Dynamical Systems Basis of Metamorphosis: Diversity and Plasticity of Cellular States in Reaction Diffusion Network

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Abstract

Dynamics maintaining diversity of cell types in a multi-cellular system are studied in relation to the plasticity of cellular states. By adopting a simple theoretical framework for intra-cellular chemical reaction dynamics and considering the division and death of cells, the development of cells is studied. Cell differentiation process is found to occur through instability in transient dynamics and cell-cell interaction. In long-term behavior, extinction of multiple cells is repeated, which leads to itinerancy over successive quasi-stable multi-cellular states consisting of different types of cells. By defining the plasticity of a cellular state, it is shown that the plasticity of cells decreases before the extinction of most cell types, from which diversity and plasticity are later recovered. In the following, a decrease of plasticity occurs again, leading to the next extinction. This cycle of diversification and extinction is repeated. Relevance of our results to development and evolution is briefly discussed.

keywords: metamorphosis, diversity, plasticity, stability

1 Introduction

In multi-cellular organisms, developmental process from a single embryonic cell or a few homogeneous cells with multi-potency leads to an organism that consists of various cell types. Furthermore, in some multi-cellular organisms such as insects, the developmental process is generally accompanied by metamorphosis. In these organisms, after a cell society consisting of a specific distribution of cell types is achieved and sustained over some time, a transition to a novel society starts through specific process. In other words, there exist several multi-cellular states, each of which is regarded to be a quasi-stable state of an ensemble of several cell types, given by some distribution of them. In the event of metamorphosis, both the number of cells and the number of cell types decrease drastically and a different multi-cellular state is realized. After this "extinction", adult organisms create embryonic cells again to form the next generation. In other words, the developmental process passes through a rather restricted state that is different from the original embryo. Through this process, the adult body of each species is

formed, from which the next generation is reproduced. With this recursive production, the life cycle is repeated.

In a natural developmental process cells successively lose their multipotency, that is their ability to produce a set of different cell types. Plasticity is the ability of one cell type to change to another cell type. As the development proceeds, the number of cells and cell types increases, while the plasticity of cells generally decreases. Through the metamorphosis, however, some cells recover the ability to produce other cell types by regaining plasticity.

In general, when considering the diversity of tissues and recursive production of a multi-cellular organism, it is important to study how several stable distributions of different cell types form, sustain, or collapse. We address the following two general questions:

1. What mechanism causes the transition between different quasi-stable states with several cell types?

2. How is the switching process related to the plasticity of cellular states?

The purpose of the present paper is to answer these questions in terms of diversity dynamics and cell type plasticity.

As for the first question, the "isologous diversification theory" has been proposed[8, 9, 4, 5] as a theoretical framework for the robust developmental process of multi-cellular organisms. In these studies, a dynamical systems approach is adopted to show that a robust developmental process with various cell types emerges as a result of interplay between intra-cellular dynamics and cell-to-cell interactions. In this approach, the state of a cell is represented by the concentrations of a set of chemicals, which can change over time through catalytic reactions. Several types of cells are formed in distinct stable states with these catalytic reaction dynamics. In the present paper, we adopt this dynamical systems approach. The dynamical systems representation of the developmental process is summarized in Table I and II.

On the other hand, as is mentioned above, in a usual multi-cellular organism there is also diversity at a tissue level, i.e., different stable distributions consisting of different cell types. So far, however, spontaneous formation of several quasi-stable states of cell type distributions have not been investigated in depth[1]. Here we study the dynamics required to maintain the diversity of cell types and the emergence of switching between several quasi-stable multi-cellular states. We show that all the cells change to drastically different states at each switching, accompanied by the deaths of multiple cells.

As for the second question, we show that the plasticity of each cell decreases with the increase of cell type diversity, throughout the developmental process. We discuss the relationship between the two and elucidate the switching mechanism in terms of diversity and plasticity.

For the above purpose, we need to define the plasticity of a cellular state. Here we define it as the susceptibility of cell's chemical compositions to an external environmental change. By choosing a specific model, plasticity is computed explicitly. By studying the developmental process of cells in the model, we also find that the switching of multi-cellular states is accompanied by extensive cell death. This switching process will be shown to be closely related to the loss of plasticity. From extensive simulations, the long-term dynamics of a cell society and the plasticity we found are summarized as follows:

As the development progresses, several cell types with low plasticity increase in number, which leads to the extinction of several other cell types. This extinction brings about a drastic change in the environment surrounding the cells. Accordingly, the internal state of all the surviving cells is changed. Then, different cell types with high plasticity are generated. From this cell society with higher plasticity and potential to differentiate into other types (similar to the initial 'undifferentiated cells'), a new cell society with a different set of cell types with lower plasticity is formed. In some case this cycle is repeated.

We show that the cycle of increasing and decreasing diversity and plasticity generally appears

[1] In ref[5], a preliminary study for a proto-type of the life cycle of multi-cellular organisms is reported.

in our dynamical systems model. We reveal a general mechanism commonly underlying such a cyclic process, and formulate it in terms of a dynamic relationship between diversity and plasticity of cell types.

For the purpose of the present study, we adopt a simple modeling framework for internal chemical reaction dynamics, a reaction-diffusion system on "chemical species space" . By adopting this model, one can link chemical concentrations to the fixed expression of genes, which is a common representation of differentiated cell types. We then discuss both the stability of the realized cell states and their diversity.

The present paper is organized as follows: in the following section we describe the details of our model. In section 3 we present the behavior of the developmental process. The condition for the present model to show cell differentiation and switching over several quasi-stable cell societies is presented there. In section 4 we reveal a feedback process leading to this switching and confirm the relationship between the switching and the plasticity of the cells. After presenting some results concerning the control of multi-cellular states by external operations in section 5, we briefly mention the generality of the presented results in section 6 and discuss their relevance to development and evolution. To clarify matters, we also show a table containing the definitions of the key concepts used in our study.

Table I. Key concepts of the developmental process in our study: basic definition, biological representation (B) and the corresponding representation in our dynamical systems model (D). Concepts at the cellular level.

Table II. Key concepts of the developmental process in our study: basic definition, biological representation (B) and the corresponding representation in our dynamical systems model (D). Concepts at the level of the cell ensemble.

2 model

In this paper we adopt a constructive modeling, by taking only some basic features of a problem into account, in order to answer general questions with regards to diversity, stability and plasticity of cell states.

The basic modeling strategy follows our previous work [8, 9, 4, 5]. (also see Table I and II for the dynamical systems translation of concepts in developmental biology). Cells with internal biochemical states compete for resources in the environment to achieve growth. Following the growth or decrease of cell contents, a cell can divide or die so that the cell number changes over time. Our dynamical systems model consists of the following three parts:

- intra-cellular chemical reaction network
- cell-cell interaction
- cell division and cell death

Next, we describe each process. In Fig.1, we show the schematic representation of our model.

- intra-cellular reaction network
  In general, intra-cellular chemical reactions involve both genes and metabolites. Genes are set to be on or off through biochemical reactions. Intra-cellular biochemical reactions constitute a network both of genes and metabolites, while the time scale for the reactions of genes is relatively slower than that of metabolites. Here, simple intra-cellular chemical reaction dynamics is chosen as an abstract model so that it satisfies the basic features described above.

  First, we assume a reaction network consisting of many products and substrate chemicals. Existence of these two types of chemicals, each constituting reaction networks, is inspired

by the genes and metabolites in a cell. The concentration of the j-th product in i-th cell is denoted by $v_i^j(t)$, while that of the substrate is denoted by $u_i^j(t)$. Here, product chemicals are synthesized autocatalytically by consuming corresponding substrate chemicals. We adopt a variant of the Gray-Scott model[7, 11] for the autocatalytic reaction scheme[2]. [3] In this paper, we mainly present the results of the case where the number of chemical species is set to $K = 30$. We take this value on the basis of the dependency of cell differentiation events on K, which is presented in Appendix.

In the model $v_i^j(t)$ is assumed to correspond to the degree of gene expression (or RNA) and $u_i^j(t)$ to the concentration of a metabolite. Besides the reaction dynamics between them, chemical concentrations may be changed by the reaction dynamics within a gene network or metabolic network. To take into account such dynamics, we assume reversible reactions for each of the product and substrate chemicals, which form two reaction networks. Each chemical is converted to other chemicals by a reversible reaction given by the network. The reaction network is represented by a reaction matrix $W(i, j)$, which is 1 if there is a reaction from chemical i to chemical j, and 0 otherwise. Since the reaction network is assumed to be reversible, the matrix is symmetric, i.e., if $W(i, j) = 1$, then $W(j, i) = 1$. Here, we adopt the same network for products and substrates and also assume that the network is composed of two reaction paths per chemical to form a single closed-loop structure for simplicity.

The rate constants of the reversible reactions are assumed to be common to all resources and all products, C_u and C_v, respectively. Moreover, considering the difference in the time scales between metabolites and genes, we also assume that C_u is larger than C_v. These values are fixed throughout the simulation. These reversible reactions are regarded as 'diffusion' in the 'chemical species space' so that intra-cellular reaction between products and substrates is regarded as a one-dimensional reaction-diffusion system[14]. In this representation, each attractor of intra-cellular reaction dynamics corresponds to a cell state with a different genetic expression pattern. Since developmental processes in real cell systems are elucidated as spontaneous cascading processes with different gene expression patterns, we study how different cellular states are selected by including the developmental process in the model.

- cell-cell interaction
  Assuming that the environmental medium is completely stirred, we can neglect spatial variations of chemical concentrations in it so that all cells share a spatially homogeneous environment.[4] Here we consider only the diffusion of resource chemicals through the medium as a simple form of interaction. In this model, we assume that only substrate chemicals are transported through the membrane as resources, in proportion to the concentration difference between the inside and the outside of a cell. All the resource chemicals have the same diffusion coefficient D_u. Each cell grows by taking up resource chemicals from the medium and transforming them to product chemicals. U^j is the concentration of j-th resource in the medium. Resource chemicals in the medium are consumed by cells and we assume that resource chemicals are supplied from an external material bath to the medium at a rate proportional to the difference between the concentration in the bath and

[2] The Gray-Scott model is a reaction-diffusion system composed of two chemicals, substrate and product. It is a simplified version of autocatalytic Selkov model that explains self-sustained oscillation of glycolysis.

[3] The necessary condition for this study is to realize local all-or-none behaviors of the chemicals whose "diffusion" is slower than the other one on the assumption of genes and metabolites. Since the Gray-Scott model is one of the candidates satisfying this condition, here we have adopted it.

[4] There is a point to notice that here we adopt reaction-diffusion system to model intra-cellular chemical reaction network(reaction-diffusion system on "chemical species space"), not to model spatial pattern formation in the system.

the medium. Again, all the resource chemicals have the same diffusion coefficient D_U in the medium. The concentrations of all resources in the material bath, U are set to be 1. The parameter Vol_0 is the volume of the medium and N is the number of cells.

- cell division and cell death
  Each cell gets resource chemicals from the medium and grows by transforming these resources into product chemicals. Here we assume that the cell volume (denoted by Vol_i for cell i) is proportional to the total amount of product chemicals. For each time step in temporal evolution, we track the change of product chemicals, that is, the change of total cell volume, and each chemical concentration is normalized by the total amount of product chemicals. When the cell volume becomes twice the original volume, then the cell is assumed to divide, while, if it is less than half the original, the cell is put to death. In real biological systems, cell division occurs after the replication of DNA (which has a smaller diffusion coefficient and cannot penetrate through the membrane). Hence these assumptions are rather natural. After cell division, each cell volume is set to be half, and each cell is divided into two almost equal cells, with some fluctuations. To be concrete, chemical concentration a (a is a representation of u, v) is divided into $(1+\eta)a_i^{(j)}$ and $(1-\eta)a_i^{(j)}$ respectively, where η is a uniform random number from $[-10^{-3}, 10^{-3}]$. These fluctuations can give rise to a small variation among cell states, which eventually lead to cell differentiation by being amplified through cell-cell interaction.

Accordingly, the concentration change of each chemical species is given by

$$\begin{aligned} \Delta u_i^j(t) &= D_u(U^j(t) - u_i^j(t)) - u_i^j(t)v_i^j(t)^2 \\ \Delta v_i^j(t) &= u_i^j(t)v_i^j(t)^2 - Bv_i^j(t) \end{aligned} \tag{1}$$

$$du_i^j(t)/dt = \Delta u_i^j(t) + C_u \sum_{k=1}^{K} W(j,k)(u_i^k(t) - u_i^j(t)) - u_i^j(t)dVol_i(t)/dt$$

$$dv_i^j(t)/dt = \Delta v_i^j(t) + C_v \sum_{k=1}^{K} W(j,k)(v_i^k(t) - v_i^j(t)) - v_i^j(t)dVol_i(t)/dt$$

$$dU^j(t)/dt = \frac{D_u}{Vol_0} \sum_{i=1}^{N}(u_i^j(t) - U^j(t)) + D_U(U - U^j(t))$$

$$dVol_i(t)/dt = \frac{\sum_{j=1}^{K} \Delta v_i^j(t)}{\sum_{j=1}^{K} v_i^j(t)} Vol_i(t)$$

In equations for $du_i^j(t)/dt$ and $dv_i^j(t)/dt$, the last term represents the dilution of the concentration by the increase of volume.

Summary of the model and choice of parameter values and initial conditions

The summary of the model is given in Table III. There are six free parameters, C_u,C_v,D_u,D_U,B,Vol_0. We set most of the parameters(C_u,C_v,D_u,B) to typical values which produce a self-replicating spot pattern in the original Gray-Scott model in one-dimensional space. With these parameter values, concentration changes by the intra-cellular reaction dynamics and by the diffusion of chemicals are of the same order. The parameter values D_U, Vol_0 concern the strength of cell-cell interaction and are related to the total number of cells at which differentiation starts. We choose these parameters so that the cell differentiation is observed at an appropriate cell number. To be specific, in almost all simulations, we set the parameters $C_u = 2.0$, $C_v = 0.020$, $D_u = 0.50$, $D_U = 1.0$, $Vol_0 = 3.0$, $B = 0.060$. In these values of $C_u \sim B$ our model also shows pattern dynamics such as in the original Gray-Scott model. The parameter values are not necessarily

fine-tuned, and the behavior to be discussed will be seen in a wide range of parameters, as long as the homogeneous chemical concentration is linearly unstable, much like the Gray-Scott equations. (If the parameters are set so that the steady state becomes a single homogeneous one, the cell differentiation to be discussed does not occur).

As mentioned above, the number of chemical species K is fixed to 30 for most simulations here. Dependence on K will be briefly mentioned in the next section and also in the appendix.

Initial condition

The initial condition of the first cell is chosen as $u_i^j(0) = 0.50$ and $v_i^j(0) = 0.250 + 0.01 \times rand(j)$, where $j = 1, 2, ..., K$ and $rand(j)$ is a uniform random variable from [-1,1], although this specific choice is not important.

Table III. Summary of our model.

3 Developmental process with cell division and cell death

First we consider the dynamics of chemical concentrations in a single cell. The behavior of a single cell state depends on the bifurcation structure of the reaction-diffusion system in the 'chemical species space' mentioned above. Parameters are selected such that a chemical species becomes unstable; a single cell state will then have multiple attractors. This is in strong contrast to the previous studies [8, 9, 4, 5], where a single cell can take only one or a few attractors. Many stable cellular states that correspond to different cell types are realized accordingly. The number of attractors is expected to increase exponentially with K, as was also verified by numerical simulations(data not shown).

Noting the nature of the above mentioned single-cellular dynamics, we discuss the developmental process with the change of cellular states under cell division and death. We study a coupled dynamical system, where the intra-cellular state and the inter-cellular interaction mutually influence each other. Without cell-cell interaction, all cells will take on identical chemical composition, given by a single attractor of the single-cell dynamics. By cell-cell interaction, this homogeneous state over cells will be destabilized so that novel states may appear. We study such cell differentiation processes here.

3.1 Cell differentiation

In the following, we show an example of the differentiation process. Fig.2 shows a typical temporal evolution of the concentrations $v^j (j = 1, .., K)$ in which cell differentiation occurs for all the cells starting from a single cell. A series of snapshots is shown by using a gray scale figure for the concentration of product chemicals. Each group of distinct cellular states with a different set of values v^j corresponds to a quasi-stable cell type with recursive production. (Time for reproduction is much longer than the typical transient time required to reach a quasi-stable state.) The figure also includes the cell states that appear at a later stage of the temporal evolution(Fig.2(f)). In this example, there exist eleven types, all of which are fixed points. If the correlations of the intra-cellular dynamics of all the chemical concentrations and the cell-cell interactions are at proper intermediate strength, under the instability of transient state of cells, small fluctuations in the cell division process are amplified through the competition for the environment's resources, which leads to cell differentiation.

From extensive simulations of the present model we have numerically obtained the necessary conditions for cell differentiation. Details on these conditions are shown in the appendix. [5]

[5] If we set our initial condition of the first cell exactly on an attractor of a single cellular state, differentiation does not occur. In this case, cells will go extinct after several divisions.

3.2 Switching between quasi-stable ensembles consisting of several cell types

Next we investigate the long-term behavior of the cell differentiation process. We show an example of typical temporal evolution by plotting existing cell types and their population at every 1000 time units in Fig.3. A multi-cellular state consisting of several cell types is formed at a very early stage and is maintained over a long period, which is much longer than the typical transient time of a single cell state. Then a sudden crisis occurs. First, the number of cell types decreases and only a few cell types survive. Then, these cells of identical type compete for the same resources, and some of them can no longer grow. Multiple cell deaths occur, leading to drastic decrease in the cell number. After a couple of generations, a new multi-cellular state with different cell types is created. This switching between diversification and extinction can occur repeatedly.

The drastic decrease in cell number is followed by a loss of diversity in cell types. The cell ensemble of identical cell types uses the same resources for growth, and a large population of cells is no longer sustainable. Although an analytic expression of the instability of the population distribution of the existing cell types is not at hand, the onset for the instability against the number of cells could be numerically estimated for a homogeneous cell type and is discussed below.

4 Further Analysis of the switching process

4.1 quantitative characterizations of the plasticity of cell types

Now we investigate the switching between several quasi-stable multi-cellular states in more detail. We define the recursiveness of each cellular state through reproduction by comparing the averaged concentrations of product chemicals in a mother cell and its daughter cell. The similarity of chemical compositions between a mother and its daughter cell is defined as follows. Let $\overrightarrow{\mathrm{V_i(n)}}$ denote the vector representation of all the averaged concentrations of the product chemicals (v_i^j) of the i-th cell between the (n-1)-th and n-th cell division, denoted by $\overline{v_i^j}$ for $j = 1, 2..., K$. As an index for the recursiveness of a cellular state, we introduce $H_i(n)$, a normalized inner product of $\overrightarrow{\mathrm{V_i(n)}}$ and $\overrightarrow{\mathrm{V_i(n-1)}}$.

$$H_i(n) = \frac{\overrightarrow{\mathrm{V_i(n)}} \cdot \overrightarrow{\mathrm{V_i(n-1)}}}{|\overrightarrow{\mathrm{V_i(n)}}||\overrightarrow{\mathrm{V_i(n-1)}}|}, \quad \overrightarrow{\mathrm{V_i(n)}} = (\overline{v_i^1}, \overline{v_i^2}, ..., \overline{v_i^K})$$

If $H_i(n) \approx 1$, the cell is regarded to maintain its type between successive cell divisions; that means recursive reproduction. In the present model, each differentiated cell type is represented by cells in a distinct chemical state. Each cell type can produce its own type recursively. The cell differentiation processes in our model are well represented with these two quantities ($\overrightarrow{\mathrm{V_i(n)}}$ and $H_i(n)$) by which we can distinguish all cell states correctly.

Next we introduce a measure of plasticity for the cell types in order to study the switching process in terms of temporal change of cell plasticity. Here we define the plasticity of each cell type as the susceptibility of the cell state to environmental fluctuations. These fluctuations are caused by changes in resource chemical concentrations in the environment due to cell division and death. We characterize the plasticity of a cell state in the following manner:
First, we take ${v(o)_i}^j$, the concentration of the j-th product chemical of the type i cell, while we define ${v(f)_i}^j$ as the concentration of the j-th product chemical of the attractor of the cell type i by eliminating cell division and death processes and checking the attractor state with the environment held constant. Then we define the **"attractor distance"** $Dist_i$ of the type i cell as the Euclid distance between ${v(o)_i}^j$ and ${v(f)_i}^j$, namely, $Dist_i = \sqrt{\sum_{j=1}^{K} ({v(f)_i}^j - {v(o)_i}^j)^2}$.

Now, we conjecture that

(1)the cell plasticity (changeability) is characterized by the attractor distance and that

(2) the potential for differentiation decreases as the attractor distance decreases.

We will demonstrate these conjectures by measuring the temporal fluctuation of cells and the frequency of differentiation.

conjecture (1): The first conjecture will be confirmed by the positive correlation between the attractor distance and the degree of temporal fluctuations of each cell state. In the present system, the environment fluctuates according to the birth and death of surrounding cells. We compute the temporal fluctuation of each cell type for an extended period over which several cell types coexist stably. (The relationship between fluctuation and response to the environment is known as the fluctuation-dissipation theorem in physics, and has been extended to biological systems[12].) This temporal fluctuation of each cell type is computed from the difference of chemical concentrations (i.e., Euclid distance) over a given time span. Here we use the time span $t = 1000$. Temporal fluctuations are calculated from the average over 100 data samples. An example of this temporal fluctuation plotted as a function of the attractor distance is shown in Fig.4, which shows a positive correlation between the two. We investigated other data over ten periods from five temporal evolutions including the example Fig.3, and all of them show positive correlations. (The correlation coefficient ranges from 0.46 to 0.84.) Those results show positive correlation between the temporal fluctuations of the chemical concentrations of a cell type and its attractor distance.

Conjecture (2): The second conjecture will be confirmed by the positive correlation between the attractor distance and the frequency of the re-differentiation event when a cell ensemble consisting of cells of a single cell type is put in a new environment. We computed the average frequency for cells within a given range of an attractor. The relationship between the frequency of re-differentiation and the attractor, thus obtained, is plotted in Fig.5, using the same data as above. [6] As shown in Fig.5, there is a sigmoidal dependence between the attractor distance and the frequency of re-differentiation. There is a threshold for the attractor distance below which the corresponding cell type loses the ability of re-differentiation drastically. [7]

By summing up these two results, it is confirmed that the attractor distance introduced above is valid as a measure of cell type plasticity.

4.2 Mechanism for switching through extinction of many cells

In the following we discuss the switching with multiple cell deaths in relationship to the loss of plasticity.

First, we summarize our results discussed here as follows: At each stage of a given quasi-stable multicellular state, cell types with different degrees of plasticity coexist. Then at each stage, cell types with relatively high plasticity (i.e., with larger attractor distance) differentiate into other cell types with lower plasticity, so that the proportional amount of cell types with lower plasticity increase gradually. Then, the distribution of cell types allowing for effective use of resource chemicals is destroyed, resulting in the extinction of many cell types. With these multiple deaths, the concentrations of environmental resource chemicals change drastically, leading to re-differentiation of some of the surviving cells with low plasticity into a new state with high plasticity. Then, the emergence of novel cell types with high plasticity gives rise to a novel multi-cellular state, and the effective use of resources becomes possible again. With this drastic change, a switch to a novel multi-cellular state follows.

[6] Here the population of each cell type is set to be 80. If the number is much larger, the homogeneous cell ensemble grown from the group goes extinct by the lack of the resources. On the other hand, if the number is much smaller, then the effect to internal cell states caused by the change of the environment is so large that the cells lose the original character when they are selected. Hence we choose this medium number for cell population.

[7] This also supports the initial condition dependence of cell differentiation event mentioned before.

In other words, for the duration of the quasi-stable multi-cellular state, there exists a balance between intra-cellular dynamics and cell-cell interaction. Hence, the proportional amount of each cell type should be within some range, lending robustness to a cell ensemble.

This scenario is verified by computing the temporal change of the following five quantities using the data for the temporal evolution of Fig.3: the total diversity of cell types (Fig.6(a)), the average recursiveness of cell types of all cells (Fig.6(b)), the total number of cells (Fig.6(c)), the average of attractor distance over all of the existing cell types at each moment(Fig.7(a)) and the number of cells in each bin of attractor distances(Fig.7(b)). As shown in Fig.6 and Fig.7, the average attractor distance as well as the number of cell types decrease at first. With these decreases, the recursiveness of cells increases on average. Thereafter, the attractor distance and the number of cell types stay at low values and almost complete recursive production is sustained since most existing cells have low plasticity. After slight decreases in attractor distance and diversity, these two values increase, accompanied by multiple cell deaths and the switching of cell types. With this, the high plasticity and diversity of cell types is recovered. After this recovery, the attractor distance and diversity again decrease gradually, until the next multiple cell deaths occur. This cycle, consisting of the decrease of diversity, extinction, and recovery, is repeated.

Although we show only one numerical example demonstrating the above scenario here, qualitatively the same behavior is generally observed at each switching event in the present model. The scenario mentioned above is rather universal.

5 Transition of states by external operations

So far, we have studied the switching process with regards to the relationship between diversity and plasticity of cell types. In this section, we study the behavior of the present cell system after external operations are applied to the cell system.

First, we add noise to the intra-cellular chemical reactions. Here the noise is regarded as the fluctuation of the number of molecules and is assumed to be Gaussian white noise. The stochastic differential equations for resource and product chemicals in a cell are expressed by adding a noise term to the ordinary difference equations (1);

$\Delta u_i^j(t) = D_u(U^j(t) - u_i^j(t)) - u_i^j(t)v_i^j(t)^2 + \eta\sqrt{u_i^j(t)}$.

$\Delta v_i^j(t) = u_i^j(t)v_i^j(t)^2 - Bv_i^j(t) + \eta\sqrt{v_i^j(t)}$

Here η is a Gaussian white noise satisfying $\langle\eta(t)\eta(t')\rangle = \sigma^2\delta(t-t')$.

The developmental process changes with the change of the noise amplitude. When the noise amplitude is too large, multi-cellular states change almost randomly over time. If the amplitude is small, stable multi-cellular states of several cell types are eventually formed, and the switching process does not appear any more. An example of such long-term behavior is plotted in Fig.8, and the corresponding change of the average attractor distance is shown in Fig.9. Starting from different initial conditions, different multi-cellular states consisting of different cell types are realized, which have different plasticity. It is now shown that multi-cellular states (with relatively low plasticity) are stabilized by the noise.

Second, we study the behavior to external change of the environment. For example, the change of multi-cellular states as a result of the restriction of some resource chemicals is shown in Fig.10. When the concentration of five chemical resources is reduced, indicated by the arrow in the figure, the original cell types that have low plasticity become unstable and some of the cell types regain plasticity. Then the cell differentiation process is restarted, leading to a novel multi-cellular state. The corresponding change of the average attractor distance is also plotted in Fig.11 which clearly shows that the plasticity is regained by the external change of the chemical concentrations of the environment. Thus, a multi-cellular state that was stable is destabilized

by an external operation which leads to a change of the environment.

6 Summary and discussion

In this paper, we have studied a dynamical systems model of a developmental process by introducing a new framework, the reaction-diffusion system on 'chemical species space' for intracellular chemical reaction dynamics. By taking the developmental process further into account, it was shown that cells differentiate into several types. The condition for the cell differentiation by cell-cell interaction was obtained.

In long-term behavior, we have found a switching over several multi-cellular states which maintain diverse cell types. In each multi-cellular state, diverse cell types coexist to reduce the competition for chemical resources while the switching is characterized by multiple cell deaths arising from the loss of diversity of cell types and competition for the resources. The discovery of this switching behavior in the present model is novel and not described elsewhere in literature.

We propose that this switching behavior is characterized by the loss of the total cell plasticity, a general consequence of our dynamical systems theory. The irreversible loss of plasticity is a general course in the developmental process, i.e., differentiation from a cell type with relatively high plasticity to one with lower plasticity so that the proportion of cell types with lower plasticity gradually increases. Thus, a suitable distribution of different cell types no longer exists resulting in an ineffective use of resource chemicals leading to multiple cell deaths. Drastic change of the environmental resource chemical composition results, and cells with low plasticity are replaced by those with high plasticity. As a result, a new multi-cellular state with novel cell types is generated. This process is repeated. Fig.12 summarizes schematically the above scenario.

It is important to consider the existence of several quasi-stable multi-cellular states when considering the origin of several tissues in multi-cellular organisms. These tissues can be thought of as a cell ensemble with a different composition of coexisting cell types, which have a common gene set. From this point of view, the switching over several multi-cellular quasi-stable states will be important in the study of the life cycle of a multi-cellular organism. As a first step toward such a study, we impose an external change on the system to make a transition between different multi-cellular states. In future, the search for a rule of transition between successive multi-cellular states will be important. The dynamics of metamorphosis can be discussed along these lines.

In our switching process, all cells with low plasticity are changed to cells with high plasticity through multiple, simultaneous cell deaths. In real metamorphosis, such "extinction" is also observed, for example, in insects. According to our results, fluctuations of cell states have positive correlation with the plasticity of cell types.

The present scenario of loss of plasticity and recovery from multiple cell deaths can be experimentally verified by measuring the gene expression as an indicator of chemical concentration. One can measure the variance of gene expressions, for example, by using fluorescent proteins and a cell sorter, during the course of the developmental process. By measuring the change of variance of the gene expression in a colony of cells[3, 1, 6] throughout the developmental process, one can examine whether there is a decrease in gene expression fluctuations, and moreover, whether there is a recovery when multiple cell deaths lead to a novel ensemble of cells, as in the event of metamorphosis.

In the present paper, we have discussed spontaneous cell differentiation and switching processes in a well-stirred medium, i.e., in a spatially homogeneous medium. To discuss morphogenesis with spatial pattern formation, it would also be interesting to include spatial inhomogeneity in the medium.

The present results also have some implications for evolution. Indeed, one could extend

our model to regard each unit as an organism, instead of a cell, and include genetic change (mutation) of the reversible reaction rate constants C_u and C_v in the model. With this extension, different units in our model can be regarded as distinct types of organisms. Indeed, a theory of sympatric speciation with using phenotypic plasticity has recently been proposed along these lines[10, 13]. In a preliminary study of the present model including the above mentioned genetic mutation process, we have observed a sympatric speciation process forming several species. With this process, the plasticity of each species defined in this paper decreases. With this extension, successive extinction events of some cell types in the present model correspond to mass extinction of species through evolution. Note that in the theory of punctuated equilibrium[2], evolution process consists of long quasi-stationary regime and rapid temporal change accompanied by extinctions, as discussed above. Here, the recovery of species plasticity of species after the extinction of many cells is relevant to open-ended evolution, as will be discussed elsewhere.

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A The condition for cell differentiation

Here we study the conditions for cell differentiation. First, we study the dependence of the differentiation events on initial conditions. As is mentioned above, it is necessary for an initial cellular state not to be exactly an attractor of a single cell. If we start precisely from an attractor of a single cell state, then the cells that are derived from its successive divisions cannot differentiate. Once the state is on an attractor, the cell division gives two identical cells, as long as the fluctuation in the new cell's chemical composition is not large to attain a different cellular states. With the increase in cell numbers, identical cells compete for the same resources and eventually reach a stage in which they cannot take in enough resources, which leads to the decrease of the chemical contents of the cell. Hence, these identical cells die, almost simultaneously. On the other hand, cell differentiation generally emerges as long as the initial condition is not chosen precisely on an attractor of a single cell state.

Second, we study the dependence of the differentiation frequency on C_v/C_u, the ratio of the reversible reaction rate constants. We changed the parameter C_v from 0.002 to 20 while fixing $C_u = 2.0$.

If C_v is much smaller than C_u, all the cells increase their number while keeping almost the same chemical composition and competition for the resources is too strong for the cells to survive. However, if C_v is of an order comparable to C_u, all the cells take the same uniform state with $u^j \neq 1$ and $v^j \neq 0$, so that an extinction event occurs. Cell differentiation events occur most probably in the intermediate cases.

Third, we study the dependence of the differentiation event on the strength of the interaction between the cell and the environment. As an index of interaction strength, we use the parameter D_u from 0.1 to 1.9. For $D_u \leq 0.4$, cell-cell interaction is too weak to amplify small differences among cell states, so that differentiation events cannot occur. Hence it is necessary that the strength of cell-cell interaction is stronger than some threshold value.

Fourth, we study the dependence of the differentiation event on the number of chemicals K. The frequency of cell differentiation rises with the increase of K. Hence it is necessary that the number of chemicals is larger than a certain level.

The necessary conditions for cell differentiation obtained in this appendix are summarized below:
(0) Initial condition is not set to be exactly on an attractor of a single cell.
(1) Inter-cellular interaction is larger than some threshold.

(2) The ratio of the reaction time scale of u^j to that of v^j is in the intermediate range.
(3) The number of chemicals is larger than a certain level.

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key concepts	definitions in our study
Cell state	State given by intra-cellular chemical composition.
biological meaning (B)	Often measured by gene expression pattern, i.e., concentrations of mRNAs which produces the corresponding proteins.
representation by dynamical systems model (D)	Given by the concentration of chemical components in the model. Represented by a point (or orbit) in the phase space showing chemical concentrations of all the components.
Change of cell state	Through intracellular reaction dynamics, chemical concentrations change over time.
Cell type	Through the above dynamics, cells of similar chemical compositions are clustered into distinct groups.
(B)	Typically given by several distinct sets of gene expressions.
(D)	The states represented in the phase space are localized in several regions. The chemical concentrations are attracted into these distinct regions through dynamics(proximity to attractors).
Cell differentiation	An event where the state of a daughter cell distinctly differs from that of the mother cell, i.e., the change of cell type.
(B)	Often detected by a change of gene expression pattern.
(D)	Given by the change of cell type (represented by the attractor).
Cell death	
(B)	Irreversible loss of cell's self-maintenance.
(D)	A cellular state in which the chemical reaction dynamics no longer maintains intracellular chemicals, resulting the loss of cell volume.
Recursiveness	Degree of similarity between states of mother and daughter cells.
(B)	Similarity between the gene expression patterns of mother and daughter cells.
(D)	Similarity of chemical composition of cells.
Plasticity	Degree of susceptibility of a cell state to external environmental change.
(B)	Change of gene expression patterns to environmental (or other external) change.
(D)	Change of chemical compositions to the change of environmental condition including the change of the number of cells.

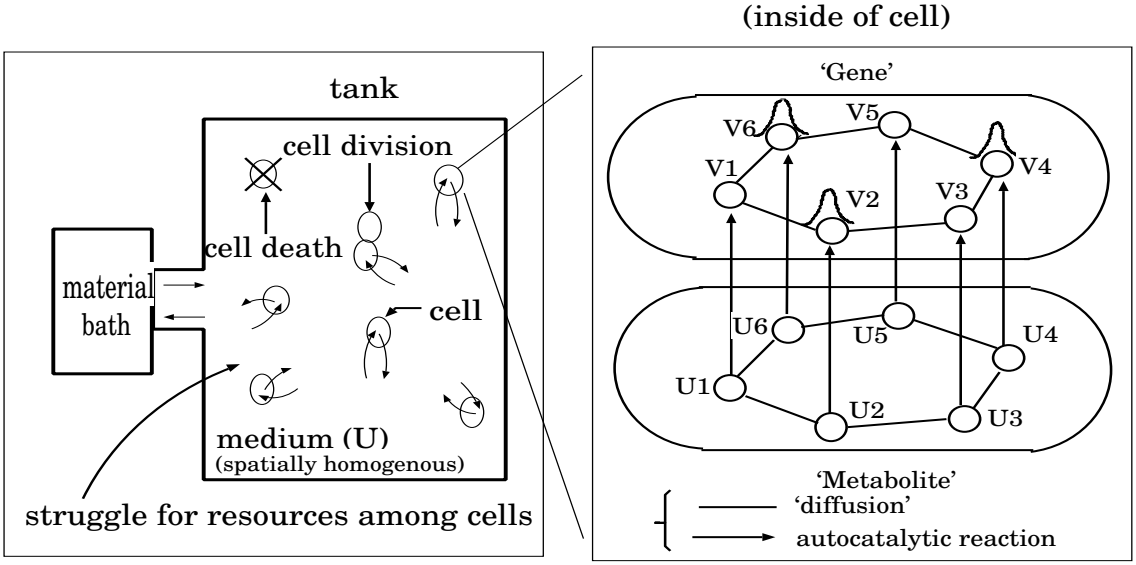
Table I. Key concepts in the developmental process in our study: basic definition, biological representation (B) and the corresponding representation in our dynamical systems model (D). Concepts at a cellular level.

key concepts	definitions in our study
Quasi-stable states for an ensemble of cells	The population of cells takes on a distinct distribution of cell types, which is stable over times much longer than that required for cell division.
(B)	Distinct tissue for multicellular organism.
(D)	Distinct distribution of cells classified by cell type.
Switching	Transition between different quasi-stable states through cell division.
(B)	Corresponding to the process of metamorphosis and life-cycle of multi-cellular organism.
(D)	Given by the drastic change of population of cell types in the model.
Diversity	The number of different cell types coexisting in each quasi-stable state.

Table II. Key concepts in the developmental process in our study: basic definition, biological representation (B) and the corresponding representation in our dynamical systems model (D). Concepts at a level of cell ensemble.

parameter	the meaning of the parameter
K	Number of species for each chemical type u and v
u_i^j	Concentration of resource chemicals (corresponding to metabolites)
v_i^j	Concentration of product chemicals (corresponding to expressions of genes)
W	Connection matrix to specify reaction (randomly given, fixed in time)
C_u,C_v	Reaction rates for resource and products respectively
D_u	Diffusion constant of resource chemicals through membrane
U^j	Concentration of resource chemicals at the medium surrounding cells
D_U	Diffusion rate of resource chemicals into the medium from the bath
B	Decay rate of product chemicals
$N(t)$	The total number of cells, that is time-dependent
Vol_0	The volume of the medium
Cell division	When the volume of a cell (given by the amount of resource chemicals) is larger than threshold 2V
Cell death	When the volume is smaller than 1/2 V

Table III. Summary of our model.



	resource	product
role in cells	metabolite(U)	gene(V)
source of supply	flow from the environment	synthesized autocatalytically
environment	exist	not exsist
interaction in each chemical species	species-exchanging reaction (symmetrical = 'diffusion')	species-exchanging reaction (symmetrical = 'diffusion')
time scale	faster	slower
cell division	————	when total V becomes twice
cell death	————	when total V become half

Figure 1: Schematic representation of our model.

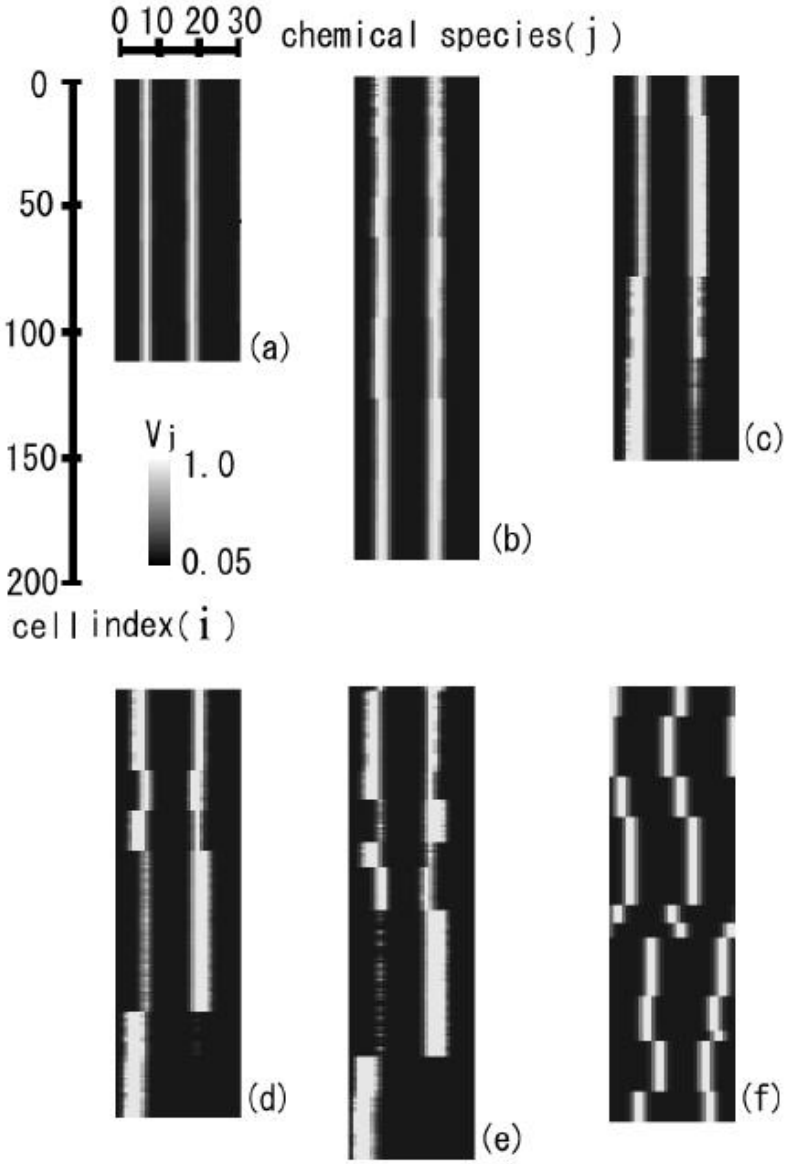


Figure 2: An example of the time series of all the cells. Snapshot patterns of the concentrations of all the product chemicals are overlaid with a gray scale at every $t = 50$((a)$\sim$(e)) except for (f)(at $t = 10000$). The horizontal axis represents the number of product chemicals, whereas the vertical axis represents the indices of cells, which are sorted so that the cells of the same type are aligned. Unless otherwise mentioned, we adopt the parameter values $A = 0.020$,$B = 0.060$, $C_u = 2.0$, $C_v = 0.020$, $D_u = 0.50$, $D_U = 1.0$, $Vol_0 = 3.0$ and $K = 30$ for later figures.

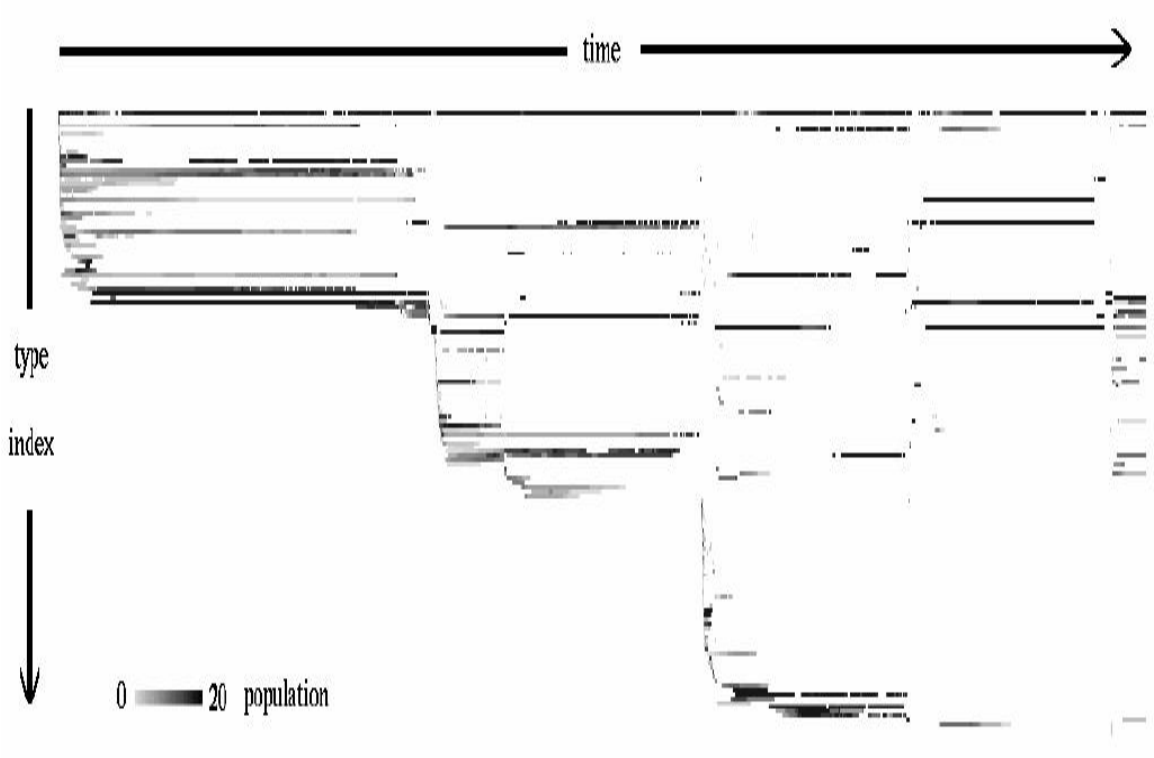


Figure 3: An example of the long time behavior of cell differentiation process. Simulation is carried out up to $t = 1000000$ starting from a single cell, while the data for the total cells are sampled by every 1000 time, to classify all cell types and to get the temporal change of population of each cell type. The method for the classification of cell types is mentioned in the text. All cell types are shown in the order of their appearance; a cell type that appears earlier in the simulation has a smaller index for the cell type. The population of each cell type is represented with a gray scale. Here the unit time of the figures is 1000.

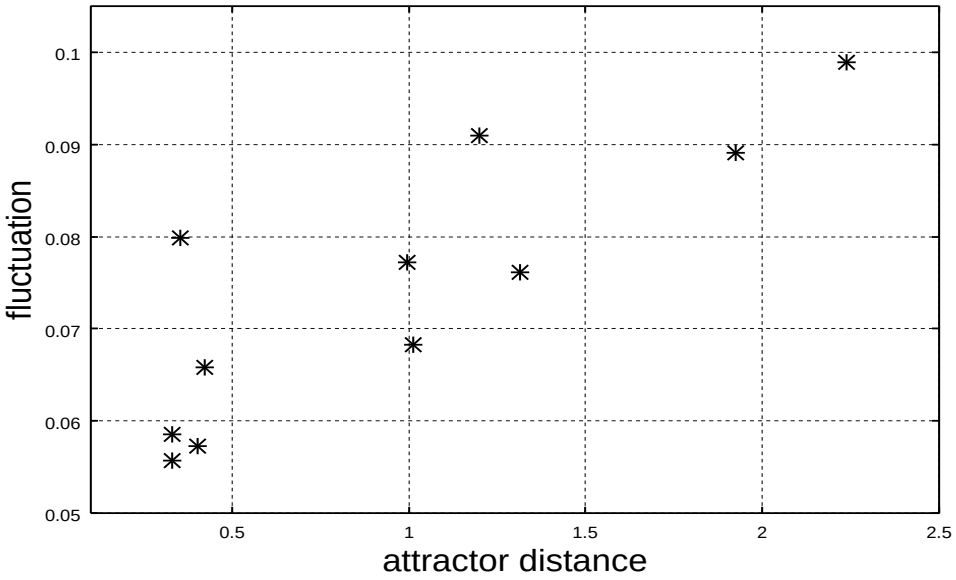


Figure 4: An example showing the relation between the attractor distance and the temporal fluctuation of each cell type. Over a period when several types coexist stably in a temporal evolution, we measured the temporal fluctuation of each cell type as the Euclid distances of chemical concentrations between two cells of the same type, chosen at different time, separated by a time span $t = 1000$. The temporal fluctuations are computed over 100 data samples, and the average over the samples are plotted.

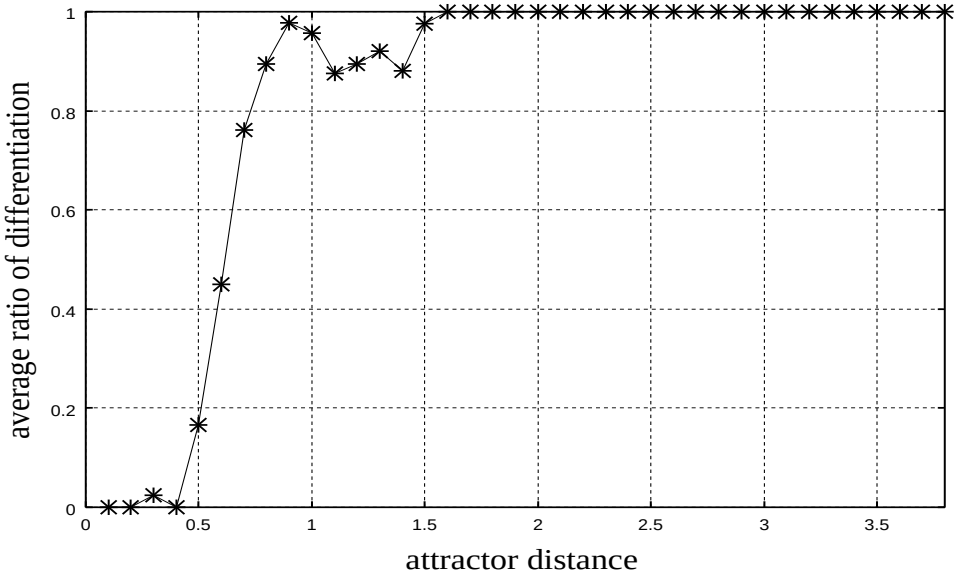


Figure 5: The relation between the attractor distance and the frequency of re-differentiation. We compute attractor distance of all the cell types appeared in each temporal evolution, and take an ensemble of cells whose members have the same initial condition. This cell ensemble is put into a new environment to check whether cell differentiation occurs or not. By sampling the data for the attractor distance by 0.1 bin size, we compute the average ratio of the frequency of re-differentiation event for each bin, to get the relationship between the differentiation ratio and the attractor distance.

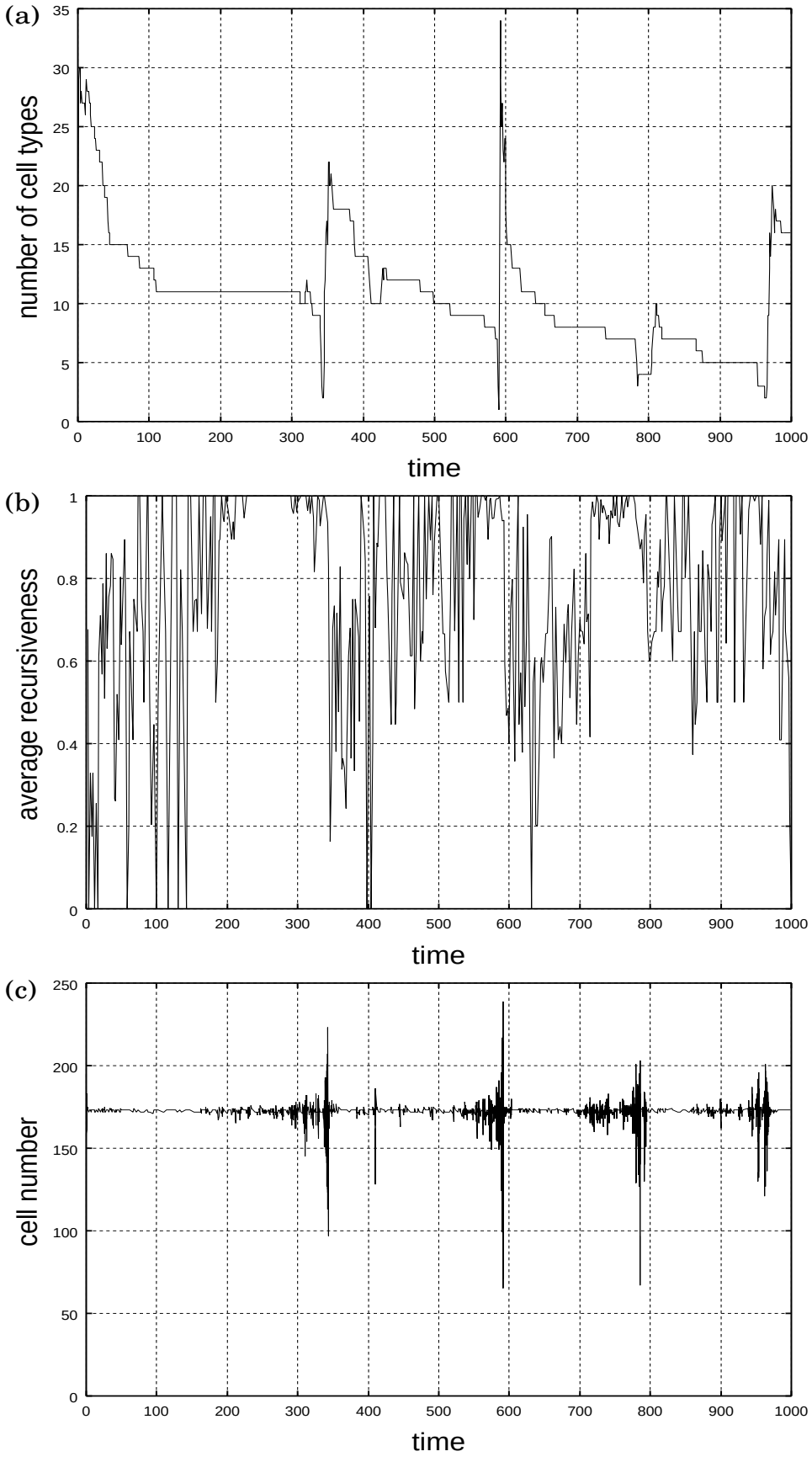


Figure 6: (a):Temporal change of the number of cell types. (b):Temporal change of the average recursiveness over all cells. (c):Temporal change of the total cell number. We computed all the quantities for the data given in the temporal evolution of Fig.3. Here the unit time of the figures is 1000.

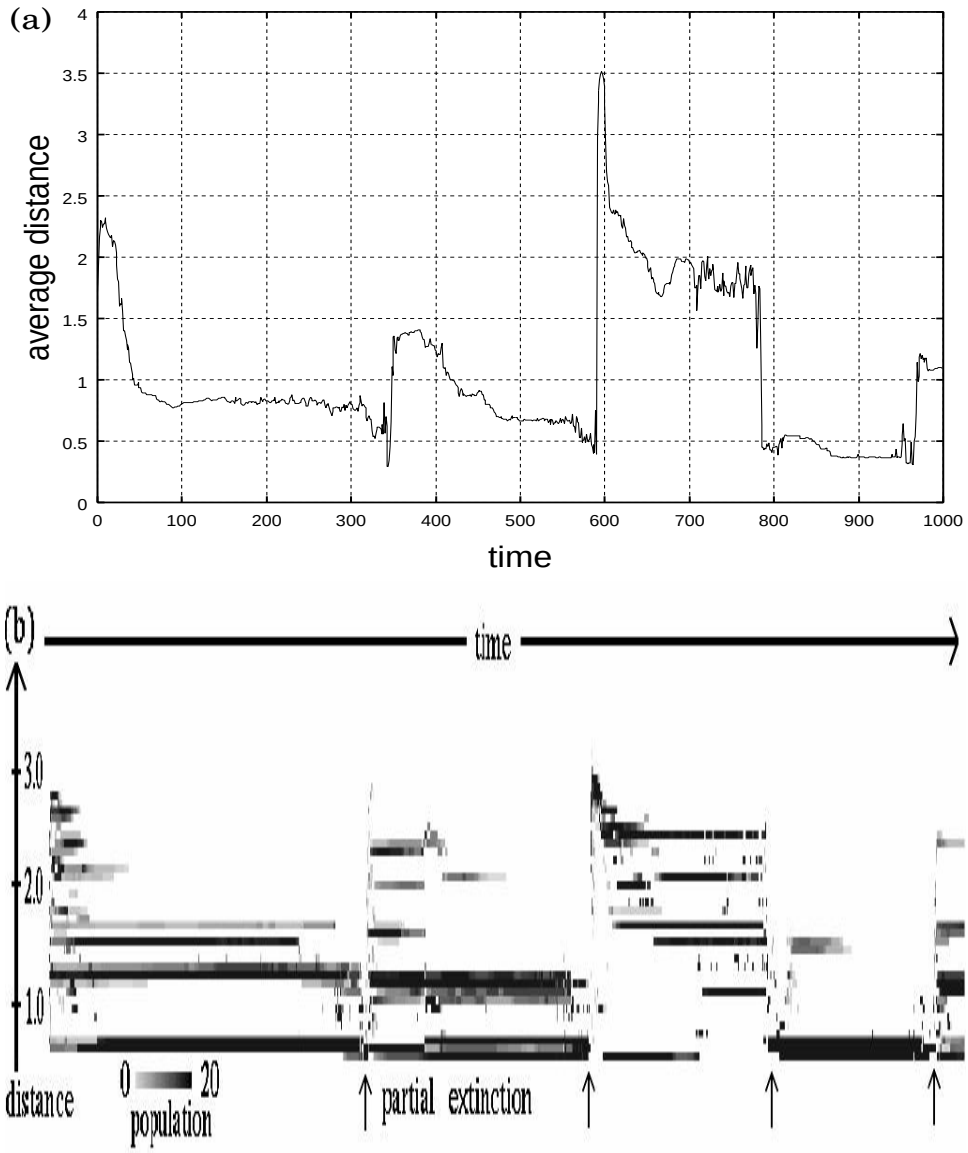


Figure 7: (a):Temporal change of the average attractor distance. The average attractor distance is obtained by averaging over all existing cells. (b):Temporal change of attractor distances of all the cell types existing simultaneously are plotted with their population. We computed all the quantities for the data given in the temporal evolution of Fig.3. Each time when extinctions of many cells occur is shown by the arrow. Here the unit time is 1000.

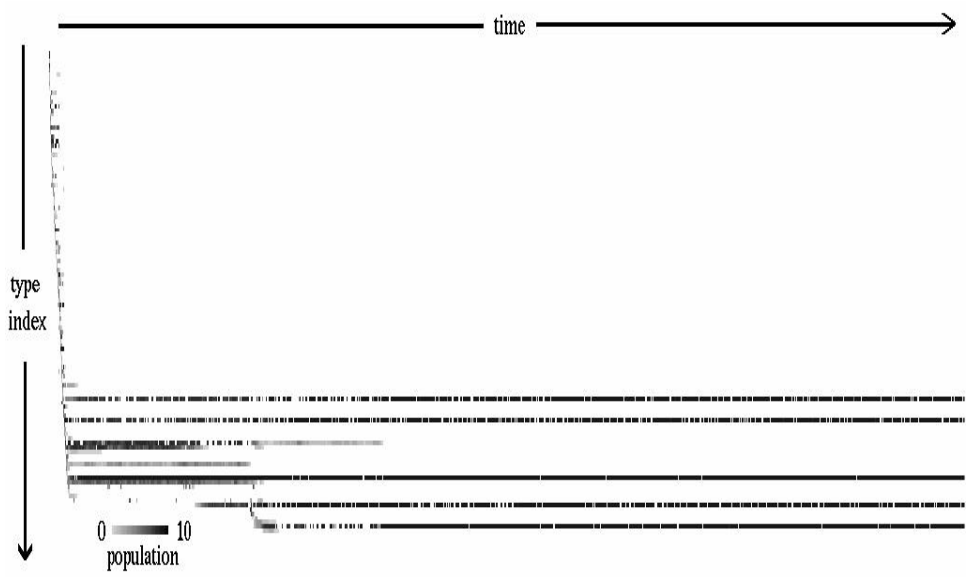


Figure 8: An example of the long time behavior of cell differentiation process by adding noise with the amplitude 0.00010. Simulation is carried out up to $t = 1000000$ starting from a single cell, while the data for the total cells are sampled by every 1000 time, to classify all cell types. The initial condition and method for the classification of cell types are mentioned in the text. All cell types are shown in the order of their appearance; a cell type that appears later in the simulation has a larger index for the cell type. The population of each cell type is represented with a gray scale. Here the unit time is 1000.

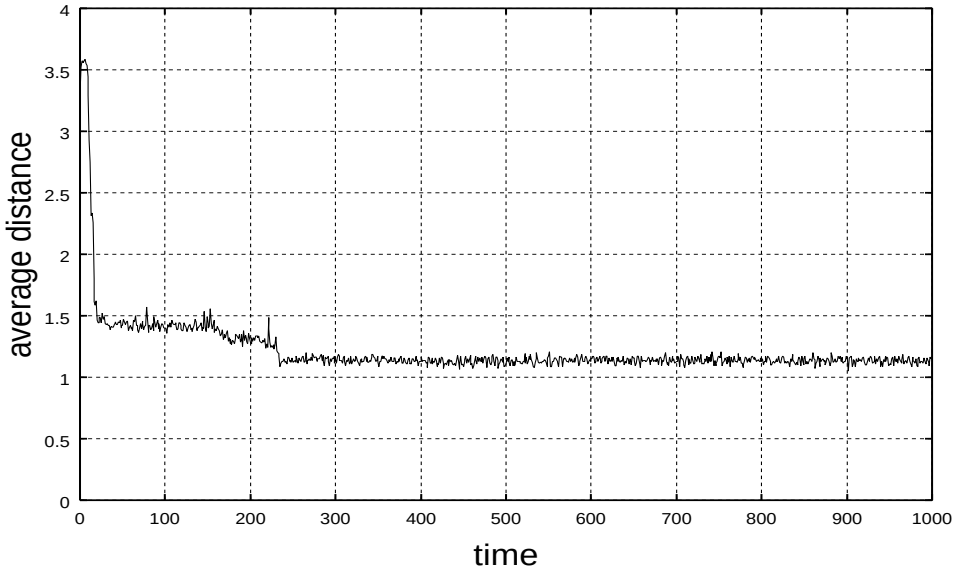


Figure 9: The time series of the average attractor distance, corresponding to the temporal evolution of Fig.8. Here the unit time of the figures is 1000.

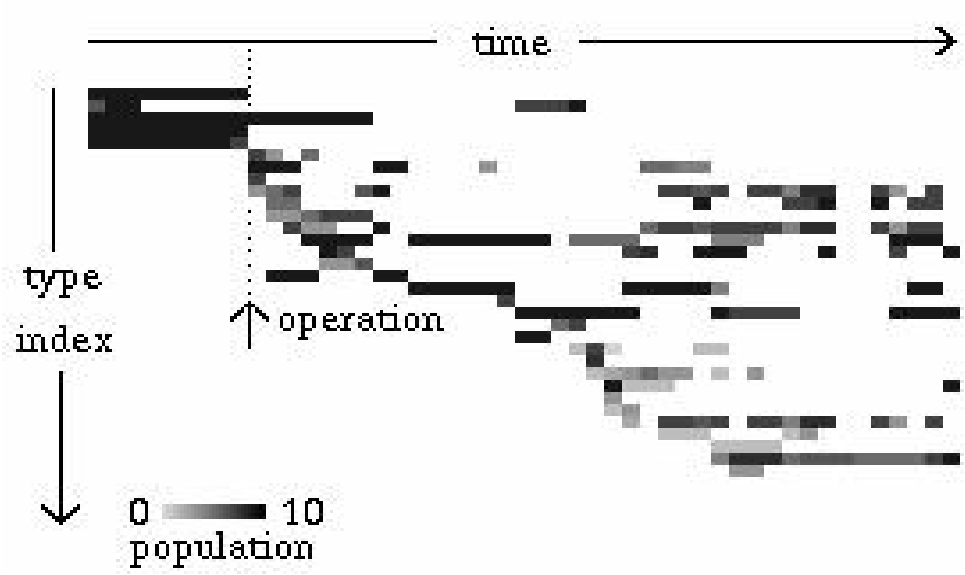


Figure 10: An example of the temporal behavior of cell differentiation process after external change of environmental resources shown by the arrow. Initially, cell distribution was given by the quasi-stable multi-cellular state at $t = 10^6$ in the simulation of Fig.8. Then, the simulation is carried out up to $t = 50000$, by reducing the supply of five resource chemicals at $t = 5000$, as shown by the arrow. The states of all cells are sampled by every 1000 time unit, to classify all cell types. The method for the classification of cell types is mentioned in the text. Here, indices of cell types are numbered in the order of their appearance. The population of each cell type at each time is represented with a gray scale.

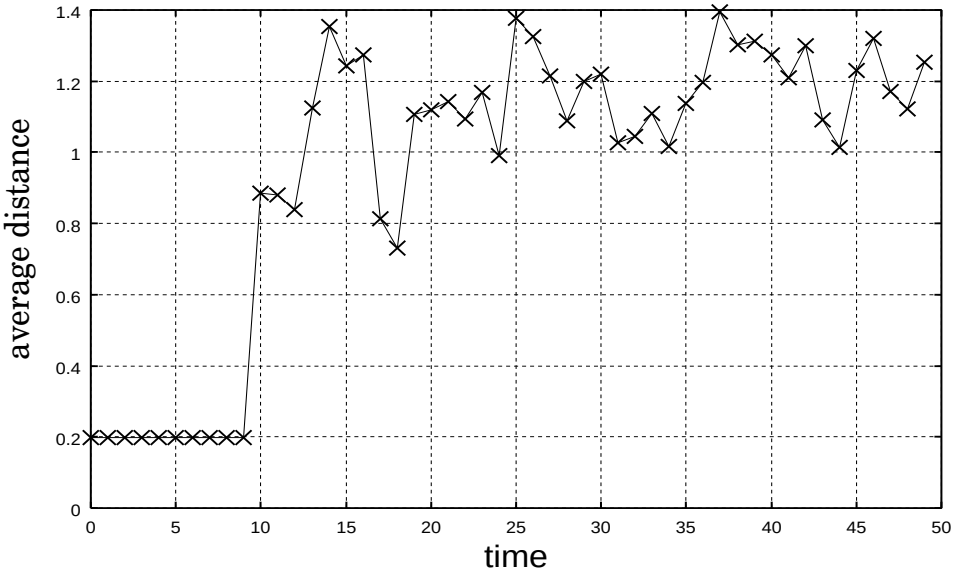


Figure 11: The time series of the average attractor distance, corresponding to the temporal evolution of Fig.10. Here the unit time of the figures is 1000.

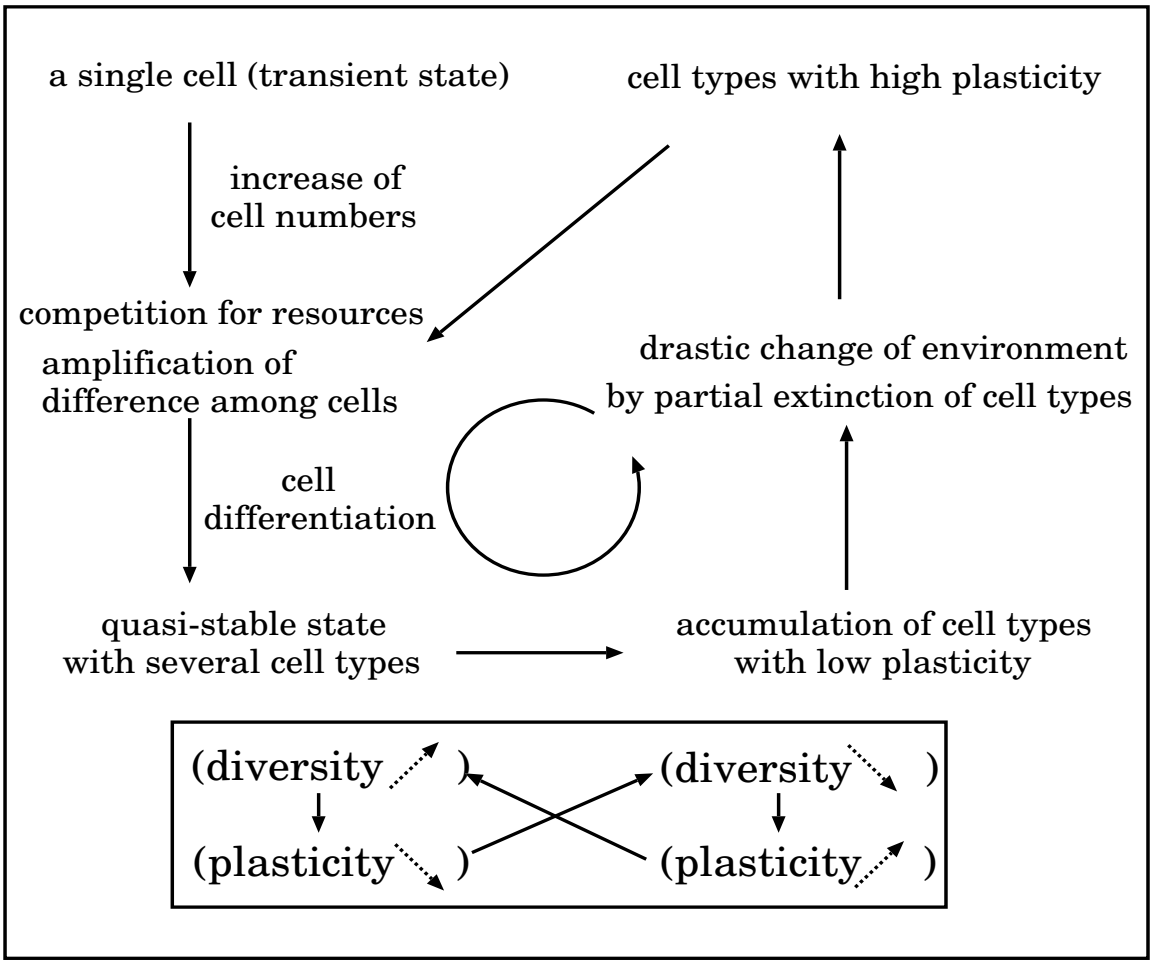


Figure 12: Schematic representation of our scenario for the mechanism to keep the diversity of cell types.

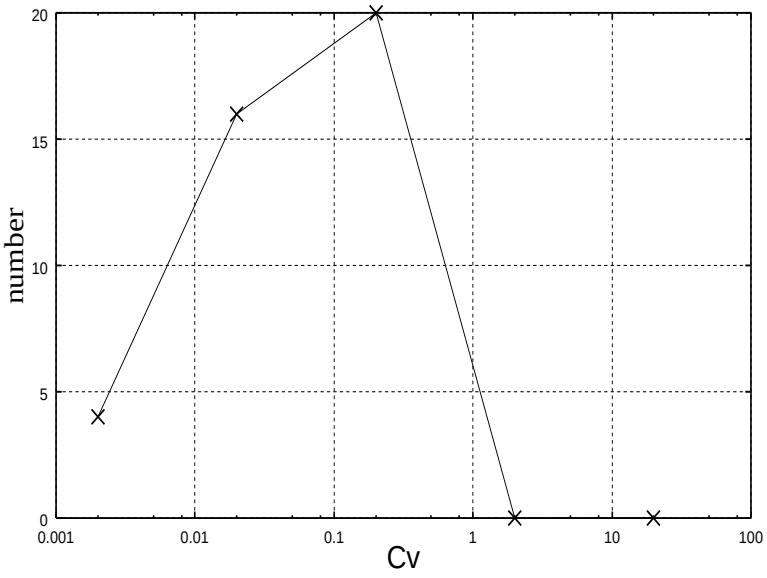


Figure 13: The frequency of cell differentiation plotted as a function of the parameter C_v with fixing $C_u = 2.0$. For each point of the figure, we took 20 different initial conditions, and carried out simulations up to $t = 20000$, to check the differentiation. Plotted are the number of events with cell differentiation.

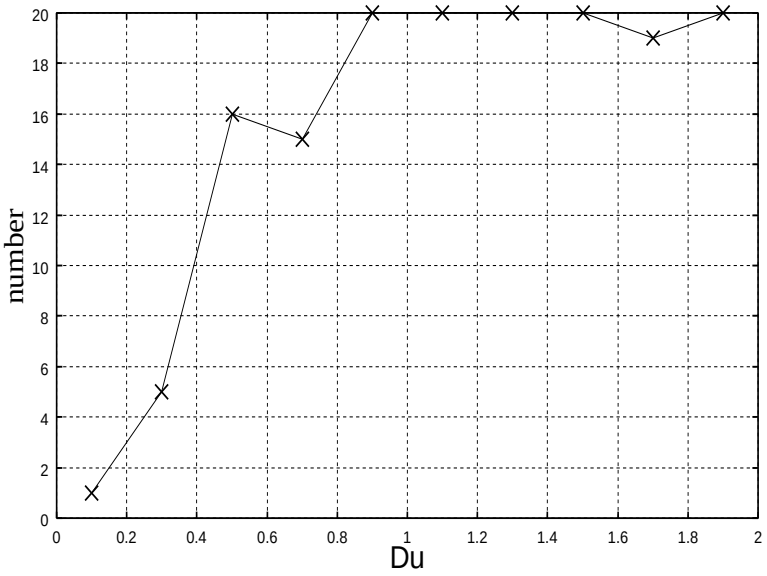


Figure 14: The frequency of cell differentiation plotted as a function of the parameter D_u. The parameter D_u is changed from 0.1 to 1.9 with the bin size 0.2. For each point of the figure, we took 20 different initial conditions, and carried out simulations up to $t = 20000$, to check the differentiation. Plotted are the number of events with cell differentiation.

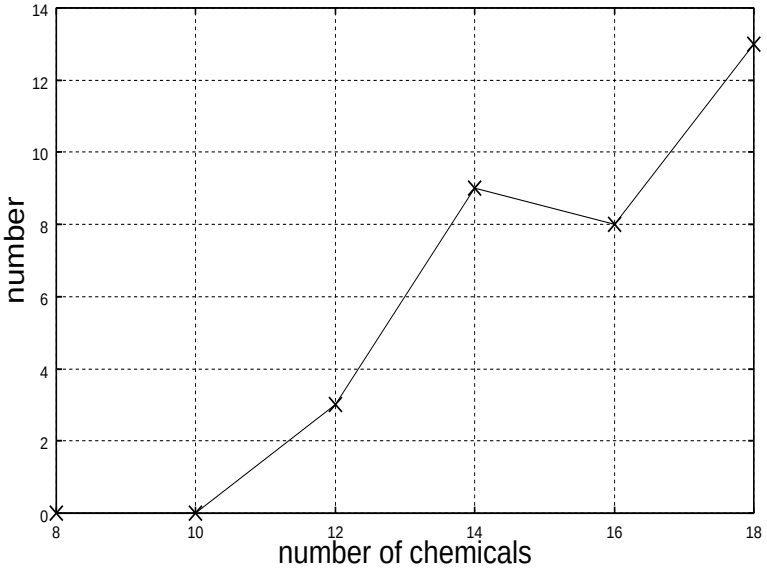


Figure 15: Dependence of cell differentiation on the number of chemicals K. We study the cases with $K = 8$, 10, 12, 14, 16 and 18. For each point of the figure, we take 20 different initial conditions and carry out a simulation up to $t = 20000$ with the same parameters mentioned in the text. Accordingly we plot the number of the event where cell differentiation occurs.