Temporal Clusters Prefer to be Equally Distributed - an example from the Yeast Cell Cycle

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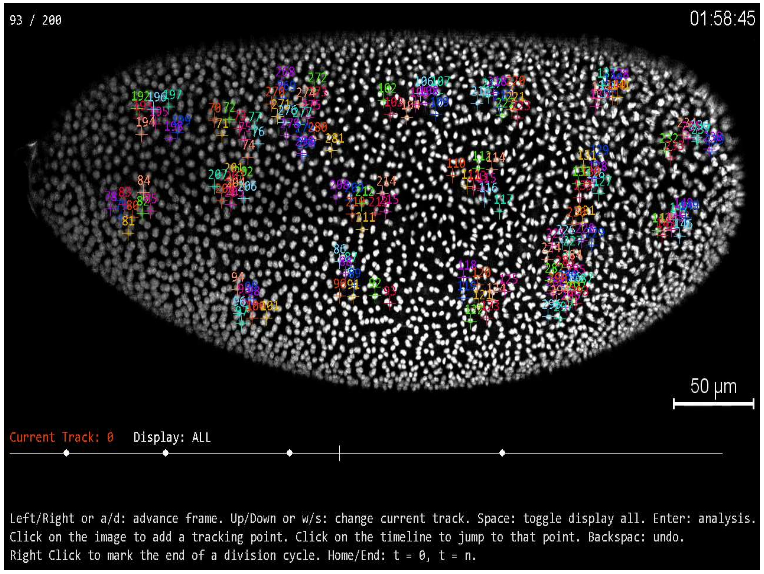
Synchrony

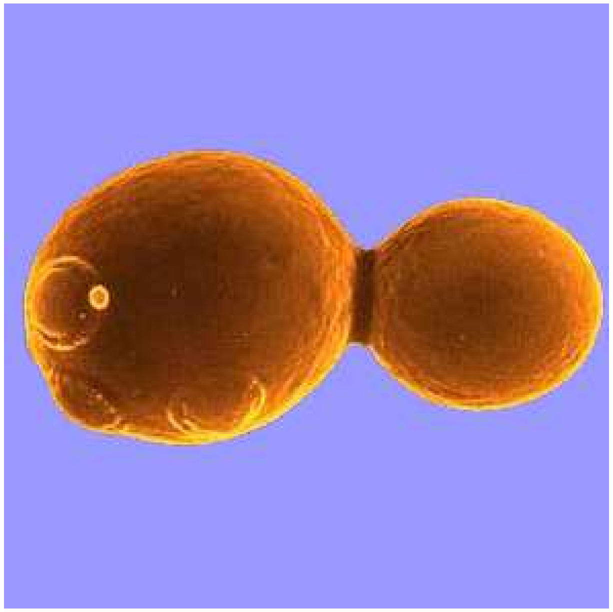


Drosophila embryo:

- Divides synchronously for first 13 divisions.

Common in Eukaryotes embryos. (Except mammals.)

Saccharomyces cerevisiae - Budding Yeast

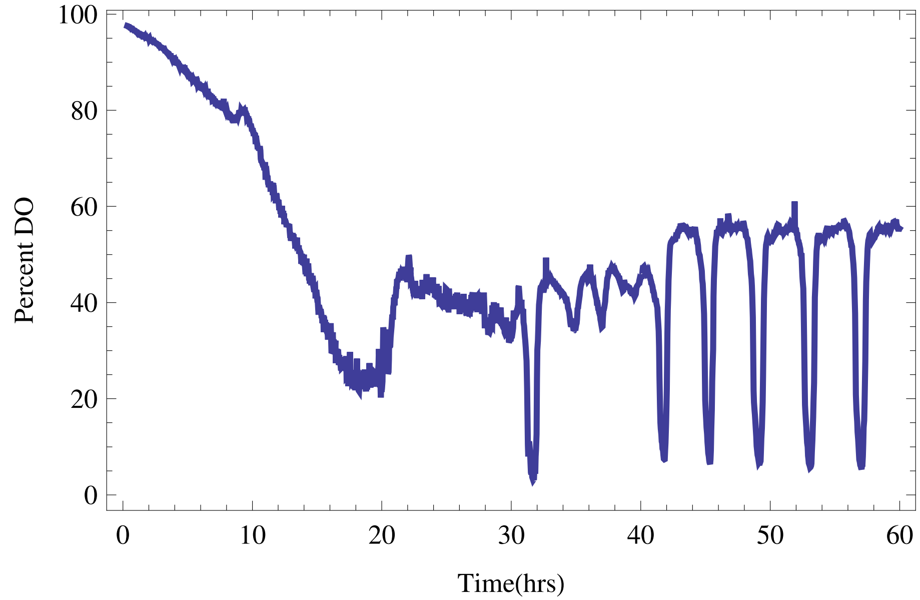
 

Photos: Wikipedia, www.kaeberleinlab.org, www.alltech.com

Brewer's, Baker's or Ale Yeast. Studied by biologists as a model eukaryotic organism.

Cell cycle synchrony is impossible to sustain in *S. cerevisiae*.

Yeast Metabolic Oscillations.



A type of synchrony? Yes, metabolic.

A connection between some of these oscillations to the cell cycle was observed long ago, but never explained.

Temporal 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 (via metabolic products?).

This feedback can reinforce the formation of clusters.

Clustering and Oscillations are intrinsically linked.

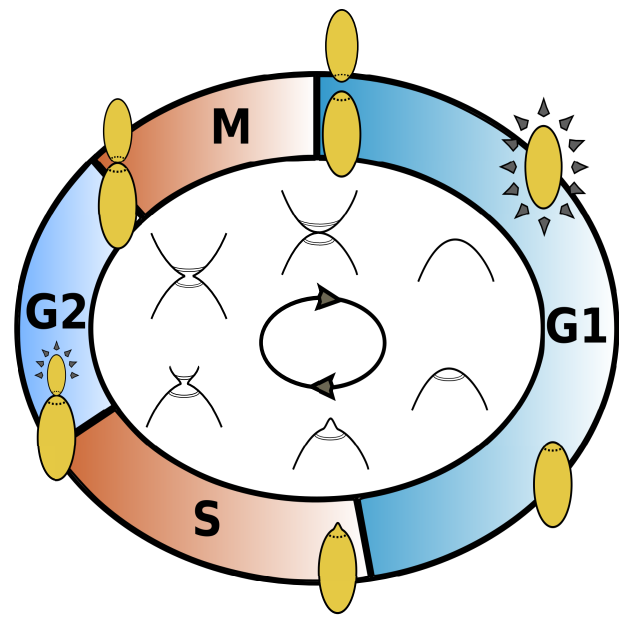
Cell Cycle of Budding Yeast

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



- *Different, complex* chemistry in each phase.
- Cells in one phase may produce chemicals that influence cells in another phase.

A Model with Phase-Specific Coupling.

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

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

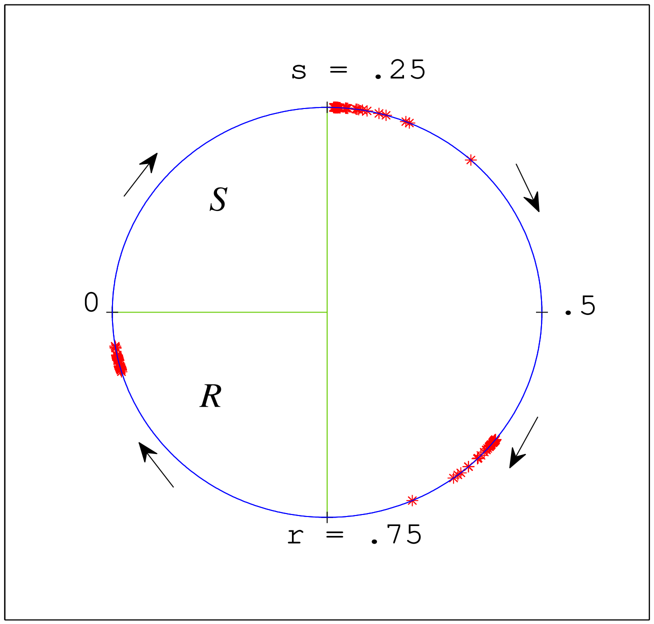
$I = \#\{\text{cells in } S\}/n$. $\qquad n \sim O(10^{10})$ - number of cells.

RS feedback model:

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

$\rho(I)$ - monotone "response" function, $+$ or $-$, *Nonlinear*.

Clusters Exist - Simulations



500 cells, Negative linear feedback & noise.

R - Responsive

S - Signaling

- Negative RS feedback almost always produces clusters.

Clusters Exist - Mathematics

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

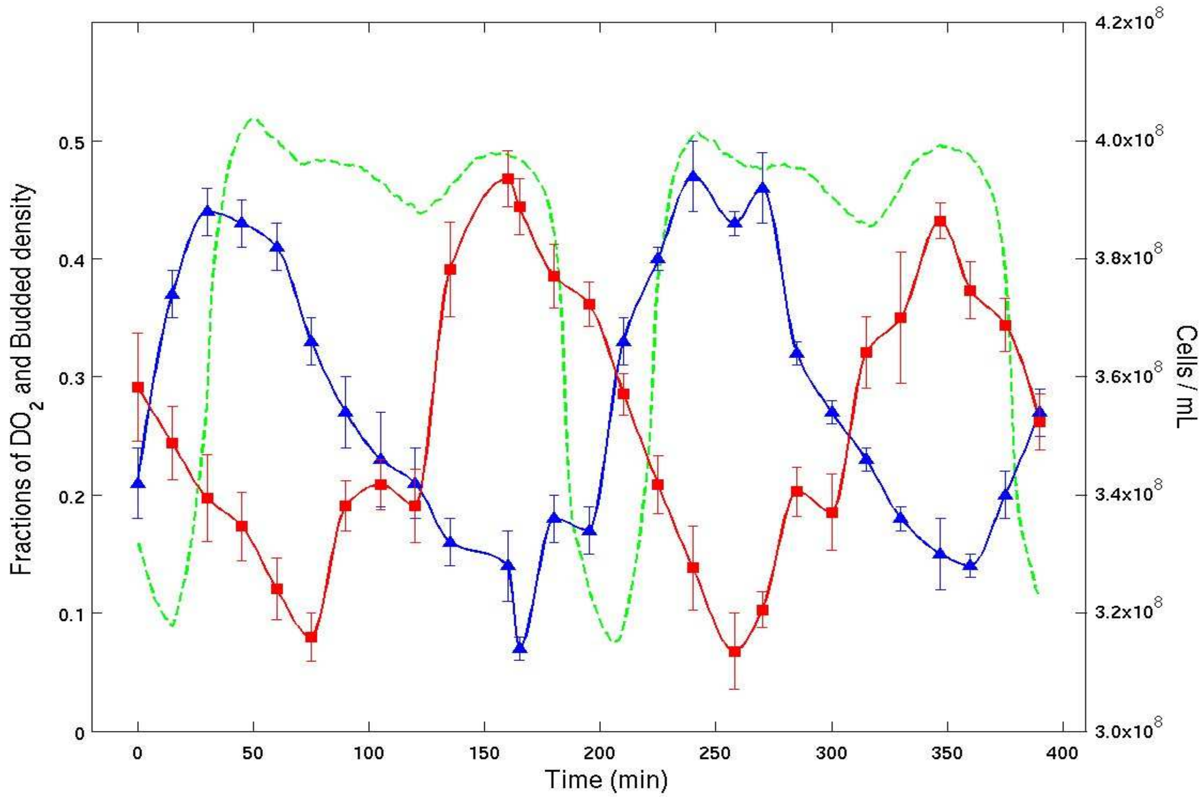
A clustered solution $\{x_i(t)\}_{i=1}^{k}$ is k **cyclic** if $\exists$ a time $d > 0$ s.t.:

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

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*.

Clusters Exists - Experiments.



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

Mathematical questions:

What are the differences between systems that Synchronize and those that Cluster?

What determines how many clusters form?

When are clusters asymptotically stable?

We answered many questions like these is a series of papers under the assumption that $k|n$ and each cluster has n/k cells.

Some Conclusions for General Cell Cycle Feedback

- Systems that Synchronize and Cluster are very different!

- Positive Feedback robustly produces Synchronization.

- Clustering is a robust phenomenon for Negative Feedback:

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

  - Not dependent on functional form of feedback.

  - It occurs for large open sets of parameter values.

  - It requires interaction among clusters.

  - All conclusions are for equal clusters.

Can clusters be unequal?

Poincaré Section and Return Map

Def: *We say that a hyper-surface* Σ *is a* **Poincaré section** *if* Σ *is transverse to a flow* $\phi^t(x)$ *at every* $x \in \Sigma$*, and, every solution reaches* Σ *in finite time.*

For any $x \in \Sigma$, $\phi^t(x)$ will return to Σ for a minimal $t^* > 0$.

Define $\Pi(x)$ to be the return point.

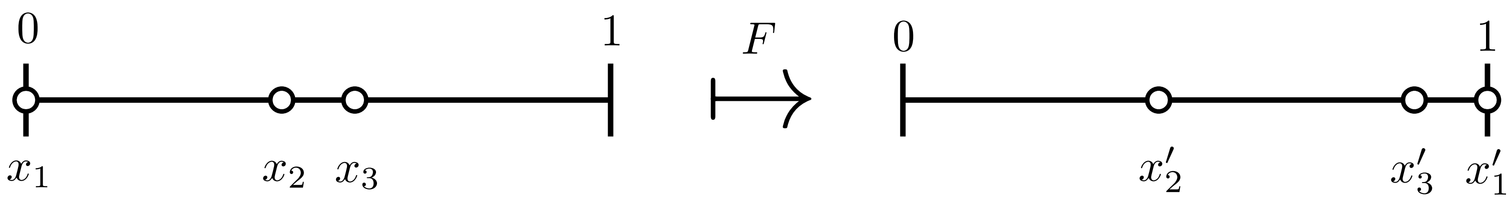
For our system, since $\dot{x}_i > 0$, any set $\{x_i = c\}$ is a Poincaré section.

We will use $\Sigma = \{x_1 = 0\}$

A Fixed Point of Π is a Periodic Orbit for the flow.

Even Cluster Systems

Strategy: Use the map F below. F^k is the Poincaré return map.

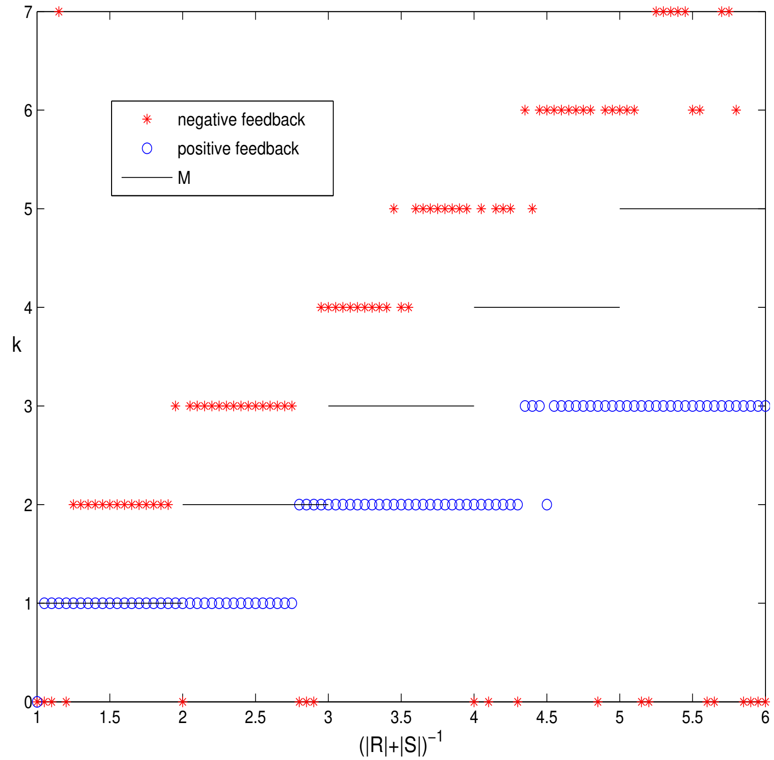


F consists of flowing until $x_k(t) = 1$, then reordering indices.
$F : S \to S$, $S = \{0 \leq x_2 \leq \ldots \leq x_k \leq 1\}$.

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

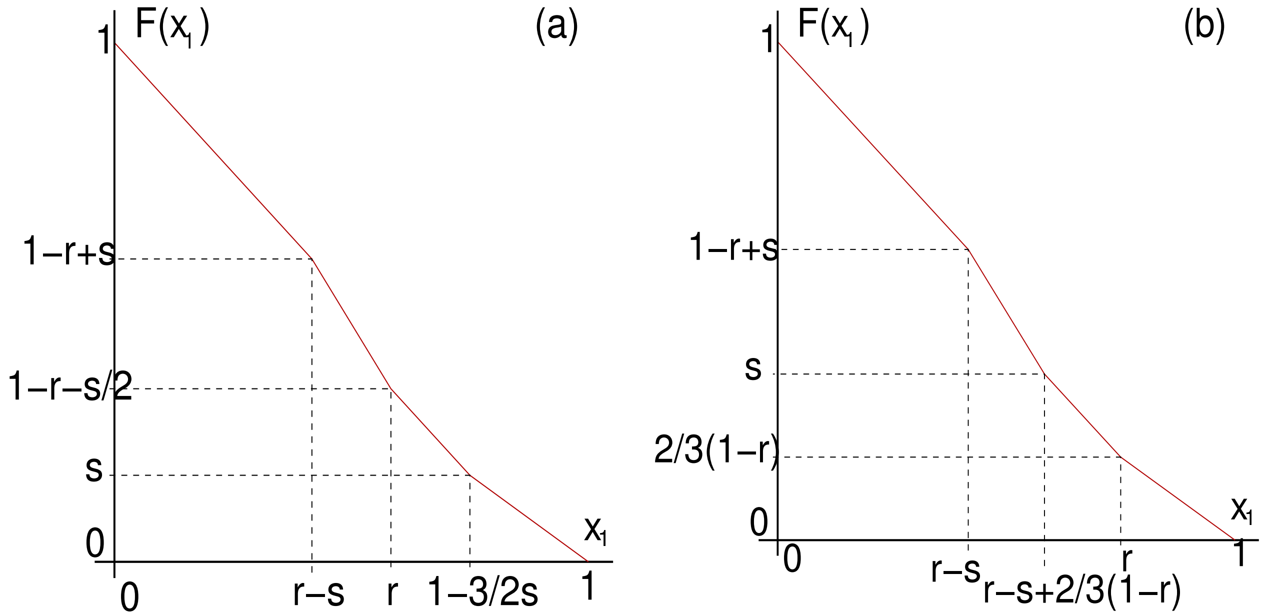
We can also use F to study solutions for small k in detail.

Negative vs. Positive Feedback



The number of clusters that form in simulations compared with $M = \lfloor (|R| + |S|)^{-1} \rfloor$. M is the number of clusters that can exist without interactions. Stable clustering requires negative feedback and interaction between clusters.

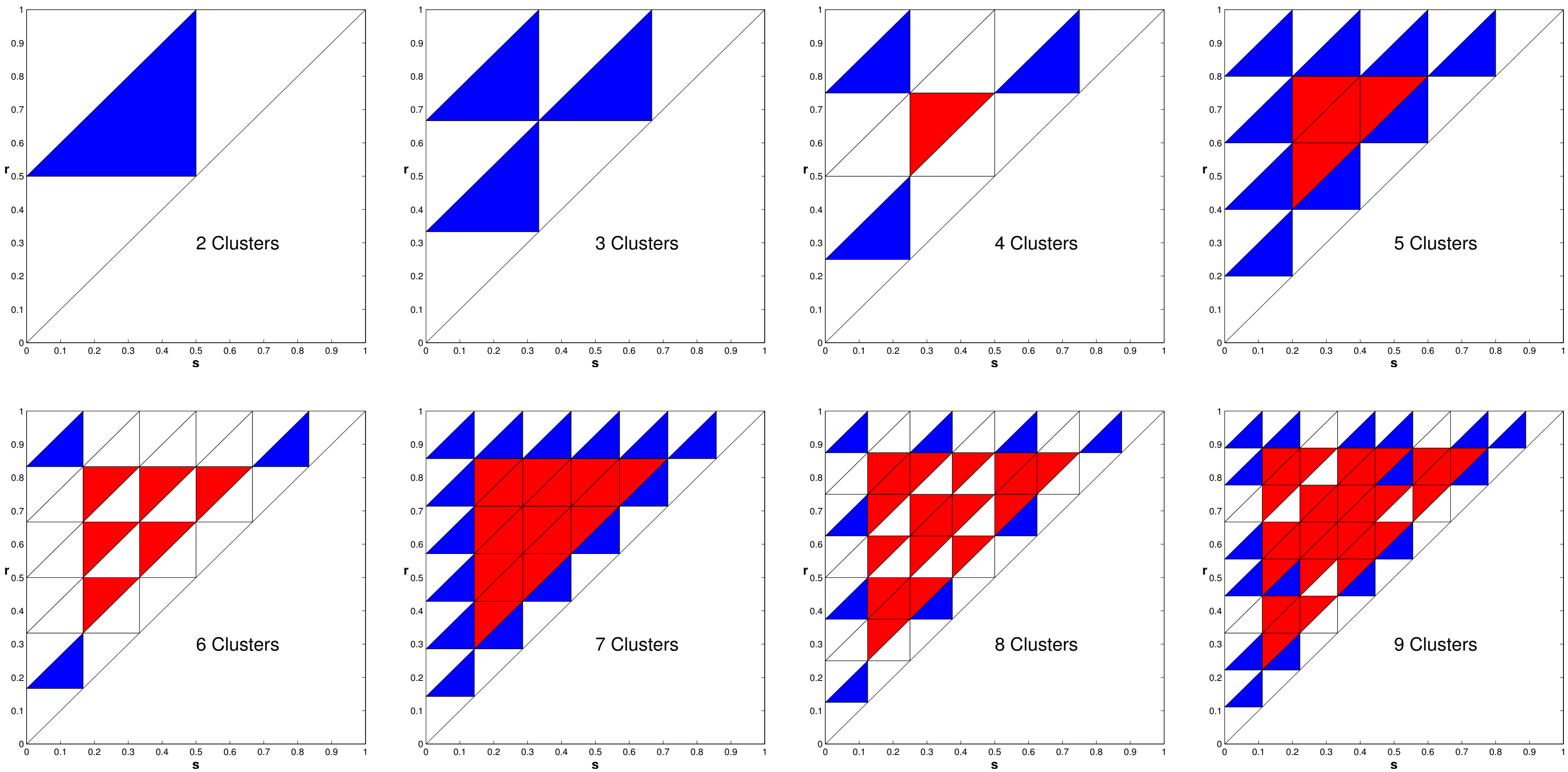
2 Cluster Systems - Detailed Analysis



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 in the 2-cluster submanifold.

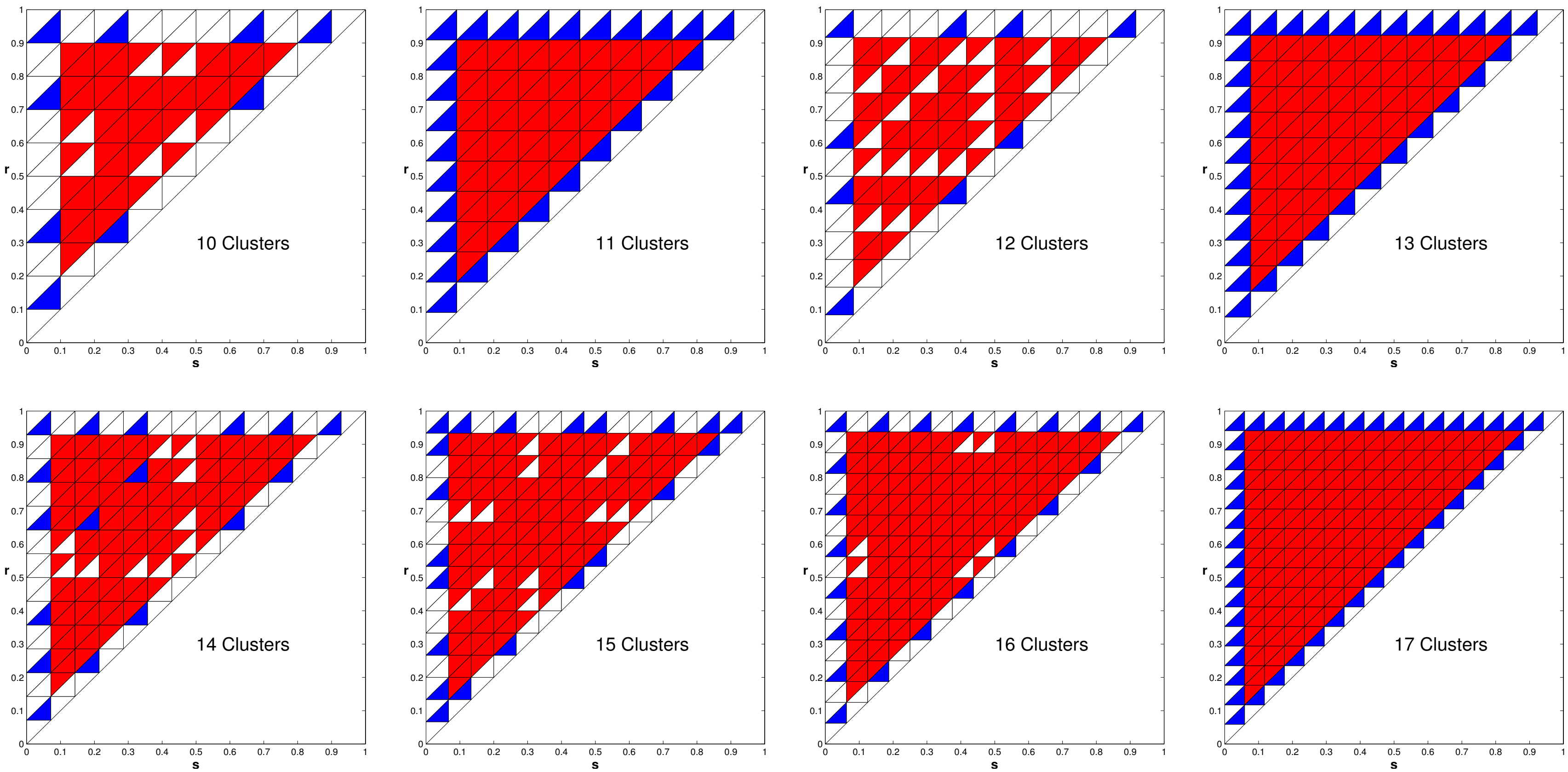
Stability of k cyclic solution in r-s parameter space



Negative Feedback, $k = 2, \ldots, 9$

Blue - Asymptotically Stable, White - Neutral, Red - Unstable.

Stability of k cyclic solutions in r-s parameter space

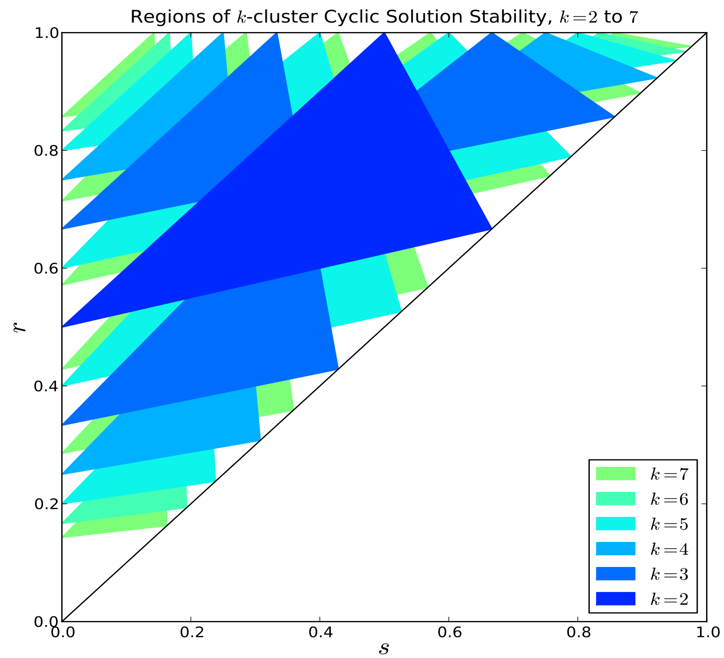


$k = 10, \ldots, 17$

Primes are Regular, Composites are Irregular!!.

Clustering is Universal for Negative Feedback

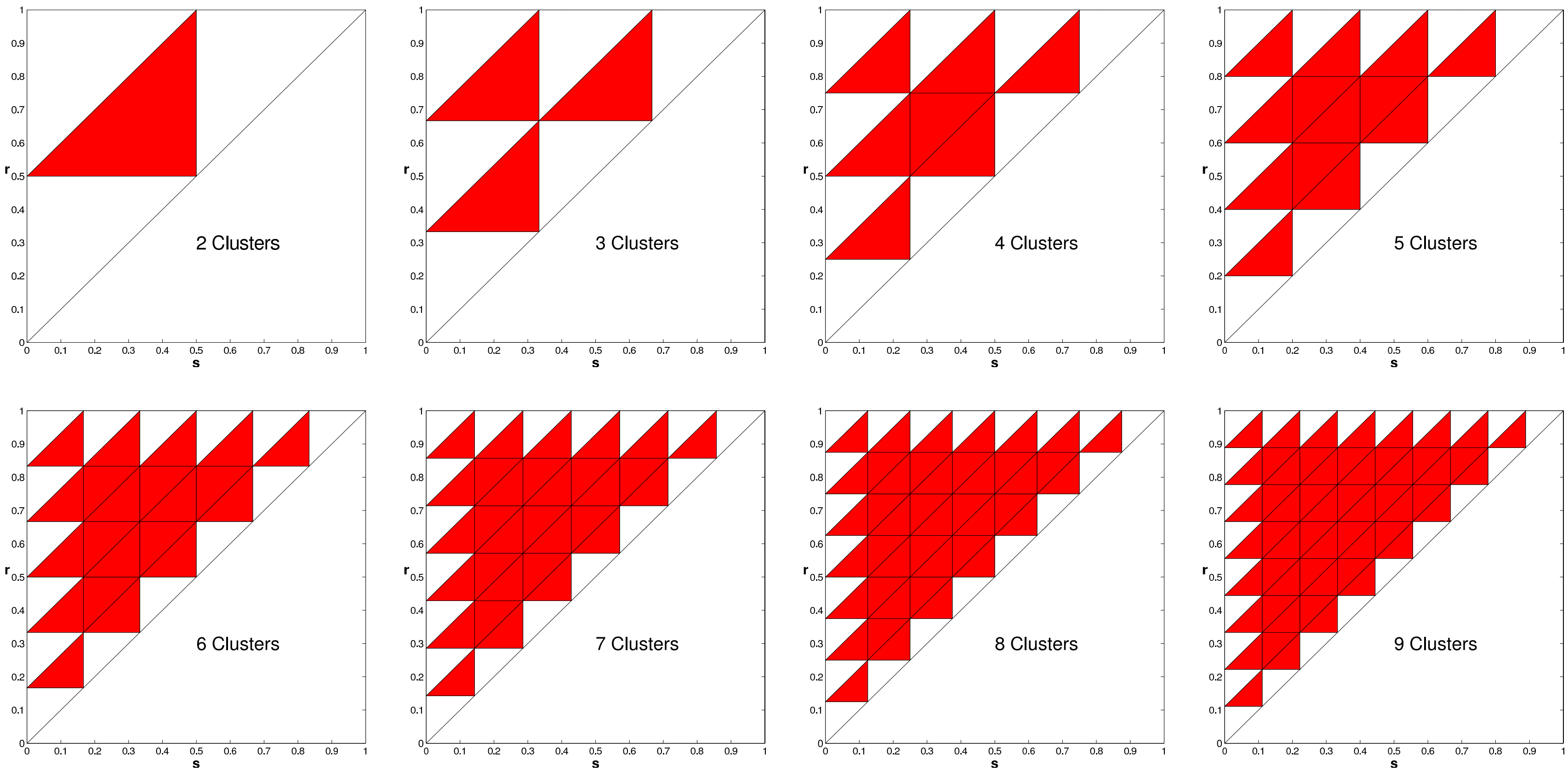
Overlay of Stable Regions:



- *Theorem:* For negative linear feedback, every interior point is covered by a stable region for some k.

- There are many regions of bi-stability or multi-stability.

(in)Stability of Cluster Solutions for Positive Feedback



$k = 2, \ldots, 9$

Multiple Clusters are NEVER Asymptotically Stable.

Instability in the Middle

Theorem: For k large:

- Interior triangles are all unstable with Positive Feedback.
- At least half of interior triangles are unstable for Neg. Feedback.

Corollary: For a fixed set of parameters and n large, the uniform solution is unstable for positive feedback.

Proof: Hard, tedious calculations and a few Linear Algebra tricks.

What about uneven clusters?

Suppose that 2 clusters are asymptotically stable.

What happens if we make one bigger than the other?

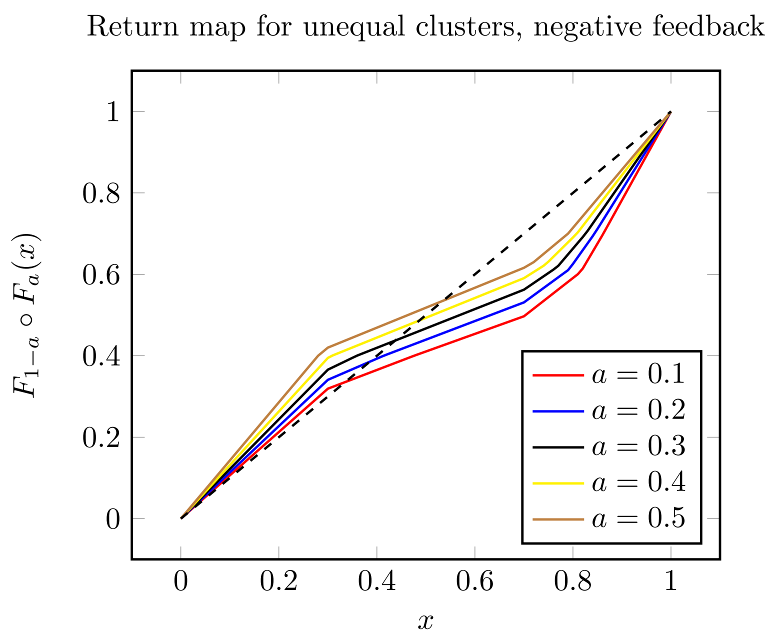
Suppose one cluster has more cells than the other.

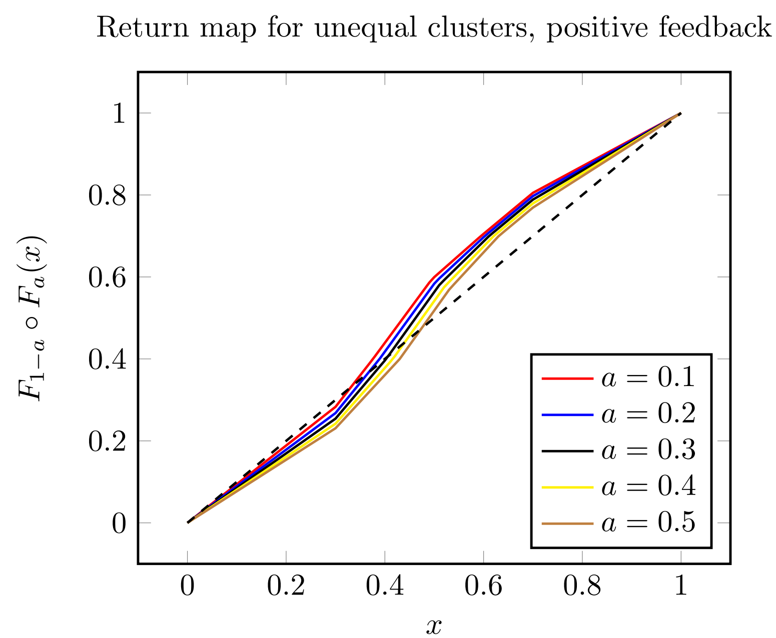
Let $0 < a < 1$ denote the fraction.

Uneven clusters are locally asymptotically stable.

Theorem: Let $\alpha = f(a)$ and $\beta = f(1-a)$ and suppose $a < 1/2$ and $\beta < \alpha < 0$. Then for $r - s \leq 1/2$, within the subspace of two cluster solutions with weightings a and $1-a$, there exists a unique, attracting periodic orbit for which the two clusters are distinct. The synchronized solution is a repelling periodic orbit in this subspace. If $r-s > 1/2$, then the Poincaré map has an interval of fixed points corresponding to two cluster solutions. This interval attracts all orbits except for the synchronized solution, which is repelling.

Dynamics in the 2 Cluster Subspace





Return map for the system with 2 unequal clusters; a is the weight of the smaller cluster and $1-a$ is the weight of the larger. Left: negative feedback, right: positive feedback. Parameters: $s = 0.5$, $r = 0.7$. For negative feedback the 2 cluster solution is always stable in the clustered subspace while the synchronized solution (at 0) is unstable.

How do cells distribute themselves into two clusters?

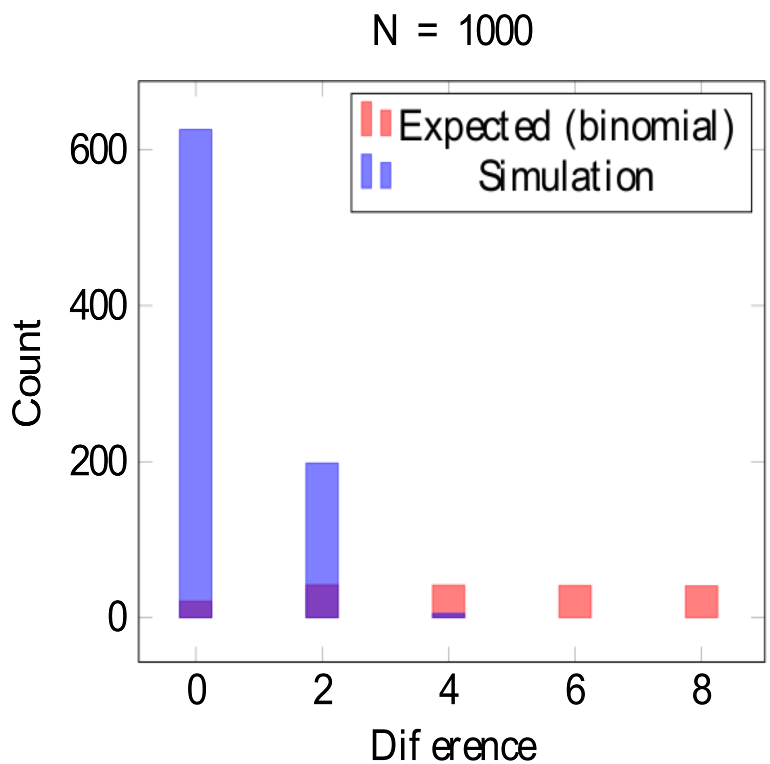
Let $f(I) = -.6I$, $s = 0.4$ and $r = 0.65$.
$\Longrightarrow$ The 2 cluster solution is stable for any a.

1000 Simulations, 1000 Cells with random initial conditions.

Count the cells in each cluster and find the absolute difference.

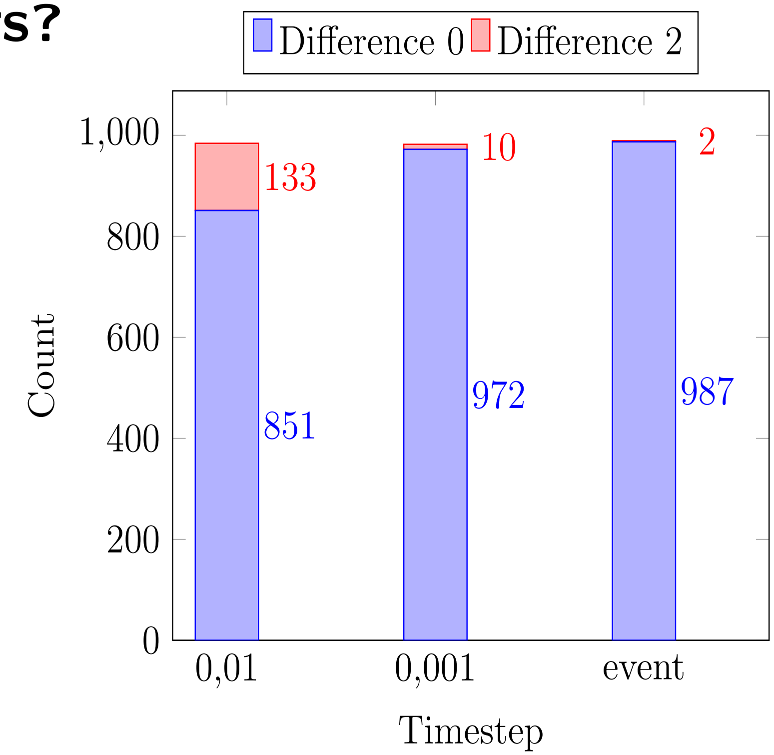
Clusters Prefer Equity

Distribution of |differences| in # of cells/cluster:



Cells are much more equally distributed than they should be.

Numerical Errors?



Histogram of the differences between clusters, for runs with the same parameter values but different time steps.

Clusters are almost exactly equal!

Local Stability Again

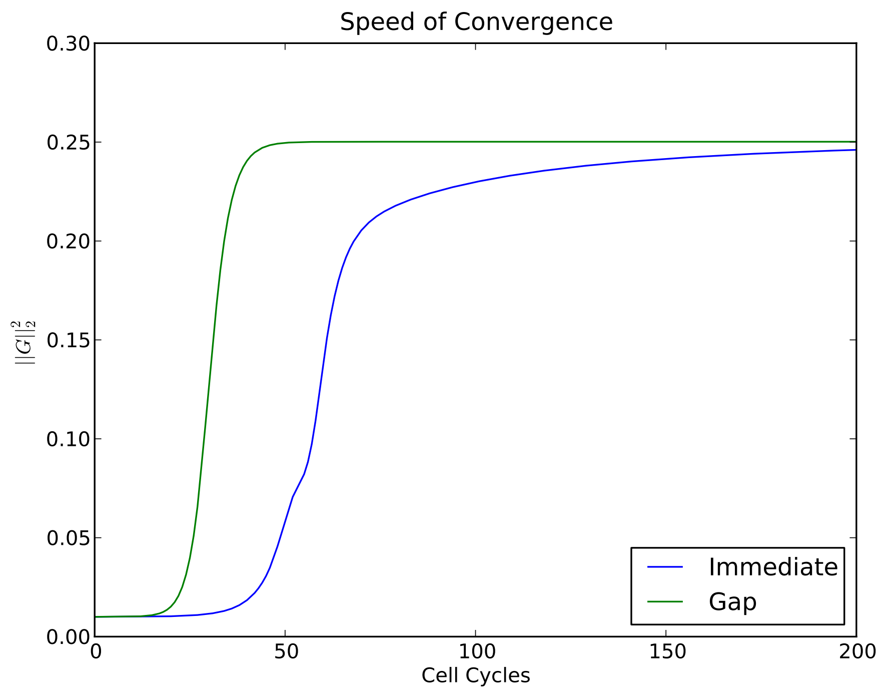
The theorem concerned local stability in the clustered subspace.

With equal clusters, subspace stability implies full stability (Moses).

But, in the full phase space:
Local stability is lost when clusters are not equal.

Model with a small gap (delay)

$S = [\epsilon, s]$ - a gap of width ϵ between R and S.



A small delay does not effect the number of clusters.

A delay enhances the stability of stable clusters.

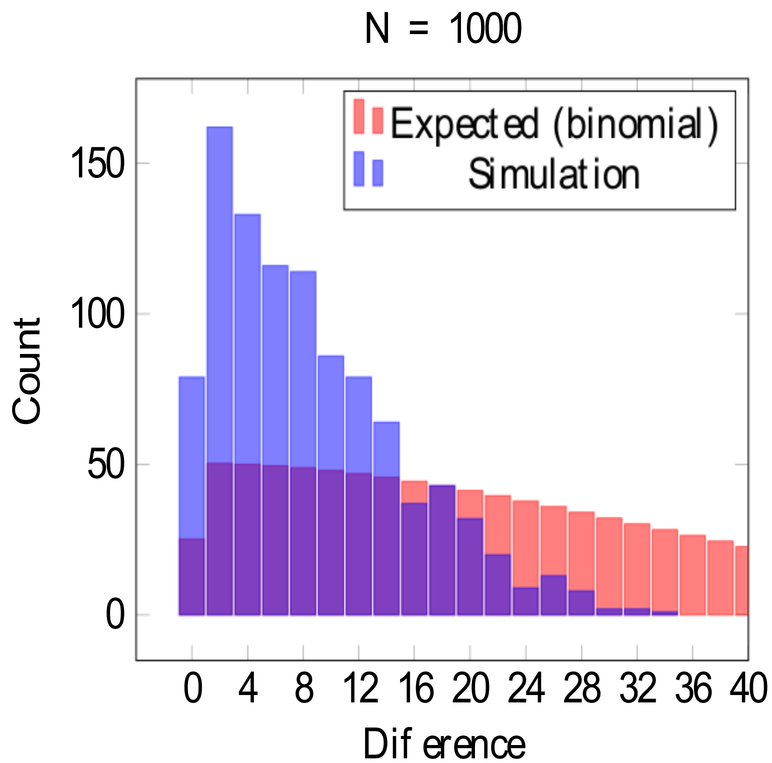
Local Stability for the Gap Model

Theorem Local asymptotic stability in the 2 cluster subspace implies the same in the full phase space.

Unequal clusters can be locally asymptotically stable.

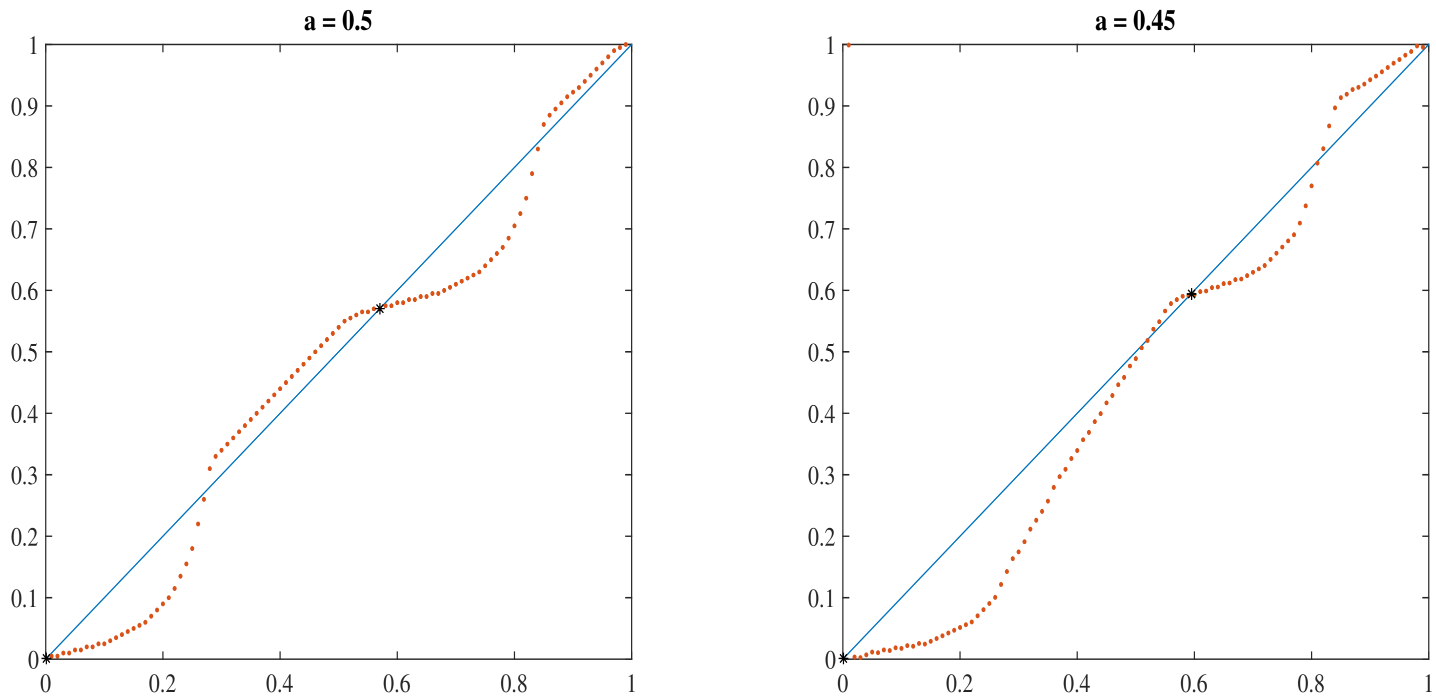
With a gap - Clusters Still Prefer Equity!

With a small gap, the uneven 2 cluster solution is locally stable, but:



Cells are still more equally distributed than they should be. Why?

Unequal clusters shift the basins of attraction

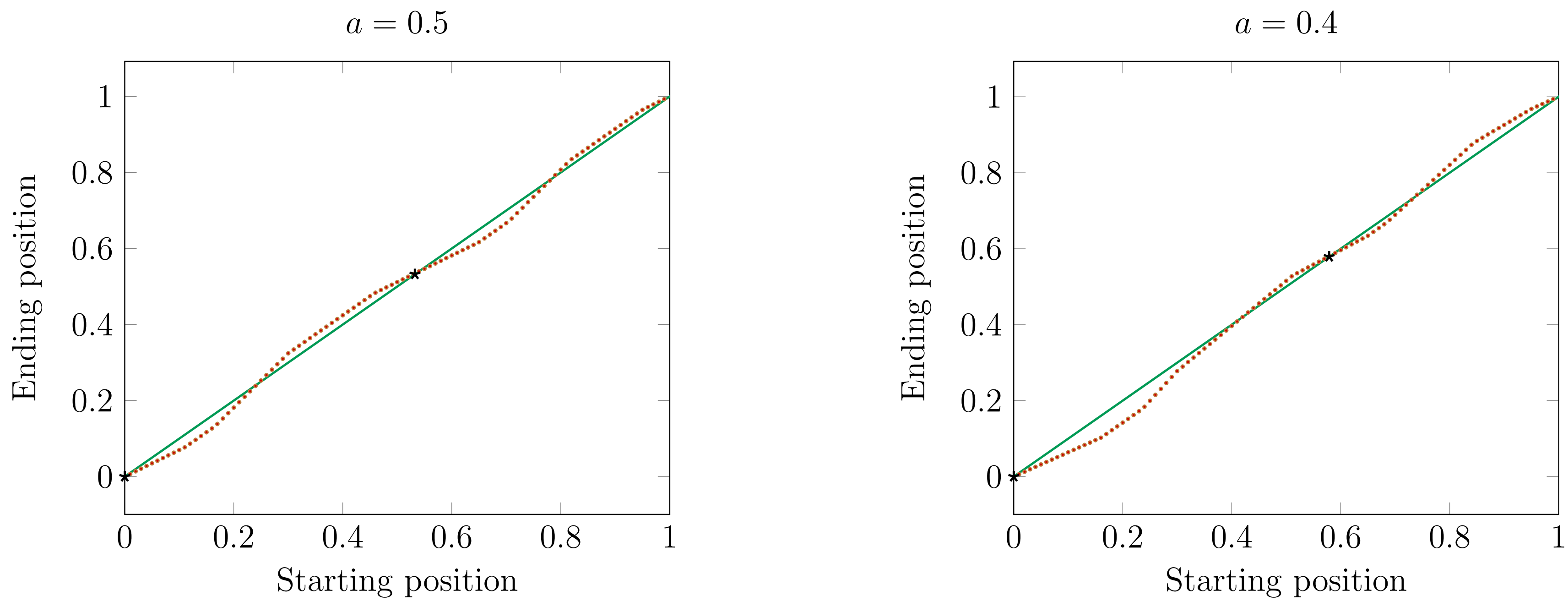


$a \sim$ fraction of cells in cluster 1 (at $x = 0$).

$a = .5$ (evenly distributed) - basins are nearly equal.

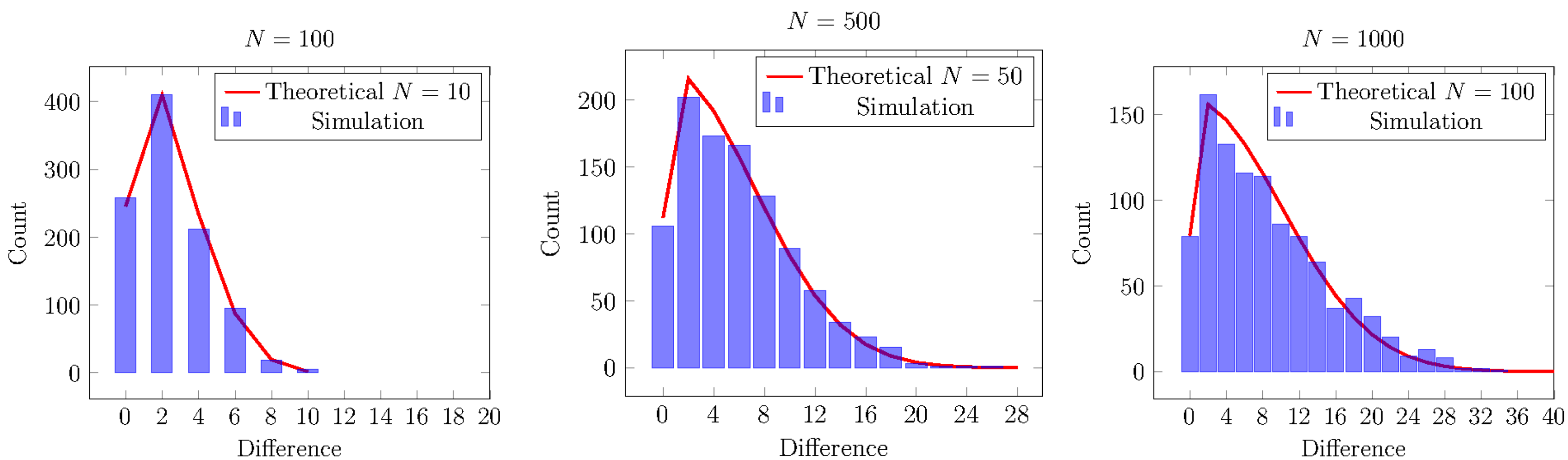
$a = .45$ - the small cluster has a much larger basin.

Basins are shifted in Gap Model Also



For $a = .5$ the basins of the two clusters are nearly equal. For $a = .4$ and smaller, the small cluster has a much larger basin.

A closer look at distribution of differences



Distribution of the difference in the gap model ($s = 0.4, r = 0.65, \epsilon = 0.05$), together with the theoretical distribution of the difference binomial with parameter equal to $N/10$.

Conclusions about Unequal Clusters

- Clustering is a robust phenomenon for Negative Feedback:

- Stable clustering requires interaction among clusters

- Interaction favors equal clusters

- Global, not Local, dynamics have a large influence.