Clustering in Cell Cycle Dynamics with General Forms of Feedback

University of Warwick
Complexity Forum, October 2014

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Acknowledgments

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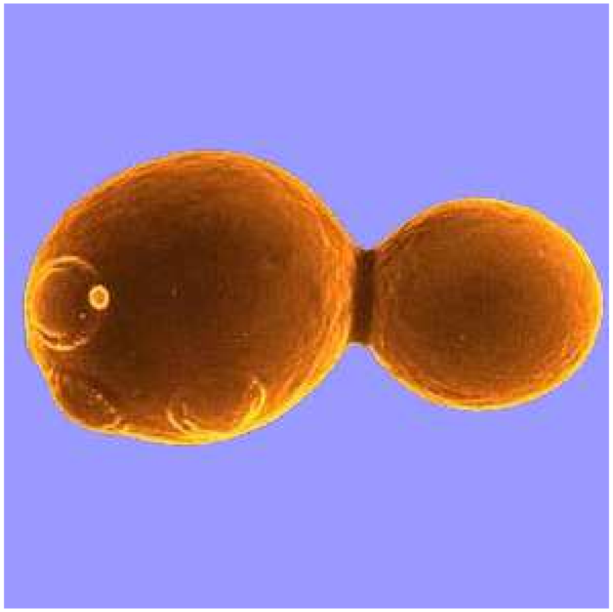
Supported by “Dynamics and Bifurcations of Population Structures Induced by Cell Cycle Feedback” NIH # R01GM090207.

Saccharomyces cerevisiae



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

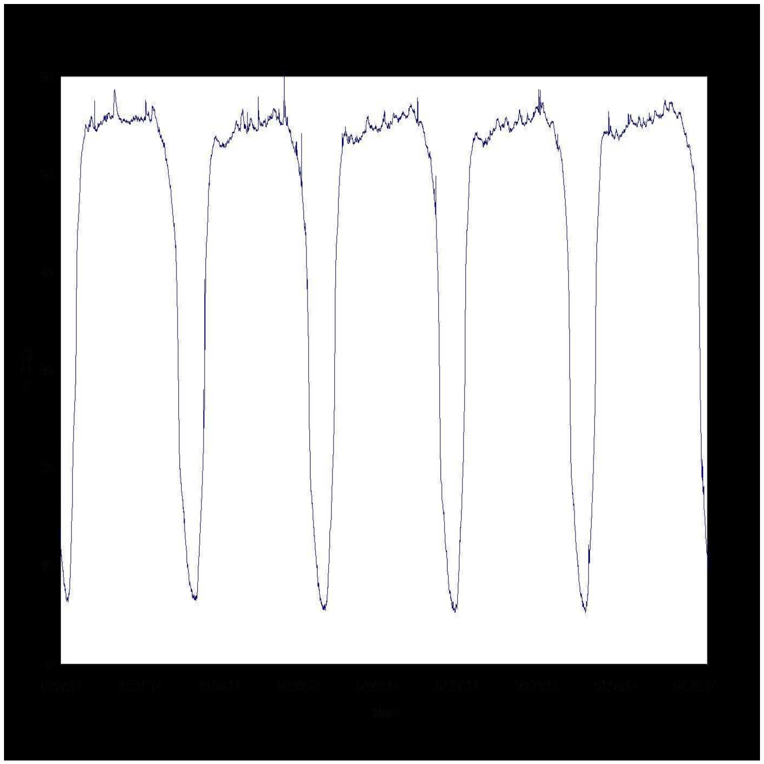
Brewer's, Baker's or Ale Yeast.

Studied by biologists as a model eukaryotic organism.

Yeast are used in many bio-engineering processes.

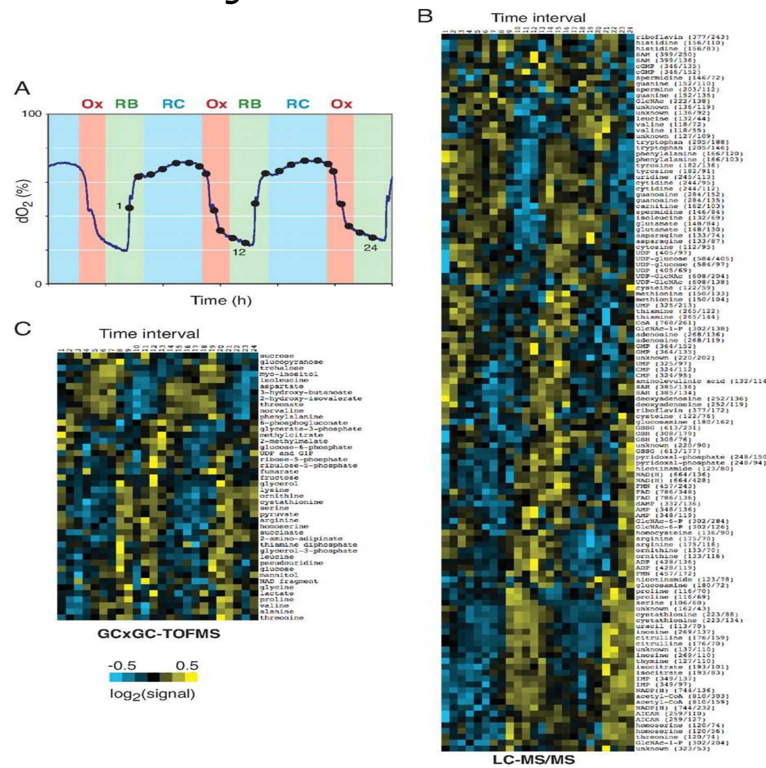
Metabolic Oxygen Oscillations

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.

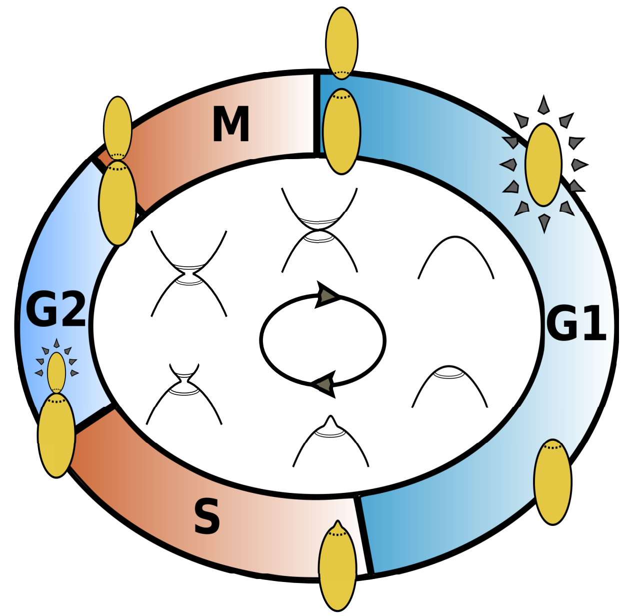
Cell Cycle of Budding Yeast

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 unstable in budding yeast. Initially synchronized cultures quickly de-synch, due to asymmetric division.

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:

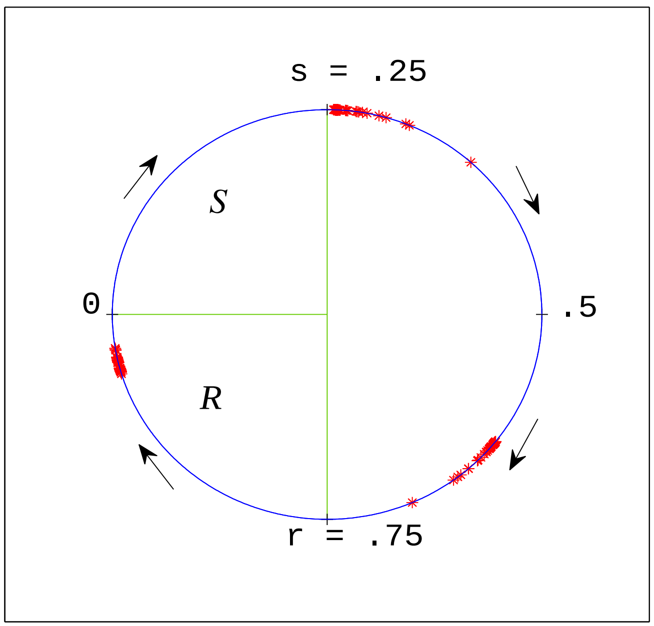
$$\frac{dx_i}{dt} = \begin{cases} 1, & \text{if} \quad x_i \notin R \\ 1 + \rho(\sigma), & \text{if} \quad x_i \in R. \end{cases} \tag{1}$$

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

Clusters Exist - Simulations

S - Signaling, R - Responsive

Simulation, 500 cells,
Negative feedback & noise:



- Simulations with any 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.:

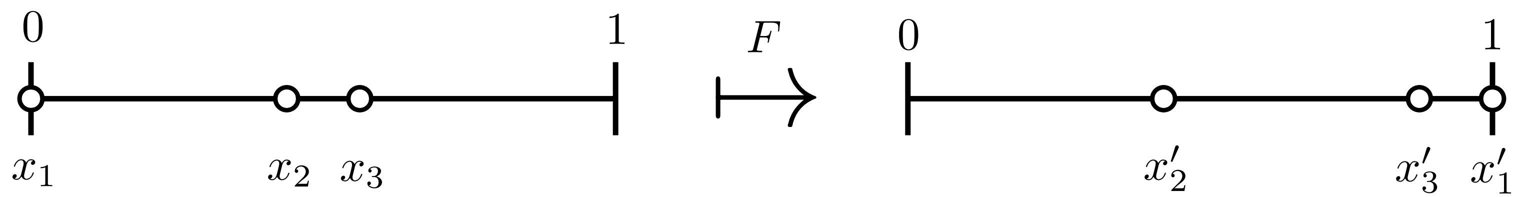
$$x_i(d) = x_{i+1}(0) \quad \forall \quad i = 1, \ldots, k-1,$$
$$\text{and} \quad x_k(d) = x_1(0) \quad \text{mod } 1.$$

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.



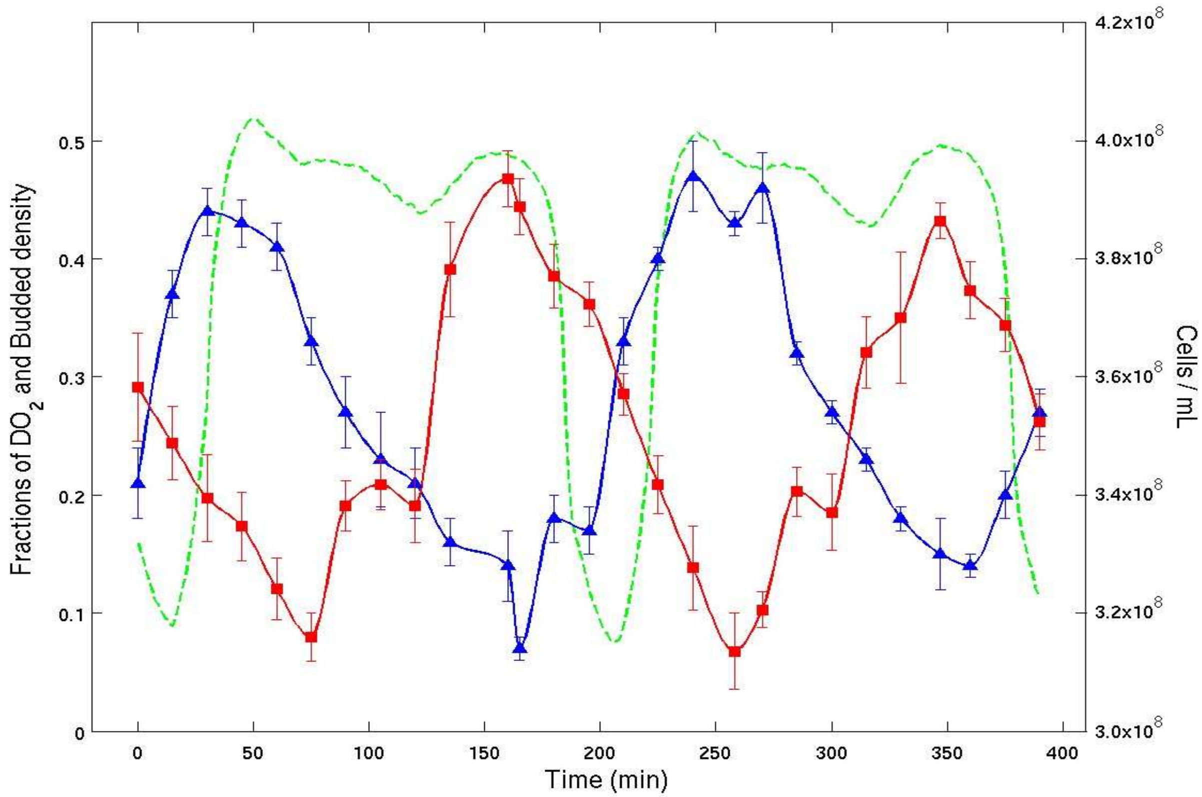
F consists of flowing until $x_k(t) = 1$, then reordering indices.
$F : S \to S$, $S = \{0 \le x_2 \le \ldots \le x_k \le 1\}$.

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 in anti-phase.

Isolated Clusters and the Geometric Constant M

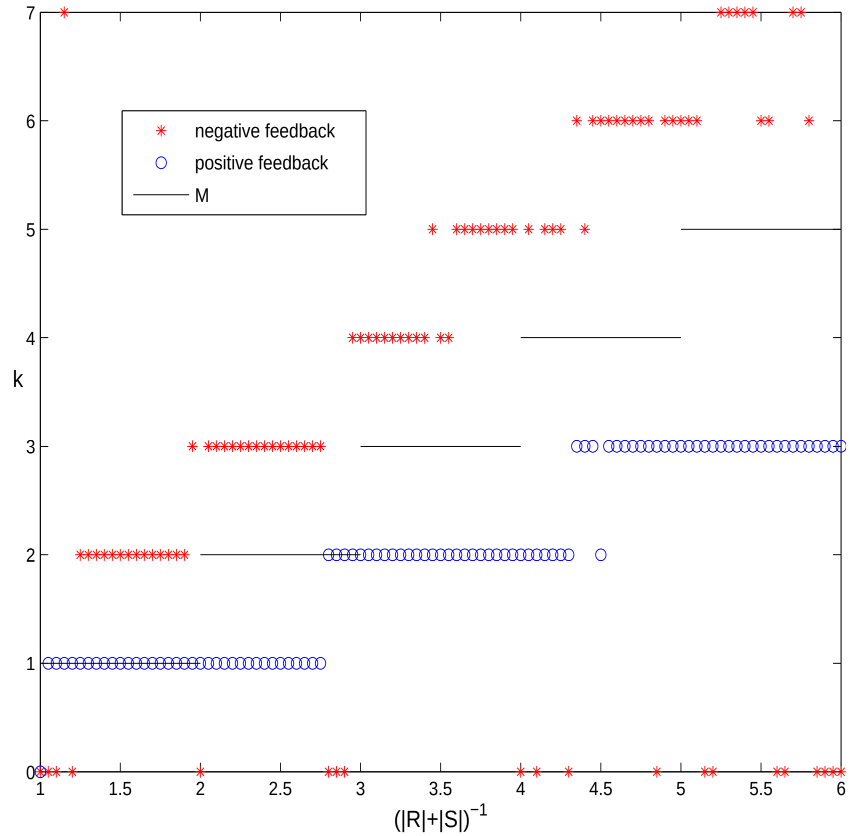
If the distance between two clusters is more that $|R| + |S|$ then those two clusters will not interact.

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

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

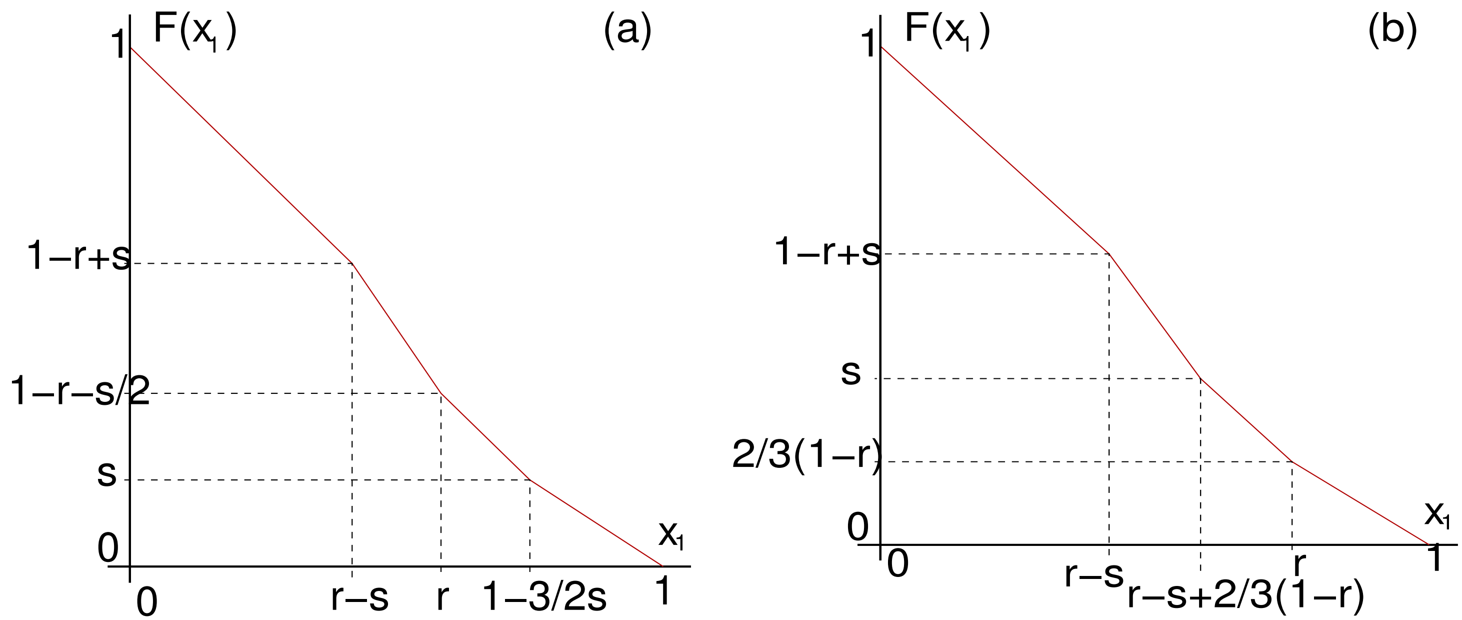
Theorem - For positive feedback the set of solutions with non-interacting clusters is locally asymptotically stable. For negative feedback it is unstable. Synchronized solution is stable for positive feedback, unstable for negative. True for general models.

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

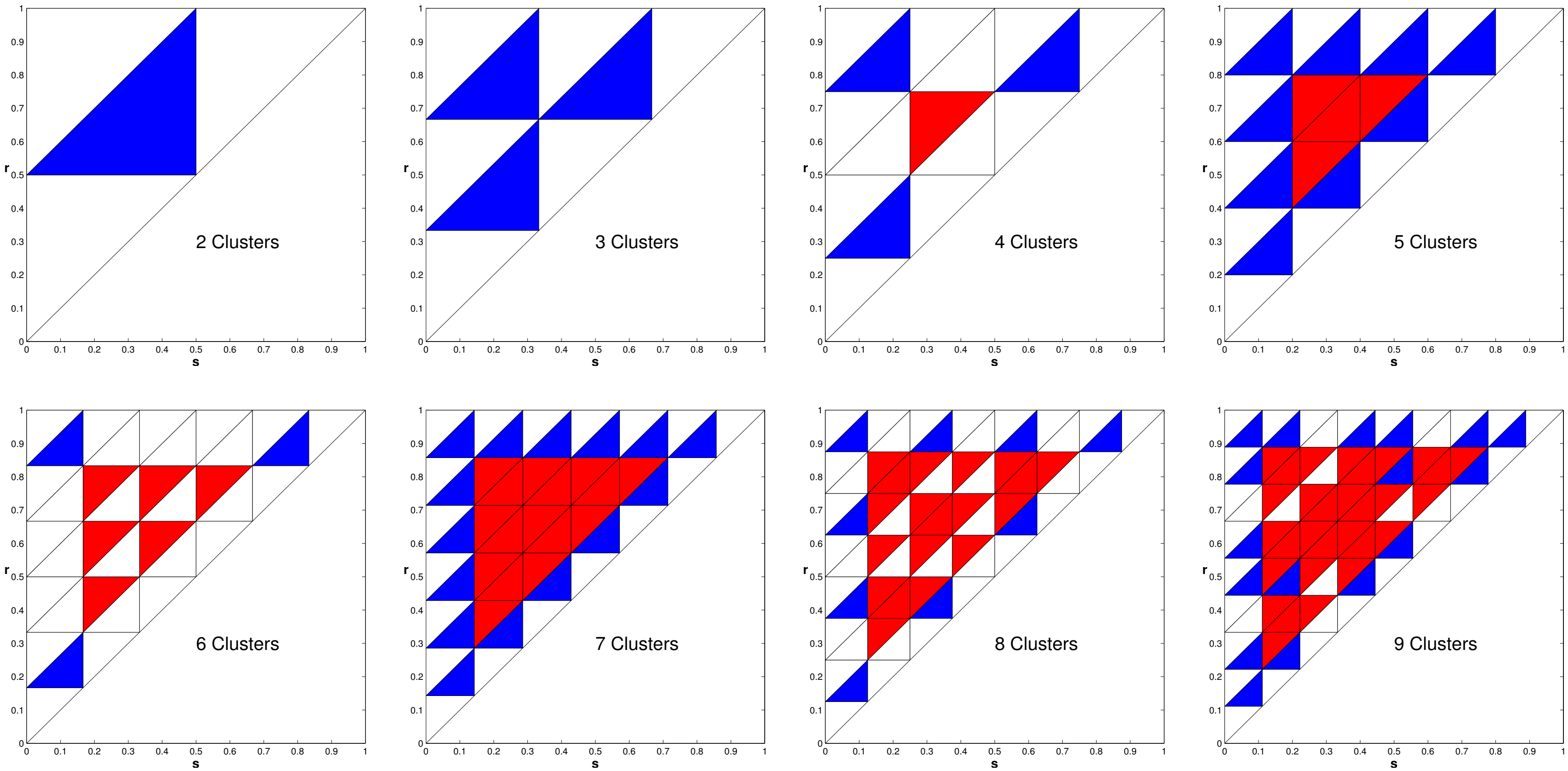


F for positive feedback, $k = 2$. (a) $r + \frac{3}{2}s < 1$. (b) $r + \frac{3}{2}s \geq 1$.

From F we can infer all dynamics of 2 cluster solutions:

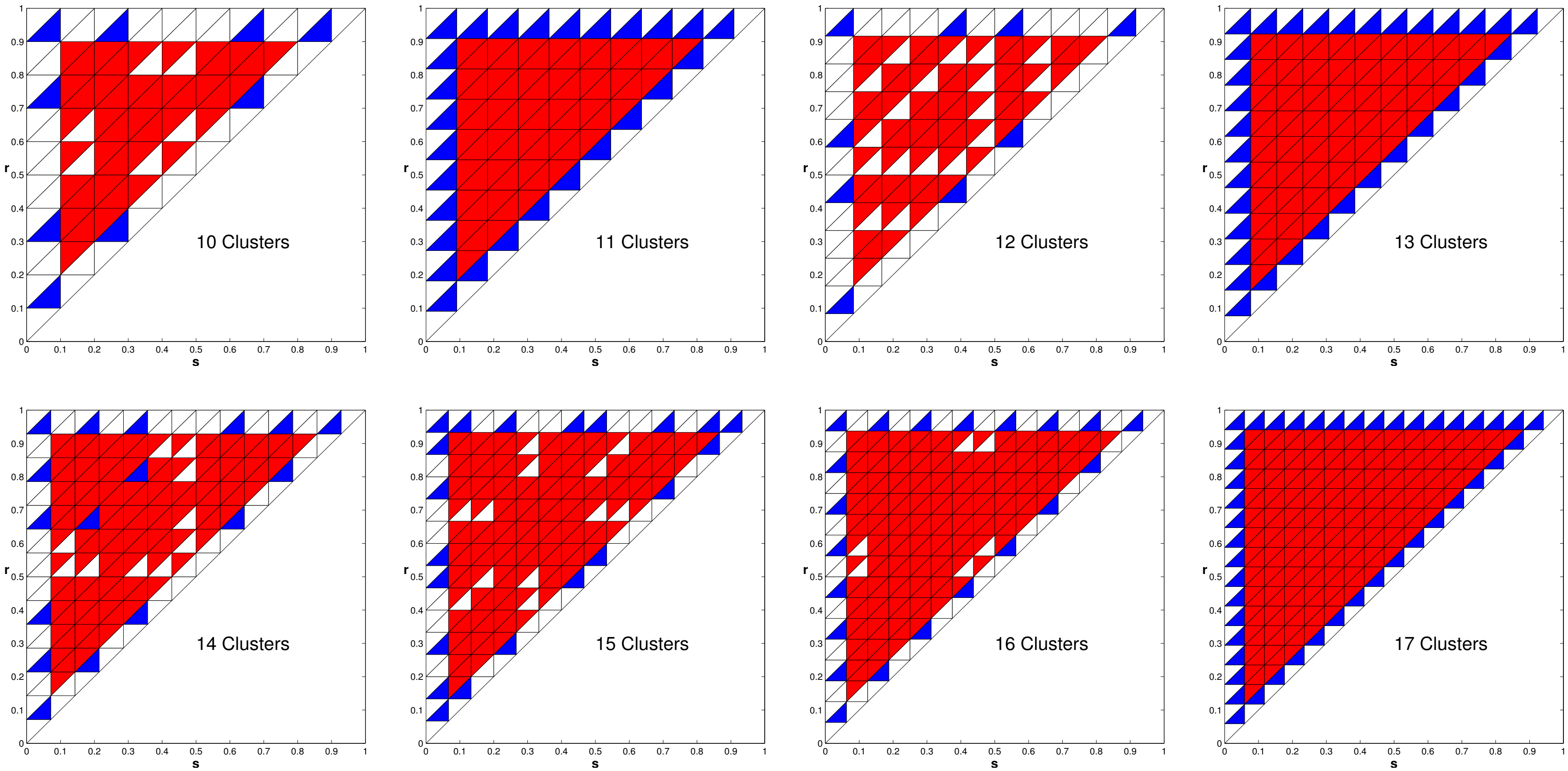
- Cyclic solution is unstable for positive feedback.
- Cyclic solution is stable for negative feedback and large set of parameter values.

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!!.

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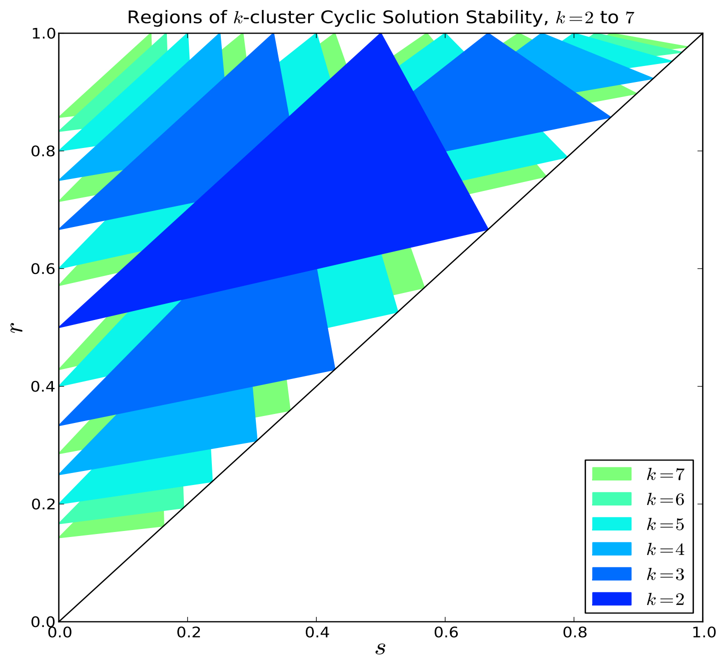
Clustering is Universal for Negative Feedback

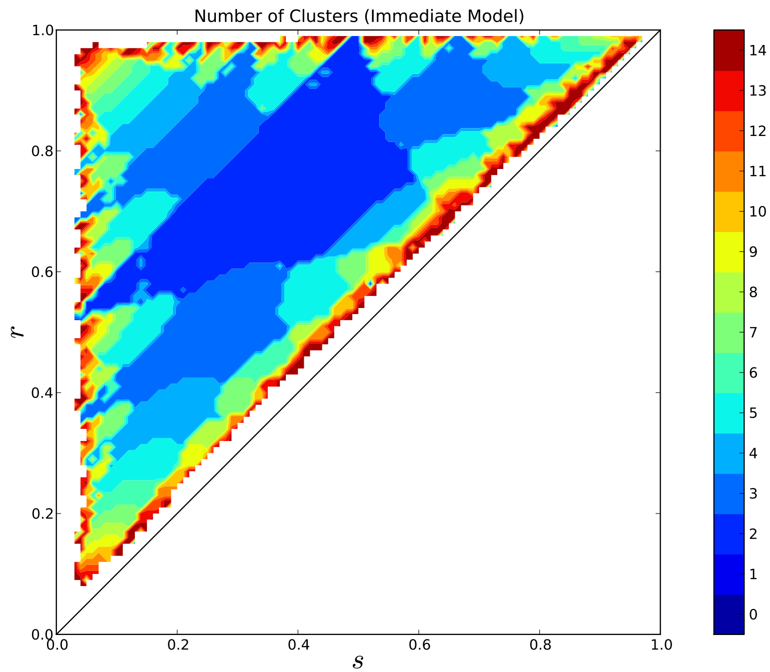
Overlay of Stable Regions:

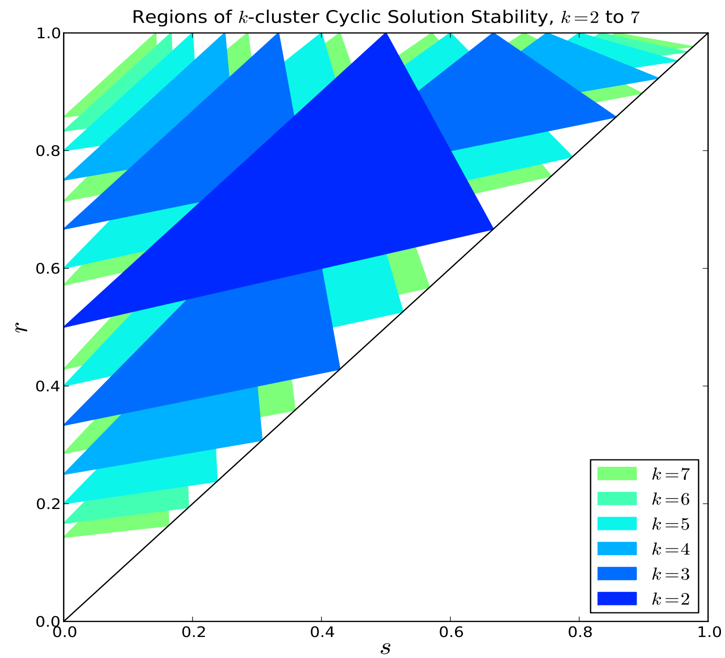


- There are many regions of Bistability.
- Conjecture: All area is covered by stable regions.

Another Nice picture

Number of clusters in simulations vs. overlay of stable regions:





$n = 720, \quad f(\sigma) = -0.6\sigma$

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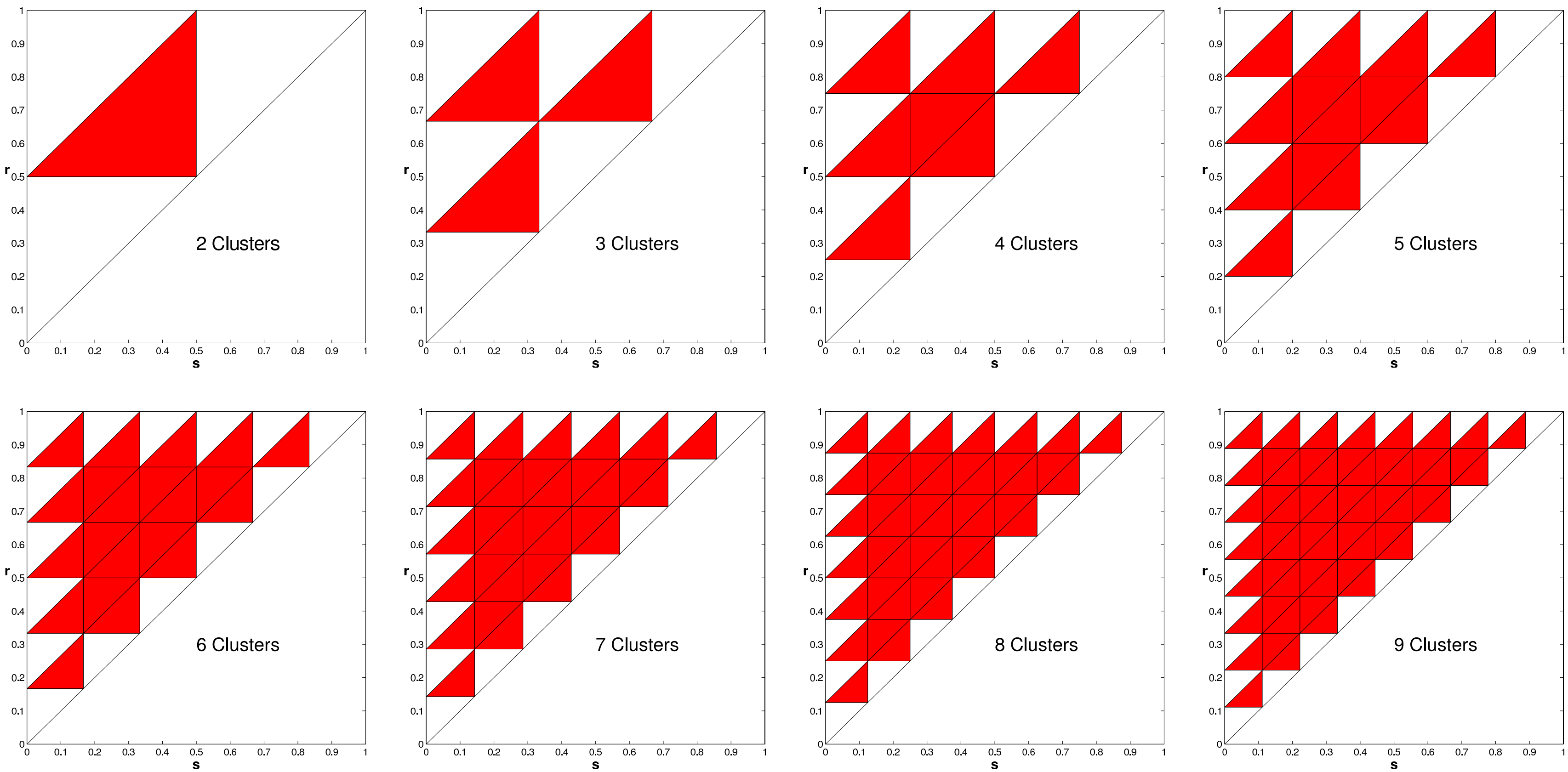
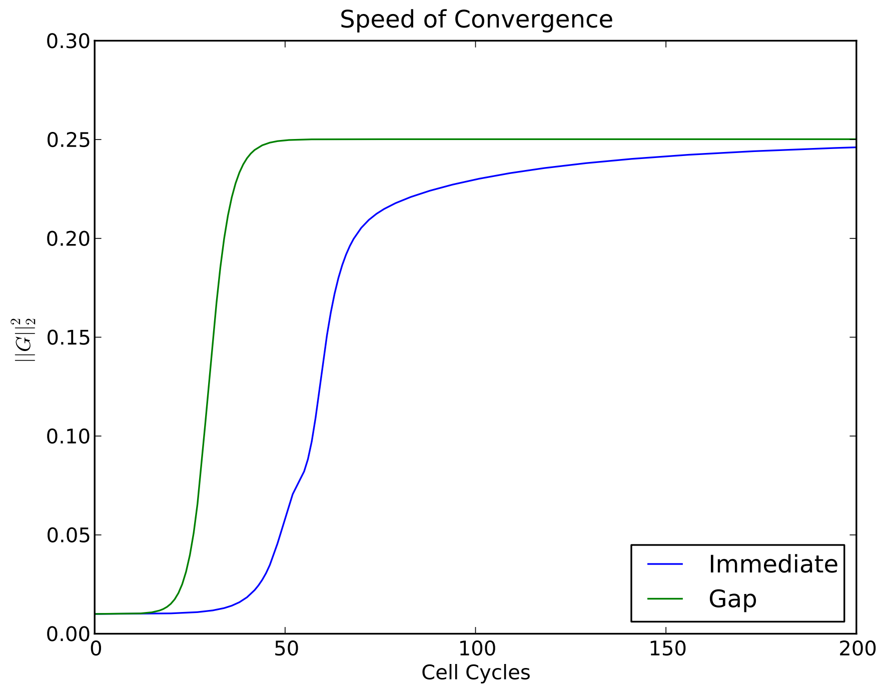
Instability of k-cyclic solutions with Positive Feedback



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

Model with a gap (delay)

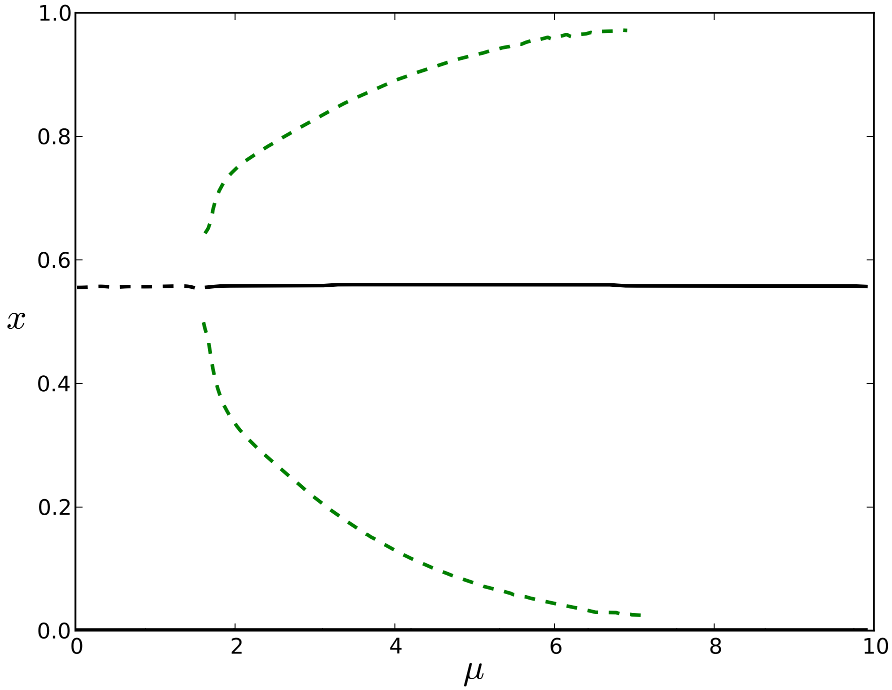


$n = 720$, $\quad k = 4$ clusters form.

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



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

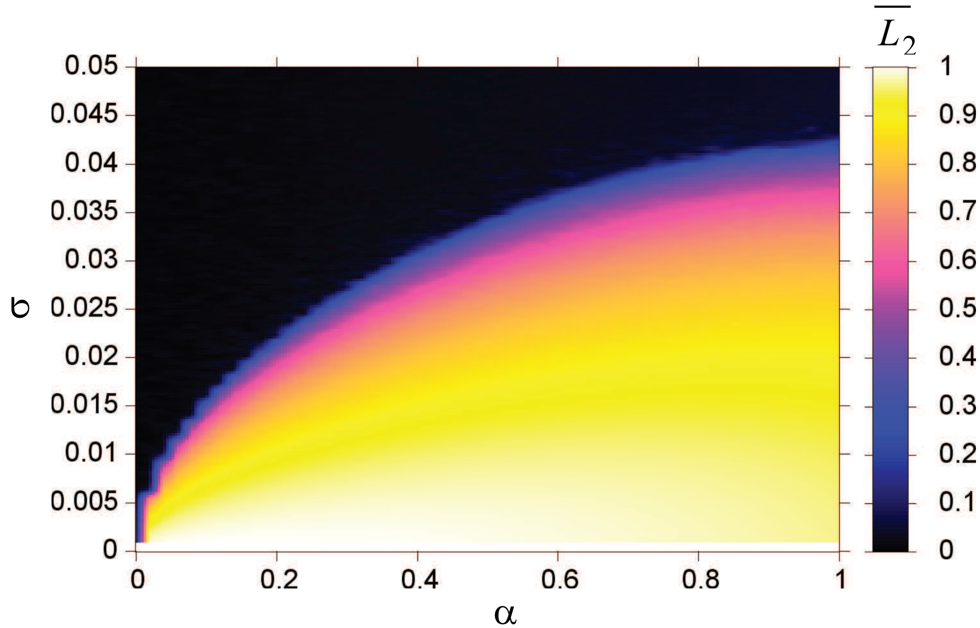
$$\frac{dz}{dt} = \mu\sigma - \gamma z$$

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

2 cluster cyclic solution always exists

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

The model with noise



Noise due to asymmetric division: $\sigma \approx 0.036$.
(estimated from oscillation experiments).
The negative feedback parameter must be at least $\alpha = .6$.
The "slowdown" is at least 1/3 of the normal rate.

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.

- Cell cycle clustering is experimentally verified.

- The biological mechanism driving clustering seems to be *large negative feedback*.

Some Open Problems

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

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

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

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

O. Diekmann and collaborators proved stability of the uniform solution for dispersive 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.