Evolution enhances the mutational robustness and suppresses the emergence of new phenotype

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Introduction

- Living systems have developed mutational robustness in the course of evolution.
- Mutational robustness can be examined experimentally by several methods such as gene knock-out or artificial addition of gene regulation.
- But the evolution of mutational robustness is not easy to investigate experimentally. Thus numerical methods play important roles.

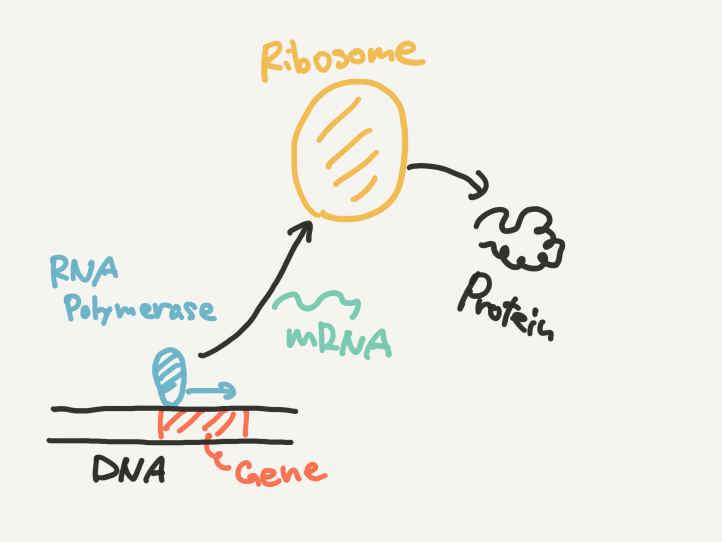
In this talk, we focus on the evolution of mutational robustness of a toy model of gene regulatory networks (GRN).

- Evolutionary simulations provide evolutional-path-dependent traits.
- In order to study the universality and the particularity of evolution, we need reference systems.
- The reference systems that we consider natural are GRNs obtained by random sampling.

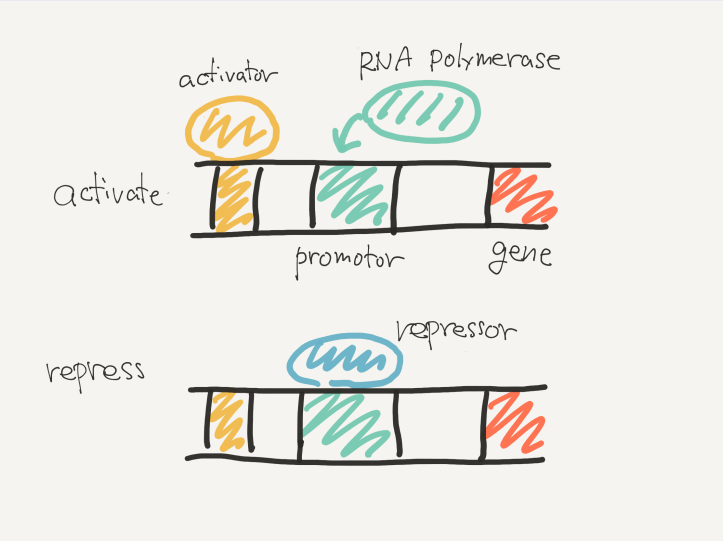
The preceding work

- S. Nagata and M. Kikuchi "Emergence of cooperative bistability and robustness of gene regulatory networks" PLoS Comput Biol 16 (2020) e1007969

Gene Regulatory Network



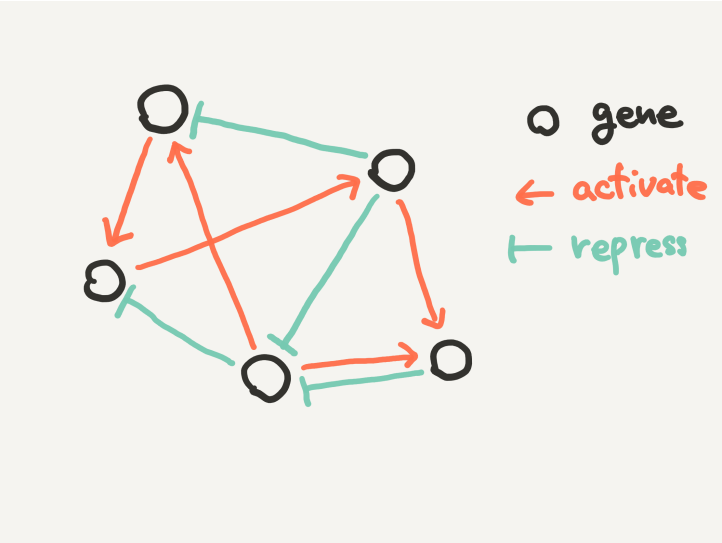
Gene expression



Gene regulation by transcription factors

Model and Methods

Model



- We ignore details of gene regulation.
- We consider neural-network-like connectionist model.
  - Node: Gene
  - Edge: Regulation

Network model

- Directed random graph
  - $N = 32$ nodes、$K = 80$ edges
- One input gene and one output gene
- All the possible networks are allowed

Discrete-time dynamics (Neural-network like)

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

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

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

Fitness

- We require that the expression of the output node for $I = 0$ and 1 differ as large as possible.
- We define fitness

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

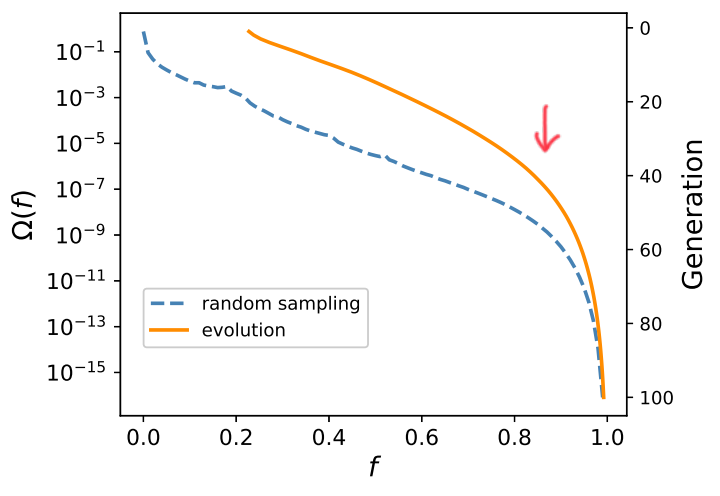
  where $\bar{x}_{out}(I)$ is the fixed-point value (or the long-time average if no fixed point is reached) of the output node for input I.
- $f \in [0, 1]$ by definition

Methods

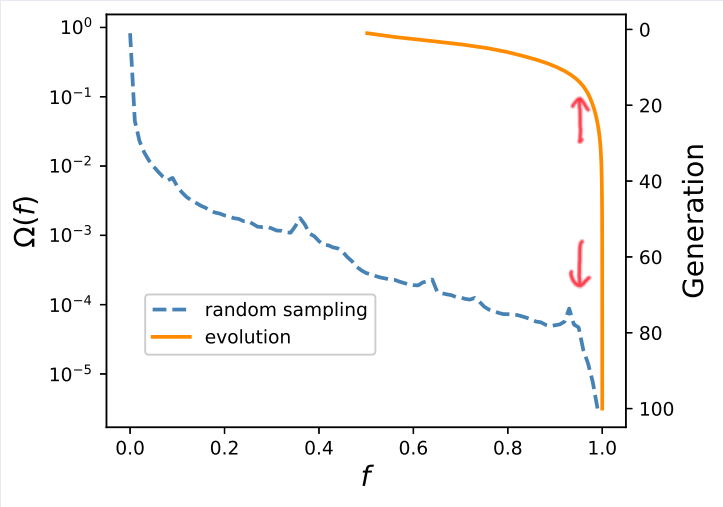
1. Random sampling by multicatnonical ensemble method
   - It enables us to sample GRNs in wide range of fitness randomly (in principle).
     - Fitness are divided into 100 bins. The weight for each bin is determined by Wang-Landau method.
     - 5M samples
2. Evolutionary simulation
   - Mutation: random reconnection of a randomly selected edge
     - 1000 GRNs; 500 are selected at each generation
     - The lineage of the highest fitness at 150 generation is sampled
     - 10000 independent runs

Results

Fitness landscape and evolutionary speed



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

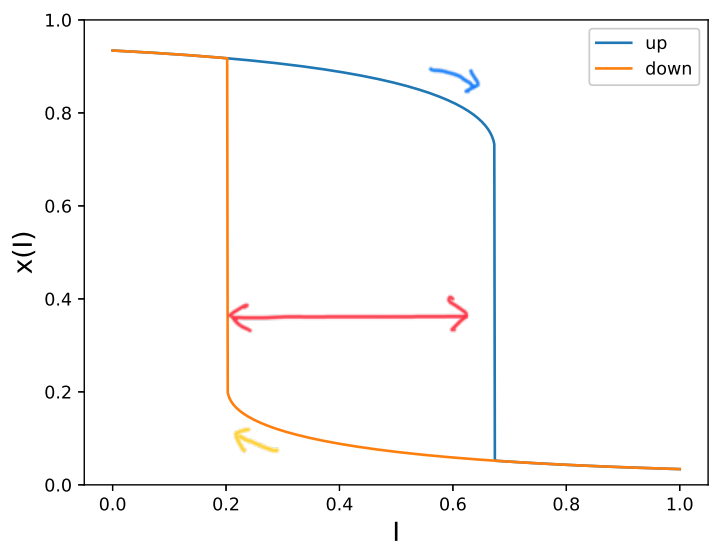


for $\beta = 4$ (only this figure)

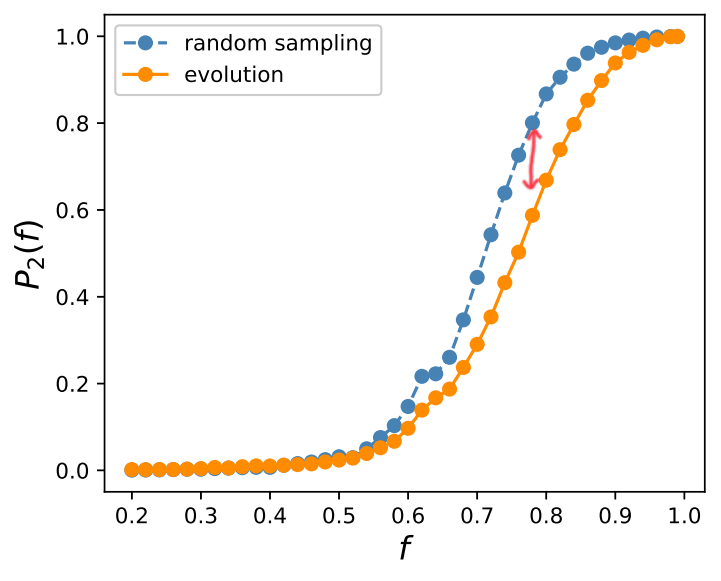
1. Most GRNs have low fitness ($f < 0.1$).
2. At intermediate fitness, GRNs become exponentially rare as fitness increases.
3. For very high fitness ($f > f^*$), GRNs become faster-than-exponentially rare.
4. Evolution slows down suddenly at $f \simeq f^*$.

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

Emergence of bistability



Example of bistability for $f = 0.9$



The fraction of the bistable GRNs vs. fitness

1. Universality of evolution:
   Bistable GRNs emerge, and their fraction grows as fitness increases. All GRNs become bistable as $f \to 1$.
   - Bistability is a new phenotype. This new phenotype that was not explicitly required by the fitness appears inevitably.
2. Particularity of evolution:
   Evolution delays appearance and growth in the fraction of the bistable GRNs.
   - Evolution is conservative!

Mutational robustness

Robustness measure

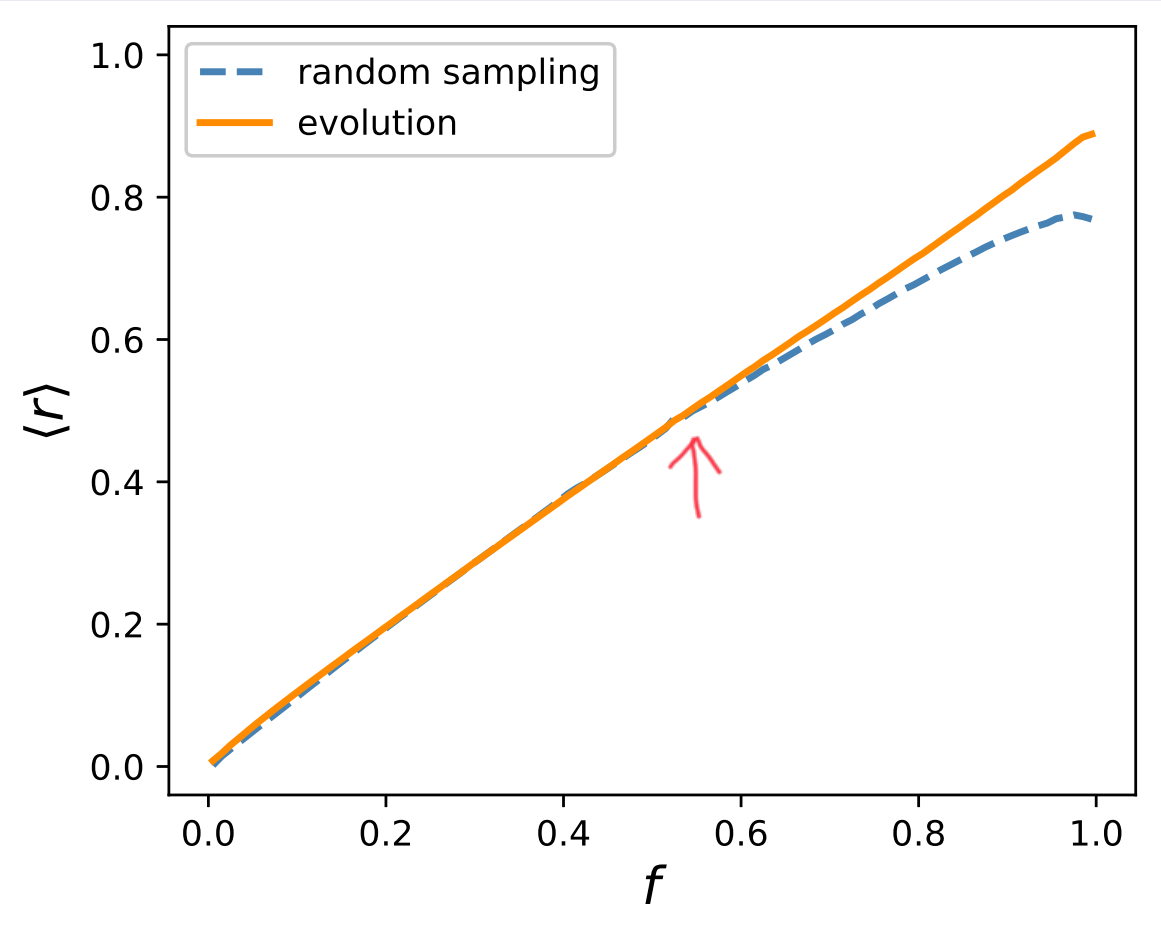
- We consider the single-edge deletion as mutation.
- Delete a single edge from GRN and compute fitness f' of mutated GRN
- Robustness measure for a given GRN is the average of f' for all the possible single-edge deletion.

$$r \equiv \frac{1}{K} \sum_i f_i'$$

  - f_i' is f' for GRN that ith edge is deleted.

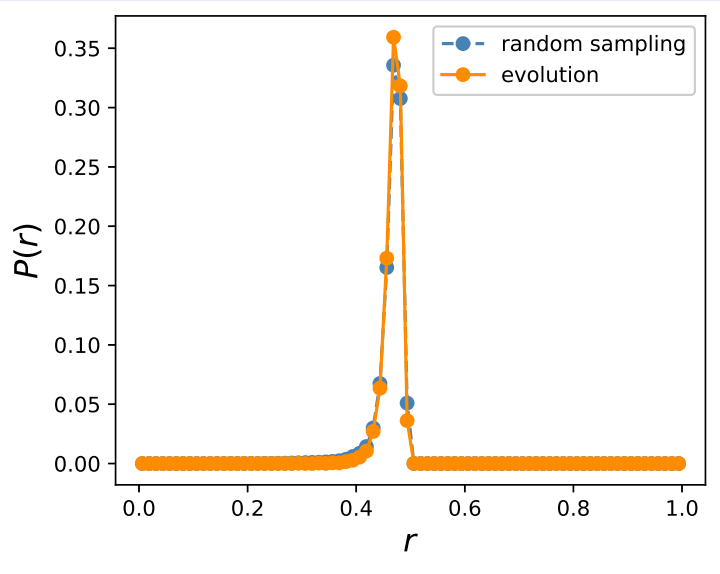
- We compute the average $\langle r \rangle$ over all GRNs in each bin.

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

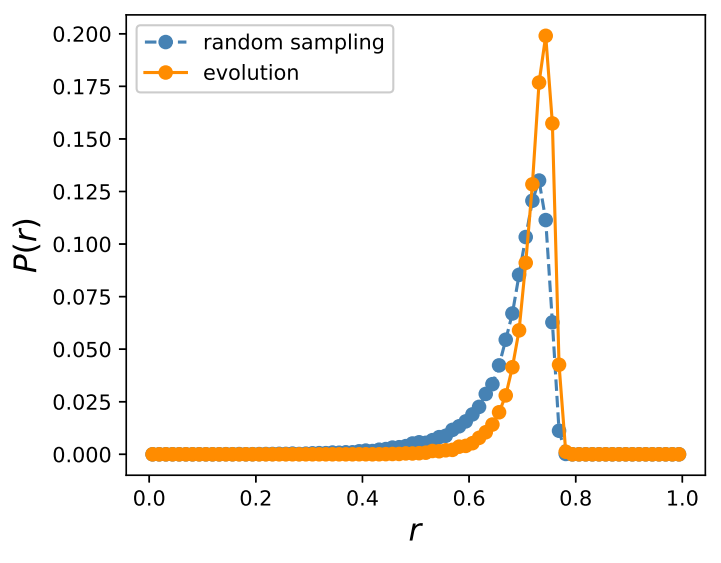


Robustness measure vs. fitness

Probability distribution of r

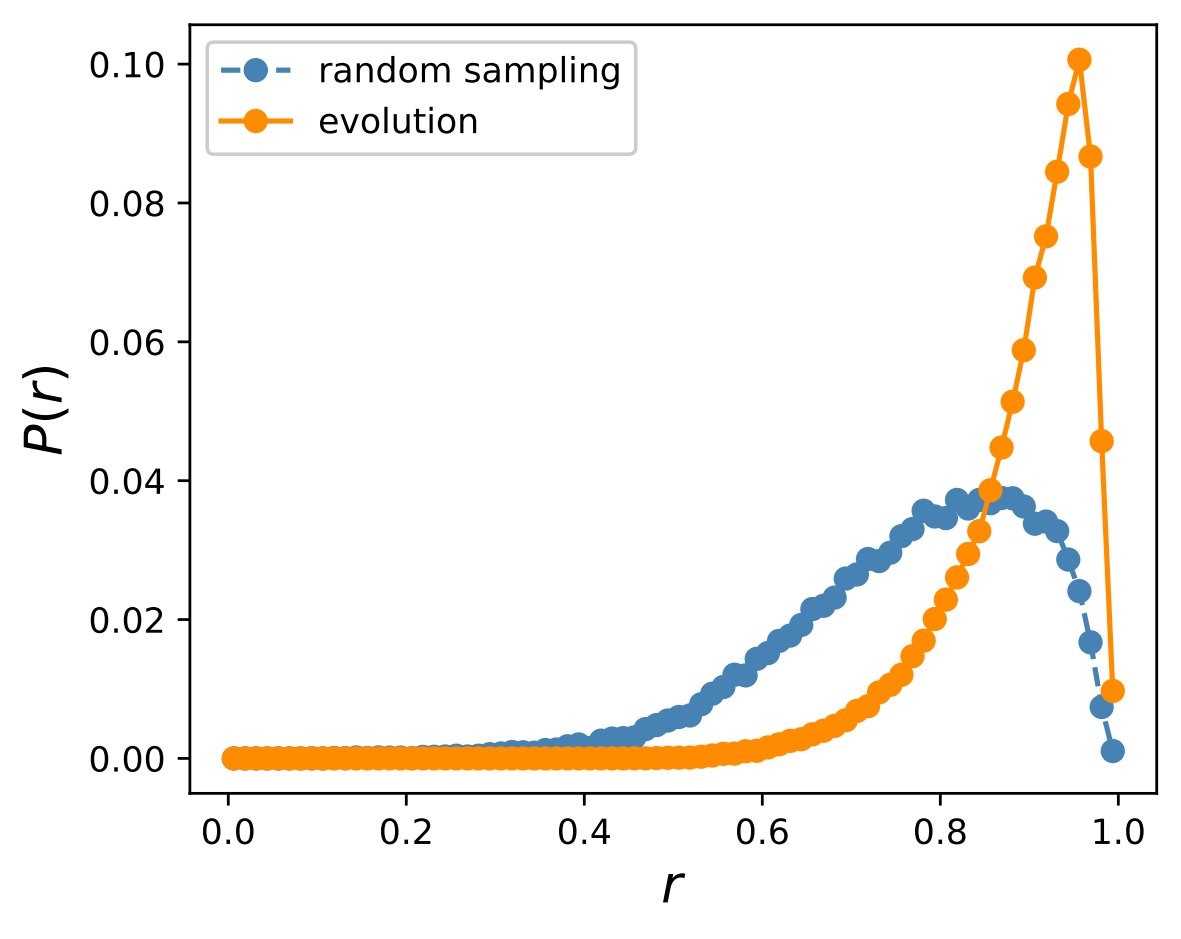


$f = 0.5$



$f = 0.8$

Probability distribution of r



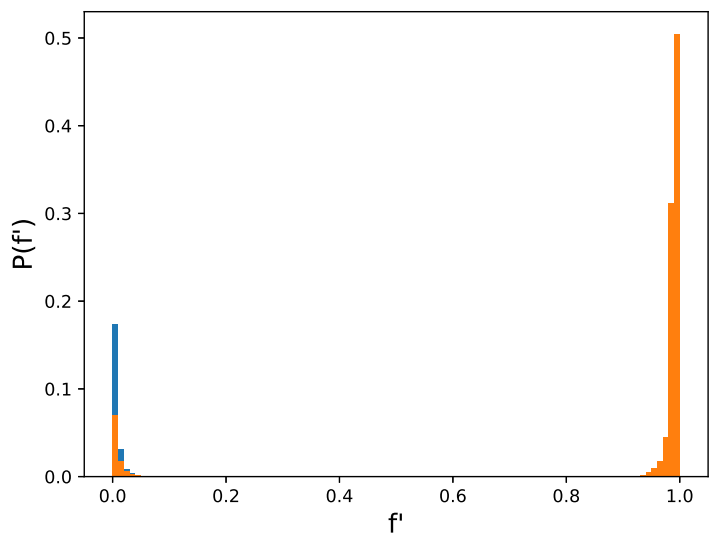
$$f = 0.99$$

Two-step evolution

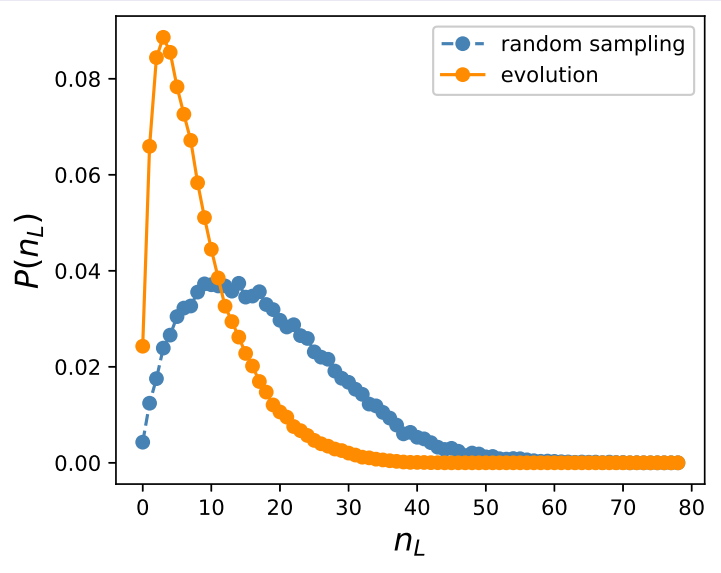
1. In the early stage of evolution, $\langle r \rangle$ coincides with random sampling.
2. In the late stage of evolution, $\langle r \rangle$ exceeds random sampling.

- Particularity of evolution:
  The evolutionally obtained probability distribution of r for high fitness is significantly biased to the robust side compared to random sampling.
  - Evolution enhances the mutational robustness!

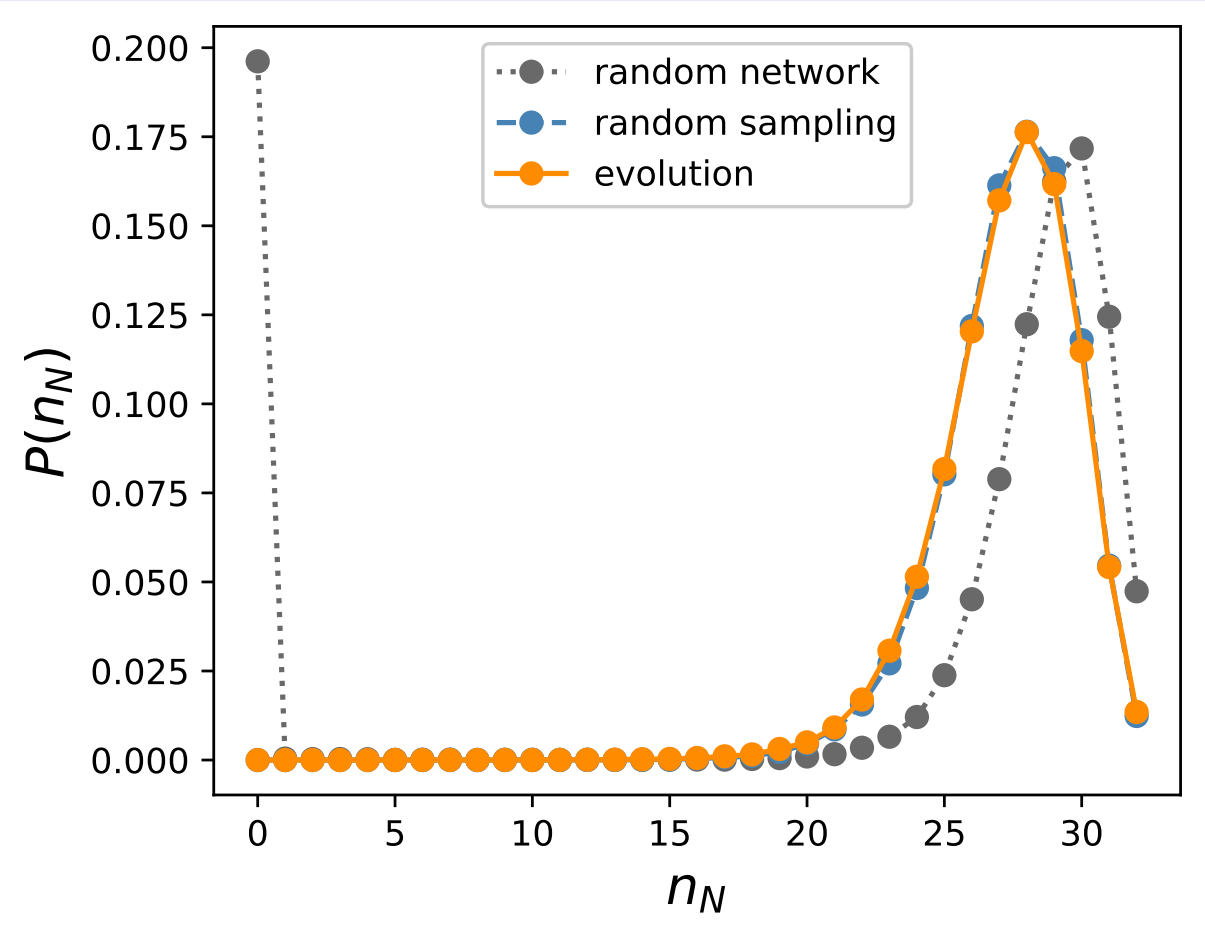
Explore the origin of robustness



Distribution of f' for $f = 0.99$



Distribution of lethal edges for $f = 0.99$



Distribution of effective network size for $f = 0.99$

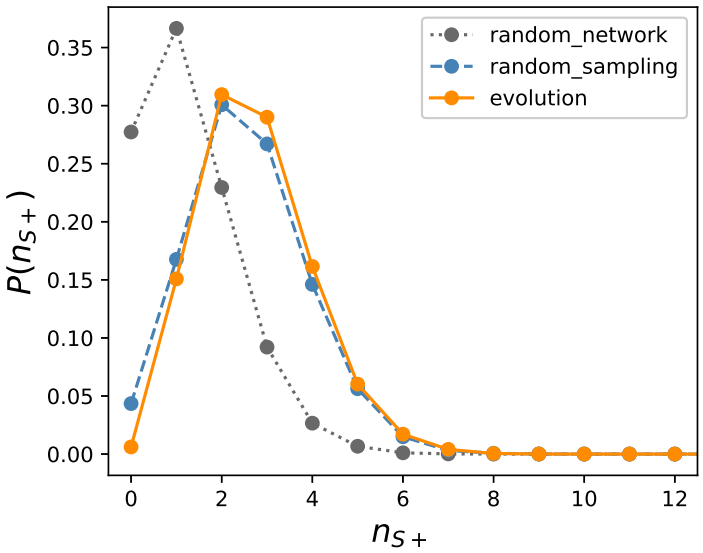
- For high fitness, edges are divided in two classes: neutral and lethal
- The number of lethal edges are significantly scarce in evolutionally obtained GRNs.
  - The origin of the difference in the mutational robustness is the difference in the number of lethal edges.
- The effective network size does not affect the mutational robustness.

Network motifs

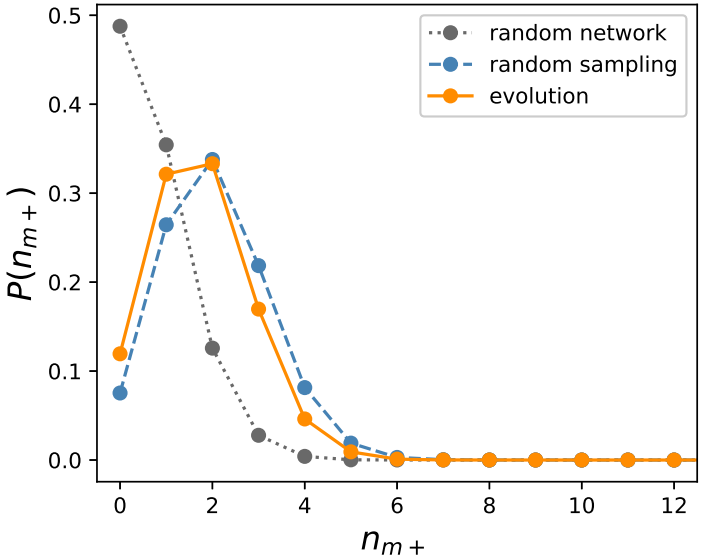
Definition of motifs

- The connection patterns appear in the given network at frequencies much higher than those found in random networks.

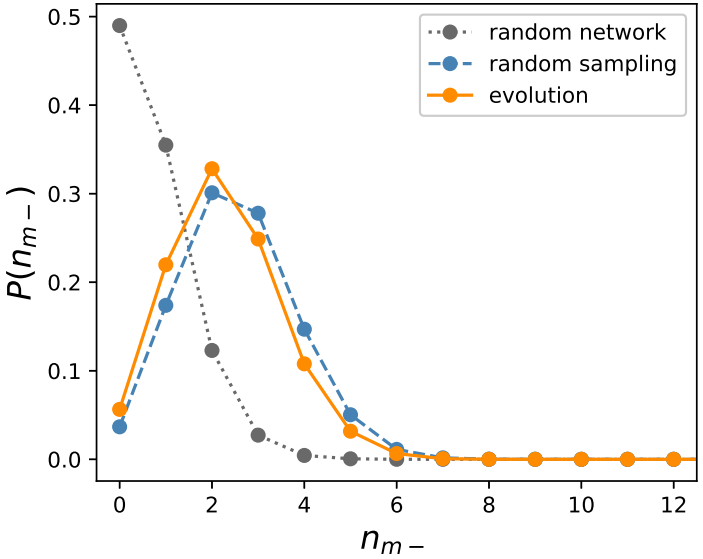
- We explore the following patterns for $f = 0.99$
  1. auto-regulation
  2. mutual regulation
  3. feedforward loop
  4. feedback loop
  5. mutual activation/repression plus auto-activation



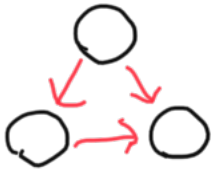
Auto-activation

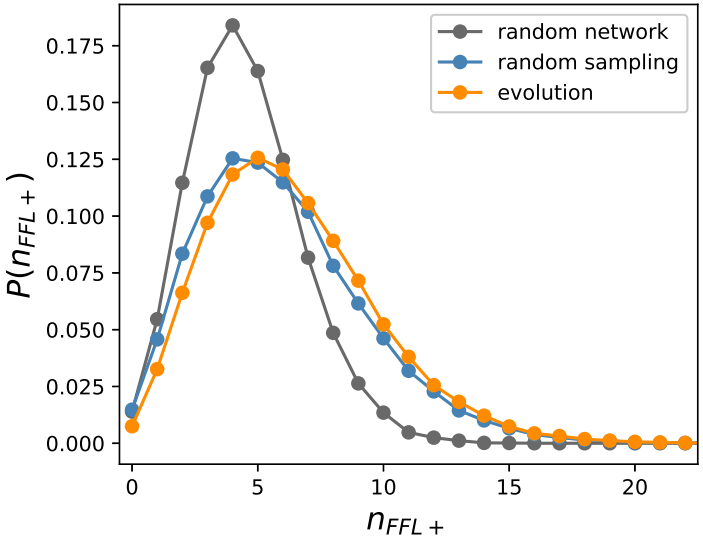


Mutual activation

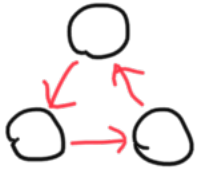


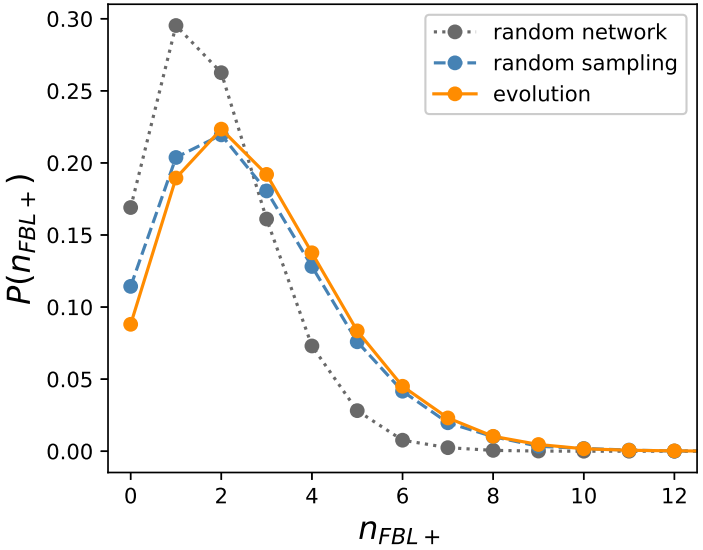
Mutual repression



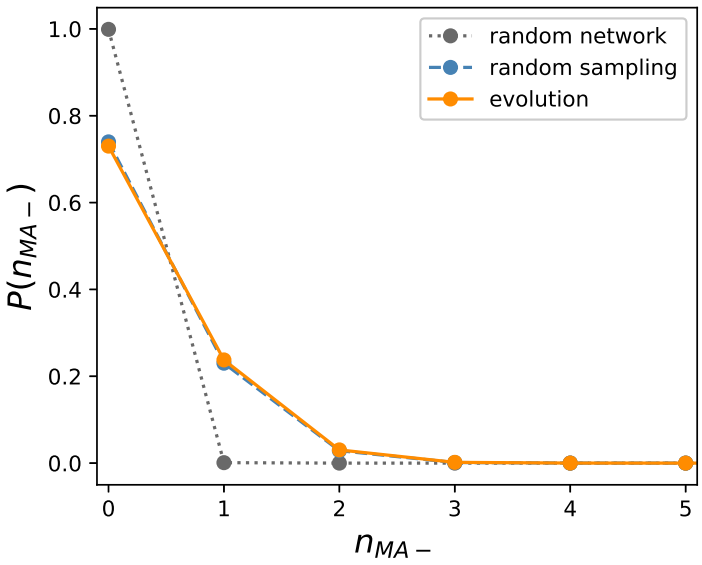


Coherent feed-forward loop





Positive feedback loop



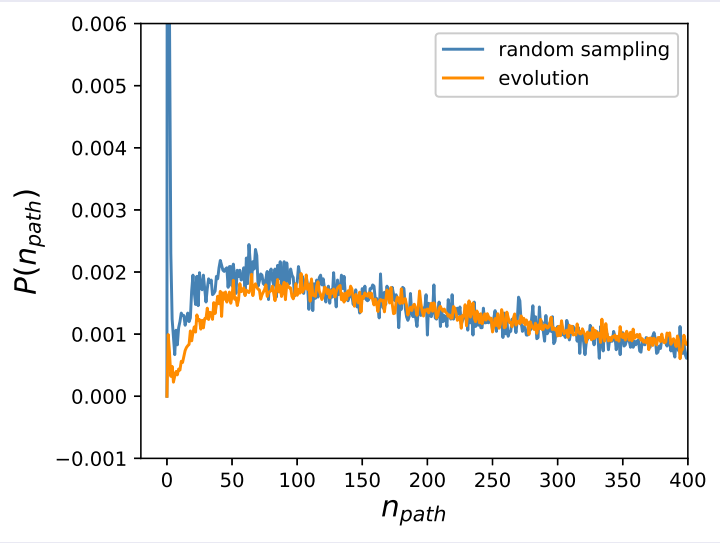
mutual repression plus auto-activation

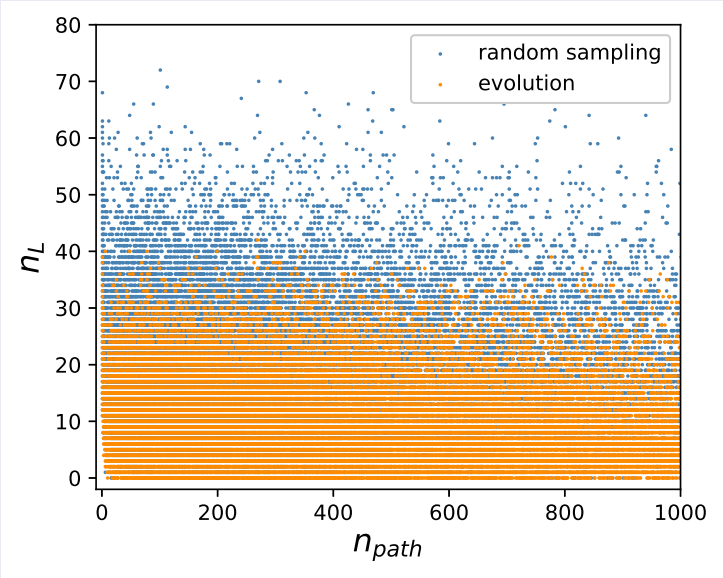
- Six patterns may be called as motif (but not so significant)
  - +FFL is ubiquitous motif in GRNs
  - Other patterns are known as motifs in multistable GRNs.

- Motif distributions do not differ for evolutionally obtained GRNs and randomly sampled 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

The number of paths connecting input and output nodes





The number of paths vs. the number of lethal edges

1. The probability distribution of the number of paths n_{path} connecting input and output nodes are significantly different for a small number of paths, $n_{path} = 1$ in particular.
2. This difference does not fully explain the mechanism for enhancement of the mutational robustness by the evolution, because the lethal edges are significantly scarce irrespective of the number of paths compared to randomly-produced GRNs.

Discussion

Mechanism

- 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.

- GRNs with many lethal edges are more likely to be eliminated than those with fewer lethal edges.
  - Second-order selection (Wagner)

Summary

Method

- We compared the evolution of GRN with random sampling achieved by the multicanonical ensemble.

Results

1. Evolution delays the emergence of a new phenotype.
   - Evolution is conservative.
2. Mutational robustness evolves.
   - 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.

The results are intuitively natural. But we were able to discuss them quantitatively by the combination of the Multicanonical Monte Carlo method and the evolutionary simulation.

Selected references

- Robustness
  - A. Wagner "Robustness and Evolvability in Living Systems" (2005) Princeton
- Method
  - Y. Iba, N. Saito N and A.Kitajima "Multicanonical MCMC for sampling rare events: an illustrative review" Ann Inst Stat Math. 66 (2014) 611
- Motifs
  - U. Alon "An Introduction to Systems Biology (second edition)" (2020) CRC Press