Emergence of multicellularity in a model of cell growth, death and aggregation under size-dependent selection

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How multicellular life forms evolved out from unicellular ones constitutes a major problem in our understanding of the evolution of our biosphere. A recent set of experiments involving yeast cell populations has shown that selection for larger aggregates leads to the appearance of stable clusters of cells that are able to split into smaller aggregates. It was suggested that the observed evolutionary patterns could be the result of evolved programs affecting cell death. Here we show, using a simple model of cell-cell interactions and evolving adhesion rates, that the observed patterns in cluster size and localized mortality can be easily interpreted in terms of diffusion-limited growth dynamics. An experimental test of this scenario is proposed. This simple mechanism would have played a key role in the early evolution of multicellular life forms based on aggregative development. The potential extensions of this work and its implications for natural and synthetic multicellularity are discussed.

Keywords: Major transitions, multicellularity, artificial life, evolution, synthetic biology, complexity

1. Introduction

One of the key major transitions of evolution involved the emergence of multicellular life forms out from single-cell systems [1,2]. The standard view is that groups of cooperating cells are able to take advantage of division of labor in order to better exploit external resources, avoid predators or improve given adaptive traits [3,4]. Yet the transition multicellularity (MC) encapsulated in this picture involves an increase in overall complexity [5] and thus increasing costs for coordinated cooperating behavior. The main problem is then to understand what makes the tradeoff between these two sides balance out.

Available phylogenetic techniques have shed light into how and when the roots of multicellularity got established [6-9]. Particularly, comparative analyses of different clades of multicellular organisms have proven to be very useful in delineating of the genetic toolkit required for multicellular existence [10]. These studies show that cell-cell communication and adhesion genes were co-opted from ancestral functions unrelated to multicellular phenotypes into robust developmental processes. In this vein, many unicellular species have the potential to behave (at least in some circumstances) as cooperative ensembles of cells [11,12].

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Figure 1. Modeling evolution of multicellular aggregates. Following the experimental setup described in (Ratcliff et al 2011) we consider a physically embodied description of aggregates growing and falling under the action of gravity (a). For simplicity, the spatial domain is confined to a two-dimensional lattice. In it, yeast cells have a limited number of potential attached cells and, in response to the local concentration of nutrients, cells can grow, divide or die. They can remain attached to daughter cells due to failure of separation, thus forming aggregates (b). Such aggregates are modeled in terms of simple repelling particles connected by springs (c). The physical displacement or breakage of these aggregates is introduced by cell death due to starvation (see text).

Two major paths towards MC have been identified [7]. The first is clonal development [6,8] which involves the evolution of a life cycle that requires all cells to display adhesion molecules capable of maintaining them together and for all cells to share the same genotype. The second is aggregative development. This alternative path does not require clonality and is present in some well known but rare systems, such as slime molds [1]. In this scenario, MC aggregates can form under some conditions and disaggregate into non-clonal individual cells. More recently, it has been found that some unicellular species display a MC pattern of development based on aggregative dynamics [13]. These remarkable findings suggest that non-clonal developmental processes might have played an important role in the early evolution of multicellular life forms [14].

In a recent set of experiments [15-17] artificial selection of cell clusters under gravity constraints was performed. The authors took advantage of the fastest sedimentation speed of cell aggregates of increasing size as a shortcut for selecting for more complex cell assemblies and potential mutations favoring them. Remarkably, after a relatively short number of generations, obtained by repeated culture transfers, the so called snowflake phenotypes appeared in a predictable way. These are rounded clusters of cells that appear attached to each other. The authors also studied the role played by cellular interactions and cluster structure on the underlying reproductive processes. It was found that clusters do not reproduce through events associated to single cells but instead involved a cluster-level set of events and -it was argued- a division of labor resulting from an apparently active control of apoptosis. The sequence of events as reported from this microcosm experiments has important consequences for our understanding of the evolution of multicellularity and potential scenarios for recreating the first steps from single cells to cooperating ensembles and organisms. The claim that evolved apoptotic paths might be at work is specially appealing.

Performing actual experiments involving physical aggregates is a necessary step
towards reconstructing the events that pervaded the rise of MC systems. Most theoretical models consider genetic traits but typically ignore embodiment: both individual cells and aggregates are mapped into non-dimensional, point objects, but including the actual embodiment makes a difference [18]. In this paper we present a computational model of Ratcliff’s et al experiments, by dealing with a simple set of assumptions that support an alternative interpretation, based on the depletion of resources that can take place inside a large cluster instead of programmed cell death. The model involves a physical, embodied implementation of cellular aggregates falling in a given medium. Our model allows to reproduce the basic experimental results and provide a computational framework to analyse alternative scenarios for the emergence of MC.

2. Methods

The experiments summarised above include a selection process obtained by suspending cells in a given medium and selecting for those displaying faster sedimentation. This approach immediately makes larger clusters of cells to be preferentially selected as in [15]. Here we examine these results under the light of a simple, embodied computational model using the NetLogo programming language, which allows to simulate Newtonian physics [19] on groups of interacting particles. Here cells are represented as objects having a given position and velocity. Cell-cell interactions are modelled by simple but physically meaningful spring-like interactions. Similarly, the interaction between cells and the fluid environment within which they move (essentially under free fall) is also introduced in a realistic manner. Additional rules related to nutrient diffusion and consumption are also introduced.

(a) Computational model

Our model considers a spatially extended description of the individuals and their interactions (figure 1a-c). For simplicity, we assume a two-dimensional spatial domain $\Gamma$. In this area, cells are described as point physical objects falling under the action of a gravitational field and interacting (figure 1b), when attached to each other, through springs (figure 1c).

The experiment starts with a population of single cells located on random positions along $\Gamma$. Cells increase in biomass through the consumption of the available nutrient to them, if a particular threshold is surpassed a cell can divide and asymmetrically split the resources between the two resulting cells. Stochastically, these two new cells can fail to separate correctly and become an aggregate, which in turn determines some of the individual properties of the cells (namely the sedimentation speed). Yeast cells are considered to have a limited number of potential attached cells due to geometrical constraints. As such, aggregates in the simulation are, in essence, Bethe lattices with $k_n$ neighbors (we consider $k_n = 4$ as the upper limit due to physical constraints). The simulated experiments include the selection protocol where, after a given time, those aggregates collected at the lower part of $\Gamma$ are used to seed back the next round of the process, to be located again randomly all over the spatial domain.

The basic components of the models presented here are cells or clusters of cells resulting from birth and death processes. At any time $t$ in a given simulation, the
Figure 2. The basic set of rules used in our model approach to the evolution experiments. The model introduces a cellular death mechanism relying in nutrient depletion. A given aggregate $A_i$, here composed by just three cells, is shown in (a). It can experience three different types of processes: cell division without (b) and with (c) an increase of aggregate size and (d) cell death from starvation. The last scenario takes place if the cell biomass of -say- the third cell $C_3i$ is below a critical threshold $\delta_c$. If the first cell, $C_1i$, has a mass larger than another threshold $M_c$ and has fewer than 4 spring-connected relatives, it will split generating an additional cell. This new cell can leave the aggregate (b) or remain attached (c) with probabilities $1 - p_{1i}$ and $p_{1i}$, respectively.

total population will be composed by a set $\mathcal{A}$ of $n(t)$ aggregates, namely

$$\mathcal{A} = \{A_1, ..., A_{n(t)}\}$$

(2.1)

Each aggregate $A_i$ is formed by a set of linked cells, i.e.

$$A_i = \{C_{1i}, C_{2i}, ..., C_{n_i,i}\}$$

(2.2)

Let us label as $|A_i|$ the size of the $i$-th aggregate. The mass of each $(i)$ cell within a given $(j)$ aggregate will be indicated as $M_{ij}$.

In our model, we will consider that cell death is a consequence of lack of enough resources in the core of the aggregate. This scenario defines a null model not incorporating a gene-regulated control of apoptosis and is thus only tied to physicochemical constrains. The key concept here is that the aggregate receives nutrients from the surrounding medium through its boundary, and passive diffusion is considered to be the main form of transport of the nutrient from the periphery of the aggregate to its core. This is of course a very simple approximation, which we keep in order to maintain the simplicity of the model and its interpretation.

To take into account this process, cells have an energy value $M_{ij}$ as well as common a division threshold $M_c$. Nutrient concentration change in the finite element $\phi_{ij}$ is given by a diffusion model represented by means of a partial differential equation:

$$\frac{\partial \phi_{ij}}{\partial t} = D_\phi \nabla^2 \phi_{ij} - \rho \Theta_{ij} \phi_{ij}$$

(2.3)
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Figure 3. The pattern of diffusion of nutrients associated and a growing aggregate. Here (a-f) six spatial snapshots are shown at six different times within the in silico selection experiment. The nutrient field appears as a continuously shaded gradient, lighter areas indicating lower concentration levels. As expected, the concentration is highly depleted inside the aggregate, eventually causing the death of cells if nutrient intake goes below a threshold level. Once cells inside the cluster die out, it breaks into several aggregates. A different view of the nutrient field of snapshot f is given in (g) where we display the concentration values close to a large aggregate. The simulation times in algorithm cycles for the snapshots are: 500, 600, 700, 800, 900 and 1000. The parameter’s values used in this simulation are: $D_\phi = 0.2$, $\rho = 0.1$, $\beta_c = 0.01$, $M_c = 100$, $\kappa = 0.1$, $\delta_c = 30$, initial nutrient concentration $\phi_0 = 22.5$ and a non-evolving adhesion probability $p = 1$.

The Heaviside function $\Theta_{ij}$ is used to indicate the presence or absence of cells in that particular patch of the lattice (so we have $\Theta_{ij} = 1$ if a cell is present and zero otherwise). Here the diffusion operator $\nabla^2\phi_{ij}$ is numerically computed (using the NetLogo libraries) by means of a standard discretization form:

$$D_\phi \nabla^2\phi_{ij} = D_\phi \left[ \phi_{ij} - \frac{1}{4} \sum_{kl} \phi_{kl} \right]$$  \hspace{1cm} (2.4)

Where $D_\phi$ accounts for the diffusion coefficient and $\rho$ the intake of nutrients from the culture medium. The energy change for $i$-th cell in the $j$-th aggregate is:

$$\frac{\partial M_{ij}}{\partial t} = \rho \phi_{ij} - \beta_c M_{ij} (1 + \kappa \Delta_{ij})$$  \hspace{1cm} (2.5)

Where $\beta_c$ represents the maintenance costs and $\Delta_{ij}$, which accounts for the number of divisions this particular cell has undergone, is used in order to introduce an aging effect, causing cells to spread more their following divisions. If the energy value of a particular cell reaches its division threshold, a new cell is created and the original energy value is split asymmetrically between the cells. Conversely, cells die if the threshold condition:

$$M_{ij} \leq \delta_c$$

is met. Where $\delta_c$ is a lower bound that cells can withstand. In figure 3 we show an example of how the nutrient field is depleted close by the growing aggregate. Six snapshots are displayed, with increasing cell numbers and decreasing nutrient content.
levels as we move towards the centre of the aggregate, as expected. We can also appreciate the fragmentation process resulting from cell death inside the aggregate (e-f).

(b) Mutation

Little is known about the genetic changes behind the establishment of the snowflake phenotype reported in Ratcliff et al. Whether it involved extensive rewiring of basic adhesion toolkit genes or slight tuning of interactions in gene networks we do not know, but experiments involving different sedimentation times clearly show that correct separation between cells is not a binary, all or nothing, process.

In order to make the less assumptions about the genetic changes taking place in Ratcliff et al., our model enables evolution of only one cell parameter: \( p_{ij} \), which stands for the probability of remaining attached to the offspring in the event of a division and condenses the effect of multiple genes related to adhesion mutating independently. As such, \( p_{ij} \) is a continuous variable constrained between zero and one. This parameter is inherited by daughter cells with very small variations.

(c) Selection process

In Ratcliff’s et al. paper, the researchers made use of gravity as the external force facilitating the differential deposition of cell aggregates [15]. Physically this corresponds to a simple property of increasingly large objects falling within a fluid medium with a given friction and a fixed gravity field. In our model, we have used a simplified sedimentation process in which the speed in the vertical axis was proportional to the square root of the aggregate size divided by the projected diameter of the aggregate. A flat noise term was added to the speed of both axis, and so, the movement of the cells effectively behaves like a biased random-walk.

At the beginning of each simulation a set of cells was created with random positions in the virtual space and fell until a fixed number simulation cycles had passed, the aforementioned settling time. After a given transient time, clusters located at the bottom, below a given critical height \( h_c \), were uplifted to new random positions leaving intact their history and traits. Moreover, the virtual medium was refreshed to a homogeneous nutrient level.

3. Results

Several traits of the multicellular aggregates emerging through the simulation can be measured with the experimental results discussed above. In our study we have followed both average values of aggregate size over generations as well as those selected traits (such as cell-cell adhesion) favouring the selection process towards larger aggregates. We can estimate the probability of finding aggregates of a given size \( |A_i| \), given by:

\[
P(|A_i|; t) = \frac{N(|A_i|; t)}{\sum_{\mu=1}^{M} N(|A_\mu|; t)}
\]

(3.1)
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Figure 4. (a) Time evolution of average aggregate sizes in the diffusion model, for a nutrient concentration $\phi_0 = 17.5$. All values shown are averages of 10 replicate experiments and the shaded area represents one standard deviation of the dataset. In (b, c) we show two snapshots of the simulation, obtained at the end of passes $T = 31$ and 78, respectively (which correspond to attachment probabilities of $\langle p \rangle = 0.25$ and $\langle p \rangle = 0.75$, respectively). In (d) we also show the size distribution (obtained from $10^4$ aggregates) for the second time step indicated by the arrow in (a), both in the selected and non-selected area (red and black respectively). The dashed line in (b, c) is a visual reference marking the boundary of the selection/non-selection regions.

In figure 4a we display the evolution of the mean aggregate size as a function of time, calculated from:

$$\langle |A|(t) \rangle = \frac{\sum_{\mu=1}^{M} |A_\mu| N(|A_\mu|; t)}{\sum_{\mu=1}^{M} N(|A_\mu|; t)}$$

We can appreciate a logistic-like growth pattern, thus exhibiting an attrition after a given number of steps. The standard deviation is also displayed. Diffusion driven death is heavily dependent on nutrient concentration at the start, increasing or decreasing such value has a considerable impact on average aggregate size. Two snapshots of the aggregate spatial distribution at $\langle p \rangle = 0.25$ (b) and $\langle p \rangle = 0.75$ (c) are shown. In figure 4d we also display the size distribution of aggregate sizes above (black) and below (red) the critical height $h_c$. It is possible to appreciate the progressive displacement towards higher aggregates as a result of the selection process.

A specially relevant result seems to support our view. In Ratcliff’s paper, it was shown that a highly nonlinear correlation exists between the size of the aggregate and the fraction of cells undergoing death within them (figure 5, inset). In a nutshell, what is observed is that little death is found under a given aggregate size whereas it rapidly grows once we cross this threshold. However, the same nonlinearity is obtained in our evolution model, as shown in figure 5 (main plot), where we display the statistics of cell death against the size of the cell aggregates. A nonlinear relationship is also found in our model, which is due to the nonlinearities associated to the thresholds of survival as well as the nonlinear relationships due to the geometric constraints imposed by our system. This nonlinear behavior can be explained by considering the interactions between cell populations within aggregates and the nutrient field. The situation considered here is close to the one
associated with avascular solid tumours, also formed by a mass of cells in contact with an external medium [20]. As we move towards the aggregate center, the decline in available nutrients and/or oxygen leads to necrosis resulting from cell death. In these spherical tumours, the core is simply an internal domain surrounded by the proliferative shell. In our context, due to the very small size of the aggregates, loss of cells in the internal core result in aggregate disintegration.

Using this approximation, we can consider aggregates as circular masses of a given radius $R$ that grow over time. If we indicate by $\phi(r)$ the spatial concentration of nutrient at a given distance $r$ from the aggregate center, a diffusion equation can be established to describe its evolution and steady states. For simplicity, we consider that the aggregate size $A(t)$ changes slowly compared to the nutrient dynamics. Assuming spherical symmetry, the qualitative dynamics can be described by a diffusion equation for the nutrient field:

$$\frac{\partial \phi}{\partial t} = -\eta \phi + D_\phi \nabla^2 \phi - \Gamma$$ (3.3)

The terms in the left-hand side correspond to (i) nutrient decay, (ii) diffusion and (iii) a chemical consumption term linked to the use made by cells of the nutrient. If we assume radial symmetry, the previous equation simply reads:

$$\frac{\partial \phi}{\partial t} = -\eta \phi + \frac{D_\phi}{r^2} \frac{\partial}{\partial r} \left( \frac{\partial^2 \phi}{\partial r^2} \right) - \Gamma$$ (3.4)

If $\phi_0$ defines the nutrient concentration outside the cell mass, it can be shown [20] that the radially symmetric solution of the previous equation, assuming that nutrient depletion is uniform, is:

$$\phi(r) = -\frac{1}{6} k D_\phi \left( R^2 - r^2 \right) + \phi_0$$ (3.5)

Cell death will occur if the radius is large enough so that a necrotic zone appears. This will occur provided that no nutrients are available and thus $\phi(r) = 0$. This situation appears for the first time when $\phi(0) = 0$. Using the last equation, this leads to find the critical aggregate size, associated to a radius $R_c$ given by:

$$R_c = \left( \frac{6D_\phi \phi_0}{\mu} \right)^{1/2}$$ (3.6)

with $R$ being the aggregate radius. This equation is valid for $r \leq R$. Beyond this point, we would have $\phi(r) = \phi_0$.

The presence of this threshold value can explain the jump in death rates observed in our previous figure. If we call $\delta_0$ a basal death rate and by $\delta_n$ the one due to lack of nutrients or oxygen, we can write an approximate death rate $\pi_d$ for an aggregate of radius $R$ as follows:

$$\pi_d \sim \delta_0 + \left( \frac{\pi r_c^2}{\pi R^2} \right) \delta_n = \delta_0 + \left( 1 - \frac{R_c}{R} \right)^2 \delta_n$$ (3.7)

for $R \geq R_c$ and $\pi_d \sim \delta_0$ otherwise. This probability of death sharply increases once we cross $R_c$, as the subset of cells in the nutrient depleted zone increases.

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Figure 5. Nonlinear relation between death rates and aggregate size. Our diffusion-driven model predicts a slow increase in death rates (here measured as the probability of cell death within an aggregate) up to certain aggregate size, from which death rapidly increases. Such a nonlinear relation was also found in the experiments (inset, adapted from Ratcliff et al 2011) and considered as evidence for a selection process for programmed cell death. However (main plot) the diffusion-limited model discussed here predicts a very similar outcome, with low death rates below a given aggregate size and a sharp increase beyond that threshold.

A final point to be made concerns how to test the diffusion model experimentally. In order to provide a source of validation, we have performed additional evolution experiments using different amounts of nutrients, which allow larger clusters to develop, as shown in figure 6. As we can see, the average cluster size grows rapidly with nutrient concentration. The correlation between both seems to follow a power law, as indicated in figure 6. Specifically, we have found that the average |A| scales with n as follows:

\[ |A| \sim n^\gamma \]  

with \( \gamma = 1.84 \pm 0.04 \). This scaling relation could be easily tested by replicating Ratcliff’s experiment with different medium conditions.

4. Discussion

Unraveling the mechanisms responsible for the emergence of multicellular life forms out from single-cell systems represents a major challenge for our understanding of biological complexity. The traditional approach to this problem was based either in data-driven, experimental and phylogenetic analysis or in mathematical and computer models of simple cell-like units and their emerging interactions. The experimental work described in [21] provides a novel way of addressing this problem through a simple and elegant design of a selection-driven experimental setup. Despite the differences existing between wild and laboratory microorganisms [22] we can safely conjecture that the mechanisms responsible for generating and disaggregating cell clusters should be universal.

Although the experimental results suggested an interpretation of the evolutionary dynamics that could be interpreted in terms of an evolved apoptotic, regulated response, the results reported here suggest a simpler, alternative interpretation in terms of a diffusion-limited process of aggregate growth where the cluster of cells
keeps growing provided that enough nutrient levels are available. Once its size is large enough, cells occupying the inner layers of the aggregate can rapidly fail to receive the minimal nourishment compatible with cell survival. Cell death is a result of starvation and stress, and not the outcome of programmed signals. This scenario can be tested, we believe, by repeating the same experiments under variable conditions of nourishment. We predict that the resulting average aggregates will rapidly increase with nutrient levels, following a power law.

Our interpretation does not diminish the relevance and implications of the experimental evolution experiments. On the contrary, we think that our interpretation suggests a potentially interesting framework concerning the steps followed by primitive aggregates predating the first multicellular life forms. Aggregates breaking up due to internal cell death through starvation result form a mechanism of splitting that clearly goes beyond the single-cell level, but is based in physical (or physico-chemical) constraints instead of actively operating regulatory mechanisms. Such role played by physics over the cell’s molecular machinery is consistent with a view of evolving multicellularity based on an early dominance of physical mechanisms over genetic ones [23,24].

Our model provides a simple computational framework that can be expanded in different ways. It also provides a useful system to design new forms of evolving multicellular aggregates. In this context, an interesting avenue can be considered here involving the use of synthetic biology, where specific engineered circuits for population size control or programmed cell death have been designed using microbial models. As a result of such work, it is fair to talk about to design cell-cell interactions in order to provide new, controlled scenarios of multicellular evolution [25]. In this context, we could take advantage of new engineered forms of cellular
aggregation that can then be evolved over time. Such synthetic multicellular approach will offer a whole pathway of inquiry into the problem of how complex life might evolve or how we can evolve them.

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