

# The Ensemble of Gene Regulatory Networks at Mutation-Selection Balance

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## Abstract

The evolution of diverse phenotypes both involves and is constrained by molecular interaction networks. When these networks influence patterns of expression, we refer to them as gene regulatory networks (GRNs). Here, we develop a quasi-species model of GRN evolution. With this model, we prove that—across a broad spectrum of viability and mutation functions—the dynamics converge to a stationary distribution over GRNs. Next, we show from first principles how the frequency of GRNs at equilibrium will be proportional to each GRN’s eigenvector centrality in the genotype network. Finally, we determine the structural characteristics of GRNs that are favored in response to a range of selective environments and mutational constraints. Our work connects GRN evolution to quasi-species models, and thus can provide a mechanistic explanation for the topology of GRNs experiencing various evolutionary forces.

## 1 Introduction

Molecular networks influence both macro- and micro-evolutionary processes [1, 2, 3, 4, 5]. But, how might they themselves evolve? A recent comparative study of regulatory networks found that their structures often exist at the edge of critically, straddling the border of chaotic and ordered states [6]. That biological regulatory networks should exhibit the kind of dynamic stability associated with near-critical networks has been theorized as adaptive, both from the perspective of functional robustness [7] and their ability to effectively process information [8]. However, there is also empirical and theoretical evidence for the importance of change in these networks, e.g., if species must evolve to meet shifting environmental or ecological selection pressures [9]. This tradeoff between robustness and evolvability is hypothesized as an explanation for the common “small-world” property in biological networks [10]. Nevertheless, foundational work on self-organized criticality and  $1/f$  noise demonstrated that dynamical systems embedded in a spatial dimension, e.g., biological regulatory networks, might naturally evolve to near-critical states [11, 12]. Therefore, one could observe near-critical networks in nature that are derived from constraints, as opposed to directly optimized by selective forces.

Focusing specifically on interactions that modulate expression, recent studies have hypothesized how various evolutionary forces shape the structure of gene regulatory networks (GRNs) [13, 14, 15, 16]. Analyses of transcription factors [17, 18], mRNA profiles [19] and comparative genomics [20] suggest that gene duplication/loss have a substantial contribution to divergent gene regulation. Moreover, several mathematical models of GRN evolution were introduced to encompass duplication events [21], selection on functional dynamics [22], horizontal gene transfer [23], correlated mutations on genomes [24], and non-genetic inheritance [25]. Force et al. [26] computationally showed that subfunction fission following duplication events can lead to a modular structure of GRNs. Similarly, Espinosa-Soto and Wagner [27] demonstrated that sequential adaptation to newly specialized gene activity patterns can increase the modularity of GRNs. Conversely, GRNs are hypothesized to emerge largely as a by-product of the progression towards some optimal state, via some combination of negative-feedback regulation [28], the rate of molecular evolution [29], tradeoffs between robustness and evolvability [6], and self-organization of functional activity [30].

In principle, existing frameworks that model evolutionary dynamics can be applied to the evolution of GRNs. Ideally, and hypothetically given “omniscience” over the genomes—including comprehension of every fundamental interaction between molecules—one can reconstruct inter-dependencies among genes and obtain GRNs from a

28 bottom-up approach. Of course, this ambition is far from practical and even sounds like a fantasy. Yet, it shows that  
29 GRNs are essentially a direct abstraction of the genotypes. This abstraction is not only central to the omnigenic  
30 perspective of complex traits [31], but it also motivates a theoretical framework of regulatory circuit evolution [32].  
31 Over the past two decades, several models of GRN evolution have been proposed [33, 34, 35, 36, 37]. The resulting  
32 models have influenced our understanding of diverse phenomena including canalization [38, 34], allopatric speciation  
33 [39, 36, 37], expression noise [40], and the structural properties of GRNs themselves [41, 27, 35].

34 However, to date, the existing models of GRN evolution remain largely computational, and the complexity of  
35 genetic interactions impedes more advanced theoretical analyses of these models. Our ambition in this work is  
36 to derive analytical conclusions for models of GRN evolution, with aids from known theoretical establishments in  
37 population genetics and quasi-species theory [42]. Population genetics describes how the frequency of different alleles  
38 change over time in a population mechanistically through evolutionary forces [43], in which mathematical models  
39 usually focus on a finite-sized population and a few loci. Quasi-species theory, on the other hand, concentrates  
40 on the balance between selection and mutation in an infinitely large population, where genotypes with a higher  
41 dimensionality are incorporated [44]. Literature has provided exact solutions for the steady distribution of genotypes  
42 along with their global convergence in quasi-species theory [45, 46, 47] under the assumption of irreducible and  
43 primitive transition matrices. This assumption was latter proposed to correspond to the mutational accessibility  
44 among genotypes with non-zero fitnesses [48]. It is thus not hard to vision that, when extended with complex  
45 genetic interactions, the stationary solution of a population-genetic or quasi-species model implicates the balanced  
46 distribution of plausible GRNs under the focal evolutionary forces. Nevertheless, it remains to be shown that such  
47 assumptions are valid for high dimensional genotype-phenotype maps associated with gene regulatory networks.

48 Here, we develop a quasi-species model describing how the structure of GRNs are shaped by a combination of  
49 selection and mutation. First, using this model we study the dynamics of GRN evolution in an infinitely large pop-  
50 ulation with non-overlapping generations in a constant environment. By depicting the mapping between genotypes  
51 and phenotypes through the GRNs [37], we mechanistically recover the key assumption in the literature mentioned  
52 above and prove that the dynamics always converge to a stationary distribution over GRNs. Then, assuming bi-  
53 nary viability, identical reproductivity, and rare mutation, we analytically show that the frequency of GRNs at  
54 mutation-selection balance is proportional to each GRN's eigenvector centrality in a sub-graph of the genotype net-  
55 work [49, 50, 51, 52]. Finally, we determine the structural motifs associated with GRNs that are favored in response  
56 to a wide variety of selective regimes and regulatory constraints. We discuss the implications of our results in the  
57 context of the evolution of complex phenotypes and the challenges of studying GRN evolution.

## 58 2 Models

### 59 2.1 Quasi-Species Model with Selection, Reproduction, and Mutation

60 We begin with a quasi-species model that incorporates selection, reproduction, and mutation: The viable individuals  
61 in the current generation reproduce and generate their offspring, which may possibly mutate, and undergo selection  
62 to form the next generation. This phenomenological modeling scheme has frequently appeared in existing literature  
63 for a deterministic dynamics or a stochastic Markovian process perspective [44, 45, 46, 48, 47]. Yet, we shall  
64 see shortly that basic probability theory assists us to construct the model in a bottom-up fashion and leads to a  
65 probabilistic interpretation of various parameters. We additionally impose a few assumptions to the model, including  
66 a.) an infinitely large population size, b.) non-overlapping generations, c.) asexual reproduction, d.) a constant  
67 reproductivity of each genotype and a fixed selective environment over time, and e.) that any single-locus mutation  
68 has a non-zero chance to occur per generation.

69 Suppose that  $I_t$  represents an individual randomly sampled from the population at generation  $t$ . Let  $g(I_t)$  and  
70  $\Psi(I_t)$  be its genotype and the event that  $I_t$  is viable respectively. We will further denote by  $I_{t-1} \rightarrow I_t$  the event that  
71 the randomly sampled individual at generation  $t - 1$ , namely  $I_{t-1}$ , reproduced and generated the randomly sampled  
72 individual at generation  $t$ , namely  $I_t$ . We will also write  $\mathcal{G}$  to represent the set of all plausible genotypes.

73 For any genotype  $g \in \mathcal{G}$ , we are interested in its prevalence in the population at a given generation  $t$  *after*  
74 selection. In other words, we would like to know the probability that we observe a randomly sampled individual at  
75 generation  $t$  with the genotype  $g$ , given the fact that the sampled individual is viable. Applying the Bayes' theorem,  
76 this focal conditional probability becomes

$$\begin{aligned}\mathbb{P}[g(I_t) = g \mid \Psi(I_t)] &= \frac{\mathbb{P}[\Psi(I_t) \mid g(I_t) = g] \mathbb{P}[g(I_t) = g]}{\mathbb{P}[\Psi(I_t)]} \\ &= \frac{\nu_g}{\nu_t} \mathbb{P}[g(I_t) = g].\end{aligned}\tag{1}$$

77 For simplicity we adapt the abbreviation  $\nu_g = \mathbb{P}[\Psi(I_t) \mid g(I_t) = g]$  and  $\nu_t = \mathbb{P}[\Psi(I_t)]$ , which are equivalently the  
78 survival probability or the **viability** of genotype  $g$ , and the average viability at generation  $t$  respectively.

79 What we have left in equation (1) is the probability that a randomly sampled individual has genotype  $g$  *before*  
80 selection. The derivation of  $\mathbb{P}[g(I_t) = g]$  relies on two observations: First, the genotype of individual  $I_t$  arose from  
81 mutation and the unique genotype of its parent; second, this parent individual must be viable. The event  $g(I_t) = g$   
82 is hence partitioned<sup>1</sup> by the joint events  $\{g(I_t) = g, g(I_{t-1}) = g' \mid I_{t-1} \rightarrow I_t, \Psi(I_{t-1})\}_{g' \in \mathcal{G}}$ . So we have

$$\begin{aligned}\mathbb{P}[g(I_t) = g] &= \sum_{g' \in \mathcal{G}} \mathbb{P}[g(I_t) = g, g(I_{t-1}) = g' \mid I_{t-1} \rightarrow I_t, \Psi(I_{t-1})] \\ &= \sum_{g' \in \mathcal{G}} \mathbb{P}[g(I_t) = g \mid g(I_{t-1}) = g', I_{t-1} \rightarrow I_t, \Psi(I_{t-1})] \mathbb{P}[g(I_{t-1}) = g' \mid I_{t-1} \rightarrow I_t, \Psi(I_{t-1})] \\ &= \sum_{g' \in \mathcal{G}} \mu_{g'g} \mathbb{P}[g(I_{t-1}) = g' \mid I_{t-1} \rightarrow I_t, \Psi(I_{t-1})].\end{aligned}\tag{2}$$

83 Again we abbreviate  $\mu_{g'g} = \mathbb{P}[g(I_t) = g \mid g(I_{t-1}) = g', I_{t-1} \rightarrow I_t, \Psi(I_{t-1})]$  that shows the **mutation probabil-**  
84 **ity** from genotype  $g'$  to genotype  $g$ .

85 It remains to resolve  $\mathbb{P}[g(I_{t-1}) = g' \mid I_{t-1} \rightarrow I_t, \Psi(I_{t-1})]$  in equation (2), which is the probability that the  
86 parent of a randomly sampled individual at generation  $t$  has genotype  $g'$ . Applying the Bayes' theorem once more,  
87 this probability becomes

$$\begin{aligned}\mathbb{P}[g(I_{t-1}) = g' \mid I_{t-1} \rightarrow I_t, \Psi(I_{t-1})] &= \frac{\mathbb{P}[I_{t-1} \rightarrow I_t \mid g(I_{t-1}) = g', \Psi(I_{t-1})] \mathbb{P}[g(I_{t-1}) = g' \mid \Psi(I_{t-1})]}{\mathbb{P}[I_{t-1} \rightarrow I_t \mid \Psi(I_{t-1})]} \\ &= \frac{\rho_{g'}}{\rho_{t-1}} \mathbb{P}[g(I_{t-1}) = g' \mid \Psi(I_{t-1})],\end{aligned}\tag{3}$$

88 where  $\rho_{g'} = \mathbb{P}[I_{t-1} \rightarrow I_t \mid g(I_{t-1}) = g', \Psi(I_{t-1})]$  is the **reproductivity** of genotype  $g'$ , and  $\rho_{t-1} = \mathbb{P}[I_{t-1} \rightarrow I_t \mid \Psi(I_{t-1})]$   
89 is the average reproductivity at generation  $t - 1$ . Note that, instead of defining reproductivity as the number of  
90 offspring of an individual, the probabilistic formulation conversely describes, when sampling from the infinitely-sized  
91 next generation, how likely we will observe an offspring of the focal individual.

92 More importantly, we see that equation (3) leads us back to the focal conditional probability that, at generation  
93  $t - 1$ , a randomly sampled individual has genotype  $g'$  given that it is viable. Combining (1) to (3), we obtain the  
94 master equation for the simple quasi-species model that integrates selection, reproduction, and mutation of genotypes:  
95

$$\mathbb{P}[g(I_t) = g \mid \Psi(I_t)] = \frac{1}{\nu_t \rho_{t-1}} \sum_{g' \in \mathcal{G}} \rho_{g'} \mu_{g'g} \nu_g \mathbb{P}[g(I_{t-1}) = g' \mid \Psi(I_{t-1})].\tag{4}$$

## 96 2.2 Pathway Framework of GRNs: Representing Genotypes by Expression Behavior

97 In existing literature of quasi-species theory, the model parameters are usually arbitrarily tunable or follow a particular  
98 distribution for simplicity. Hypothetically, these parameters depend on the resultant phenotypes of the genotypes,  
99 and any genotype-phenotype mapping reflects constraints and provides information of the model parameters. Our  
100 previous work has proposed a modeling approach, termed the **pathway framework**, to describe how the structure  
101 of GRNs varies due to genetic changes and how they respond to a given selective pressure [37] (which we summarize  
102 below; see its formal mathematical formulation in Appendix A). In the current work, we apply the pathway framework  
103 of GRNs to the quasi-species model (4) as a simple genotype-phenotype mapping for parameterization.

<sup>1</sup>A set  $A$  is said to be *partitioned* by  $\{A_i\}_{i \in I}$  if  $A = \bigcup_{i \in I} A_i$  and  $A_i \cap A_j = \emptyset$  for two distinct  $i, j \in I$ .

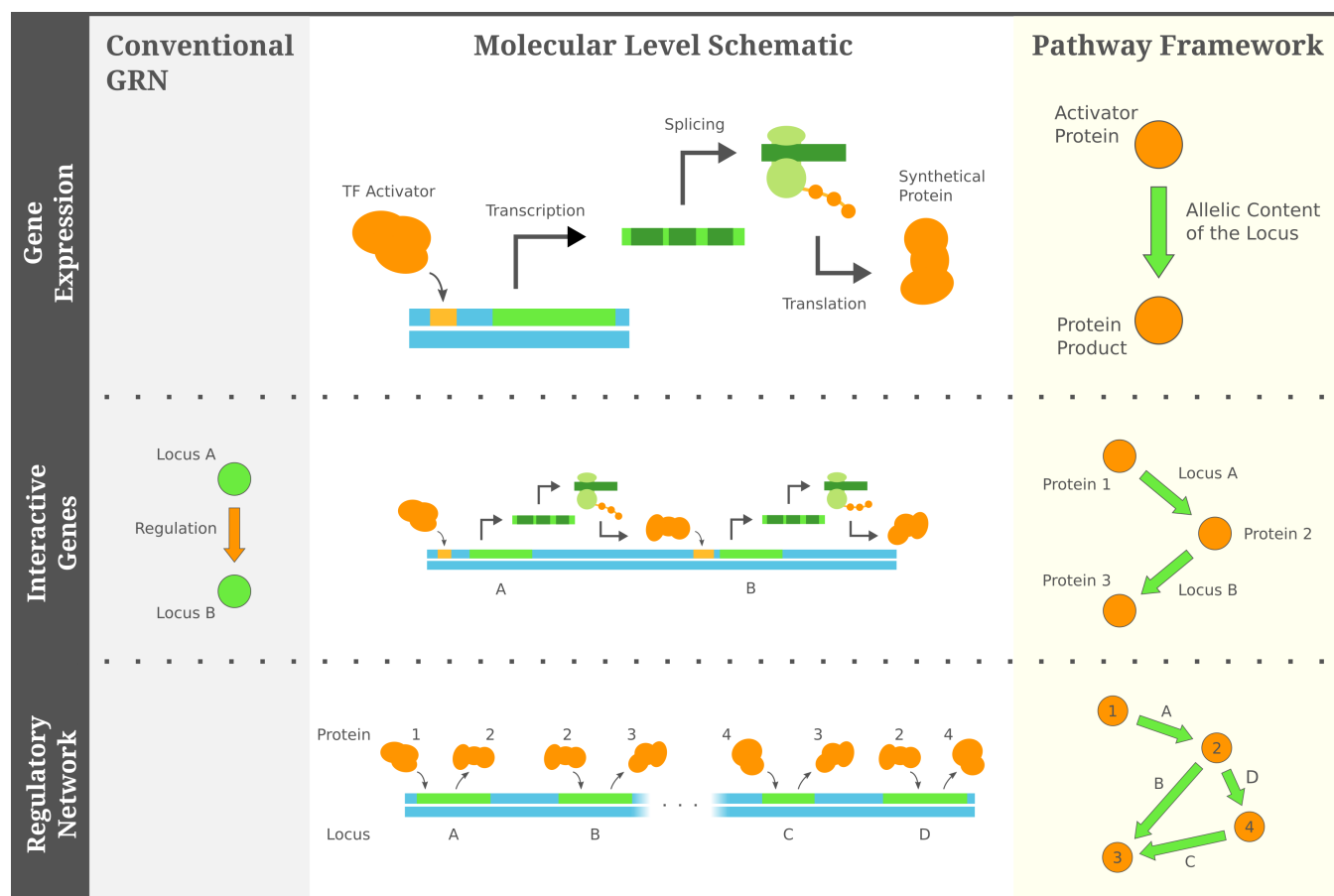


Figure 1: The pathway framework interprets a GRN as an abstraction of the expression behavior of the genotype. In this framework a GRN consists of edges indicating the input-output pair of a gene’s expression, from which transcriptional regulation between genes can be recovered, and it is arguably a more compact representation than the conventional notion of GRNs.

104 We note that the pathway framework is not without a handful of presumptions and thus restricts to rather  
 105 specific GRNs. Nevertheless, the pathway framework GRNs play the role of an informative genotype-phenotype  
 106 mapping which evokes some mechanistically interpretable parameterization for models in quasi-species theory. As a  
 107 teaser, we shall in later sections that this naive and particular genotype-phenotype mapping through gene regulation  
 108 surpasses the key assumption in existing quasi-species theory when proving the global convergence to a stationary  
 109 solution. The pathway framework may seem an arbitrary and perhaps oversimplified choice to encapsulate the  
 110 genotype-phenotype mapping; future works can indeed incorporate more realistic modeling frameworks to GRNs to  
 111 strengthen the conclusion of global convergence.

112 The key of the pathway framework is to conceptualize alleles of genes as “black boxes” that encapsulate their  
 113 expression behavior. Expression of a gene is triggered by some protein called the transcription factor, which is  
 114 followed by a series of procedures to synthesize the protein product. The pathway framework of GRNs extracts  
 115 the allele of a gene through this input-output relation, i.e., the activator protein(s) of gene expression and the  
 116 protein(s) it produces. Regulation between two genes naturally arises once one gene’s protein product involves  
 117 in the activation of the other’s expression (see Figure 1). Furthermore, these input-output relations of gene  
 118 expression serve as the “inherited” reactions through which external environmental stimuli and internal chemical  
 119 signals of proteins propagate to develop the phenotype. The pathway framework hence represents the genotype  
 120 as the input-output relation of each gene’s expression behavior, where the corresponding GRN is constructed  
 121 accordingly, and it considers the collective state of proteins as the resulting phenotype.

122 In this work, we focus on a minimal pathway framework of GRNs which integrates a few additional assumptions:  
 123 First, we presume there is a constant collection of proteins  $\Omega$  that can *possibly* appear in the organisms, and the state  
 124 of a protein is binary, which indicates whether the protein is present or absent in an organism. Second, assuming

125 that any gene’s expression is activated a single protein and produces a single protein product, the allele of the gene  
126 becomes the ordered pair of protein activator/product. If the protein activator is in the present state, the allele  
127 of the gene turns the state of the protein product to presence. Third, there is a fixed collection of genes  $\Gamma$  in the  
128 organisms, and the allele of each gene can be any pair of activator/product in the constant collection of proteins.  
129 Forth, the external environmental stimuli, if any, specify some activator proteins in the constant collection  $\Omega$  and  
130 turn their state to presence.

131 Under these assumptions, a GRN can be transformed from its conventional notion, where nodes in the network  
132 represent genes and the edges shows regulation among them, into a more compact format such that the nodes are  
133 exactly the constant collection of proteins and the directed edges describe the expression behavior of alleles of genes  
134 (see Figure 1). Hereafter, if not otherwise specified, we refer the term GRNs to those in the compact format under  
135 the pathway framework, yet it is noteworthy that the two constructions are merely different representations of the  
136 expression behavior of the same underlying genotype. While the set of all possible genotypes is denoted by  $\mathcal{G}$  in  
137 section 2.1, we abuse the notation  $\mathcal{G}$  for their corresponding GRNs as well, and we write  $g \in \mathcal{G}$  to refer to a possible  
138 genotype/GRN. Given the constant collection of proteins  $\Omega$  and genes  $\Gamma$ , the set of all possible GRNs is determined:  
139 A possible GRN is a network among  $\Omega$  with  $|\Gamma|$  directed edges, each of which is labeled by a gene in  $\Gamma$  and points  
140 from any protein activator to any protein product in  $\Omega$ .

141 The pathway framework provides an approach to model evolutionary mechanisms, such as random mutation and  
142 natural selection, through graphical operations and structural characteristics on the GRNs. Mutation at a gene  
143 changes its allele stochastically, which is essentially a random process over all possible pairs of protein activator/  
144 product in the constant collection  $\Omega$  excluding the original allele. In the corresponding GRN, mutating the allele of  
145 a gene is equivalent to rewiring the directed edge labeled by the focal gene. On the other hand, selection is usually  
146 characterized as some phenotypic response against the environment. Specifically, since a phenotype is developed  
147 through the cascading of internal signal of protein appearances starting from the external environmental stimuli, the  
148 binary state of a protein in the resulting phenotype corresponds to its reachability from the stimulated proteins in the  
149 GRN. The viability and the reproductivity of a genotype can therefore be modeled as functions of node reachability  
150 in the GRN. For example, in the case study in section 3.2, we will consider a simple scenario where the mutation at  
151 each gene is independent and the outcome is uniform among all possible alleles, and that the viability is 1 if some  
152 phenotypic constraint is satisfied or 0 otherwise. We explore more complex scenarios in later sections.

### 153 2.3 Genotype Network: a Space of Mutational Relationship between GRNs

154 Previous literature has developed the concept of the genotype network, which captures how various genotypes tran-  
155 sition from one to another through mutations (not necessarily just point mutations) and/or recombination [49, 53].  
156 Here, we adopt the genotype network to describe the mutational connection between GRNs. The **genotype net-**  
157 **work** of GRNs is a undirected network of networks, where every possible GRN becomes a mega-node, and two  
158 mega-nodes are connected if the two corresponding GRNs only differ by the allele at a single locus. In other words,  
159 an edge between two mega-nodes in the genotype network represents a single-locus mutation between GRNs (Fig-  
160 ure 2). Sometimes, instead of concentrating on all possible GRNs, we focus the mutational relationship between a  
161 subset of them. A particularly remarkable scenario is to constrain the GRNs on the binary state of proteins of the  
162 resulting phenotype. For instance, one common phenotypic constraint is to focus on the GRNs with equal fitness  
163 under selection, and the consequent induced subgraph of the genotype network is known as the **neutral network**  
164 [51, 53], which captures mutational transition between GRNs that are selectively neutral.

165 We emphasize two important properties of a genotype network of GRNs and its induced subgraphs under the  
166 pathway framework. First, because the underlying collections of proteins and genes are fixed, and a mutation at any  
167 gene can lead to a mutant allele that points from any protein activator to any protein product, each GRN has the  
168 same number of mutational neighbors. As a result, the genotype network of GRNs is in fact a regular graph. Second,  
169 for any phenotypic constraint, we show that the resulting induced subgraph of the genotype network is connected.  
170 In other words, there always exists a sequence of single-locus mutations between two GRNs such that the involved  
171 GRNs all satisfy the arbitrary given constraint on their phenotype. The guaranteed connectedness also applies to the  
172 neutral network of GRNs, where the phenotypic constraint corresponds to protein states leading to the same fitness.

173 We leave to Appendix C the detailed proof for the connectedness of a subgraph of the genotype network induced  
174 from arbitrary phenotypic constraint, and we only provide a brief outline here. The proof is based on a few ob-  
175 servations of the pathway-framework GRNs. Under the presumption of binary protein state, there naturally exist  
176 some protein activator/product pairs that are “redundant” in terms of the resulting phenotype. Such redundancy  
177 manifests when the product is simply the activator itself, or when multiple genes the same activator/product pair.



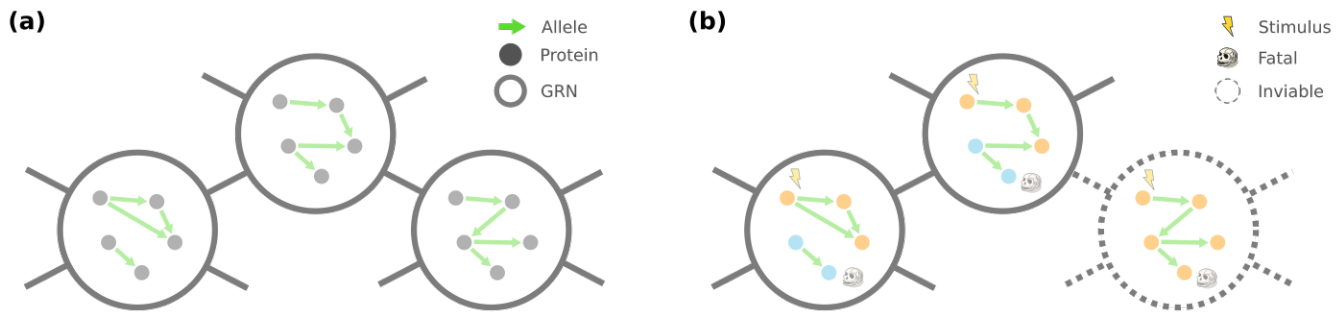


Figure 2: (a) Genotype network of GRNs, where, under the pathway framework, two mega-nodes (GRNs) are connected if and only if they differ by one edge rewiring. (b) Neutral network of GRNs, where inviable mega-nodes are removed from the genotype network. In this illustrative example inviability is modeled as a regulatory pathway from the stimulus to the protein product with a fatal effect.

178 Furthermore, given a phenotypic constraint, one can come up with a family of “naive” GRNs that satisfy the con-  
 179 straint. Specifically, such a “naive” GRN is constructed by (a) for each required-present protein, assign it as the  
 180 product of a gene with an external stimulus as the activator, and (b) assign the rest of genes with redundant acti-  
 181 vator/product pairs. Our proof in Appendix C systematically finds a mutational trajectory between two GRNs  $g_s$   
 182 and  $g_t$  satisfying the phenotypic constraint. This trajectory consists of three segments — between  $g_s$  and a naive  
 183 GRN  $g'$ , between  $g'$  and another naive GRN  $g''$ , and finally between  $g''$  and  $g_t$  — and all GRNs traversed by the  
 184 mutational trajectory also satisfy the given phenotypic constraint.

### 185 3 Analyses

#### 186 3.1 Convergence to a Stationary Distribution of GRNs

187 Our main result shows the convergence of the quasi-species model (4) under the pathway framework, and we derive  
 188 the stationary distribution over possible GRNs. We begin with noting some groups of GRNs whose probability  
 189 to be observed is relatively straightforward in the model. First, for any GRN  $g$  with a zero viability, i.e.,  $\nu_g =$   
 190  $\mathbb{P}[\Psi(I_t) | g(I_t) = g] = 0$ , the probability to observe  $g$  from a randomly sampled individual that has survived selection  
 191 is also zero. Formally speaking, denoting those GRNs with a non-zero viability by  $\mathcal{G}_v = \{g \in \mathcal{G} | \nu_g > 0\}$ , we  
 192 have  $\mathbb{P}[g(I_t) = g | \Psi(I_t)] = 0$  for each  $g \in \mathcal{G} \setminus \mathcal{G}_v$  and at any time  $t$ . Second, denote the GRNs with a zero  
 193 reproductivity by  $\mathcal{G}_s = \{g \in \mathcal{G} | \rho_g = 0\}$ . Since GRNs  $\mathcal{G}_s$  do not contribute to the offspring, their probability to  
 194 be observed solely depends on the other GRNs  $\mathcal{G} \setminus \mathcal{G}_s$ . In particular, for each  $g \in \mathcal{G}_s$  and at any time  $t$ , we have  
 195  $\mathbb{P}[g(I_t) = g | \Psi(I_t)] = \frac{1}{\nu_t \rho_{t-1}} \sum_{g' \in \mathcal{G} \setminus \mathcal{G}_s} \rho_{g'} \mu_{g'g} \nu_{g'} \mathbb{P}[g(I_{t-1}) = g' | \Psi(I_{t-1})]$ .

196 It is thus useful to only keep track of the GRNs with a non-zero viability and a non-zero reproductivity. Hereafter,  
 197 we consolidate the focal conditional probability for every  $g \in \mathcal{G}_v \setminus \mathcal{G}_s$  at generation  $t$  through a column vector  $\mathbf{p}^{(t)}$ .  
 198 We write the  $i_g$ -th entry of  $\mathbf{p}^{(t)}$  as the one that corresponds to  $g$ , namely  $[\mathbf{p}^{(t)}]_{i_g} = \mathbb{P}[g(I_t) = g | \Psi(I_t)]$ . The master  
 199 equation (4) can also be rewritten in a matrix format. Specifically, we denote by  $\mathbf{T}$  a semi-transition matrix whose  
 200 entry at the  $i_g$ -th row and the  $i_{g'}$ -th column is  $\rho_{g'} \mu_{g'g} \nu_{g'}$ , for any pair of  $g, g' \in \mathcal{G}_v \setminus \mathcal{G}_s$ . We in addition have another  
 201 matrix  $\mathbf{R}$  to capture the transition from  $g' \in \mathcal{G}_v \setminus \mathcal{G}_s$  to  $g \in \mathcal{G}_v \cap \mathcal{G}_s$ , whose entry at the  $i_g$ -th row and the  $i_{g'}$ -th column  
 202 is again  $\rho_{g'} \mu_{g'g} \nu_{g'}$ . With these matrix notations, and along that the product of the average viability and the average  
 203 reproductivity  $\nu_t \rho_{t-1} = \sum_{g \in \mathcal{G}_v} \sum_{g' \in \mathcal{G}} \rho_{g'} \mu_{g'g} \nu_{g'} \mathbb{P}[g(I_{t-1}) = g' | \Psi(I_{t-1})]$  since  $\sum_{g \in \mathcal{G}_v} \mathbb{P}[g(I_t) = g | \Psi(I_t)] = 1$ , the  
 204 master equation (4) therefore becomes

$$\mathbf{p}^{(t)} = \frac{\mathbf{T} \mathbf{p}^{(t-1)}}{\mathbf{1}^\top \mathbf{T} \mathbf{p}^{(t-1)} + \mathbf{1}^\top \mathbf{R} \mathbf{p}^{(t-1)}}, \quad (5)$$

205 where we use the notation  $\mathbf{1}^\top$  for the row vector of ones with the proper length.

206 The matrix  $\mathbf{T}$  plays a key role in the master equation (5), and it has a nice property that all its entries are  
 207 positive. Since  $\mathbf{T}$  corresponds to transition between GRNs  $g, g' \in \mathcal{G}_v \setminus \mathcal{G}_s$ , the relative reproductivity  $\rho_{g'}$  and the

208 viability  $\nu_g$  are both positive. Next, we must show that the mutation probability  $\mu_{g'g}$  is positive as well. Recall that,  
 209 when constructed through the pathway framework of GRNs, the subgraph of the genotype network induced by any  
 210 phenotypic constraint is connected (see section 2.3 and Appendix C). More formally, the connectedness among GRNs  
 211 constrained by a non-zero viability and reproductivity implies that, for any  $g, g' \in \mathcal{G}_v \setminus \mathcal{G}_s$ , there exists a sequence of  
 212 mutations which transforms  $g'$  to  $g$  through GRNs in  $\mathcal{G}_v \setminus \mathcal{G}_s$ . Since we presume that any single-locus mutation can  
 213 occur with a non-zero probability (recall from section 2.1), there is a non-zero chance for  $g'$  to mutate to  $g$  within  
 214 one generation<sup>2</sup>, i.e.,  $\mu_{g'g} > 0$ . As a result, we observe that  $\mathbf{T}$  is a positive matrix.

215 For the ease of presentation, we next show the convergence of equation (5) when the matrix  $\mathbf{T}$  is symmetric and  
 216 leave the proof for a non-symmetric  $\mathbf{T}$  in Appendix D. In this case, the eigenvectors  $\{\mathbf{v}_i\}_{i=1}^n$  of the symmetric matrix  
 217  $\mathbf{T}$  are linearly independent and form a basis of  $n$ -dimensional vectors, where  $n = |\mathcal{G}_v \setminus \mathcal{G}_s|$ . We order the eigenvectors  
 218 such that the magnitudes of their corresponding eigenvalues  $\{\lambda_i\}_{i=1}^n$  are non-increasing. The initial distribution can  
 219 then be rewritten as a linear combination of the eigenvectors of  $\mathbf{T}$

$$\mathbf{p}^{(0)} = \sum_{i=1}^n a_i \mathbf{v}_i. \quad (6)$$

220 In addition, because  $\mathbf{p}^{(t)}$  is proportional to  $\mathbf{T} \mathbf{p}^{(t-1)}$  for  $t > 0$ , we have  $\mathbf{p}^{(t-1)}$  proportional to  $\mathbf{T}^{t-1} \mathbf{p}^{(0)}$  and  
 221 consequently

$$\begin{aligned} \mathbf{p}^{(t)} &= \frac{\mathbf{T}^t \mathbf{p}^{(0)}}{\mathbf{1}^\top \mathbf{T}^t \mathbf{p}^{(0)} + \mathbf{1}^\top \mathbf{R} \mathbf{T}^{t-1} \mathbf{p}^{(0)}} \\ &= \frac{\sum_{i=1}^n a_i (\lambda_i)^t \mathbf{v}_i}{\sum_{i=1}^n a_i \left[ (\lambda_i)^t (\mathbf{1}^\top \mathbf{v}_i) + (\lambda_i)^{t-1} (\mathbf{1}^\top \mathbf{R} \mathbf{v}_i) \right]} \\ &= \frac{a_1 \mathbf{v}_1 + \sum_{i=2}^n a_i (\lambda_i/\lambda_1)^t \mathbf{v}_i}{a_1 \left( \mathbf{1}^\top \mathbf{v}_1 + \frac{\mathbf{1}^\top \mathbf{R} \mathbf{v}_1}{\lambda_1} \right) + \sum_{i=2}^n a_i \left[ (\lambda_i/\lambda_1)^t (\mathbf{1}^\top \mathbf{v}_i) + (\lambda_i/\lambda_1)^{t-1} \left( \frac{\mathbf{1}^\top \mathbf{R} \mathbf{v}_i}{\lambda_1} \right) \right]}, \end{aligned} \quad (7)$$

222 where  $\mathbf{v}_1$  and  $\lambda_1$  are the leading eigenvector and the leading eigenvalue of  $\mathbf{T}$  respectively. Since  $\mathbf{T}$  is a positive  
 223 matrix, by the Perron-Frobenius theorem, we have  $|\lambda_1| > |\lambda_i|$  for every  $i > 1$ , which guarantees the convergence of  
 224 equation (5)

$$\lim_{t \rightarrow \infty} \mathbf{p}^{(t)} = \frac{\mathbf{v}_1}{\mathbf{1}^\top \mathbf{v}_1 + \frac{\mathbf{1}^\top \mathbf{R} \mathbf{v}_1}{\lambda_1}}. \quad (8)$$

225 For a general and potentially non-symmetric matrix  $\mathbf{T}$ , we can first factor  $\mathbf{T}$  by its generalized eigenvectors and  
 226 its Jordan normal form and then an analogous derivation follows (see Appendix D). Therefore, under the pathway  
 227 framework of GRNs, the master equation (5) converges to a stationary distribution that is proportional to the leading  
 228 eigenvector of  $\mathbf{T}$ . Combined with the GRNs with a zero viability/reproductivity, whose probability to be observed  
 229 under the limit  $t \rightarrow \infty$  can be easily computed given (8), the stationary distribution of GRNs describes the balanced  
 230 scenario between selection, mutation, and reproduction.

### 231 3.2 Case Study: Binary Viability, Identical Reproductivity, and Independent Muta- 232 tion

233 We next turn to a case study to validate our predicted stationary distribution of GRNs. We will examine a more  
 234 specific version of the quasi-species model (4) with assumptions on the viability, reproduction, and mutation of a  
 235 GRN. First, a GRN  $g$  either always survives the selection or becomes inviable, i.e., it has a binary viability  $\nu_g \in \{0, 1\}$ .  
 236 It also implies that for any GRN  $g \in \mathcal{G}_v$  with a non-zero viability, we have  $\nu_g = 1$ .

<sup>2</sup>To be more precise, this argument is only valid when the joint probability for any combination of multiple single-locus mutations is non-zero per generation. Otherwise, we can modify the master equation (5) by extending the time scale from 1 to  $\Delta t$ , where  $\Delta t$  is the diameter of the subgraph of the genotype network constrained by a non-zero viability and reproductivity. The modified transition matrix is now proportional to  $\mathbf{T}^{\Delta t}$ , which is a positive matrix since mutation events at different generations are independent. Replacing  $\mathbf{T}$  by  $\mathbf{T}^{\Delta t}$  we have an analogous derivation to prove the convergence of the master equation.

237 Second, we assume that each GRN  $g \in \mathcal{G}$  produces the same number of offspring and there is no sexual selection.  
 238 Equivalently, the probability that an individual randomly sampled from an infinitely large offspring population is  
 239 reproduced by a viable parent with GRN  $g$  is a constant for any viable GRN  $g \in \mathcal{G}_v$ . We denote this uniform  
 240 reproductivity by  $\rho_g = \mathbb{P}[I_{t-1} \rightarrow I_t \mid g(I_{t-1}) = g, \Psi(I_{t-1})] = \rho$ , which is asserted to be non-zero.

241 Third, given the underlying collection of proteins  $\Omega$  and genes  $\Gamma$ , the per-generation occurrence of mutation at  
 242 every  $\gamma \in \Gamma$  is assumed an independent identically distributed Bernoulli random variable with a constant success  
 243 probability  $\mu$ . Moreover, if it occurs, a mutation at  $\gamma$  randomly changes  $\gamma$ 's expression behavior to any other pair  
 244 of protein activator/product encoded in  $\Omega$  with an equal probability. Under this assumption of independent and  
 245 uniform mutation, the per-generation probability that a GRN  $g'$  mutates to  $g$  becomes

$$\mu_{g'g} = \left( \frac{\mu}{|\alpha(\Omega)| - 1} \right)^{d(g',g)} (1 - \mu)^{|\Gamma| - d(g',g)}, \quad (9)$$

246 where we denote by  $\alpha(\Omega)$  the set of possible pairs of protein activator/product in  $\Omega$ , and  $d(g',g)$  is the number of  
 247 genes with different expression behavior between  $g'$  and  $g$ .

248 For this more specific model, we can rewrite the semi-transition matrix  $\mathbf{T}$  into a series

$$\mathbf{T} = \mathbf{T}_0 + \mathbf{T}_1 + \mathbf{T}_2 + \dots + \mathbf{T}_{|\Gamma|}, \quad (10)$$

249 where the entry at the  $i_g$ -th row and the  $i_{g'}$ -th column of matrix  $\mathbf{T}_k$  is  $\rho\mu_{g'g}$  if  $d(g',g) = k$  and 0 otherwise. Observe  
 250 that  $\mathbf{T}_0$  is proportional to the identity matrix  $\mathbf{I}$  (of a proper size), and  $\mathbf{T}_1$  is proportional to the adjacency matrix  
 251 of the neutral network of GRNs (see section 2.3), which we denoted by  $\mathbf{A}$ . Writing  $\tilde{\mathbf{T}}_k = \mathbf{T}_k / \mu^k$ , whose entries are  
 252 finite even for a zero per-generation, per-locus mutation probability  $\mu$ , equation (10) becomes

$$\mathbf{T} = \rho(1 - \mu)^{|\Gamma|} \mathbf{I} + \rho \left( \frac{\mu}{|\alpha(\Omega)| - 1} \right) (1 - \mu)^{|\Gamma| - 1} \mathbf{A} + \sum_{k=2}^{|\Gamma|} \mu^k \tilde{\mathbf{T}}_k. \quad (11)$$

253 We further consider the scenario that mutations are rare events, specifically, under the limit  $\mu \rightarrow 0$ . Since the  
 254 eigenvectors of  $\mathbf{I} + c\mathbf{A}$  are exactly the eigenvectors of  $\mathbf{A}$  for any scalar  $c$ , and  $\{\mathbf{T}_k\}_{k=1}^{|\Gamma|}$  are symmetric matrices because  
 255  $d(g',g) = d(g,g')$ , the theory of eigenvalue perturbation [54, 55] ensures that the leading eigenvector of  $\mathbf{T}$  converges  
 256 to the leading eigenvector<sup>3</sup> of  $\mathbf{A}$ :

$$\lim_{\mu \rightarrow 0} \mathbf{v}_1 = \mathbf{v}_1(\mathbf{A}). \quad (12)$$

257 From equation (8), we have

$$\lim_{\mu \rightarrow 0} \lim_{t \rightarrow \infty} \mathbf{p}^{(t)} = \frac{\mathbf{v}_1(\mathbf{A})}{\mathbf{1}^\top \mathbf{v}_1(\mathbf{A})}. \quad (13)$$

258 In network science, entries of the leading eigenvector of the adjacency matrix of a connected, undirected graph is  
 259 known as the eigenvector centrality [56, 57] of the nodes. As a result, under the assumptions of binary viability,  
 260 identical reproductivity, and rare, uniform mutation, *the probability distribution of viable GRNs converges to a*  
 261 *stationary distribution that is proportional to their eigenvector centrality in the neutral network.*

262 To validate the predicted probability distribution of GRNs under mutation-selection balance, we simulate the  
 263 evolution of  $10^7$  parallel populations. The simulations are parametrized with the constant sets of  $|\Gamma| = 4$  genes and  
 264  $|\Omega| = 6$  proteins. We further presume that two proteins can not be the product of any expression behavior, whose  
 265 presence state can only be stimulated externally and hereafter they are referred to the *input* proteins. We also presume  
 266 that two other proteins only have direct physiological effects and they can not serve as the activator of any expression  
 267 behavior, which we call the *output* proteins. Under this minimal setup, there are in total  $|\alpha(\Omega)| = 16$  potential pairs  
 268 of expression activator/product, which leads to  $|\mathcal{G}| = 65536$  plausible GRNs. We evolve the populations under  
 269 the environmental condition such that one of the input proteins is externally stimulated, and one of the output  
 270 proteins shows a fatal effect which is required absent for an individual's viability, resulting in  $|\mathcal{G}_v| = 45389$  possible  
 271 viable GRNs altogether. Our simulated GRNs are indeed small to avoid data scarcity in sampling the empirical  
 272 distribution since the number of possible GRNs grows super-exponentially with the number of genes and proteins.  
 273 We leave simulation of more realistic GRNs in future work.

<sup>3</sup>Here we abuse the notation  $\mathbf{v}_1(\mathbf{A})$  and  $\lambda_1(\mathbf{A})$  for the leading eigenvector and the leading eigenvalue of  $\mathbf{A}$  respectively.



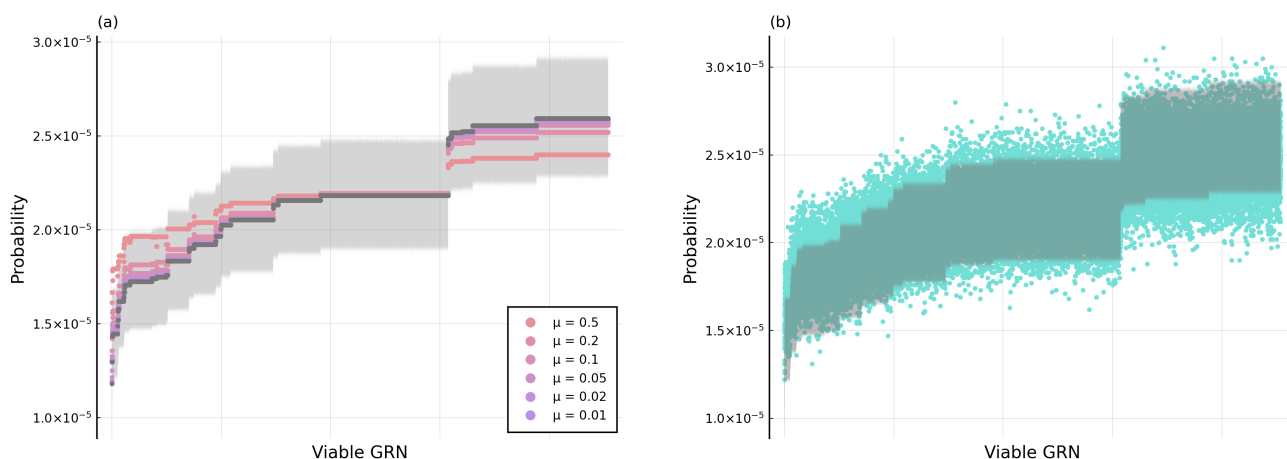


Figure 3: Validation that the evolutionary dynamics of GRNs converges to the derived stationary distribution (13). We compare the predicted stationary distribution of viable GRNs under the rare-mutation approximation with (a) the exact leading eigenvector of the transition matrix (11) with various per-locus mutation probability  $\mu$  (colored by a red-purple gradient from large to small), and (b) the distribution of GRNs sampled from their simulated evolutionary dynamics with  $\mu = 0.1$  (blue). The predicted distribution is colored in gray, and the shaded area shows its 95% confidence band that accounts for the uncertainty of finite-sized sampling in the simulations. In both panels, the viable GRNs are ordered increasingly by their predicted probability to be observed.

274 The evolution of parallel populations are simulated using a Wright-Fisher model [58]. Specifically, we fix a  
 275 number of 16 individuals for all populations, and given a current generation, the next generation is generated  
 276 through randomly choosing viable GRNs from the current generation without replacement followed by potential  
 277 mutations with a per-locus mutation probability  $\mu = 0.1$ . We begin with 10,000 different initial populations where  
 278 the GRN of every individual is chosen uniformly at random from all possibilities  $\mathcal{G}$ , and 1,000 lineages are evolved  
 279 from each initial population. Each of the  $10^7$  parallel populations are evolved for a constant number of generations,  
 280 from this ensemble of lineages we randomly sample a viable GRN to form the simulated distribution of GRNs. This  
 281 fixed length of evolution is determined through the temporal lower bound such that the resulting GRN distribution  
 282 is theoretically closed enough to the stationary distribution regarding to a given level of error tolerance (detailed in  
 283 Appendix E).

284 Moreover, in order to account for the uncertainty of finite-sized sampling in the simulated distribution, we  
 285 also draw the same number of  $10^7$  independent samples from the predicted distribution (13) to form an empirical  
 286 distribution. Repeating the sampling procedure 1,000 times, we obtain an ensemble of empirical distributions that  
 287 captures the effect of finite-sized sampling over the predicted probability that GRNs are to be observed. We further  
 288 use the averaged variation distance between the empirical distribution and the predicted distribution as the error  
 289 tolerance from which the number of generations to be simulated is calculated such that convergence of the model is  
 290 theoretically guaranteed (Appendix E).

291 In Figure 3a, we compare the exact, properly normalized leading vector of the transition matrix  $\mathbf{T}$  (11) along  
 292 with the predicted stationary distribution of viable GRNs under the rare-mutation approximation (13). Observe  
 293 that even a moderate per-locus mutation probability  $\mu$  leads to a GRN distribution well aligned with the predicted  
 294 one, especially, with respect to the uncertainty arising from finite-sized sampling in the simulations. Moreover,  
 295 Figure 3b shows the simulated distribution of viable GRNs after long-term evolution. We see that, despite a little  
 296 overdispersion, the simulated distribution agrees with the derived stationary distribution of GRNs. Direct comparison  
 297 between the simulated distribution and the exact solution, i.e., the leading eigenvector of the transition matrix  
 298 (11) shows no significant difference as well (see Supplementary Figure 5). Combined, our simulations indicate  
 299 computational evidence that, when viability is assumed rugged and mutations are rare, the topology of the neutral  
 300 network, particularly the eigenvector centrality of mega-nodes, serves as a informative predictor of the prevalence of  
 301 GRNs under mutation-selection balance.

	Env. 1	Env. 2	Env. 3	Env. 4	Env. 5	Env. 6	Env. 7
Stimuli	{1}	{1}	{1, 2}	{1}	{1}	{1, 2}	{1}
Essentials	$\emptyset$	$\emptyset$	$\emptyset$	{6}	{5, 6}	{6}	{5}
Fatals	{6}	{5, 6}	{6}	$\emptyset$	$\emptyset$	$\emptyset$	{6}

Table 1: Different environmental conditions specified by the sets of stimulated, essential, fatal proteins.

	No spare genes	No redundant genes	All genes activated	No direct selection
Group (i)				
Group (ii)	✓			
Group (iii)	✓	✓		
Group (iv)	✓		✓	
Group (v)	✓	✓	✓	
Group (vi)		✓		
Group (vii)				✓
Group (viii)		✓		✓

Table 2: Groups of GRNs by imposing constraints on their structural properties.

### 3.3 Prevalent GRNs under Mutation-Selection Balance

We now apply our prediction in the case study of binary viability, identical reproductivity, and rare mutation to further investigate the structure of GRNs that have a higher probability to be observed than others under different environmental conditions. Here we again consider GRNs with a constant collection of 6 proteins and 4 genes. In addition, for the ease of presentation, we label the genes by uppercase letter  $\Gamma = \{A, B, C, D\}$  and the proteins by numerals  $\Omega = \{1, 2, 3, 4, 5, 6\}$ , where protein 1 and 2 are the input proteins and protein 5 and 6 are the output proteins respectively (see section 3.2). Under the pathway framework of GRNs, an environment can be jointly described by a.) a set of stimuli proteins that are externally stimulated to be in the presence state, b.) a set of essential proteins whose absence state leads to inviability of the individual, and c.) a set of fatal proteins whose presence state also causes inviability. We will focus on seven distinct environments listed in Table 1 that showcase the scenarios of single versus multiple stimulated/essential/fatal proteins and their combinations.

For each of the focal environmental conditions, we examine the prevalent regulatory structure among various groups of GRNs. These groups consist of GRNs satisfying different constraints on their structural properties, which correspond to a few artificially enforced scenarios of interests. Here the focal topological constraints originate from patterns observed in the most prevalent GRNs, and progressively adding constraints offers a rough ranking of regulatory patterns for their inducing prevalence. We arrange groups of GRNs based on the following four constraints: First, GRNs with a gene of “spare” functionality are excluded, where the spareness of a gene refers to its negligible consequence on the resulting phenotype. This includes self-regulating genes due to the binary state assumption and genes that are activated by an input protein which is not externally stimulated or that produce an output protein without an essential/fatal effect under the given environment. Second, we exclude GRNs with multiple genes of the same, redundant expression behavior. Third, we only consider those GRNs where all the genes are functionally activated. This constraint mimics the scenario that genes with active expression behavior are more likely to be observed empirically than inactive ones. Forth, we exclude GRNs where a gene is directly activated by a stimulus and produces an essential protein to enforce selection on regulation rather than individual genes. Combinations of these four constraints lead to eight distinct groups where the prevalent GRNs are investigated (see Table 2).

In Figure 4, we plot the GRNs that have the largest predicted probability to be observed among the various groups and environments, i.e., the GRNs with the greatest eigenvector centrality in the neutral network under each scenario. Note that such GRNs may not be unique; in fact, one can find multiple alike GRNs through transformations that preserve their roles in the neutral network, e.g., exchanging the expression behavior of two genes  $A$  and  $B$ . Yet, these GRNs all share the common structural features, and we only show a random sample from the GRNs with the same, maximal probability to be observed in our prediction. Moreover, Figure 4 demonstrates the prevalent GRNs in both the representation of the pathway framework that manifests expression activator/product of each gene (labeled arrows between circles) and that of the conventional notion showing the regulation between genes (unlabeled arrows among rectangles).

A few intriguing observations arise from the prevalent regulatory structure in Figure 4. For the environmental

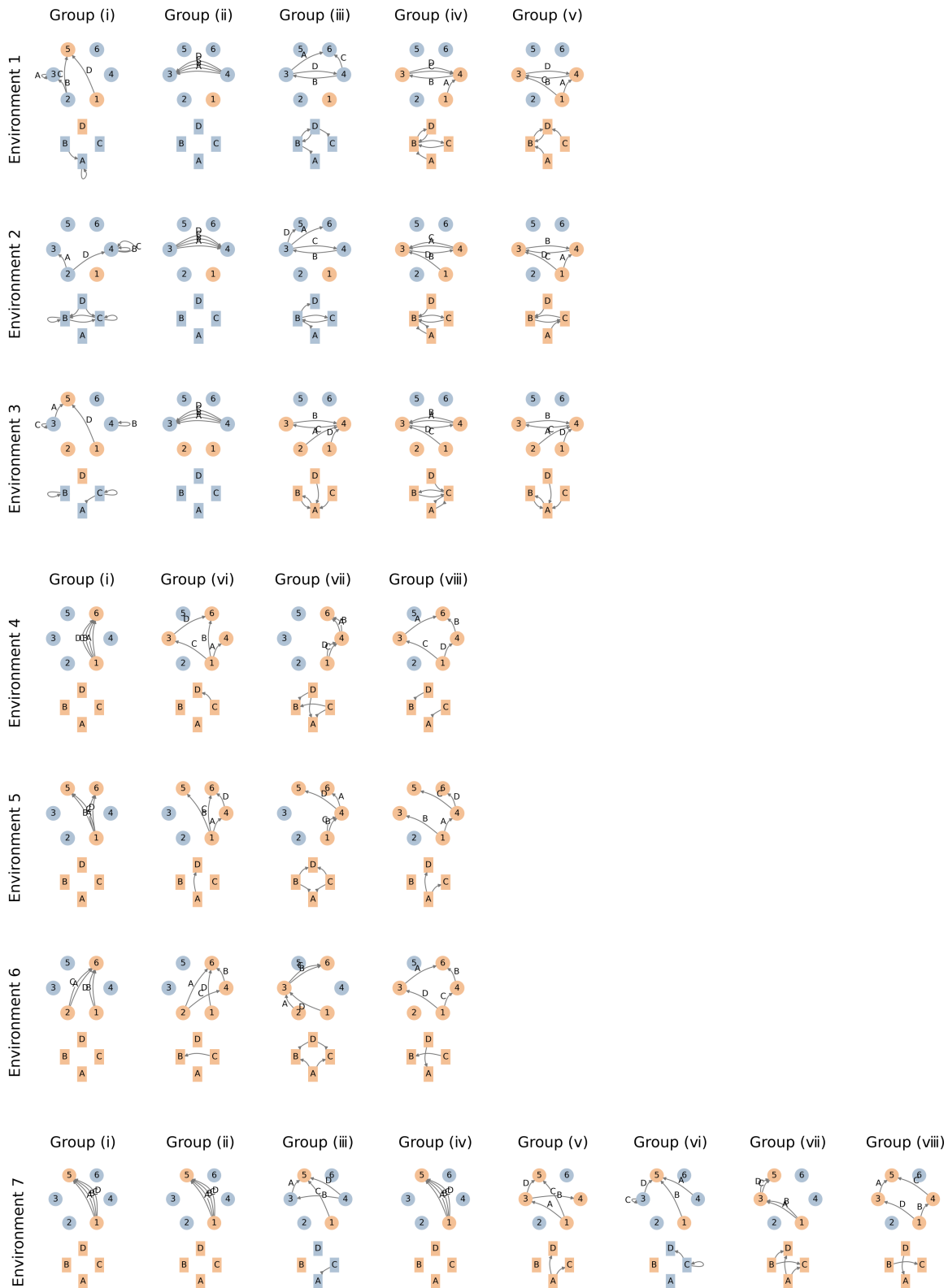


Figure 4: GRN that has the largest eigenvector centrality in the neutral network for different environmental conditions (Table 1) and among different constrained groups of GRNs (Table 2). For each prevalent GRN, its pathway framework representation is plotted by the circles and the labeled arrows, while its conventional representation is drawn through the rectangles and the unlabeled arrows. A node is colored in orange if the protein/gene is present/activated and in blue otherwise.

337 conditions where only the fatality of protein products is imposed (environment 1, 2, and 3), the GRNs with the largest  
338 probability to be realized under mutation-selection balance are the ones in which spare genes dominate (group (i)).  
339 Once constrained by the absence of the spare genetic functionality (group (ii)), we see prevalent GRNs favoring all  
340 genes sharing the same expression behavior which does not involve any stimulated or fatal proteins. If we further  
341 exclude redundant genes or enforce all genes to be activated (group (iii) and (iv) respectively), the prevalent GRNs  
342 demonstrate a structure which seemingly avoids expression activated by the stimulated protein or producing the  
343 fatal proteins as much as possible, and imposing both constraints leads to a similar outcome. Interestingly, for the  
344 environment with multiple stimuli and constraining on no spare and redundant genes (environment 3 and group  
345 (iii)), the functional activeness of all genes naturally emerges.

346 On the other hand, for the environmental conditions where only the essentiality of protein products is obligated  
347 (environment 4, 5, and 6), the most prevalent GRNs are the ones where several redundant genes are directly activated  
348 by a stimulus and produces an essential protein, and they are evenly split if multiple essential targets or stimuli  
349 exist (group (i)). When redundant genes are artificially excluded (group (vi)), the prevalent GRNs turn into a  
350 structure that manifests multiple pathways between the stimuli and the essential proteins. While constrained by  
351 no direct gene expression activated by a stimulus and producing an essential target (group (vii)), the prevalent  
352 GRNs similarly show multiple pathways yet each of which involves at least two genes, and these pathways share the  
353 same intermediate protein that serves as the product of one and the activator of another. Jointly imposing the two  
354 constraints mentioned above (group (viii)) results in the prevalent GRN structure that maintains multiple regulatory  
355 pathways and simultaneously triggers the presence state of the underlying proteins, if plausible. Notice that for these  
356 environmental condition, the prevalent GRNs take advantages of the functionality of every gene, and all the genes  
357 are activated.

358 Last but not least, for the environmental condition where both essential and fatal proteins exists (environment  
359 7), the most probable GRNs favor redundant genes that are directly activated by the stimulus and synthesize the  
360 essential target when the genetic redundancy is not constrained (group (i), (ii), and(iv)). Otherwise, the prevalent  
361 regulatory structure leaves one gene to maintain its essentiality, whereas others are capable to generate the essential  
362 protein but their activators remain absent (group (iii) and (vi)). If we further artificially require the activation of  
363 genes or exclude direct selection on individual genes (group (v), (vii), and (viii)), we begin to see multiple pathways  
364 in the prevalent GRNs.

365 We discover that most of the prevalent structures of GRNs in Figure 4 follow an intuitive pattern: these GRNs  
366 have the least plausible, subsequent inviable mutants under the diverse environmental conditions and structural  
367 constraints. When selection is enforced by the fatality of proteins, any regulatory pathway from the stimulus to the  
368 fatal protein is prohibited. The prevalent GRNs keep the fewest proteins in their presence state that can serve as the  
369 potential expression activators, since this minimizes the ways for subsequent mutations to create a lethal regulatory  
370 pathway. As a result, the mutation-selection balance drives the dominance of genes with a spare functionality, and  
371 secondly the appearance of redundant genes whose expression involves neither the stimulus nor the fatal protein. On  
372 the contrary, when selection acts through the essentiality of proteins, regulatory pathways from the stimulus to the  
373 essential target become critical for an individual's viability. The prevalent GRNs show the structure of redundant  
374 genes or multiple pathways such that the chance of eliminating the essential pathways through subsequent mutations  
375 is most mitigated. Therefore genes in these prevalent GRNs are expected to be functionally active. Moreover, even if  
376 a gene does not participate in an essential pathway, its expression behavior will involve the stimulus or the essential  
377 protein to potentially form a pathway with latent mutations. In the case where both the fatal and the essential target  
378 exist, the prevalent GRNs demonstrate structures as a superposition of the two patterns we previously discussed,  
379 which alternatively display the characteristics of the fatality-/essentiality-driven scenario under different structural  
380 constraints of gene regulation.

## 381 4 Discussion

382 In this work, we analyze the evolutionary dynamics of GRNs under a quasi-species model with selection, muta-  
383 tion, and asexual reproduction. Integrating with the pathway framework of GRNs that abstracts the alleles of  
384 genes through their expression behavior, we analytically show that the population dynamics always converges to  
385 a stationary distribution of GRNs given any arbitrary viability function and stochastic mutational transition as  
386 long as no mutation is prohibited. This stationary distribution characterizes the ensemble of regulatory circuits  
387 under mutation-selection balance, and it implicates the structural features of GRNs to be predicted favorable under  
388 long-term evolution. Next, we investigate a case study assuming binary viability, identical reproductivity and rare

389 mutation, and find that the stationary distribution of GRNs can be derived from the topology of the genotype net-  
390 work. Specifically, the probability to observe a GRN under mutation-selection balance is proportional to the GRN's  
391 eigenvector centrality in the neutral network, which is a subgraph of the genotype network consisting of all viable  
392 GRNs.

393 We advocate that our contribution to the theory of evolutionary dynamics provides a mechanistic explanation  
394 for the key assumption of irreducible transition matrices in existing literature [45, 46, 47]. As we mentioned in the  
395 Introduction, Moran relates this assumption, which leads to the global convergence to a single quasi-species, to the  
396 scenario that viable genotypes are mutually accessible through mutations [48]. In a similar spirit, a recent review  
397 [53] concludes that a population genetic model situating on a genotype network always evolves into a stationary  
398 solution once we retreat to the regime of non-zero fitnesses. Our work takes an alternative route to recover the  
399 same assumption; instead of the absent knowledge between genotypes and their fitnesses, we consider a minimal  
400 modeling framework of how genotypes develop into phenotypes via the mechanisms of gene regulation [37]. Despite  
401 the simplicity of this modeling framework, when encapsulating the genotype-phenotype mapping through GRNs, the  
402 mutational accessibility between genotypes with non-zero fitnesses naturally emerges due to the high dimensionality  
403 of GRNs. This result relaxes the global convergence in quasi-species theory to the cases with extreme fitness values  
404 such as a holey adaptive landscape [59].

405 Our derivation sheds light on how we may interpret the prevalence of GRNs under rare mutation and strong  
406 selection on the resulting phenotypic functionality. When first introduced [56], the eigenvector centrality was designed  
407 to capture an individual's global "importance" as measured by their social ties in a communication network. In  
408 particular, the eigenvector centrality is computed based on the idea that a node's importance is proportional to the  
409 sum of its neighbors' importance scores. This interpretation is nicely translated to the content of the neutral network  
410 of regulatory circuits: Under mutation-selection balance, our derivation predicts that the probability to observe a  
411 GRN is proportional to the total likelihood to find its viable, mutational neighbors in the population. Intriguingly,  
412 the interpretation of eigenvector centrality leads to some emerging concept of robustness [60], where the prevalence  
413 of a GRN is not only due to its selective advantage but also the overall prevalence of its mutational neighboring  
414 GRNs.

415 Moreover, the observed prevalent structures of GRNs in our analyses also provides a possible alternative expla-  
416 nation for evolutionary robustness. We inductively find that these prevalent regulatory structures follow the same  
417 pattern to achieve a minimal number of plausible inviable mutants (see section 3.3). Since the genotype network is  
418 a regular graph under the pathway framework of GRNs (recalling from section 2.3), i.e., every GRN has the same  
419 amount of mutational neighbors, minimizing the number of inviable mutants optimally increases the viable mutants  
420 for a GRN. In other words, the observed prevalent GRNs under various environmental conditions appear to show  
421 the regulatory structures with the maximal number of neighbors in the neutral network, and indeed the degree of  
422 a node is known to be strongly correlated with its eigenvector centrality in the network science literature [57]. We  
423 emphasize that these concepts of robustness naturally emerge from the mechanistic, quasi-species model of GRN  
424 evolution rather than an *a priori* assumption about prevalent regulatory circuits.

425 Previous work often focused on relating the topological features of genotype networks to evolutionary processes of  
426 interest. For example, evolvability has been approximated by the size of the genotype network of a given phenotype  
427 [61], as well as the number of "neighboring" phenotypes inferred from the genotype network [62]. Robustness has  
428 been modeled as the node degree in the genotype network [62], and [63] adopted the average path length in the  
429 genotype network as a proxy for genetic heterogeneity. To our best knowledge, Van Nimwegen et al. was the first  
430 to bridge between the asymptotic abundance of different genotypes under a population genetic model and their  
431 eigenvector centrality in the neutral network [60]. Our case study resonates Van Nimwegen et al.'s conclusion and  
432 differentiates a quasi-species perspective from a model lacking of genetic variation in a population. For instance, if a  
433 population fixes a single genotype at all time and its evolution is modeled as a random walk on the neutral network,  
434 network science guarantees the fixation probability at a given genotype to be proportional to its *degree* instead of  
435 the eigenvector centrality in the neutral network [57].

436 The current scope of the work presented here is not without a few noteworthy limitations. First, we assume a  
437 constant, static surrounding in which the population evolves, whereas populations certainly experience shifting or  
438 alternating environmental conditions [64, 65, 66]. Second, our model mainly focused on the joint forces of selection  
439 and mutation. Although this simple model can indeed be extended through more sophisticated mechanisms known  
440 to play a role in evolutionary dynamics such as recombination [67, 68], gene duplication [69, 70], and demographic  
441 information [71], we leave such extensions—along with their possible implications—to future work. Third, when the  
442 time scale of environmental changes is much faster than that of the evolutionary dynamics (see Appendix E), the  
443 transient constitution of GRNs in a population shall acquire more attention than their stationary distribution at



444 mutation-selection balance [72, 73, 74]. Put simply, it remains an open question whether real-world populations  
445 should ever be conceptualized as at equilibrium (even dynamic) as opposed to existing in some far-from equilibrium  
446 state [75]. As a result, further investigation should focus on the transient distributions and/or trajectories of GRNs  
447 under various population genetic models. Finally, despite confirmation between the derived stationary distribution of  
448 GRNs in an infinitely large population and the long-term numerical simulations, we also find that a finite population  
449 size moderately influences the transient evolutionary dynamics. Developing a richer understanding of the role drift  
450 plays in structuring the evolution of GRNs is an important extension of our work.

451 The observed structure of molecular interaction networks is a result of myriad evolutionary forces. By analyzing  
452 such topologies using a network-science approach, it may be possible to construct a mechanistic theory for how  
453 evolution shapes and is constrained by higher-order interactions. Across a broad scope of genotype/neutral networks—  
454 with applications ranging from RNA sequences to metabolic reactions—our work rigorously shows that the neutral  
455 network of GRNs must be connected (in agreement with existing computational work [76]) and that the relative  
456 frequency at equilibrium of various GRNs can be predicted from first principles. Therefore, our work connects  
457 the evolutionary forces/mechanisms embedded in a population genetic model with the accordingly favorable GRN  
458 structure through the topology of the neutral network. Clearly, our predicted prevalent regulatory structures under  
459 mutation-selection balance may not capture all the features in empirical GRNs [77, 78]; however, we establish a null  
460 expectation for how GRNs are shaped by mutations and selection [26, 27]. Critically, this null expectation appears to  
461 recapitulate many of the topological features of molecular interaction networks currently associated with evolvability  
462 and robustness. Perhaps, more broadly speaking, the emergence of complex fitness landscapes can result from simple  
463 evolutionary rules.

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467 Hold.

## 468 Conflicts of Interest

469 The authors declare no competing interests exist.

## 470 Data Availability

471 The authors affirm that all data necessary for confirming the conclusions of the article are present within the article,  
472 figures, and tables.

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## 473 Appendices

### 474 A Mathematical Formulation of the Pathway Framework of GRNs

475 In this study, we assume there is a constant set of proteins that can *possibly* appear in the organisms. We clarify  
476 that this constant collection is not necessarily the proteins which we have observed in the certain species to date;  
477 contrarily, these proteins are the plausible options of the activators and products of gene expression, and they are  
478 better acknowledged as all (or a reasonable subset of) the proteins under our awareness. We will refer to the *state*  
479 over this protein set for their actual appearance in the organisms, with a more detailed discussion later.

480 We furthermore divide the constant set of proteins into three categories: *input proteins* that can only be supplied  
481 through external stimuli but not through any internal gene expression, *output proteins* that are products of gene  
482 expression which affect physiological traits of the organisms but can not serve as activators of gene expression, and  
483 the remaining *internal proteins* with neither constraints. The input and internal proteins form the set of plausible  
484 activators for gene expression, whereas the internal and the output proteins become the set of products. This  
485 completes the underlying backbone of GRNs under the pathway framework.

486 **Definition A.1.** We denote by  $\Omega_s$  and  $\Omega_t$  be the fixed underlying **activator set** and **product set** of gene expression  
487 respectively. And we call their union  $\Omega = \Omega_s \cup \Omega_t$  the underlying **protein set**.

488 The three categories of proteins can be recovered easily from the notion of expression activators and products.  
489 In particular, the input, output, and internal proteins are  $\Omega - \Omega_t$ ,  $\Omega - \Omega_s$ , and  $\Omega_s \cap \Omega_t$  respectively.

490 With a pre-specified underlying backbone  $(\Omega_s, \Omega_t)$  of the regulatory structures, a gene regulatory network is  
491 a graphical abstraction of the expression behavior for the whole genotype. We will assume that the collection of  
492 genes of the organisms remains the same over evolutionary time, i.e., there is no duplication and deletion of the  
493 loci. A GRN is then uniquely determined by the activator and the product of every gene, and we have the following  
494 formulation:

495 **Definition A.2.** Denote by  $\Gamma$  the fixed set of genes, or the **gene set**. We define a **gene regulatory network**  
496 (**GRN**) as a mapping  $g : \Gamma \rightarrow \Omega_s \times \Omega_t$ . We further denote by  $\mathcal{G}$  the set of all such gene regulatory networks.

497 Definition A.2 may seem an unusual way to describe a network. To illustrate that the definition is appropriate,  
498 recall that a directed edge in a GRN under the pathway framework represents the input-output relation of a gene's  
499 expression. Every edge in the GRN is thus labeled by the gene whose allelic content is abstracted as the edge. With  
500 the given protein set  $\Omega$  as nodes, the GRN can be described as its edgelist representation — a table where each  
501 row stands for an edge (and its corresponding gene) and the two columns entails its source and target (i.e., the  
502 corresponding activator and protein product respectively). This table, and therefore the GRN, is equivalent to a  
503 mapping  $g$  from the finite set of genes  $\Gamma$  to the pairs of activators and products  $\Omega_s \times \Omega_t$ , where  $g(\gamma)$  is the expression  
504 input-output pair of gene  $\gamma \in \Gamma$ .

505 We also have the notion of projection from the input-output relations of a genotype. For any gene  $\gamma \in \Gamma$ , these  
506 projections explicitly point to its activator protein  $s_g(\gamma)$  and its protein product  $t_g(\gamma)$ :

507 **Definition A.3.** The **activator projection** and the **product projection** of a gene regulatory network  $g$  are  
508 defined by  $s_g = p_s \circ g$  and  $t_g = p_t \circ g$ , where  $p_s$  and  $p_t$  are the set projection from  $\Omega_s \times \Omega_t$  onto  $\Omega_s$  and  $\Omega_t$   
509 respectively.

510 We next introduce how the two evolutionary forces we will consider in a population genetic model of GRNs,  
511 mutation and selection, can fit into the pathway framework.

512 Mutating the allele of a gene can alter the expression behavior of the gene. Since under the pathway framework  
513 a genotype is conceptualized as a GRN on the expression functional level, we will model mutation to be changing  
514 the input-output relation of gene expression. Specifically, a mutation randomly rewires a single edge in the GRN  
515 and results in a mutant GRN. Equivalently we can find all the possible mutants, namely, those only differ by one  
516 input-output pair from the original GRN, and a mutation can be defined as a random process over the mutants.

517 **Definition A.4.** Let  $g_1, g_2$  be two gene regulatory networks. The set of genes with different alleles between  $g_1$  and  
518  $g_2$  is

$$\Delta(g_1, g_2) = \{\gamma \in \Gamma \mid g_1(\gamma) \neq g_2(\gamma)\} \quad (14)$$

519 The **edit distance** between  $g_1$  and  $g_2$  is defined by  $d(g_1, g_2) = |\Delta(g_1, g_2)|$ .

520 **Definition A.5.** Let  $g$  be a gene regulatory network. We denote the set of **mutants** from  $g$  by

$$N(g) = \{g' \in \mathcal{G} \mid d(g, g') = 1\} \quad (15)$$

521 i.e., those gene regulatory networks that are 1-edit-distant from  $g$ .

522 **Definition A.6.** A **mutation** of gene regulatory network  $g$  is a random process with a probability measure over its  
523 mutants  $N(g)$ , which we denote by  $\mu_g$ .

524 On the other hand, natural selection can be regarded as a phenotypic response to the surrounding environment,  
525 where the phenotype is derived from the genotype. We presume that the physiological traits of an organism are  
526 uniquely determined by the actual appearance of proteins within it, and that they are conditionally independent of  
527 the external environments. The phenotype is thus the collective state over the underlying protein set  $\Omega$ . And this  
528 collective state is the outcome of external environmental stimuli and internal chemical signals propagating on the  
529 gene regulatory networks.

530 For simplicity we adapt the chemical state of proteins to be binary, i.e., that a protein is present in the organism  
531 versus that it is absent. Additionally, assuming that the environmental condition directly triggers the presence state  
532 of some proteins, the binary state of a protein is determined by its reachability from those stimulated ones on the  
533 GRN. We let the set of proteins with the presence state to represent the phenotype derived from the GRN:

534 **Definition A.7.** Let a  $g$  be a gene regulatory network, and let  $\Omega_0 \subset \Omega - \Omega_t$  be the set of environmentally stimulated  
535 proteins. The **phenotype** of  $g$  is the function  $\chi_g : \mathcal{P}(\Omega - \Omega_t) \rightarrow \mathcal{P}(\Omega)^4$ , where for any protein  $\omega \in \Omega$ ,  $\omega \in \chi_g(\Omega_0)$  if  
536 and only if there exists a sequence of genes  $\{\gamma_i \in \Gamma\}_{i=1}^k$  such that  $t_g(\gamma_k) = \omega$ ,  $s_g(\gamma_{i+1}) = t_g(\gamma_i)$  for  $i = 1, 2, \dots, k-1$ ,  
537 and  $s_g(\gamma_1) \in \Omega_0$ .

538 The phenotypic response to the environmental condition, or namely the individual viability under natural selec-  
539 tion, becomes a function of the collective binary state of the underlying proteins  $\Omega$ . We again for simplicity adapt the  
540 viability to be the binary variable that whether the individual organism survives or not. Moreover, we suppose that  
541 this binary viability solely depends on two collections of proteins: those proteins which are essential for the organism  
542 to survive, and those having fatal effects to the organism. The selective environment is then explicitly specified by  
543 the sets of stimulated, essential, and fatal proteins respectively. We describe the outcome of selection through the  
544 viable GRNs, i.e., those with which a organism will survive natural selection:

545 **Definition A.8.** Let  $\Omega_0 \subset \Omega - \Omega_t$  and  $\Omega_+, \Omega_- \subset \Omega - \Omega_s$  be the stimulated, essential, and fatal proteins in the  
546 environmental condition respectively. The selective environment, or simply **selection**, is the triplet  $\mathbb{S} = (\Omega_0, \Omega_+, \Omega_-)$ .  
547 We define the set of **viable** gene regulatory networks under selection  $\mathbb{S}$  by

$$\mathcal{G}_{\mathbb{S}} = \{g \in \mathcal{G} \mid \Omega_+ \subset \chi_g(\Omega_0), \Omega_- \subset \Omega - \chi_g(\Omega_0)\} \quad (16)$$

548 Notice that we have implicitly exerted the assumption that the stimulated proteins must be a subset of the input  
549 proteins, and the essential and fatal proteins must be a subset of the output proteins (recall Definition A.1).

## 550 B Formal Definition of the Genotype Network and the Neutral Net- 551 work of GRNs

552 With the constant sets of activators  $\Omega_s$ , products  $\Omega_t$ , and genes  $\Gamma$ , and the pre-determined sets of stimulated proteins  
553  $\Omega_0$ , essential proteins  $\Omega_+$ , and fatal proteins  $\Omega_-$ , we have the following definitions:

554 **Definition B.1.** Recall from Definition A.2 and A.5 that  $\mathcal{G} = \{g : \Gamma \rightarrow \Omega_s \times \Omega_t\}$  is the set of all plausible gene  
555 regulatory networks, and  $N(g)$  are mutants from gene regulatory network  $g$ . The **genotype network** is a graph  $G$   
556 whose nodes  $V(G) = \mathcal{G}$  and whose edges  $E(G) = \{(g, g') \in \mathcal{G} \times \mathcal{G} \mid g' \in N(g)\}$ .

557 **Definition B.2.** Let  $\mathbb{S} = (\Omega_0, \Omega_+, \Omega_-)$  be a given selection, and recall from Definition A.8 that  $\mathcal{G}_{\mathbb{S}}$  is the set of viable  
558 gene regulatory networks under  $\mathbb{S}$ . The **neutral network** subjected to  $\mathbb{S}$  is a graph  $G_{\mathbb{S}}$  whose nodes  $V(G_{\mathbb{S}}) = \mathcal{G}_{\mathbb{S}}$   
559 and whose edges  $E(G_{\mathbb{S}}) = \{(g, g') \in \mathcal{G}_{\mathbb{S}} \times \mathcal{G}_{\mathbb{S}} \mid g' \in N(g)\}$ . Note that  $G_{\mathbb{S}}$  is the induced subgraph of the genotype  
560 network  $G$  on nodes  $\mathcal{G}_{\mathbb{S}}$ .

<sup>4</sup>We denote by  $\mathcal{P}(S)$  the *power set* of a set  $S$ , which is the set of all possible subsets of  $S$ .

## C Structural Properties of the Genotype Network and the Neutral Network of GRNs

We begin with analyzing the structural properties of the genotype/neutral network of GRNs under the pathway framework, as well as highlighting those that are relevant to deriving the stationary distribution in the generalized population genetic model. First of all, the genotype network  $G$  shows an intuitive and nicely ordered structure. Since the mega-nodes in  $G$  consist of all the plausible GRNs  $\mathcal{G}$  given the constant activators  $\Omega_s$ , products  $\Omega_t$ , and genes  $\Gamma$ , every mega-node is equivalent to a tuple of  $|\Gamma|$  entries, each of which takes a discrete value from  $\Omega_s \times \Omega_t$ . Two mega-nodes/GRNs are connected in  $G$  if and only if they differ by the allele of a single gene, namely that the two corresponding tuple only differ by one entry, and as a result, the genotype network  $G$  is essentially a high-dimensional lattice.

The lattice-like nature of the genotype network  $G$  implies several structural properties. The genotype network  $G$  must be a connected graph, which agrees with the intuition that any two genotypes (at least on their gene expression level, i.e., the GRNs) can be mutually reached by a sequence of mutations under zero selection pressure. In addition, the distance between two GRNs  $g_1$  and  $g_2$  in  $G$  is, recalling from Definition A.4, exactly their edit distance  $d(g_1, g_2)$  because the shortest paths correspond to the scenarios to mutate the genes with different alleles  $\Delta(g_1, g_2)$  sequentially. Furthermore, we also see that any GRN has the same number of mutational neighbors in  $G$ :

**Lemma C.1.** *The genotype network  $G$  is a regular graph.*

*Proof.* Given an arbitrary gene regulatory network  $g \in \mathcal{G}$  and for any gene  $\gamma \in \Gamma$ , there are  $|\Omega_s \times \Omega_t| - 1$  other gene regulatory networks that only differ from  $g$  by the allele at  $\gamma$ . The number of mutants is

$$|N(g)| = |\Gamma| (|\Omega_s \times \Omega_t| - 1) \quad (17)$$

for any gene regulatory network  $g \in \mathcal{G}$ , and hence every mega-node in  $G$  has the same degree.  $\square$

On the other hand, although the neutral network  $G_{\mathbb{S}}$  subjected to a pre-determined selection  $\mathbb{S} = (\Omega_0, \Omega_+, \Omega_-)$  is a subgraph of the genotype network  $G$  (see Definition A.8), its structure is more disordered. There is no guarantee that  $G_{\mathbb{S}}$  is regular, and in fact one can easily find some counter-examples (e.g., see Figure ). The distance between two GRNs in  $G$  may not be preserved in  $G_{\mathbb{S}}$  either. For example, consider the case where  $\Omega_s = \{1, 2, 3\}$ ,  $\Omega_t = \{2, 3, 4\}$ ,  $\Gamma = \{a, b\}$ ,  $\Omega_0 = \{1\}$ ,  $\Omega_+ = \{4\}$  and  $\Omega_- = \emptyset$ , and two GRNs  $g_1$  and  $g_2$  such that  $g_1(a) = (1, 2)$ ,  $g_1(b) = (2, 4)$ ,  $g_2(a) = (1, 3)$  and  $g_2(b) = (3, 4)$ . In the genotype network  $G$ , there are two length-2 paths between  $g_1$  and  $g_2$ , either through GRN  $g_3$  or  $g_4$  where  $g_3(a) = g_2(a)$ ,  $g_3(b) = g_1(b)$ ,  $g_4(a) = g_1(a)$  and  $g_4(b) = g_2(b)$ . However, neither  $g_3$  nor  $g_4$  satisfy the selection criterion, and thus they are excluded from the neutral network  $G_{\mathbb{S}}$ , in which the distance between  $g_1$  and  $g_2$  is greater than 2.

Nevertheless, it turns out that, in most scenarios, any two GRNs are mutually reachable through some mutational trajectory in the neutral network  $G_{\mathbb{S}}$ :

**Lemma C.2.** *If  $|\Gamma| > |\Omega_+|$ , then the neutral network  $G_{\mathbb{S}}$  under selection  $\mathbb{S} = (\Omega_0, \Omega_+, \Omega_-)$  is a connected graph.*

*Proof.* To show that  $G_{\mathbb{S}}$  is a connected graph, our strategy follows: For any two viable gene regulatory networks  $g_s, g_t \in V(G_{\mathbb{S}}) = \mathcal{G}_{\mathbb{S}}$ , we will find a sequence of viable GRNs  $g_s = g_0, g_1, \dots, g_{k-1}, g_k = g_t \in \mathcal{G}_{\mathbb{S}}$  that form a mutational trajectory from  $g_s$  to  $g_t$ , i.e.,  $(g_{i-1}, g_i) \in E(G_{\mathbb{S}})$  for every  $i = 1, 2, \dots, k$ . More specifically, we will uncover the sequence of mutations through a few general “steps” of edge rewiring in GRNs and ensure that two invariants hold in each of these steps:

- (I) There is a path from the stimulated proteins  $\Omega_0$  to each of the essential proteins  $\Omega_+$  in the GRNs.
- (II) There is no path from the stimulated proteins  $\Omega_0$  to any of the fatal proteins  $\Omega_-$  in the GRNs.

Here we would like to introduce a few notations for the ease to illustrate the edge-rewiring steps in the GRNs. First, we put the genes into different groups with respect to  $g_s$  and  $g_t$ . Let  $\Gamma_s^{(+)} = \{\gamma \in \Gamma \mid t_{g_s} \in \Omega_+\}$  and  $\Gamma_t^{(+)} = \{\gamma \in \Gamma \mid t_{g_t} \in \Omega_+\}$  be the genes that directly produce the essential proteins in  $g_s$  and  $g_t$  respectively. Similarly, let  $\Gamma_s^{(-)} = \{\gamma \in \Gamma \mid t_{g_s} \in \Omega_-\}$  and  $\Gamma_t^{(-)} = \{\gamma \in \Gamma \mid t_{g_t} \in \Omega_-\}$  be the genes that directly produce the fatal proteins in  $g_s$  and  $g_t$  respectively. Graphically, these genes correspond to the incoming incident edges of either  $\Omega_+$  or  $\Omega_-$ . In

605 addition, denote by  $\Pi_g(u, v)$  the set of genes that are involved in paths from protein  $u$  to protein  $v$  in the GRN  $g$ , and  
 606 let  $\Pi_s = \{\gamma \in \Pi_{g_s}(u, v) \mid u \in \Omega_0, v \in \Omega_+\}$  and  $\Pi_t = \{\gamma \in \Pi_{g_t}(u, v) \mid u \in \Omega_0, v \in \Omega_+\}$  be the genes that are involved  
 607 in pathways from  $\Omega_0$  to  $\Omega_+$  in  $g_s$  and  $g_t$  respectively.

608 Second, when  $\Gamma \setminus \Pi_s$  or  $\Gamma \setminus \Pi_t$  is non-empty, there exists some “safe” allele among all the plausible allelic contents  
 609  $\Omega_s \times \Omega_t$ . We will denote such a tuple as  $\alpha$ . If  $\Omega_s \cap \Omega_t$  is non-empty, then there is a protein  $\omega'$  that can serve both an  
 610 activator and a product, and we will take  $\alpha = (\omega', \omega')$ . On the other hand, if  $\Omega_s \cap \Omega_t = \emptyset$ , we must have a non-empty  
 611  $\Omega_s \setminus \Omega_0$ , otherwise  $\Gamma \setminus \Pi_s = \Gamma \setminus \Pi_t = \emptyset$ . Hence there is a protein  $u' \in \Omega_s \setminus \Omega_0$  of the absence state, and we will take  
 612  $\alpha = (u', v')$  for some  $v' \in \Omega_-$ . Note that the allele  $\alpha$  is said “safe” in the sense that introducing  $\alpha$  will never break  
 613 invariant (II).

614 Now we state in details the five steps of edge rewiring that mutate  $g_s$  into  $g_t$  through viable GRNs:

- 615 1. Rewire edges (alleles of genes) in  $g_s$  to generate a viable GRN  $g'_1$  such that  $g'_1(\gamma) = \alpha$  for any gene  $\gamma \in \Gamma_s^{(-)}$  and  
 616  $g'_1(\gamma) = g_s(\gamma)$  for  $\gamma \notin \Gamma_s^{(-)}$ . During this rewiring process, invariant (I) holds since the alleles of  $\Pi_s \subset \Gamma \setminus \Gamma_s^{(-)}$   
 617 remain unchanged, and invariant (II) holds because this step simply introduces the safe allele  $\alpha$ .
- 618 2. Rewire edges in  $g'_1$  to generate another viable GRN  $g'_2$  such that  $g'_2(\gamma) = (u, t_{g_s}(\gamma))$  for any gene  $\gamma \in \Gamma_s^{(+)}$  and  
 619 some  $u \in \Omega_0$ , and  $g'_2(\gamma) = g'_1(\gamma)$  for  $\gamma \notin \Gamma_s^{(+)}$ . Since this step only creates length-1 pathways from  $\Omega_0$  and  $\Omega_+$ ,  
 620 both invariant (I) and (II) are guaranteed.
- 621 3. Rewire edges in  $g'_2$  to generate another viable GRN  $g'_3$  such that  $g'_3(\gamma) = g_t(\gamma)$  for any gene  $\gamma \notin \Gamma_s^{(+)} \cup \Gamma_t^{(-)}$   
 622 and  $g'_3(\gamma) = g'_2(\gamma)$  for  $\gamma \in \Gamma_s^{(+)} \cup \Gamma_t^{(-)}$ . Invariant (I) holds because the length-1 pathways introduced in Step 2  
 623 remain unchanged. Since in  $g'_1$  (and thus  $g'_2$ ) proteins  $\Omega_-$  have no incoming incident edges, and no rewiring  
 624 leads to an incoming edge of  $\Omega_-$  in this step, invariant (II) is also ensured.
- 625 4. Rewire edges in  $g'_3$  to generate another viable GRN  $g'_4$  such that  $g'_4(\gamma) = g_t(\gamma)$  for any gene  $\gamma \in \Gamma_s^{(+)} \setminus \Gamma_t^{(-)}$   
 626 and  $g'_4(\gamma) = g'_3(\gamma)$  for  $\gamma \notin \Gamma_s^{(+)} \setminus \Gamma_t^{(-)}$ . In particular, for a gene  $\gamma \in \Gamma_s^{(+)} \cap \Gamma_t^{(-)}$ , the rewiring process can be  
 627 achieved via an intermediate gene  $\gamma' \in \Gamma$  due to the pre-condition that  $|\Gamma| > |\Omega_+|^5$ . Since for each  $\gamma \in \Pi_t \setminus \Gamma_s^{(+)}$ ,  
 628 Step 3 has properly rewired its edge/allele to  $g'_3(\gamma) = g_t(\gamma)$ , the essential pathways formed by  $\Pi_t$  are gradually  
 629 completed throughout the process of rewiring edges corresponding to  $\Gamma_s^{(+)} \setminus \Gamma_t^{(-)}$ . And similar to Step 3, no  
 630 edge is rewired to be an incoming edge of  $\Omega_-$  in this step, and thus invariant (II) holds as well.
- 631 5. Rewiring edges corresponding to  $\Gamma_t^{(-)}$  in  $g'_4$  to generate  $g_t$  completes the viable mutational trajectory. Because  
 632 edges of  $\Pi_t \subset \Gamma \setminus \Gamma_t^{(-)}$  remain unchanged in this step, invariant (I) still holds. Furthermore, since  $g_t$  is viable,  
 633 rewiring edges of  $\Gamma_t^{(-)}$  also preserves invariant (II).

634 □

635 Note that since for a GRN to satisfy the selection criterion, every protein in  $\Omega_+$  must be produced by a gene,  
 636 so we must have  $|\Gamma| \geq |\Omega_+|$ . As a result, the only case that Lemma C.2 has excluded is that of  $|\Gamma| = |\Omega_+|$ , where  
 637 the GRNs form  $|\Omega_+|!$  components in the neutral network, each of size  $|\Omega_0|^{|\Omega_+|}$ . Moreover, the proof we provide here  
 638 is general enough such that Lemma C.2 holds even if one adapts additional constraints and defines a mutation as  
 639 changing either the protein activator or the protein product of a gene but not both.

## 640 D Convergence to a Stationary Distribution with a Non-symmetric 641 Transition Matrix

642 Here we show a general, analogous proof for (8) in the case that the semi-transition matrix  $\mathbf{T}$  is non-symmetric.  
 643 Any square matrix can be factored by its general eigenvectors and its Jordan normal form. In particular, we have

<sup>5</sup>Here such a rewiring process through an intermediate gene is necessary for most scenarios. Specifically, in the case where  $t_{g_s}(\gamma) \neq t_{g_t}(\gamma)$ , directly rewiring  $g'_3(\gamma)$  to  $g'_4(\gamma)$  may break the essential pathway to  $t_{g_s}(\gamma)$ . One can alternatively find a gene  $\gamma'$  whose allele does not produce  $t_{g_s}(\gamma)$  or  $t_{g_t}(\gamma)$  in  $g'_3$ , where the existence of  $\gamma'$  is guaranteed since  $|\Gamma| > |\Omega_+|$ . Rewiring  $g'_3(\gamma')$  to  $\tau = (u, t_{g_s}(\gamma))$  for some  $u \in \Omega_0$ , applying the direct rewiring between  $g'_3(\gamma)$  and  $g'_4(\gamma)$ , and then rewiring  $\tau$  back to  $g'_3(\gamma')$  avoids the potential break of the essential pathways.

644  $\mathbf{T} = \mathbf{P}\mathbf{J}\mathbf{P}^{-1}$  (or equivalently  $\mathbf{TP} = \mathbf{PJ}$ ), where  $\mathbf{P}$  is a matrix consisting of linearly independent column vectors, and  
 645  $\mathbf{J}$  is a block diagonal matrix such that

$$\mathbf{J} = \begin{bmatrix} \mathbf{J}_1 & & \\ & \ddots & \\ & & \mathbf{J}_m \end{bmatrix},$$

$$\mathbf{J}_k = \begin{bmatrix} \lambda_k & 1 & & \\ & \lambda_k & \ddots & \\ & & \ddots & 1 \\ & & & \lambda_k \end{bmatrix}, \quad \text{for } k = 1, 2, \dots, m. \quad (18)$$

646 The diagonal entries of  $\mathbf{J}$  are the eigenvalues of  $\mathbf{T}$  with multiplicities. The matrix  $\mathbf{J}$  is called the **Jordan normal**  
 647 **form** of  $\mathbf{T}$ , and the column vectors of  $\mathbf{P}$  are called the **generalized eigenvectors** of  $\mathbf{T}$ . Similar to the derivation  
 648 in section 3.1, we will again arrange the eigenvalues of  $\mathbf{T}$ , i.e., the diagonal entries of  $\mathbf{J}$ , in non-increasing order.

649 There are a few noteworthy points about factoring the matrix  $\mathbf{T}$  by its generalized eigenvectors and its Jordan  
 650 normal form. First, note that the generalized eigenvectors are linearly independent and form a basis for  $n$ -dimensional  
 651 vectors, where  $n$  is the size of  $\mathbf{T}$ . We denote by  $n_k$  the size of the Jordan block  $\mathbf{J}_k$ , and let  $\{\mathbf{v}_i^{(k)}\}_{i=1}^{n_k}$  be the generalized  
 652 eigenvectors corresponding to the eigenvalues in  $\mathbf{J}_k$ . Recalling from the notation in section 3.1, the initial distribution  
 653 over the GRNs with a non-zero viability and a non-zero relative reproductivity  $\mathcal{G}_v \setminus \mathcal{G}_s$  can be written as a linear  
 654 combination of the generalized eigenvectors

$$\mathbf{p}^{(0)} = \sum_{k=1}^m \sum_{i=1}^{n_k} a_i^{(k)} \mathbf{v}_i^{(k)}. \quad (19)$$

655 Second, since  $\mathbf{T}$  is a positive matrix (see section 3.1), by the Perron-Frobenius theorem, the size of the first Jordan  
 656 block  $n_1$  equals to 1. Specifically, the only entry in  $\mathbf{J}_1$  is the leading eigenvalue  $\lambda_1$ , and  $|\lambda_1| > |\lambda_k|$  for any  
 657  $k = 2, 3, \dots, m$ . For convenience, we abuse the notation and write  $\mathbf{v}_1 = \mathbf{v}_1^{(1)}$ .

658 From the matrix form of the master equation (5), we know that  $\mathbf{p}^{(t)}$  is proportional to  $\mathbf{T} \mathbf{p}^{(t-1)}$ , and consequently

$$\begin{aligned} \mathbf{p}^{(t-1)} &\propto \mathbf{T}^{t-1} \mathbf{p}^{(0)} \\ &= \mathbf{T}^{t-2} \cdot \sum_{k=1}^m \sum_{i=1}^{n_k} a_i^{(k)} \mathbf{T} \mathbf{v}_i^{(k)} \\ &= \mathbf{T}^{t-2} \cdot \sum_{k=1}^m \sum_{i=1}^{n_k} a_i^{(k)} \left[ \lambda_k \mathbf{v}_i^{(k)} + (1 - \delta_{i,1}) \mathbf{v}_{i-1}^{(k)} \right] \\ &= \mathbf{T}^{t-2} \cdot \sum_{k=1}^m \sum_{i=1}^{n_k} \left[ \lambda_k a_i^{(k)} + (1 - \delta_{i,n_k}) a_{i+1}^{(k)} \right] \mathbf{v}_i^{(k)} \\ &\quad \vdots \\ &= \sum_{k=1}^m \sum_{i=1}^{n_k} \left[ \lambda_k^{t-1} a_i^{(k)} + (1 - \delta_{i,n_k}) \left( \sum_{s=0}^{t-2} \lambda_k^s \right) a_{i+1}^{(k)} \right] \mathbf{v}_i^{(k)}. \end{aligned} \quad (20)$$

659 Plugging (20) into the master equation (5), we have



$$\begin{aligned}
 \mathbf{p}^{(t)} &= \frac{\mathbf{T} \cdot \sum_{k=1}^m \sum_{i=1}^{n_k} \left[ \lambda_k^{t-1} a_i^{(k)} + (1 - \delta_{i,n_k}) \left( \sum_{s=0}^{t-2} \lambda_k^s \right) a_{i+1}^{(k)} \right] \mathbf{v}_i^{(k)}}{\mathbf{1}^\top (\mathbf{T} + \mathbf{R}) \cdot \sum_{k=1}^m \sum_{i=1}^{n_k} \left[ \lambda_k^{t-1} a_i^{(k)} + (1 - \delta_{i,n_k}) \left( \sum_{s=0}^{t-2} \lambda_k^s \right) a_{i+1}^{(k)} \right] \mathbf{v}_i^{(k)}} \\
 &= \frac{\sum_{k=1}^m \sum_{i=1}^{n_k} \left[ \lambda_k^t a_i^{(k)} + \frac{(\lambda_k^t - 1)(1 - \delta_{i,n_k})}{\lambda_k - 1} a_{i+1}^{(k)} \right] \mathbf{v}_i^{(k)}}{\sum_{k=1}^m \sum_{i=1}^{n_k} \left\{ \left[ \lambda_k^t a_i^{(k)} + \frac{(\lambda_k^t - 1)(1 - \delta_{i,n_k})}{\lambda_k - 1} a_{i+1}^{(k)} \right] \left( \mathbf{1}^\top \mathbf{v}_i^{(k)} \right) + \left[ \lambda_k^{t-1} a_i^{(k)} + \frac{(\lambda_k^{t-1} - 1)(1 - \delta_{i,n_k})}{\lambda_k - 1} a_{i+1}^{(k)} \right] \left( \mathbf{1}^\top \mathbf{R} \mathbf{v}_i^{(k)} \right) \right\}} \\
 &= \frac{f_1^{(1)}(t) \mathbf{v}_1 + \sum_{k=2}^m \sum_{i=1}^{n_k} f_i^{(k)}(t) \mathbf{v}_i^k}{\left( f_1^{(1)}(t) \mathbf{1}^\top \mathbf{v}_1 + f_1^{(1)}(t-1) \frac{\mathbf{1}^\top \mathbf{R} \mathbf{v}_1}{\lambda_1} \right) + \sum_{k=2}^m \sum_{i=1}^{n_k} \left( f_i^{(k)}(t) \mathbf{1}^\top \mathbf{v}_i^{(k)} + f_i^{(k)}(t-1) \frac{\mathbf{1}^\top \mathbf{R} \mathbf{v}_i^{(k)}}{\lambda_1} \right)}, \quad (21)
 \end{aligned}$$

660 where

$$f_i^{(k)}(t) = \left( \frac{\lambda_k}{\lambda_1} \right)^t a_i^{(k)} + \frac{(\lambda_k^t - 1)(1 - \delta_{i,n_k})}{\lambda_1^t (\lambda_k - 1)} a_{i+1}^{(k)}. \quad (22)$$

661 Since  $f_1^{(1)}(t) = a_1^{(1)}$  for any  $t$  and  $\lim_{t \rightarrow \infty} f_i^{(k)}(t) = 0$  for  $k > 1$ , we hence recover equation (8) and show the  
662 convergence of the master equation (5) for a non-symmetric matrix  $\mathbf{T}$ .

## 663 E Convergence Rate to the Stationary Distribution of GRNs

664 In this section, we provide an estimate of the rate that the master equation (4) converges to its stationary distribution,  
665 using the technique known as the uniform minorization condition of Markov chains [79]. Specifically, given a sequence  
666 of probability distribution  $\{p_t\}_{t=1}^\infty$  over a finite discrete space  $\mathbb{X}$  which converges to  $\pi = \lim_{t \rightarrow \infty} p_t$ , we will find an  
667 upper bound of the variation distance

$$\|p_t - \pi\| = \sup_{A \subset \mathbb{X}} |p_t(A) - \pi(A)|. \quad (23)$$

668 An upper bound of  $\|p_t - \pi\|$  will then lead us to estimating a large enough  $t$  such that  $\|p_t - \pi\| < \epsilon$  for any arbitrary  
669 tolerance  $\epsilon$ .

670 To begin, for any genotype/GRN  $g, g' \in \mathcal{G}$ , we introduce the notation

$$F(g', g, t) = \frac{1}{\nu_t \rho_{t-1}} \rho_{g'} \mu_{g'} \nu_g. \quad (24)$$

671 Since  $\nu_t = \mathbb{P}[\Psi(I_t)]$  and  $\rho_{t-1} = \mathbb{P}[I_{t-1} \rightarrow I_t \mid \Psi(I_{t-1})]$  are always less than or equal to 1, observe that

$$F(g', g, t) \geq \beta \zeta(g), \quad (25)$$

672 where

$$\beta = \sum_{g \in \mathcal{G}} \min_{g' \in \mathcal{G}} \rho_{g'} \mu_{g'} \nu_g \quad (26)$$

673 and  $\zeta$  is a probability distribution over  $\mathcal{G}$  such that  $\zeta(g) = \frac{1}{\beta} \min_{g' \in \mathcal{G}} \rho_{g'} \mu_{g'} \nu_g$  for any  $g \in \mathcal{G}$ . The inequality (25) is  
674 a uniform minorization condition, which has been recognized to elegantly estimate the convergence rate of Markov  
675 chains. We will adapt the derivation for Markov chains as reviewed by [79] and only summarize the key steps in  
676 what follows.

677 Let  $X_1, Y_1$  be two independent random variables, whose probability distribution are

$$p(X_1) = \{\mathbb{P}[g(I_1) = g \mid \Psi(I_1)]\}_{g \in \mathcal{G}}, \quad (27)$$

$$p(Y_1) = \left\{ \lim_{t \rightarrow \infty} \mathbb{P}[g(I_t) = g \mid \Psi(I_t)] \right\}_{g \in \mathcal{G}} \quad (28)$$

678 respectively. Next, given random variables  $X_t$  and  $Y_t$ , let  $X_{t+1}$  and  $Y_{t+1}$  be two random variable such that

- 679 (i) With probability  $\beta$ , set  $X_{t+1} = Y_{t+1}$  which follows the probability distribution  $\zeta$ ;  
 680 (ii) Otherwise,  $X_{t+1}$  and  $Y_{t+1}$  are independent random variables such that

$$\mathbb{P}[X_{t+1} = g \mid X_t = g'] = \frac{F(g', g, t) - \beta\zeta(g)}{1 - \beta}, \quad (29)$$

$$\mathbb{P}[Y_{t+1} = g \mid Y_t = g'] = \frac{F(g', g, t) - \beta\zeta(g)}{1 - \beta} \quad (30)$$

681 for  $g, g' \in \mathcal{G}$ .

682 Note that the probability distribution of  $\{X_t\}_{t=1}^{\infty}$  reconciles with the solution of (4) with initial condition  $p(X_1)$ ,  
 683 and the probability distribution of  $\{Y_t\}_{t=1}^{\infty}$  remains to be the stationary distribution of (4). We write  $p(X_t) =$   
 684  $\{\mathbb{P}[g(I_t) = g \mid \Psi(I_t)]\}_{g \in \mathcal{G}} = p_t$  and  $p(Y_1) = \{\lim_{t \rightarrow \infty} \mathbb{P}[g(I_t) = g \mid \Psi(I_t)]\}_{g \in \mathcal{G}} = \pi$  respectively.

685 Suppose random variable  $T$  to be the first time step that scenario (i) occurs so  $X_T = Y_T$ . By construction,  
 686 we have  $\mathbb{P}[T > t] = (1 - \beta)^t$ . Let  $\{Z_t\}_{t=1}^{\infty}$  be another sequence of random variables such that  $Z_t = Y_t$  for  $t \leq T$   
 687 and  $Z_t = X_t$  for  $t > T$ . We observe that the probability distribution of  $\{Z_t\}_{t=1}^{\infty}$  also remains to be the stationary  
 688 distribution of (4). It is not hard to see that the variation distance between two probability distributions is bounded  
 689 from above by the probability that the two corresponding random variables are not equal (for details, see [79]), and

$$\begin{aligned} \|p_t - \pi\| &= \|p(X_t) - p(Z_t)\| \\ &\leq \mathbb{P}[X_t \neq Z_t] \\ &\leq \mathbb{P}[T > t] \\ &= (1 - \beta)^t. \end{aligned} \quad (31)$$

690 Therefore, for an arbitrary tolerance  $\epsilon$  and in the case that there is no  $g \in \mathcal{G}$  with a zero  $\rho_g$  (so  $\beta > 0$ ), a sufficient  
 691 condition for  $\|p_t - \pi\| < \epsilon$  is

$$t > \frac{\log(\epsilon)}{\log(1 - \beta)}. \quad (32)$$

## 692 F Supplementary Figures

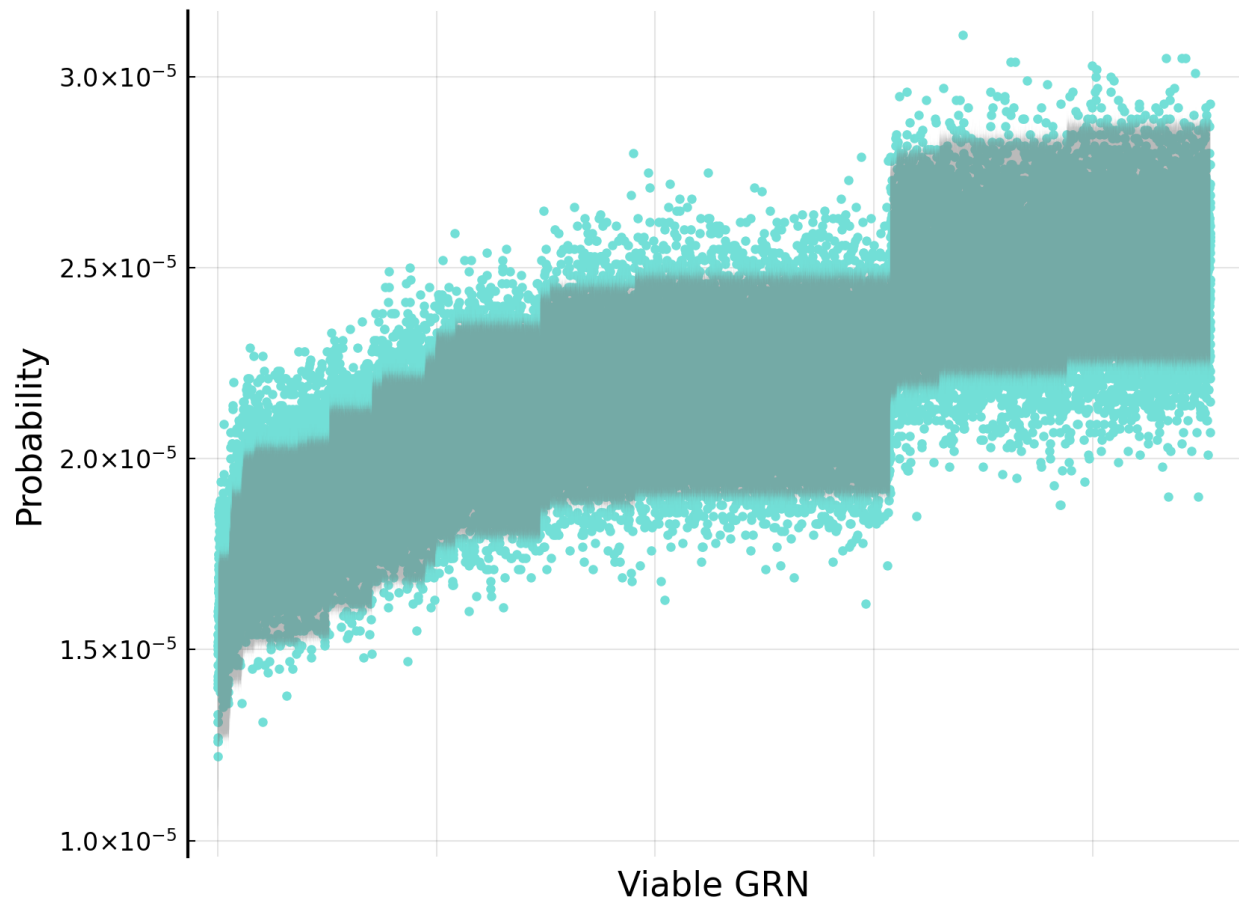


Figure 5: Validation that the evolutionary dynamics of GRNs converges to the derived stationary distribution. We compare the distribution of GRNs sampled from their simulated evolutionary dynamics in subsection 3.2 (blue) with the exact leading eigenvector of the transition matrix (11) with the same  $\mu = 0.1$  (gray). The shaded area shows its 95% confidence band that accounts for the uncertainty of finite-sized sampling in the simulations. No significant deviation was found.