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Selection of initial conditions for recursive production of multicellular organisms

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Abstract

The development of a multicellular organism is a dynamic process. Starting from one or a few cells, the organism becomes a set of cells with different types that form well-determined patterns. It is rather surprising that differentiation in cell types and formation of controlled patterns are compatible, because the former gives morphogenetic diversification whereas the latter implies recursive production of a cell ensemble, reducing individual differences. We studied this problem by taking a simple cell model with intracellular reaction dynamics of chemical concentrations, cell–cell interactions, and increase in cell numbers. We observed successive differentiation from a cell type with diverse chemicals and chaotic concentration dynamics to cell types with oscillatory or fixed-point dynamics, leading to morphogenetic diversity in a spatial pattern. We further show that, by starting from an initial object consisting of both the former cell type with diverse chemicals and the latter differentiated cell type, the recursive production of a multicellular organism with morphogenetic diversity is possible. By relating the former type to a cell in the vegetal pole and the latter to one in the animal pole, classic experimental results with separation of blastomeres in sea urchin eggs are coherently explained, while some theoretical predictions are made for in vitro morphogenesis from embryonic stem cells.

Keywords: Recursive production; Morphogenetic diversity; Development; Cell differentiation

1. Introduction

In a multicellular organism, a single cell—an egg—or a group of cells develops into a prospectively determined pattern correctly. This morphogenesis is robust against environmental perturbations, and the same pattern is always generated from an egg. In other words, recursive production is repeated. At the same time, the developmental process in a multicellular organism produces a variety of cell types. Morphogenetic diversity is a common feature. The compatibility of these two points is surprising, because 'recursive production' is the reproduction of the same pattern of an individual cell, whereas 'morphogenetic diversity' is the existence of various patterns, namely various cell types within an individual. (Indeed, it would be much easier to achieve recursive production without morphogenetic diversity, like crystal growth.)

Of course, compatibility between recursive production and morphogenetic diversity is not always possible, when starting from any type of cell. In a natural condition of a multicellular organism, a somatic cell can produce itself, but a recursive pattern with morphogenetic diversity does not appear in general. By starting only from a specific type of cell, i.e. germ cells, recursive production with morphogenetic diversity is realized. The question we address here is therefore the selection of initial cell(s), to allow for compatibility. When one regards development as a dynamic process, the question of concern is the selection of an initial state (condition) that allows for compatibility. Our aim in this paper is a

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systematic investigation of this problem, by clarifying which subset of cells in a parent multicellular organism can be selected as a starting object (an egg or a blastomere) to satisfy compatible conditions for such contradictory attributions.

In recursive production of an individual multicellular organism, an egg or a group of cells divides repeatedly to form a determined pattern. The initial condition for recursive production of a multicellular organism was studied in two classic experiments. Roux showed that the frog embryo is a mosaic of self-differentiating parts, and a 2- or 4-cell frog embryo cannot produce a normal adult body. On the other hand, Driesch showed that each isolated blastomere taken from the 4-cell stage of an embryo develops into a complete larva of a sea urchin (Gilbert, 2003, Chapter 3, Fig. 3.15). In this case, the cells still provide the initial conditions to allow normal development. To date, which or why a certain stage of blastomere is mosaic or regulative remains unexplained. In this paper, we approach this question by searching for conditions on initial cell state(s) that allow recursive production of a multicellular pattern.

The problems of recursive production and morphogenetic diversity have been studied from theoretical viewpoints over decades. John von Neumann constructed an abstract model for recursive production of self-replicating automata (von Neumann, 1966), where each 'cell' can take a finite number of states, which are updated synchronously in discrete time steps according to prescribed rules. If each cell state is considered as a cell type, this system can be interpreted as a model for recursive production, while finely tuned transition rules between cell types must be implemented in advance. Indeed, as a result of this assumption of elaborate rules, the reproduction in the model is not robust against perturbations. Furthermore, selection of initial cell states that allow for recursive production was not studied.

Theoretical study of cell differentiation and morphogenesis was pioneered by Alan Turing, who showed that a reaction–diffusion system can produce an inhomogeneous stable pattern (Turing, 1952). Independent of initial conditions, concentrations of chemicals form a stripe or wave pattern, and this pattern formation process is robust against perturbations. Turing's theory gives a dynamic systems basis for morphogenesis and potentiality of cell differentiation. On the other hand, it cannot explain the dependence of the developmental process on the initial cell state(s). Embryogenesis with *increases of cell numbers* was not studied, and the intracellular dynamics were not sufficiently complex. In fact, resource chemicals are transported into a cell and a complex catalytic reaction network within the cell changes the cell's state over time. Genes are expressed on or off with this intracellular dynamics.

Kauffman proposed that each cell type should be regarded an attractor of such intracellular dynamics (Kauffman, 1993, pp. 467–499, pp. 482–484), where each cell type is represented as an attracting state of a genetic network. Again, neither morphogenetic processes with cell differentiation nor the initial conditions (starting object) required to satisfy recursive production were studied.

By considering both the cell-to-cell interaction in Turing's study and intracellular dynamics, in conjunction with the cell division process to increase the cell numbers, one of the authors (K. K.) and T. Yomo proposed *isologous diversification* (Kaneko and Yomo, 1997, 1999). This process allows spontaneous cell differentiation through cell division processes. In contrast to Kauffman's viewpoint, cell types that cannot exist as attractors of single-cell dynamics are stabilized through cell–cell interactions. The cell types as well as the distribution of each cell type are robust against perturbations. Later, two of the authors (C. F. and K. K.) demonstrated that interacting cells with complex intracellular reaction dynamics lead to irreversible, hierarchical cell differentiation (Furusawa and Kaneko, 1998, 2002).

These studies may give a basis for recursive production as well as morphogenetic diversity of a multicellular organism. However, they do not define the initial cell states that would make recursive production of a multicellular pattern possible. In this paper we answer this question, based on the previous studies. By modifying the previous model (Furusawa and Kaneko, 2002) to take into account direct cell–cell diffusion processes, we discuss the pattern formation of a one-dimensional chain of cells. With this model, we perform a systematic investigation to identify the subsets of initial cell states that allow both recursive production and morphogenetic diversity.

This paper is organized as follows. In Section 2, we introduce a simple model of a multicellular organism consisting of one-dimensional cells with complex intracellular chemical reaction dynamics. Results of numerical simulations are given in Section 3, which shows recursive production and morphogenetic diversity of a multicellular organism. We show that an initial cell state with diverse chemicals and chaotic intracellular dynamics leads to cell differentiation into several types, whereas cells with fixed chemical concentrations do not allow cell differentiation to occur. By defining measures for recursive production ('recursiveness', in short) and morphogenetic diversity ('diversity', in short), we then establish the types of initial cells that allow compatibility between the two attributes. In particular, we show that an initial condition consisting of cells with chaotic concentration dynamics and fixed concentrations satisfies both recursiveness and diversity. Change of growth speed through successive differentiation of cell types is

also discussed. Finally, in Section 4, we compare our results with the development in real organisms such as *sea urchins*. Surveying embryogenesis from some types of cells, we suggest that the cell state of the vegetal hemisphere has diverse chemicals and chaotic dynamics, thereby promoting diversity, while that of the animal hemisphere has less diverse chemical concentrations with stationary concentration, thereby promoting recursiveness. A few predictions are made from the present theory.

2. Model

In this section, we present an abstract model of a multicellular organism. See Fig. 1 for a schematic illustration of our model. Within each cell, catalytic and autocatalytic chemical reactions maintain the cell itself and synthesize some chemicals for the cell membrane.

The concentration of the lth chemical species in the ith cell at time t is denoted by $x_i^l(t)$. We assume that outside the cells a source material with a constant concentration, denoted by X^0, is supplied. The corresponding concentration of the chemical within a cell is denoted by x_i^0, which plays the role of a source to produce other chemicals. There are catalytic reactions within each cell, represented by a set of Michaelis–Menten reactions as follows:

$$Met_i^l(t) = e \sum_{m,j} Con(m,l,j) \frac{x_i^j(t) x_i^m(t)}{1 + x_i^m(t)/x_M} - e \sum_{m,j} Con(l,m,j) \frac{x_i^l(t) x_i^j(t)}{1 + x_i^l(t)/x_M}, \quad (1)$$

where x_M is a parameter for the Michaelis–Menten form and e is the coefficient for the reaction. The reaction network is represented by $Con(m,l,j)$, which is 1 when there is a path from chemical m to l catalysed by chemical j, and 0 otherwise. It should be noted that

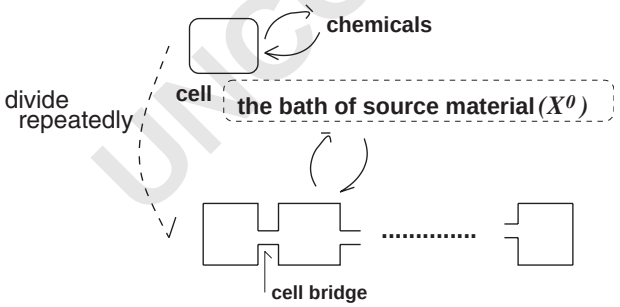


Fig. 1. Schematic representation of our model. Cells are surrounded by a bath of source material with a constant concentration denoted by X^0. After a division, cells are connected with one another by forming a cell bridge (plasmodesma). Cells are thus connected with one another as a one-dimensional chain.

$Con(m,0,0)$ is 0 because the source material x_i^0 is not autocatalytic.

Now, we denote by $V_i(t)$ the volume of the ith cell at time t. We assume that some chemicals produce the cell membrane materials, and that the chemicals decay linearly with a coefficient γ. This term is expressed by $\gamma P(l) x_i^l(t)$ where $P(l) = 1$ when x_i^l produces the membrane, otherwise $P(l) = 0$. To sum up, $V_i(t)$ grows as follows:

$$dV_i(t)/dt = V_i(t) k_m \gamma \sum_{l=1} P(l) x_i^l(t), \quad (2)$$

where k_m denotes the coefficient of the membrane material's contribution to the growth of $V_i(t)$.

The ith cell divides into two when

$$V_i(t) > 2V_0 \quad (3)$$

is satisfied, where V_0 is a prescribed initial volume. When this happens, the daughter cell (numbered $i+1$ here) has almost the same concentration of chemicals as the mother cell i. To be specific, x_i^l and x_{i+1}^l, after a division, become $(1+\varepsilon)x_i^l(t)$ and $(1-\varepsilon)x_i^l$, respectively, where ε represents a small fluctuation caused by the cell division. In this paper, ε is taken as a random number over $[-10^{-4}, +10^{-4}]$, but this magnitude is not important for cell differentiation (as long as it is not zero). After a division, the two cells have the same volume, namely $V_i(t)$ and remain connected with each other by forming a cell bridge (plasmodesma). Through this cell bridge, the chemicals of the two cells spread out with normal diffusion and its coefficient D_{pd}. Following cell divisions, cells are connected with one another as a one-dimensional chain. (Such a chain of cells connected by cell bridges (plasmodesmata) is seen, e.g. in *Anabaena* (Balkwill et al., 1984; Ehlers et al., 1999; Ehlers and Kollmann, 2001)).

Only x_i^0 is supplied by a flow from the bath of source material X^0 with normal diffusion and its coefficient D_{in}, while the other chemicals spread out slightly into the bath with the diffusion coefficient D_{out}. This flux of each chemical is assumed to be proportional to the volume of the cell. Summing up all these processes, we study the following model equation:

$$dx_i^l(t)/dt = Met_i^l(t) + D_{pd}/V_i(t) \times \sum_{NN=\text{Nearest neighbour}} (x_{NN}^l(t) - x_i^l(t)),$$

$$\begin{cases} +D_{in}(X^0 - x_i^0(t)), & l = 0 \text{ the source material,} \\ -D_{out} x_i^l(t) - \gamma P(l) x_i^l(t), & l > 0 \text{ the others,} \end{cases} - x_i^l(t)(dV_i(t)/dt)/V_i(t). \quad (4)$$

3. Simulation results

In this section, we study some conditions for recursiveness and diversity of a multicellular organism consisting of cells of the model in Section 2. We also discuss some relationships between the growth speed and cell types.

3.1. Choice of reaction network

We have carried out numerical computations for a variety of randomly chosen reaction networks, i.e. *Con* of Eq (1) and parameters of Eqs. (2) and (4). For most of the networks we studied, there appears to be no cell differentiation. The chemical dynamics fall onto fixed points, and after a cell divides, the chemical concentrations of the two cells remain almost identical. For networks consisting of 20 chemical species with four autocatalytic and three non-autocatalytic paths from each on average, about 99.9 per cent of randomly chosen networks exhibit this fixed-point behaviour. The remaining 0.1 per cent of reaction networks show spontaneous cell differentiation through development, in the sense that the chemical compositions of cells start to take a few, clearly separated sets of values.

In this paper, we study the latter cases with cell differentiation. The first reason we study these rare cases is that we are interested in multicellular organisms with cell differentiation. Another reason for this choice is that the cases with differentiation have higher growth speeds as an ensemble of cells, as already discussed in (Furusawa and Kaneko, 2002).

The number of networks allowing cell differentiation could be increased by choosing a suitable network topology or by including positive or negative feedback loops. Although study of a network structure that allows cell differentiation is important, it remains a future problem. To study the topology of such a network, the number of chemicals should be increased. Here we study the case with 20 chemicals only, to see clearly the dynamics of chemical concentrations in the 20-dimensional phase space and to focus on dynamics and differentiation in the phase space. This set of chemicals can of course be understood as a 'core' part, essential to cell differentiation, within a much higher-dimensional reaction network.

We have studied all the examples with the cell differentiation we observed. In terms of diversity and recursiveness, all six cases we studied have common features. Here, we present simulation results from only two of the reaction networks, but the results discussed here are also valid for the other four networks.

3.2. Differentiation of chemical compositions

An example of the course of differentiation is shown in Fig. 2, where the time series of x^6 from a certain initial condition is plotted. (We term this network example 'I' in this paper). The network structure of Example I is depicted in Fig. 3, where the nodes and arrows denote the chemical species and the paths of chemical changes, respectively. During cell division and the increase of cell numbers, the chemical state of some cells switches to a different type. This differentiation originates from the instability of a homogeneous state and clustering, as has been theoretically studied in coupled dynamical systems (Kaneko, 1990) generally. Each cell type has different compositions of chemicals, as well as different type of dynamics.

These different types are plotted in the phase space (to be specific as a projection to the plane x^6 and x^{18}) in Fig. 4(a) with different colours: red, green and blue. The cell types can be discretely discriminated in the phase space and the transition from one type to another occurs in a rather short span compared with a cycle for cell division. In Example I, only the three types appear, while only the transitions from 'red' to 'green' and 'green' to 'blue' occur. As for the dynamics, the red type shows chaotic chemical concentration dynamics, the green one shows a periodic motion and the blue a fixed point, respectively, as depicted in Fig. 4(a).

The difference in dynamics can be discerned from the time series of the concentrations, and the plot in the phase space. Whether or not the motion is chaotic is well

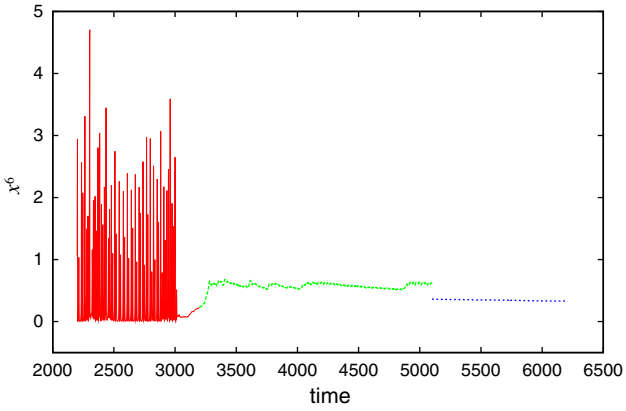


Fig. 2. Example I: the time series of x^6 when initiated from a single cell with a certain initial condition of its chaotic intracellular dynamics. Three types of dynamics can be observed: red, green and blue. Parameters are $X^0 = 1.5$, $e = 0.3$, $x_M = 5.0$, $k_m = 0.1$, $\gamma = 0.001$, $V_0 = 5.0$, $D_{in} = D_{pd} = 0.01$ and $D_{out} = 0.0001$. The chemicals producing cell membrane materials are $x^5, x^{11}, x^{13}, x^{18}$ and x^{19}. For Example II (the time series is not shown), the parameters which are not the same as those of Example I are $D_{pd} = 0.1$ and *Con* (network structure). The chemicals producing cell membrane materials are x^4, x^5, x^{11}, x^{12} and x^{16}.

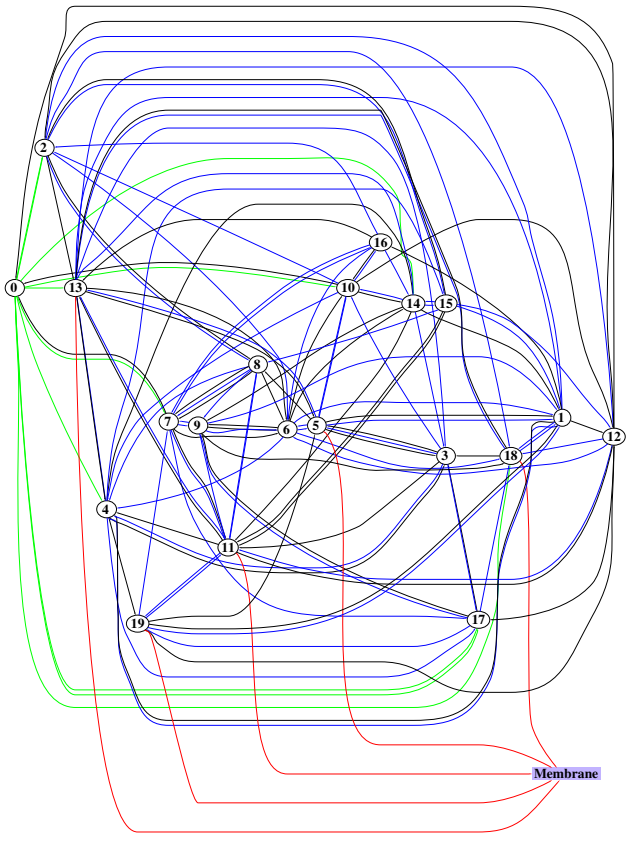


Fig. 3. Biochemical network adopted in Example I. Each node denotes the chemical numbered in the unshaded portion. The green arrows denote the flows from the source chemical 0. The blue and black arrows denote autocatalytic and non-autocatalytic paths, respectively. Each red arrow denotes a membrane-producing chemical by its root node number (i.e. $P(l) = 1$).

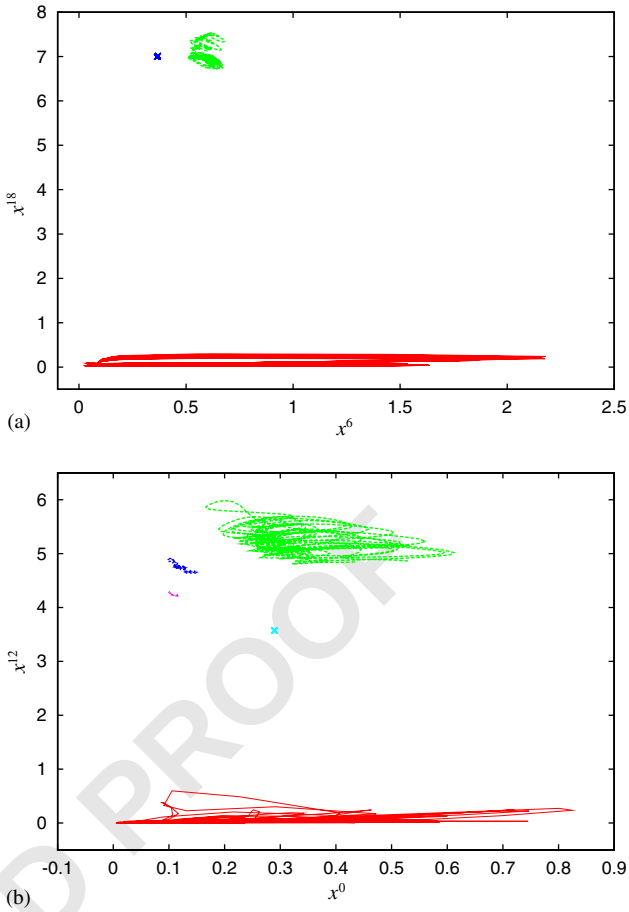


Fig. 4. (a) and (b) Dynamics of Examples I and II respectively. The orbits of chemical concentrations are plotted by using (x^6, x^{18}) and (x^0, x^{12}), respectively. (a) The three colours red, green and blue denote a chaotic attractor, a periodic *partial* attractor, and a fixed point, respectively, corresponding to the cell types. (b) The five colours (red, green, blue, magenta and cyan) denote a chaotic attractor, a quasiperiodic *partial* attractor, a periodic *partial* attractor, a periodic *partial* attractor , and a fixed point, respectively.

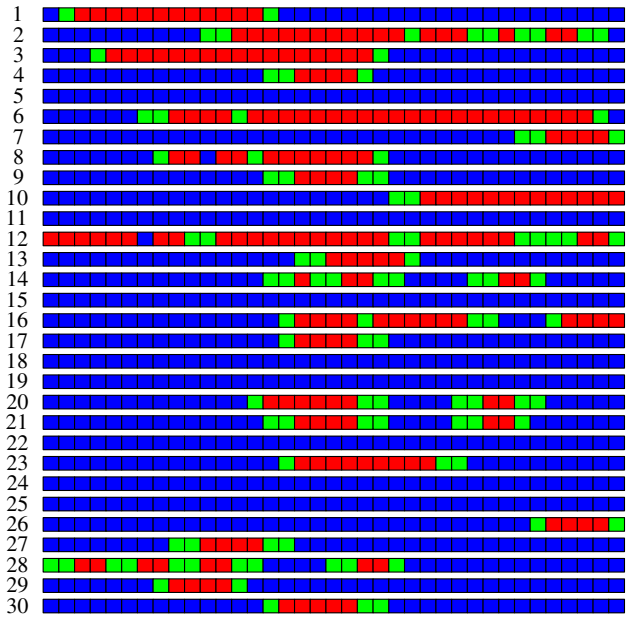
discriminated by the signs of the Lyapunov exponents.[1] In Example I, the (maximal) Lyapunov exponent for the 'red' type is +0.072, whereas it is zero for other types. The types can also be distinguished by their power spectra, where the chaotic type has a continuous spectrum.

Notice that the motion corresponding to the 'green' cell type cannot exist in single-cell dynamics. It appears only as a transient before it falls onto the 'blue' fixed point from the initial condition of the 'green' cell. Coexistence with 'red' cells is necessary for the maintenance of 'green' cells. The cell state that exists by single-cell dynamics is an attractor by itself, and there appear to be cell types (like 'green' here) that do not exist as an attractor in single-cell dynamics, but can exist through interaction with other cell types ('red' here). With the interaction with other cells, this state is stable, and is attracted by the dynamics. Following Furusawa and Kaneko (2002), we call such a state a *partial* attractor.

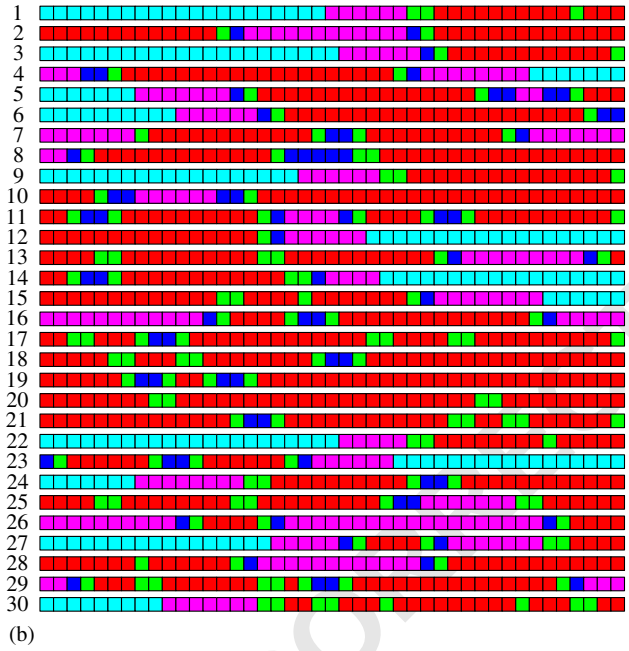
In the beginning, a single cell is put on the bath of source material and divides into two, and then the cell number increases. When initiated from a single 'red' cell, a variety of patterns of cell types are formed depending on the initial condition of the chemical concentration, as depicted in Fig. 5(a). One sample of a chain of cells is also depicted by the *cell-lineage* diagram shown in Fig. 6(a), as a branching process of cell division and differentiation.

Corresponding to the variety of patterns in Fig. 5(a), there are different cell-lineage diagrams, depending on the initial condition of the 'red' cell. For some examples, the sequence of cell types is disordered, as illustrated in Fig. 6(b), while for others all the cells fall onto the same fixed point at a certain time, leading to a homogeneous pattern, as in Fig. 6(c). Starting from one or more cells consisting only of 'green' or 'blue' cells, this homo-

[1]To be precise, we computed Lyapunov exponents over a finite time step before a cell divides.



(a)



(b)

Fig. 5. (a) and (b) Examples I and II, respectively. When initiated from a single red cell, a variety of patterns of cell types are formed depending on the initial condition of the chaotic intracellular dynamics as shown by 30 samples. In these figures the cell-patterns are depicted when the number of cells reaches 37 in (a) and 43 in (b).

geneous pattern solely with blue cells is reached at a certain time.

Similarly, we have plotted the cell differentiation process for Example II, in Fig. 4(b), where five cell types are formed as distinct chemical composition states, and are represented by the colours red, green, blue, magenta and cyan. They correspond to a chaotic attractor, a quasiperiodic motion, a periodic motion and a fixed point, respectively. In this case, four transitions, 'red' to 'green', 'green' to 'blue', 'blue' to 'magenta' and 'magenta' to 'cyan' occur. Similarly to Example I, when initiated from a single 'red' cell, a variety of patterns of cell types are formed, as shown in Fig. 5(b). One cell-lineage diagram is depicted in Fig. 7(a). Again, some sequences of cell types are disordered, whereas from some other initial conditions, cells converge to a homogeneous pattern consisting only of all 'cyan' cells, after a certain time. Indeed, by starting from patterns without red cells, this homogeneous pattern is reached. Here, 'green', 'blue' and 'magenta' cells are not single-cell attractors, but *partial* attractors.

3.3. General rule for differentiation

In this model, cell differentiation starts from an initial chaotic attractor type, which we call C. Then the cell differentiates to several intermediate types $I_1 \to I_2 \to \ldots \to I_m$, before it differentiates into the final type F. The differentiation occurs as $C \to I_1 \to I_2 \to \ldots \to I_m \to F$. Each cell state C or I_j either proliferates to form the same type or differentiates to the next type. The number of intermediate steps m depends on each network. For example, $m = 1$ in Example I and $m = 3$ in Example II. The chemical concentrations of the first type C always show chaotic dynamics, and the complexity in dynamics decreases during this intermediate step (for example, from quasiperiodic-to-periodic), and the chemical concentration of the final type F falls onto fixed-point dynamics. C and F are attractors in single-cell dynamics terms, while I_j are not single-cell attractors, but are stabilized through interactions with other cell types (*partial* attractors). In this subsection, diversity in chemical composition and its relationship with *growth-speed* are discussed.

Depending on the cell type, the chemical composition is different. For particular cell types, the composition is biased to particular chemicals. To characterize the *chemical diversity* of a certain type, we use the following measure:

$$S = -\sum_{l=0} p(l) \ln p(l)$$

with $p(l) = \langle x^l(t)/\sum_{l=0} x^l(t) \rangle$, where $\langle \cdots \rangle$ represents the temporal average. This form is borrowed from Shannon entropy (Furusawa and Kaneko, 2001), but the specific form is not important, as long as it reaches a maximum if all chemicals coexist equally, and becomes smaller when the composition is biased.

In all the examples, this chemical diversity decreases as the cell differentiation progresses. It is largest for cell type C (with chaotic attractor), and decreases as it differentiates into I_j with increasing j, and takes the smallest value for type F with fixed-point dynamics. The *growth-speed* of a cell type is computed as the inverse of

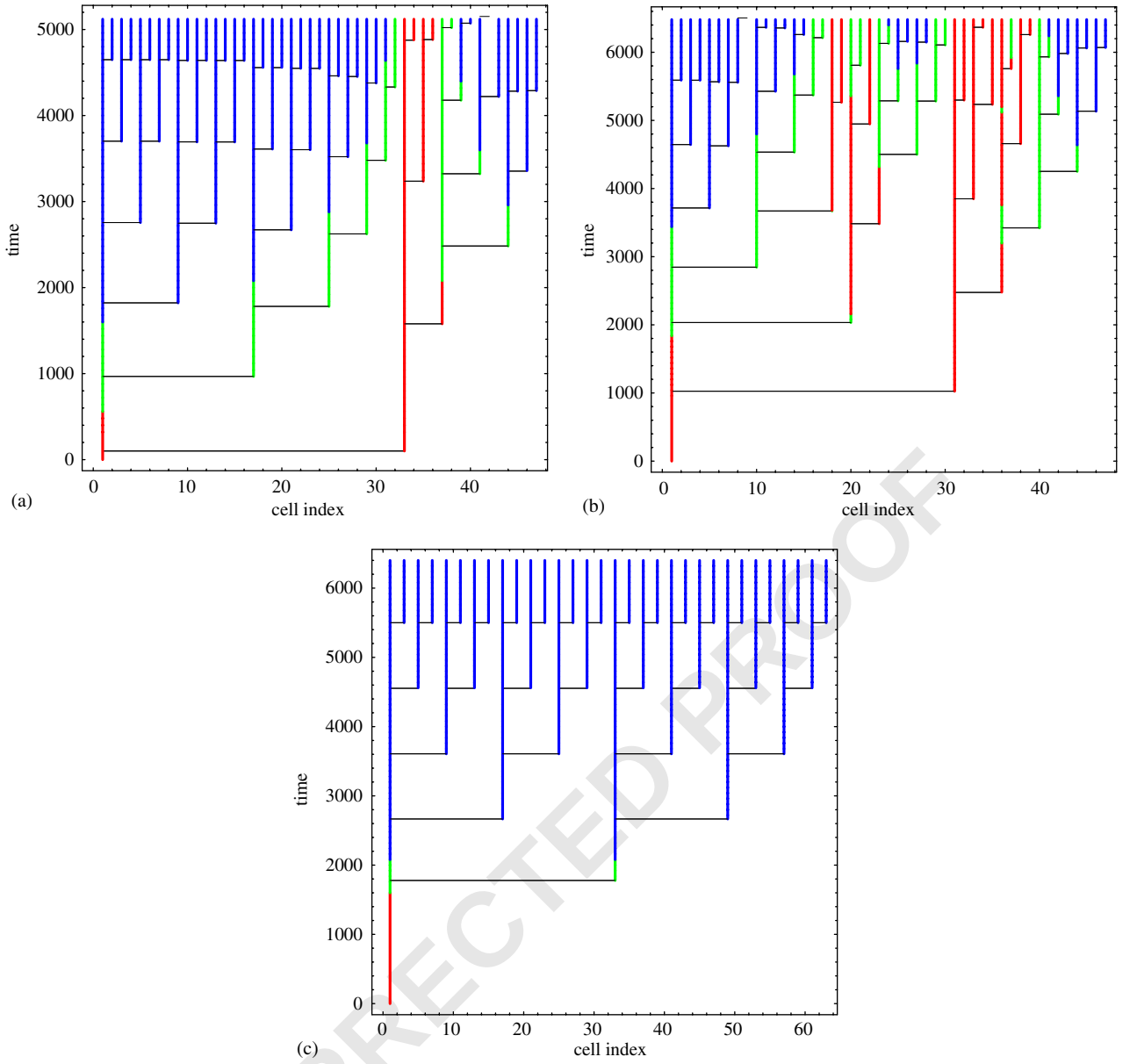


Fig. 6. (a) An example of cell-lineage diagrams for Example I. Initially, a single 'red' cell divides into two at the first branch point. The colour corresponds to each cell type, and the branch gives each cell division event. (b) Another example of cell-lineage diagrams from Example I, when initiated from a single chaos-dynamical (red) cell and (c) Another cell-lineage diagram from Example I when initiated from a single red cell. Around time 2200, two blue cells (with fixed-point dynamics) appear, and replication of the same type of cell is repeated to form a homogeneous pattern.

the time interval between successive cell divisions of the cell type.

The relations between *chemical diversity* and *growth-speed* of cell types in Examples I and II are shown in Fig. 8, where the cell differentiation progresses as red (C) → green (I_1) → blue (F) (Example I), and red (C) → green (I_1) → blue (I_2) → magenta (I_3) → cyan (F) (Example II). In all the examples, the growth speed of the first type (with diversity in chemical composition) is slow, and the next few types derived from it show faster growth, with a peak at some intermediate type (I_l), after which the speed decreases for the later types. In the two examples above, the green type (I_1) has the highest growth speed, and the speed decreases successively for later types. This rule—slow growth for the first type with diverse chemicals, the peak speed of growth in an intermediate type, and the decrease of the speed for the later types—is generally observed in all the examples we have studied. Indeed, this rule is also observed in related models we have studied previously (Furusawa and Kaneko, 2001; Kaneko and Yomo, 1999), and seems to be universal.

3.4. Recursiveness and diversity

Now, we investigate systematically the recursiveness and diversity of a multicellular organism. To investigate

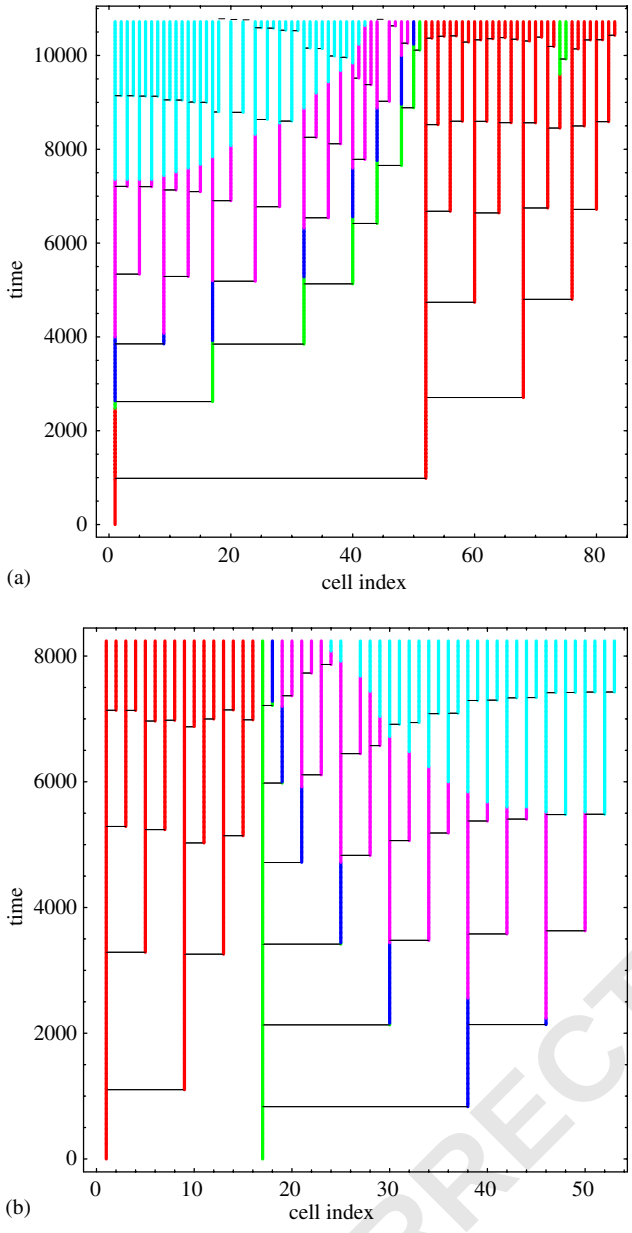


Fig. 7. (a) A cell-lineage diagram for Example II. The colours correspond to cell types. (b) A cell-lineage diagram of Example II that achieves recursive production. In this case, two initial cells, a chaotic attractor coloured red and a quasiperiodic *partial* attractor coloured green are put in the bath of source material.

the compatibility of these two aspects, we first introduce measures characterizing them. Then, we discuss the relationship between diversity and recursiveness, by using these measures.

3.4.1. *Definitions of recursiveness and diversity of a multicellular organism*

Recursive production of an ensemble of diverse cells is essential for a multicellular organism. The diversity, here, means the existence of plural cell types in a multicellular organism—within an individual. If a group

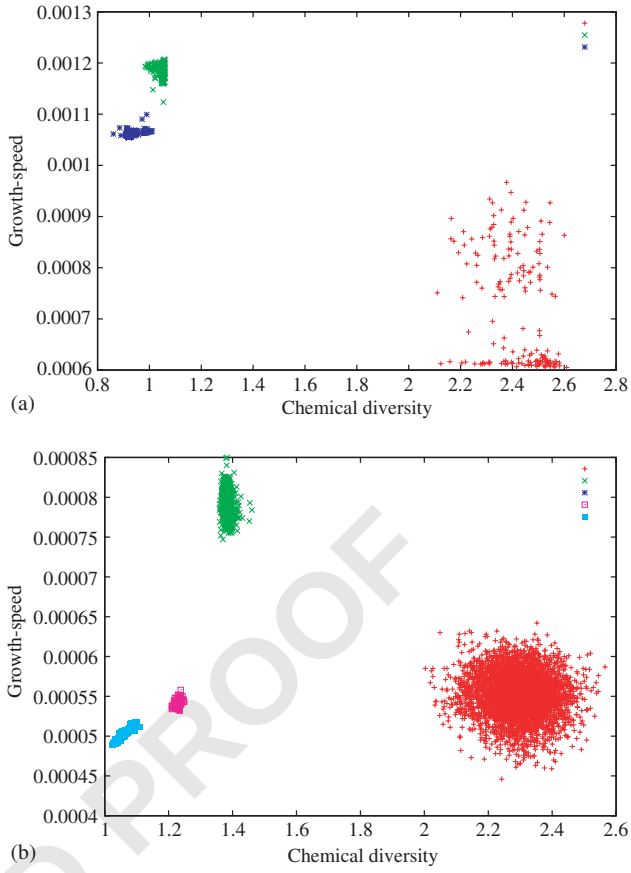


Fig. 8. The relation between *chemical diversity* and *growth-speed* for each cell type plotted by distinct colours in Examples I (a) and II (b). *Chemical diversity* of a cell type is computed as $-\sum_{l=0} p(l) \ln p(l)$ with $p(l) = \langle x^l(t)/\sum_{l=0} x^l(t) \rangle$, where $\langle \cdots \rangle$ represents the temporal average. *Growth-speed* is the inverse of the time interval between successive cell divisions. Each point corresponds to each cell, sampled over about 500 cells: (a) Example I: the transition red → green → blue and (b) Example II: the transition red → green → magenta → cyan.

of cells consisted only of a single type, it would not be called a standard multicellular organism, even though trivial recursiveness is then achieved. Recursiveness, on the other hand, means the development into the same (or a similar) organism, i.e. with the same set of cell types, and same (or similar) spatial arrangement of these cell types. We must understand the origin of such recursiveness and how it combines with diversity of cells.

In our model, recursiveness is represented by similarity between a mother and daughter. To be specific, a subset of cells of the mother is taken away from the mother at a certain time t, as a chain of cells connected by cell bridges. The subset of cells developed from this chain of cells is called a *daughter* of the mother organism. Then we check the similarity between the daughter and mother. For the check, we define two measures of recursiveness. One is a rough measure characterizing the similarity by comparing the daughter's cell lineage diagram with that of the mother's. If the diagrams are the same except for a few shifts, we

consider that the production is recursive. The frequency of such recursive production is then computed as a first measure.

The other measure is relatively more accurate, namely the *Levenshtein distance* (Sankoff and Kruskal, 1983) between the patterns of a daughter and the mother, after reproduction as mentioned above. Following *Levenshtein*, this distance, denoted by L, is defined as follows: let P and D denote the cell-type pattern (string) P of the parent at time t_p and that (D) of a daughter of the same size at time t_d, respectively. Then L is the minimum number of steps to match the two patterns P and D. It is given by the minimum number of steps over all possible transformations of P into D, consisting of substitution, insertion and deletion of a letter, corresponding here to each cell type. The minimum value is easily obtained by dynamic programming. Considering that there is no distinction between left and right in our model, the mirror images of the pattern are assumed to be identical and the minimum is computed by matching to the original or to the mirror image pattern. As L becomes smaller, the sequences between the mother and its daughter become more similar, and recursiveness is increased.

The diversity of a multicellular organism M, on the other hand, is simply defined here as the number of different three-letter sequences that exist as three contiguous cells. For instance, for a sequence 0000122333 (with 0, 1, 2, 3 as distinct cell types), the diversity of M is 7 because we can identify the distinct patterns 000, 001, 012, 122, 223, 233 and 333. We could use some other measure such as an entropy of a sequence, or the diversity of a longer sequence. For the present study, however, this simple measure with three symbols is sufficient to characterize the diversity of cell-type sequences.

3.4.2. Initial state conditions for recursiveness and diversity

To compare a mother and daughter, we take out some cells at a certain stage of development, and put them in the original environment in a bath of material, and start the developmental simulation again. We describe the results when some cells are taken at a certain time from the two examples corresponding to Fig. 6(a) or 7(a), but the results presented here apply to all the other simulations for cells at other time stages or by adopting other networks.

Before describing the results, we explain the terms *disordered* and *uniform* used below. A pattern is *disordered* if the cell pattern consists of mingled cell types without some periodic patterns, as depicted in Fig. 6(b). A pattern is *uniform* if it eventually becomes uniform with one cell type, as depicted in Fig. 6(c).

Depending on the choice of initial cell types, the recursiveness and diversity are summarized as follows:

(1) Starting from a cell type C showing chaotic dynamics of the mother, the resulting pattern is diverse, and varies in each run. Often, the pattern is *disordered* with all possible cell types, but sometimes the pattern is *uniform* with a single cell type F. In the disordered case, there is no periodicity or regular structure. Diversity of cell types and their sequence is supported for most cases, but recursiveness in the pattern is not supported.

(2) Starting from a single cell of types other than C, i.e. starting from I_j or F, the cell pattern of a daughter is *uniform*, consisting only of the fixed-point type F. Then the same pattern of a single cell type arises as $FF...FFF$. Whenever the cell type is started from F, the same pattern arises. In this sense, high recursiveness and low diversity result.

(3) Starting from multiple cells consisting solely of cell types C with chaotic attractor dynamics, the resulting cell-pattern of a daughter is *disordered* with diverse cell types. Thus, the cell type sequence is diverse, but a recursive production is not achieved.

(4) Starting from multiple cells consisting of types other than the chaotic one, such as a combination of cells I_j and F (i.e. with periodic, quasiperiodic or fixed-point (*partial*) attractors), the resulting cell-pattern of a daughter is *uniform* with the single cell type F.

(5) Starting from two cells, one of type C, and the other of I_j or F, the resulting pattern consists of all the cell types, and has some spatial structure, as shown in Fig. 7(a). The sequences of cell types are similar, and satisfy recursiveness relatively well, as depicted in Fig. 7(b) (note again that there is no distinction between left and right).

Summarizing the results, we obtain

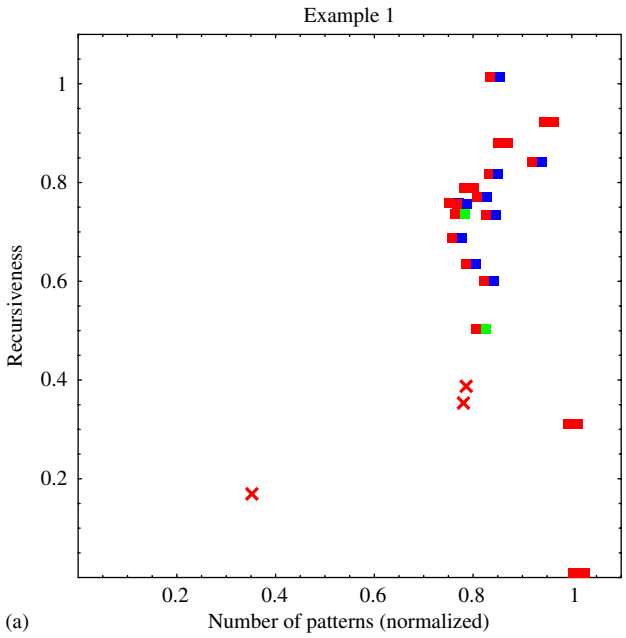
(i) Homogeneous patterns without diversity, when started from cells without C.

(ii) Random patterns without recursiveness, when started from C cell(s) alone.

(iii) Complex patterns with some regularity and recursiveness, when started from a C cell and another cell of type I_j or F.

Hence, the recursive production of a multicellular organism with a diversity of cell types is possible only when two cells with chaotic dynamics and non-chaotic dynamics are taken as the initial condition. For example, considering Example II, even if the two cells are 'red' (chaotic attractor) and 'cyan' (a fixed point), the recursive pattern as depicted in Fig. 7(b) is produced eventually, that is, 'green', 'blue', and a few 'magentas' are produced between 'red' and 'cyan.' As shown in the figure, the sequence of cell types is regular, and this pattern is reproducible from the initial condition with one C-type cell and the other I_j or F.

To confirm the statement above quantitatively, the measures of recursiveness and diversity are plotted in Figs. 9(a) and (b), where each mark gives the average value of recursiveness and diversity for a different choice of initial conditions. The colour of the mark corresponds to the cell type. For single-cell initial conditions, the measure is plotted with a cross with a different colour, while for two-cell initial conditions, it is shown as a pair of squares with different colours. The vertical axis shows the frequency of recursive production (by adopting the first measure of recursiveness), and the horizontal axis shows the diversity, measured from 100 samples of the initial condition.



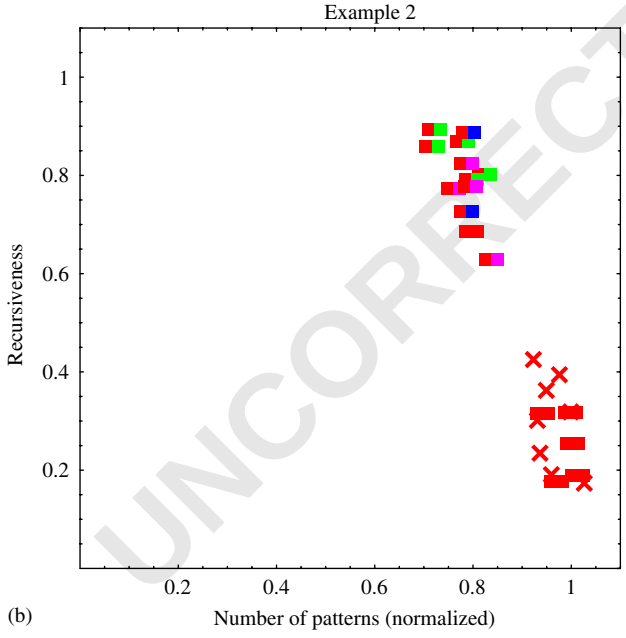


Fig. 9. (a) The diversity (normalized) and recursiveness (frequency) of the cell pattern illustrated in Fig. 6(a). Each cross denotes the values starting from a single cell with its colour corresponding to a cell type. Each pair of squares denotes the values from two cells with the cell types corresponding to the colours, connected by a cell bridge. (b) The diversity (normalized) and recursiveness (frequency) of the cell pattern illustrated in Fig. 7(a). Plotted as in (a).

In Fig. 10, the distribution of the *Levenshtein distance* (the second measure of recursiveness) and the diversity is plotted, again from 100 samples. Here, the *Levenshtein distance* is measured between the cell-type sequences of the mother and the daughter when the number of cells reaches 26 in Example I and 49 in Example II. The two Figs. 9(a), (b) and 10 support items (1)–(5). Starting from a cell of type C, diverse cell types are generated, but it is quite difficult to make such a pattern recursive. On the other hand, from a cell of type I_j or F, the cell state is so stable that a resulting pattern is homogeneous, consisting solely of a single cell type. A balance between C and I_j/F makes compatibility possible, even when cell numbers and cell–cell interactions increase. Concepts developed in coupled dynamical systems (Kaneko, 1990) may be relevant to analyse such a balance.

From several (i.e. more than two) cells consisting of both chaotic and non-chaotic types, the diversity and recursiveness change depending on the ratio between the two. Generally, the compatibility of diversity and recursiveness is difficult with this choice.

4. Discussion

In this section, we first discuss the relevance of our result to the development of real multicellular organisms. In particular, correspondence of the results with separation experiments of blastomeres in sea urchin eggs is discussed in detail. Last, we give some predictions to be confirmed in experiments.

The key issue in this paper is compatibility between recursive production of multicellular organisms and their morphogenetic diversity. In particular, we showed that starting from two cells composed of a cell state with diverse chemicals with chaotic attractor and the other with less diversity and non-chaotic (*partial*) attractor, this compatibility is achieved.

Starting from a cell with chaotic dynamics, diverse cell types are generated, as well as complex patterns of cell configurations. However, it is quite difficult to make such a complex pattern recursive, even by selecting initial condition of chemical compositions of a cell. On the other hand, from a cell type with regular ((quasi)-periodic or fixed-point) dynamics, the cell state is so stable that a resulting pattern is homogeneous, consisting solely of a single cell type. Through several trials we found that one straightforward solution to make the

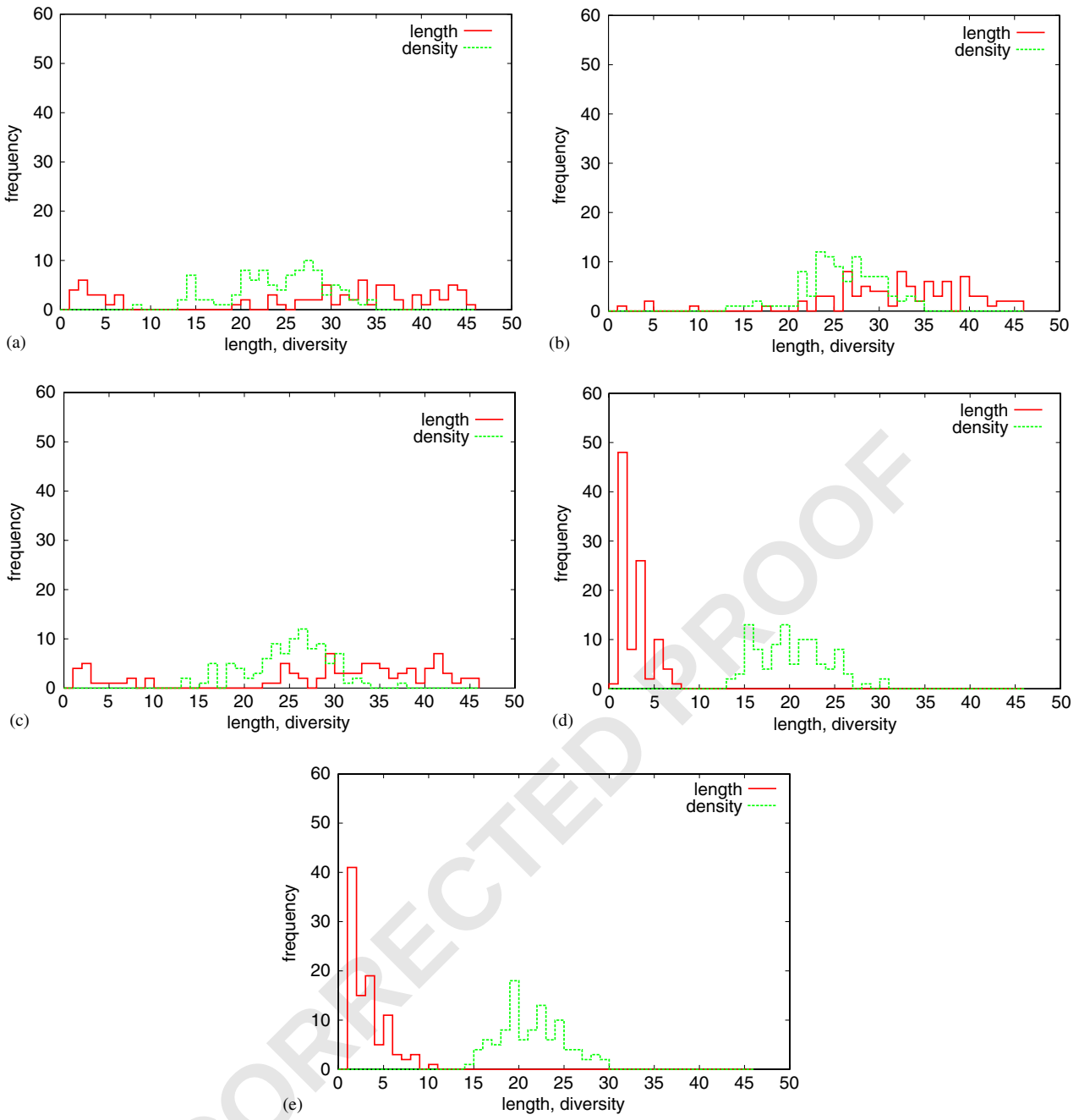


Fig. 10. The distributions of *Levenshtein distance* (red) and the diversity (green) over 100 initial conditions for the given initial type(s) of cells. We measured the *Levenshtein distance* when the daughter reaches the same generation number (49) as the mother: (a) one cell with random initial values, (b) two cells, one a chaotic attractor and one a chaotic attractor (*CC*), (c) One cell with a chaotic attractor (*C*), (d) two cells, one a chaotic attractor and one a fixed point (*CF*) and (e) two cells, one a quasiperiodic *partial* attractor and one a chaotic attractor (*CI*).

recursiveness and diversity compatible is to use two cells with these two distinct features in the beginning.

It is not obvious that recursiveness and diversity are compatible even under this choice of initial conditions. If chaotic dynamics were too strong, recursiveness might not be achieved. If the cell state of the regular dynamics is an attractor by itself, it might not be influenced by the neighbouring cell, and a homogeneous cell-pattern would be formed at least on one side of the chain. A balance between a chaotic attractor and a regular partial attractor makes the compatibility possible, even with an increase of cell numbers and cell–cell interactions.

Of course, development starts from a single cell. Hence, at the very initial cell division process, the cell must be divided into two cells with distinct behaviours. For example, by a gradient of some chemical, or localization of some chemical only at one edge, such differentiation to two types is easily achieved. Recipro-

cal signalling between an egg and follicle cells can also trigger such differentiation. Hence, the choice of two cells (or two parts in a single cell) with distinct natures is not so difficult. Our prediction here is that, if and only if these two cells consist of one cell type with chaotic dynamics and the other with regular partial attractor dynamics, recursiveness and diversity are compatible.

There may be other solutions that make recursiveness and diversity compatible. For instance, the cells may be arranged in a specific manner when the number of cells is larger (e.g. 8) as in Fig. 7(b). However, it would be difficult for an organism to select such highly specific initial condition as a starting object with many cells. In this sense, 'two cells' with chaotic and regular dynamics is a straightforward and reasonable choice, and rather easy to control.

4.1. Possible relationship with the differentiation between animal and vegetal poles

We now discuss the coexistence of two distinct features in the embryogenesis of real multicellular organisms. Here, it is quite difficult to measure all the dynamics of chemicals (or the dynamics of gene expressions) quantitatively during development. Hence, direct demonstration of the theory would be quite difficult. Therefore, we discuss the relevance of our result to multicellular organisms mainly at a qualitative level, focusing on the two distinct natures required to achieve the compatibility between recursiveness and diversity.

We discuss classical experiments on the embryo of the sea urchin. This embryo has animal and vegetal poles. As shown by Driesch, each of four cells isolated from an embryo's fertilization envelope forms a smaller, but normal, pluteus larva (Gilbert, 2003, Chapter 3, Fig. 3.15). Note that in this experiment each of the four isolated cells includes animal and vegetal poles (Gilbert, 2003, Chapter 8, Fig. 8.7). On the other hand, cells isolated from the animal hemisphere only develop into a ball consisting solely of ectodermal tissue (Gilbert, 2003, Chapter 8, Fig. 8.11; Hörstadius, 1939), and only an animal tuft is formed. In contrast, isolated cells from vegetal poles only develop a tissue ovoid in shape, and the archenteron forms oesophagus, stomach and intestine, and sometimes gives rise to dwarf plutei (Hörstadius, 1939, pp. 148–150; Hörstadius, 1973, Chapter 6).

These experiments show

- Starting from cells with both animal and vegetal poles, normal development is possible, with recursive production and diversity in cell types and tissues.
- Starting from cells only from animal poles, a tissue with relatively homogeneous cell types is produced. The resultant tissue is almost the same in each trial, and diverse cell types or tissues are not generated.
- Starting from cells only from vegetal poles, a variety of tissues are generated with diverse cell types. On the other hand, the developmental process is not robust, and the formed tissue varies with each trial.

It is also interesting to recall experiments with amphibians. When an egg is divided along the plane of the first cleavage into two blastomeres, so that each of them has both animal and vegetal hemispheres, each of the separated cells develops into a normal embryo, whereas when an egg is divided into animal and vegetal hemispheres (along the third cleavage), each cell develops into a belly piece (Kageura and Yamana, 1983, 1984).

These results are consistently explained with our numerical results by assuming that cells at the vegetal pole correspond to the chaotic type with diverse chemicals and those at the animal pole correspond to the regular dynamics type with less chemical diversity. The compatibility between recursiveness and diversity in cell types by the combination of two distinct cell types in our model corresponds to the normal development process from cells with both animal and vegetal poles. The diverse tissue without recursiveness from cells only from the vegetal pole is completely analogous to what we observed in the development from chaotic cells only, while the absence of diversity from the animal pole corresponds to the development from cells with regular dynamics.

This remarkable coincidence is also supported by the fate map and cell lineage of the sea urchin (Wray, 1999), wherein an animal hemisphere becomes only ectodermal cells but a vegetal hemisphere becomes ectodermal, endodermal, skeletogenic cells, secondary mesenchyme and coelom. In addition, an 8-cell embryo after the third cleavage is mosaic as seen above, whereas an animal hemisphere recombined with the veg2-tier or micromeres—a part of developed vegetal hemisphere—produces an embryo resembling a normal pluteus larva (Hörstadius, 1939; Logan and Mcclay, 1999). This suggests that the combination of two cell types, i.e. veg2-tier/micromeres and an animal hemisphere, is necessary for normal development.

As a conventional explanation for the above developments of animal and vegetal halves from the sea urchin, the assumption of *two opposite gradients* is often adopted since Runnström's proposition. However, this assumption by itself cannot explain the origin of asymmetry between vegetal and animal poles with regards to the diversity of tissues. From our standpoint, such asymmetry is coherently explained, even without assuming *gradients* of two chemicals in advance.

With regards to the development from the two distinct types of cells, one might argue that an egg is

just one cell, and the recursiveness and diversity should be realized by starting with one cell in the model. To answer this question, we have carried out various simulations starting from one cell, separated at various stages from a mother. In principle, if the two cells developed from the single egg consist of the chaotic and regular types, the argument so far completely follows. Indeed, such an event occurs with some probability (e.g. 20% in Example I). If the initial chemical concentrations of the first cell were selected very precisely, it would be possible for the two cells always to become such types. In the present case, however, such selection is rather difficult, because of the chaotic dynamics and fluctuations. On the other hand, if by some external stimulus, the homogeneity in the single cell is lost to produce the required two types of cells, the resultant two cells easily satisfy the postulate here. Indeed, the symmetry in an egg is usually broken by external signals from follicle cells, and the animal and vegetal poles are formed (Rotoli et al., 1998). With this external signalling, the choice of initial conditions is stabilized.

4.2. Predictions

It is possible to make some experimental predictions based on the differentiation of cells with chaotic and regular dynamics, even though at present it is hard to measure the dynamics of chemical concentrations (or gene expressions) for each cell, while the diversity of chemicals can be estimated by the data from gene expression. Because the initial cell type with chaotic dynamics always produces a richer variety than that from differentiated cells with regular dynamics, our theory can be tested by measuring the diversity in chemicals. Indeed, in our model and in our previous work (Furusawa and Kaneko, 2001), we have shown that a cell with chaotic intracellular dynamics always has a larger diversity of chemicals than a cell with regular dynamics, which has a rather biased concentration to specific chemicals, and a few chemicals have much larger concentration than others. This correlation between chaotic dynamics and chemical diversity is also true in our model, and is believed to be rather universal (for details, see Furusawa and Kaneko, 2001).

Now, it is predicted that, at the very first stage of development, cells are differentiated into two types: those with high chemical diversity and temporal variation and those with much lower values. The former type provides the cellular heterogeneity, and the latter type maintains the recursive production over generations. According to the correspondence we discussed, the cells from vegetal poles must have a higher variety in chemicals, and those from animal poles must have lower variety. In particular, this difference between vegetal and animal poles should be directly measurable by a global pattern of gene expressions and protein abundances. We predict that diverse genes are weakly expressed in cells around vegetal poles, while fewer genes are strongly expressed, and the expression pattern is more biased in cells around animal poles. This prediction can be examined by using suitable microarrays.

Next, we make a novel proposal on experiments with ES cells. A collective of ES cells is a starting object capable of producing diversity, but it is impossible to make a recursive production from it. In view of the results in this paper, the ES cells in such a collective must be chaotic cells with diverse chemicals. Thus, by combining them with highly differentiated cells with less variability, more stable and recursive production should be possible. Indeed, there is a related experimental observation, that a co-aggregate culture with bone morphogenic protein (BMP)4-producing cells (highly differentiated cells) induces in vitro differentiation of the ES cells into germ-line cells (Toyooka et al., 2003).

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