Mathematical Models in Cell Cycle Biology and Pulmonary Immunity

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This dissertation titled

Mathematical Models in Cell Cycle Biology and Pulmonary Immunity

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ABSTRACT

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Mathematical models are used to study two biological systems: pulmonary innate immunity and autonomous oscillation in yeast.

In order to better understand the dynamics of an early infection of the lungs, we construct a predator-prey ODE model of pulmonary innate immunity which describes several observed properties of the pulmonary innate immune system. Under reasonable biological assumptions, the model predicts a single nontrivial equilibrium point with a stable and unstable manifold. Trajectories to one side of the stable manifold are asymptotic to the disease-free equilibrium and on the other side are unbounded in the size of the infection. The model also reproduces a phenomenon observed in [24] whereby the innate response to an infectious challenge reduces the ability of further infections to take hold. The model may be useful in analyzing and understanding time series data obtained by new methods in pathogen detection in ventilated patients.

We also examine several models of autonomous oscillation in yeast (YAO), called the Immediate, Gap, and Mediated models. These models are based on a new concept of Response / Signaling (RS) coupled oscillator models, where feedback signaling and response are phase-dependent. In all three models, clustering of the type seen in YAO is a robust and generic phenomenon. The Gap and Mediated models add a dynamical delay, the latter by modeling a signaling agent present in the culture. For dense populations the Mediated model approximates the Immediate model, but the Mediated model includes dynamical quorum sensing where clustered solutions become stable through density-dependent bifurcations. A partial differential equations model is also examined, and we demonstrate existence and uniqueness of solutions for most parameter values.
The road to wisdom? Well, it’s plain
And simple to express:
Err
and err
and err again,
but less
and less
and less.

-Piet Hein
Acknowledgements

I have been incredibly lucky, both personally and professionally, to be surrounded by wonderful, supportive people. I must thank my Mother and Father, my Grandparents, my Brother and Sister and the rest of my family for helping to make me who I am. I am proud of my achievements and I am thankful to have had the many opportunities that allowed me to achieve them.

For supporting me unfailingly I must thank my wife Anna Rose. She is always there when I need a patient and discerning ear.

To my colleagues and coworkers: thank you all for helping me when I needed help and making me work when I needed that. Your companionship has been invaluable and I hope you’ll always be a part my life.

Lastly I owe a tremendous debt to my academic advisors and mentors: to David Craft, who helped me to succeed my second time around, to Winfried Just, whose sharp questions and high expectations inspire me, and to Todd Young, who has been more generous with his patience and advice than I deserve. I do not exaggerate when I say that without Todd I would not have had it in me to complete this work, and I will always be grateful.

I hope that in turn I might use the wisdom and knowledge I have gained in pursuit of my PhD to help others to be happy and successful in their lives and work, as all of the people I mention here have done for me. Thank you all.
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<tr>
<td>BAL</td>
<td>Bronchoalveolar Lavage</td>
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<tr>
<td>CDC</td>
<td>Cell Division Cycle</td>
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<tr>
<td>HCH</td>
<td>Hygroscopic Condenser Humidifier</td>
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<tr>
<td>IVP</td>
<td>Initial Value Problem</td>
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<tr>
<td>MPF</td>
<td>Mitosis Promoting Factor</td>
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<tr>
<td>ODE</td>
<td>Ordinary Differential Equation</td>
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<tr>
<td>PDE</td>
<td>Partial Differential Equation</td>
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<tr>
<td>PMN</td>
<td>Polymorphonuclear Leukocyte</td>
</tr>
<tr>
<td>VAP</td>
<td>Ventilator Associated Pneumonia</td>
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<td>YAO</td>
<td>Yeast Autonomous Oscillation</td>
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1 Mathematical Modeling in Biology

1.1 Complex Systems in Biology

Many of the phenomena that biologists are interested in, including those in this work, are emergent properties of complex systems: they arise from a large number of simple interactions among individual elements at a smaller scale. The defining characteristic of a complex system is that the emergent phenomena can not be deduced by studying a single individual; the large scale properties of the system are fundamentally due to its connected nature. Complex systems are ubiquitous, but as a formal category of study are relatively new.

A classic example of a complex system is an ant colony [3]. Individual ants have a limited repertoire of behavior, and in isolation are largely inconsequential to the surrounding ecosystem. Nonetheless an ant colony performs a vital role that no single ant can. Perhaps the most compelling example of a complex system in biology is the brain, whereby a large number of neurons, interacting in (what are presumed to be) simple ways, produces thought, emotion, and consciousness.

This work concerns the study of two complex systems in biology: the pulmonary immune system and a population of yeast cells (Saccharomyces cerevisiae) in a bioreactor. Each of these systems has a long history of scientific study, and it is hoped that the projects detailed in this work can contribute in some way to that study by helping to describe and understand them.

1.2 Modeling Philosophy

The subject of Mathematical Modeling in Biology encompasses two broad approaches, which can be called mechanistic and phenomenological. This distinction is especially sharp in the context of complex systems, where a mechanistic model is a mathematical description of the individuals and their interactions, and a phenomenological model is a description of the emergent properties of the system.
Depending on the goal of the research project, either method may be appropriate. In this work, both approaches are used: the immunity model is purely phenomenological, abstracting the many and varied cells and chemicals involved in a pneumonia infection into just two variables representing the pathogen and the immune response. In this project, the goal is to assess risk of pneumonia and predict health outcomes of hospital patients, for which the behavior at the scale of individual cells is largely irrelevant. On the other hand, the *S. cerevisiae* project involves an attempt to explain a population-scale phenomenon by modeling the individual interactions that give rise to it.

For a mathematical model to be biologically relevant, it should be *robust*. Mathematically speaking, a model is robust if it is structurally stable, i.e. if a small change in either the formulation of the model, or its related parameters, will not produce any qualitatively different behaviors. The biological interpretation of this is: the model is by definition not exactly correct, but as long as it is a reasonable approximation its results are meaningful.
2 PULMONARY INNATE IMMUNITY

2.1 Methods for Detecting Pneumonia in Ventilated Patients

Mechanically ventilated patients are at risk of developing ventilator associated pneumonia (VAP), an infection of the lungs that is often acquired in hospitals [25]. These infections cause increased time on ventilators, lengthened stays in critical care units, added expense to patients and hospitals, and worsened health outcomes [15]. For these reasons, early diagnosis and treatment of VAP is a high priority.

Current methods of diagnosis are invasive, slow, and / or unreliable. The state of the art is the bronchoalveolar lavage (BAL) technique, where a fluid sample is squirted into the lung and then collected for analysis. The process is invasive, and the analysis takes several days as the sample is cultured until the pathogen levels become detectable (recent results indicate that PCR may be a viable alternative to this lengthy culture process). Other tests are regularly performed on ventilated patients, for example x-rays are often performed daily. Although an x-ray may indicate the need for further testing, on its own this method is unreliable, nonspecific, and may generate both false negatives and false positives. Because a definite diagnosis can only be made some time after pneumonia is already suspected, there can be substantial delay between infection and diagnosis [25].

In [41], a non-invasive method for detecting pneumonia pathogens in ventilated patients was introduced. Ventilator systems include a hygroscopic condenser humidifier (HCH) filter in the air pathway near the endotracheal tube. These filters are designed to limit moisture and heat loss, and to filter outside contaminants. The authors of [41] showed that the HCH filters, which are normally discarded every 12 hours, contain significant, uncontaminated samples of aerosolized fluid from the patient’s lungs. Such fluid can then be analyzed easily using polymerase chain reaction (PCR) techniques to detect pathogens [41].

Because HCH filters are changed regularly, and because PCR analysis is relatively inexpensive and fast, it is hoped that these advances will lead to a method of detecting pneumonia that is significantly
more effective than current techniques. To make such a system effective, medical workers need to be able to interpret data from the filters to detect a nascent infection. Mathematical modeling of the underlying process is intended to identify the diagnostic variables which will be useful in predicting the course of an infection before it is amenable to traditional methods of diagnosis.

Formal criteria are often used to aid in the diagnosis of pneumonia, but they are not based on the kind of time series data that this new technique would make available. New criteria should be found that take into account the predator-prey type dynamics of an immune response to a pathogenic infection. This is the basis for the work detailed in this chapter.

2.2 Modeling the Innate Immune Response to a Pathogen

2.2.1 Prior Work

Low dimensional models of immune response to a pathogen exist, but do not include the unique characteristics of the immune response in the lungs. Because the lungs are continually exposed to microorganisms, a particularly effective and fast-acting innate system has evolved to clear small infections.

Pugliese and Gandolfi [32] studied the following model of a host-pathogen interaction:

\[
\begin{align*}
    x' &= \alpha x - \frac{mx}{1 + \beta u x} - \frac{x}{1 + \beta_s x} y \\
    y' &= -y + \frac{x}{1 + \gamma x} y + \eta.
\end{align*}
\]

This model is notable for its predator-prey type dynamics, where \( x \) (the ‘prey’) represents the pathogen load and \( y \) (the ‘predator’) the response from the immune system. The immune response limits the growth of the pathogen via the mass action term \( \frac{-xy}{1 + \beta_s x} \), while the immune response is proportional to the size of the pathogen load via the term \( \frac{xy}{1 + \gamma x} \). The variable \( y \) represents the adaptive immune system, but the innate response is partially captured in the term \( -\frac{mx}{1 + \beta_u x} \), where some fixed immune capacity immediately goes to work decreasing the growth rate of the pathogen.
Note however that in this model, the innate immune response decreases in effectiveness as $x$ grows, and in the lungs this is not the case [33].

It was shown in [24], which we call the two-hit experiment, that in addition to the immediate effect of ever-present ‘background’ immune cells, there is a rapid (but not immediate) response to bacterial infection as other, more effective, immune cells (such as PMNs) from nearby reservoirs are recruited to aid in the fight [5, 6, 16]. This allows the innate immune system to clear more and larger infections that would otherwise become chronic and require the intervention of the adaptive system. The model in the next section is intended to capture the dynamics of the innate immune response to a pathogen in the early stages of an infection, and to predict whether the infection will be cleared by the innate system or require the adaptive system, leading to the possible necessity of medical intervention.

### 2.2.2 The Model

In [42], we considered a model of the form:

\[
\begin{align*}
    x' &= \alpha x - \frac{mx}{1 + bx} - \beta x z \\
    z' &= \delta x - \gamma x z + \eta - \mu z \\
\end{align*}
\]  

(2.2.2)

where, as in the Pugliese-Gandolfi model $x$ represents the bacterial load. Here $z$ represents the level of response from the rapidly-acting innate immune system.

The terms $\alpha x - \frac{mx}{1 + bx}$ mirror the earlier model, as we still assume exponential growth and a low, ever-present capacity to clear very small infections. The chief method of bacterial elimination is phagocytosis, which is captured in the term $\beta x z$.

The innate immune response $z$ satisfies the following physiological considerations:

- The rate of recruitment is proportional to the bacterial challenge (the $\delta x$ term),
- Individual phagocytic cells have a limited capacity for destroying invader cells (the $-\gamma x z$ term),
• there is a small, constant background level (the $\eta$ term), and

• it is self-limiting (the $-\mu z$ term).

Additionally, physiological knowledge limits the possibilities for the parameter values. For example, the carrying capacity of a bacterial population in the lungs is very large; the limiting factor is the survival of the host rather than physical limitations on growth of the pathogen. On the other hand, the innate immune response capacity is finite. Although we do not specify exact units for $x$ and $z$, they should be considered as having the same order of magnitude. Thus the carrying capacity of $x$, given by $\frac{\alpha}{\beta}$, should be larger than the carrying capacity of $z$, given by $\frac{\delta}{\gamma}$. On the other hand, we know that there is significant capacity in the innate immune system to respond to infection above its background level $\frac{\eta}{\mu}$. Thus the parameters should satisfy:

$$\frac{\eta}{\mu} \ll \frac{\delta}{\gamma} < \frac{\alpha}{\beta}. \quad (2.2.3)$$

We also know that a very small bacterial challenge should be cleared by the immediate portion of the response, and so we assume:

$$\alpha < m. \quad (2.2.4)$$

There is an equilibrium solution at $x = 0$, $z = \frac{\eta}{\mu}$. This represents the disease-free state, and the linearization of the system at this point is:

$$DF(0, \eta/\mu) = \begin{pmatrix} \alpha - m - \frac{\beta \eta}{\mu} & 0 \\ \delta - \frac{\gamma \eta}{\mu} & -\mu \end{pmatrix}. \quad (2.2.5)$$

Provided that $\alpha < m + \frac{\beta \eta}{\mu}$, this matrix will have two negative eigenvalues. But since the parameters are assumed to be positive and since we require $\alpha < m$, the inequality holds. Therefore the disease-free equilibrium is linearly stable, thus locally attracting, and small infections will be cleared.

Other nontrivial equilibrium points can exist when the portions of the nullclines given by the two curves (2.2.6) intersect.
\[ z_1 = \frac{1}{\beta} \left( \alpha - \frac{m}{1 + bx} \right) , \tag{2.2.6} \]
\[ z_2 = \frac{\delta x + \eta}{\gamma x + \mu} . \]

The existence and characteristics of equilibrium points thus depends on whether and how they intersect. To determine how many intersections exist, we set the equations in (2.2.6) equal to each other and simplify, obtaining:

\[ \frac{b (\alpha \gamma - \beta \delta) x^2 - \left( \beta (\delta + \eta b) + \gamma (m - \alpha) - \alpha \mu b \right) x - \mu (m - \alpha) - \beta \eta}{\beta (1 + bx) (\gamma x + \mu)} = 0 \tag{2.2.7} \]

The LHS has a nonzero denominator and its numerator is of the form \( Ax^2 + Bx + C = 0 \), where

\[ A = b(\alpha \gamma - \beta \delta) , \]
\[ B = \beta(\delta + \eta b) + \gamma(m - \alpha) - \alpha \mu b , \tag{2.2.8} \]
\[ C = -\mu(m - \alpha) - \beta \eta . \]

Note that \( C < 0 \), and so the number of solutions (and thus the number of equilibrium points) is determined in part by which of the following holds:

I: \( \alpha \gamma > \beta \delta \), or

II: \( \alpha \gamma < \beta \delta \).

Case I corresponds to the carrying capacity assumption (2.2.3) and is therefore the most biologically relevant case. In Case I there is a unique positive real root. Case II includes the possibility of zero or two positive real roots. We still analyze Case II, but it is less biologically relevant; in particular the set \( \alpha \gamma = \beta \delta \) is an algebraic variety of codimension 1.

### 2.2.2.1 Case I

In Case I the curves (2.2.6) have a unique point of intersection \( x^* \) given by taking the positive square root in:
Theorem 2.2.1. The equilibrium \((x^*, z_1(x^*)) = (x^*, z_2(x^*))\) in Case I is a saddle point. Its stable manifold partitions phase space into two components; trajectories to the left of the stable manifold are asymptotic to the disease-free equilibrium and trajectories to the right are unbounded in \(x\).

**Proof.** Recall that the equilibrium point occurs at the intersection of the curves \(z_1\) and \(z_2\). Since in Case I there are two real roots, the nullclines must cross. In fact, the crossing must be transversal, because a tangency would require a root of multiplicity at least 3, and so \(z_1' > z_2'\) at \(x^*\). Recall that for a vector field \(F(x, z) = (f(x, z), g(x, z))\), the level curves \(f = 0\) and \(g = 0\) satisfy:

\[
z_1' = \frac{f_z}{f_x} \quad \text{and} \quad z_2' = \frac{g_z}{g_x}
\]

Therefore \(\det(DF) = f_xg_z - g_xf_z < 0\). And since the eigenvalues of \(DF\) solve \(\lambda^2 - \text{Tr}(DF)\lambda + \det(DF) = 0\), there are in fact two real eigenvalues, one positive and one negative. This proves that the equilibrium is a saddle point, and so it has a stable and unstable manifold.

Now, because the nullclines cross exactly once in the first quadrant, they divide the quadrant into 4 sectors which we label I, II, III, and IV, as in Figure 2.1. It can be seen from (2.2.2) that above the \(x\)-nullcline the \(x\)-component of \(F\) is negative and below the nullcline it is positive. Similarly, above the \(z\)-nullcline the \(z\) component is negative and it is positive below the nullcline. This means that in each sector, the vector field points in the following directions:

- I: southeast (↘),
- II: southwest (↙),
- III: northwest (↖),
- IV: northeast (↗).

Now because the vector field is smooth, so are the stable and unstable manifolds \(W^s\) and \(W^u\). It is easy to check that they are distinct from the nullclines. We now show that they must be entirely contained in sectors II and IV, for suppose it were otherwise. If either manifold is in sector I then
its graph must have positive slope near the equilibrium. But as the manifolds are invariant sets, this
would require the flow to be to the northeast (↗) or southwest (↙), which possibilities have already
been eliminated above. A similar argument holds for sector III. This proves that locally the stable
and unstable manifolds are arranged as pictured in Figure 2.2.

Globally the picture is much the same. First consider the component of $W^s$ that locally intersects
sector IV. It has positive slope and there are no other equilibrium points, so it must intersect either
the $x$-axis or the $x$-nullcline. The second cannot happen because $W^s$ cannot meet sector III, so it
must intersect the $x$-axis, as in Figure 2.3.

Now we consider the component of $W^s$ in sector II. It must be contained entirely in sector II with
positive slope for all time, by similar arguments. Thus it continues indefinitely in the northeast
direction and partitions the first quadrant into left and right components.

The unstable manifold $W^u$ is similar, having components in sector II and sector IV. The sector II
component terminates at the stable equilibrium $(0, \eta/\mu)$ and the sector IV component is asymptotic
to $z = \delta/\gamma$ as $x \to \infty$. 

\[\square\]
From this theorem we have a complete characterization of the dynamics in Case I, summarized in Figure 2.3. In biological terms, all infections end in one of two outcomes: the innate immune system clears the infection (initial conditions to the left of the unstable manifold) or the infection grows beyond the capacity of the innate system (initial conditions to the right of the stable manifold).

2.2.2.2 Case II

If $\alpha \gamma < \beta \delta$, there are three possibilities for the solutions to (2.2.7), which we call Case IIa, IIb, and IIc, corresponding to no real roots, a single positive real root, and two positive real roots, respectively.

In Case IIa, the nullclines do not intersect, so the disease free equilibrium is the only equilibrium point.

**Theorem 2.2.2.** In Case IIa, the disease free equilibrium is globally attracting.

**Proof.** The nullclines divide the first quadrant into three regions, I, II, and III, as in Figure 2.4. In sector I, $z'$ is positive and bounded away from zero, including at the boundary. Since region II is
bounded above and below, and since the slope field outside a neighborhood of the $x$-nullcline has positive $z$-component, any initial condition starting in this region will leave in finite time. Further, such a solution has negative $x$ velocity, also bounded uniformly away from zero, so it must intersect either $z_2$ or the $z$-axis. The latter can only happen at the equilibrium $(0, \eta/\mu)$, so trajectories starting in the interior of sector II either reach equilibrium or cross into sector III.

Once a trajectory enters sector III, the only possibility is that it continues toward the disease free equilibrium. Since this exhausts the possibilities, every trajectory is asymptotic to $(0, \eta/\mu)$.
Figure 2.4: Phase plane diagram showing the global dynamics in Case IIa.

In Case IIb, (2.2.7) has a single real root corresponding to a tangency between the nullclines. The resulting equilibrium point is a bifurcation point between Cases IIa and IIc.

Theorem 2.2.3. The second equilibrium point in Case IIb is a saddle-node.

Proof. Recall that this case corresponds to $z'_1 = z'_2$ which implies det($DF$) = 0. Therefore there is a zero eigenvalue, and the other eigenvalue is given by Tr($DF$). To determine its size, we consider the linearization:

$$DF = \begin{pmatrix} \frac{\alpha - \beta z - \frac{m}{(1 + bx)^2}}{\delta - \gamma z} & -\beta x \\ -\beta x & -(\gamma x + \mu) \end{pmatrix}$$

(2.2.11)

which has characteristic polynomial
\lambda^2 + \left( \gamma x + \mu - \alpha + \beta z + \frac{m}{(1 + bx)^2} \right) \lambda + \left( \gamma x + \mu \right) \left( -\alpha + \beta z + \frac{m}{(1 + bx)^2} \right) + \beta \lambda (\delta - \gamma z) = 0. \quad (2.2.12)

We can simplify (2.2.12) along the \( x \)-nullcline \( \alpha - \beta z = \frac{m}{1 + bx} \) getting

\lambda^2 + \left( \gamma x + \mu - \frac{mbx}{(1 + bx)^2} \right) \lambda - \left( \gamma x + \mu \right) \frac{mbx}{(1 + bx)^2} + \beta x (\delta - \gamma z) = 0 \quad (2.2.13)

and then along the \( z \)-nullcline to get:

\lambda^2 + \left( \gamma x + \mu - \frac{mbx}{(1 + bx)^2} \right) \lambda - \left( \gamma x + \mu \right) \frac{mbx}{(1 + bx)^2} + \beta \frac{\delta \mu - \gamma \eta}{\gamma x + \mu} = 0, \quad (2.2.14)

which is valid at the equilibrium point. The roots of (3.1.42) depend on \( x \), but we can make the simplifying assumption that the third term is negligible for reasonable parameter values. The \( \lambda \) coefficient is by definition equal to \( -\text{Tr}(DF) \), which will be negative and so we expect the saddle-node equilibrium point to have one stable direction, as pictured in Figure 2.5.

\[\square\]

In Case IIc, the \( z \)-nullcline is initially much steeper than the \( x \)-nullcline, and so there are two intersections as in Figure 2.6, which we call the first and second, counting from the left. Here the analysis of the first equilibrium point is the same as Case IIa. The other equilibrium requires a closer look. Since \( z_1' < z_2' \) at the second intersection, \( \text{det}(DF) \) is positive. Thus there are two eigenvalues, which may be real or complex, leading either to a stable or unstable node or a stable or unstable focus. Because of the stable saddle-node equilibrium at the bifurcation point, near the bifurcation we expect the new equilibrium to be a stable node. We know that no other equilibrium points can exist, so the only way to lose this stability is through a Hopf bifurcation into a periodic orbit.

Biologically Case IIc corresponds to the possibility of a chronic infection that is not cleared by the innate immune system.
Figure 2.5: Phase plane diagram showing the global dynamics in Case IIb.

**Theorem 2.2.4.** The flow described by (2.2.2) in Case I, IIa, and IIc is of Morse-Smale type, and thus structurally stable.

**Proof.** A flow is of Morse-Smale type if it has a finite number of periodic trajectories and equilibrium points, and if each of them is locally structurally stable. In this case, there are no periodic orbits and only finitely many equilibrium points. In the above analysis it is shown that the stable and unstable manifolds of the equilibrium points are transverse, and this is sufficient for local structural stability.
2.3 The Two-Hit Experiments

In [24], the authors demonstrated an unexpected property of the immune response in the lungs of murine models to microaspirated bacterial infection. They prepared two strains of *K. pneumoniae* to be identical except for staining properties and then administered doses of each in sequence to mice, four hours apart. The infections were allowed to progress, then the lungs were cultured 24 hours later and the sizes of the resulting bacterial populations were compared. Remarkably, the first of the two strains administered proliferated while the second languished, appearing in the final culture in a ratio of 24:1. This time span is too short to include the adaptive immune system [16] and so the conclusion is that the innate system quickly ramps up in response to an initial challenge, creating
an environment where it is hard for new infections to take hold. This is significant because earlier models of the pulmonary immune system fail to account for this behavior.

To compare the results of our model to those of the two-hit experiments, we performed numerical simulations using an adapted form of the model (2.2.2):

\[
\begin{align*}
    x'_1 &= f(x_1, x_2, z) = ax_1 - \frac{mx_1}{1 + b(x_1 + x_2)} - \beta x_1 z, \\
    x'_2 &= f(x_1, x_2, z) = ax_2 - \frac{mx_2}{1 + b(x_1 + x_2)} - \beta x_2 z, \\
    z' &= g(x_1 + x_2, z) = (\delta - \gamma z)(x_1 + x_2) + \eta - \mu z.
\end{align*}
\] (2.3.1)

In these modified equations, the two pathogens \( x_1 \) and \( x_2 \) appear identical to the immune system response which is a function of their sum only, reflecting the assumption in the original experiment that the two strains were pathologically identical.

The doubling time of \( K. \) pneumoniae is on the order of 1 hour [39], so we chose the time unit to be 1 hour and we set \( \alpha = 1 \). We took \( \beta = 0.2 \) which we consider a conservative estimate. The time scale of the PMN response is around 4 hours [16], and we assume that bacterial growth is faster than immune cell recruitment, leading to values of \( \delta \approx 0.04 \) and \( \gamma \approx 0.01 \). The self-limiting parameter \( \mu \) is known to be approximately 0.087 [5], and to keep the background rate of immune activity low we set \( \eta = 0.04 \). Because the doses in the two-hit experiments were large enough to overwhelm the immediate response, we chose \( m = 4 \) and \( b = 3.7 \) to make the immediate response negligible. This choice of parameters places the system in Case I as described above. We chose these parameter values for the simulation based on a combination of biological knowledge and guessing. Because the model is robust, the results are valid for an open set of parameter values around the ones we chose.

The system was integrated on the time interval \( t \in [0, 12] \) with initial condition \( x_1(0) = d_1, \)
\( x_2(0) = 0, \) and \( z_1(0) = \eta/\mu. \) At time \( t = 4, \) \( x_2 \) was set to \( d_2 = 5 \) CFU and then allowed to progress normally. This corresponds to the second inoculum administered after 4 hours. As in [24],
we varied the first dose \( d_1 \) between 5 and 15 CFU. These initial conditions place the system well to the right of the stable manifold.

At time \( t = 12 \), first order growth rates were determined for each strain via the formula

\[
k_i = \frac{\ln(x_i(12)) - \ln(d_i)}{T_i}
\]  

(2.3.2)

where \( T_1 = 12 \) and \( T_2 = 8 \). The results are shown in Figure 2.7. They match closely with experimental values in several ways: first, we see that \( k_1 \) is much larger than \( k_2 \), with \( k_1 - k_2 \approx 0.25 \), close to the observed difference of 0.22. Second, the actual values for the growth constants are close to those observed experimentally. And third, the growth rate \( k_2 \) is inversely related to the size of the initial inoculum while \( k_1 \) is less strongly affected. The conclusion is that the model is adequate to capture the dynamics reported in [24].

We can also use the experimental data to obtain parameter estimates for our model by making two small simplifying assumptions: because the first dose overwhelms the immediate response, we ignore the term \( \frac{m x_1}{1 + b x_1} \). And because of the response time of approximately 4 hours, we assume \( z = 0 \) for \( t \) between 0 and 4, then assume that it quickly reaches its capacity so that \( z = \frac{\delta}{\gamma} \) for \( t \) from 4 to 24.

With these assumptions, \( x_1(t) \approx x_1(0)e^{\alpha t} \) for \( t \in [0, 4] \), and for \( t \in [4, 24] \),

\[
x_1(t) = x_1(0)e^{\alpha t}e^{(\alpha - \beta \delta / \gamma)(t-4)},
\]

\[
x_2(t) = x_2(4)e^{(\alpha - \beta \delta / \gamma)(t-4)}.
\]  

(2.3.3)

From the experiments we have \( x_1(24) = x_1(0)e^{24k_1} \) and \( x_2(24) = x_2(4)e^{20k_2} \), which when combined with (2.3.3) gives:

\[
k_1 = \alpha - \frac{5\beta \delta}{6\gamma},
\]

\[
k_2 = \alpha - \frac{\beta \delta}{\gamma}.
\]  

(2.3.4)
We immediately see that the system is in Case I since $\alpha = \frac{\beta \delta}{\gamma} + k_2 > \frac{\beta \delta}{\gamma}$. We can also treat (2.3.4) as a linear system in $\alpha$ and $\beta$, obtaining the estimates
\[ \alpha \approx 1.49 \text{ and } \frac{\beta \delta}{\gamma} \approx 1.38. \]  \hspace{1cm} (2.3.5)

The estimate for \( \alpha \) corresponds well with known values for \( K. pneumoniae \). The immune parameters are only given as a non-dimensional ratio, and need to be scaled appropriately before being used in the model.

2.4 Using Clinical Data to Assess Infection Dynamics

As mentioned above, this work is being done in conjunction with an experimental effort to detect pneumonia in mechanically ventilated patients before traditional methods of diagnosis are effective. The model was developed for two main reasons: to reproduce phenomena of the pulmonary innate immune system that previous models could not, and to develop criteria for classifying time series data obtained from patients' HCH filters as either problematic (pre-pneumonia) or acceptable.

Based on the comparison with the two-hit experiments we believe that this work goes some way toward achieving the first goal. We await more data from anticipated clinical trials, but in the meantime we have some ideas for further work toward the second goal.

Because aerosolized fluids have turned out to be a rich source of information, showing not just bacterial load but a number of biomarkers for immune activity as well [41], the model may require more detail, for instance including several variables to represent the immune response. Because too much detail in the model works against the philosophy of low-dimensional modeling, there will likely need to be analysis to determine which of the biomarkers are essential to detecting nascent infections and which are superfluous.

There also remains the task of using the model to create a diagnostic procedure to help medical professionals interpret the data from HCH filters. For such a method to be effective, it must be simple, and should not require the use of computer simulations. Ideally, simple yes/no questions would be employed in a short decision tree leading to an actionable conclusion. Working entirely from the model, this could be achieved by dividing phase and parameter space into convex regions
(based on measurement uncertainty) which would be classified in various ways, for instance as healthy, worrisome, or pre-pneumonia.

In addition to considerations from the model, if the data set is large enough it may be feasible to use machine learning techniques to construct such a decision tree. Such a method applied to a more complex model might also take the place of traditional analysis in the case that the model becomes too unwieldy for calculations by hand.
3 Response / Signaling Models of Cell Cycle Feedback

Response / signaling (RS) models are a type of coupled oscillator model; that is, they are described by a graph where each node is an oscillator and nodes which share an edge may influence one another’s speed of oscillation. Coupled oscillator approaches have been used successfully in many applications, including in physics, biology, and engineering [40]. In an RS model, the feedback between nodes is phase dependent in both the source and the target nodes of the feedback. This makes RS models a natural choice for modeling the cell division cycle (CDC) because of the latter’s segmentation into distinct phases.

The main phases of the CDC are denoted G0, G1, S, G2, and M. Certain proteins, for instance Cyclin B, are associated with one or more specific phases [9]. Similarly, certain genetic pathways are only active during specific phases, causing the sensitivity of the cell to various proteins and chemical signals to vary depending on phase.

An RS oscillator is a point on the circle $S^1 = [0, 1] / (0 ∼ 1)$ that usually moves with velocity 1. Two subsets, $S$ and $R$, are distinguished as the signaling and responsive regions, respectively. When one such oscillator is in the $S$ region and a neighbor oscillator is in the $R$ region, then the latter’s velocity may be regulated up or down in any way so long as it remains positive and bounded away from zero.

RS type models were developed to address the problem of autonomous oscillation in yeast (YAO) but because of their generality, and because of their biological relevance, we expect them to find use in many types of biological models, including Drosophila embryogenesis, described in the the section on future work.

3.1 Autonomous Oscillation in Yeast

Saccharomyces cerevisiae (baker’s or brewer’s yeast) is a model organism and has been the target of intense research for many years. As a result much is known about it. S. cerevisiae cells reproduce
through budding and mitosis; this process leaves a scar on the mother cell and so the age of an \textit{S. cerevisiae} cell can be determined visually.

When modeling a yeast cell as an RS oscillator, we usually model the biological S phase as the signaling region $S$ and some portion of the G1 phase for the responsive region $R$. This leads to the mathematical definition $S = (0, s)$ and $R = (r, 1)$ for $0 < s < r < 1$, with $0 \equiv 1$ corresponding to the onset of DNA replication. We make this choice because the S phase of the CDC is vital and resource intensive [19], and there is a known checkpoint at the end of the G1 phase, where the cell decides whether or not to enter S and begin division [27].

\textit{S. cerevisiae} populations are often cultured in bioreactors, containers with various inputs and outputs meant to keep a population of microorganisms homogeneous. They range in size from less than a liter to many thousands of liters, and are used for research, fermentation, and protein production.

When a population in a well-mixed bioreactor reaches a high density, observable quantities such as dissolved oxygen, pH, and many other physiological variables will begin to oscillate spontaneously, with a period that approximately divides the length of the CDC [1, 14, 20, 28]. These autonomous oscillations have been a subject of much study for more than 40 years, and there is not yet consensus on its causes [21]. A better understanding of the phenomenon of YAO is desired and would contribute to increased stability and efficiency in bioengineering applications where such populations are used. An understanding of YAO would also contribute to the study of metabolism and the cell cycle generally, a topic of much interest in the biological community.

Some progress has been made recently in determining the cause of these oscillations: in [31] and [37] direct measurements were made of an oscillating population of yeast and the authors showed that the population was \textit{clustered}, meaning that two subpopulations were each somewhat synchronized in the CDC, but 180° out of phase with one another.

Based on previous knowledge of the yeast cell cycle, the authors of [37] introduced several response / signaling type models for continuously cultured yeast populations and showed that such models
predict clustering over a broad range of parameter values and modeling assumptions. Subsequently, several refinements and generalizations [46, 47, 48] have been made to these models, and the results point very strongly toward a response / signaling feedback mechanism as the causal agent for autonomous oscillation in yeast. This chapter will discuss four of these, called the Immediate (and related Gap), Mediated, and Partial Differential Equation Models.

### 3.1.1 Immediate and Gap Models

Because YAO occurs in well-mixed populations, we can make several simplifying assumptions for our model. First and most significantly, we can discard the spatial component of the coupling between cells and consider all-to-all coupling. Second, we consider a population that has grown to the approximate carrying capacity of a bioreactor, so we can assume that \( n \) is constant. Third, because of the mixing and because of the long time span of the CDC (several hours), we can assume that the time span of the feedback signals between cells is negligible (this assumption is confirmed later, through analysis of the Gap and Mediated models).

In the Immediate and Gap models, we consider a population of \( n \) cells \( c_i, i = 1 \ldots n \), where each \( c_i \in S^1 \) represents a cell’s position in the CDC. The system is represented by the system of coupled ODEs:

\[
\dot{c}_i = \begin{cases} 
1 & c_i \not\in R \\
1 + f(I) & c_i \in R
\end{cases}
\]

\[
I = \frac{\#\{i : c_i \in S\}}{n}
\]

\[
f : [0, 1] \rightarrow (-1, \infty)
\]

\[
S = [\varepsilon, s)
\]

\[
R = [r, 1)
\]

(3.1.1)

where \( f \) is monotone and \( 0 \leq \varepsilon < s < r < 1 \). The case \( \varepsilon = 0 \) is the Immediate model and \( \varepsilon > 0 \) is the Gap model, so called because this case adds a gap between the \( S \) and \( R \) phases. Unless otherwise noted, the analysis that follows will be for the Immediate model.
We distinguish between positive and negative feedback, which correspond to the conditions \( f \geq 0 \) and \( f \leq 0 \) respectively. In numerical experiments, both types of feedback produce clustering generically, although the number of clusters that arise depends on the type of feedback as well as the parameters \( \varepsilon, s, \) and \( r \). Remarkably, the strength of the feedback is largely unrelated to the number of clusters that form in simulation.

Additionally, reversing the order of \( S \) and \( R \), i.e. setting \( S = [s, 1) \) and \( R = [\varepsilon, r) \) with \( 0 \leq \varepsilon < r < s < 1 \) has the same effect as reversing the type of feedback. We have only considered this effect informally and through numerical experimentation, but the mechanism is clear: in an R-S configuration, positive feedback moves cells in \( R \) closer to \( S \), while in an S-R configuration the opposite is true, and similarly for negative feedback.

### 3.1.1.1 Clusters and Cyclic Solutions

Although the model is formulated in terms of \( n \) interacting cells, we are chiefly interested in configurations where many of the \( c_i \) coincide, called *clustered* solutions. We define:

**Definition 3.1.1.** A group of cells is a set \( x = \{c_i\}_{i \in J} \) with diameter equal to \( \varepsilon \) for some \( 1 \gg \varepsilon \geq 0 \).

**Definition 3.1.2.** A cluster is a group of diameter 0.

Because the system is symmetric with respect to labeling, and because the nodes are identical, we see that if \( c_i(t) = c_j(t) \) then they will remain equal for all time, thus we can treat a cluster as a fundamental object and consider the \( k \)-dimensional subspace of \( \mathbb{T}^n \) consisting of \( k \) clusters \( x_1, \ldots, x_j \). Although in reality a cluster will not be perfectly synchronized, such analysis is nonetheless very useful. For example, in [48] the authors show that the clustering predicted by RS models remains robust even under several types of relatively large noise.

In fact, mathematically there is no difference between a clustered solution and an unclustered solution; a system with \( k \) equally sized clusters is formally equivalent to a system with \( k \) cells. Biologically however these represent very different situations; the former represents an instance of autonomous oscillation, while for large \( k \) the latter represents a completely uncoordinated population. For this reason, we will continue to distinguish between the two types of solution.
Also important to our analysis is the synchronized solution, that is a configuration where \( c_i = c_j \) for every pair \( i, j \). One of the first results we obtained in [43] is the following.

**Proposition 3.1.1.** The synchronized solution is locally stable for positive feedback and unstable for negative feedback.

**Proof.** A perturbation of the synchronized solution is a group of cells all within a small distance of one another. We distinguish the leading cell \( c_\ell \) and the trailing cell \( c_t \) of the group and consider the distance between them \( \delta(t) = c_\ell(t) - c_t(t) \pmod{1} \). We can assume that the perturbation is sufficiently small so that \( \delta(0) < \min\{s, 1 - r\} \).

When \( c_\ell \) reaches 1 and enters the signaling region \( S \), \( c_t \) is still in \( R \), which causes its speed to increase (positive feedback) or decrease (negative). Thus when \( c_t \) reaches 1 and exits \( R \), \( \delta \) will have decreased (positive feedback) or increased (negative feedback). This will repeatedly occur until the group size converges to zero (positive feedback) or grows to \( \max\{s, 1 - r\} \) (negative).

\[ \square \]

The essential property of a synchronized solution that leads to this result is that unperturbed, the cluster always travels with speed 1, as there are no cells in \( S \) when it is in \( R \). This property can be generalized.

**Definition 3.1.3.** An isolated cluster is one that never experiences feedback and thus travels with constant speed 1.

**Definition 3.1.4.** An isolated group is a group consisting of cells which never experience feedback.

Isolated clusters play a large role in the observed dynamics of positive feedback systems. Of course a system with exactly one cluster, i.e. a synchronized solution, represents a trivial example of a system consisting of isolated clusters. In fact, nontrivial solutions consisting of isolated clusters exist for many choices of parameters. To see this, for any \( 0 < s < r < 1 \), define:

\[
M = \left| \frac{1}{s + 1 - r} \right|. \tag{3.1.2}
\]
Figure 3.1 illustrates the regions of parameter space that correspond to \( M \) for each integer \( M \geq 1 \). The number \( M \) represents the maximum number of isolated clusters that can exist simultaneously, and there is always a periodic solution consisting of \( M \) isolated clusters, which has as a representative member the solution with initial condition given by:

\[
x(0) = (0, 1/M, 2/M, \ldots, (M - 1)/M).
\] (3.1.3)

The clusters in this solution will remain isolated for all time because the distance between them is \( 1/M \) which is no less than \( s + 1 - r \) by definition. We further define:

**Definition 3.1.5.** A strictly isolated cluster is a cluster such that the nearest cell in front and behind the cluster is strictly greater than \( s + 1 - r \) away.

In numerical simulations the solutions we observe are often related to the number \( M \): with positive feedback, evenly distributed initial conditions tend toward solutions with \( M \) clusters, and with negative feedback, we usually see \( M + 1 \) clusters form. Under negative feedback, interaction between clusters is the mechanism that causes clusters to form, and \( M + 1 \) is the minimum number of clusters which guarantees that interaction will occur. As this mechanism is not necessary for clustering under positive feedback, cells that are not isolated from one another tend to cluster, leading to solutions consisting of isolated clusters. Also notable from simulations is that synchronization only occurs with positive feedback.

In Figure 3.3, the results of numerical experiments are pictured, where the color corresponds to how many clusters formed after 300 time units for the given \( s \) and \( r \) values. The \( M \)-bands are apparent, but do not completely characterize the number of clusters that actually form. A more thorough explanation of the observed dynamics has been provided in [46].

Every solution consisting of \( k \leq M \) isolated clusters is periodic. There is a special class of periodic solution which we call a \( k \)-cyclic solution:

**Definition 3.1.6.** A \( k \)-cyclic solution is a system of \( k \) clusters \( x_j, \ j = 0, \ldots, k - 1 \) such that for some positive time \( d \), \( x_j(d) = x_{j+1}(0) \) and \( x_{k-1}(d) = x_0(0) \).
In other words, in time $d$, the clusters permute cyclically and the system is indistinguishable from the initial condition after relabeling. Note that we allow for the possibility of $n$ clusters containing a single cell each; we sometimes refer to this as the uniform solution, because the clusters are spread out as uniformly as possible for a periodic solution. The $k$-cyclic solutions are closely related to a discretization of the system (3.1.1) called the return map $F$, which is the $n$th root of the natural Poincaré map defined as follows.

Consider the subspace of phase space $P$ consisting of all configurations of cells such that $c_0 = 0$. Because a cell’s velocity is bounded away from zero, every orbit starting from an initial condition in $P$ will return in finite time. Thus $P$ defines a Poincaré section of the phase space and we denote the corresponding Poincaré map by $P$.

For an initial condition in $P$ consisting of $k$ clusters, we can assume that the clusters are ordered so that $0 = x_0 < x_1 < \ldots < x_{k-1}$. We define the return map $F$ by starting with an initial condition $x \in P$ and running time forward until $x_{k-1}(t^*) = 1$. The new value of $x_{j+1}$ is set to be $x_j(t^*)$ for each $j = 0, \ldots, k-2$ and $x_0$ is set to be 0. Figure 3.4 illustrates $F$ for a three-cluster system.
To extend this definition rigorously to the boundary of the space, we consider 0 to be equivalent to but distinct from 1, so that $x_{k-1}$ may reach 1 in zero time, but it will be relabeled. For instance, if $x = \langle 0, 0.3, 0.7, 1, 1 \rangle$, then $F(x) = \langle 0, 0, 0.3, 0.7, 1 \rangle$ and $F^2(x) = \langle 0, 0, 0.3, 0.7 \rangle$.

Now because $x_0$ is always 0, we can ignore it and formally define $F$ to map the $(k - 1)$-dimensional subspace $\{x \in [0, 1]^k : x_0 = 0\}$ into itself. This is useful for the analysis of 2-cluster systems to follow. It is also useful to note that with this convention, and considering the preceding example, $F$ permutes the hyperplanes $\{x : x_j = 1\}$ that make up the boundary of the space. A fixed point of $F$ corresponds in 1-1 fashion to a $k$-cyclic solution, which we use in the proof of the following theorem.

**Theorem 3.1.1.** There exists a $k$-cyclic solution for every $k$ dividing $n$ and for any $0 < s < r < 1$. If $f$ is a negative feedback function, then the $k$-cyclic solution is unique.

**Proof.** Considering $F$ as a function on $[0, 1]^{k-1}$, we note that it maps a compact set into itself. Thus $F$ has at least one fixed point, corresponding to the desired solution. Now the boundaries of the

---

**Figure 3.2:** Regions of stability / instability for $(k + 1)$-cyclic solutions, illustrated for positive feedback.
Figure 3.3: For each point in parameter space, a system with 420 cells was integrated for 300 cell cycles. The feedback function was taken to be $f(I) = -0.6I$. The number of clusters that formed is pictured.

Figure 3.4: An illustration of the map $F$ for the 3-cluster system. Here $x_i \mapsto x'_i$.

domain of $F$ are the points where one of the clusters is at 1, and as noted above, $F$ permutes these sets, so there can be no fixed points on the boundary. Therefore the guaranteed fixed point must be in the interior of the set, proving existence.

From the definition we see that a $k$-cyclic solution must be of the form:

$$x = \langle 0, d, \ldots, ad, 1 - be, \ldots, 1 - e \rangle$$

for nonnegative integers $a$ and $b$ with $a + b + 1 = k$. 
For uniqueness under negative feedback, we use a monotonicity argument. In brief, a choice of \( d \) uniquely determines the value of \( e \) in (3.1.4). We will show that there is exactly one value of \( d \) for which the resulting initial condition (3.1.4) represents a \( k \)-cyclic solution.

If \( f \) is a negative feedback function, a cluster’s speed is bounded above by 1. Therefore \( 1/k \) is a lower bound for \( d \). For a given choice of \( d \geq 1/k \), we define \( \delta = s \pmod{d} \), the time at which the first cluster leaves \( S \). This allows us to define \( I(t) \) to be \( \lfloor s/d \rfloor + 1 \) for \( t \in [0, \delta] \) and is \( \lfloor s/d \rfloor \) for \( t > \delta \). This definition is motivated by the fact that in a \( k \)-cyclic solution, exactly one cluster must leave \( S \) during an application of \( F \).

With this definition of \( I(t) \), we can then define \( e \). The form of this definition depends on the values of \( d \) and \( r \). We have:

\[
e = \begin{cases} 
\int_0^d 1 + f(I(t)) \, dt, & \text{if } d < r \text{ and the integral is less than } 1 - r, \\
1 - (k - 1)d, & \text{if } r \geq (k - 1)d, \text{ or} \\
1 - \frac{d}{k - 1}, & \text{if } d \geq r.
\end{cases} \tag{3.1.5}
\]

The first form intuitively corresponds to ‘large enough’ \( k \), the second to a ‘small’ responsive region, and the third to a ‘large’ responsive region.

Once \( d \) and \( e \) are defined, the integers \( a \) and \( b \) from (3.1.4) are determined as follows: \( a = \lfloor r/d \rfloor \), and \( b = \lfloor (1 - r)/e \rfloor \). Note that in the second case of the definition of \( e \), \( b = 0 \), and in the third, \( a = 0 \).

Consider the set \( D = \{ d \geq 1/k : a + b + 1 = k \} \). A \( k \)-cyclic solution must be of the form (3.1.4) for some \( d \in D \). We define a function \( \phi \) on this set whose roots correspond to cyclic solutions in a 1-1 fashion.

Let \( m \) be the largest integer such that \( md < r \). Then \( x_m \) is the cluster in (3.1.4) that is closest to \( r \) on the left, i.e. the clusters to the left of \( x_m \) are evenly spaced at a distance of \( d \) from each other, but the cluster to the right of \( x_m \) (if any exists) is not.
Define:

\[ \phi(d) = \begin{cases} 
  x_m(d) - x_{m+1}(0), & \text{if } m < k - 1, \\
  x_m(d) - 1, & \text{if } m = k - 1.
\end{cases} \tag{3.1.6} \]

Then a root of \( \phi \) corresponds to a cyclic solution, and vice versa. What’s more, \( \phi \) is strictly increasing. This can be seen from the definition of \( e \) and noting that increasing \( d \) serves to decrease the total amount of feedback, thus increasing \( e \).

Since a \( k \)-cyclic solution is guaranteed to exist, \( \phi \) must have a root. And since \( \phi \) is strictly monotone, that root is unique, and the result is proved.

Of course, as noted above, when \( k \leq M \) the cyclic solution consists of isolated clusters. It is sometimes useful to consider dynamics not in the full phase space, but in the subspace of \( k \)-cluster solutions with \( k << n \). In this setting, which is formally the space \( \mathbb{T}^k \), there is a natural distance defined as the max of the mod 1 distances between corresponding clusters.

**Proposition 3.1.2.** If a solution consisting of \( k \) isolated solutions under positive feedback is perturbed, it will converge to another solution with \( k \) isolated clusters. The set of all such solutions, if any exist, is locally repelling at the boundary as a subset of the \( k \) cluster solutions.

**Proof.** Suppose \( x \) is a \( k \) cluster solution in a small neighborhood of the isolated cluster solutions. Then for each pair of clusters that are distance less than \( s + 1 - r \) apart, the positive feedback will shorten the gap between them indefinitely, i.e. the system will converge to the boundary of the isolated \((k - 1)\)-cluster solutions.

If the feedback is positive, then the set of strictly isolated clusters is locally attracting in the full phase space.

**Proposition 3.1.3.** Suppose \( f \) is a positive feedback function. For any initial condition \( x \) of strictly isolated clusters, there is an \( \varepsilon > 0 \) so that in an \( \varepsilon \)-neighborhood of the initial condition in the full
phase space, every initial condition tends toward a solution with strictly isolated clusters (which
may be distinct from $x$).

**Proof.** If $\varepsilon$ is small enough, then any solution in an $\varepsilon$ neighborhood of a solution with strictly isolated clusters must consist of $k$ groups which are each strictly isolated from one another; see Figure 3.5. As an easy corollary of Prop 3.1.1, the width of each group must tend to zero, so that the system approaches a configuration with $k$ strictly isolated clusters.

\[ \varepsilon \]

\[ R \]

\[ S \]

\[ 0 \equiv 1 \]

**Figure 3.5:** Perturbing isolated clusters results in isolated groups.

Negative feedback is a different story, in fact the results are essentially opposite.

**Proposition 3.1.4.** If a solution with $k$ isolated clusters under negative feedback exists for a given $s$ and $r$, then the set of all such solutions is locally stable in $k$-cluster subspace.

**Proof.** Suppose $x$ is a $k$ cluster solution in a small neighborhood of the isolated cluster solutions. then for each pair of clusters that are distance less than $s + 1 - r$ apart, the negative feedback will
lengthen the gap between them until it is exactly $s + 1 - r$, i.e. the system will converge to the boundary of the isolated cluster solutions.

\[\square\]

**Proposition 3.1.5.** Suppose $f$ is a negative feedback function. If $x$ is an initial condition consisting of strictly isolated clusters, then for any $\varepsilon > 0$, there is an initial condition $y$ with $\|x - y\| < \varepsilon$ such that the trajectory of $y$ is away from the set of $k$-cluster solutions.

**Proof.** Choose an $\varepsilon$-perturbation in the full phase space that consists of isolated groups of cells each with width no more than $\varepsilon$. Again following Prop 3.1.1, we see that the width of each group will grow until they are no longer isolated. Even if the resulting configuration tends toward clusters, there will be more than $k$ such clusters.

\[\square\]

To rephrase the Proposition, if a solution with $k$ strictly isolated clusters exists, then the set of all such solutions is locally unstable in the full phase space.

### 3.1.1.2 Cyclic Solutions with $k = M + 1$

Under negative feedback, numerical simulations very often lead to systems with $k \geq M + 1$ clusters. In the case with $k$ exactly equal to $M + 1$ we can determine the stability of the solution analytically.

**Theorem 3.1.2.** Let $\beta = f(1/k)$. The $k = (M + 1)$-cyclic solution will be unstable for positive $\beta$ and stable for negative $\beta$ if

\[
s < \frac{1}{k} \left( \frac{1 + \beta r}{1 + \beta} \right) \quad \text{and} \quad r > \frac{k - 1}{k} (1 - s\beta),
\]

(3.1.7)

and neutrally stable otherwise, where stability is within the set of $k$ cluster solutions.

**Proof.** In $s, r$ parameter space, the regions where $M$ is constant are diagonal bands. The inequalities in (3.1.7) are linear inequalities that separate each band into three regions which we denote I, II, and III. These regions are illustrated in Figure 3.1.
Suppose \( \mathbf{x} = \langle 0, x_1, \ldots, x_{k-1} \rangle \) is a \( k \)-cyclic solution. Note that because \( k = M + 1 \), at any time that \( R \) is nonempty there is at most one cluster in \( S \), which is necessarily \( x_0 \). Recall that \( F(\mathbf{x}) \) is defined by letting time run forward until \( x_{k-1} \) reaches 1. During that time, \( x_0 \) must reach \( s \) and if \( x_{k-1}(0) < r \) then \( x_{k-1} \) must reach \( r \). The order in which these events happens is determined by \( s \) and \( r \), and we classify the possibilities into three exhaustive cases, given by:

\[
\begin{align*}
\text{Case 1: } & x_{k-1} \mapsto r, \ x_0 \mapsto s, \ x_{k-1} \mapsto 1, \\
\text{Case 2: } & x_0 \mapsto s, \ x_{k-1} \mapsto 1, \\
\text{Case 3: } & x_1 \mapsto s, \ x_{k-1} \mapsto r, \ x_{k-1} \mapsto 1.
\end{align*}
\] (3.1.8)

It is easy to see that Case 1 corresponds to parameter values in region I, Case 2 to values in region II, and Case 3 to values in region III, i.e. in Case 2 we do not require the first inequality in (3.1.7) to hold and in Case 3 we do not require the second to hold.

In each case, the amount of time required for each event to occur can be calculated exactly, leading to a total time \( d \) until \( x_{k-1} \) reaches 1. These values are as follows:

\[
\begin{align*}
\text{Case 1: } d &= \frac{1 + \beta (r - s)}{k + \beta (k - 1)}, \\
\text{Case 2: } d &= \frac{1 - s \beta}{k}, \\
\text{Case 3: } d &= \frac{1 + r \beta}{k(1 + \beta)}.
\end{align*}
\] (3.1.9)

Using the above calculations, the map \( F \) can be explicitly represented as an affine transformation in a neighborhood of \( \mathbf{x} \), given by \( F(\mathbf{x}) = A \mathbf{x} + \mathbf{b} \), where the form of the matrix \( A \) again depends on the order of events. For Case 1 we have:

\[
A = \begin{pmatrix}
0 & 0 & 0 & \cdots & 0 & -(1 + \beta) \\
1 & 0 & 0 & \cdots & 0 & -(1 + \beta) \\
0 & 1 & 0 & \cdots & 0 & -(1 + \beta) \\
\vdots & \vdots & \ddots & \ddots & \vdots & \vdots \\
0 & 0 & 0 & \cdots & 0 & -(1 + \beta) \\
0 & 0 & 0 & \cdots & 1 & -(1 + \beta)
\end{pmatrix}.
\] (3.1.10)

Stability of the cyclic solution is determined by the eigenvalues of \( A \), which has characteristic equation:

\[
-\lambda^{k-1} - (1 + \beta) \left( \lambda^{k-2} + \lambda^{k-3} + \cdots + \lambda + 1 \right) = 0.
\] (3.1.11)
It is immediately clear that $\lambda = 1$ is not a possibility. For $\lambda \neq 1$, we can rewrite this as:

$$\frac{1}{1 + \beta} \lambda^n + \sum_{i=0}^{n-1} \lambda^i = \frac{\lambda^n - 1}{\lambda - 1} = 0.$$  \hfill (3.1.12)

Therefore $\lambda \neq 1$ is an eigenvalue of $A$ if and only if

$$\left(\frac{\lambda + \beta}{1 + \beta}\right)^n = 1. \hfill (3.1.13)$$

Now, suppose the system has positive feedback, i.e. $\beta > 0$. If $|\lambda| \leq 1$ (and $\lambda \neq 1$), then $|\lambda^n| \leq 1$ and $|\lambda + \beta| < |1 + \beta|$. Thus

$$\frac{|\lambda + \beta|}{1 + \beta} \cdot |\lambda^n| < 1, \hfill (3.1.14)$$

i.e. $\lambda$ cannot satisfy (3.1.13). Thus for positive feedback all of the eigenvalues lie outside the unit disk and so the map is unstable at the cyclic solution. In fact it is unstable in every direction, which is not surprising because of the symmetry of the system.

If instead we have $\beta < 0$, suppose that $|\lambda| > 1$. By the reverse triangle inequality, $|\lambda + \beta| = |\lambda - (-\beta)| \geq |\lambda| - |\beta| = |\lambda| + |\beta| = |\lambda| + \beta > 1 + \beta$. Thus

$$\frac{|\lambda + \beta|}{1 + \beta} \cdot |\lambda^n| > 1 \hfill (3.1.15)$$

and again (3.1.13) is not satisfied, so $\lambda$ cannot be an eigenvalue of $A$.

Note also that if $|\lambda| = 1$ but $\lambda \neq 1$ then $|\lambda + \beta| < 1 + \beta$, i.e. in a negative feedback system all the eigenvalues lie on the interior of the unit disk and the map is stable at the $k = (M + 1)$-cyclic solution in Case 1. Plots of the eigenvalues of $A$ for various $k$ are given in Figure 3.6.

In Cases 2 and 3, the matrix $A$ at $x$ is given by

$$A = \begin{pmatrix} 0 & 0 & 0 & \cdots & 0 & -1 \\ 1 & 0 & 0 & \cdots & 0 & -1 \\ 0 & 1 & 0 & \cdots & 0 & -1 \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & 0 & -1 \\ 0 & 0 & 0 & \cdots & 1 & -1 \end{pmatrix}. \hfill (3.1.16)$$

which has characteristic equation $\lambda^{k-1} + \lambda^{k-2} + \ldots + \lambda + 1 = 0$, the roots of which all have magnitude 1. Thus the map is neutrally stable in Cases 2 and 3, and the theorem is proved.
Figure 3.6: For negative feedback, the \((M+1)-\)cyclic solutions are stable, and for positive feedback they are unstable. Here the eigenvalues of the linearization at the \(k = (M + 1)\)-cyclic solution are plotted for \(k = 2, \ldots, 12\) as the feedback parameter \(\beta\) varies on \((-0.5, 0.5)\). For negative feedback, the spectral radius is plotted, and for positive feedback, the minimum modulus eigenvalue is plotted.

\[ k = 2 \quad k = 4 \quad k = 6 \quad k = 8 \quad k = 10 \quad k = 12 \]

3.1.1.3 Dynamics for \(k = 2\)

Here we present an overview of the system with \(k = 2\), for two reasons. One, two-cluster solutions are the ones we most often see in nature, and fully understanding the dynamics in this case may find application in non-mathematical settings. Second, many of the concepts introduced above are given more concrete meaning in this context, and specific instances of the above theorems regarding stability are shown. Throughout we will use the convention that \(\alpha = f(1/2)\).
First we will construct the map $F$, using the principle of order of events introduced earlier. Note that when $k = 2$, the equation $\dot{x}$ in (3.1.1) simplifies to:

$$
\dot{x}_j = \begin{cases} 
1 + \alpha & x_j \in R \text{ and } x_i \in S \text{ for } i \neq j, \\
1 & \text{otherwise}. 
\end{cases}
$$

(3.1.17)

Recall that $F$ is a function of a single variable since we require $x_0 = 0$, and $F(x_1) = x_0(t)$ where $x_1(t) = 1$. The form of $F$ depends on the parameters $s$ and $r$; specifically on whether $(1 + \alpha)s$ greater or less than $1 - r$. Note that these two cases actually represent two different orders of events; in the first, the cyclic solution follows $x_0 \mapsto s$ before $x_1 \mapsto r$, and the reverse is true in the second case. In both cases $F$ is continuous, piecewise linear, and decreasing.

When $r + (1 + \alpha)s < 1$, we have:

$$
F(x_1) = \begin{cases} 
1 - x_1, & 0 \leq x_1 \leq r - s \\
1 - (1 + \alpha)x_1 + \alpha(r - s), & r - s < x_1 < r \\
1 - x_1 - \alpha s, & r \leq x_1 < 1 - (1 + \alpha s) \\
\frac{1 - x_1}{\alpha + 1}, & 1 - (1 + \alpha)s < x_1 \leq 1 
\end{cases}
$$

(3.1.18)

and when $r + (1 + \alpha)s \geq 1$, we have:

$$
F(x_1) = \begin{cases} 
1 - x_1, & 0 \leq x_1 < r - s \\
1 - (1 + \alpha)x_1 + \alpha(r - s), & r - s < x_1 \leq 1 + \frac{ar}{\alpha + 1} - s \\
r - x_1 + \frac{1 - r}{\alpha + 1}, & 1 + \frac{ar}{\alpha + 1} - s < x_1 \leq r \\
\frac{1 - x_1}{\alpha + 1}, & r < x_1 \leq 1 
\end{cases}
$$

(3.1.19)

Both cases are shown in Figure 3.7, and the resulting possibilities for the Poincaré map $P = F^2$ are shown in Figure 3.8. The dynamics of $P$ for $k = 2$ are completely determined by $F$, and can be summarized as follows: for positive feedback there is either a unique unstable fixed point with $x_1 \neq 0$ or an interval of such fixed points whose boundary is repelling. Every other orbit is asymptotic to the synchronized solution $x_1 = 0$. For negative feedback, there is either a unique
stable fixed point $x_1 \neq 0$ or an interval of such points whose boundary is globally attracting on the interior of the phase space $(0, 1)$.

Note that the intervals of fixed points occur for two reasons. First, as noted above, if the clusters are isolated then a small perturbation will leave them isolated. Second, if $S$ or $R$ is so large that either $x_0$ is in $S$ for the entire time that $x_1$ is in $R$ or vice versa, then the unique fixed point is surrounded by an interval of neutral period 2 points of $F$.

![Figure 3.7: The two cases for $F$ in the 2-cluster system, pictured for positive feedback. Stability of the fixed point is determined by the slope of the line segment that intersects the diagonal $r = s$.](image)

### 3.1.1.4 The Gap Model

In [47] we considered the system (3.1.1) with $\epsilon > 0$, that is with a positive gap. In general, $\epsilon$ is assumed to be small but for completeness we considered a large gap as well. To explore this possibility, we conducted numerical experiments as follows.

We first set $0 < s < r < 1$ so that $s > 1/2 > 1 - r$. We then allowed $\epsilon$ to vary on the interval $(0, s)$ and performed a numerical simulation for each value of $\epsilon$, using 100 equidistributed cells as the
Figure 3.8: The four possible dynamics for the map $P = F^2$ in the 2-cluster system. Clockwise from top left: an attracting interval of neutrally stable fixed points, a unique repelling fixed point, a repelling interval of neutrally stable fixed points, and a unique attracting fixed point.

initial condition. At the end of the simulation the location of each cell was plotted along a vertical line segment, and the results (with $s = 0.5$, $r = 0.8$, and negative feedback) can be seen in Figure 3.10. For small values of $\varepsilon$, two clusters emerge as predicted. As $\varepsilon \to 0.3$, a bifurcation occurs and disordered behavior is apparent at $\varepsilon = 0.3$ (see Figure 3.9). Beyond the critical value $\varepsilon = 0.3$,
the dynamics are identical to that of a positive feedback system and we see a single group of width 
0.3 = r – s form, again as predicted.

A similar experiment was performed with s = 0.5 and r = 0.9. Figure 3.10 shows time series plots 
for values of ε before, at, and after the critical value. When ε < 0.4, there are M + 1 = 2 clusters as 
predicted in a negative feedback system. At ε = 0.4 the behavior is unpredictable and unclustered. 
When ε > 0.4, exactly M clusters form, again as expected in a positive feedback system.

The critical value of ε is such that the signaling and responsive regions are exactly equal in size 
and symmetrically placed on the circle. The reason that clustering does not occur for this specific 
configuration is as follows. For time t < ε, the feedback effect on cells in R causes an increase 
in density in that region, and a corresponding decrease in density on either side. This group of 
dense cells reaches S just as the last cell which began in S reaches R. There is again an increase 
in density in the R region, but this dense group never interacts with the original one because of the 
equal spacing.

Figure 3.9: The dynamics in the Gap model are very predictable for even relatively large values 
of ε. As the gap reaches and crosses a critical threshold, the system undergoes a bifurcation and 
becomes equivalent to a system with the feedback reversed. Here the final positions of 100 cells 
are plotted as ε varies on (0, 0.32) with s = 0.5, r = 0.8.
Figure 3.10: Time series plots of the Poincaré map $P$ for values of $\varepsilon$ below, equal to, and above the critical value. Here $s = 0.5$ and $r = 0.9$.

3.1.2 Mediated Model

The Gap model introduced a biologically motivated delay in the action of the feedback. The existence of some form of delay is a biological necessity, as signaling of the kind we consider in yeast must be done through intermediary chemicals or proteins with finite rates of diffusion. Because of the homogeneous environment inside a bioreactor, and based on the results detailed in [47], we expect the immediate model to be a reasonable approximation to the true dynamics of the system, but it is worthwhile to investigate a more biologically accurate model as well.

To this purpose, in [50] we extended the Immediate model to include a new variable $z$, which represents the level of the signaling agent in the culture. We assume that $z$ is created by an
individual signaling cell at a constant (positive) rate and that the total concentration is self limiting with coefficient $\mu$. The resulting model is called the Mediated model and is given by:

$$
\dot{c}_i = \begin{cases} 
1 & c_i \notin R \\
1 + f(z) & c_i \in R 
\end{cases}
$$

$$
\dot{z} = \alpha I - \mu z
$$

$$
I = \#\{i : c_i \in S\}/n
$$

$$
f : [0, 1] \to (-1, \infty)
$$

$$
S = [0, s)
$$

$$
R = [r, 1)
$$

where $\alpha$ and $\mu$ are positive, and $s$, $r$, and $f$ are defined as before. Note that $z$ is driven by the proportion of signaling cells, and not the number of such cells. This means that $\alpha$ and $\mu$ should be considered as density dependent variables; more concentrated signaling cells should lead to a faster increase in signaling agent and a larger $\alpha$, while more responsive cells should cause a greater rate of cell process-related degradation, and thus a larger $\mu$. Although biologically we expect different values for $\alpha$ and $\mu$, the following result allows us to make an immediate simplification to the model:

**Proposition 3.1.6.** For any positive $\alpha$ and $\mu$, the system (3.1.20) is equivalent to one with $\alpha = \mu$.

**Proof.** The equivalence is achieved by the change of variables $\phi = I - (\alpha I - \mu z)^{\alpha/\mu}$. Then:

$$
\frac{d}{dt} \phi = \phi'(z) \dot{z}(t) = \alpha (\alpha I - \mu z)^{\alpha/\mu - 1} \cdot (\alpha I - \mu z) = \alpha (\alpha I - \mu z)^{\alpha/\mu} = \alpha I - \alpha \phi.
$$

\[\Box\]

For the remainder of this work we assume $\alpha = \mu$, and modify (3.1.20) by letting $\dot{z} = \alpha(I - z)$. Then $z(0) \in [0, 1]$ implies that $z(t)$ will remain in $[0, 1]$ for all times $t > 0$. This makes the state space for the system $\mathbb{T}^n \times [0, 1]$. 
Similar to the Immediate model, there is a natural Poincaré section \( P = 0 \times \mathbb{T}^{n-1} \times [0, 1] \). The Poincaré map takes an initial configuration of cells in \( P \) along with a value of \( z \) in \([0, 1]\) and runs time forward until \( c_0 \) reaches 1. The return map \( F \) is also defined similarly to before.

Lemma 3.1.1 says that for time intervals with constant \( I \), \( z(t) \) approaches \( I \) and the rate of convergence depends on the parameter \( \alpha \). We will use it in the next section to show that when \( \alpha \) is large, the Mediated model approximates the Immediate model arbitrarily closely.

**Lemma 3.1.1.** Suppose \( I(t) = I \) is constant for \( t \in [t_1, t_2] \). For any \( \delta > 0 \) there is a large enough \( \alpha \) so that \( |z(t) - I(t)| < \delta \) for every \( t \) with \( t_1 + \delta < t \leq t_2 \).

**Proof.** Note that on \([t_1, t_2]\), \( z(t) \) is given by:

\[
z(t) = I + (z(t_1) - I) e^{-\alpha(t-t_1)},
\]

and in particular,

\[
|z(t_1 + \delta) - I| = |z(t_1) - I| e^{-\alpha \delta}
\]

Since \( |z(t) - I| \) is bounded by 1, the desired result follows by choosing \( \alpha > \frac{1}{\delta} \ln \left| \frac{z(t_1) - I}{\delta} \right| \).

\( \square \)

### 3.1.2.1 Comparison with the Immediate Model

Since the Mediated model is an extension of the Immediate model, we expect some things that are true about the latter to remain true for the former. For example, we have the following analogue of Theorem 3.1.1:

**Theorem 3.1.3.** Fix \( 0 < s < r < 1, \alpha > 0 \), and \( k > 0 \). Then there exists a cyclic solution consisting of \( k \) clusters. If in addition \( f \) is a negative feedback function, that solution is unique.

**Proof.** The proof of existence is almost identical to that of Theorem 3.1.1. The addition of \( z \) requires only that we verify that \( F \) still has no fixed points on the boundary. But the state space for \( z \) is \([0, 1]\) and its vector field points inward on the boundaries 0 and 1, for any value of \( I \).
Proof of uniqueness under negative feedback is also by a similar argument; each choice of \( d \geq 1/k \) uniquely determines a distance \( e \), which in turn determines \( a \) and \( b \) that define the admissible set \( D \). The function \( \phi \) is similarly defined, but in this case monotonicity of \( \phi(d) = x_a(d) - x_{a+1}(0) \) is less obvious because of the addition of \( z \). It is still true, but we provide the calculations only for the main case; the special cases \( a = 0 \) and \( b = 0 \) are similar.

Starting from an initial configuration of the form (3.1.4), there is a unique \( \xi_d \) satisfying \( z(d) = \xi_d = z(0) \) given by

\[
\xi_d = \frac{(1/k)e^{\alpha \delta} + (I(0) - 1/k)e^{\alpha d} - I(0)}{e^{\alpha d} - 1},
\]  
(3.1.24)

where \( I \) and \( \delta \) are defined as in Theorem 3.1.1. Consider \( \frac{d}{dd} \xi_d \); \( I(0) \) is constant in \( d \) except for separated jump discontinuities where it decreases. On \( d \) intervals of constant \( I(0) \), we note that \( \frac{d}{dd} \delta = -1 \). Therefore \( \frac{d}{dd} \xi_d < 0 \) wherever the derivative is defined.

Now, a pair \( d, \xi_d \) uniquely determines \( e \) by:

\[
e = \int_0^d 1 + f(z(t; \xi_d)) \, dt.
\]  
(3.1.25)

Both the integrand and the upper limit are increasing in \( d \), since \( f \) is decreasing in \( z \). Thus \( e \) is a strictly increasing function of \( d \). At this point, the argument for monotonicity of \( \phi \) from Theorem 3.1.1 carries through, and the result is proved.

\[\square\]

In contrast to the previous result, one of the first results from the Immediate model is reversed. We will resolve this seeming contradiction later, in the section on the small \( \alpha \) domain.

Because \( z \) is nonzero, a cell in \( R \) will experience feedback even when there are no signaling cells. For a group of positive width, there is thus always an \textit{entrance effect} that serves to widen or focus the group as it crosses the boundary into \( R \), and a similar \textit{exit effect} as the group exits \( R \). These effects are determined by the trajectory of \( z \) during the time intervals when the group is entering and leaving the responsive region; because \( f \) is monotone then it is useful to think of the strength of these effects as synonymous with the definite integral of \( z \) over these same integrals.
Definition 3.1.7. Suppose \( x = \{c_i\} \) is a group of cells of diameter \( \varepsilon << 1 - r \), contained entirely in \([0, r]\) and positioned so that the leading cell is equal to \( r \). On the time interval \([0, \varepsilon]\), the leading cell will experience feedback and the trailing cell will not. Let \( \delta \) be the diameter of the group at time \( \varepsilon \). We define the entrance effect on \( x \) to be \( \delta - \varepsilon \).

Definition 3.1.8. For a group of cells of diameter \( \varepsilon \) positioned so that the leading cell is equal to \( 1 \), we similarly define the exit effect on \( x \) to be the difference \( \delta - \varepsilon \) where \( \delta \) is the diameter of the group at the time the trailing cell reaches \( 1 \).

It should be noted that entrance and exit effects are local, that is they will not remain the same even for the same group of cells over multiple cell cycles. The complex interactions between the group, other cells in the system, and between those other cells may lead to chains of effects that are difficult to quantify. Nonetheless, the concept is useful because a prerequisite for a particular clustered solution to be stable is that for each of its clusters, the entrance and exit effects must combine to focus a perturbation of that cluster into a group back together. These concepts also provide language for analyzing the synchronized solution.

Proposition 3.1.7. The synchronized solution is stable under negative feedback and unstable under positive feedback.

Proof. With positive feedback, the entrance effect increases group width and the exit effect decreases it, and the reverse is true for negative feedback. Local stability of the synchronized solution is equivalent to saying that the focusing effect is stronger than the defocusing one for a sufficiently small perturbation.

Refer to Figure 3.11. Suppose the synchronized solution is perturbed to a group of width \( \varepsilon << 1 - r \), contained entirely in \([0, r]\). At any time when the trailing cell \( x_t \) is not experiencing feedback, \( z \) will be decreasing. As soon as the leading cell \( x_\ell \) enters \( S \), \( z \) begins to increase while \( x_t \) is still in \( R \).

Now since for an individual cell \( c_i \in R \), \( c_i(t) = c_i(0) + \int_0^t 1 + f(z(t)) \, dt \), and because the trailing cell travels with speed 1 while the group enters \( R \), the entrance effect on the group is given by:

\[
\int_{r-x_\ell(0)}^{r-x_t(0)} [1 + f(z(t))] \, dt - \varepsilon. \tag{3.1.26}
\]
Note that the domain of integration has width $\varepsilon$. If $t_\ell$ is the time at which $c_\ell = 1$ and $t_t$ is the time when $c_t = 1$, then the diameter of the group after exiting $R$ is given by:

$$
\int_{t_t}^{t_\ell} \left[ 1 + f(z(t)) \right] \, dt - \int_{t - x_{\ell}(0)}^{t - x_t(0)} \left[ 1 + f(z(t)) \right] \, dt + \varepsilon,
$$

(3.1.27)

where the first integral is taken over an interval of width $\delta$, the final diameter of the group. The value of $\delta$ will be smaller than $\varepsilon$ provided the difference of the integrals is negative, and larger if it is positive. We thus show that if $\varepsilon$ is small enough, the difference is always negative for negative feedback and positive for positive feedback.

Recall that for either type of feedback $f$ is monotone so $1 + f$ is as well. Thus the integral

$$
\int 1 + f(z(t)) \, dt
$$

is also monotone in $z$. Now because $z(r - x_\ell) > z(t_\ell)$, as long as $\lim_{\varepsilon \to 0} \delta/\varepsilon < \infty$, there is a small enough $\varepsilon$ to guarantee

$$
\int_{t_t}^{t_\ell} \left[ 1 + f(z(t)) \right] \, dt - \int_{t - x_{\ell}(0)}^{t - x_t(0)} \left[ 1 + f(z(t)) \right] \, dt
$$

(3.1.28)

is negative for negative feedback and positive for positive feedback. But the limit must be finite because $\dot{z}$ is bounded above, so we are done.

When $\alpha$ is small and the convergence is slow, there is a large delay between a signal and the corresponding response. On the other hand, as $\alpha \to \infty$, the delay shortens and the feedback becomes nearly instantaneous, as illustrated in Figure 3.12. We can thus think of the Immediate model as the limiting case of the Mediated model as $\alpha \to \infty$. This idea is made formal in Theorem 3.1.4.

For a given choice of parameters $s, r, \text{ and } \alpha$, and a given initial configuration $c_0$ and initial value $z_0$, we define $c_{\text{imm}}(t)$ to be the solution to (3.1.1) and $c_{\text{med}}(t)$ to be the solution to (3.1.20). The fraction of signaling cells at time $t$ in each system is denoted by $I_{\text{imm}}(t)$ and $I_{\text{med}}(t)$, respectively.

**Theorem 3.1.4.** Fix $0 < s < r < 1$ and $\alpha > 0$. Let $c_0$ be an initial configuration of cells and let $z_0 \in [0, 1]$. For any $T > 0$ and any $\varepsilon > 0$, there exists an $\alpha$ large enough so that for all $0 \leq t \leq T$, we have

$$
\|c_{\text{med}}(t) - c_{\text{imm}}(t)\| < \varepsilon.
$$

(3.1.29)
**Figure 3.11:** A plot of $z(t)$ demonstrating the entrance and exit effects on a perturbation of the synchronized solution.

**Figure 3.12:** As $\alpha \to \infty$, $z(t)$, shown in increasingly dark shades of grey, approaches $I(t)$, in red.

**Proof.** The number of discontinuities in $I_{imm}$ is bounded above by $\left\lceil \frac{2nT}{r} \right\rceil$, since only $2n$ jumps can occur in each cell cycle (each cell enters and leaves $S$). Label the points of discontinuity $\{t_i\}_{i=1}^K$. 
Choose \(\{\delta_j\}_{j=0}^K\) so that:

1. the \(\delta_i\) satisfy:
   \[
   \delta_0(1 + t_1) + \left(\sum_{i=1}^{K-1} \delta_i(1 + t_{i+1} - t_i)\right) + \delta_K(1 + T - t_K) < \frac{\varepsilon}{K + 1},
   \]
   and

2. each of the following integrals is taken over an interval of positive width.

Because there are a finite number of intervals \([t_i, t_{i+1}]\), the second condition can always be satisfied by choosing the \(\delta_j\) small enough.

We now partition each interval \([t_i, t_{i+1}]\) into subintervals to control the signaling discrepancy \(d(t) = |z(t) - I(t)|\). For each \(i = 1, \ldots, (K - 1)\) there is an \(\alpha_i\) from Lemma 3.1.1 so that:

\[
\int_{t_i}^{t_{i+1}} d(t) \, dt = \int_{t_i}^{t_i + \delta_{i-1}(1+t_i)} d(t) \, dt + \int_{t_i + \delta_{i-1}(1+t_i)}^{t_{i+1} + \delta_{i-1}(1+t_i)} d(t) \, dt + \int_{t_{i+1}}^{t_{i+1} + \delta_{i}} d(t) \, dt. \tag{3.1.30}
\]

The limits of integration are chosen so that once \(t = t_i + \delta - (1 + t_i)\), \(I_{med}\) is guaranteed to be equal to \(I_{imm}\). The integral on the whole interval \([t_i, t_{i+1}]\) is thus bounded by:

\[
\int_{J_{t_i}}^{t_{i+1}} d(t) \, dt \leq \delta_{i-1}(1 + t_i) + \delta_i(1 + t_{i+1} - t_i) = \varepsilon_i. \tag{3.1.31}
\]

In general,

\[
\varepsilon_i = \varepsilon_{i-1} + \delta_i(1 + t_{i+1} - t_i), \tag{3.1.32}
\]

for appropriate choices of \(\alpha_i\). Repeating in this fashion, we have

\[
\varepsilon_K = \delta_0(1 + t_1) + \left(\sum_{i=1}^{K-1} \delta_i(1 + t_{i+1} - t_i)\right) + \delta_K(1 + T - t_K) < \frac{\varepsilon}{K + 1}, \tag{3.1.33}
\]

(by construction), and so in fact \(\varepsilon_i < \frac{\varepsilon}{K + 1}\) for each \(i = 1, \ldots, K\). The first interval \([t_0, t_1]\) can be treated similarly; there is an \(\alpha_0\) so that:

\[
\int_{0}^{t_1} d(t) \, dt = \int_{0}^{\delta_0} d(t) \, dt + \int_{\delta_0}^{t_1} d(t) \, dt \leq \delta_0(1 + t_1) = \varepsilon_0. \tag{3.1.34}
\]

Combining all the above and setting \(\alpha = \max_{i \leq K} \{\alpha_i\}\) gives, for any \(t \in [0, T]\), that

\[
||c_{med}(t) - c_{imm}(t)|| \leq \sum_{i=0}^{K} \frac{\varepsilon}{K + 1} = \varepsilon. \tag{3.1.35}
\]
The similar dynamics of the two models for large $\alpha$ are especially apparent in Figure 3.13. Here the same numerical experiment was performed for each model, where an equidistributed initial condition was chosen and then integrated for 300 cell cycles. The number of clusters present at the end of the simulation were counted and are plotted in the figure. The value of $\alpha$ for this experiment was chosen to correspond to the hypothesized signaling chemical acetaldehyde.

![Figure 3.13](image)

**Figure 3.13:** The results of the same numerical experiment for the Immediate model, left, and the Mediated model with $\alpha = 720$. An initial condition of 420 equidistributed cells was integrated for 300 cell cycles, and the number of clusters present was counted.

### 3.1.2.2 The Small $\alpha$ Domain

Having established that for large $\alpha$ the Mediated model approximates the Immediate model, we turn to the natural question: are the dynamics different in the small $\alpha$ domain? The answer is yes, and the differences are best illustrated in the context of two cluster solutions. All of the following results generalize (see the discussion at the end of this section, and Figure 3.20), but the concepts
are especially clear for two clusters since in this case $F$ and $P$ are both two dimensional. A complete classification of two cluster dynamics is also useful for the same reasons as in the Immediate model: two cluster solutions are expected to have the concrete applications, and they illustrate many of the more abstract ideas presented above.

Recall that the parameter $\alpha$ is increasing with relation to the population density. Thus the question of large $\alpha$ vs. small $\alpha$ can be interpreted in terms of population density: when the population of cells in a culture is very dense, the Immediate model is a good approximation to the dynamics. Also note that although we assume a more or less constant population, this is not true for a culture that has just been introduced to a bioreactor; such populations will grow until they reach the carrying capacity of their environment. Thus $\alpha$ will be small when a yeast culture is first introduced, and it will grow with the population.

Autonomous oscillation is a property of dense populations of yeast, and is not observed until a population reaches a periodic limit state, i.e. its onset is due to a type of quorum sensing, a phenomenon that is well known to biologists, chemists, and even psychologists (for example, [26, 34, 35, 36]). A quorum sensing behavior is a qualitative change in behavior that manifests once a population reaches a critical size or density. Mathematically speaking, quorum sensing is related to bifurcations. It is not surprising then that the Mediated model describes a series of bifurcations as $\alpha$ grows.

Figure 3.14 is a plot of the two dimensional Poincaré map $P(x, z)$ with $\alpha = 6$ and negative feedback. In addition to the synchronized solution $x = 0$ there are three other fixed points, marked with black disks, and an invariant one-dimensional manifold, colored red. This is a typical plot for several reasons:

1. the system is highly contractive in the $z$-direction,
2. the dynamics on the invariant manifold capture the global dynamics,
3. the cyclic solution ($x \approx 0.55$) and the synchronized solution ($x = 0$) are both stable and locally attracting, and
4. the fixed points near $x = 0$ and $x = 1$ are 2-periodic in $F$ and unstable.

These dynamics are not the only ones possible, however. As detailed in this section, there is another possibility, illustrated by Figure 3.15. Here $s$ and $r$ are the same as in Figure 3.14, but $\alpha = 2$ and we see that only the synchronized and cyclic solutions exist, with the synchronized solution globally attracting. Between these values of $\alpha$, the 2-cyclic solution undergoes a subcritical bifurcation and the 2-periodic solutions are born. As $\alpha$ continues to increase, these new fixed points move toward 0 and 1, approaching them asymptotically. A numerically generated bifurcation plot of this phenomenon is shown in Figure 3.16.

![Figure 3.14: The Poincaré map $P$ for $k = 2$ and $\alpha = 6$.]
Note that this behavior provides the explanation for a discrepancy that was mentioned in the previous section: although in the limit as $\alpha \to \infty$, the Mediated model approaches the Immediate model, we nonetheless have that stability of the synchronized solution is opposite in the two models, for any value of $\alpha$. The resolution of this seeming contradiction is that the basin of attraction for the synchronized solution is vanishingly small as $\alpha \to \infty$. If we consider $\alpha$ to vary on the extended interval $[0, \infty]$, these new fixed points are swallowed by the synchronized solution in another bifurcation at the point $\infty$. This is because, as mentioned in the proof to Prop 3.1.7, the upper bound on the derivative $\dot{z}$ determines the maximum perturbation size for which the exit effect
Figure 3.16: As $\mu = \alpha$ grows past a critical value, the 2-cyclic solution switches from unstable to stable in a pitchfork-like bifurcation. The two new fixed points approach 0 and 1 as $\alpha \to \infty$. Here $s = 0.3$ and $r = 0.7$.

This bifurcation does not occur for every choice of $s$ and $r$, however. We can partition $s$, $r$ space into two cases, I, and II, depending on the order of events of the 2-cyclic solution. In case I, $x_1 \mapsto r$, $x_0 \mapsto s$, and then $x_1 \mapsto 1$. We also include in case I solutions where $x_1$ begins in $R$ (case I corresponds to the condition $M = 1$ in the Immediate model). In case II, we have the order of events $x_0 \mapsto s$, $x_1 \mapsto r$, $x_1 \mapsto 1$. This is the analog of $M \geq 2$; without the mediating agent $z$ the clusters would be isolated.

Figure 3.17 shows the stability properties of the 2-cyclic solution in each case. The eigenvalues of the linearization $DF$ of $F$ at the cyclic solution are pictured. In case I, the bifurcation is apparent as the eigenvalue in the $x$-direction starts positive and then becomes negative at a critical value of $\alpha$. In contrast, a case II solution will always be stable as the $x$-eigenvalue is always less than 1. Note however that the eigenvalue is asymptotic to 1 and is never very small; thus although the solution
is locally stable it is only very weakly so and in numerical simulations we do not see the 2-cyclic solutions occur in case II. This corresponds to the neutral stability of these solutions in what we called regions II and III in the Immediate model. Also note in the figure that the eigenvalue in the z direction is asymptotic to zero and decreases very quickly, indicating the strong contraction in this direction in the full phase space.

The reason for the bifurcation can be explained fairly easily, and is not specific to the two-cluster system. In Figure 3.18, a typical trajectory of z(t) for the synchronized solution over one application of F is pictured. Note that the intervals on which x₀ and x₁ are each affected by feedback coincide. By perturbing x₁ slightly forward, we cause the intervals to no longer coincide, as seen in Figure 3.19. The total amount of feedback experienced by each cluster is determined by the integral of f(z(t)) over the respective intervals, and the difference in feedback between them is thus determined by the difference in the integrals, which is a monotone function of the difference of the areas of the regions I and III in the figure. As the initial position of x₁ increases, region III increases in area faster than region I does, because x will reach S while x₀ is still in R. But region III also starts out smaller than region I because z is strictly decreasing on the time interval [s, t₁], where x₁(t₁) = 1. If α is large enough, then there will be some point at which the two integrals are equal, and this point corresponds to the new equilibrium near 0. By symmetry (relabeling x₀ := x₁ and vice-versa) this also describes the new fixed point near 1.

**Figure 3.17:** The eigenvalues of DF at the 2-cyclic solution. Left: for a system in case I. Right: for a system in case II.
A perturbation away from the synchronized solution in a $k$-cluster system for larger $k$ will have similar properties, and so we expect subcritical bifurcations to occur for every $k = 2, \ldots, \infty$. We also expect the critical values of $\alpha$ to be increasing with the number of clusters, since the larger $k$ is, the smaller the effect of a perturbation of a single cluster on the trajectory of $z$. In fact such bifurcations are apparent in Figure 3.20. Each image in the figure represents a still frame taken from an animation created in the following way: for 100 values of $\alpha$ on the interval $[0, 10]$, a simulation similar to that described in Figure 3.13 was performed and the results plotted. In general no clusters form when $\alpha$ is very small, but as $\alpha$ crosses various threshold values, solutions with 2, 3, and more clusters begin to appear.

![Figure 3.18: A plot of $z(t)$ for the synchronized solution. The time interval when $x \in R$ is shaded. Here $\alpha = 2$, $s = 0.3$ and $r = 0.7$.](image)

### 3.1.2.3 Conclusions and Future Work

The Mediated model is a natural extension of the Immediate model of cell cycle feedback in yeast. Although the addition of the signaling agent $z$ makes the model harder to study analytically, it introduces rich, population dependent dynamics. These new dynamics only occur for relatively
Figure 3.19: A plot of $z(t)$ near the synchronized solution, here with $x_1 = 0.1$. Region I indicates feedback felt only by $x_1$, region II indicates feedback felt by both clusters, and region III indicates feedback felt only by $x_0$. Increasing $x_1$ increases the size of region III relative to region I.

small values of $\alpha$, which confirms that the Immediate model is sufficient to explain the dynamics of dense yeast populations undergoing autonomous oscillation. However, the new complexity of the Mediated model provides more knowledge about the system, and allows us to make biological predictions which may guide the search for genetic pathways of response / signaling type in \textit{S. cerevisiae}.

In particular, we make the following claims based on the above analysis:

- Since the order of events for the cyclic solution determines whether the 2-cyclic solution undergoes a subcritical bifurcation, we expect the biological phases corresponding to $R$ and $S$ to be arranged so that they place the system in case I.

- Because the 2-cyclic solution is stable only for large enough values of $\alpha$, we expect the rate of the relevant biological processes (e.g. transcription or cell cycle related degradation) for producing and responding to the signaling agent to place the value of $\alpha$ above the critical threshold for the $k = 2$ bifurcation in a mature culture.
Figure 3.20: Four frames of an animation demonstrating the successive bifurcations of $k$-cluster solutions as $\alpha$ increases through the corresponding critical values. The plots are of $s$-$r$ parameter space and indicate the number of clusters which eventually form for each value of $\alpha$, as in Figure 3.13

- We expect the lengths of the biological signaling and responsive phases to be relatively long, placing the system in the region $M = 1$, because cultures with more than two clusters have not been confirmed experimentally, and there are significant regions of bistability inside the parameter domain where 2 cluster solutions are stable.

These claims could be verified in several ways, but they all rely on identifying the signaling agent and/or the biological phases corresponding to $R$ and $S$. There is a wealth of existing literature on chemically influencing the cell cycle, and from this literature we have identified acetaldehyde as a likely candidate, although other compounds such as alcohol or extracellular proteins should also be considered.
Based on the identity of the signaling agent, portions of the genetic regulatory system which are sensitive to it can be determined, either experimentally or once again by examining existing literature. Our predictions for the sizes of $S$ and $R$, plus the rates of production and metabolization, can guide this search.

### 3.1.3 Comparison of the ODE Models

The motivation for adding the gap in the Gap model and the signaling agent in the Mediated model was twofold: to make the model more biologically realistic, and to introduce a source of dynamical delay. Biologically this is necessary because signaling cannot be immediate; the signaling agent must diffuse through the environment before affecting responsive cells. Introducing a delay into the model also makes sense for the reason that systems with moderate dynamical delay often show a stronger tendency toward synchronized behavior.

In order to measure the effect on clustering of introducing a delay, we construct a measure of ‘clustered-ness’ $\text{clust}(c) = \|g\|^2_2$, where $g$ is the vector of intercellular gaps, defined as follows:

**Definition 3.1.9.** Assume a configuration of cells $c = (c_0, c_1, \ldots, c_{n-1})$ is ordered so that $0 \leq c_0 \leq c_1 \leq \ldots \leq c_{n-1} \leq 1$. Then the vector $g$ is given by

$$
g = (c_0, c_1 - c_0, c_2 - c_1, \ldots, c_{n-1} - c_{n-2}, 1 - c_{n-1} + c_0). \tag{3.1.36}
$$

The sum $\sum_{i=0}^{i=n-1} g_i$ is always equal to 1, and so the squared $\ell^2$ norm of $g$ provides a good (relative) measure of how clustered the systems are. The maximum value of $\text{clust}(c)$ is 1 when the cells are completely synchronized, and the minimum is equal to $1/n$ when the cells are equidistributed on the circle.

Figure 3.21 compares the values of $\text{clust}(c)$ over time for the Immediate, Gap, and Mediated models, each starting from the same random initial configuration and two choices of parameter values. For a wide range of parameter values, the Gap model converges much more quickly to a clustered solution, and approaches its asymptotic value more sharply.
Figure 3.21: The speed of convergence of the Immediate, Gap, and Mediated models as measured by clust(c). In each plot, the three solutions have the same initial configuration of 40 cells chosen uniformly at random, and share parameter values. For the left plot, $\varepsilon = 0.05$, $s = 0.35$, $r = 0.65$, and $\alpha = 15$. For the right plot, $\varepsilon = 0.1$, $s = 0.35$, $r = 0.65$, and $\alpha = 5$.

3.1.4 Partial Differential Equation Model

For the populations of yeast cells that we are interested in, $n$ is very very large. In the context of modeling large populations, a population density approach often simplifies matters, and this is somewhat true in this case as well.

We suppose that the local density of cells at position $x \in S^1$, at time $t$, is given by a smooth function $u(x)$, i.e. the number of cells in an interval $[x, x + \delta]$ is approximately equal to $\int_x^{x+\delta} u(x, t) \, dx$. We will then construct a PDE for $u$ that embodies response / signaling feedback.

As in the Immediate and Gap models, we let $S = [\varepsilon, s)$ and $R = [r, 1)$ for $0 \leq \varepsilon < s < r < 1$. To include the possibility that signaling strength is phase dependent, we define the signaling profile $\sigma(x)$ to be any integrable function having the following properties:

- $\sigma : S^1 \to [0, 1]$, and
- $\text{supp} \sigma = [\varepsilon, s]$. 
The strength of signaling at time $t$ is denoted $\Sigma(t)$ and is defined as:

$$\Sigma(t) = \int_{S^1} \sigma(x)u(x, t) \, dx.$$  \hfill (3.1.37)

Similarly, we define the response profile $\rho(x)$ to satisfy the following:

- $\rho$ is integrable,
- $\rho : S^1 \to (-1, \infty)$, and
- $\text{supp}\rho = [r, 1]$.

Example signaling and response profiles are pictured in Figure 3.22. Note that the definition of $\rho$ includes the possibility of positive or negative feedback.

As before, we assume that cells outside of $R$ will move with velocity 1, so that $u_t(x, t) + u_x(x, t) = 0$ for $x \in [0, r)$. For $x \in R$, the velocity is modified by the product of signaling strength and response sensitivity, so that it is equal to $1 + \Sigma(t)\rho(x)$. Note that since $\text{supp}\rho = [r, 1]$, this expression is equal to 1 outside of $R$ and so we can define the velocity function $a(x, t)$ for any point $x \in S^1$ at any time $t \geq 0$ by:

$$a(x, t) = 1 + \rho(x)\Sigma(t).$$ \hfill (3.1.38)

The density function then satisfies the advection equation with velocity $a$:

$$u_t(x, t) + [a(x, t)u(x, t)]_x = 0.$$ \hfill (3.1.39)

If we make the particular choices of $\sigma(x) = 1_S$ and $\rho(x) = 1_R$, we recover the Immediate model in the limiting case as $n \to \infty$.

It is important to note that $\Sigma(t)$ depends on the value of $u(x, t)$ for every $x \in S^1$ at time $t$, and so it is more properly understood as a linear operator on $u$. In this context, (3.1.39) becomes an initial value problem on a space $X$ of functions on $S^1$ that contains $u$:

$$u_t = F(t, u).$$ \hfill (3.1.40)
For convenience, we collect all the required definitions of the model here.

\[ u_t(x, t) + [a(x, t)u(x, t)]_x = 0 \]
\[ u(x, 0) = u_0(x) \] (3.1.41)
\[ a(x, t) = 1 + \rho(x)\Sigma(t) = 1 + \rho(x) \int_0^1 \sigma(x)u(x, t) \, dx \]

![Figure 3.22: Example signaling and response profiles \( \sigma \) and \( \rho \). For this choice of \( \rho \) the system would have negative feedback.](image)

### 3.1.4.1 Solution via Conservation Law Methods

The first of our results come from the recognition that except for the functional dependence on \( u \) in the \( \Sigma \) function, (3.1.39) has the form of a conservation law. Conservation laws are well understood, so if we can decouple \( \Sigma \) from its dependence on \( u \) and treat it as merely a function of \( t \), there are many things we can say about the solutions to (3.1.41).

It is the configuration of \( S \) and \( R \) that allow us to achieve this decoupling in the case \( \epsilon > 0 \), via the method of characteristics. Recall from the theory of characteristics that for a conservation law of the form \( u_t(x, t) + a(x, t)u(x, t) + c(x, t, u) = 0 \), the characteristic equations are given by: \( \dot{x}(t) = a(x, t) \).
and \( \hat{z}(t) = -c(x, t, z) \). Thus for (3.1.41), we have:

\[
\begin{align*}
\dot{x}(t) &= a(x, t) = 1 + \rho(x)\Sigma(t), \\
x(0) &= x_0, \\
\dot{y}(t) &= -a_x(x, t)y(t) = -\rho'(x)\Sigma(t)y(t), \\
y(0) &= u_0(x_0).
\end{align*}
\]

(3.1.42)

These equations are not well defined because they rely on the solutions for many neighboring initial conditions simultaneously. However, if we restrict the time interval of the solutions, (3.1.42) can be solved for initial conditions on a subset of \([0, 1]\). Due to the configuration of \( S \) and \( R \) when \( \epsilon > 0 \), this in turn allows us to define \( \Sigma \) independently as a function of \( t \), achieving the decoupling and enabling the solution to (3.1.42) for any initial condition. This solution can then be extended indefinitely forward in time.

Refer to (3.1.37). Under the present assumptions, this can be simplified to:

\[
\Sigma(t) = \int_{s-\epsilon}^{s} \sigma(x)u(x, t) \, dx.
\]

(3.1.43)

For \((x, t) \in [\epsilon, s] \times [0, \epsilon]\), \( a(x, t) = 1 \). Thus the characteristic projections \( x(t) \) with initial condition \( 0 \leq x(0) \leq s \) will satisfy \( \dot{x} = 1 \) for \( t \leq \epsilon \), as pictured in Figure 3.23. On the same time interval, we also have \( \dot{y} = 0 \), so that \( y \) is constant along these projections for \( t \leq \epsilon \). This means that the portion of \( u(x, t) \) on \( S \) is just a shift of the initial density \( u_0 \), so we can rewrite (3.1.43):

\[
\Sigma(t) = \int_{\epsilon}^{s} \sigma(x)u_0(x - t) \, dx, \quad 0 \leq t \leq \epsilon.
\]

(3.1.44)

Using this formulation of \( \Sigma \), the characteristic equations (3.1.42) are well defined for every initial value \( x_0 \), and standard methods provide a corresponding solution to (3.1.41) for \( t \leq \epsilon \).

Note that the interval of solution is of positive width and does not depend on \( u_0 \). Thus the above solution can be extended to the interval \([\epsilon, 2\epsilon]\) by repeating the same construction, using \( u(x, \epsilon) \) as the initial density. Continuing inductively in this way, a solution to (3.1.41) can be constructed on arbitrarily large time intervals.
Figure 3.23: Because of the configuration of $S$ and $R$ when $\varepsilon > 0$, the characteristics in the shaded region are completely determined by the initial condition, allowing the signaling function $\Sigma$ to be decoupled from the solution $u$.

**Theorem 3.1.5.** Let $u \in C^\infty S^1$, and let $\sigma$ and $\rho$ be integrable functions as described above with $\varepsilon > 0$. Then there is a unique (weak) solution $u(x, t)$ to (3.1.41), for $x, t \in S^1 \times [0, T]$ for any $T > 0$.

**Proof.** The above arguments constitute a partial proof, following chapter 3 of [38]. We must still show that the initial data $u_0$ is noncharacteristic, but this is immediate: $u_0$ will be noncharacteristic provided that $\dot{x}(0) = a(x, 0) \neq 0$, which is true by definition.

Uniqueness is a consequence of the uniqueness of solutions to 3.1.42.

Figure 3.24 shows several snapshots of a numerical solution to (3.1.41) using the method of characteristics.\textsuperscript{*} Here $\sigma$ and $\rho$ are chosen so that $\varepsilon = 0$, $s = 0.3$, and $r = 0.7$. The initial condition

\textsuperscript{*} For the solution pictured in Figure 3.24, we solved the characteristic equations (3.1.42) using a simple Euler’s method with step size $h = 0.001$. We chose this method, and this small step size, because of the interdependence of the
is chosen to be the equilibrium solution \( u(x) = \frac{1}{1 + \Sigma \rho(x)} \), where \( \Sigma = \int_{0}^{s} \sigma(x) \, dx \). Rounding error is sufficient to perturb the solution away from the equilibrium and toward a clustered solution with \( k = 2 \).

The above methods do not work when \( \varepsilon = 0 \). It may be possible to construct a solution in this case by taking the limit of the solutions as \( \varepsilon \to 0 \).

Our goal with the PDE model was to find a setting in which the ubiquitous formation of clusters has a simple mathematical explanation, by considering the linearization of the equation at the stationary solution given by \( u_t(x, t) = 0 \). Conservation law methods may be insufficient for the task due to the complications mentioned above, but there is another natural setting in which to study (3.1.41) where characteristic solutions on one another. Since the solutions exhibit exactly the clustering behavior we expect, we believe this method should be adequate for numerical solution of (3.1.41).
we hope to have more success, which we describe, along with other directions for future work, in
the following section.

### 3.1.5 Directions for Future Work

The complications from the functional dependence of $\Sigma$ on $u$, described in Section 3.1.4.1, disappear
if we consider instead the form (3.1.40), since $F(u, t) = -(au)_x$ is well defined as a nonlinear
operator on $C^\infty$. It may be possible to show that a solution to (3.1.40) exists by demonstrating that
$F$ is Lipschitz and applying standard theory.

The claims made in Section 3.1.2.3 concerning the likely values of the model parameters might be
verified both experimentally and theoretically.

In addition to questions about yeast oscillation, we are also interested in whether RS type models
may be used elsewhere in cell biology to help explain cell cycle dependent phenomena. The
erly development of a *Drosophila melanogaster* embryo is one such setting. The first 13 nuclear
divisions all occur inside a single syncytial cell, meaning that for much of the mitotic cycle the
nuclear proteins and other chemicals are free to diffuse in the shared cytoplasm, creating the
potential for internuclear feedback similar to that we described in yeast.

In particular, we suspect a protein called Cyclin B might be a signaling agent for such feedback.
Cyclin B diffuses throughout the embryo, first as a maternal gradient, and later after it is expressed
by the nuclei. It is also a factor in many processes within the nuclei that are cell cycle-dependent.
In particular, Cyclin B is expressed in the S phase, at a ratio of 50:1 compared with the rest of the
cell cycle, and it is highly active in the G2/M phase, at a ratio of 25:1 [10]. This makes a reaction-
diffusion model for Cyclin B, combined with an RS type model for the nuclei, a natural choice for
studying the *Drosophila* embryo.
REFERENCES


APPENDIX A: NUMERICAL METHODS

A.1 Integration Methods for The Immediate, Gap, and Mediated Models

For the simulations reported in Chapter 3, an efficient and accurate method of integrating (3.1.1) and (3.1.20) was required. Large time step methods such as Runge-Kutta are not useful for these models because the form of the equations may change during a time step, when a cell either enters or exits the signaling region $S$. To address this problem, we developed exact methods for integrating each system, based on the concept of order of events used elsewhere.

For a given initial configuration of cells $c(0)$, we distinguish as before the three special cells $c_{\sigma}$, $c_{\rho}$, and $c_{n-1}$. Define $t_\sigma = s - c_\sigma$ and $t_\rho = c_\rho$. Define $t_1$ by assuming that $I$ remains constant at its current value and setting $t_1$ to be the time until $c_{n-1}$ reaches 1. Note that in the Immediate model, $t_1$ is just $(1 - r)/(1 + f(I))$, but in the Mediated model $t_1$ is given by:

$$\int_{0}^{t_1} 1 + f(z(t)) \, dt = 1. \quad (A.1.1)$$

For the simulations, we made the simplifying assumption that $f(z) = -z$ so that an exact form of (A.1.1) could be given. It then takes the form:

$$At_1 + Be^{Ct_1} = D. \quad (A.1.2)$$

Now no closed-form solution exists to (A.1.2) using elementary functions, but after a change of variables one can use the special Lambert $W$ function, also known as the product log, to solve it. In [13] the authors give an efficient modification of Newton’s method for solving the equation $W(x) = 0$. We use this algorithm to find $t_1$ and in practice we can still produce results that are arbitrarily accurate.

Now, once $t_\sigma$, $t_\rho$, and $t_1$ are defined, we let $t_{\text{step}} = \min\{t_\sigma, t_\rho, t_1\}$. It is guaranteed that $I$ will remain constant on the time interval $[0, t_{\text{step}}]$, and that no cells will enter or leave $R$ on that interval. Thus we can calculate the distance $d$ a cell in $R$ will travel during $[0, t_{\text{step}}]$ and integrate exactly on this
interval by setting:
\[
c_i(t_{\text{step}}) = \begin{cases} 
  c_i(0) + t_{\text{step}} & c_i(0) \notin R, \\
  c_i(0) + d & c_i(0) \in R, 
\end{cases} \quad (A.1.3)
\]
and also calculating \( z(t_{\text{step}}) = I + (z(0) - I)e^{-\alpha t_{\text{step}}} \) in the case of the Mediated model. Now the algorithm can be iterated using \( c(t_{\text{step}}) \) as the new initial configuration of cells. The \( F \) map is taken by iterating this process until \( c_{n-1} = 1 \), and repeating this algorithm \( n \) times yields the Poincaré map \( P \).

This exact method compares favorably to Euler’s or other approximate methods when \( n \) is relatively small. For example, if \( n = 10 \) and \( h = 0.01 \), then this method is approximately an order of magnitude more efficient, while at the same time being exact. The efficiency advantage is lost for larger values of \( n \), especially in highly vectorized environments such as Matlab or Numpy.
APPENDIX B: CONTRIBUTIONS

Much of the work reported in this dissertation was done jointly with colleagues. In this Appendix I will describe in detail the individual contributions I have made to each project. I generated all figures used herein except for Figure 3.7.

Chapter 1 is of course all mine.

The impetus for the innate immunity project in Chapter 2 was a collaboration between my advisor, Todd Young, and his longtime collaborator Erik Boczko. The project was already underway when I began working with Todd, so I did not create the model (2.2.2). I did contribute to the analysis in varying capacity; in particular the comparison with the two-hit experiment includes much of my work. Section 2.4 is original to this dissertation.

Chapter 3 is a large chapter and encompasses several projects. The introduction and Section 3.1 consist of information which is generally understood among our research group. I did not create the Immediate model (3.1.1) but I did contribute to its extension to the Mediated model (3.1.20).

Section 3.1.1.1 was developed collaboratively with Todd and Erik.

Section 3.1.1.2 is largely my own work, including the concept of order of events, which figures prominently in much of the analysis that follows. Erik deserves credit for the neat trick that produced (3.1.13) and allowed the rest of the stability analysis to carry through.

Section 3.1.1.3 is largely a summary of the work of others, especially Gregory Moses, although I double checked the calculations that led to the formulations of $F$ given in (3.1.18) and (3.1.19).

Section 3.1.1.4 is a short section and along with 3.1.3 includes the totality of my concrete contributions to that paper, although I also performed a ‘consultant’s role’ for some of the analysis of the Gap model.

Section 3.1.2 is entirely my own work, including each of its subsections.

Section 3.1.3 was developed collaboratively with Todd.