Modeling Pathways of Cell Differentiation in Genetic Regulatory Networks With Random Boolean Networks

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Topics

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High Level View

- Can cells be modeled as non-linear dynamical systems -> cell differentiation?
- We chose ensembles of random Boolean networks to be the model.
- Ensembles of networks allowed us to find the typical behavior of the model.
- Results indicate phenomena which might occur in cell differentiation.
- These results are testable using gene arrays.

Introduction

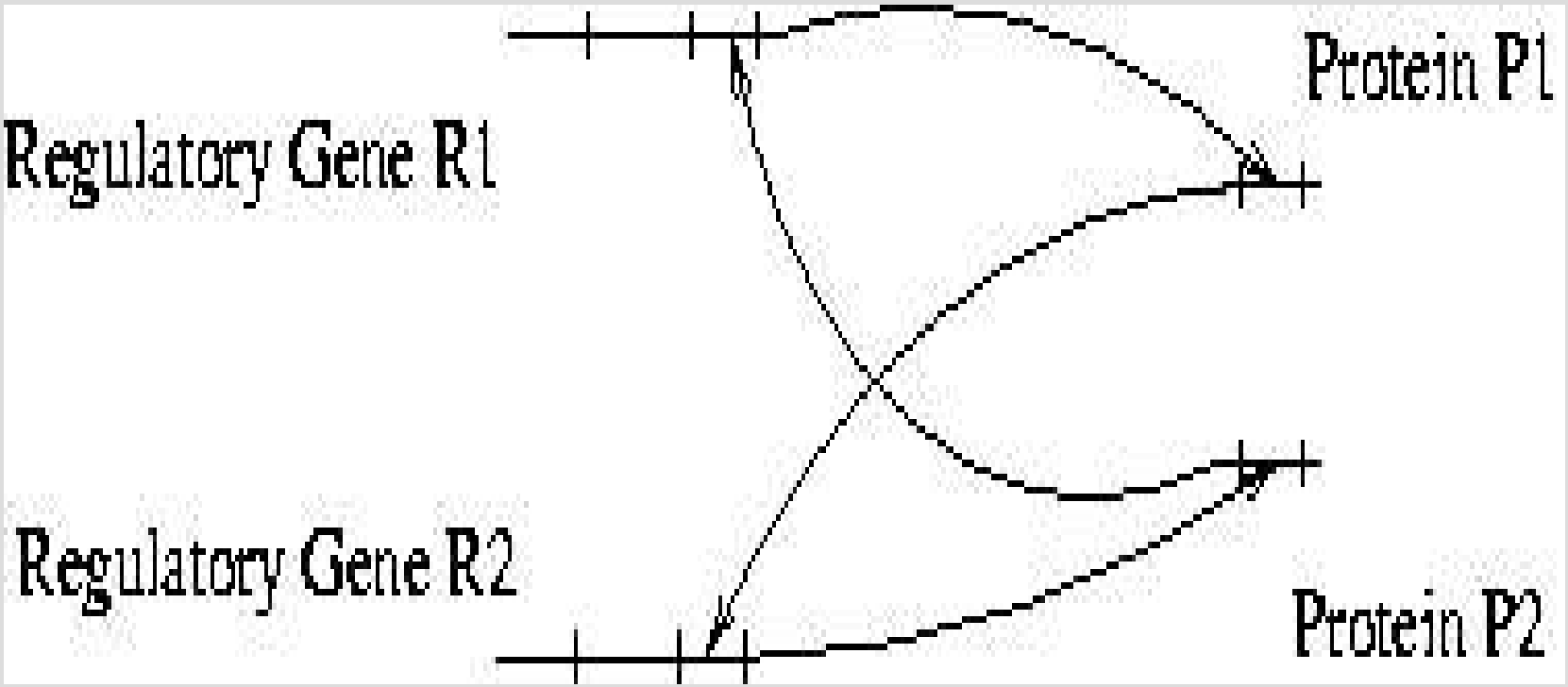
- If cells can be modeled using non-linear dynamical systems, then cell types are attractors.
- If a cell type is an attractor, then the steps from one attractor to another is a pathway of cell differentiation.
- Hypothesis: Boolean networks share some key behaviors of cells in the process of differentiation.

Introduction cont.

- Findings suggest Boolean networks are able to support some predictions about pathways of differentiation.
- Predictions should be testable using gene array techniques.
- Challenge: How to map the continuous nature of cell dynamics to the discrete steps of a synchronous Boolean network?

Background

- Early 1960's, Jacob and Monod proposed a “genetic circuit” to explain mechanics of cell differentiation.



Background cont.

- If we restrict a gene's activity to being either on or off, then a cell can be modeled with a Boolean network.
- Such a network having N genes has 2^N possible states.
- The human genome has an estimated 25,000 genes.
- A model network would then have 2^25,000 possible states!

Biology

- Each gene is a segment of DNA.
- Interaction between genes induce them to express proteins which in turn can modify their own or another gene's behavior.
- These interactions form the connections of a genetic regulatory network.
- Genes in cells are in a continuous level of activation.
- Gene expression levels determines the cell's type.

Cell Differentiation

- Process where a cell of one type becomes a cell of another type.
- Cells differentiate as part of a normal process: embryonic stem cells can differentiate into one of 265 different cell types.
- Cells may also be induced to differentiate through exposure to certain chemicals.
- Gene arrays are used to take measurements of cell differentiation.

Random Boolean Networks (RBNs)

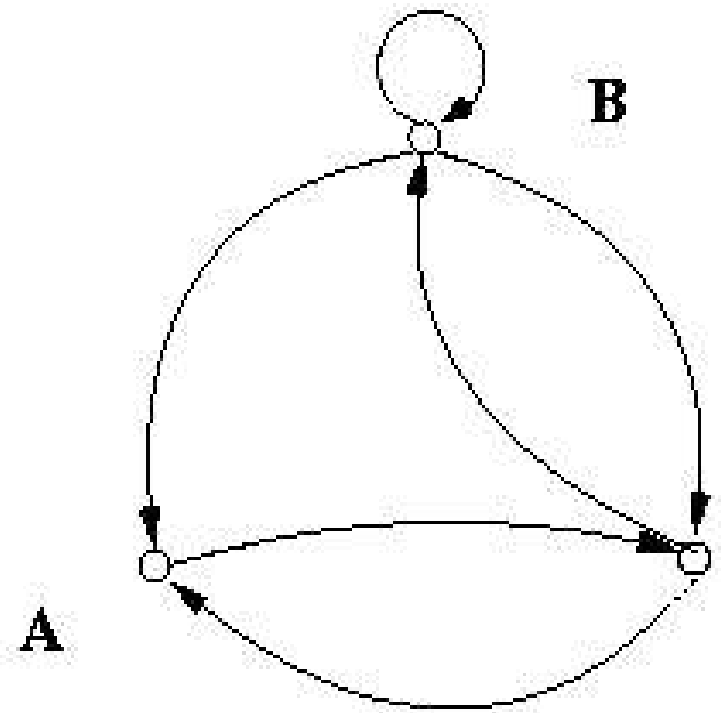
- RBNs are directed graphs of N nodes with K inputs to each node.
- Wiring of network is chosen at random.
- Boolean functions: Rules which allow for state transitions in the network.
- State of network is a snapshot of all node values.
- Each node evaluates to 1 or 0, on or off.
- Subsequent states generated synchronously by feeding the network state through a set of Boolean functions.

Model cont.

A simple K=2, N=3 Boolean network

B	C	B
0	0	1
0	1	1
1	0	0
1	1	1

B	C	A
0	0	1
0	1	1
1	0	0
1	1	1



A	B	C
0	0	0
0	1	0
1	0	0
1	1	1

Model cont.

- A sequence of states which repeats itself is called a state-cycle or attractor.
- All Boolean Networks have state cycles.
- If cell types are attractors, then we can model them with RBNs.
- As previously stated, getting from one attractor to another becomes a model for a pathway of differentiation.
- In RBNs, a pathway of differentiation, or transient, is initiated by perturbing a state.

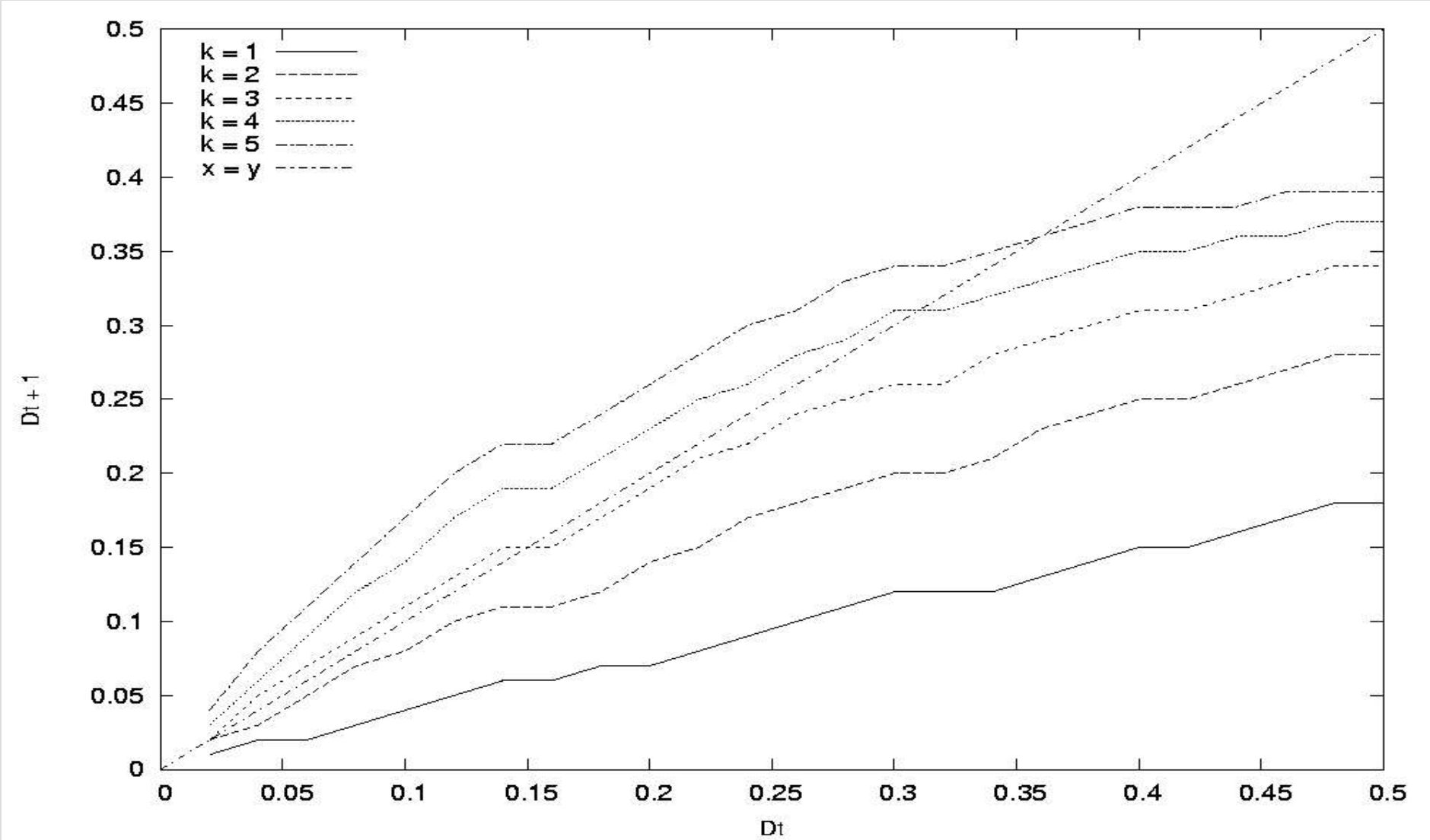
Testing Network Dynamics

- Needed to confirm the network dynamics for ordered, critical, and chaotic networks.
- The standard way to measure the complexity of a Boolean network was discovered by Derrida and Pommeau in 1986.
- Idea: compare the Hamming distance between pairs of states (dt) and their successor states (dt+1) in a random Boolean network.

Dynamics cont.

- If the Hamming distances are greater, then nearby states are diverging.
- If the Hamming distances are less, nearby states are converging.
- If the Hamming distances are about equal, then nearby states are neither converging nor diverging.
- Repeat for different pairs of states and plot the results.

Derrida plot



Experimental Methods

- Networks generated for various N,K values.
- For each state-cycle found, all single node perturbations generated to create transients.
- Statistics were gathered for:
  - Homeostatic versus non-homeostatic behavior.
  - Transient fusion, where pairs of transients join and flow together.
  - Hamming distances between states along the transient.
  - Number of times a node (gene) changes state over the length of a transient.

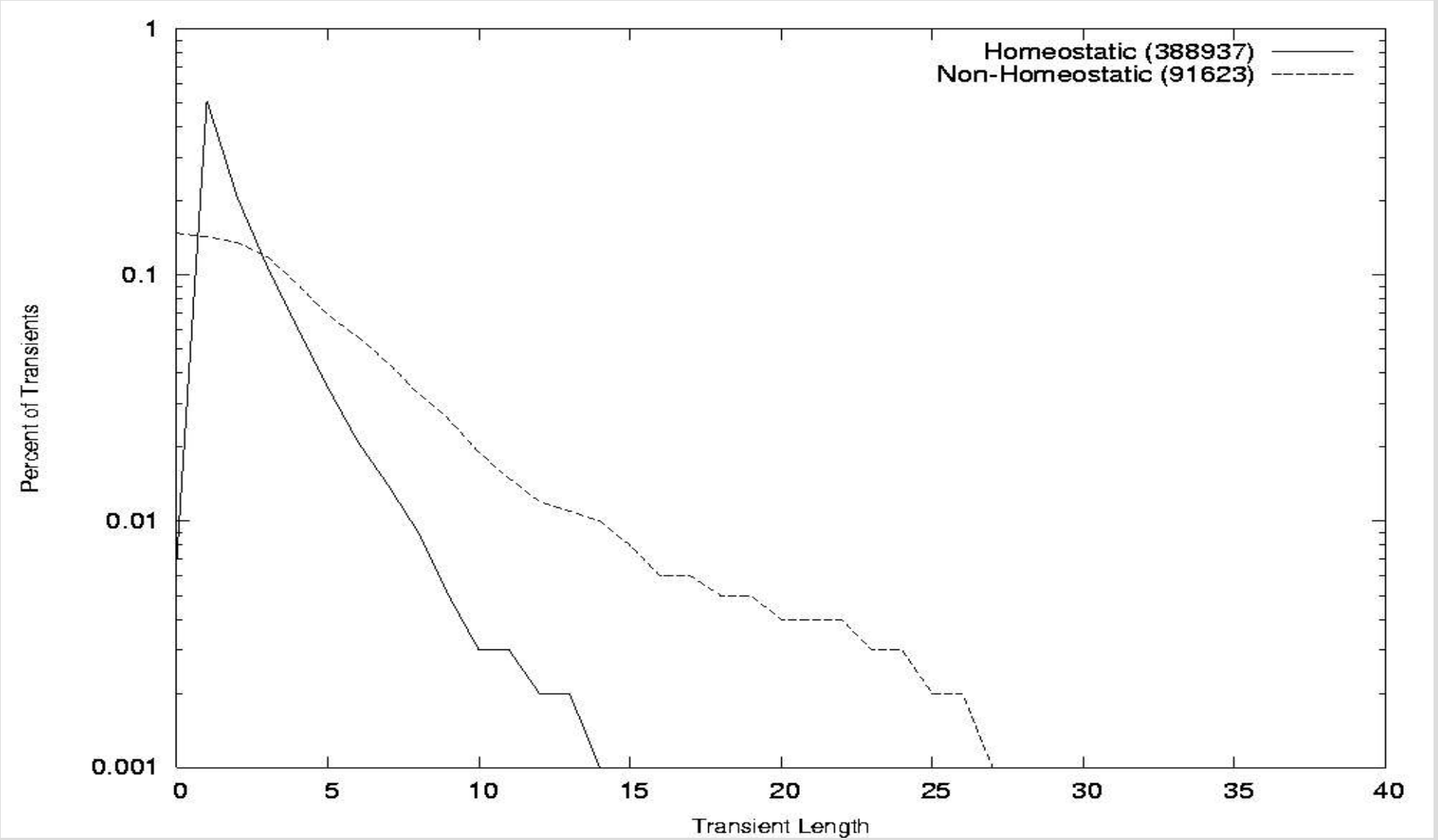
Homeostatic versus non-homeostatic behavior

- Homeostasis is a sign of stability.
- Networks with a single attractor excluded.
- For N=10-40, percentages of homeostatic transients decrease monotonically as K increases for K=1,2,3,4.
- This is consistent with the idea that cell types are stable attractors.

Transient Length vs. Percentage of Transients

- Transient length is the number of state transitions required to reach an attractor.
- As K increases, the average transient length becomes longer.
- Homeostatic, but not non-homeostatic transients show a pronounced peak in transients of a single state transition.
  - We can predict the same for living cells.

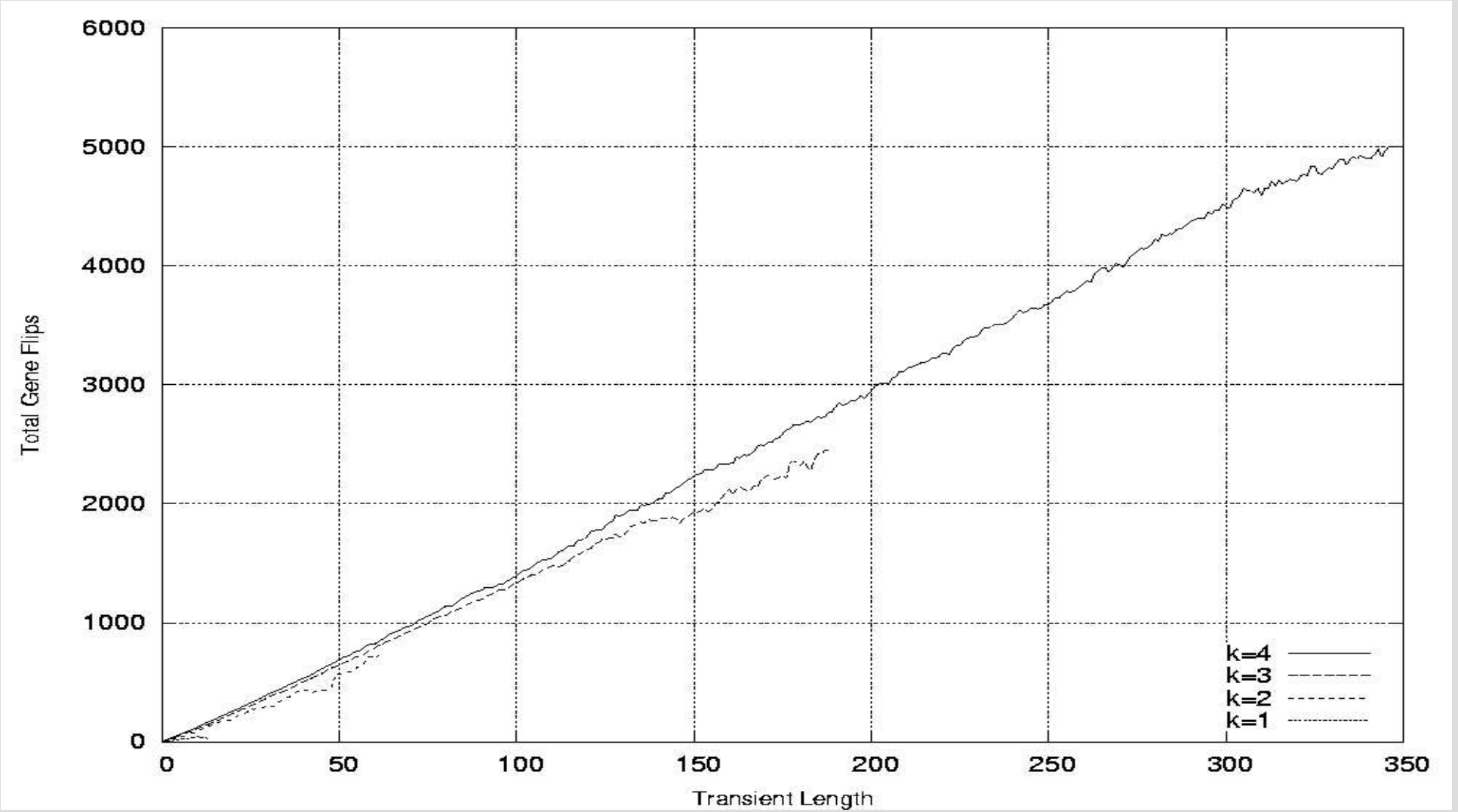
Transient length vs homeostasis, K=2



Mapping the discrete to the continuous

- Goal: Achieve a credible mapping between Boolean networks and genetic regulatory networks.
- Idea: Track the incidence of gene change or “flips” over a transient.
- For K=2,3,4 the number of gene flips was found to increase monotonically.

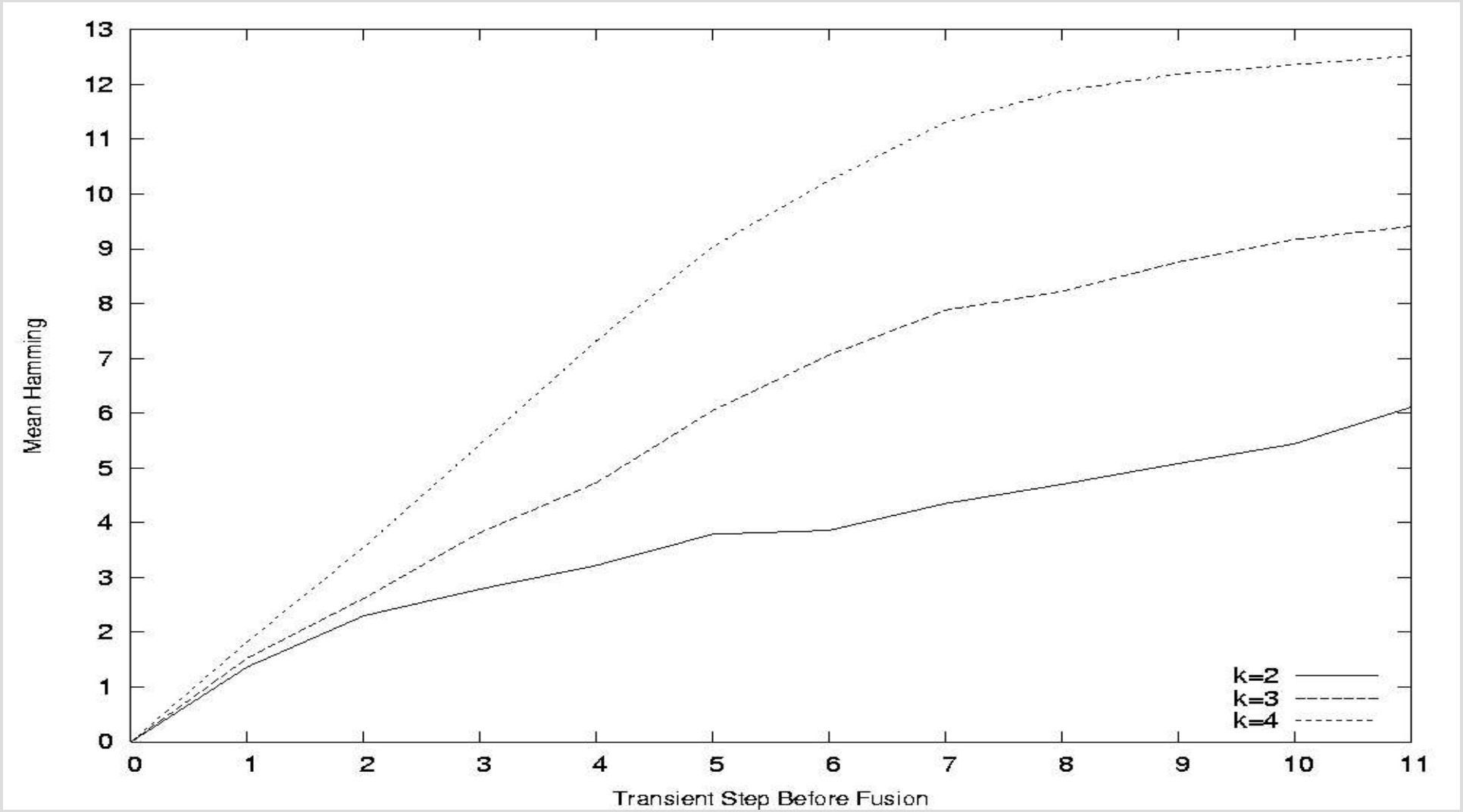
Gene flips as a function of transient length



Transient fusion

- Fusion: Where transient paths merge and flow together.
- Is the process of convergence a smooth or sudden process?
- It was found that between pairs of transients which fuse, the Hamming distances converged smoothly with proximity to the point of fusion.
- Note: this cannot happen for K=N.

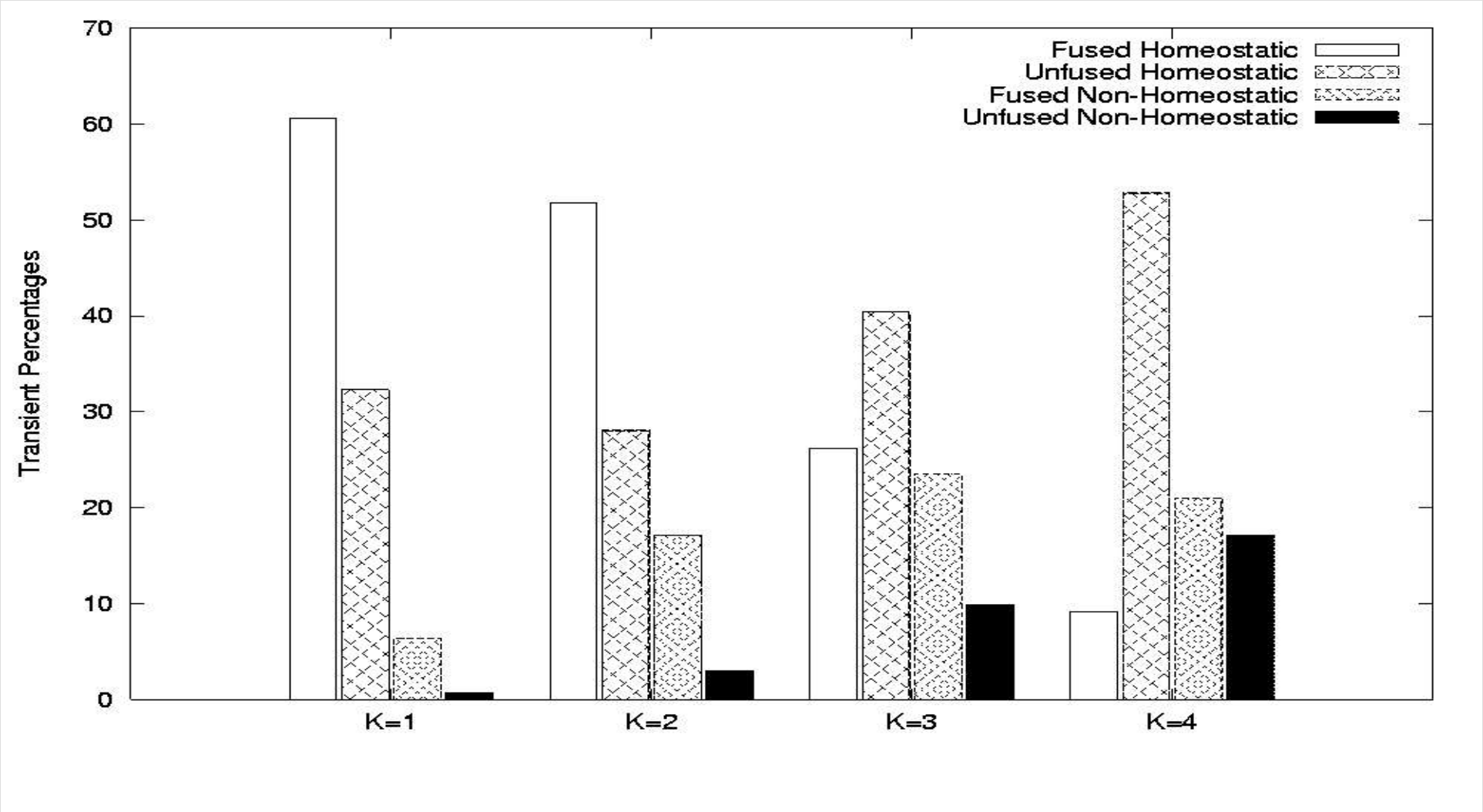
Convergence with transient fusion



Fusion Statistics

- It was found that as K grows larger, the number of fused homeostatic transients grows smaller.
  - This indicates a higher convergence in state space for networks in the ordered and critical regime than for networks in the chaotic regime.
- It is hypothesized that the ratio of fused homeostatic to unfused homeostatic transients is a marker of the critical regime.

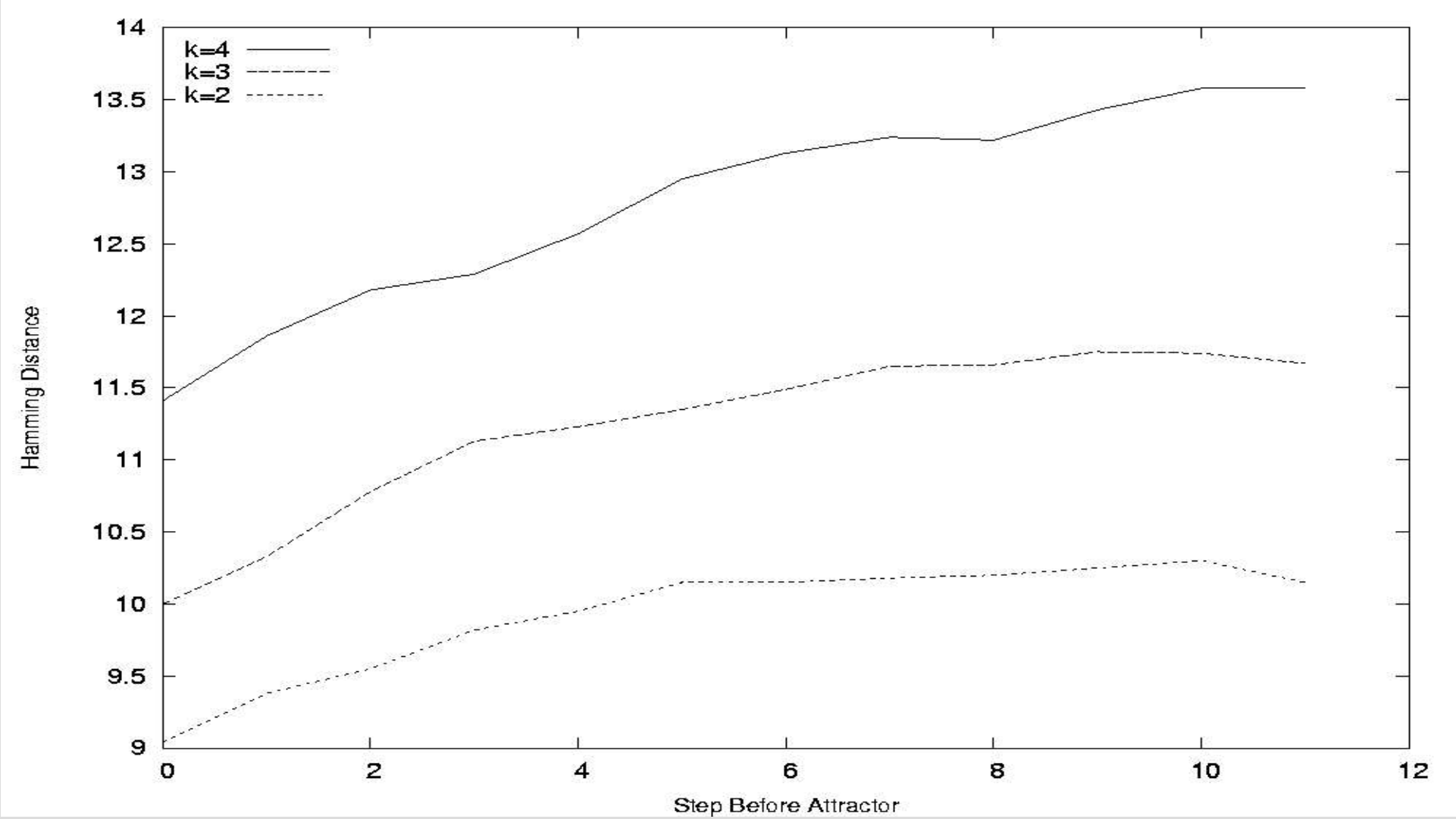
Fused and unfused transients



Hamming distances along the trajectory

- It was hypothesized that the hamming distance between successive states along the trajectory would monotonically decrease.
- This behavior was indeed found to exist for all K-values explored.
- Findings support a study done at Harvard Children's Hospital.
- Unfortunately the standard deviation is large, so the reduction in Hamming distance is difficult to notice.

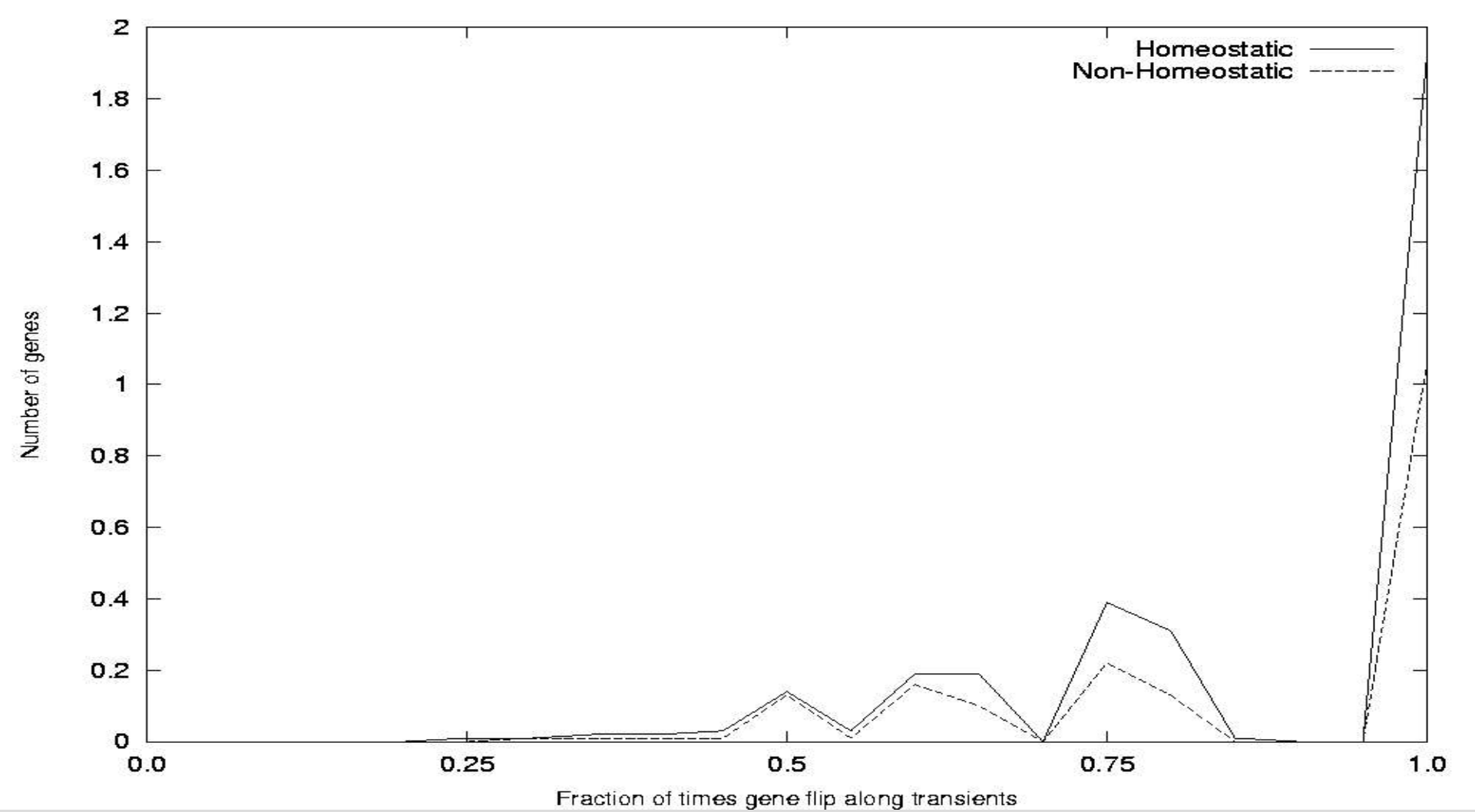
Hamming distance along trajectory



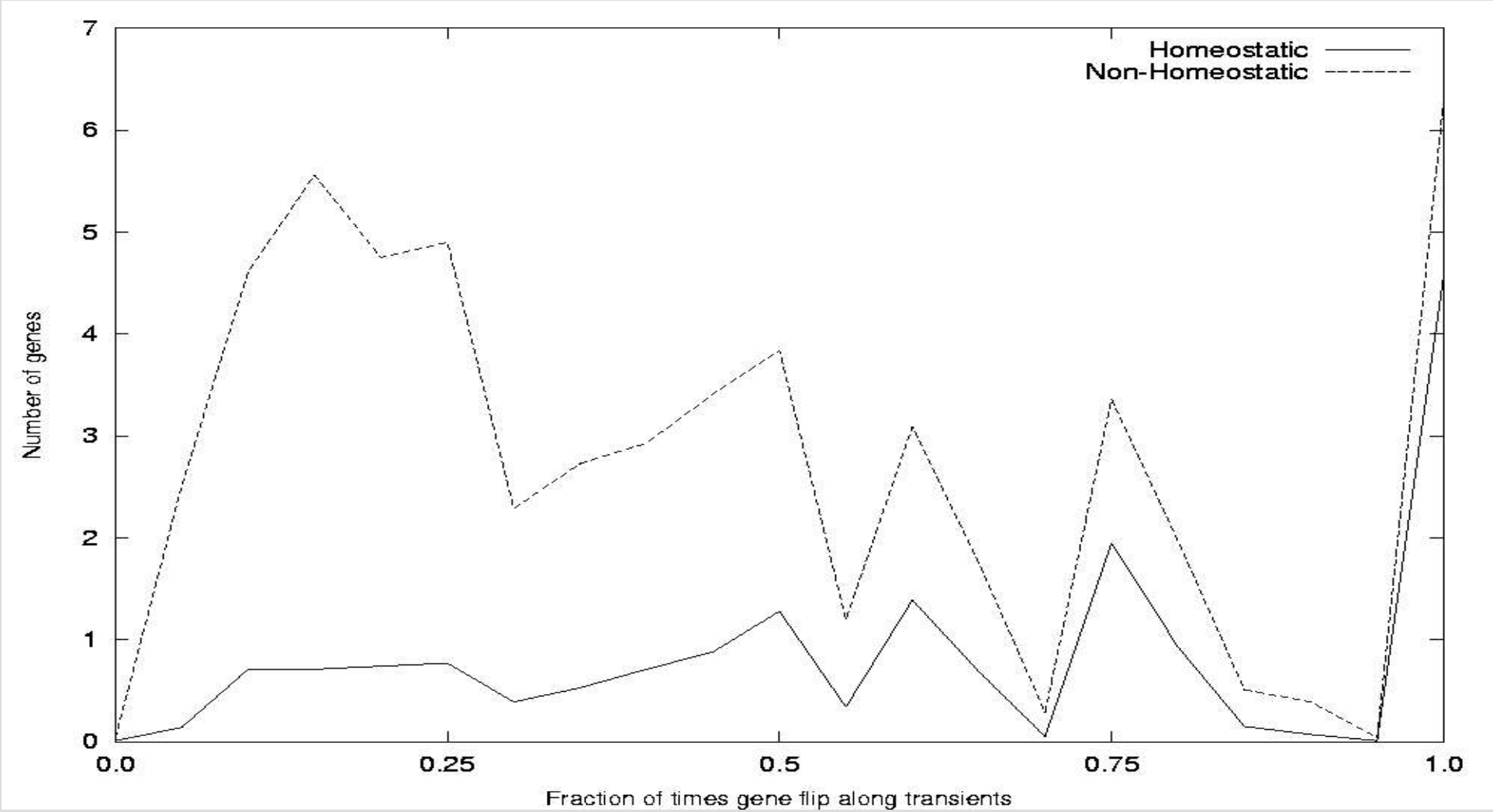
Distribution of gene flips as a function of transient length

- The fraction of times along a transient that a gene changes state.
- K=1, 2 shows a wide distribution of the fraction of times genes change along transient.
- K=3, 4 shows that a large fraction of genes never change state.
- These features should be experimentally testable using a gene array.

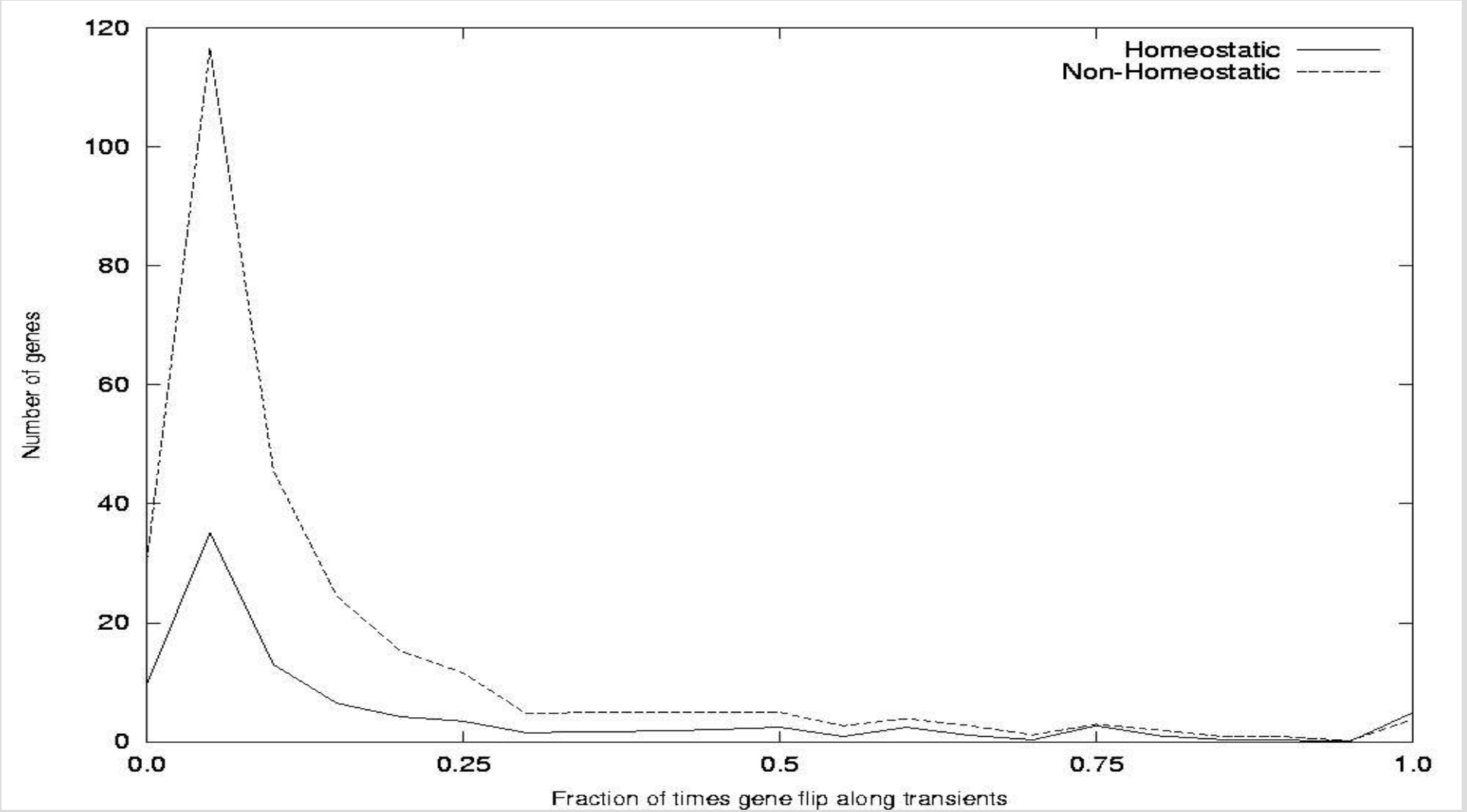
Fraction of time genes flip along transient, K=1



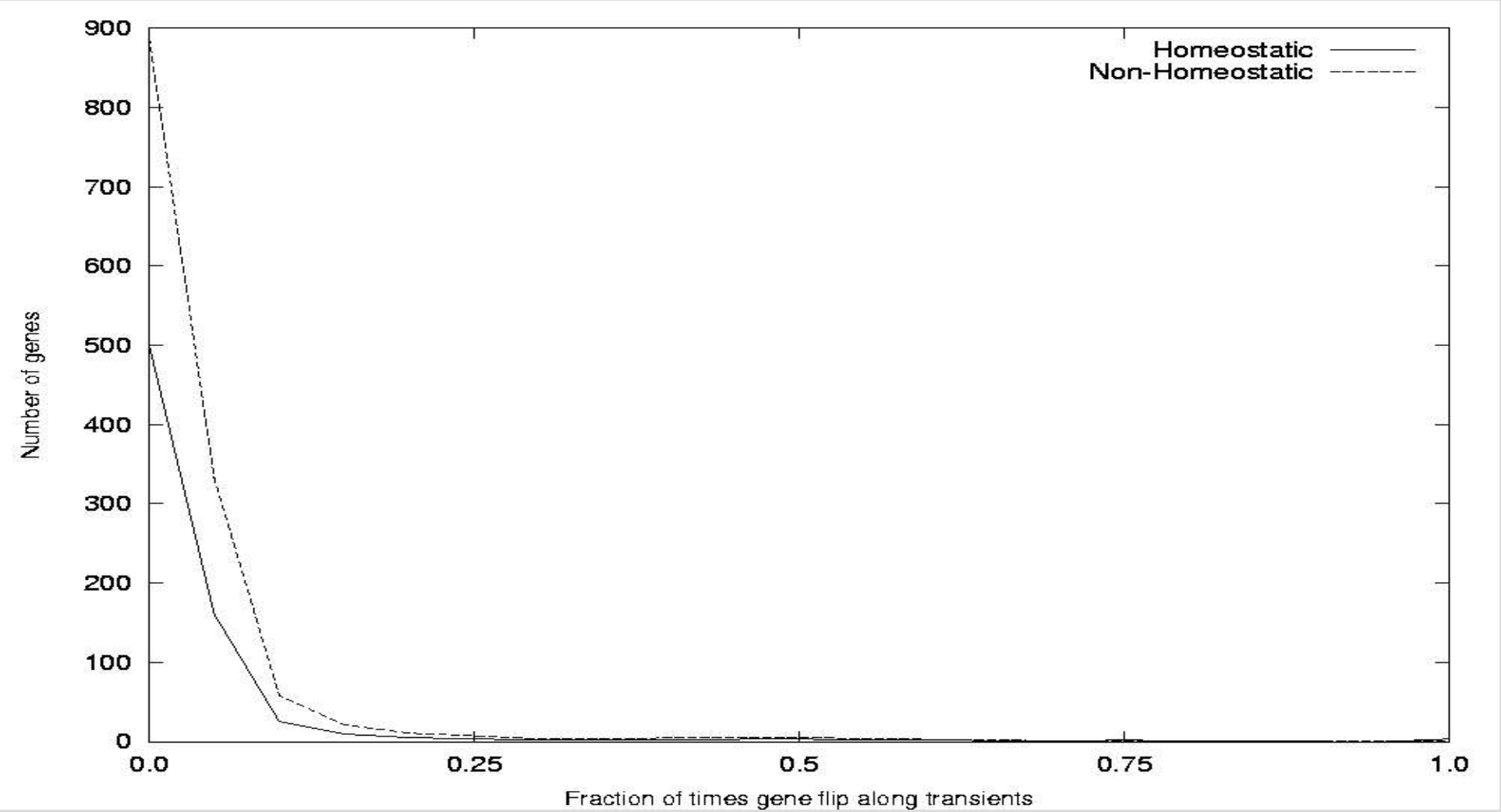
Fraction of time genes flip along transient, K=2



Fraction of time genes flip along transient, K=3



Fraction of time genes flip along transient, K=4



Discussion

- The number of fusing pairs of transients discovered in RBNs suggests that fusing pathways of differentiation can be found.
- A large percentage of genes in transients of chaotic networks never change state where the reverse is true in the ordered and critical regimes.
  - If cells are stable attractors, we would expect to find a wide distribution in the percentage of time that genes change state.

Discussion
Discrete vs. Continuous

- The mapping of gene flips as a function of transient length suggests a linear relation between transient lengths in RBNs and the number of times a gene alters activity.
- If the mapping is valid, it should be possible using gene arrays to measure the number of gene variations over a series of closely timed intervals.

Summary

- This is the first examination of pathways of differentiation under the hypothesis that cell types correspond to attractors.
- It was not expected that RBNs would yield a comprehensive model of living cells.
- However, some behaviors in RBNs were found which should be measurable in living cells.

Summary cont.

- Expected measurable features:
  - The amount of gene activity over time.
  - The ratio of fused homeostatic to unfused non-homeostatic transients.
  - The Hamming distance between successive states on a transient as it approaches a cell type.
  - The Hamming distance between pairs of transients as they approach a common cell type.

Future Directions

- Medusa networks
  - Small group of regulator genes, “head”.
  - Large group of regulated genes, “tail”.
  - Follows some behavior seen in living cells.
- Asynchronous RBNs
  - Updates genes one at a time, randomly.
  - Adds non-determinism to the model.
  - ARBNs may more closely approximate gene expression in genetic regulatory networks.
    - But nobody really knows

Acknowledgements

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