Modeling the Role of the Cell Cycle in Regulating \textit{Proteus mirabilis} Swarm-Colony Development

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SMU Math Report 2005-03

DEPARTMENT OF MATHEMATICS
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**Abstract**

We present models and computational results which indicate that the spatial and temporal regularity seen in *Proteus mirabilis* swarm-colony development is largely an expression of a sharp age of dedifferentiation in the cell cycle from motile swarmer cells to immotile dividing cells (also called swimmer or vegetative cells.) This contrasts strongly with reaction-diffusion models of *Proteus* behavior that ignore or average out the age structure of the cell population and instead use only density-dependent mechanisms. We argue the necessity of retaining the explicit age structure, and suggest experiments that may help determine the underlying mechanisms empirically. Consequently, we advocate *Proteus* as a model organism for a multiscale understanding of how and to what extent the life cycle of individual cells affects the macroscopic behavior of a biological system.

**Key words:** *Proteus mirabilis*, swarm colony, age structure, space structure, partial differential equations, cell cycle.

1 **Introduction**

We present structured multiscale models and computational results of *Proteus mirabilis* swarm-colony development which indicate that the spatial and temporal regularity of the colony development is largely due to the regularity of the cell cycle, namely a sharp age of dedifferentiation from motile swarmer cells with a multinuclear structure to single-nucleus dividing cells (also referred to as swimmer cells or vegetative cells elsewhere in the literature). We refer to our models as “structured multiscale” because they link the cellular scale to the...
colony scale, and represent the age dynamics of individual cells using a population structure variable for age and an explicit aging term in the differential equation.

This explanatory mechanism precludes the use of reaction-diffusion models that either average over the age variable, such as the models by Medvedev, Kaper, and Kopell [21], or ignore the age structure from the outset, such as the models by Czirók, Matsushita, and Vicsek [7].

The necessity of explicitly retaining the cell cycle through an age variable, rather than using density-dependent processes alone, makes *Proteus* an ideal organism for a multiscale understanding of how the kinetics at the individual cell level determines the macroscopic behavior of a biological system.

The models in this paper are based upon those presented in [2]. The previous models assumed a sharp age of dedifferentiation between motile swarmer cells and immotile dividing cells. This was incorporated into the model using an upper bound on the age domain and then assuming that the swarmer-cell population density was zero above this maximum age.

The models in [2] used the critical insight of Esipov and Shapiro in [10] that the age dynamics of individual swarmer cells underly the observed colony regularity and should be incorporated into the model explicitly, using a population structure variable in the partial differential equation for the swarmer-cell population. The models in [2] differed substantially from those in [10] by using a simpler diffusivity which depended only on the swarmer-cell density rather than an elaborate and unnecessary memory field – one that proved to be insufficient in generating the desired colony behavior when accurate numerical methods were applied to the model equations. The models in [2] also incorporated the known mechanism of a density-dependent lag in differentiation between dividing cells and swarmer cells. Other differences included the use of a radially-symmetric geometry rather than a linear geometry, diffusion based on isotropic random motion rather than Fickian (symmetric) diffusion, and
accurate numerical methods. The models in [10], when solved accurately, can generate a
regular temporal cycle and contain a control of the ratio of swarm time to consolidation time,
but are unable to generate the regularity of the spatial structure, namely the bulls-eye pattern
with equally spaced concentric terraces. An appendix in [2] contains a detailed discussion
and computations of the Esipov-Shapiro model, and the manuscript as a whole address in
more detail than here the differences between the two modeling approaches. The focus in
this paper is the need to represent the age structure of the swarmer-cell population explicitly,
rather than being able to use a reaction-diffusion framework to obtain an understanding of
the mechanisms that underly the observed macroscopic behavior of Proteus.

This paper is organized as follows. We provide a brief discussion of Proteus biology,
followed by a section going into more detail on the critical differences and implications
between the structured multiscale approach used in this paper and the reaction-diffusion
models used elsewhere. We state the model equations and their biological meaning. We
then discuss the numerical methodology used to solve the equations, and present the results
along with a discussion. We close with our conclusions and suggestions how the validity of
the underlying assumptions of the structured multiscale models and the reaction-diffusion
models can be determined empirically.

2 Proteus mirabilis Swarm-Colony Development

When inoculated onto an agar surface, Proteus begin formation of strikingly regular spatio-
temporal patterns that begin with three initial phases: a lag phase, a first swarming phase,
and a first consolidation phase; these form what appear to be the center circle and first
ring of a bulls-eye. This is followed by repeating cycles of ring, or terrace, formation that
consist of a swarming phase followed by a consolidation phase. These subsequent terraces
are equal in width and time of formation. Perhaps most interestingly, the time of terrace
formation is invariant under changes in the glucose or agar concentration of the substrate, although changes in agar concentration do affect the ratio of time spent swarming versus consolidating, and changes in glucose increase terrace width. Terrace formation does vary by temperature [22].

Varying glucose concentration greatly alters biomass production without strongly affecting the spatial or temporal aspects of the swarm and the consolidation cycle of terrace formation. Changes in agar concentration do not change the total time of terrace formation, but higher agar concentrations shorten the time spent swarming and the width of the terraces, and lengthen the time spent in consolidation [10].

A short summary of the physiology of Proteus cells can be found in a section similar to this one in [2], and in more detail in [22, 26]. The critical aspect for understanding the macroscopic behavior is the transition between the two cell types: mononuclear cells that are motile in a liquid environment and immotile on an agar surface, called “dividing” cells in this paper and “swimmer” or “vegetative” cells elsewhere in the literature; and multinuclear filament cells which possess longer flagella and are able to move on an agar surface by grouping together with other swarmer cells in what are called “rafts”. The process in which dividing cells become swarmer cells is called “differentiation.” Differentiation only occurs above one dividing-cells density and below another.

When the multinuclear swarmer cells approach a maximum size, they rapidly break down into mononuclear dividing cells. This process is called “dedifferentiation.” Since mitosis results in exponential growth, the size of a swarmer cell is an exponential weight times its age. Thus, dedifferentiation occurs when swarmers reach a maximum age.

We suggest that the differentiation-dedifferentiation cycle of Proteus cells, including a density-lag before differentiation occurs and a sharp age near which dedifferentiation occurs, is a critical mechanism for generating the macroscopic colony behavior, and thus density-
dependent mechanisms alone are insufficient.

A deeper theoretical and empirical understanding of how deviations from this cell cycle affect the macroscopic behavior is needed to resolve the issue. The models presented in this paper aim to advance the theoretical understanding, and experiments, perhaps similar to those by Hay, Tipper, Gygi, and Hughes [14] where a mutated strain of *Proteus* was unable to swarm due to a lack of sufficiently long flagella, can provide a more sound empirical basis for the models.

3 Critical Differences with Reaction-Diffusion Models

In this section we discuss the critical difference between the structured multiscale models in this paper and reaction-diffusion models of *Proteus* swarming. We focus on the work by Czirók, Matsushita, and Vicsek [7]. This work makes explicit claims of the sufficiency of a reaction-diffusion framework, utilizing only density-dependent mechanisms, to answer all questions of interest concerning *Proteus* swarm-colony development. We direct less attention to the paper by Medvedev, Kaper, and Kopell [21] because their work focused more on the interesting mathematical phenomenon of a reaction-diffusion equation with periodic front dynamics and did not make such claims about fully understanding the biology of interest. The Medvedev, Kaper, and Kopell [21] paper was discussed in more detail in [2].

The models in [7] consist of a reaction-diffusion equation for the swarmer cells and an ordinary differential equation at each point in space for the “vegetative” cells. The term “vegetative” cells was initially used in [7] with the same meaning as “dividing” cells in this paper, and later used to mean dividing cells and swarmer cells which are unable to contribute to swarming. The term “consolidation” has generally been used in the literature to mean the macroscopic-scale colony phase when the front is not moving and the newly formed terrace is being built up. Czirók, Matsushita, and Vicsek use the term to mean the cellular-scale
process of “dedifferentiation”. This use is confusing given the differences in scale, and that dedifferentiation involves the breakup of multinuclear filament cells into several mononuclear dividing cells.

The models in [7] use a minimum and maximum population density of dividing cells for which differentiation into swarmer cells can occur. The upper limit has been used in all models of *Proteus* swarm-colony development we are aware of to date [2, 7, 10, 21]. Medvedev, Kaper, and Kopell first saw the importance of the density lag in differentiation from dividing to swarmer cells in the biological literature [26] and incorporated it into a model. The lower limit is used here and in [2, 7], but not in [10]. One difference between the results presented here and in [2] and those in [7] is that Czirók, Matsushita, and Vicsek get a loss of regularity in their results if the density window for swarmer cell production becomes too wide. This loss of regularity takes the form of the consolidation phase becoming shorter after each cycle. In the models here and in [2], the dividing-cell population density is scaled by the density window for swarmer cell production in the nondimensionalization. The nondimensionalization as described in [2] shows why this is natural. Instead we vary the center of the interval, denoted by $v_c$. As $v_c$ increases, so does total cycle time and consolidation time, while swarm time remains invariant. Although we can remove the consolidation phase by changing $v_c$, the result of each simulation retains the temporal and spatial regularity.

The diffusivity used in [7] is simpler than those used in [10] or [21], but is more complicated than the diffusivity used here and in [2].

The models in this paper extend those developed in [2] to examine the necessity of a sharp age of dedifferentiation from swarmer to dividing cells. The critical difference between the models in this paper and in [2] and those in [7] is the mechanism of dedifferentiation. We argue that the assumptions underlying the models are mutually exclusive. The models in this paper require that dedifferentiation occur almost exclusively near a critical age. The
models in [7] use a constant rate of dedifferentiation.

The important differences and commonalities between the age explicit frameworks used here and those of Esipov and Shapiro [10] were discussed in much detail in [2]. A crucial difference relevant to this discussion is that we dispense with a memory field in the diffusion, which constitutes a built in hysteresis. Unfortunately, Czirók, Matsushita, and Vicsek [7] focus on the memory field, rather than the critical insight of Esipov and Shapiro of including the cell cycle in the model, in their discussion of [10]. The major deficiencies of the models and computations in [10] are covered in much detail in [2]. However, in the context of [7], this paper and [2] constitute a defense of the need to explicitly represent age and the cell cycle in a model of *Proteus* swarm-colony development.

The models in [2] reproduce three major aspects of *Proteus* swarm-colony development: temporal regularity of overall cycle time, spatial regularity, and control of the ratio of swarm time to consolidation time. By retaining the explicit age structure in the mathematics, the models reproduce the colony behavior by relying on two main mechanisms: a density-lag in differentiation from dividing cells to swarmer cells, and a sharp age of dedifferentiation from swarmer cells to dividing cells. The reaction-diffusion models of both in Czirók, Matsushita, and Vicsek [7] and Medvedev, Kaper, and Kopell [21] reproduce only the temporal regularity of the overall cycle time and the spatial regularity. No attempt is made to address the ratio of swarm time to consolidation time. Given that this is controlled in the models in [2] by an age threshold, it is not clear how such a control can be inserted into one of the reaction-diffusion modeling frameworks. However, we concede that a control within a reaction-diffusion framework is within the realm of the possible.

Instead we argue the necessity of including the cell cycle explicitly by showing that moving from a sharp age of dedifferentiation to a broader distribution of dedifferentiation ages causes a breakdown in regularity, which would exclude density-dependent mechanisms
alone from explaining *Proteus* swarm-colony and indicate the need to include age explicitly. The models would be verified if experiments with strains of *Proteus* which do not have a sharp age of dedifferentiation give rise to irregular colony formation.

4 The Models

The structured multiscale models in this paper are based on a mathematical framework with some history behind it. At the colony or population scale, Skellam [24] considered the effects of diffusion in his classic work of 1951. At the individual scale, Sharpe and Lotka in 1911 and McKendrick in 1926 considered population models with linear age structure [25]. More recently, Gurtin and MacCamy [12] considered models with nonlinear age structure. Rotenberg [23] and Gurtin [11] posed models dependent on both age and space. Gurtin and MacCamy [13] differentiated between two kinds of diffusion in these models: diffusion due to random dispersal, and diffusion toward an area of less crowding. Existence and uniqueness results can be found for various forms of these models in Busenberg and Iannelli [4], di Blasio [8], di Blasio and Lamberti [9], Langlais [17], and MacCamy [19]. Further analysis has been done by several authors [15, 16, 18, 20].

We present in this section dimensionless equations, variables, and parameters. We assume the colony is radially symmetric and scale space so that radius goes from zero to one. We scale time and age by the time it takes a cell to subdivide (typically 1.5 hours.) A complete discussion of the nondimensionalization is contained in [2]. The dimensionless variables \( r, a, \) and \( t \) represent radius in two-dimensional space, age, and time, respectively. The function \( u(r, a, t) \) represents the swarmer-cell population density at radius \( r \), age \( a \), and time \( t \). The functions \( p(r, t) \) and \( v(r, t) \) represent the biomass density of swarmer cells and the dividing-cell population density, respectively, at radius \( r \) and time \( t \).
The nondimensional model consists of the age-structured nonlinear diffusion system

\[
\begin{align*}
\partial_t u + \partial_a u &= \frac{1}{r} \partial_r \left( r \partial_r (D(p) u) \right) - \mu(a) u, \quad 0 \leq r < 1, \ a > 0, \ t > 0, \\
\partial_t v &= (1 - \xi(v)) v + \int_0^\infty \mu(a) u e^a \, da, \quad 0 \leq r \leq 1, \ t > 0, \\
u(r, 0, t) &= \xi(v) v(r, t), \quad 0 \leq r \leq 1, \ t > 0
\end{align*}
\]

with boundary and initial conditions

\[
\begin{align*}
\partial_r \left( D(p(1, t)) u(1, a, t) \right) &= 0, \quad a > 0, \ t > 0, \\
u(r, a, 0) &= 0, \quad 0 \leq r \leq 1, \ a \geq 0, \\
v(r, 0) &= v_0(r), \quad 0 \leq r \leq 1.
\end{align*}
\]

The total motile swarmer cell biomass is given by

\[
p(r, t) = \int_{a_{\text{min}}}^\infty u(r, a, t) e^a \, da, \quad 0 \leq r \leq 1, \ t \geq 0.
\]

Proteus move through a process of raft building that requires two things: sufficient maturity in swarmer cells to contribute to raft building, and a sufficient biomass of mature cells to form the rafts. The lower limit of integration, \(a_{\text{min}}\), is the minimum age when a swarmer cell is sufficiently large, with sufficiently long flagella, to contribute to motion on the agar surface. This parameter controls the ratio of swarm time to consolidation time without changing the total time of ring formation. The parameter \(a_{\text{min}}\) is related to agar concentration. The higher the concentration, the drier the surface, raising the value of \(a_{\text{min}}\). Higher agar concentration, and thus higher \(a_{\text{min}}\), shortens the swarming phase and lengthens the consolidation phase in terrace formation.

The diffusivity has the form

\[
D(r, t) = D_0 \max \left\{ (p(r, t) - p_{\text{min}}), 0 \right\}.
\]
The parameter $p_{\text{min}}$ is the minimum biomass needed for swarvers to build rafts capable of moving on the agar surface.

We use a differentiation function with a lag phase that is a $C^1$ piecewise cubic with support of length 2:

$$
\xi(v) = \begin{cases} 
\xi_0 (2|v-v_c|^3 - 3(v-v_c)^2 + 1), & |v-v_c| \leq 1, \\
0, & \text{otherwise},
\end{cases} (4.1i)
$$

The interval $[v_c - 1, v_c + 1]$ is the swarmer-cell production window. The interval width of 2 was obtained in the nondimensionalization. The parameter $v_c$ represents the lag phase in swarmer-cell production [21]. We use a compactly supported cubic function because it is smooth. The important aspects of the functional form of $\xi$ are that it is zero above and below certain thresholds. An appendix in [2] shows that the nature of the results do not depend strongly on the shape of the curve. The shape of the curve does help with numerical issues concerning the degenerate diffusion. The parameter $v_c$ has no analog in [10].

We take the initial condition to be

$$
v_0(r) = \begin{cases} 
v_0 \left( 2 \left( \frac{r}{r_0} \right)^3 - 3 \left( \frac{r}{r_0} \right)^2 + 1 \right), & 0 \leq r \leq r_0, \\
0, & r > r_0.
\end{cases} (4.1j)
$$

Again, it is mass, not shape, that matters qualitatively. Shape affects numerical efficiency.

The major difference in the models in this paper and those in [2] is the dedifferentiation modulus, $\mu(a)$, and the resulting behavior of the system to changes in dedifferentiation from swarmer to dividing cells. In [2], we used a form that was the sum of a Heaviside function and a Gaussian distribution so that in the limit of the spread parameter $\sigma \to 0$ it would be transparent that we obtain a model with no explicit function $\mu$ but could instead represent the sharp age of dedifferentiation by an upper limit in the age domain. Only the limiting case was treated in [2].

Our goal in this paper is to show the necessity of retaining explicit space structure and a sharp age of dedifferentiation. Consequently, we examine the change in swarm-colony
regularity as dedifferentiation become less sharp. We use a function with similar shape but a simpler expression than the dedifferentiation modulus used in [2],

\[ \mu(a) = \frac{\mu_0}{\sigma} \left( \tanh \left( \frac{a - a_{\text{max}}}{\sigma} \right) + 1 \right), \]

where \( \sigma \) is the spread parameter and \( \mu_0 \) is a height parameter. This function is equivalent to a Fermi distribution. For a fixed \( \mu_0 \) and taking the limit \( \sigma \to 0 \), we get a situation where swarmer cells dedifferentiate into their component dividing cells all at the same age, \( a_{\text{max}} \). For \( \sigma \) small but not equal to zero, this dedifferentiation modulus represents a situation where the probability of dedifferentiation is low for young swarmers, increases rapidly as they approach \( a_{\text{max}} \) in age, and remains high afterwords. What is important is the rapid change in the dedifferentiation rate. Simulations carried out with a piecewise continuous linear function \( \mu \), that is zero below \( a_{\text{max}} - \sigma \), \( \mu_0 \) above \( a_{\text{max}} + \sigma \), and increases linearly in between, show no qualitative and minor quantitative differences with the form in equation (4.1k).

It may be unclear at first why the magnitude coefficient of \( \mu \) (the ratio \( \mu_0/\sigma \)) should increase as the hyperbolic tangent approaches a step function. The intent is to model the effect on colony behavior of moving away or toward a sharp age of dedifferentiation in the model. We show that if we keep \( \mu_0/\sigma \) fixed as we vary \( \sigma \), we converge to something other than a sharp age of dedifferentiation as \( \sigma \to 0 \). The probability that a swarmer cell has not dedifferentiated by age \( a \) is given by

\[ \Pi(a) = \exp \left( - \int_0^a \mu(\omega) \, d\omega \right) \quad (4.2) \]

(see pg. 82 of [3].) Using the specific form of \( \mu \) in equation (4.1k), we obtain

\[ \Pi(a) = \exp \left( \frac{-\mu_0 a}{\sigma} \right) \left( \frac{\cosh \frac{a - a_{\text{max}}}{\sigma}}{\cosh \frac{-a_{\text{max}}}{\sigma}} \right)^{-\mu_0} \quad (4.3) \]
Figure 1: The probability $\Pi(a)$ that a swarmer cell has not dedifferentiated by age $a$. In figure 1(a) $\mu_0 = 1.0$ in equation (4.3). In figure 1(b) $\mu_0 = \sigma$ in equation (4.3). These figures illustrate the need to increase the magnitude of the dedifferentiation modulus to approximate a sharp age of dedifferentiation from swarmer cell to dividing cell in the models.

Figure 1 illustrates how keeping $\mu_0$ fixed as $\sigma \to 0$ yields convergence of $\Pi$ to a step function, whereas keeping $\mu_0/\sigma$ fixed gives convergence to a function with decay to zero rather than a discontinuity.

The correct way to represent a sharp age of dedifferentiation has proven to be nontrivial. Models in [10] (specifically “Model A” as defined on pp. 252-253) conflate the desired probability density function (pdf) for dedifferentiation ages (a Dirac delta function centered at the sharp age of dedifferentiation) with the dedifferentiation modulus. As can be seen by setting $\mu(\omega) = \delta(\omega - a_{\text{max}})$ in equation 4.2 a Dirac delta function as the dedifferentiation modulus would give rise to a situation where there is no dedifferentiation until $a_{\text{max}}$, at which point the population is reduced by a factor of $1/e$. This remaining swarmer-cell population
never dedifferentiates, and continues to age (and hence grow), resulting in no consolidation period whatsoever.

Esipov and Shapiro [10] attempted to examine the importance of a sharp age of dedifferentiation by treating two models, “Model A” with dedifferentiation as mentioned above, and a “Model B” with a constant dedifferentiation modulus. As discussed in [2], “Model A”, when solved accurately, does not reproduce the observed *Proteus* colony behavior, which limits the utility of that treatment. Just as important, only considering the extreme cases leaves open several questions. All swarmer cells do not dedifferentiate at precisely the same age; this is a construct useful for modeling. Does even a minor deviation from a sharp age of dedifferentiation lead to colony irregularity? If so, this would invalidate the model. Also left open is the nature of the transition from regularity to irregularity of colony formation. We attempt to answer these question using mechanisms in our dedifferentiation function absent from those in [10].

5 Computation and Results

In this section we briefly describe the computational methods used to obtain our solutions and then present those solutions with their biological interpretations.

The computational methods and software used in this paper are essentially those used to solve the model equations in [2]. The main exception is the use of a modulus, $\mu$, rather than a finite age domain truncated at the septation age, to model dedifferentiation from swarmer to dividing cells. This required changes in how information was passed from the dividing-cell equation to the swarmer-cell equation. Previously, the population of cells undergoing dedifferentiation could be determined from the oldest age cohort at each point in space at the end of the time step and passed to the dividing-cell equation at the start of the next time step. For the models in this paper, the population of dedifferentiating swarmer cells
had to be computed across all age cohorts at each point in space and then aggregated for passing to the dividing-cell equation.

A more detailed description of the numerical methodology as specifically applied to Proteus can be found in [2], and the original formulations and analyses can be found in [1, 3, 4]. To summarize, the software uses a moving-grid Galerkin method in the age variable to decouple age from time, which results in a degenerate nonlinear parabolic partial differential equations in space and time for each age cohort. The parabolic system is then solved using a step-doubling extrapolation method [4].

We emphasize the importance of reliable numerics. In the systems presented in this paper and in [2], the regularity in terrace formation is due to the regularity in the aging of the swarmer cells. A coarse, stage-structured numerical approximation to the continuous aging term, such as that used in [10], can constitute a qualitatively different swarmer-cell life cycle than that of the original continuous model. Also, the degenerate diffusion can be altered by the choice of the time step. The numerical computation may then show periodic front dynamics that are not obtained by an accurate solution of the original continuous equations, but are induced by a regularity in the numerics. An appendix in [2] details how accurate computations change the behavior of the Esipov-Shapiro system from what is presented in [10].

A uniform spatial discretization of size $\Delta x = 1/300$, and a uniform age discretization of $\Delta a = 1/40$, using piecewise constant basis functions, was sufficient to solve the system within a relative error in the $L^2$-norm of less than 1%. A uniform age discretization in the context of a moving grid method means that all but the first and last age intervals are constant in length, and that a new age interval is introduced at the birth boundary when the old birth interval reaches $\Delta a$ in length. Convergence in time was obtained by adjusting a tolerance parameter for the adaptive time-stepping in the step-doubling algorithm so that the relative
error in the $L^2$-norm was also less than 1%.

The goal of this paper is to model the effects of a non-sharp dedifferentiation age on colony regularity. Consequently, we focus on the behavior of the system defined by equations (4.1a)-(4.1k) as we vary $\mu_0$ and $\sigma$. For the computations in this paper, we set $a_{\text{max}} = 2.67$, $v_c = 8.0$, $D_0 = 2 \times 10^{-3}$, $p_{\text{min}} = 0.5$, $\xi_0 = 0.5$, and $a_{\text{min}} = 0$.

The response of the system to changes in these six parameters was investigated in detail in [2] (we have dropped the “hat” or capital notation in this paper, which was used in [2] to denote the nondimensional version of a variable or parameter.) In summary, $a_{\text{max}}$ is the nondimensional sharp age of dedifferentiation (in this paper its meaning changes to become the “center” of a mollified step function.) As $a_{\text{max}}$ increases, so does the time spent swarming, the terrace width, and the overall cycle time. The time spent in consolidation decreases. The parameter $v_c$ is the nondimensionalized lag in swarmer cell production, $\xi$. As $v_c$ increases, consolidation time, total cycle time, and terrace width increase, where as swarming time remains nearly invariant. The constant of diffusion, $D_0$, represents changes in glucose concentration and has a significant effect only on terrace width. The parameter $\xi_0$ is the constant of the differentiation ratio $\xi$, and lies between zero and one. As $\xi_0$ increases, so does swarming time and terrace width, whereas total cycle time and consolidation time decrease.

The parameter $a_{\text{min}}$ is the minimum age at which a swarmer cell can contribute to raft building and movement and controls the ratio of swarm time to consolidation time within a cycle. It only has meaning in the context of an age-explicit model. As agar concentration increases, so does the difficulty of moving on the substrate, which is represented by increasing $a_{\text{min}}$. As $a_{\text{min}}$ increases, swarming time and terrace width decrease, consolidation time increases, and total cycle time remains the same.

The parameter $p_{\text{min}}$ is the minimum swarmer biomass needed for raft formation and the
onset of swarming. It is thus measured in the same units as population density, and differs in an important respect from $a_{\text{min}}$, which is measure in the same units as age. This difference is worth noting given that we argue the necessity of including both density-dependent processes and the cell cycle in a model, rather than relying on density-dependent mechanisms alone to explain the observed *Proteus* behavior. The response of the system to changes in $p_{\text{min}}$ differs from changes to $a_{\text{min}}$. As $p_{\text{min}}$ increases, swarming time, total cycle time, and terrace width decrease, whereas consolidation time increases. The parameter $p_{\text{min}}$ is needed to create a distinct consolidation phase.

To understand the breakdown of colony regularity as the age of dedifferentiation become less sharp, we examine first the response to changes in $\mu_0$. Decreasing $\mu_0$ for a fixed $\sigma$ has the effect of decreasing the amount of dedifferentiation after $a_{\text{max}}$, which can ultimately result in a continuation of swarming and a loss of a consolidation phase. As shown in figure 2, this breakdown can be quite irregular. The temporal breakdown occurs before an apparent breakdown in spatial regularity. Not shown in the figure is the effect of increasing $\mu_0$. Increasing $\mu_0$ above what is shown results first in regular colonies with narrow terrace widths, and then the loss of regularity through the loss of any meaningful swarming phase. This is intuitive since increasing $\mu_0$ results in earlier and more frequent dedifferentiation, which in turn results in a lack of large swarmer cells to contribute to raft building and the consequent swarming.

We next examine the response to changes in $\sigma$. This parameter study corresponds to the change in the probability function $\Pi(a)$ that a swarmer cell has not dedifferentiated by age $a$ shown in figure 1(a). As illustrated in figure 3, colony regularity breaks down as the age of dedifferentiation becomes less sharp. It is important to note that the colony remains regular within an interval around $\sigma = 0$. As mentioned above, the swarming cycle becoming irregular for $\sigma$ near zero, but not equal to zero, would invalidate the model. It is interesting
Figure 2: Computed *Proteus mirabilis* swarm colonies showing a breakdown of spatial and temporal regularity as the dedifferentiation function becomes less sharp. Note that temporal regularity breaks down before the apparent spatial regularity. Figure 2(a) consists of the 3D plots of the swarmer- and dividing-cell biomass viewed from directly above with a combination of ambient light, diffuse reflection, and specular reflection. The time of the snapshot is the earlier of \( t = 30 \) or when it hits the boundary. Figure 2(b) shows the corresponding radius of the swarm colonies as functions of time. Areas where the function has zero slope correspond to consolidation periods in the swarm-colony development. The dedifferentiation parameter \( \mu_0 \) varies for the three colonies, left to right, from \( \mu_0 = 0.1 \) to \( \mu_0 = 0.01 \) to \( \mu_0 = 0.001 \). Parameters common to all three swarm colonies are \( \sigma = 0.01 \), \( a_{\text{max}} = 2.67 \), \( v_c = 8.0 \), \( D_0 = 2 \times 10^{-3} \), \( p_{\text{min}} = 0.5 \), \( \xi_0 = 0.5 \), and \( a_{\text{min}} = 0 \).
Figure 3: Swarm-colony front dynamics showing the breakdown of spatial and temporal regularity as the dedifferentiation age becomes less sharp. Parameters common to all three swarm colonies are $\mu = 1.0$, $a_{\text{max}} = 2.67$, $v_c = 8.0$, $D_0 = 2 \times 10^{-3}$, $p_{\text{min}} = 0.5$, $\xi_0 = 0.5$, and $a_{\text{min}} = 0$.

to note that the breakdown of colony regularity is nearly hysteretic as a function of $\sigma$. In the results shown in figure 3 we set $\mu_0 = 1$. Results do not change qualitatively for $\mu_0$ near this choice, however, as shown in figure 2 $\mu_0$ is an important parameter in determining colony regularity. Fortunately, we have the shape of $\Pi(a)$ as a means to determine which parameter choices represent a sharp versus a mollified age of dedifferentiation.

The last parameter exploration we present is that of fixing the ratio $\mu_0/\sigma$ and varying $\sigma$. This corresponds to the change in the probability function $\Pi(a)$ shown in figure 1(b). As $\sigma \to 0$, the colony formation never becomes truly regular. This irregularity corresponds to a lack of sharpness in $\Pi(a)$ as $\sigma \to 0$. Figure 4 illustrates our results for $\mu_0 = \sigma$. Setting $\mu_0 = 3\sigma$ results in a loss of a swarming phase as $\sigma$ increases rather than the loss of a consolidation phase.
Figure 4: Swarm-colony front dynamics showing irregularity of all colonies when $\mu_0/\sigma$ is fixed. For the colonies shown in this figure, $\mu_0 = \sigma$. Parameters common to all three swarm colonies are $a_{max} = 2.67$, $v_c = 8.0$, $D_0 = 2 \times 10^{-3}$, $p_{min} = 0.5$, $\xi_0 = 0.5$, and $a_{min} = 0$.

6 Conclusions

This paper constitutes an argument that the regularity in the Proteus cell cycle of differentiation and dedifferentiation between immotile dividing cells and motile filament swarmer cells, along with mechanisms that depend on cell density, underlies the temporal and spatial regularity in swarm-colony development. This is at odds with the implications of reaction-diffusion models which attribute Proteus behavior to density-dependent mechanisms alone.

The first conclusion to make is that the question of the correct modeling approach might be answered empirically by studying the colony regularity of strains of Proteus that do not have a sharp age of dedifferentiation. The probability that a swarmer cell has not dedifferentiated by age $a$, denoted by $\Pi(a)$, can be used as a guide to determine experimentally the forms of the dedifferentiation modulus $\mu$ in equation (4.1a).

Density-dependent effects do matter in understanding Proteus swarm-colony develop-
ment. Experiments to determine how differentiation from dividing cells to swarmer cells
depends on local population density would be particularly valuable in finding the forms of
the differentiation function, $\xi$.

We, along with the other scientists modeling Proteus swarm-colony development of whom
we are aware, used diffusion to model movement. Diffusion may not be the best representa-
tion of the spatial dynamics. Areas of future research are the specifics of how the much
larger and broader issue of biological motion relates to Proteus motion on an agar surface,
and extending the models to explicit two-dimensional space to understand the interaction of
multiple colonies.

We close by noting that the likely importance of the cell cycle in determining the macro-
scopic behavior makes Proteus a model system for a multiscale understanding of other bio-
logical systems where the spatial or temporal behavior is a manifestation of local kinetics.

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