Clustering in Cell Cycle Dynamics with General Forms of Feedback

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Acknowledgments

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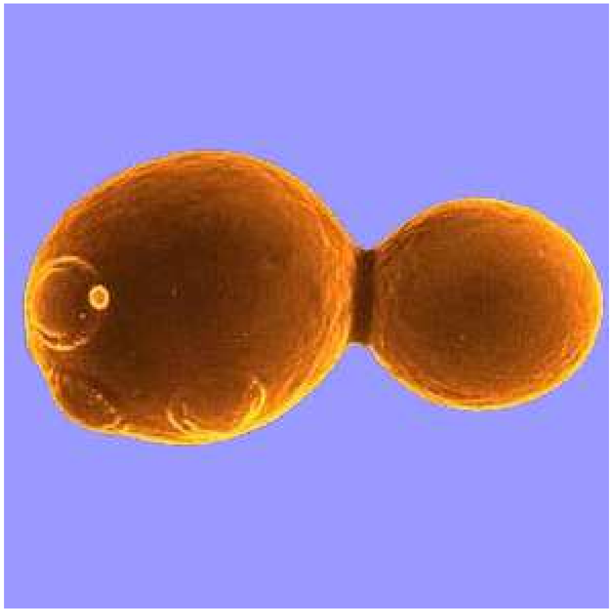
Saccharomyces cerevisiae



Photos: Wikipedia, www.kaeberleinlab.org, www.alltech.com

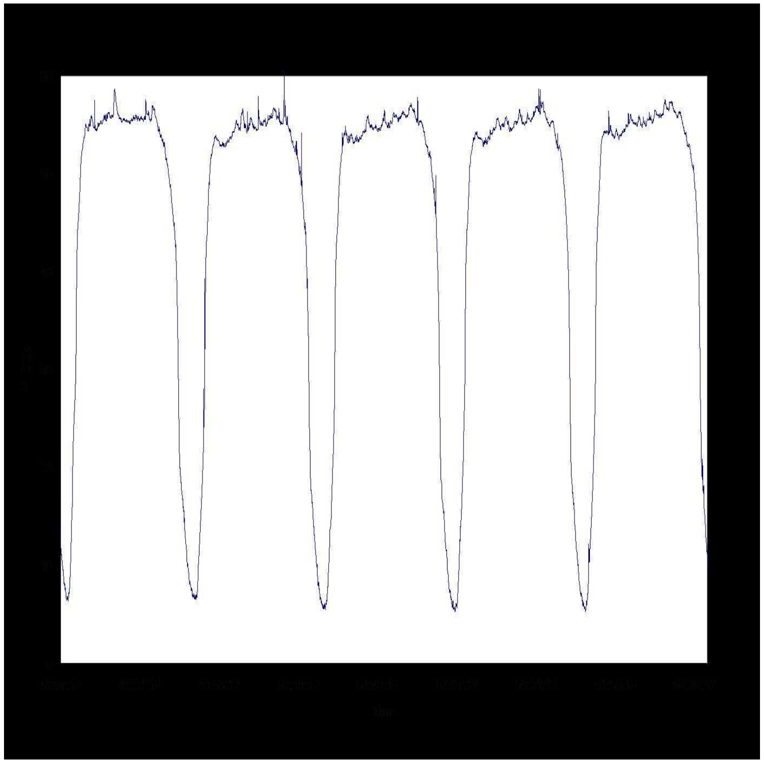
Brewer's, Baker's or Ale Yeast.

Studied by biologists as a model eukaryotic organism.

Yeast are used in many bio-engineering processes.

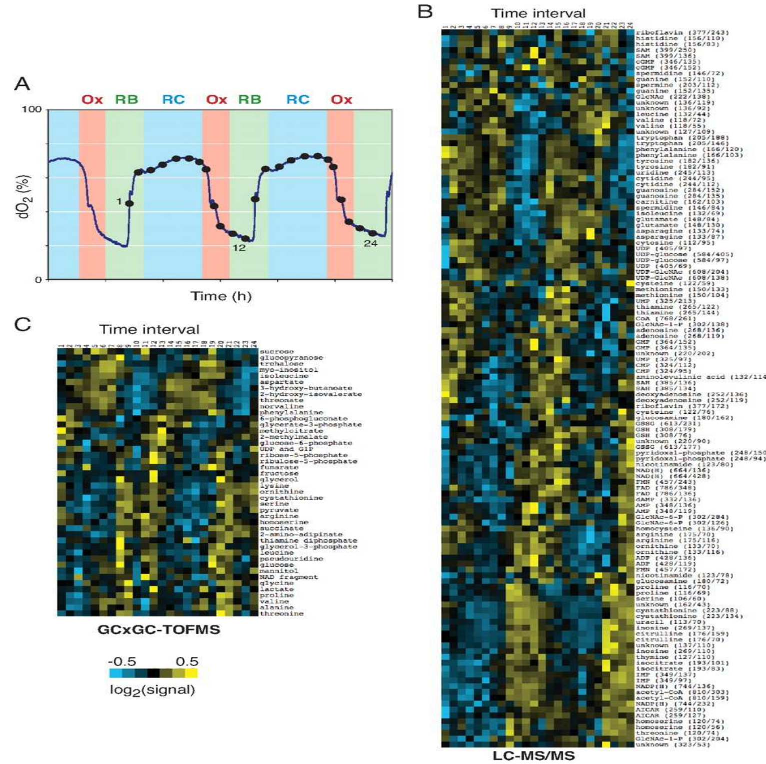
Metabolic Oxygen Oscillations

Disolved O_2 vs. time:



20 hrs. Range 5% - 65%.

Microarray time-series:



Z.Chen et. al. *Science* **316** (2007).

Oxygen Oscillations

Oscillations occur under the following conditions:

- Well-mixed bioreactor.
- Slow input and output.
- Highly oxygenated media
- High cell density.
- Boczko observed: The period of oscillation is always nearly an integer fraction of the culture's doubling time.

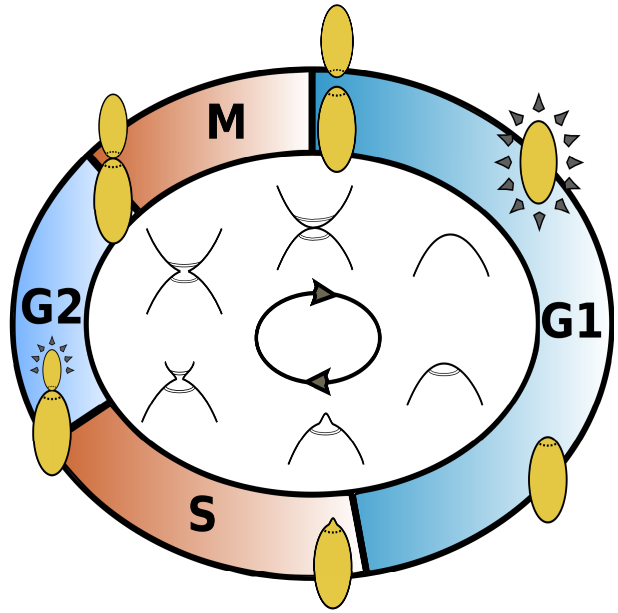
Cell Cycle of Budding Yeast

G1: growth phase, begins
with cell division

S: replication phase,
begins with budding

G2: second growth phase

M: narrowing or "necking",
ends in cell division



Cell cycle synchrony is impossible to sustain in the lab. Initially synchronized cultures quickly de-synch.

A casual link between O_2 oscillations and the cell cycle was dismissed in one early paper without data.

Clustering

By *Cluster* we mean a group of cells traversing the cell cycle in near synchrony. (Not spatial clustering.)

Hypotheses:

A large cluster of cells in one part of the cell cycle might influence the progress of cells in another part (thru metabolic products?).

This feedback might reinforce the formation/stability of clusters.

Clustering and Oscillations are intrinsically linked.

Model of RS Feedback

$x_i(t) \in [0,1]$ - state of i-th cell, $x_i = 1 \mapsto x_i = 0$ (division).

Signaling region $S = [0, s)$. Responsive region $R = [r, 1)$.

$\sigma = \#\{\text{cells in } S\}/n$.

RS feedback model:

$$\frac{dx_i}{dt} = \begin{cases} 1, & \text{if} \quad x_i \notin R \\ 1 + \rho(\sigma), & \text{if} \quad x_i \in R. \end{cases} \tag{1}$$

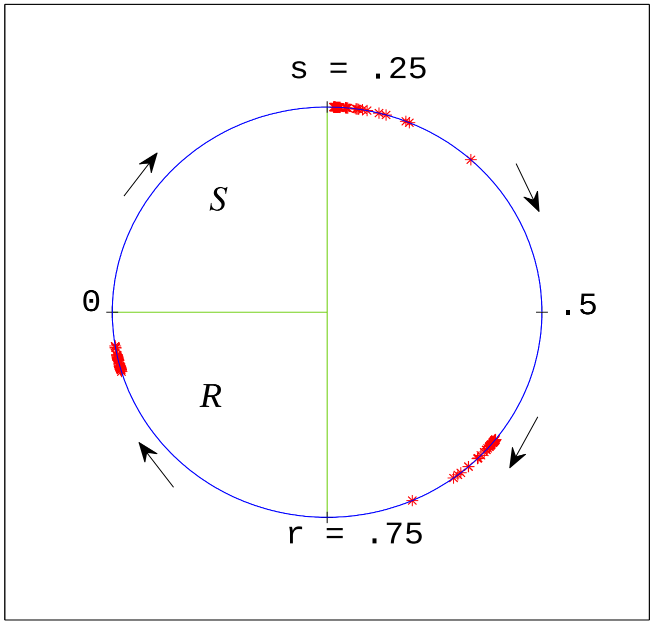
$\rho(\sigma)$ is a "response" function. Assume $+/-$ monotone.

Proposition. Synchronized solution is stable for positive feedback, unstable for negative.

Clusters Exist - Simulations

S - Signaling, R - Responsive

Simulation, 500 cells,
Negative feedback & noise:



- Simulations with RS feedback almost always form clusters.

- Analysis of simple RS feedback confirms clustering is robust.

- We began looking for clustering in yeast experiments.

Clusters Exist - Mathematics

n - number of cells, $n \sim O(10^{10})$. Phase space is $\mathbb{T}^n$.

In the model (1), a synchronized cluster of cells will persist, so we may reduce the dimension to k, the number of clusters.

A clustered solution $\{x_i(t)\}_{i=1}^k$ is *cyclic* if $\exists$ a time $d > 0$ s.t.:

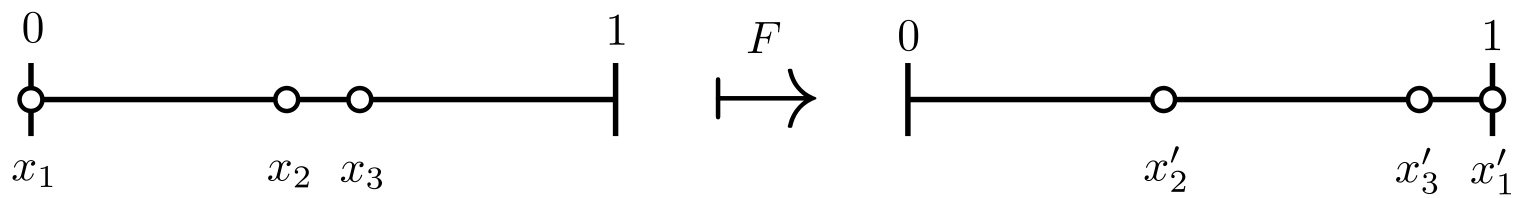
$$x_i(d) = x_{i+1}(0) \quad \forall \quad i = 1, \ldots, k-1,$$
$$\text{and} \quad x_k(d) = x_1(0) \quad \text{mod } 1.$$

Theorem. If k is a divisor of n, then a cyclic k cluster solution exists consisting of n/k cells in each cluster.

Special Cases: $k = 1$ - *synchronized*. $k = n$ - *uniform*.

Cluster Systems

Strategy: Study solutions consisting of k clusters. Use the map F below. F^k is the Poincaré return map.

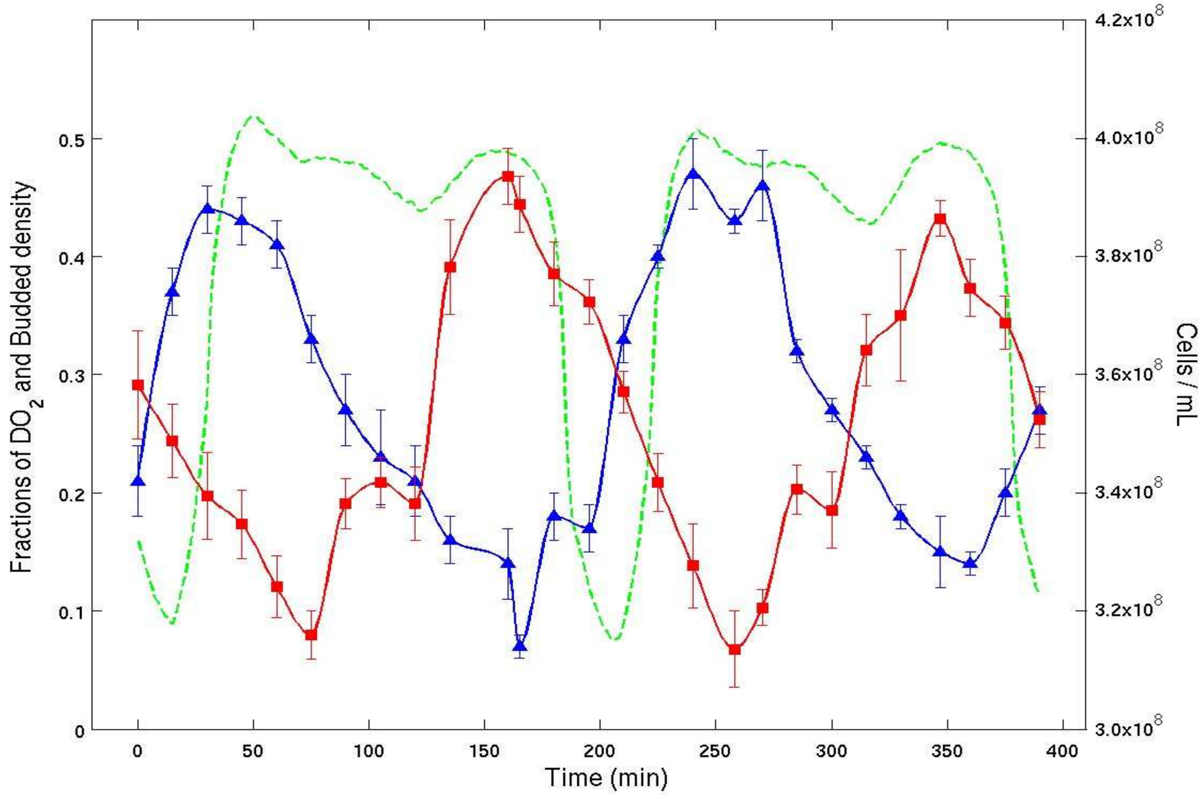


F consists of flowing until $x_k(t) = 1$, then reordering indices.
$F : S \to S$, $S = \{0 \le x_2 \le \ldots \le x_k \le 1\}$.

Proof: F permutes the boundary of S + Brouwer $\Rightarrow$
F has interior fixed point $\iff$ k-cyclic solution.

We can also use F to study solutions for small k in detail.

Clusters Exist - Experiments



Oxygen dilution (green), bud index (blue) and cell density (red) over one cell cycle period. There are 2 clusters.

Isolated Clusters and the Geometric Constant M

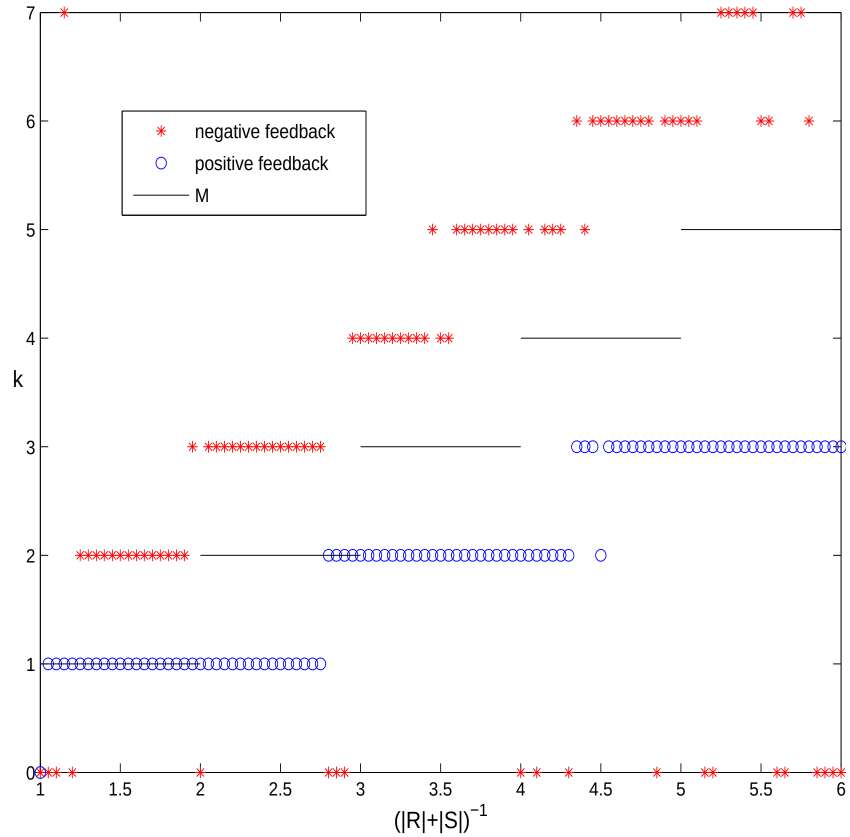
If the distance between two clusters is more that $|R| + |S|$ then those two clusters will not interact.

$M = \lfloor (|R| + |S|)^{-1} \rfloor$.
- max # of clusters that can exist without interactions.

Solutions with $k \leq M$ noninteracting clusters will be periodic.

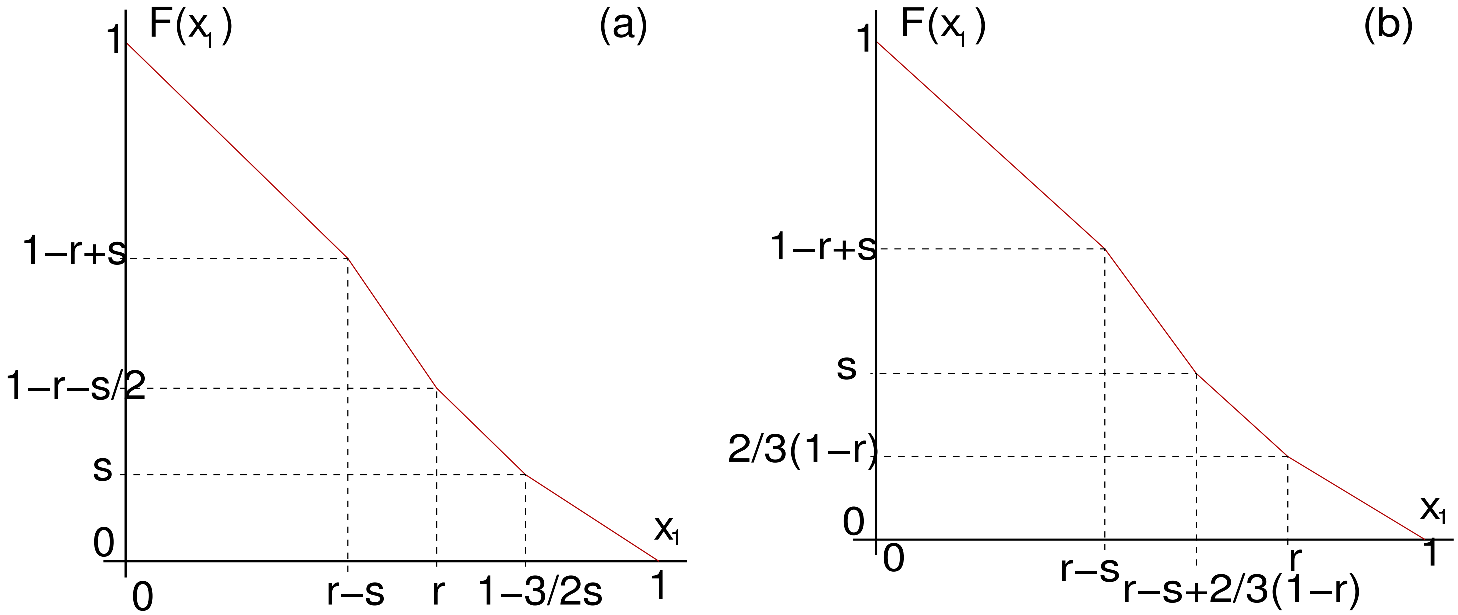
Theorem - For positive feedback (1) the set of solutions with non-interacting clusters is locally asymptotically stable. For negative feedback it is unstable.

Negative vs. Positive Feedback



The number of clusters that form in simulations compared with $M = \lfloor (|R| + |S|)^{-1} \rfloor$.

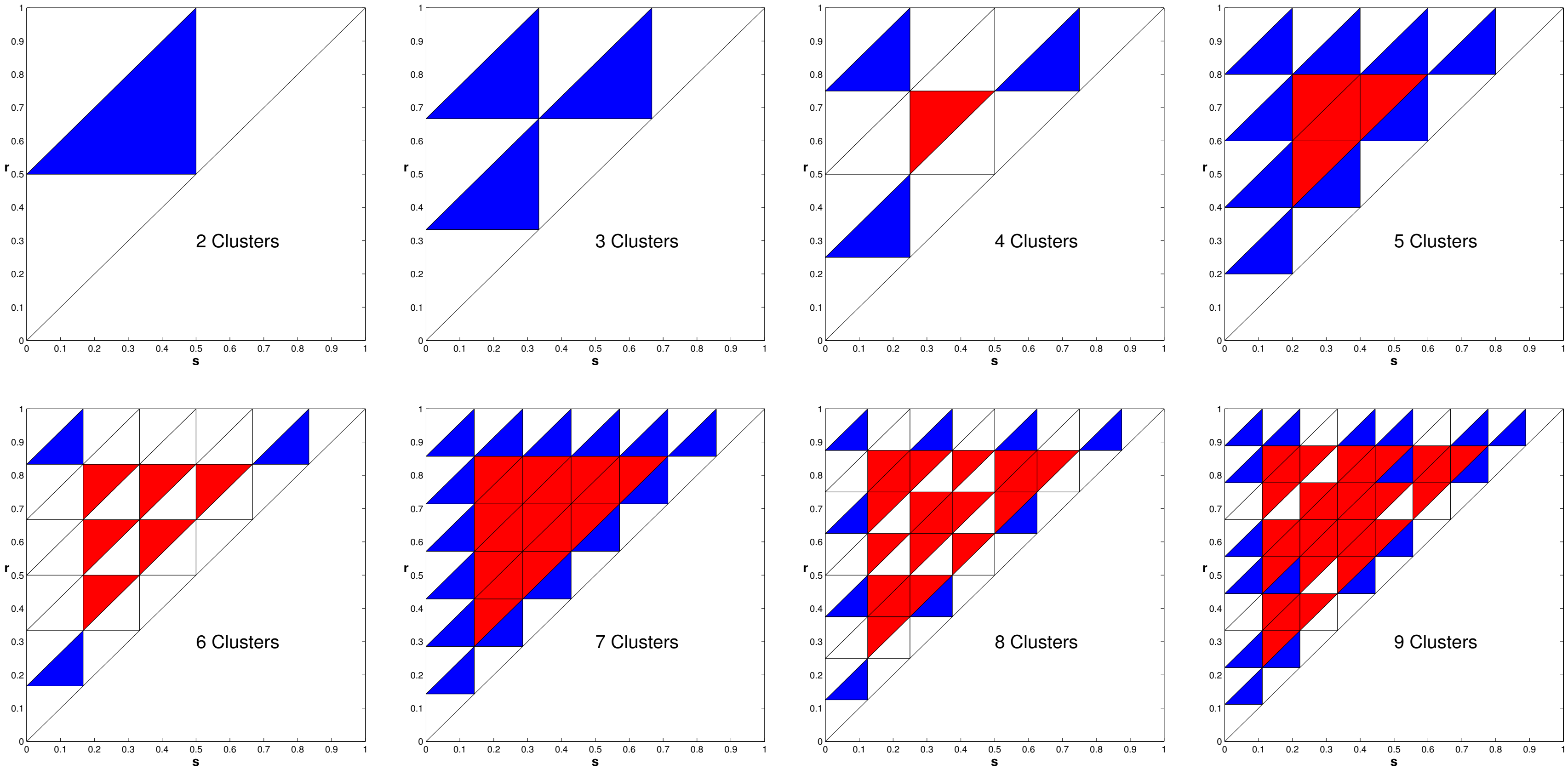
2 Cluster Systems - Detailed Analysis



F for positive feedback, $k = 2$. (a) $r + \frac{3}{2}s < 1$. (b) $r + \frac{3}{2}s \geq 1$.

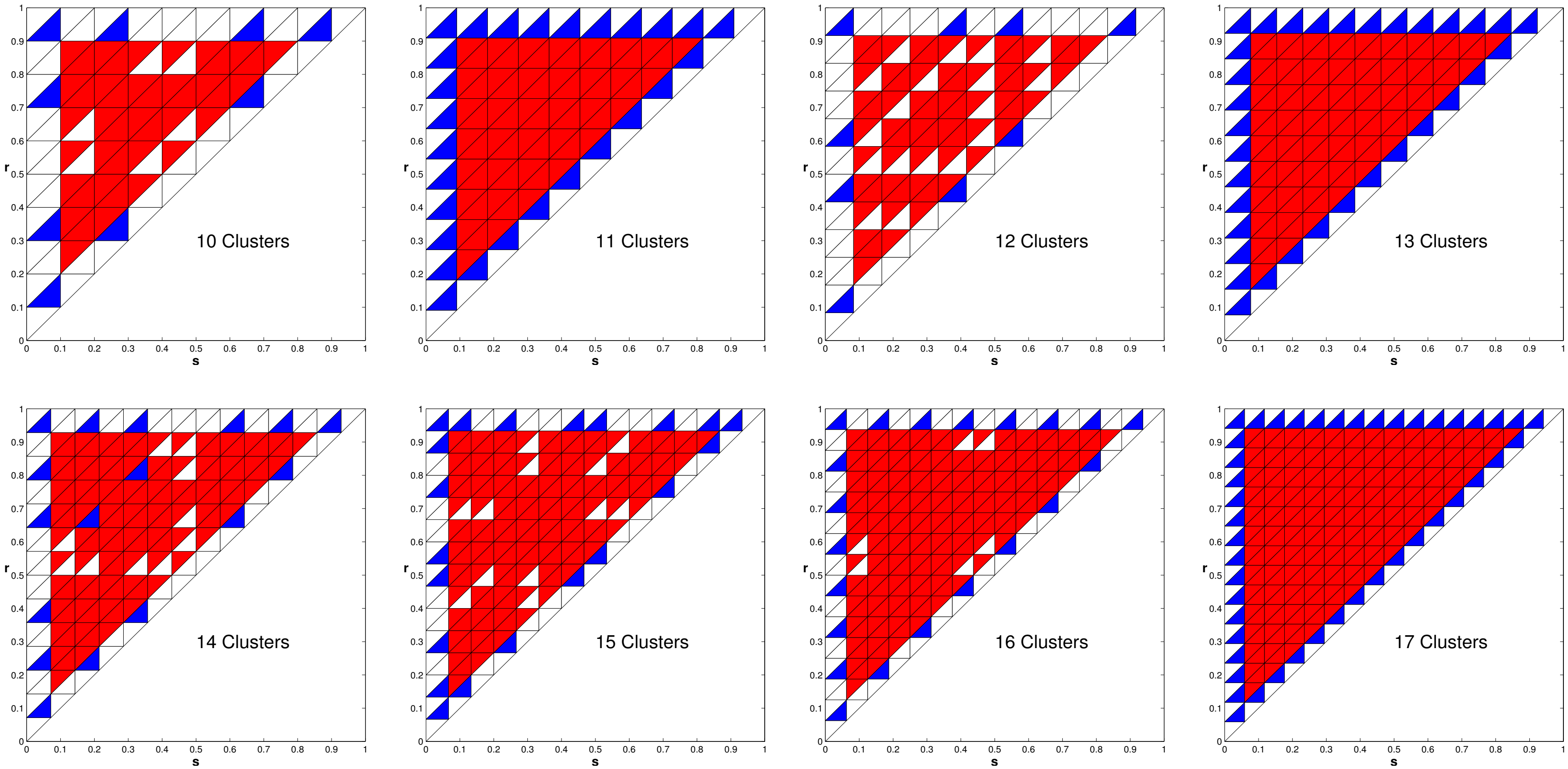
From F we can infer all dynamics.

Stability of k-cyclic solution in r-s space - Neg. Feedback



$k = 2, \ldots, 9$. In subtriangles the "order of events" is invariant.
Blue - Stable, White - Neutral, Red - Unstable.

Stability of k-cyclic solutions in r-s parameter space

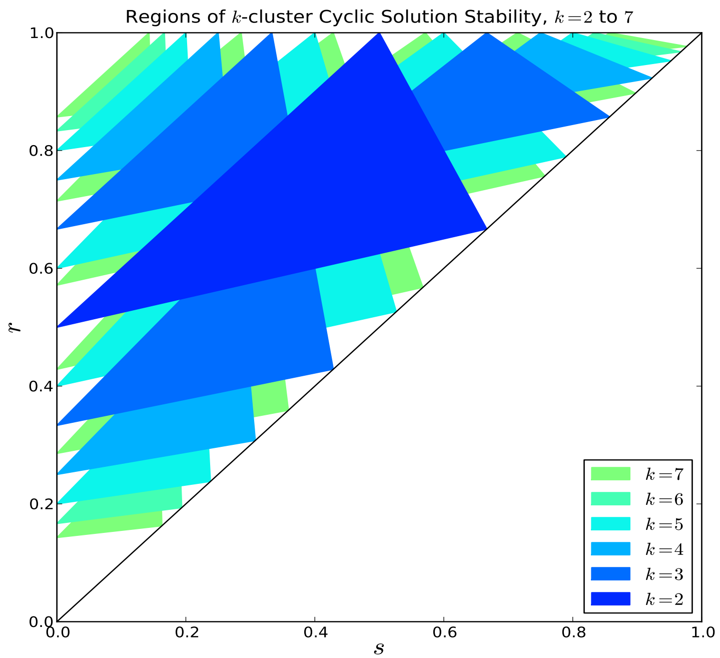


$k = 10, \ldots, 17$

Primes are Regular, Composites are Irregular!!.

Clustering is Universal for Negative Feedback

Overlay of Stable Regions:

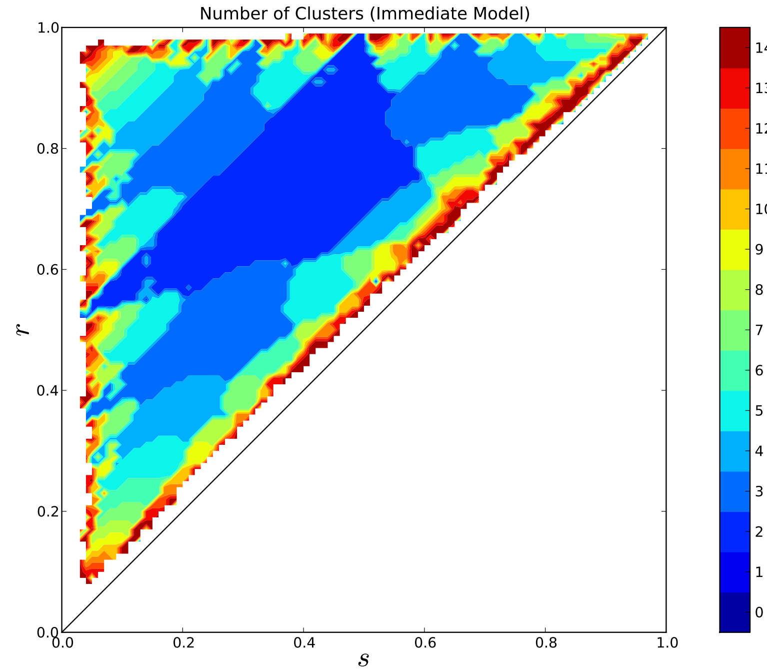


- Conjecture: All area is covered by stable regions.

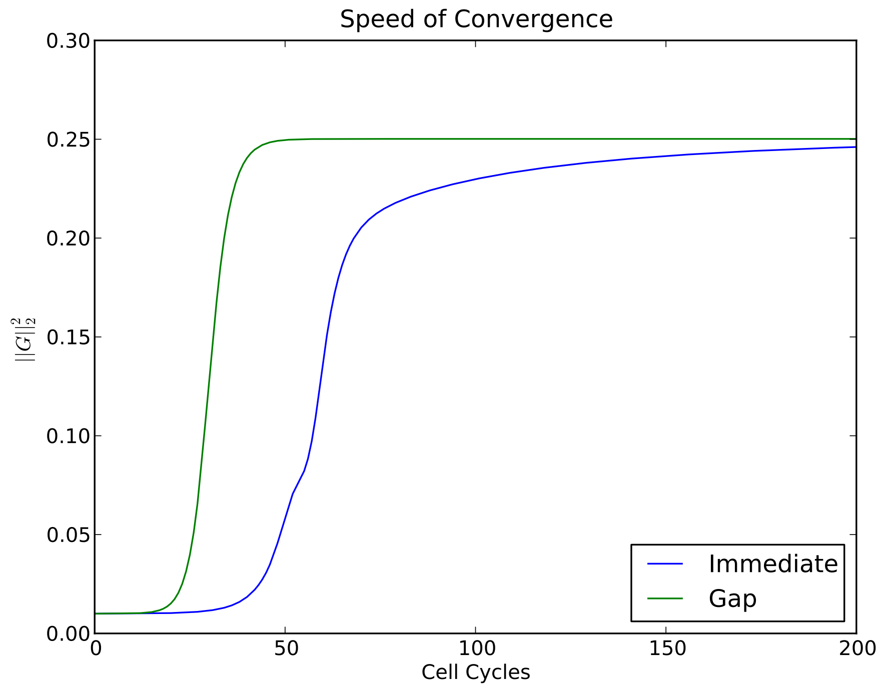
- There are many regions of Bistability.

Another Nice picture

Actual number of clusters:



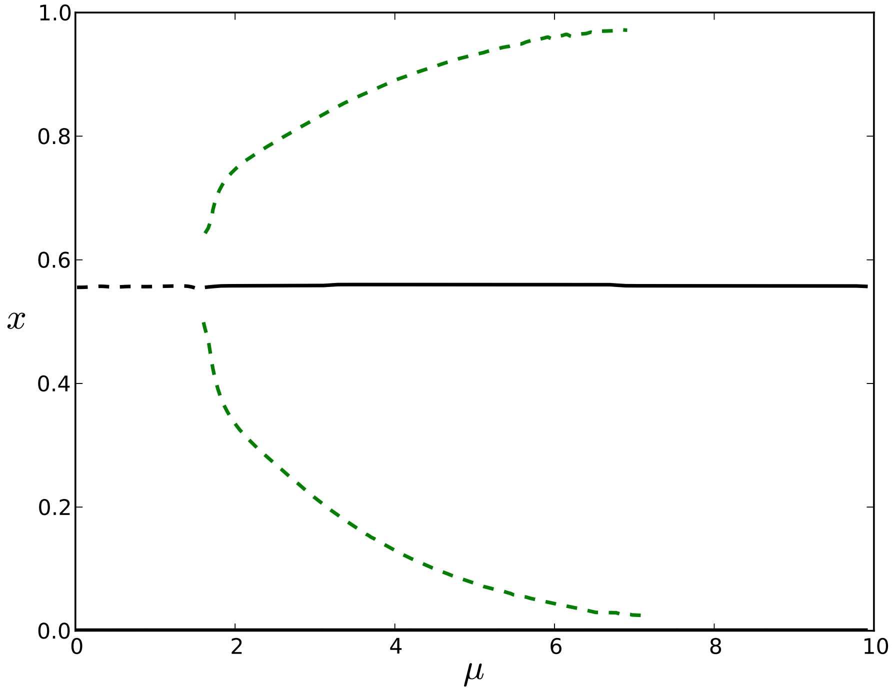
Model with a gap (delay)



A small delay does not effect the number of clusters.

A delay enhances the stability of stable clusters.

Model with an explicit signaling agent z



$$\frac{dx_i}{dt} = \begin{cases} 1, & \text{if} \quad x_i \notin R \\ 1 + \rho(z), & \text{if} \quad x_i \in R. \end{cases}$$

$$\frac{dz}{dt} = \mu\sigma - \gamma z$$

$$\sigma = \#\{\text{cells in } S\}/n.$$

2 cluster cyclic solution always exists

- *unstable* at lower cell density
- it becomes *stable* via pitchfork
- at high density basin is large.

Conclusions for General RS feedback

• Clustering is a robust phenomenon for negative feedback:
  - Not dependent on functional form of feedback.
  - It occurs for large open sets of parameter values.

• Positive feedback tends to produce Synchronization. $2 \leq k \leq M$ clusters are only neutrally stable.

• Number of clusters depends heavily on *size* of S and R.

• Clustering is experimentally verified in oscillating cultures.

• The biological mechanism driving Clustering seems to be negative feedback.

Some Open Problems

- Show that feedback makes the *uniform solution* unstable.

- Analyze PDE versions of the feedback model, such as:

$$\frac{\partial u}{\partial t} + \frac{\partial}{\partial x}\left(a(x,[u])\,u\right) = 0, \qquad a(x,[u]) = 1 + f\left(\int_0^1 k(x,y)u(y)\,dy\right),$$

with $k(x,y)$ supported on $\{x \in R\} \times \{y \in S\}$, e.g. $h(x,y) = \chi_{R\times S}$.

O. Diekmann and collaborators proved stability of the uniform solution for some cell-cycle PDE models *without* feedback.

- Connect these results with detailed modeling and biology. We are pursuing this in the context of Fruit Fly embryos.

E Boczko, C Stowers, T Gedeon, TY, ODE, RDE and SDE Models of Cell Cycle Dynamics and Clustering in Yeast, *J. Biol. Dynamics* **4** (2010), 328-345.

N Breitsch, G Moses, E Boczko, TY, Cell cycle dynamics: clustering is universal in negative feedback systems, to appear in *J. Math. Biology*.

O Diekmann, H Heijmans, H Thieme, On the stability of the cell size distribution, *J. Math. Biology* **19** (1984), 227248.

X Gong, G Moses, A Neiman, TY, Noise-induced dispersion and breakup of clusters in cell cycle dynamics, *J. Theor. Biololgy* **335** (2014), 160-169.

X Gong, R Buckalew, TY, E Boczko, Cell cycle dynamics in a response/signalling feedback system with a gap. *J. Biol. Dynamics* **8** (2014) 79-98.

RR Klevecz, D Murray, Genome wide oscillations in expression, *Molec. Biol. Reports* **28** (2001), pp. 73-82.

R Buckalew, Cell Cycle Clustering in a Nonlinear Mediated Feedback Model, *DCDS B* **19**(4), (2014), 867-881.

JB Robertson, CC Stowers, EM Boczko, CH Johnson, Real-time luminescence monitoring of cell-cycle and respiratory oscillations in yeast, *PNAS USA*, **105**(46) (2008), 17988-93

C Stowers, TY, E Boczko, The structure of populations of budding yeast in response to feedback. *Hypoth. Life Science* **1** (2011), 71-84.

TY, B Fernandez, R Buckalew, G Moses, E Boczko. Clustering in cell cycle dynamics with general response/signalling feedback, J Theor. Biology 292 (2012) 103-115.