Clustering in Cell Cycle Dynamics with General Forms of Feedback

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Todd Young
Department of Mathematics
Ohio University

Acknowledgments

Collaborators:

E. Bozcko, Vanderbilt Medical Center

B. Fernandez, CNRS Luminy, Marseille

T. Gedeon, Mathematics, Montana State

C. Stowers, Dow Chemical, Indianapolis

R. Klevecz, City of Hope Medical Center

At Ohio University:

S. Aizicovici, W. Just, A. Neiman, T. Savin

N. Breitsch, R. Buckalew, N. Burk, K. Finley, X. Gong, G. Moses

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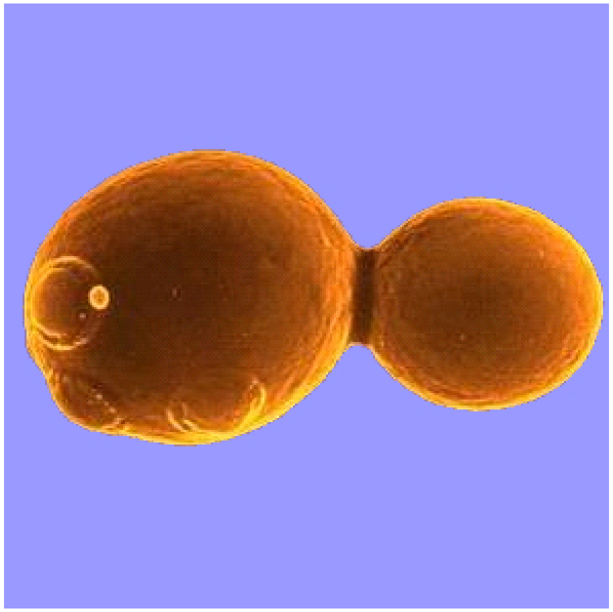
Saccharomyces cerevisiae

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

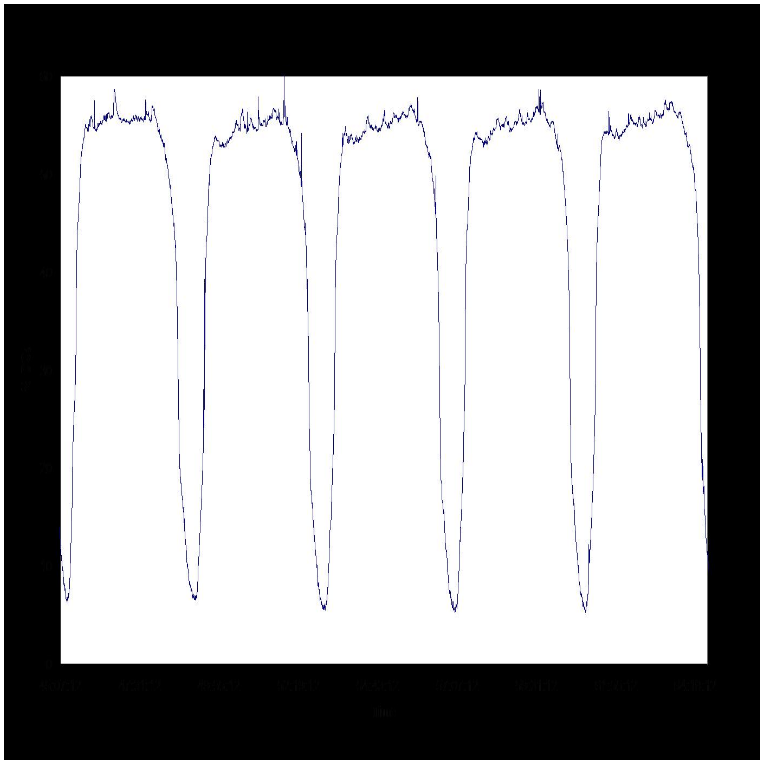
Brewer's, Baker's or Ale Yeast.

Studied by biologists as a model eukaryotic organism.

Yeast are used in many bio-engineering processes.

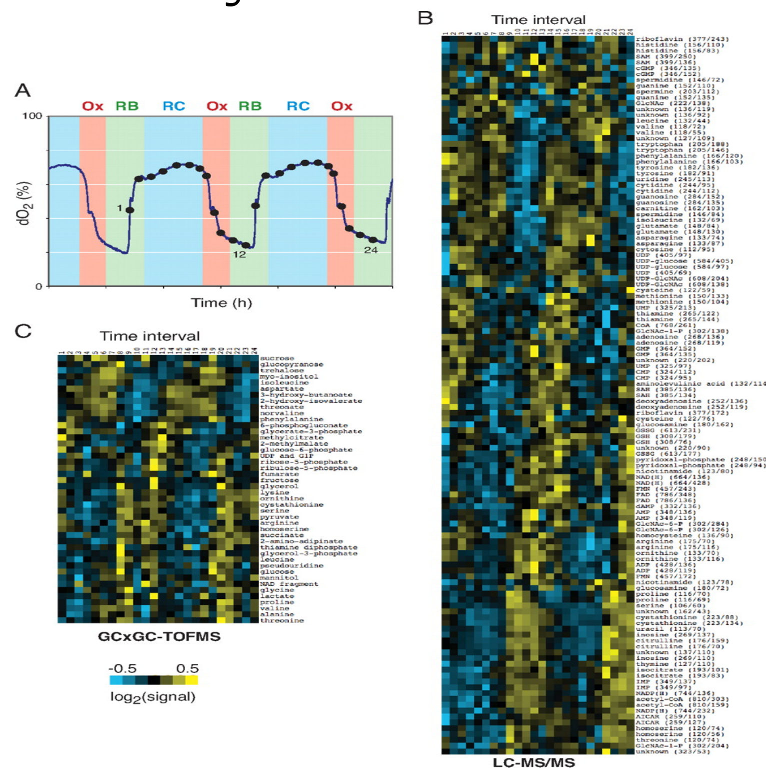
Oxygen oscillations

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.
- The period of oscillation is always nearly an integer fraction of the doubling time.

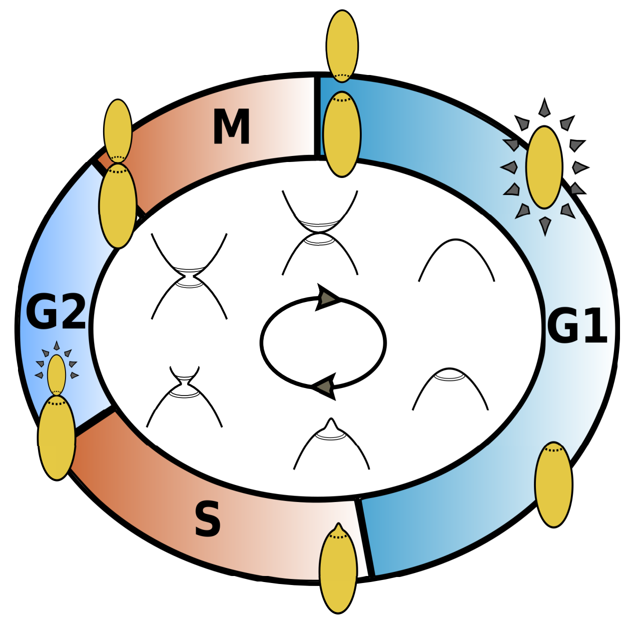
Cell Cycle of Budding Yeast

G1: gap phase, begins with cell division

S: replication phase, begins with budding

G2: second gap 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 can reinforce the formation 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:

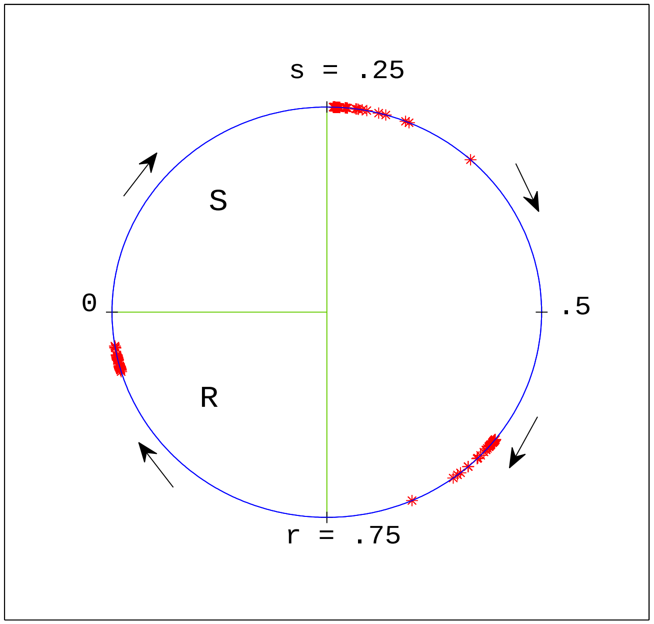
$$\frac{dx_i}{dt} = \begin{cases} 1, & \text{if} \quad x_i \notin R \\ 1 + \rho(\sigma), & \text{if} \quad x_i \in R. \end{cases} \tag{1}$$

$\rho(\sigma)$ is a monotone "response" function.

Clusters Exist - Simulations

S - Signaling, R - Responsive

Simulation, 500 cells,
Neg. 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, $n \sim O(10^{10})$.

In the model (1), a synchronized cluster of cells will persist.

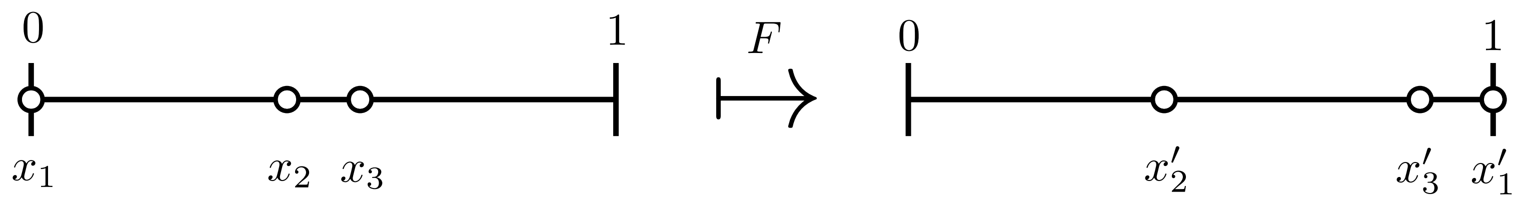
A clustered solution $\{x_i(t)\}_{i=1}^{k}$ is k **cyclic** if $\exists$ a time $d > 0$ s.t.:

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

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

Cluster Systems

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

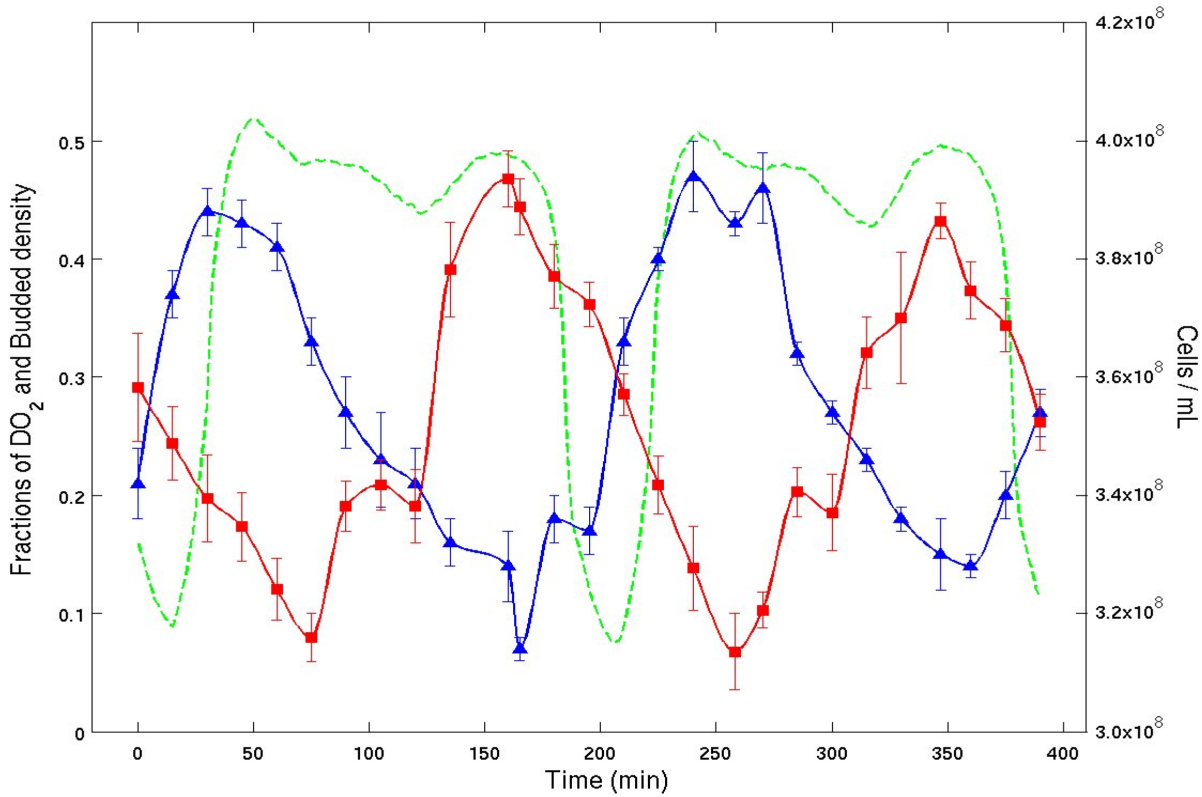


$F : S \to S$, $S = \{0 \leq x_1 \leq x_2 \leq \ldots \leq x_k \leq 1\}$.

Proof: Brouwer FPT $\implies$ F has a fixed point.
F permutes the boundary of S.

We can also use F to study solutions for $k = 2, 3$ 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.

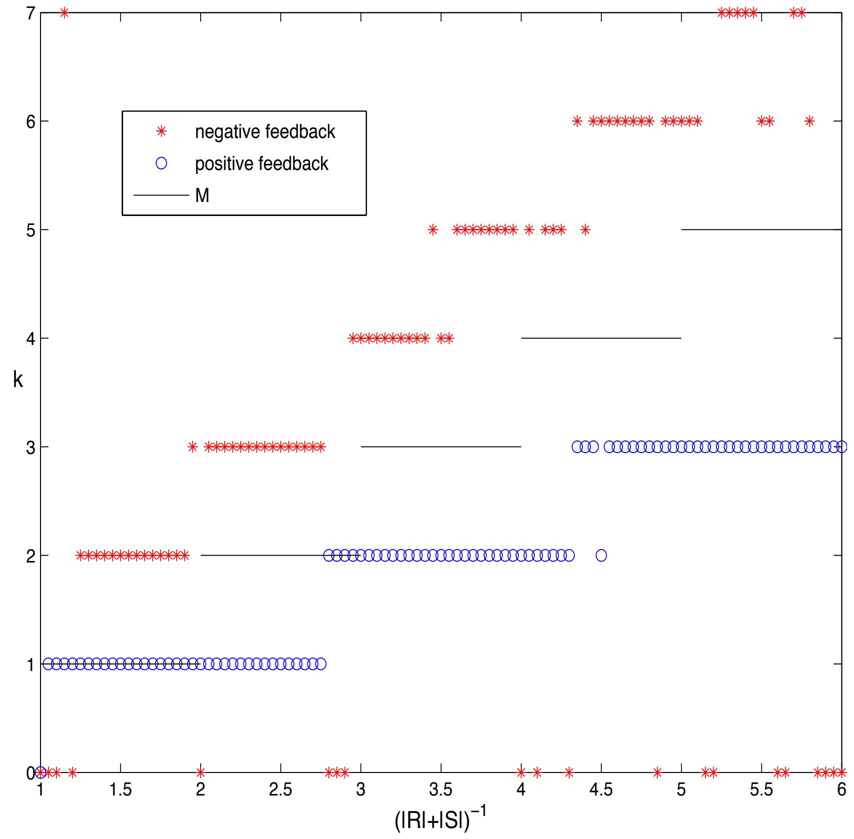
$M = \lfloor (|R| + |S|)^{-1} \rfloor$.

M - max # of clusters that can exist without interactions.

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

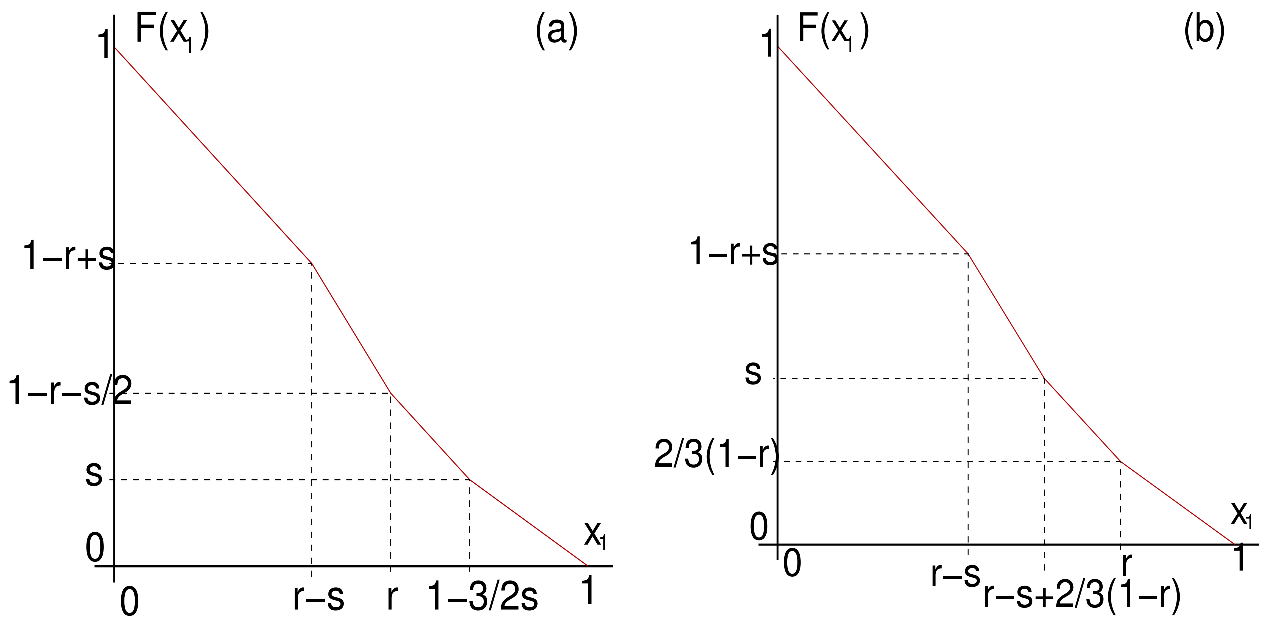
Theorem - For positive feedback (1) the set of solutions with non-interacting clusters solutions is locally asym. stable. For negative feedback it is unstable.

Negative vs. Positive Feedback



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

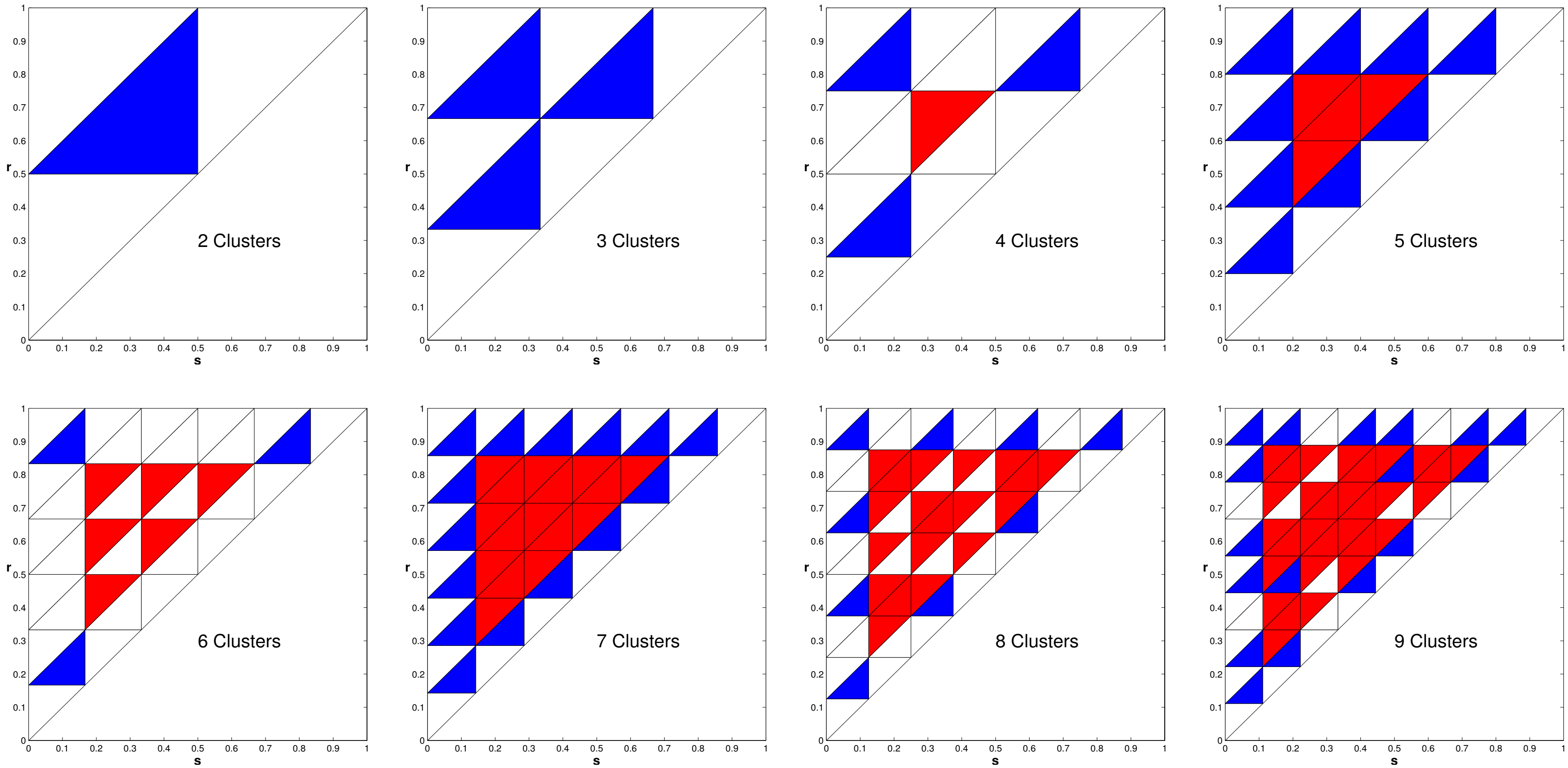
2 Cluster Systems - Detailed Analysis



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

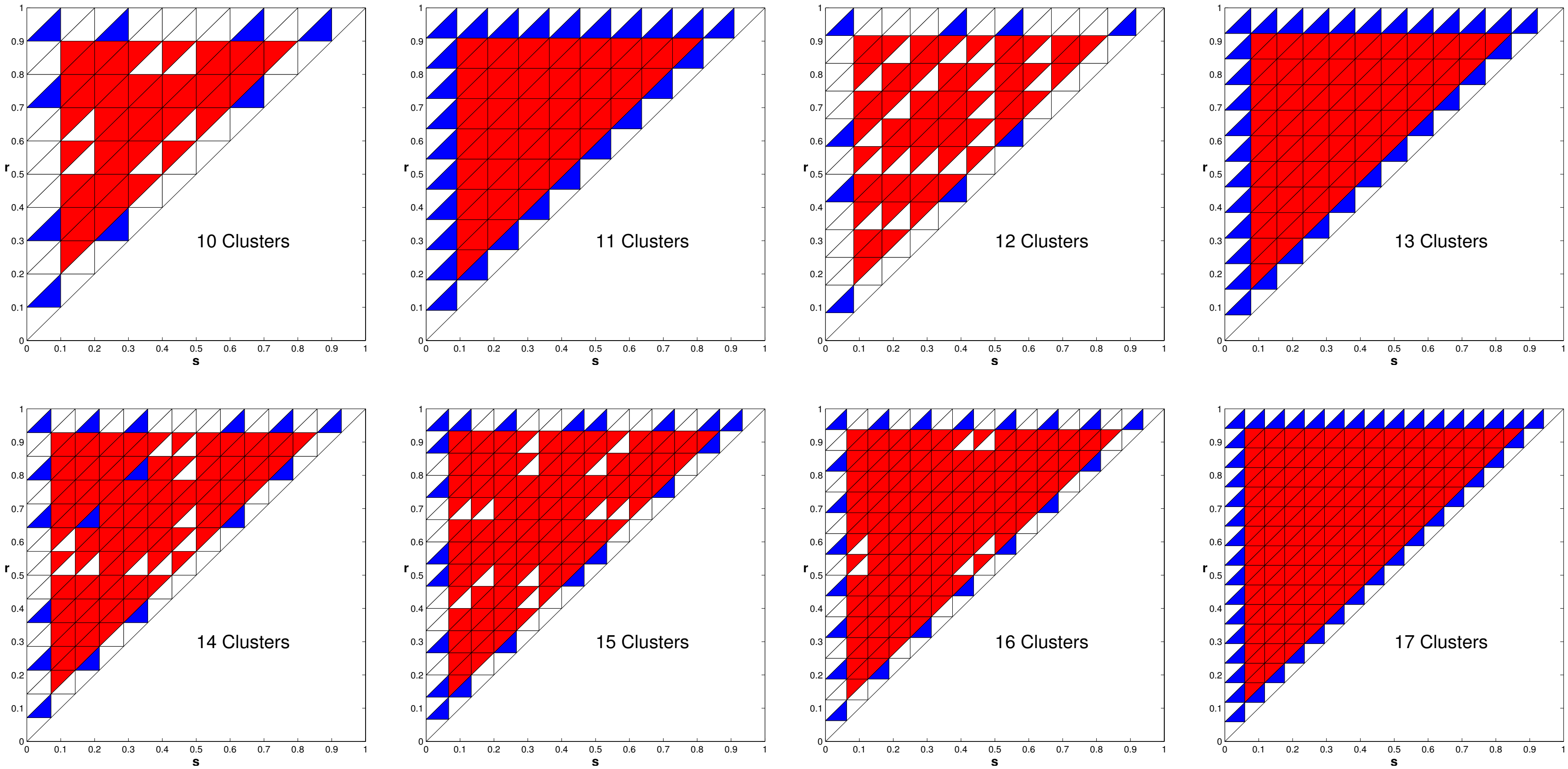
From F we can infer all dynamics.

Stability of the k cyclic solution in r-s parameter space



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

Stability of k cyclic solutions in r-s parameter space

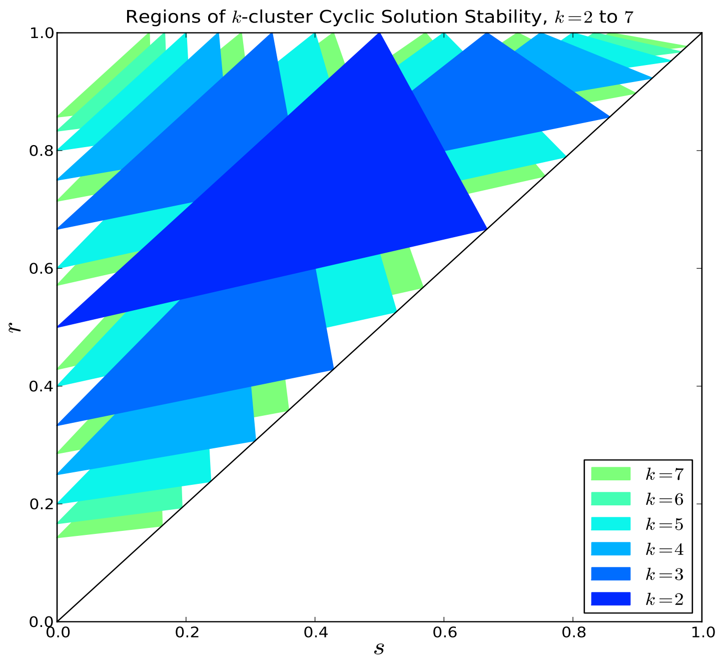


$k = 10, \ldots, 17$

Primes are Regular, Composites are Irregular!!.

Clustering is Universal for Negative Feedback

Overlay of Stable Regions:

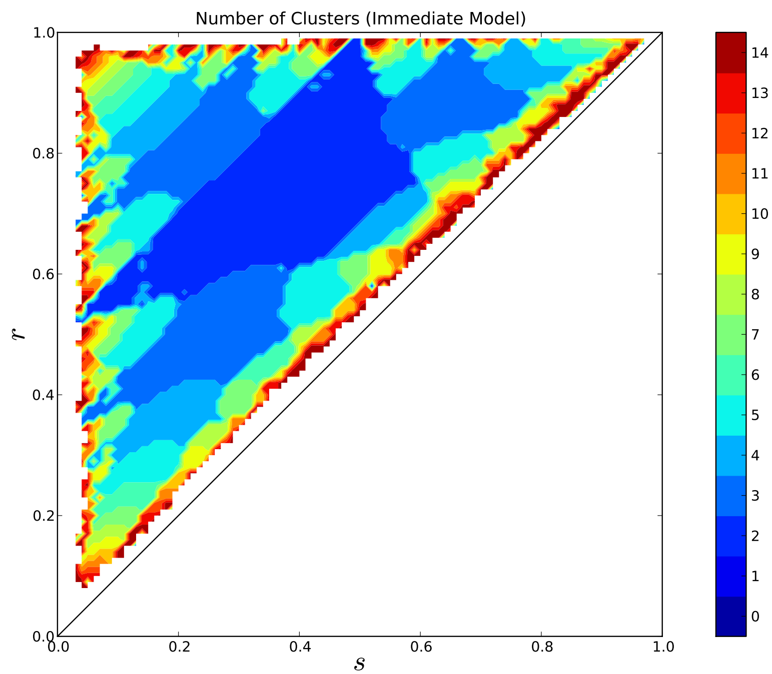


- Conjecture: All area is covered by stable regions.

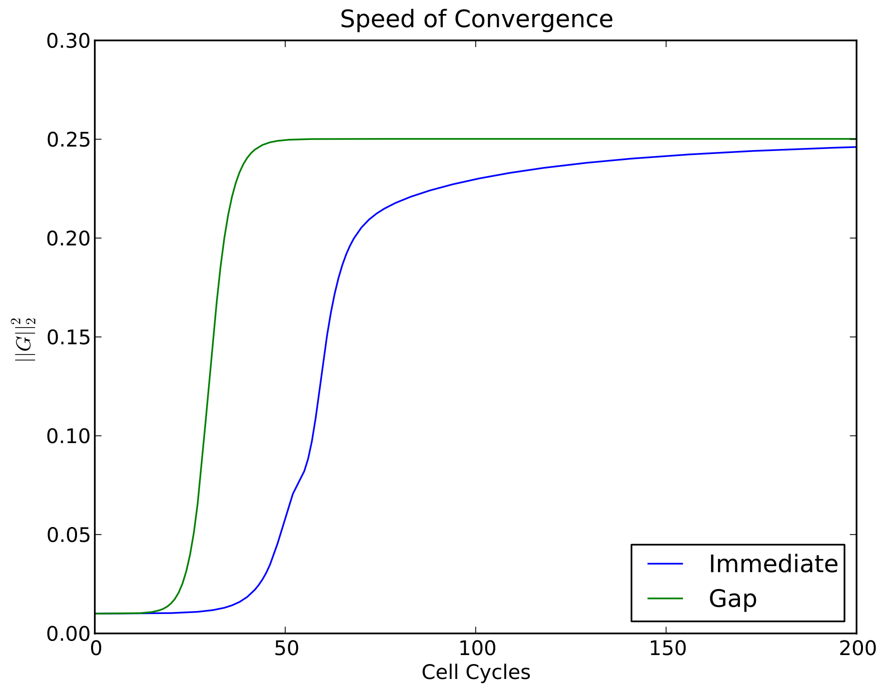
- There are many regions of Bistability.

Another Nice picture

Actual number of clusters:



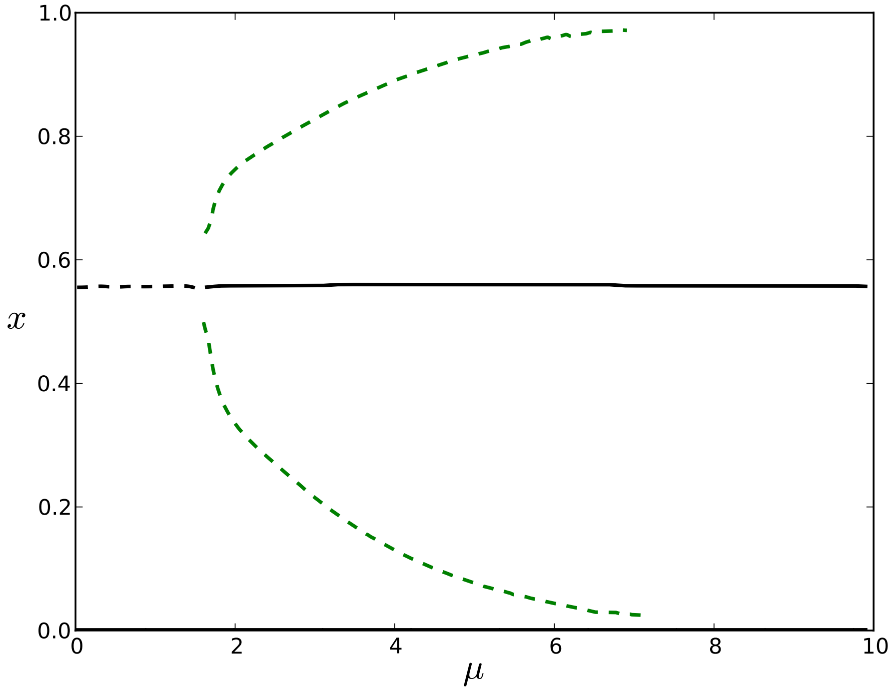
Model with a gap (delay)



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

Some Results for General RS Feedback

1. For positive feedback, interacting cluster solutions are unstable and solutions tend to $k \leq M$ isolated clusters. Only $k = 1$, *Synchronization*, is asymptotically stable.

2. For negative feedback, synchronization is unstable, interaction is necessary to enforce coherence. Most solutions tend to $k \gtrsim M$ interacting clusters. $k \neq 1$!

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 neutral-stable; small perturbation may cause clusters to merge.

• 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.