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A Comparative Analysis on Introduction to Multicellular Organisms with Active Differentiation and Spatial Pattern

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Abstract – The processes by which multicellular organisms first emerged from their unicellular ancestors are fundamental to the biology of complex, differentiated life forms. Previous work suggests that reproductive division of labor between specialized germ and soma cells was central to this evolution in some cases. Here, we assess the potential of the digital life platform Avida to examine the trade-off between survival and replication in multicellular organisms. Avida uses a grid of selfreplicating computer programs capable of mutation and evolution to address biological questions computationally. We model our digital organisms after the Volvocales, a flagellated order of photosynthetic green algae that includes both unicellular and multicellular species. We show that, given selective pressures similar to those experienced by the Volvocales in nature, digital organisms are capable of evolving multicellularity within the Avida platform. The strategies we observed that best handled the trade-off between survival and replication involved germ cells producing sterile, somatic offspring.

These strategies are similar to those observed in volvocine algae, which suggests that digital platforms, such as Avida, are appropriate to use in the study of reproductive altruism. The origin of multicellular organisms and the mechanism of development in cell societies are studied by choosing a model with intracellular biochemical dynamics allowing for oscillations, cell–cell interaction through diffusive chemicals on a two-dimensional grid, and state-dependent cell adhesion. Cells differentiate due to a dynamical instability, as described by our "isologous diversi.cation" theory. A .xed spatial pattern of differentiated cells emerges, where spatial information is sustained by cell–cell interactions. This pattern is robust against perturbations. With an adequate cell adhesion force, active cells are released that form the seed of a new generation of multicellular organisms, accompanied by death of the original multicellular unit as a halting state. It is shown that the emergence of multicellular organisms with differentiation, regulation, and life cycle is not an accidental event, but a natural consequence in a system of replicating cells with growth.

Development is the powerful process involving a genome in the transformation from one egg cell to a multicellular organism with many cell types. The dividing cells manage to organize and assign themselves special, differentiated roles in a reliable manner, creating a spatio-temporal pattern and division of labor. This despite the fact that little positional information may be available to them initially to guide this patterning.

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INTRODUCTION

The development of multicellular organisms from a single fertilized egg cell has fascinated humans at least since Aristotle's speculations more than 2000 years ago (34). In the more recent past our understanding of how interacting genes direct developmental processes has greatly increased (31; 12; 34). Cell differentiation, the inducing effects of intercellular signaling, changes in cell form like contraction, the self-organizing properties of adhesion and cell sorting in animal morphogenesis (13) are among the important principles better understood now. And although every cell is controlled by a Genetic Regulatory Network (GRN), the resulting multicellular dynamics are also strongly influenced by physical constraints.

Development has also caught the attention of computer scientists. Traditionally their evolutionary algorithms (EAs) would neglect development for a relatively direct mapping from genotype to phenotype. To overcome problems with these EAs, many models have been proposed that incorporate development in some way – reviews of these are given in Stanley and Miikkulainen (28), Kumar and Bentley (21).

One of the earliest researchers looking for a theoretical explanation of how cells in a developing embryo could establish their different roles was

Turing (30). He proposed a general symmetry breaking mechanism via the setting up of chemical gradients with reaction diffusion systems. Somewhat later, Wolpert (32, 33) came up with the very illustrative French flag model as an attempt to explain how morphogen gradients could give cells positional information as a general biological process. “Stem cells” placed along a given morphogen gradient would only have to read the morphogen contraction at their position and react to threshold values to decide whether they are in the blue, white or red part of the flag. Note that this assumes the existence of a gradient but does not explain how such a gradient could be set up by the cells. Jaeger and Reinitz (16) proposed a revised French flag model which to some degree takes the dynamic, feedback-driven nature of pattern formation into account.

How and why multicellular organisms developed are central questions in developmental biology. In life’s history, multicellularity has emerged from unicellularity on at least 25 separate occasions (Grosberg and Strathmann, 2007). The fact that this type of specialization and cooperation between cells has emerged independently and repeatedly in organisms ranging from algae to fungi suggests that this phenomenon is not a statistically unlikely event, but is the result of selective pressures experienced by various types of life. Previous theoretical and experimental work has shown multicellularity to be selectively advantageous in several circumstances (Rokas, 2008). In *Chlorella vulgaris*, for example, multicellular forms have evolved from their unicellular counterparts in the presence of a predator within 100 generations, suggesting that a multicellular existence might be advantageous to combat predation (Boraas et al., 1998). Here, we focus on the potential benefits of reproductive division of labor in multicellular forms.

Both reproduction and survival are vital for life to propagate. Differentiation between reproductive germ cells and purely functional soma cells is observed in the Volvocales, a flagellated order of photosynthetic green algae (Kirk, 2001). We chose to model our experimental parameters after the Volvocales specifically because they include multicellular organisms of varying colony size, each of which displays a different degree of complexity and specialization (Koufopanou, 1994).

The primary trade-off Volvocales address is between mobility and reproduction. An algae colony’s ability to photosynthesize effectively is dependent upon its depth within the water column, and vertical traversals of entire colonies are common (Sommer and Gliwicz, 1986). A colony’s capacity for mobility is primarily determined by the total functionality of its members’ flagella.

In the Volvocales, however, cell division damages flagella. When a cell replicates, its flagella continue to function, but “not as strongly or as well coordinated as when [a cell is] not dividing” (Marchant, 1977). After a cell replicates several times its flagella become completely nonfunctional. The number of divisions until a cell loses all flagellar function is generally assumed to be about five (Koufopanou, 1994; Michod et al., 2006). Previous literature suggests that differentiation between germ and soma “may have evolved as a solution to this problem: by denying reproduction to some cells, a parental colony can maintain functional flagella on these cells, which will enable it to maintain its position in the water column while the rest of its cells are dividing” (Koufopanou, 1994). We refer to this constraint as the flagellation constraint.

The development of multicellular organisms is one of the most elegant and interesting processes in biology. Cells that contain the same set of genomes differentiate to several types with exact order and exact location. The determination of cell type is somewhat robust, even though the development process occurs in a thermodynamic environment with molecular fluctuations. Three mechanisms are necessary to sustain a robust developmental process in multicellular organisms. First, an external field, which provides information to control differentiation and proliferation, must be maintained through the interaction among cells. Second, each cell must detect and interpret such external information. Finally, the internal state of each cell must be changed according to this interpreted information, leading to differentiation. Recent advances in molecular biology provide us with a molecular basis for these mechanisms. The gradient of morphogen concentration giving positional information can be identi.ed experimentally, the existence of a signaling pathway from receptor protein on the membrane to the nucleus is veri.ed, and the internal states of cells are reduced to regulations of protein synthesis from DNA molecules.

However, when we focus on the emergence of multicellular organisms in the evolutionary process, it is dif.cult to argue that such elaborate mechanisms appear independently at the same time. On the other hand, the fossil record shows that the transition to multicellularity has occurred at least three times in fungi, plants, and animals [12].

This suggests that the evolution to multicellularity is not a chance event but a *necessity* in evolution. The three mechanisms mentioned above must be tightly incorporated, at least at the .rst stage of multicellularity. Thus, to understand the transition to multicellular organisms, the interplay between interactions among cells and intracellular dynamics must be studied.

The motivation behind this work is not restricted to the origin of multicellularity. Even if it might be possible to describe all detailed molecular processes of the present organism, this does not answer why such a developmental process is robust in spite of the considerable thermodynamic fluctuations occurring at the molecular level, which seems to make

machinelike functions such as a “clock” almost impossible. Any rule with a threshold given by a signal molecule’s concentration is accompanied by fluctuations and therefore cannot proceed correctly. We need to construct a logic for the development process that, in general, works even under molecular • uctuations. Such a logic is relevant to understanding the level of multicellularity in present organisms, from primitive structures such as *Dictyostellum discoideum* and *Volvox*, to higher organisms.

To understand the emergence of multicellularity as a general consequence of the interplay between inter- and intradynamics of cell societies, we have earlier proposed the “isologous diversi.cation” theory [4,10,11]. This theory is rooted in the “dynamic clustering” observed in globally coupled chaotic systems [8,9]. It provides a general mechanism of spontaneous differentiation of replicating biological units, where the cells (which have oscillatory chemical reactions within) differentiate through interaction with other cells, as their number increases through divisions. This differentiation is due to the separation of orbits in phase space that is not attributed to a speci.c chemical substance but rather is represented through the dynamic relationships of several chemicals. While the differentiation is triggered by the instability of a nonlinear system, the differentiation process as a whole is shown to be robust against uctuations.

MULTICELLULAR ORGANISMS AND DIFFERENTIATION

In the work of [7], the focus is on the creation of emergent spatial patterns of differentiated cells. The model for differentiation is based on biochemical reactions within each individual cell, cellcell interactions in a medium containing diffusing chemicals, cell division, and cell adhesion. Cells divide and grow by using one of the chemicals as nutrient to build the cell body. Cell adhesion occurs between two cells of the same type that are within a specified distance threshold.

Differentiation results from the amplification of fluctuations and the increasing number of cells. The system becomes stabilized with the coexistence of different cell types. One simulation involves the emergence of a layered cluster of cells. The structure begins as a single cell of type 0, which divides and the structure develops into a type 0 cluster. As the structure grows, the central cells differentiate into type 1 and type 2 cells. The type 2 cells further differentiate into type 3 cells, which form the core of the structure. A ring pattern consisting of 3 layers results, as shown in Figure 1. The formation of the structure is not based on diffusion of chemicals, rather, it is based on growth over time. The inner core continues to grow and eventually the ring pattern is broken. A small cluster is broken off and moves away from the mother structure. The process is repeated, thus establishing a life cycle of a multicellular organism. Another simulation demonstrates the robustness of the system, as illustrated in Figure 2. A damaged part of the structure is regenerated. The growth in the damaged area is stronger. Death of the organism is implemented by a halting of the system. At this point, a life cycle is completed, and the next generation can begin.

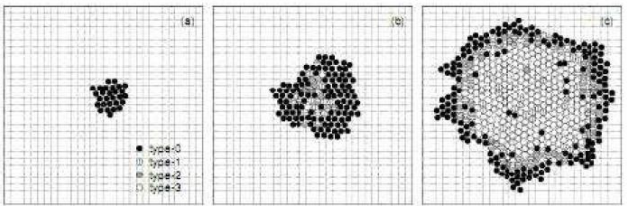


Figure 1. Creation of cell cluster of 4 different cell types.

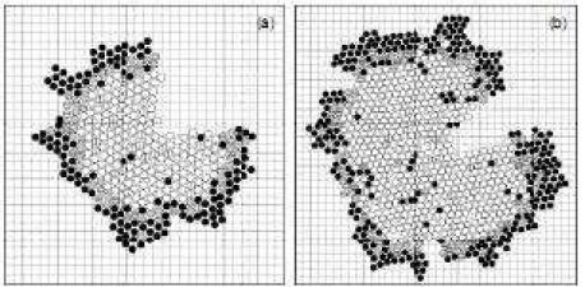


Figure 2. Repairing a damaged cluster.

MULTICELLULARITY AND DIGITAL SYSTEMS

The origins of multicellularity have previously been investigated in several contexts using artificial life models. Furusawa and Kaneko (1998) used an artificial chemistry model to examine multicellular emergence on a simulated twodimensional grid. Their focus was largely on exploring the mechanisms that cause cell differentiation during development.

Other researchers have studied task-related division of labor, wherein individuals cells cooperate to perform specialized tasks efficiently, as a mechanism by which multicellularity can arise (Michod, 2007). Goldsby et al. (2010) investigated task-related division of labor within the Avida platform and found that digital organisms are capable of selecting specialized roles in groups using both spatial information and inter-organism communication.

In this paper we focus solely on the potential benefits of *reproductive*, as oppose to task-related, division of labor. Finally, Schlessinger et al. (2006) provided an excellent investigation into the emergence of multicellularity in which they extended the Mosaic

World software system to allow for optional organism aggregation into multicellular units.

While the authors do include the ability for organisms to forfeit their reproductive capacity, individuals that join a multicellular unit lose their autonomy entirely, as tasks carried out by an aggregation are decided "democratically" through a poll of all constituents. Our investigation, in this context, is orthogonal, utilizing forced aggregation and optional autonomy.

EMERGENCE OF A ROBUST DEVELOPING METHOD

In this section, we discuss how the order of a cell society with a variety of cell types emerges, by showing that cellular differentiation and developmental processes form organized patterns. This ordered development of cell society is a common feature in existing multicellular organisms. As mentioned above, in our model we adopt a simple intracellular reaction dynamic whose rules of reaction are determined randomly and fixed throughout the simulations.

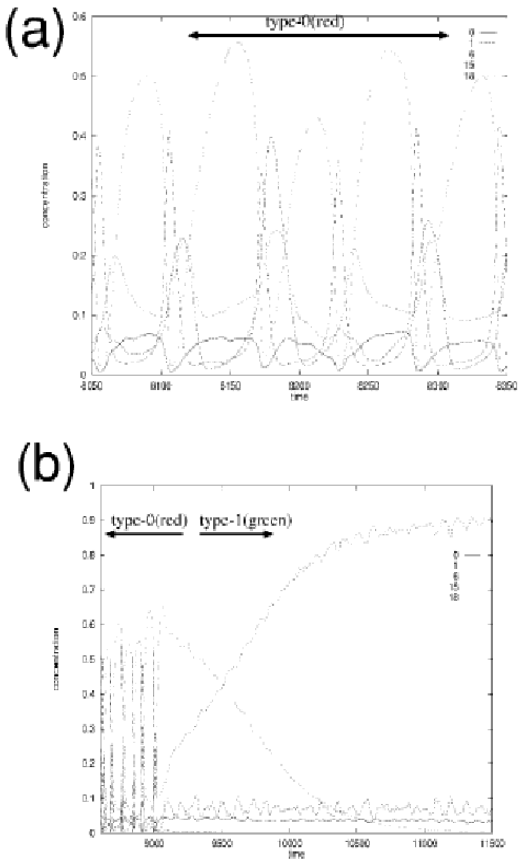
The behavior of the cellular system depends on the choice of the random reaction network. To extract the universal features of the system, which are independent of the detailed structure of network and parameters, we performed simulations using thousands of different reaction networks and parameters. As a result, we found that differentiations due to the cell–cell interactions and a robust developmental process toward an ordered spatial pattern of differentiated cells are commonly observed for some of the randomly generated reaction networks.

In the present model, cellular diversification processes are observed when intracellular chemical reaction dynamics show oscillatory behavior, as shown in Figure 3a. For other cases without oscillatory dynamics, in which the concentrations of chemicals are fixed over time, the cells keep an almost identical state, and a cell society of homogeneous cells appears. To investigate the emergence of developmental process in multicellular organisms, we assume that the intracellular dynamics exhibits oscillations.

The reasons for studying the networks that give rise to such oscillatory dynamics are as follows. First, we have found that robust developmental processes with spontaneous differentiation and spatial patterns commonly emerge only if the cells exhibit intracellular reaction dynamics. Second, as discussed below, a cell system characterized by oscillatory intracellular dynamics has a higher growth speed as an ensemble. Since the cells are crowded, only in a cell system with such dynamics can the number of cells continue to increase effectively. For this reason it is expected to be selected through evolution.

In real biological systems, such oscillatory dynamics are often observed in chemicals, such as Ca, NADH, cyclic AMP, and cyclins (Alberts et al., 1994; Hess and Boiteux, 1971; Tyson et al., 1996). Such an oscillation generally appears in a system with positive feedback reactions, which are observed ubiquitously in real biological systems. Indeed, the replication process requires the amplification of molecules, for which a positive feedback process is required. Thus, it is natural to postulate the existence of such oscillatory dynamics in our model system. Next we present the developmental process by considering two specific reaction networks that exhibit different types of spatial patterns, i.e., the concentric ring pattern and the stripe pattern of differentiated cells.

Developmental Process for the Ring Pattern : In this section, we present numerical results demonstrating the development of the concentric ring pattern of differentiated cells. This spatial pattern is most frequently observed in simulations carried out by taking a variety of randomly generated reaction networks. As the initial state, we put a single cell, whose internal state (i.e., the chemical concentrations in the initial cell) is determined randomly. In Figure 3a we show a time series of the concentrations of the chemicals for a single, isolated cell. Here, the intracellular dynamics show complex oscillatory dynamics. In this section, we call this initial type of cell "type 0". This state is the only stable state of intracellular dynamics when the cell is isolated in the medium.



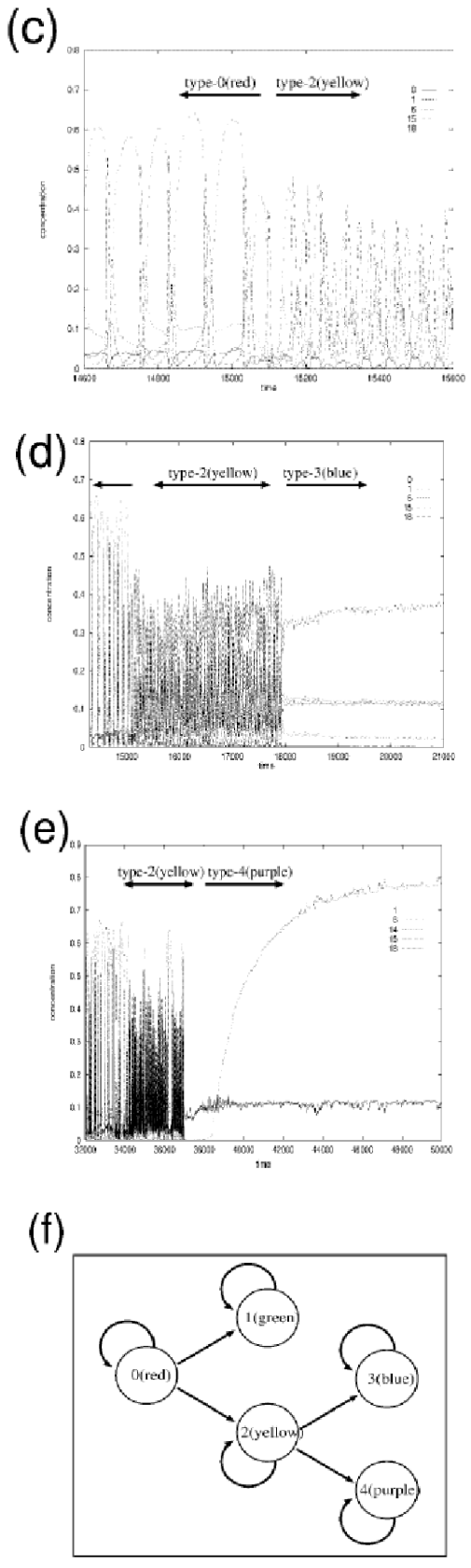


Fig. 3. a: Time series of concentrations for the type 0 cell in an example of a ring pattern. The ordinate represents concentrations of chemicals, plotted as a function of time. For clarity, we have plotted the time series of only six of the 32 internal chemicals. b–e: Time series of concentrations in a cell, representing the course of differentiation to cell types 1–4, respectively. f: The rules of differentiation. The path back to the original cell type represents the reproduction of the same type, while the paths to other types represent the potential for differentiation to the corresponding cell type.

Differentiation Process for the Stripe Pattern: In our model simulation, possible spatial patterns of differentiated cells are not restricted to the ring pattern as presented in the previous section. In this section, we present an example of the development for the stripe pattern, which is obtained by simulations of the model using a different reaction network that is also randomly generated. In this example, a single cell placed at the center of the medium shows oscillatory reaction dynamics. We call this a type 0 cell.

SIMULATION OUTCOMES WITH SPATIAL INFORMATION

Here, we present results of simulations including the motion of cells and diffusive chemicals on a two-dimensional grid. The reaction matrix and the parameters of internal dynamics are the same as those in the previous section, while the parameters related to the dynamics of the surrounding medium are tuned so that the same set of cell types is obtained with almost identical reaction dynamics and rules for differentiation. For cell types, the same nomenclature is adopted as in the previous section.

Spatial Pattern of Differentiated Cells : In this subsection, we assume that all cells adhere to each other with the same strength, irrespective of their type, when their distance is within a given threshold. The .rst cell, initially placed in the medium, shows type-0 dynamics and divides into two almost identical daughter cells, in the manner described in the previous section. These two daughter cells then make a new connection and adhere. With further divisions, a cluster of type-0 cells is formed.

When the size of the cell cluster exceeds a threshold value, some cells located at the inside of the cluster start to differentiate to type-1 and type-2 cells. As the cell number further increases, type-2 cells at the inside differentiate to type-3 cells, to form the inner core of the cluster. At this stage, a ring pattern consisting of three layers is formed. The ring of type-2 cells lies between peripheral cells with type-0 dynamics and an inner core consisting of type-1 and type-3 cells. Positional information giving rise to such a spatial pattern naturally appears through competition for nutrients, without any sophisticated programs implemented in advance. Note that the pattern formation originates from temporal

differentiation. It is not a diffusion-induced pattern like Turing's mechanism.

Emergence of Multicellularity: In this section, we change the condition of adhesion between the cells, to see continuous growth in our cell society. As is mentioned, the ring pattern with three layers is formed when all cell types can connect to each other. The growth, however, stops at a certain stage, and new cell clusters are not formed. Thus, such a cellular system cannot be sustained for long. If a change in the adhesion properties allows for the continuous growth and formation of a new generation of cell clusters, such cellular systems will come to dominate.

To study this problem we introduce a dependence of the adhesion force on cell types. Because the force of adhesion should depend on the membrane proteins on the cell surface, it is natural to include dependence of adhesion on the relative internal states of two adjacent cells. As a simple example, we assume that no connection is allowed between a type-2 cell and a type-3 cell, while the connections for all other combinations are preserved. This restriction on the connection implies that the second layer of type-2 cells and the inner core lose their capability to adhere to each other.

Death of Multicellular Organisms : After the release of peripheral cells, the remnant core with type-1 and type-3 cells stops cell divisions after intracellular chemical oscillations cease. This determines the lifetime of the replicating multicellular unit, given by its cell con. gurations and the decency of nutrition. This fact provides an interesting point of view with respect to the death of multicellular organisms. As is well known, the death of a multicellular organism is not identical with the death of cells in the organism but rather coincides with the death of the organism as a "system." For example, cells in a dead body often survive for a while. Thus, the emergence of Multicellularity must be accompanied with such a "halting" state of the system. This halting state limits the size and the lifetime of an organism. Such emergence of limitation is required to complete a life cycle and to give rise to a new generation. Indeed it is expected that when the size reaches a critical value, such a halting state is brought about by the lack of nutrition, at the first stage of Multicellularity, where no special organ for transportation of nutrition is yet developed. In fact, our results show that there is a halting state in a cell cluster when it reaches a size where even cells at the boundary of the cluster lose their activity and stop reproducing.

CONCLUSION

We have shown that digital organisms are capable of evolving Multicellularity as a solution to the flagellation and enlargement constraints within the Avida platform. The wide range of effective strategies involving germ-soma specialization we observed indicates that digital platforms can be appropriate for studying reproductive altruism. Avida, specifically, was well suited for our experiments because it offered detailed insight into novel strategies digital organisms might use to accomplish germ-soma differentiation.

In the present work, we have shown that salient features of multicellular organisms naturally emerge as the number of very primitive cells increases. Here the primitive cells have internal reaction dynamics (for example, metabolic reaction or genetic expression) and simple cell–cell interaction, and potential for division.

At this point, it is interesting to note that all multicellular organisms satisfying the condition of reclusiveness as a colony (i.e., property I) consist of eukaryotic cells. Indeed, a colony of bacteria cannot establish multicellular organisms to satisfy the requirement of reclusiveness as a colony (I). On the other hand, the differentiation itself (stated as the property II) has a broader generality (Ko et al., 1994; Shapiro and Dworkin, 1997). When bacteria are put into a condition with very strong cell–cell interaction, they can be differentiated into distinct types of enzyme activities (i.e., they satisfy property II) (Ko et al., 1994).

Still, for prokaryotes, individuality by each cell is so strong that they cannot reach stage I (reclusiveness as a colony). This review has presented several different simulation techniques for generating biological-like structures. Most of the simulations involve hundreds or thousands of cells, and the majority of them are two-dimensional, although several of the experimenters are confident that it is straightforward to adapt the simulations to 3D. Many typical cell behaviors have been simulated, including cell growth, cell adhesion, cell movement, cell differentiation, cell division, cell death, chemo taxis, and haptotaxis. Most of the simulations involve cells that interact locally with neighboring cells, and chemicals diffusing throughout an environment. The cell behaviors are often based on cellular automata or genome-like models, and the chemical environment is typically represented by a reaction-diffusion model with partial differential equations. Many of the shapes generated by these simulators are rather simple, such as a sphere or diamond, although the CompuCell3D project has involved more complex structures, such as the avian limb, somitogenesis, and vasculogenesis. Increasing the complexity of the simulated structures will likely lead to a deeper understanding of the morphogenesis of biological-like structures, that may ultimately result in the ability to move beyond simulation and create physical biologically inspired structures for use in real-world applications.

In the present article, we have studied a dynamical model to show that a prototype of cell differentiation occurs as a result of internal dynamics, interaction, and division. We have made several simulations choosing several chemical networks, with a different

number of chemical species, and were able to observe the same scenario for cell differentiation. With the same parameters as used in the previous example [4], approximately 40% of randomly chosen chemical networks show oscillatory behavior in our system, while others fall into .xed points.

A developmental genotype has been described that can under evolution, and solve tasks with phenotypes of arbitrary size capable of self-repair and adaptation under global environmental change. In the future, coordinated cell growth and movement will be investigated. Careful work remains to be undertaken to examine the role of chemicals. Future investigations will consider the chemical as energy so that cell growth and death might become an emergent phenomenon, this might also be helpful in establishing a more open ended evolution, where multi-cellular organisms fight for survival, thus linking morphology with behavior.

REFERENCES

- Furusawa, C. and Kaneko, K. (1998). Emergence of multicellular organisms with dynamic differentiation and spatial pattern. Artificial Life, 4(1):79–93.
- Grosberg, R. K. and Strathmann, R. R. (2007). The evolution of multicellularity: A minor major transition? Annu. Rev. Ecol. Evol. Syst., 38:621–654.
- Rokas, A. (2008). The origins of multicellularity and the early history of the genetic toolkit for animal development. Annu. Rev. Genet., 42:235–351.
- Eggenberger, P. (1997). Evolving morphologies of simulated 3D organisms based on differential gene expression. In P. Husbands & I. Harvey (Eds.), Proceedings of the Fourth European Conference on Arti.cial Life, (pp. 205-213). Cambridge, MA: MIT Press.
- Fleischer, K., & Barr, A. H. (1994).A simulation testbed for the study of multicellular development: The multiple mechanisms of morphogenesis. In C. G. Langton (Ed.), Arti.cial life III, (pp. 389-416). Redwood City, CA: Addison-Wesley.
- Furusawa, C., & Kaneko, K. (1998). Emergence of rules in cell society: Differentiation, hierarchy, and stability. Bulletin of Mathematical Biology, 60, 659–687.
- Goldbeter, A. (1996). Biochemical oscillations and cellular rhythms. New York: Cambridge University Press.
- Furusawa C, Kaneko K. 1998b. Emergence of multicellular organisms with dynamic differentiation and spatial pattern. Artif Life 4:78–89.
- Shapiro JA, Dworkin M, editors. 1997. Bacteria as multicellular organisms. Oxford: Oxford University Press.
- Fleischer, K., Barr, A. H. A Simulation Testbed for the Study of Multicellular Development: The Multiple Mechanisms of Morphogenesis, Artificial Life III, 1994.
- Fleischer, K. Investigations with a Multicellular Developmental Model, Artificial Life V, 1997.