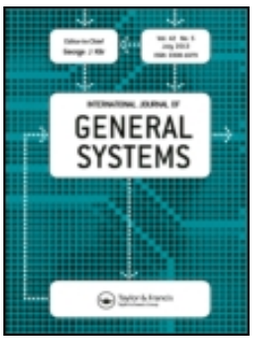

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Construction of living cellular automata using the *Physarum* plasmodium

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The plasmodium of *Physarum polycephalum* is a unicellular and multinuclear giant amoeba that has an amorphous cell body. To clearly observe how the plasmodium makes decisions in its motile and exploratory behaviours, we developed a new experimental system to pseudo-discretize the motility of the organism. In our experimental space that has agar surfaces arranged in a two-dimensional lattice, the continuous and omnidirectional movement of the plasmodium was limited to the stepwise one, and the direction of the locomotion was also limited to four neighbours. In such an experimental system, a cellular automata-like system was constructed using the living cell. We further analysed the exploratory behaviours of the plasmodium by duplicating the experimental results in the simulation models of cellular automata. As a result, it was revealed that the behaviours of the plasmodium are not reproduced by only local state transition rules; and for the reproduction, a kind of historical rule setting is needed.

Keywords: *Physarum polycephalum*; nature-inspired computing; two-dimensional cellular automata; Conway's game of life; growth model

1. Introduction

The plasmodium of *Physarum polycephalum* is a unicellular and multinuclear giant amoeba. The plasmodium is a predatory stage in the life cycle of true slime mould *P. polycephalum*, and the plasmodium grows by feeding from the microscopic cellular scale to the macroscopic scale that is visible to the naked eye (Figure 1). Though the plasmodium is a unicellular organism with very large scale, every part of the cell body behaves cooperatively and in the behaviour of the plasmodium we can find a kind of sociality that is similar to that of animal and insect swarms. Furthermore, the plasmodium can sense many kinds of environmental stimuli. Namely, the plasmodium has various taxes such as chemotaxis (Nakagaki, Yamada, and Tóth 2000; Shirakawa and Gunji 2007), phototaxis (Aono and Hara 2008), thermotaxis (Shirakawa, Gunji, and Miyake 2011), geotaxis (Wolke, Niemeyer, and Achenbach 1987), electrotaxis (Anderson 1951) and magnetotaxis (Shirakawa, Konagano, and Inoue 2012); it changes its behaviour by these taxes and adapts itself to its environment.

These properties of the plasmodium attract a lot of attention in the field of computer science, especially in the context of nature-inspired unconventional computing. The previous studies demonstrated that the plasmodium has an ability of spatial optimization. For example, if two nutrient sources are given to the plasmodium that is fully spread in a maze, the plasmodium gives a solution to the maze (Nakagaki, Yamada, and Tóth 2000). Furthermore, if several nutrient sources are given to the plasmodium that is spread out in a closed space, the

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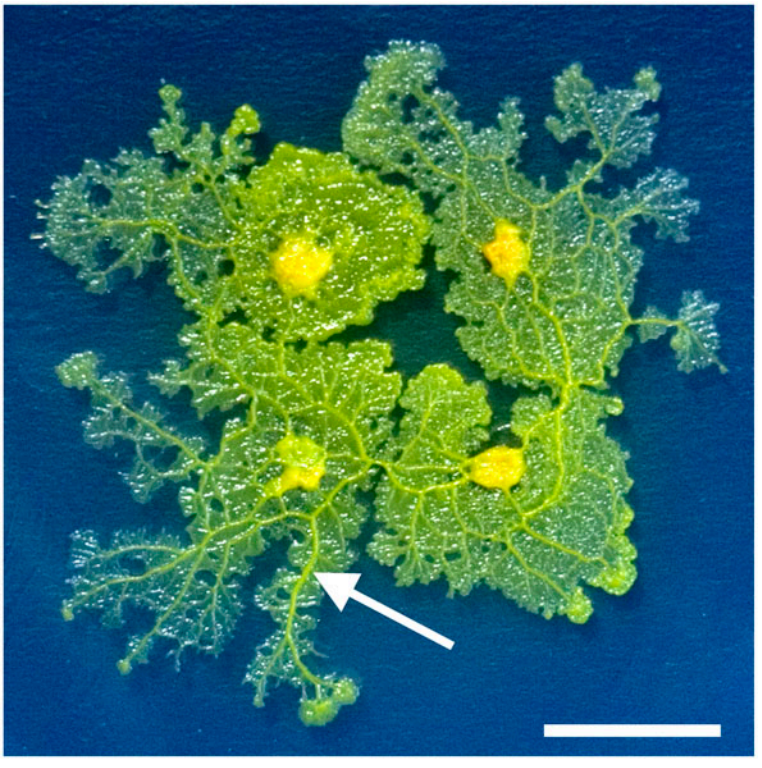

Figure 1. The plasmodium of *Physarum polycephalum* crawling on a 1.5% agar plate. The arrow indicates the tubular structure of the plasmodium, which is used for spatial optimization between food sources that is exemplified in the text. (Bar: 1 cm).

plasmodium forms an optimized network that connects all of the nutrients (Shirakawa and Gunji 2007). Additionally, some studies demonstrated that the plasmodium is able to duplicate the transportation networks in the UK (Adamatzky 2010) and in Japan (Tero et al. 2010), in the experiments that have similar settings. All of the studies indicated above have observed the optimization behaviour of the plasmodium with the experimental setups roughly consisting of three steps: in the first step, the plasmodium fully searches the given space; in the second step, some sources of attractant or repellent stimuli are given to the plasmodium that is fully and homogeneously spreading in the space; and in the third step, the plasmodium optimizes the connection between the sources. There are many studies on the computational abilities of the plasmodium in this manner; however, few studies have been done for the plasmodium that is exploring an open space.

As mentioned above, the majority of the studies focus on the behaviour of the plasmodium in a closed space. On the contrary, we believe that we can find more enhanced biological characteristics of the biological entities by observing the behaviours of them in an open and unknown environment. In this study, we thus tried to investigate the exploratory behaviour of the plasmodium in an open space and to understand how the organism makes its decision in exploration. To accomplish this goal, we developed an experimental setup to discretize the motility of the plasmodium.

The body of the plasmodium is amorphous, and when the plasmodium crawls on a space, the direction of the motility is omnidirectional. Therefore, in observing the motility of the

plasmodium, there is some difficulty in defining the motile state of the plasmodium at each time, and in defining the state transition from past to present. To realize these definitions, we discretized the motility of the plasmodium in our experimental setup. In our experimental system, the motility of the plasmodium was limited to four directions, and the motility was forced to be a stepwise one.

In our experiment, the motility of the plasmodium was successfully discretized, and the behaviour of the plasmodium was similar to that of two-dimensional cellular automata. We furthermore extracted the transition rules of the time development of the plasmodial morphology from our experimental results, and reconstructed the cellular automata model based on the real plasmodium. In our model, how the plasmodium decides its movement was analysed.

2. Experiment

2.1. Methods

We developed an experimental system to discretize the motility of the plasmodium. In this experiment, a polycarbonate plate (30 cm × 30 cm × 5 mm) was used as a basic substrate of the plasmodium. The polycarbonate plate had 3 mm holes in diameter that were arranged on the plate to form two-dimensional lattice with 5 mm intervals. Namely, the gaps between the edges of two holes were 2 mm. Every hole penetrates the plate. The plate was fixed in the middle space of a heat-resistant plastic box (32.5 cm × 32.5 cm × 5.4 cm) by using four plastic spacers, and the plate was kept level. A boiled solution of 1.5% plain agar was poured into the box until the surface of the agar solution coincided with the surface of the polycarbonate plate. In such a way, we constituted the non-uniform substrate that consisted of an agar surface and a polycarbonate surface (Figure 2).

In this substrate, 40 or 80 mg of the plasmodium that was cultivated by the method of Camp (1936) was inoculated at the centre. The plasmodium was repelled by the hydrophobic surface, and therefore the plasmodium rapidly went through the polycarbonate surface and tended to stay on the agar gel surface for a long time. This makes the motion of the plasmodium stepwise and it takes 10–30 min for one transition (Figure 3). Furthermore, when the plasmodium in the middle of the polycarbonate surface touches the other agar gel surface, the motion of the plasmodium is immediately attracted to the new agar surface. By this mechanism, the direction of the plasmodial motion is limited to four directions, that is, the motion is limited to from one cell to the four neighbours (Figure 3). The whole experimental system was illuminated by a surface light source of 600 nm LED (custom-order item, AITEC SYSTEM, Japan) under the experimental setup, and the motility of the plasmodium was photographed directly above by a digital camera (EOS Kiss X2, Canon, Japan) at 10 min intervals.

2.2. Results and discussion

The experimental setup was designed by referring to the previous study with similar setup (Halvorsrud and Wagner 1998). The difference is that in the previous study, the authors used droplets of nutrient-containing agar on a hydrophobic plastic plate. They also observed the heterogeneous motion of the plasmodium on the heterogeneous substrate; however, the motion of the plasmodium has not been discretized. To develop our experimental setup, we tried several kinds of plastic substrate and several settings for the hole size, and finally the setup described above gave the best score. In our non-uniform substrate, the plasmodium showed stepwise movement and the direction of the motion was limited to the Von Neumann-type four neighbours. However, though it is very rare, the plasmodium sometimes

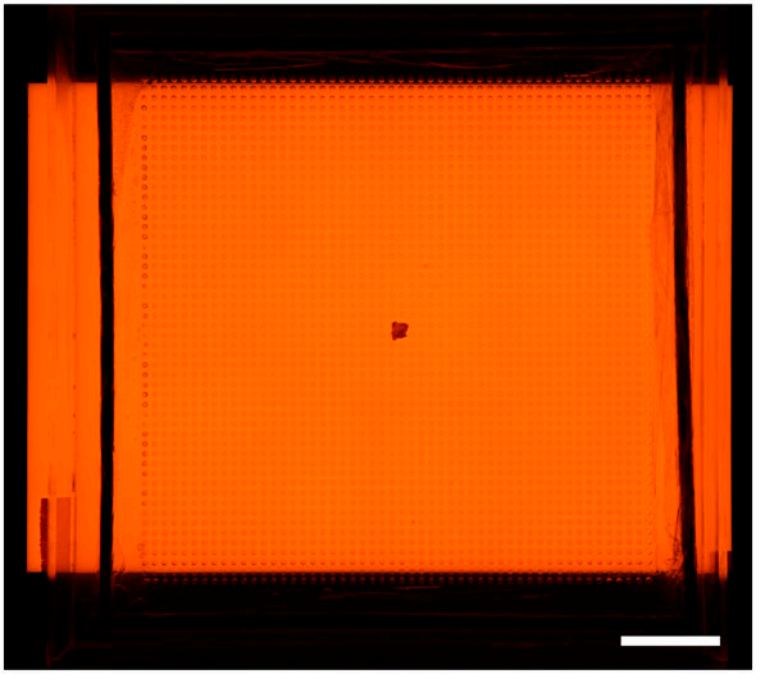

Figure 2. A whole image of the experimental setup. This is an initial condition of the experiment and there is a plasmodium inoculated at the centre. (Bar: 5 cm).

shows diagonal movement and fails to reach four cell neighbourhoods. Our experimental setting with a polycarbonate plate and 3 mm holes minimize the probability of this kind of failure to about 3%.

By using the experimental system described above, we succeeded to observe the pseudo-discretized motility of the plasmodium. In this setup, the morphology of the plasmodium is defined in a discrete manner and the state transition of the plasmodium with the progress of its motility is also clearly defined. Therefore, in the future studies, the experimental setup developed here will be used in many kinds of experiments to observe how the plasmodium makes decisions on its motile and exploratory behaviour. In the following section, we describe the results from simulations based on the experimental results. From the experimental results, we tried to extract the rules which determined the motility of the plasmodium in a plain condition without attractant and repellent stimuli, and we also tried to reconstruct the motile activities of the plasmodium using models of two-dimensional cellular automata.

3. Simulation

In this chapter, the results from the experiments described in Section 2.1 were analysed and based on them the simulations in the following sections were performed. For the analysis, we used four experimental data, two from the experiments using 40 mg plasmodium and two from 80 mg plasmodium. The whole experimental space was regarded as two-dimensional cellular automata with two states, the agar surfaces as cells, absence of the plasmodium as state 0 and the presence as state 1. Von Neumann neighbourhood was used for the analysis of the experimental results and in the reconstruction of the plasmodial movement in the

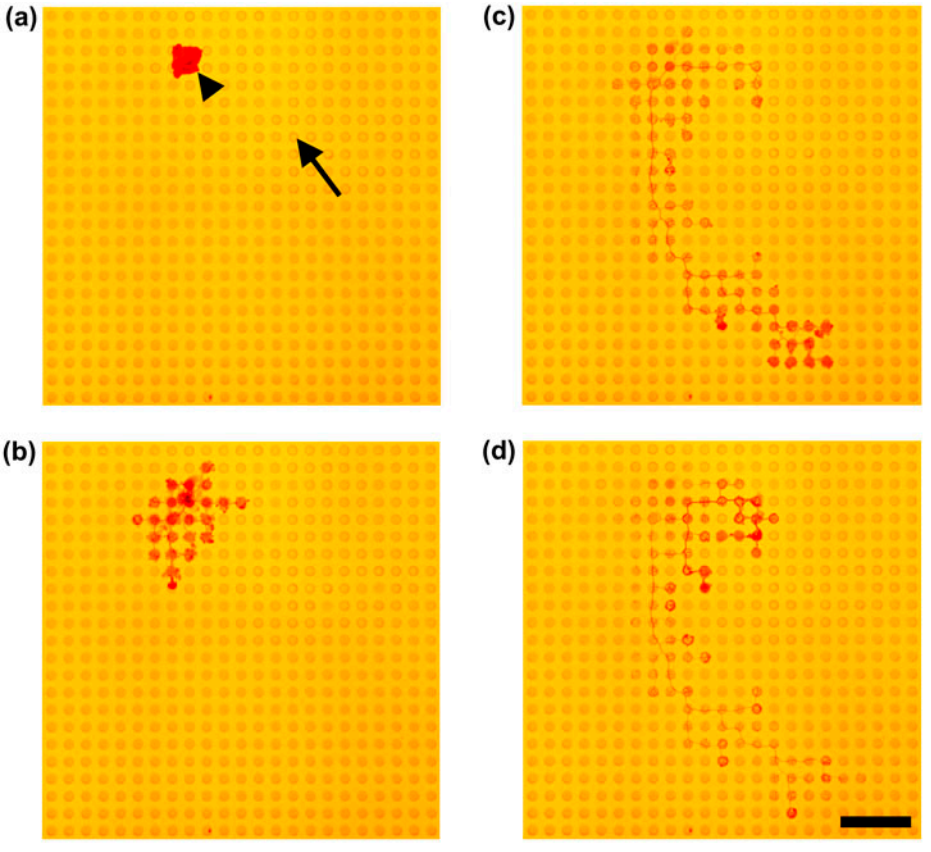

Figure 3. Enhanced images of the time development of plasmodial morphology. Each figure indicates the morphology of the plasmodium at (a) 0 min (b) 1000 min (c) 2000 min (d) 3000 min after the inoculation. These figures show the trajectory of the stepwise and four-neighbour-limited movement of the plasmodium. Arrow indicates the circular gel surface and arrowhead indicates the body of the plasmodium. (Bar: 2 cm).

simulations. As indicated in Figure 4, image-based analysis was performed to define the state of cellular automata in the experiment and based on this analysis, two-dimensional cellular automata were reconstructed in a lattice space with 50 cells by 50 cells. In the analysis of experimental results, the time developments of the plasmodia during 4500 min were analysed and about 120 K times of state transitions were observed. In the simulation of reconstructed cellular automata, the distance between the cells corresponds to 5 mm and one-time step corresponds to 10 min in the real world. We tried four models of cellular automata to duplicate the behaviour of the plasmodium.

3.1. Model 1

In model 1, from the experimental results we extracted the transition rules those are similar to Conway's game of life. In this model, the next state of each cell is determined by the present state of the cell itself and its four neighbours (Table 1). For example, if the present state of a cell is 0 and there are three state-1 cells in the neighbourhood, the next state of the cell will

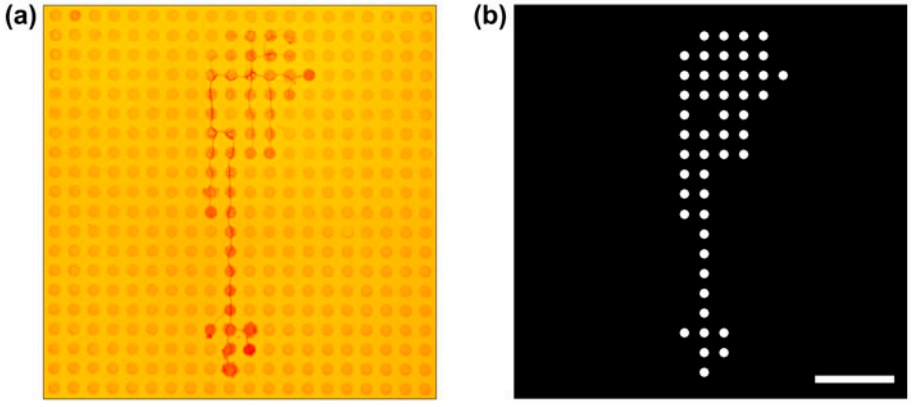

Figure 4. (a) An image from the experiment. (b) A reconstructed image from (a). The agar surfaces in the experiment were regarded as cells in the simulation of cellular automata, and if the plasmodium was present in one cell, the cell was assigned state 1. In (b), cells with state 1 are indicated by white circles. For the detection of the state-1 cells, a simple thresholding was effective, because in the experiment secreted materials or remnant of the plasmodium was hardly detectable. (Bar: 2 cm).

be 1 with the probability of 0.046 and the cell remains 0 with the probability of 0.954. The example of time development in this model is illustrated in Figure 5. Compared with the real plasmodium and models 2–4 in the following section, the morphological growth of model 1 is obviously slow and this rule setting did not seem to duplicate the behaviours of the real plasmodium.

3.2. *Model 2*

In model 2, we adopted more detailed rule settings compared with model 1. Namely, 32 states were defined for a cell and its four neighbours, and though some patterns have symmetry between them, they were distinguished (Table 2). From the defined 32 states to the next 32 states, the probabilities of the state transition were extracted from the experiments, and implemented in the simulations in model 2 (Table 3). Model 2 was successful in that it realized the sufficient growth speed of morphology; however, the body of the automata was separated into many clusters and this is not consistent with the real plasmodium (Figure 6). Therefore, we incorporated a new setting into the model.

Table 1. Settings for the probability of state transitions used in model 1.

State transition	Number of state 1 cells in 4-neighbours				
Present to next	0	1	2	3	4
0 to 0		0.987	0.976	0.954	0.979
0 to 1		0.013	0.024	0.046	0.021
1 to 0	0.124	0.041	0.016	0.008	0.002
1 to 1	0.876	0.959	0.984	0.992	0.998

Note: The values of the state transition probability are configured according to the actual behaviours of the plasmodium in the experiments.

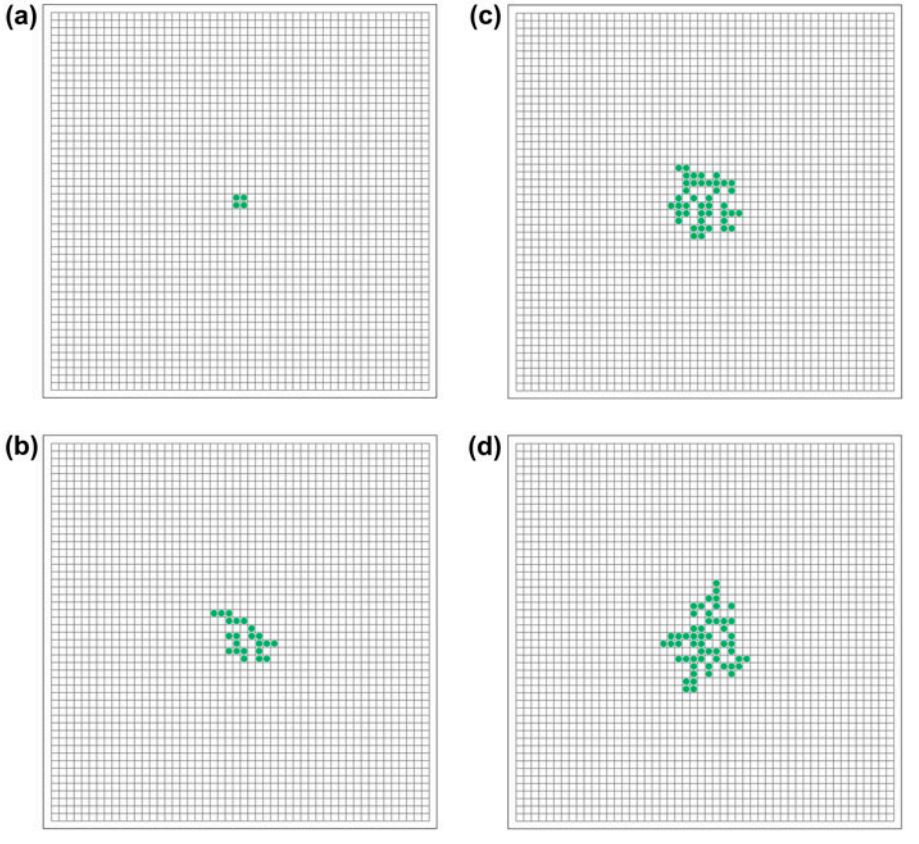

Figure 5. Time development of model 1 cellular automata. State-1 cells are indicated by green circles and state-0 cells are indicated by blanks. Each figure indicates the state of the automata at (a) 0th (b) 100th (c) 200th (d) 300th time step.

Table 2. The definition of 32 states of a cell and its four neighbours in models 2–4.

0	1	2	3	4	5	6	7
							
8	9	10	11	12	13	14	15
							
16	17	18	19	20	21	22	23
							
24	25	26	27	28	29	30	31
							

Table 3. The probabilities of state transition from state 7. Similar lists of state transition probabilities were also defined for the other 31 states.

Present state: 7 Next state	Probability	Next state	Probability
0	0.000246	16	0
1	0.000246	17	0
2	0.001474	18	0
3	0.005895	19	0.001228
4	0.001719	20	0
5	0.005650	21	0
6	0.024073	22	0.000737
7	0.930238	23	0.013265
8	0	24	0
9	0	25	0
10	0	26	0
11	0	27	0
12	0	28	0
13	0	29	0
14	0	30	0
15	0.014001	31	0.001228

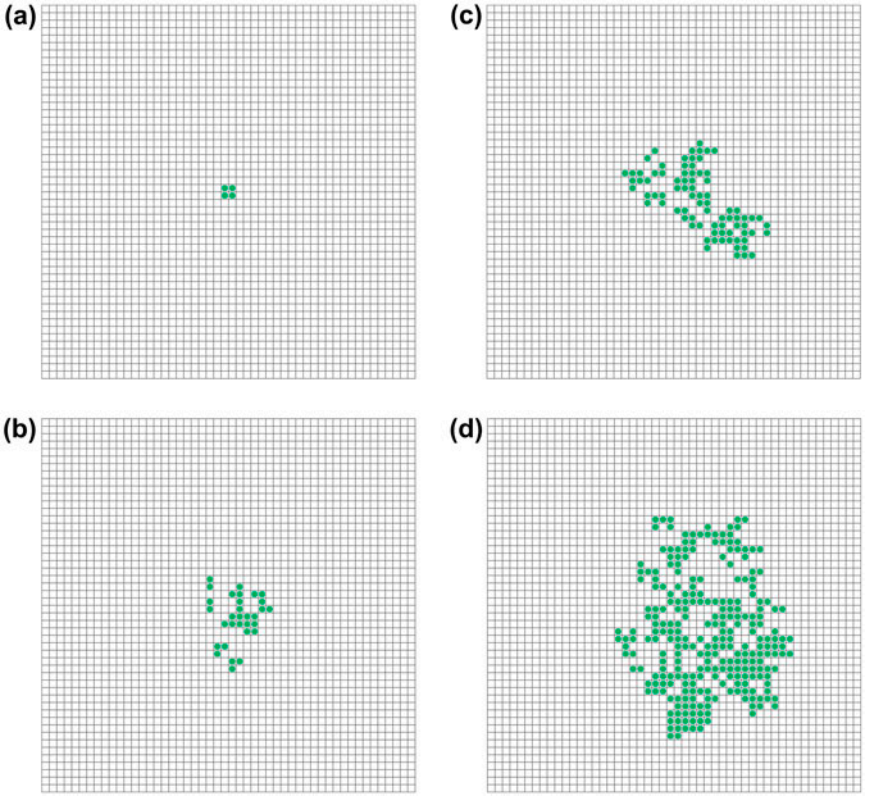

Figure 6. Time development of model 2 cellular automata. State-1 cells are indicated by green circles and state-0 cells are indicated by blanks. Each figure indicate the state of the automata at (a) 0th (b) 100th (c) 200th (d) 300th time step.

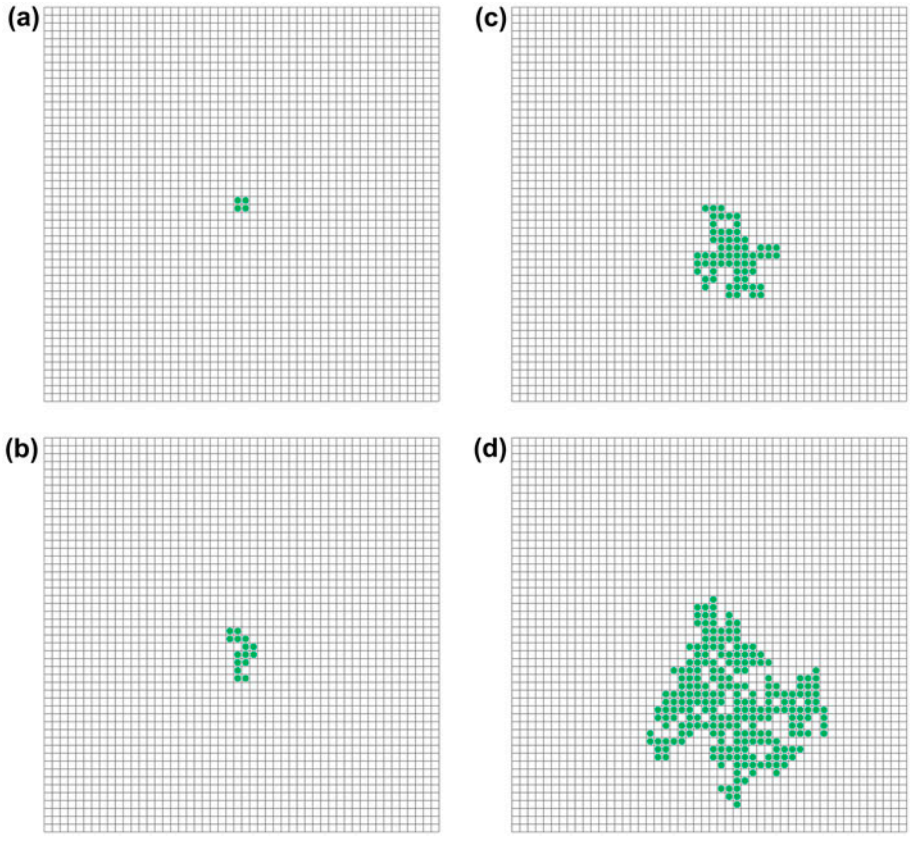

Figure 7. Time development of model 3 cellular automata. State-1 cells are indicated by green circles and state-0 cells are indicated by blanks. Each figure indicate the state of the automata at (a) 0th (b) 100th (c) 200th (d) 300th time step.

3.3. Model 3

In model 3, the rule settings of state transition were the same as model 2, but there was an additional rule. In this model, if some of the state-1 cells were separated from the main body of the state 1 cluster as a result of state transition, the transition was cancelled and the probabilistic processes of the state transition were repeated until the transition was performed without the separation. By the introduction of this rule in model 3, the morphology of the automata body achieved a unity that is similar to the real plasmodium (Figure 7). However, the growth pattern of model 3 was relatively homogeneous and this is not consistent with the heterogeneous morphology of the real plasmodium. In the next model, we further added a new rule to the model.

3.4. Model 4

Model 4 is a model 3 with one modification. In model 4, if one type of state transition occurs, the same type of state transition is repeated five times. For example, provided that a transition from state 7 to 15 occurred in one place, this type of transition is applied to the other state-7 cells five times and the other types of transition from state 7 were prohibited.

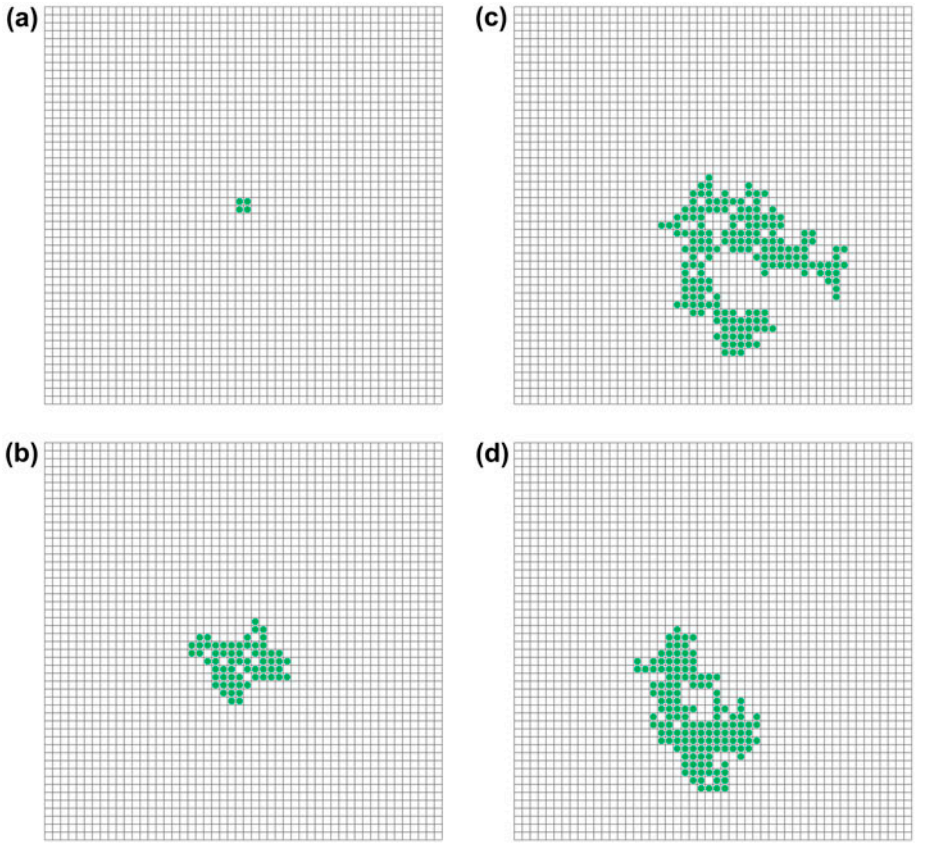

Figure 8. Time development of model 4 cellular automata. State-1 cells are indicated by green circles and state-0 cells are indicated by blanks. Each figure indicates the state of the automata at (a) 0th (b) 100th (c) 200th (d) 300th time step.

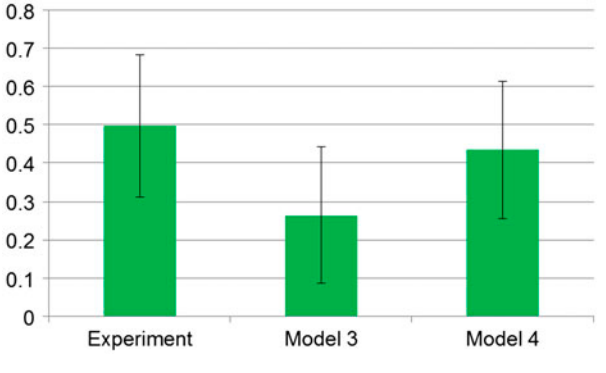

Figure 9. Average values of flatness F in the experiment, model 3 and model 4 at 300th time step. The values in the experiments come from four samples, and those in the models come from eight trials of simulation. Error bars indicate standard deviation.

If this setting causes a separation of the automata body, then the cell remains in the original state. By introducing this rule, we implemented a bias and historical effect in the growth patterns of the model. Model 4 automata formed a heterogeneous structure that was similar to the real plasmodium (Figure 8(c)). Furthermore, a degeneration of such structure was also observed (Figure 8(d)), as in the real plasmodium (Figure 3(d)). The morphologies of automata in model 3, model 4, and of the plasmodium in the experiments were analysed by using principal component analysis. The analyses were performed on the two-dimensional distribution of state-1 cells, and the heterogeneity of the morphology was evaluated based on the eigenvalues in major (λ_1) and minor (λ_2) axes. Flatness F of the morphology was defined based on these values as $F = 1 - \lambda_2/\lambda_1$ according to Takamatsu, Takaba, and Takizawa (2009). In Figure 9, the average values of F in model 3, model 4, and in the experiment are shown, and if F is equal to 0 ($\lambda_1 = \lambda_2$), the morphology is isotropic, and if the value is near 1 ($\lambda_1 \gg \lambda_2$), the morphology is anisotropic. The average value of F in model 4 showed similar value with that in the experiment, and was much higher than that in model 3 (Figure 9). By this analysis, we confirmed that the morphology of model 4 has more similar heterogeneity to the real plasmodium compared with model 3.

3.5. Discussion

In the simulations, we extracted the state transition rules from the experimental results and constructed the models. In model 1 with game of life-like transition rules, the growth patterns of the real plasmodium were not reproduced. This is because the transition rules in this model are isotropic, and thus the rules do not match the anisotropic growth patterns of the plasmodium. In model 2, we introduced the distinction between some symmetric states of a cell and its four neighbours to duplicate the anisotropic growth patterns of the plasmodium; and in model 3, another additional rule was incorporated into the model to maintain the unity of automata body. The morphology of the automata in model 3 partly reproduced the behaviour of the plasmodium; however, there was no heterogeneous growth pattern that was observed in the real plasmodium. We thus further introduced an additional rule in model 4 and implemented a historical effect in the state transition rules. By this setting, the model duplicated the heterogeneous morphology of the real plasmodium. The results from model 3 and model 4 indicate that the settings of the local state transition rules are not enough for duplicating the behaviours of the plasmodium and an additional rule that refers to the past is needed. In other words, with only local state transition rules of cellular automata, the growth patterns of the plasmodium are not reproduced.

4. Concluding remarks

In this study, we developed an experimental system to discretize the movement of the plasmodium. By using our experimental system, we succeeded in limiting the motility of the plasmodium to four neighbours and making it stepwise. In the future studies, this experimental system will be useful in observing the plasmodium that is exploring an unknown space and how the organism makes its decision. Especially, in our experimental system, the state of the plasmodium and its state transition are clearly defined; thus, it is also possible to clearly observe the correlationship between the present state of the plasmodium and its behaviours in the future, or how the history of one behaviour affects the next.

Actually, we performed this kind of observation. In the simulations in this study, we constructed a two-dimensional cellular automata model based on the experimental results and tried to duplicate the behaviours of the plasmodium in the models. As a result, we found that

to reproduce the behaviours of the plasmodium, the local state transition rules are insufficient and a kind of historical effect is needed. This result implied that there is a similar effect from history in the real plasmodium. As our simulation results demonstrated, our experimental system is useful in the studies of the plasmodial growth patterns and the plasmodial exploratory behaviour. Furthermore, the substrate used in this study was only plain agar; however, the whole or parts of agar components are replaceable with attractant or repellent containing agar. Therefore, in the future study, it will be also made possible to clearly observe in the discrete experimental system how the plasmodium is affected by such environmental stimuli and how the plasmodial exploratory behaviour is changed. The experimental system will contribute to the understanding of cellular exploratory behaviour and its adaptability to the environment.

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