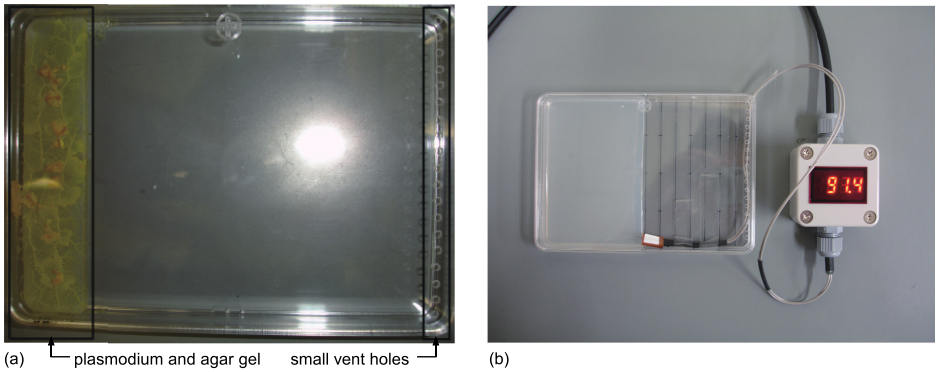

Fig. 4. Thin plastic container used in experiment B. (a) Detail of the container; plasmodium and agar gel on the left end, small vent holes on the right end. (b) Local humidity measured inside the container. (Note, in this particular picture a larger agar band was put in the container.)

2.2 Experiment B: spatial distribution of fruiting bodies

To observe a moving behavior of the amoebae in a spatially sloped humidity condition, experiment B was carried out. The experiment setup is essentially the same to the one of experiment A, but only the glass container (Fig. 2) was replaced with a thin plastic container; sterile petri plate AW2000, EIKEN Co., size: 14 cm × 10 cm × 1.5 cm (shown in Figs. 3 and 4).

In experiment A, the amoebae were allowed to freely move along the two dimensional surface inside

the glass container (Fig. 2). In contrast, here in experiment B the amoebae were confined in a thin plastic container (shown in Figs. 3 and 4), and the moving behavior of the amoebae is expected to take place in the two dimensional bottom of the container. (In fact, this expectation was verified in all (more than one hundred) trials shown in Section 3.2.) In addition, the local humidity inside the container is easily controlled and measured (Fig. 4(b)), which is difficult for the case of experiment A.

The mechanism by which the local humidity was spatially sloped in the thin container, is explained as follows. As shown in Fig. 4(a), initially an agar gel band (14 cm × 2 cm × 0.6 cm) was cut out and put on the left end in the container. The container has 19 small vent holes on the right end; 2 mm diameter, 5 mm distance with each other, and the container was set in a constant humidity in the incubator. As the result, after a short transient, a spatially sloped local humidity; higher humidity on the left and lower one on the right, was formed. This local humidity was precisely measured (as shown in Fig. 4(b)) by a humidity transducer (TA503-RGS, Toplas Engineering Co.). It is noted that this spatially sloped local humidity was stably maintained during each trial.

Under the above static humidity pattern, the cultured and prepared amoebae of 0.01 g and foods (oat flakes, 0.1 g) (see Section 2.1 for detail) were uniformly put on the agar gel band. The behaviour of amoebae was continuously monitored by a CCD camera as in experiment A.

3. Results

All results obtained from experiments A and B are shown in Table I.

3.1 Experiment A: climbing and gathering behavior

By analyzing the video records obtained in experiment A (Section 2.1), the following patterns (i) and (ii) are observed. For verifying each pattern multiple trials were carried out (as shown in Table I) under the mentioned static condition in experiment A.

(i) With probability 87.5% (*i.e.*, 7 out of 8 trials), the amoebae exposed to the strong light crawled upward and gathered at the upper part of the container before they form their fruiting bodies, (Fig. 5(a)). In contrast, if the amoebae were not exposed to the strong light, they swept uniformly inside the con-

Table I. Statistics of experiments A and B, that examined fruiting body formations respectively.

Experiment	A	A	A	A	B	B	B	B	B	B
Light exposure time [min]	360	None	360	None	360	None	360	None	360	None
Humidity [% RH]	50	50	Sealed	Sealed	90	90	50	50	30	30
#(Trials)	11	10	18	10	25	16	25	18	33	18
#(Sporulation)	8	0	8	0	25	1	24	0	26	0

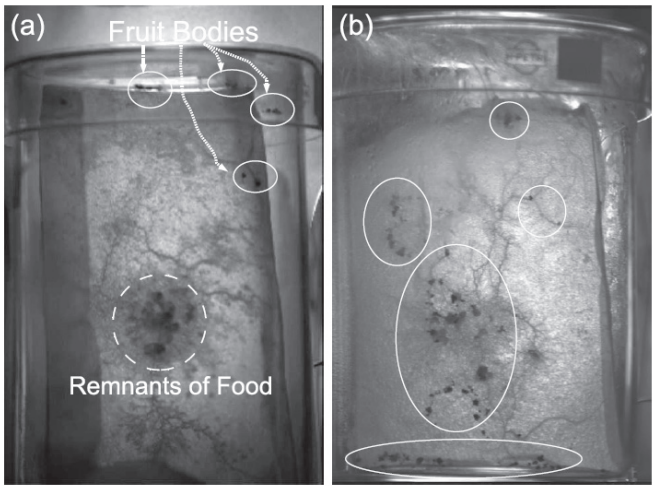

Fig. 5. Fruiting bodies of *Physarum* formed in (a) the container with a small loophole on the top, and in (b) the completely sealed container. White loops indicate the regions where fruiting bodies are formed.

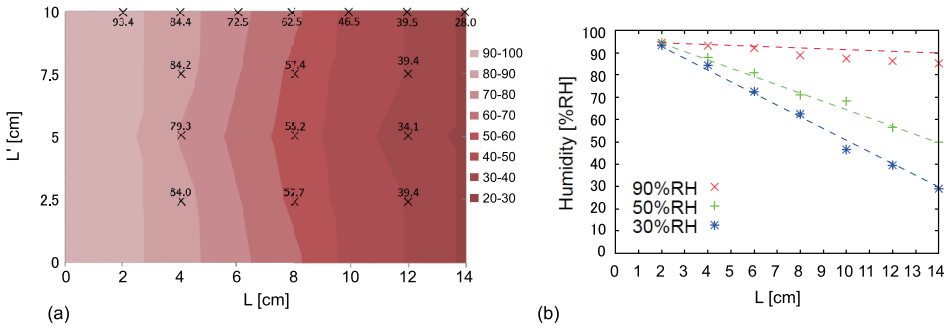

Fig. 6. Measured local humidity. (a) Two dimensional static pattern of local humidity. Numbers indicate the local humidity at each point. (b) Local humidity distributions in one dimension; data sets ×, +, and ∗ are respectively obtained under 90, 50, and 30% RH humidity in the incubator.

tainer and did not show any particular 'climbing' or 'gathering' behaviour.
(ii) In contrast to (i), if the gap of the container is completely sealed, the amoebae exposed to strong light did not show any particular 'climbing' and 'gathering' behaviour before their sporulation (Fig. 5(b)). Instead, they formed fruiting bodies almost uniformly in the container for each trial.

3.2 Experiment B: spatial distribution of fruiting bodies

Figure 6 shows the measured local humidity inside the thin plastic container for experiment B. For this measurement, only an agar gel band ($14 \times 2 \times 0.6$ cm) was put on the left end in the container; the amoebae was not put in the container at this stage. A static, spatially sloped humidity pattern is obtained as shown in Fig. 6(a), for instance, for 30% RH humidity condition in the incubator. As we see in Fig. 6(a), two dimensional spatial patterns can be reduced to one dimentional distributions, since the local humidity is almost constant along the vertical axis for any given L. Hence, we measured a reduced one dimensional distributions of the local humidity respectively for cases of 90, 50, and 30% RH humidity in the incubator as shown in Fig. 6(b), after we have checked that the two dimensional local humidity pattern is uniformly sloped (as shown in Fig. 6(a)) in each case. These three local humidity distributions show a clear linear characteristics in the range of $2 \leq L \leq 14$ cm as shown in Fig. 6(b).

Figure 7(a) shows a typical spatial pattern of fruiting bodies formation in this experiment B. As shown in Fig. 7(a), a fine 0.5×0.5 cm mesh is introduced, and for each 0.5×0.5 cm small square the following quantification procedure is applied for counting fruiting bodies; if no fruiting body exist it gives 0, if the area of fruiting bodies image occupies less than 30% it gives 1, if the area occupies less than 60% larger than 30% it gives 2, if the area occupies less than 90% larger than 60% it gives 3, and if the area occupies larger than 90% it gives 4. This procedure was automatically done by an image processing software. Note that this procedure is reliable since fruiting bodies can be densely formed but still separated with each other.

Figures 7(b), (c), and (d) respectively show the two dimensional histogram for multiple instances of sporulation (shown in Table I) for the case of 90, 50, and 30% RH humidity in the incubator, which is obtained through the above quantification procedure. Figures 8(a), (b), and (c) show one dimensional spatial distributions of fruiting bodies respectively reduced from two dimensional histograms Figs. 7(b), (c), and (d). The reduction to one dimensional distributions is made simply by summing up the elements in the two dimensional histogram for each L (*i.e.*, the horizontal axis of the two dimensional histogram, $0 < L < 14$ cm) and then by normalizing the total of the elements to unity. Note that this reduction is reasonable since the values of the elements for a given L are not widely spread over L' (the vertical axis) as observed in Figs. 7(b), (c), and (d).

From the above two dimensional histograms (Fig. 7) and one dimensional distributions of fruiting bodies (Fig. 8) the following observations are obtained.
(iii) If the amoeba exposed to strong light was put in a thin, flat square container in which the local humidity was almost spatially uniform between 95% RH and 90% RH, fruiting bodies were uniformly

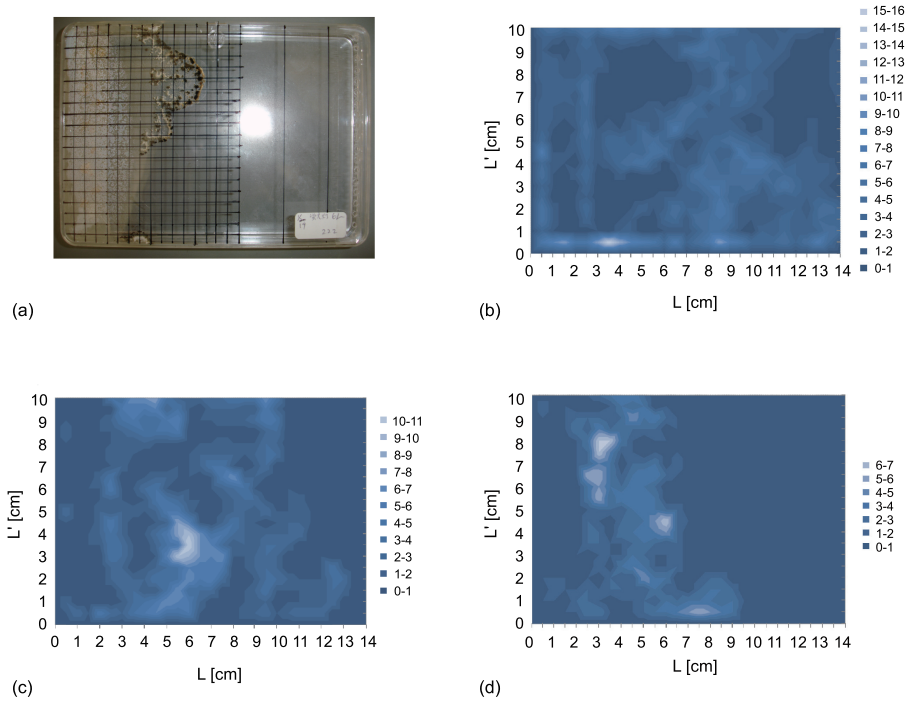

Fig. 7. Spatial pattern of fruiting bodies formation for three different local humidity patterns. (a) A typical formation pattern of fruiting bodies. (b), (c), (d) Two dimensional histograms of fruiting bodies distribution over multiple instances; (b) sums 25 instances for the case of 90% RH humidity in the incubator, (c) 24 instances for the case of 50% RH humidity in the incubator, and (d) 26 instances for the case of 30% RH humidity in the incubator.

distributed in the container.
(iv) In contrast to (iii), if the local humidity was spatially sloped between 95% RH and 50% RH (or 30% RH), most fruiting bodies were formed in a range of the local humidity between around 75% RH and 95% RH.

Finally, we investigate a time evolution of the network of the plasmodium. Figure 9 shows a typical time evolution of a network for the normal plasmodium (not exposed to strong light). As is observed in Fig. 9 the network was always connected to the wet agar gel band on the left end and the plasmodium stayed around the gel band, although its network was constantly transforming. In contrast to the normal plasmodium, Fig. 10 shows a typical time evolution of a network for the plasmodium exposed to the strong light. Initially, the network was connected to the agar gel (as in the snapshots 1, 2, 3, 4, and 5 of Fig. 10). Then, right before the sporulation (the snapshot 8) the network was rapidly extended (the snapshot 6). Eventually, the connection to the gel band was lost (the snapshot 7) and the fruiting bodies were formed in a relatively dry area. It is reasonable to assume this observed network dynamics explains how the spatial pattern in the observation (iii) is realized.

4. Discussion and conclusions

It is known that *Physarum* in the normal condition (not exposed to the strong light) prefers wet environments (*i.e.*, hygrotaxis). Actually, part of the observations (i) and (ii) in Section 3.1, and the observation in Fig. 9 seem consistent to this fact, *i.e.*, the amoebae not exposed to the strong light always stayed around the wet places. In contrast, the amoebae exposed to the strong light clearly lost this characteristic and eventually left from the wet places, as observed in (i), (ii) in Section 3.1, and in Fig. 10. Now, the following questions naturally arise.
(a) Do the amoebae 'intentionally' search for a relatively dry place and head for there, right before their sporulation? Or, do they merely lose their hygrotaxis before their sporulation? And,
(b) how does the surrounding environment affect a spatial pattern of fruiting bodies formation?

One possible, decent answer to the question (a) might be to attribute the sporulation of fruiting

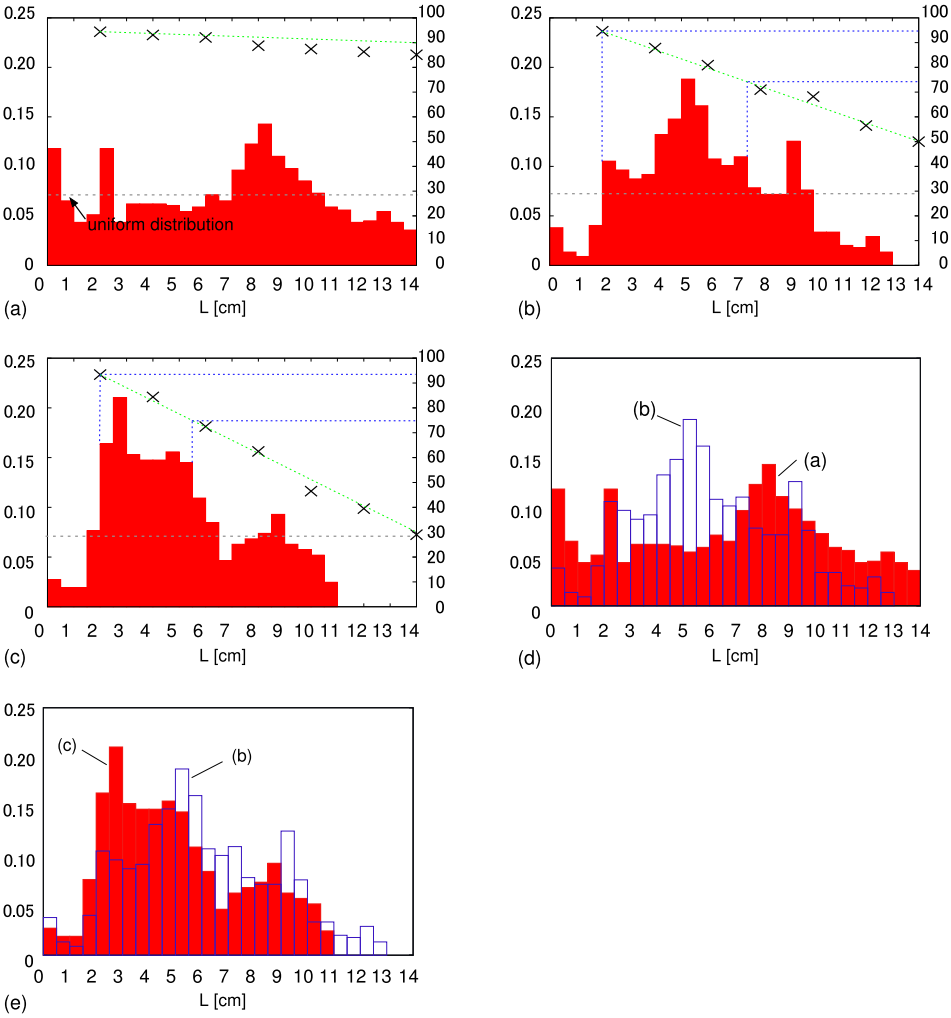

Fig. 8. Comparison of one dimensional spatial distributions of fruiting bodies. Graphs show one dimensional distributions of fruiting bodies over multiple instances. (Note that these distributions are the probability distribution functions, and the uniform distribution gives the value of $1/14 \simeq 0.0714$ which is shown in Figs. 8(a), (b), and (c).) Data sets $\times$ indicate the associated local humidity patterns respectively shown in Fig. 6(b). (a) Distribution for the case of 90% RH in Fig. 6(b). (b) Distribution for the case of 50% RH in Fig. 6(b). (c) Distribution for the case of 30% RH in Fig. 6(b). (d) Comparison of distribution (a) and distribution (b). (e) Comparison of distribution (b) and distribution (c).

bodies to a putative 'threshold detector' incorporated in the organism, which makes *Physarum* sporulate if a certain local humidity is sensed. Nevertheless, only such a 'threshold detector' does not clearly explain our observations in this study, because the network is not disconnected and goes back and forth until the sporulation (as shown in Fig. 10).

Although the question (a) seems difficult to be answered at this stage, the observation (iii) in Section 3.2 gives an answer to the question (b). Namely, only certain spatially sloped local humidity condition in Section 3.2 is enough for *Physarum* to concentrate their fruiting bodies in a particular range with moderate humidity from 75% RH to 95% RH.

One interpretation of the above answer in a real environment is as follows. After sporulation, *Physarum* has to leap over the air as spores to increase the chance of survival. From this fact, the observations (i), (ii), (iii), and (iv) suggest the following reasonable tactics of *Physarum* optimizing the efficiency of sporulation. For instance in experiment A, after *Physarum* senses the local humidity in the container, it concentrates its fruiting bodies around the gap, and this increases the chance of survival after the sporulation. Similarly, in experiment B *Physarum* decreases the risk of death due to dryness before sporulation, and at the same time it increases the chance of survival as spores after

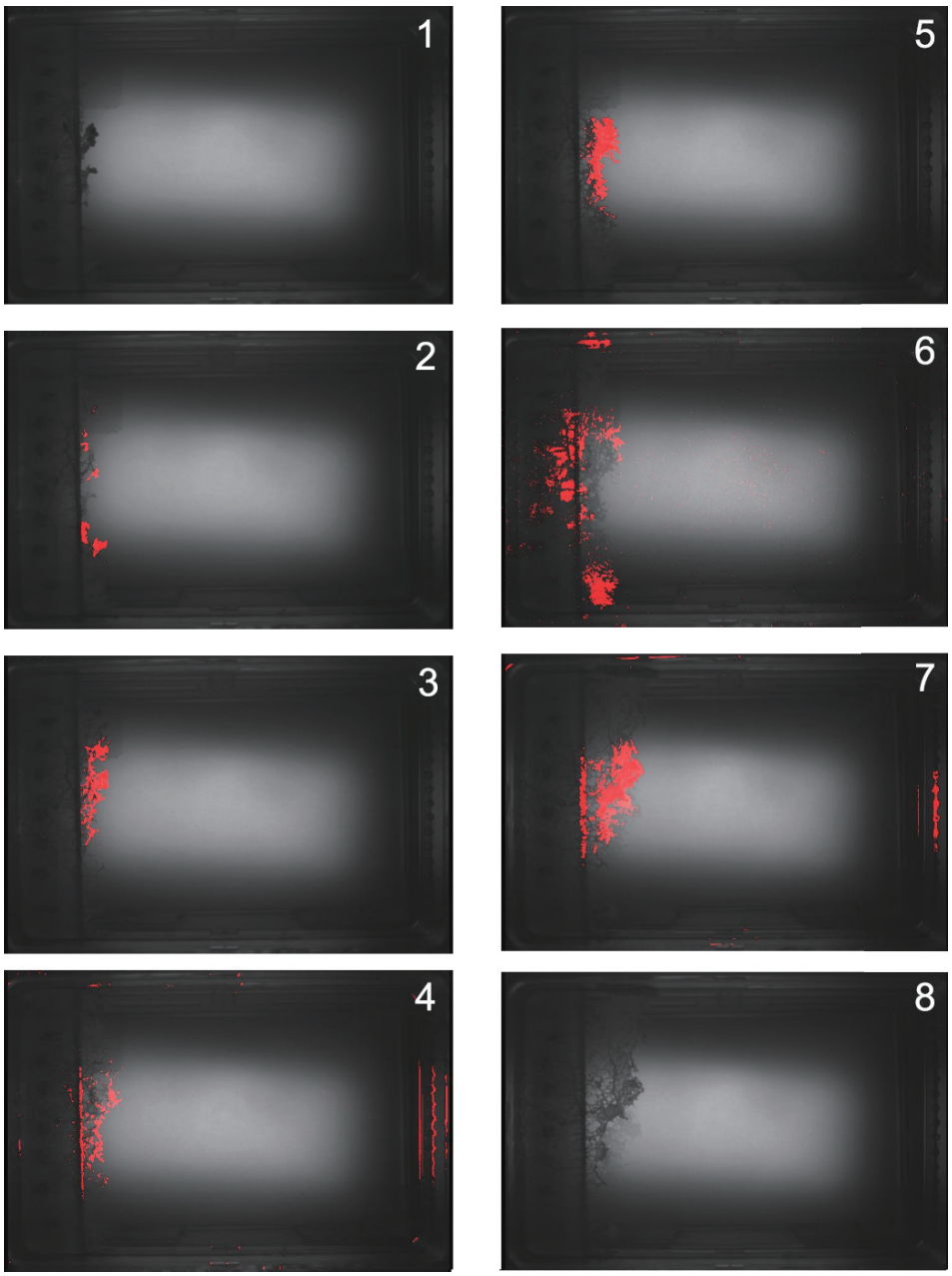

Fig. 9. Time evolution of a network for the normal plasmodium. Snapshots are taken every 2 hours. In the snapshots 2, 3, 4, 5, 6, and 7, red areas indicate the new additions to the previous snapshot obtained by an image processing software, showing a newly swept area by the plasmodium network. Note that thin red lines on the right in the snapshots 4 and 7 are due to a temporal moisture and they are nothing to do with the plasmodium.

sporulation by forming fruiting bodies at a relatively dry place, *i.e.*, by climbing up to a relatively higher place in the real environment of woods, without using information from the sun light during the night. To obtain a deeper understanding of the network behavior, further experiments and analysis are required. For instance, in experiment B, a different size of the container or different local humidity patterns are worth for investigating to see how the distribution of fruiting bodies formation is altered.

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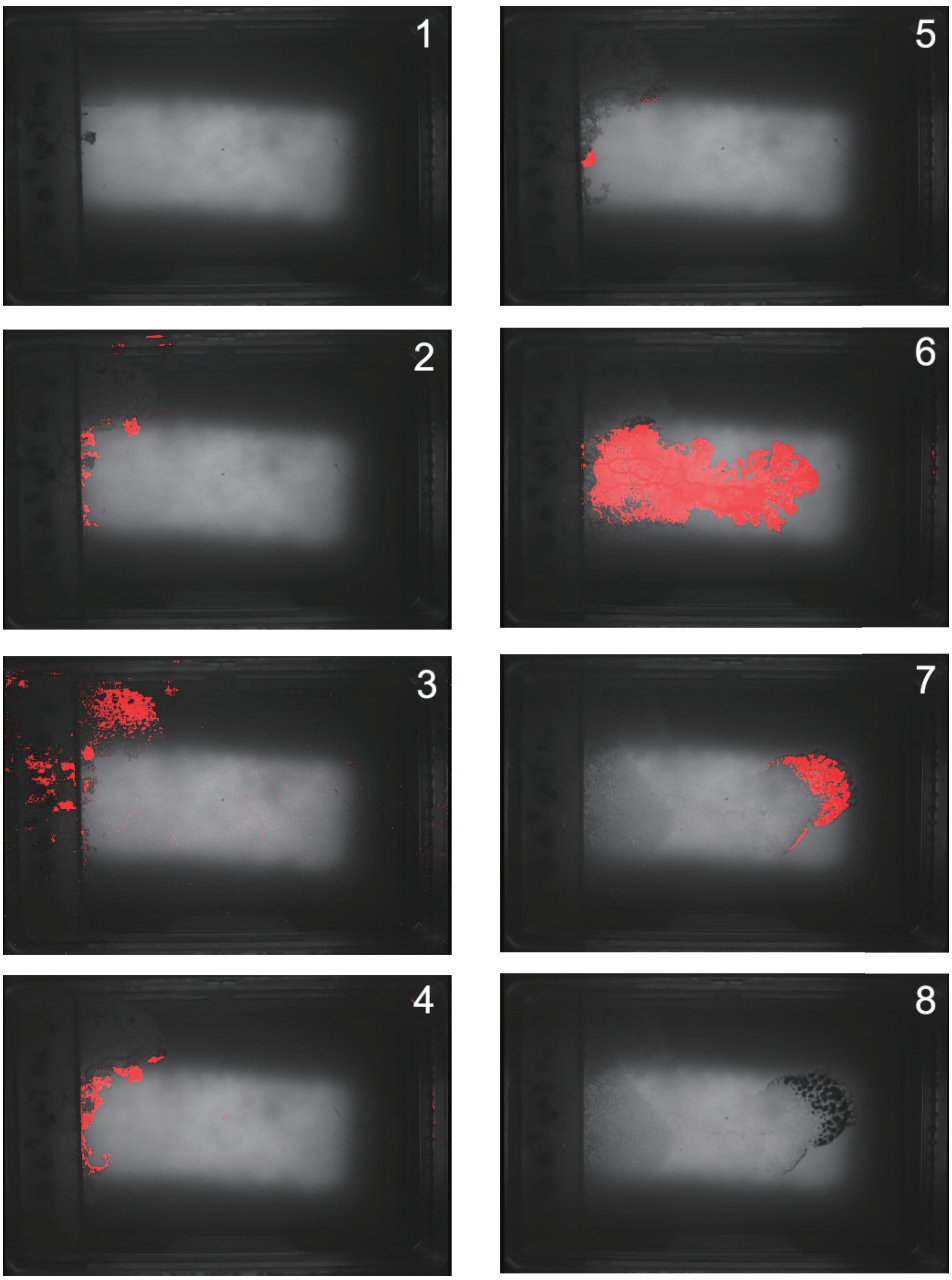

Fig. 10. Time evolution of a network for the plasmodium exposed to the strong light. Snapshots are taken every 2 hours. In the snapshots 2, 3, 4, 5, 6, and 7, red areas indicate the new additions to the previous snapshot obtained by an image processing software, showing a newly swept area by the plasmodium network. In the snapshot 8, fruiting bodies are formed.

References

[1] T. Nakagaki, H. Yamada, and A. Toth, "Maze-solving by an amoeboid organism," *Nature*, vol. 407, p. 470, 2000.

[2] T. Nakagaki, M. Iima, T. Ueda, Y. Nishiura, T. Saigusa, A. Tero, R. Kobayashi, and K. Showalter, "Minimum-risk path finding by an adaptive amoebal network," *Phys. Rev. Lett.*, vol. 99, no. 6, 068104, 2007.

[3] T. Saigusa, A. Tero, T. Nakagaki, and Y. Kuramoto, "Amoebae anticipate periodic events," *Phys. Rev. Lett.*, vol. 100, no. 1, 018101, 2008.

[4] L. Zhu, M. Aono, S.J. Kim, and M. Hara, "Amoeba-based computing for traveling salesman problem: Long-term correlations between spatially separated individual cells of *Physarum polycephalum*," *BioSystems*, vol. 112, no. 1, pp. 1–10, 2013.

[5] T. Nakagaki, S. Umemura, Y. Kakiuchi, and T. Ueda, "Action spectrum for sporulation and photoavoidance in the plasmodium of *Physarum polycephalum*, as modified differentially by temperature and starvation," *Photochem. Photobiol.*, vol. 64, pp. 859–862, 1996.
[6] W.H. Auden, *Selected Poems*, Edward Mendelson eds., Faber & Faber, London, 2010.