

Evolution enhances mutational robustness and suppresses the emergence of a new phenotype – A study on gene regulatory network –

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1. Introduction

- robustness, gene regulatory network

2. Model and Methods

- network model, multicanonical MC, evolutionary simulation

3. Results

- fitness landscape, bistability, mutational robustness

4. Discussions

Introduction

Central question

Mutational Robustness

- Living systems do not easily lose their viability even when some of the genes are mutated.
- This trait of **mutational robustness** has been developed through the long history of evolution.
- Mutational robustness can be examined experimentally by several methods such as gene knockout or artificial addition of gene regulation.
 - *e.g.* Isaran *et al.* 2008.
- But the answer to the question, **“Does mutational robustness evolves?”** remains unclear because experimental investigations are difficult.
 - Thus numerical methods play an indispensable role.

New methods are required

We investigated the evolution of mutational robustness for **gene regulatory network** (GRN) model.

Problem

- Evolutionary simulations only provide evolutionary-path-dependent traits.
- To study **the universality and the characteristics** of evolution, we need a reference system.
- The reference system that we consider appropriate is a set of **randomly sampled** GRNs.
- A simple random sampling is useless because highly fit GRNs are **rare**.

McMC to study evolution

Solution

- Rare event sampling using the [multicanonical Monte Carlo](#) (McMC) method
 - Random sampling over wide range of fitness

The preceding works applying McMC to GRN

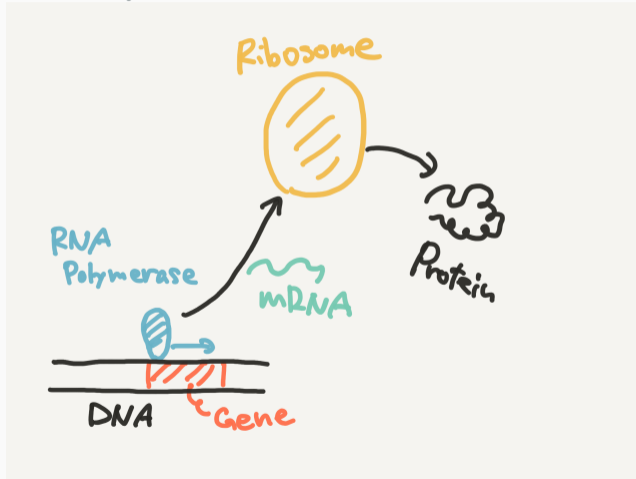
- N. Saito and M. Kikuchi: New J Phys 15 (2013) 053037
- S. Nagata and M. Kikuchi: PLoS Comput Biol 16 (2020) e1007969

This talk

- T. Kaneko and M. Kikuchi: arXiv:2012.03030

Gene Regulatory Network 1

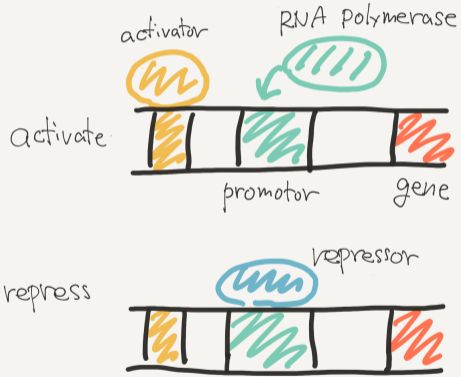
Gene expression



- Information coded in a gene is transcribed to mRNA.
- Ribosome assembles amino acids according to mRNA sequence.
 - Protein is produced.

Gene Regulatory Network 2

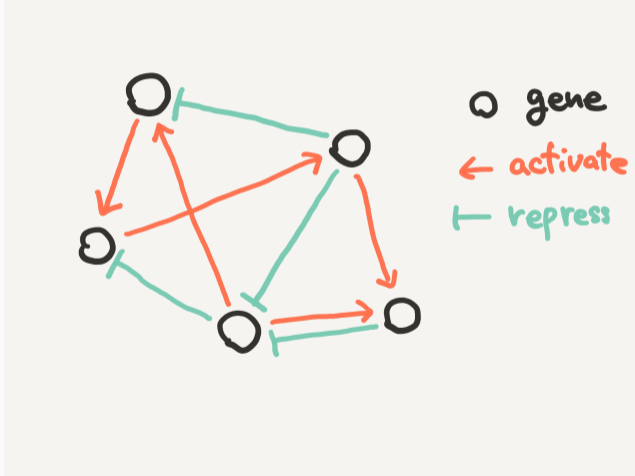
Gene regulation



- A category of proteins calls **transcription factor** regulate expression of other genes working either as activator or repressor.

Gene Regulatory Network 3

Gene regulatory network



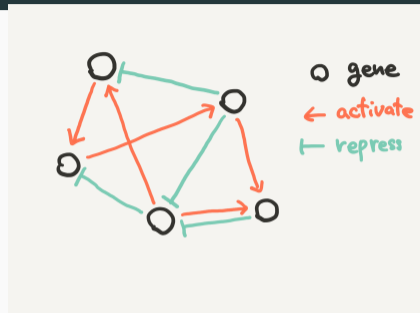
- Genes regulate mutually and form a complex network to control the cell states.
 - Adaptation to environmental change
 - Maturation, differentiation

Model and Methods

Model 1

Connectionist model

- We ignore details of gene expression and take into account only regulatory interactions.
 - Node: Gene
 - Edge: Regulation



Details

- GRNs are represented by directed random graphs
 - $N = 32$ nodes, $K = 80$ edges
- One input gene and one output gene
- All the possible networks are allowed

Model 2

Discrete-time dynamics

$$x_i(t+1) = R \left(I\delta_{i,0} + \sum_j J_{ij}x_j(t) \right)$$

$$R(x) = \frac{1}{1 + e^{-\beta(x-\mu)}}$$

- x_i : Expression of i th gene ($[0, 1]$)
- J_{ij} : Regulation of i th gene by j th gene($0, \pm 1$)
 - $+1$: activation, -1 : repression
- I : Input from exterior world ($[0, 1]$)
- $\mu = 0, \beta = 2$ (Hopfield-Tank)

Model 3

Fitness

- We require that the expression of the output node for $l = 0$ and 1 differ as large as possible.
- Fitness is defined as follows:

$$f = |\bar{x}_{out}(0) - \bar{x}_{out}(1)|$$

- $\bar{x}_{out}(l)$: the fixed-point value (or the long-time average if no fixed point is reached) of the output node for input l .
- $f \in [0, 1]$ by definition

Two methods

1. Random sampling by multicanonical Monte Carlo method
2. Evolutionary simulation

Method 2

Multicanonical Monte Carlo

- Originally developed in statistical physics to sample **energies** evenly.
 - Berg and Neuhaus 1991,1992
- Later, it was realized that McMC can be used also for **non-physical** systems.
 - Review: Y. Iba, N. Saito, and A. Kitajima: Ann Inst Stat Math 66 (2014) 611.

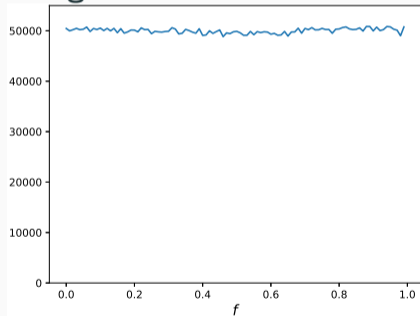
Application to GRN

- It enables us to sample GRNs in wide range of fitness **randomly** (in principle).
- Fitness are divided into 100 bins.
- The weight for each bin is determined by **Wang-Landau** method.
- 5M samples

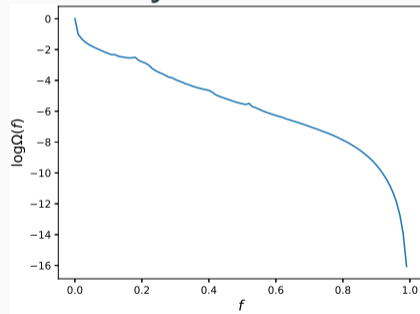
Method 3

Example of McMC

Histogram



Probability

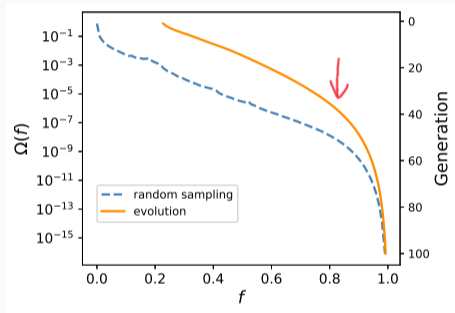


Evolutionary simulation

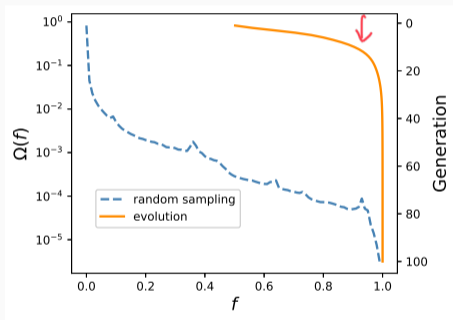
- Mutation
 1. Delete a randomly selected edge
 2. Add a new edge to a randomly selected node pair
- Initial population: 1000 random GRNs.
- 500 are selected at each generation based on fitness.
- The lineage of the highest fitness at 150th generation is sampled.
 - 100,000 independent runs: 100,000 lineages

Results

Fitness landscape and evolutionary speed 1



Probability of fitness and fitness vs. generation for $\beta = 2$



for $\beta = 4$ (only this figure)

Fitness landscape and evolutionary speed 2

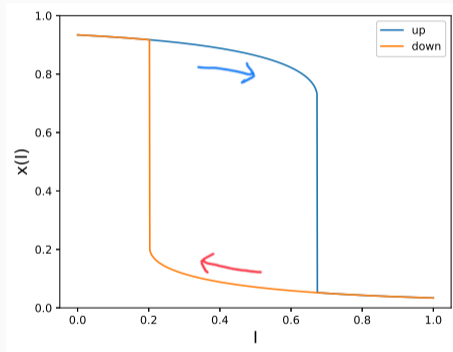
Observations

1. Most GRNs have low fitness ($f < 0.1$).
2. At intermediate fitness, GRNs become exponentially rare as fitness increases.
3. For very high fitness ($f > f^*$), GRNs become **faster-than-exponentially** rare.
4. Evolution slows down substantially at $f \simeq f^*$.

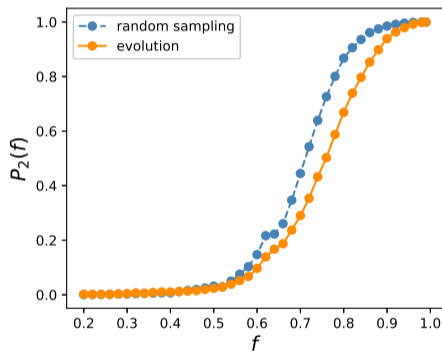
Genotypic entropy matters

- The evolutionary speed is determined mainly by the number of available GRNs (or **genotypic entropy**).

Emergence of bistability 1



An example of bistability for $f = 0.9$



The fraction of the bistable GRNs

Emergence of bistability 2

Universality of evolution

- Bistable GRNs emerge, and their fraction grows as fitness increases. All GRNs become bistable as $f \rightarrow 1$.
 - Bistability is a new phenotype. This new phenotype that was not explicitly required by the fitness appears inevitably.

Particularity of evolution

- Evolution delays the appearance and growth of the fraction of bistable GRNs

Evolution is conservative!

Mutational robustness 1

Robustness measure

- We consider the single-edge deletion as mutation.
- Delete a single edge from GRN and compute fitness f' after the mutation.
- Robustness measure for a given GRN is the average of f' for all the possible single-edge deletion.

$$r \equiv \frac{1}{K} \sum_i f'_i$$

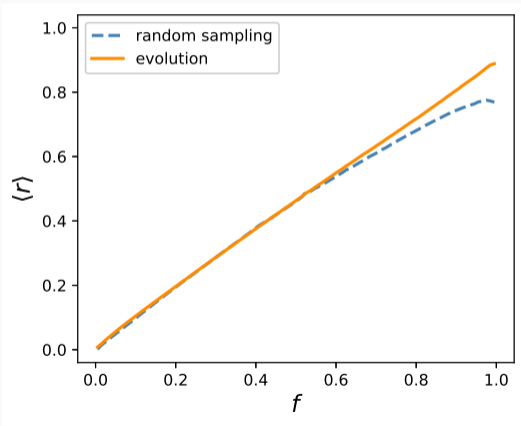
- f'_i is f' for GRN that i th edge is deleted.

Mutational robustness 2

- Since $\langle r \rangle$ increases with f , it is not suitable to compare the robustness measure of different fitness f .
- We focus on difference in $\langle r \rangle$ of GRNs having the same f obtained by random sampling and evolution.

Mutational robustness 3

Robustness measure

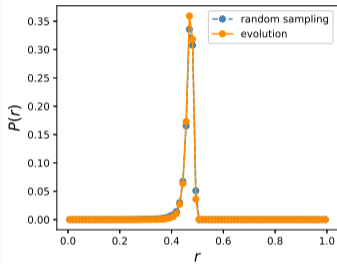


Two-step evolution

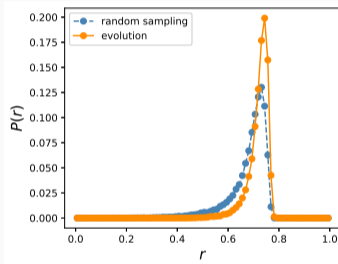
1. Early: $\langle r \rangle$ coincides with random sampling.
2. Later: $\langle r \rangle$ exceeds random sampling.

Mutational robustness 4

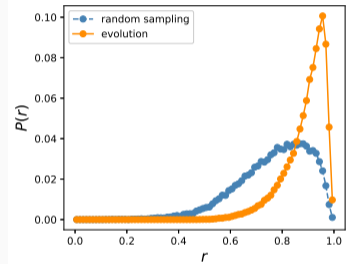
Probability distribution of r



$$f = 0.5$$



$$f = 0.8$$

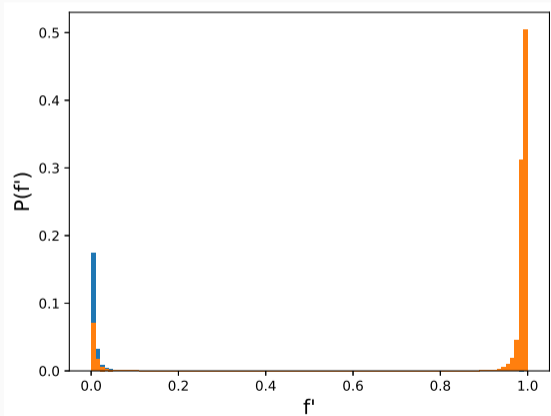


$$f = 0.99$$

Evolution enhances mutational robustness!

Mutational robustness 5

Lethal edges

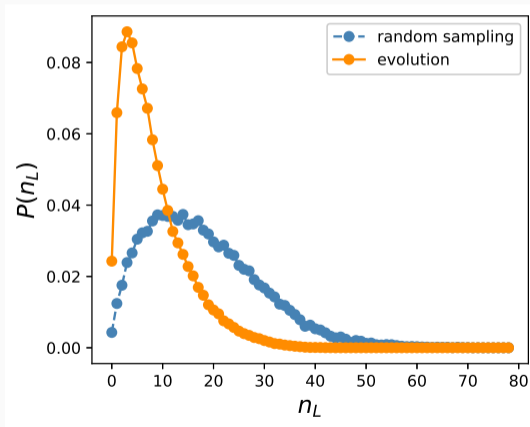


Distribution of f' for $f = 0.99$

- Edges are divided in two classes: neutral and lethal
 - Intermediate edges are scarce.

Mutational robustness 6

Distribution of lethal edges

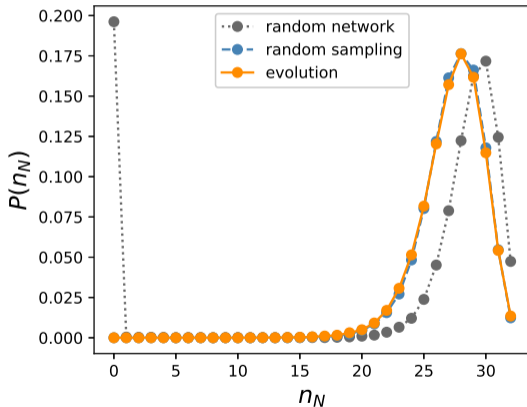


$$f = 0.99$$

- Lethal edges are significantly scarce in evolutionally obtained GRNs.
 - The origin of the difference in the mutational robustness is the difference in the number of lethal edges.

Mutational robustness 7

Effective network size



$$f = 0.99$$

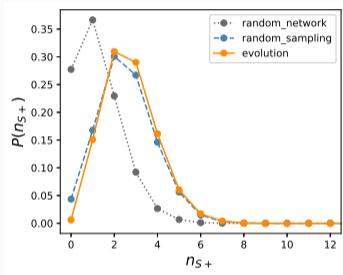
- We counted the number of nodes having at least one path to the output node.
 - The effective network size does not affect the mutational robustness.

Network motifs 1

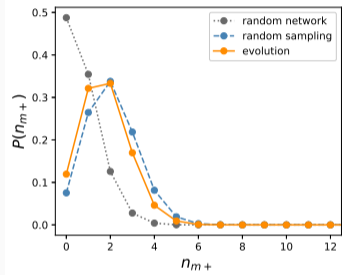
Definition of motifs

- The connection patterns appear in the given network at frequencies much higher than those found in random networks.
- We counted the following patterns for $f = 0.99$
 1. auto-regulation
 2. mutual regulation
 3. feedforward loop
 4. feedback loop
 5. mutual activation/repression plus auto-activation

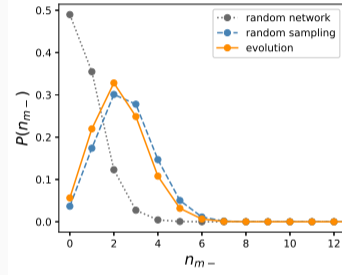
Network motifs 2



Auto-activation



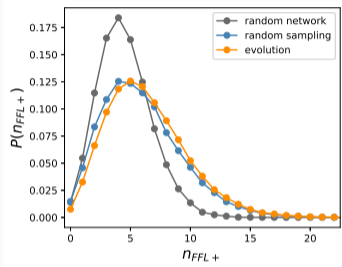
Mutual activation



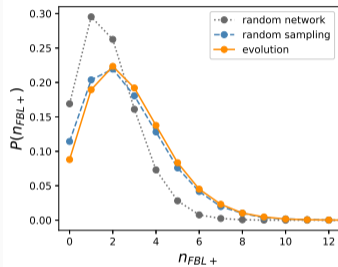
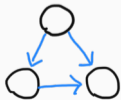
Mutual repression



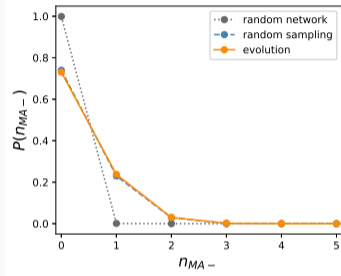
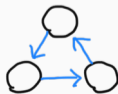
Network motifs 3



Coherent feed-forward loop



Positive feedback loop



mutual repression plus auto-activation

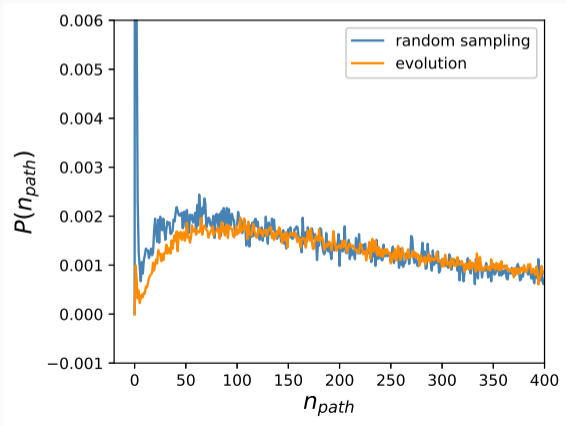


Network motifs 4

- Six patterns may be called as motif (but not so significant)
 - +FFL is ubiquitous motif in GRNs
 - Other patterns are known as motifs in multistable GRNs.
- Motifs are related to the function but not to the mutational robustness.
 - Global structures are considered to be relevant to the mutational robustness.

Path distribution 1

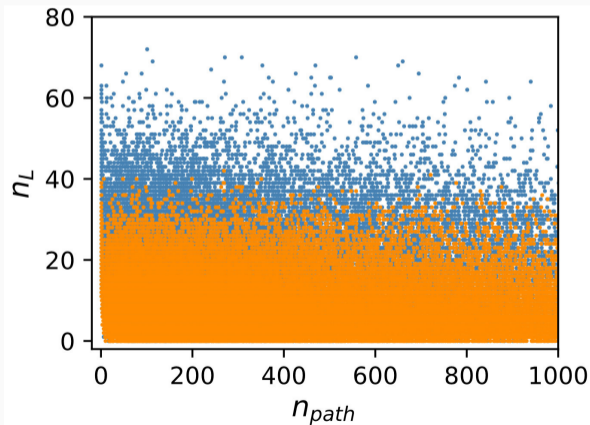
The number of paths connecting the input and output nodes



- n_{path} are significantly different for a small number of paths
 - $n_{path} = 1$ in particular.

Path distribution 2

The number of paths vs. the number of lethal edges



- The lethal edges are significantly scarce in evolution, irrespective of the number of paths.
 - The difference n_{path} does not fully explain the mechanism for enhancement of the mutational robustness.

Discussions

Mechanism for enhancement of mutational robustness

Mutation consists of two successive processes:

1. Deletion of a randomly selected edge
 2. Addition of a new edge
- When a lethal edge is deleted in the first process, the fitness of the GRN drops down to almost zero, and the possibility is very low that the fitness recovers by the second process.

Second-order selection (Wagner)

- GRNs with many lethal edges are more likely to be eliminated than those with fewer lethal edges in evolution.

Evolutionary speed

Entropy effect

- Speed of evolution is in large part determined by the number of available GRNs.
 - Effect of **genotypic entropy**.

Question

- Sato *et al.*(2003) showed that evolution slowed down as fitness becomes high.
 - Consistent with the entropic effect
- They found that evolution speed is in association with phenotypic divergence.
 - Relationship between the phenotypic divergence and the genotypic entropy?

Summary

New method

- We compared the evolution of GRNs with a randomly sampled set generated by the multicanonical Monte Carlo.

Results

1. Mutational robustness does evolve.
 - GRNs with fewer lethal edges are selected by evolution.
 - Motifs are not relevant to mutational robustness.
 - The number of paths has some relevance to mutational robustness.
2. Evolution delays the emergence of a new phenotype.
 - Evolution is conservative.
3. Evolutionary speed is determined mainly by genotypic entropy.