The Population Genetics of Gene Regulatory Networks

Chia-Hung Yang[1,†] and Samuel V. Scarpino[1,2,3,4,‡]

[1]Network Science Institute, Northeastern University, Boston, MA, USA
[2]Roux Institute, Northeastern University, Boston, MA, USA
[3]Santa Fe Institute, Santa Fe, NM, USA
[4]Vermont Complex Systems Center, University of Vermont, Burlington, VT, USA
[†]yang.chi@northeastern.edu
[‡]s.scarpino@northeastern.edu

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

Here, we develop a population genetic model describing how the structure of GRNs are shaped by a combination of selection and mutation. First, using this model we study the dynamics of GRN evolution in an infinitely large population with non-overlapping generations that experiences constant selection and mutation. Generalizing these results to arbitrary viability functions and rules for mutational transition, we prove that the dynamics always converge to a stationary distribution over GRNs. Then, assuming binary viability, identical reproductivity, and rare mutation, we analytically show that the frequency of GRNs at mutation-selection balance is proportional to each GRN's eigenvector centrality in a sub-graph of the genotype network [42, 43, 44, 45]. Finally, we determine the structural motifs associated with GRNs that are favored in response to a wide variety of selective regimes and regulatory constraints. We discuss the implications of our results in the context of the evolution of complex phenotypes, and the challenges of studying GRN evolution.

2 Models

2.1 Population Genetic Model with Selection, Reproduction, and Mutation

We begin by demonstrating how a single locus population genetic model can be generalized to an arbitrarily complex genotype using basic probability theory. Here, we focus on a simple population genetic model that incorporates selection, reproduction, and mutation: The viable individuals in the current generation reproduce and generate their offspring, which may possibly mutate, and undergo selection to form the next generation. We additionally impose a few assumptions to the model, including a.) an infinitely large population size, b.) non-overlapping generations, c.) asexual reproduction, d.) a constant reproductivity of each genotype and a fixed selective environment over time, and e.) that any single-locus mutation has a non-zero chance to occur per generation. We relax many of these assumptions in future sections and in the Appendix.

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

For any genotype $g \in \mathcal{G}$, we are interested in its prevalence in the population at a given generation t *after* selection. In other words, we would like to know the probability that we observe a randomly sampled individual at generation t with the genotype g, given the fact that the sampled individual is

viable. Applying the Bayes' theorem, this focal conditional probability becomes

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

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

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

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

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

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

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

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

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

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

2.2 Pathway Framework of GRNs: Representing Genotypes by Expression Behavior

As we discuss in the Introduction, a GRN can be constructed through a bottom-up fashion assuming we have all knowledge of how the underlying genetics works, and this hypothetical ideal implies that a GRN is

[1] 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 $i, j \in I$.

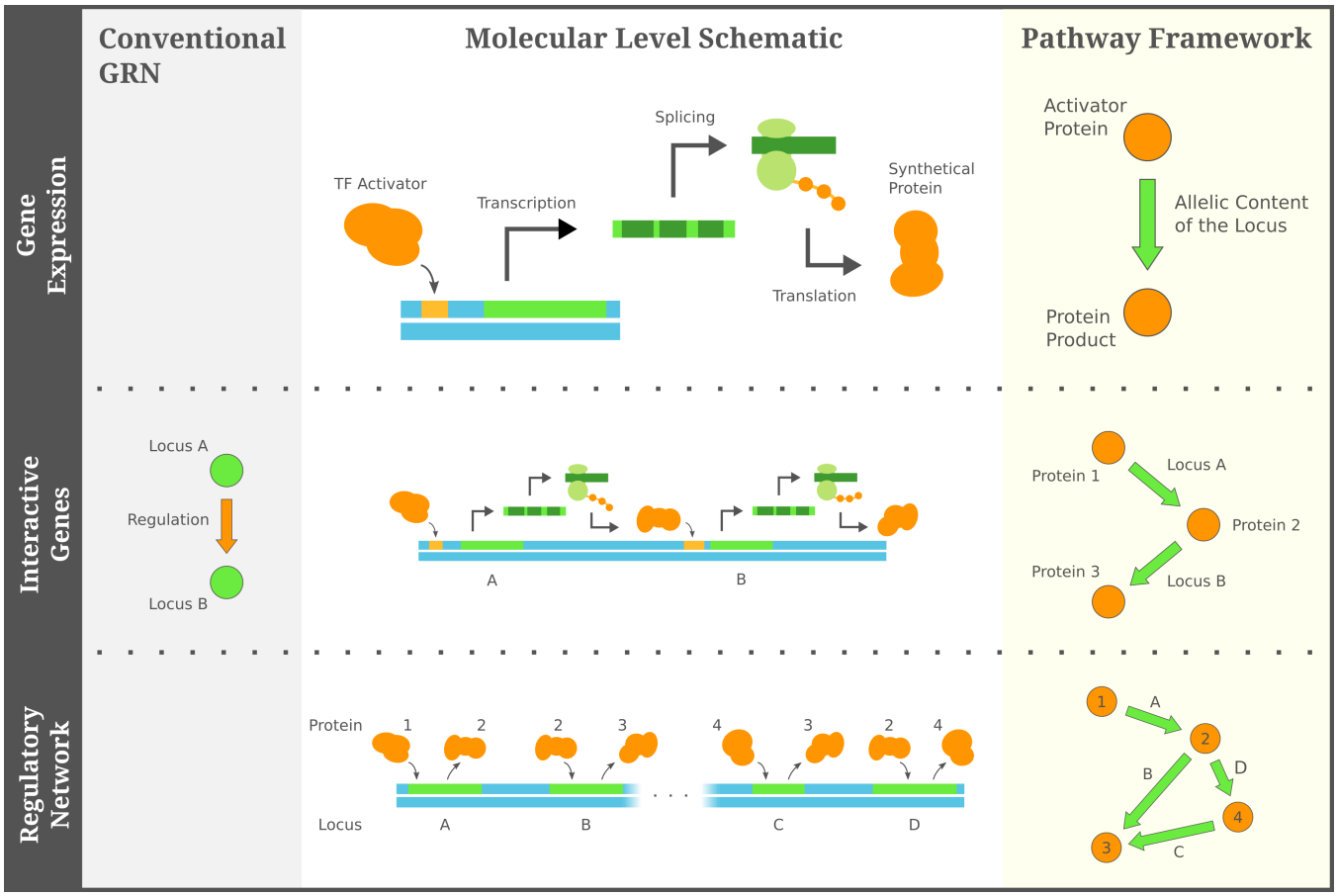

Figure 1: The pathway framework interprets a GRN as an abstraction of the expression behavior of the genotype. In this framework a GRN consists of edges indicating the input-output pair of a gene's expression, from which transcriptional regulation between genes can be recovered, and it is arguably a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

essentially a representation of the genotype on the level of gene regulation. Previous work has adopted this idea and proposed a modeling approach, termed the **pathway framework**, to describe how the structure of GRNs varies due to genetic changes and how they respond to a given selective pressure [37]. We use the pathway framework to establish the specific connection between a genotype and its derived GRN (which we summarize below; see it formal mathematical formulation in Appendix A). Combining the pathway framework of GRNs and the generalized population genetic model developed in section 2.1 provides a mechanistic understanding of the variation of GRNs under evolution.

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

In this work, we focus on a minimal pathway framework of GRNs which integrates a few additional assumptions: First, we presume there is a constant collection of proteins Ω that can *possibly* appear in the organisms, and the state of a protein is binary, which indicates whether the protein is present or absent in an organism. Second, assuming that any gene's expression is activated a single protein and produces a single protein product, the allele of the gene becomes the ordered pair of protein activator/product. If the protein activator is in the present state, the allele of the gene turns the state of the protein product to presence. Third, there is a fixed collection of genes Γ in the organisms, and the allele of each gene can be any pair of activator/product in the constant collection of proteins. Forth, the external environmental stimuli, if any, specify some activator proteins in the constant collection Ω and turn their state to presence.

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

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

2.3 Genotype Network: a Space of Mutational Relationship between GRNs

Previous literature has developed the concept of the genotype network, which captures how various genotypes transition from one to another through mutations (not necessarily just point mutations) and/or recombination [42, 46]. Here, we generalize the genotype network to describe the mutational connection between GRNs. The **genotype network** of GRNs is a undirected network of networks, where every possible GRN becomes a mega-node, and two mega-nodes are connected if the two corresponding GRNs only differ by the allele at a single locus. In other words, an edge between two mega-nodes in the genotype network represents a single-locus mutation between GRNs (Figure 2). We shall see in the Analyses that the structure of the genotype network and its induced subgraph not only plays an crucial role in proving the convergence of the population genetic model introduced in section 2.1, but it also encodes information about the stationary distribution of GRNs under evolution.

We emphasize two important properties of the genotype network of GRNs under the pathway framework. First, because the underlying collections of proteins and genes are fixed, and a mutation at any gene

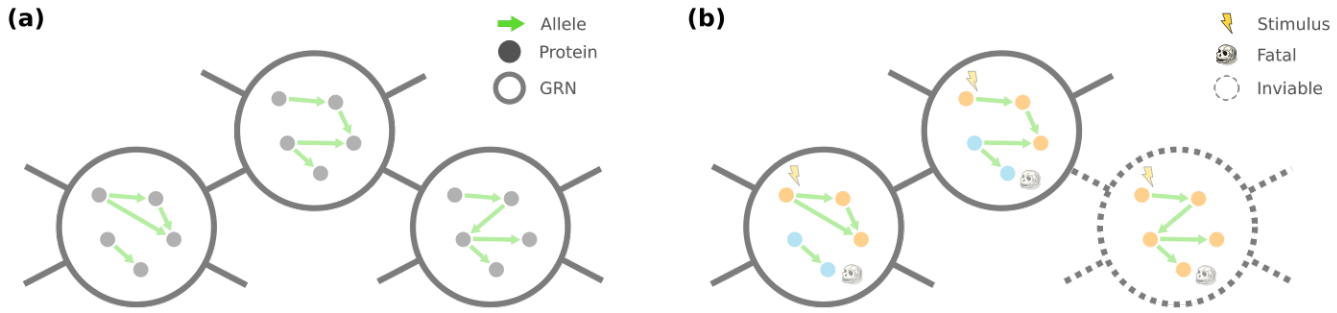

Figure 2: (a) Genotype network of GRNs, where, under the pathway framework, two mega-nodes (GRNs) are connected if and only if thy differ by one edge rewiring. (b) Neutral network of GRNs, where inviable mega-nodes are removed from the genotype network. In this illustrative example inviability is modeled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

can lead to a mutant allele that points from any protein activator to any protein product, each GRN has the same number of mutational neighbors. As a result, the genotye network of GRNs is in fact a regular graph. Second, instead of concentrating on all possible GRNs, one may be interested in the mutational relationship between a subset of them. A particularly remarkable scenario is to constrain the GRNs on the binary state of proteins of the resulting phenotype, i.e., to only retain the GRNs in whose phenotype some pre-specified proteins are present/absent. In Appendix C, we show that the induced subgraph of the genotype network from any phenotypic constraint is connected. There always exists a sequence of single-locus mutations between two GRNs such that the involved GRNs all satisfy the arbitrary given constraint on their phenotype.

When the viability of GRNs is binary, such that a GRN either always survives the selection pressure or is always eliminated, one common phenotypic constraint is to focus on the viable GRNs. Following the literature of protein sequences [44, 46], we call the induced subgraph of the genotype network of all viable GRNs the **neutral network**. This network of networks captures the mutational transition between GRNs that are selectively neutral. Note that, unlike the genotype network, the neutral network is not necessary a regular graph; yet it is always connected graph.

3 Analyses

3.1 Convergence to a Stationary Distribution of GRNs

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

It is thus useful to only keep track of the GRNs with a non-zero viability and a non-zero relative reproductivity. Hereafter, we consolidate the focal conditional probability for every $g \in \mathcal{G}_v \setminus \mathcal{G}_s$ at generation t through a column vector $\mathbf{p}^{(t)}$. We write the i_g-th entry of $\mathbf{p}^{(t)}$ as the one that corresponds to g,

namely $\left[\mathbf{p}^{(t)}\right]_{i_g} = \mathbb{P}\left[g\left(I_t\right) = g \mid \Psi\left(I_t\right)\right]$. The master equation (4) can also be rewritten in a matrix format. Specifically, we denote by $\mathbf{T}$ a semi-transition matrix whose 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 $g, g' \in \mathcal{G}_v \backslash \mathcal{G}_s$. We in addition have another matrix $\mathbf{R}$ to capture the transition from $g' \in \mathcal{G}_v \backslash \mathcal{G}_s$ to $g \in \mathcal{G}_v \cap \mathcal{G}_s$, whose entry at the i_g-th row and the $i_{g'}$-th column is again $\rho_{g'}\mu_{g'g}\nu_g$. With these matrix notations, and along that the average viability $\nu_t = \sum_{g\in\mathcal{G}_v}\sum_{g'\in\mathcal{G}}\rho_{g'}\mu_{g'g}\nu_g\mathbb{P}\left[g\left(I_{t-1}\right) = g' \mid \Psi\left(I_{t-1}\right)\right]$ since $\sum_{g\in\mathcal{G}_v}\mathbb{P}\left[g\left(I_t\right) = g \mid \Psi\left(I_t\right)\right] = 1$, the master equation (4) therefore becomes

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

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

The matrix $\mathbf{T}$ plays a key role in the master equation (5), and it has a nice property that all its entries are positive. Since $\mathbf{T}$ corresponds to transition between GRNs $g, g' \in \mathcal{G}_v \backslash \mathcal{G}_s$, the relative reproductivity $\rho_{g'}$ and the viability ν_g are both positive. Next, we must show that the mutation probability $\mu_{g'g}$ is positive as well. Recall that, when constructed through the pathway framework of GRNs, the subgraph of the genotype network induced by any phenotypic constraint is connected (see section 2.3 and Appendix C). More formally, the connectedness among GRNs constrained by a non-zero viability and relative reproductivity implies that, for any $g, g' \in \mathcal{G}_v \setminus \mathcal{G}_s$, there exists a sequence of 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 occur with a non-zero probability (recall from section 2.1), there is a non-zero chance for g' to mutate to g within one generation[2], i.e., $\mu_{g'g} > 0$. As a result, we observe that $\mathbf{T}$ is a positive matrix.

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

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

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 consequently

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

where $\mathbf{v}_1$ and λ_1 are the leading eigenvector and the leading eigenvalue of $\mathbf{T}$ respectively. Since $\mathbf{T}$ is a positive matrix, by the Perron-Frobenius theorem, we have $|\lambda_1| > |\lambda_i|$ for every $i > 1$, which guarantees the convergence of equation (5)

$$\lim_{t\to\infty}\mathbf{p}^{(t)} \;=\; \frac{\mathbf{v}_1}{\mathbf{1}^\top\mathbf{v}_1 \;+\; \frac{\mathbf{1}^\top\mathbf{R}\mathbf{v}_1}{\lambda_1}}\,. \tag{8}$$

[2]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 Δt, where Δt is the diameter of the subgraph of the genotype network constrained by a non-zero viability and relative 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.

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

3.2 Case Study: Binary Viability, Identical Reproductivity, and Independent Mutation

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

Second, we assume that each GRN $g \in \mathcal{G}$ produces the same number of offspring and there is no sexual selection. Equivalently, the probability that a individual randomly sampled from an infinitely large offspring is reproduced by a viable parent with GRN g, $\mathbb{P}\left[I_{t-1} \to I_t \;\mid\; g\left(I_{t-1}\right) = g,\; \Psi\left(I_{t-1}\right)\right]$, is a constant for any viable GRN $g \in \mathcal{G}_v$. The relative reproductivity thus equals to 1 for every $g \in \mathcal{G}_v$

$$\rho_g \;=\; \frac{\mathbb{P}\left[I_{t-1} \to I_t \;\mid\; g\left(I_{t-1}\right) = g,\; \Psi\left(I_{t-1}\right)\right]}{\sum_{g \in \mathcal{G}_v} \mathbb{P}\left[I_{t-1} \to I_t \;\mid\; g\left(I_{t-1}\right) = g,\; \Psi\left(I_{t-1}\right)\right] \;\mathbb{P}\left[g\left(I_{t-1}\right) = g \mid \Psi\left(I_{t-1}\right)\right]} \;=\; 1\,. \tag{9}$$

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

$$\mu_{g'g} \;=\; \left(\frac{\mu}{|\alpha(\Omega)| - 1}\right)^{d(g',g)} (1-\mu)^{|\Gamma| - d(g',g)}\,, \tag{10}$$

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

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

$$\mathbf{T} \;=\; \mathbf{T}_0 + \mathbf{T}_1 + \mathbf{T}_2 + \ldots + \mathbf{T}_{|\Gamma|}\,, \tag{11}$$

where the entry at the i_g-th row and the $i_{g'}$-th column of matrix $\mathbf{T}_k$ is $\mu_{g'g}$ if $d(g', g) = k$ and 0 otherwise. Observe 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 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 finite even for a zero per-generation, per-locus mutation probability μ, equation (11) becomes

$$\mathbf{T} \;=\; (1-\mu)^{|\Gamma|}\,\mathbf{I} \;+\; \left(\frac{\mu}{|\alpha(\Omega)| - 1}\right)(1-\mu)^{|\Gamma|-1}\,\mathbf{A} \;+\; \sum_{k=2}^{|\Gamma|} \mu^k\;\tilde{\mathbf{T}}_k\,. \tag{12}$$

We further consider the scenario that mutations are rare events, specifically, under the limit $\mu \to 0$. Since the 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

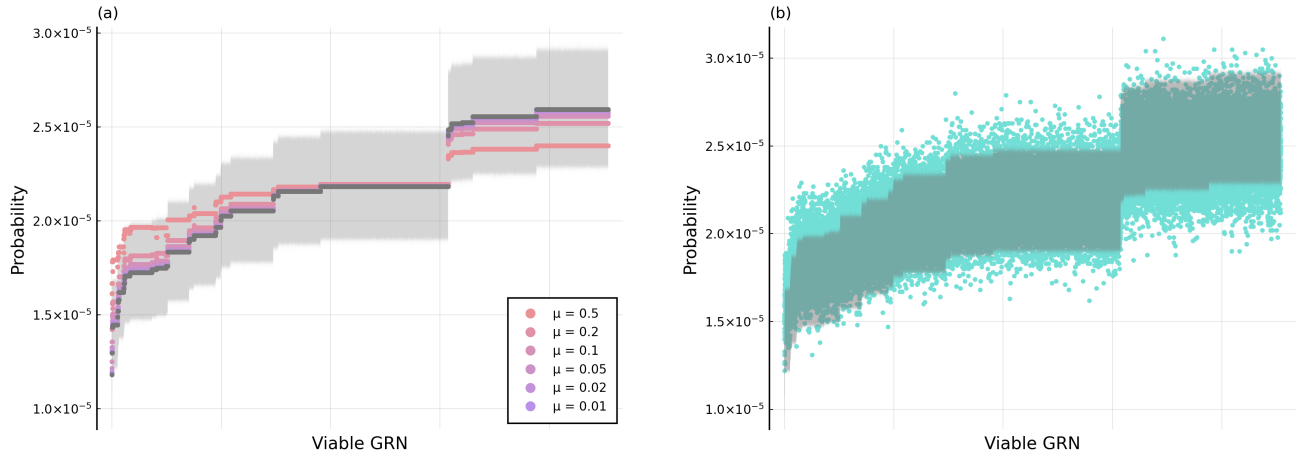

Figure 3: Validation that the evolutionary dynamics of GRNs converges to the derived stationary distribution (14). We compare the predicted stationary distribution of viable GRNs under the rare-mutation approximation with (a) the exact leading eigenvector of the transition matrix (12) with various per-locus mutation probability μ (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.

symmetric matrices because $d(g', g) = d(g, g')$, the theory of eigenvalue perturbation [47, 48] ensures that the leading eigenvector of $\mathbf{T}$ converges to the leading eigenvector[3] of $\mathbf{A}$:

$$\lim_{\mu \to 0} \mathbf{v}_1 \;=\; \mathbf{v}_1 (\mathbf{A}) \; . \tag{13}$$

From equation (8), we have

$$\lim_{\mu \to 0} \lim_{t \to \infty} \mathbf{p}^{(t)} \;=\; \frac{\mathbf{v}_1 (\mathbf{A})}{\mathbf{1}^\top \mathbf{v}_1 (\mathbf{A})} \; . \tag{14}$$

In network science, entries of the leading eigenvector of the adjacency matrix of a connected, undirected graph is known as the eigenvector centrality [49, 50] of the nodes. As a result, under the assumptions of binary viability, identical reproductivity, and rare, uniform mutation, *the probability distribution of viable GRNs converges to a stationary distribution that is proportional to their eigenvector centrality in the neutral network.*

To validate the predicted probability distribution of GRNs under mutation-selection balance, we simulate the evolution of 10^7 parallel populations. The simulations are parametrized with the constant sets of $|\Gamma| = 4$ genes and $|\Omega| = 6$ proteins. We further presume that two proteins can not be the product of any expression behavior, whose presence state can only be stimulated externally and hereafter they are referred to the *input* proteins. We also presume that two other proteins only have direct physiological effects and they can not serve as the activator of any expression behavior, which we call the *output* proteins. Under this minimal setup, there are in total $|\alpha(\Omega)| = 16$ potential pairs of expression activator/product, which leads to $|\mathcal{G}| = 65536$ plausible GRNs. We evolve the populations under the environmental condition such that one of the input proteins is externally stimulated, and one of the output proteins shows a fatal effect which is required absent for an individual's viability, resulting in $|\mathcal{G}_v| = 45389$ possible viable GRNs altogether.

The evolution of parallel populations are simulated using a Wright-Fisher model [51]. Specifically, we fix a number of 16 individuals for all populations, and given a current generation, the next generation

[3]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.

	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.

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

Moreover, in order to account for the uncertainty of finite-sized sampling in the simulated distribution, we also draw the same number of 10^7 independent samples from the predicted distribution (14) to form an empirical distribution. Repeating the sampling procedure 1,000 times, we obtain an ensemble of empirical distributions that captures the effect of finite-sized sampling over the predicted probability that GRNs are to be observed. We further use the averaged variation distance between the empirical distribution and the predicted distribution as the error tolerance from which the number of generations to be simulated is calculated such that convergence of the model is theoretically guaranteed (Appendix E).

In Figure 3a, we compare the exact, properly normalized leading vector of the transition matrix $\mathbf{T}$ (12) along with the predicted stationary distribution of viable GRNs under the rare-mutation approximation (14). Observe that even a moderate per-locus mutation probability μ leads to a GRN distribution well aligned with the predicted one, especially, with respect to the uncertainty arising from finite-sized sampling in the simulations. Moreover, Figure 3b shows the simulated distribution of viable GRNs after long-term evolution. We see that, despite a little overdispersion, the simulated distribution agrees with the derived stationary distribution of GRNs. Combined, our simulations indicate computational evidence that, when viability is assumed rugged and mutations are rare, the topology of the neutral network, particularly the eigenvector centrality of mega-nodes, serves as a informative predictor of the prevalence of GRNs under mutation-selection balance.

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

	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.

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

On the other hand, for the environmental conditions where only the essentiality of protein products is obligated (environment 4, 5, and 6), the most prevalent GRNs are the ones where several redundant genes are directly activated by a stimulus and produces an essential protein, and they are evenly split

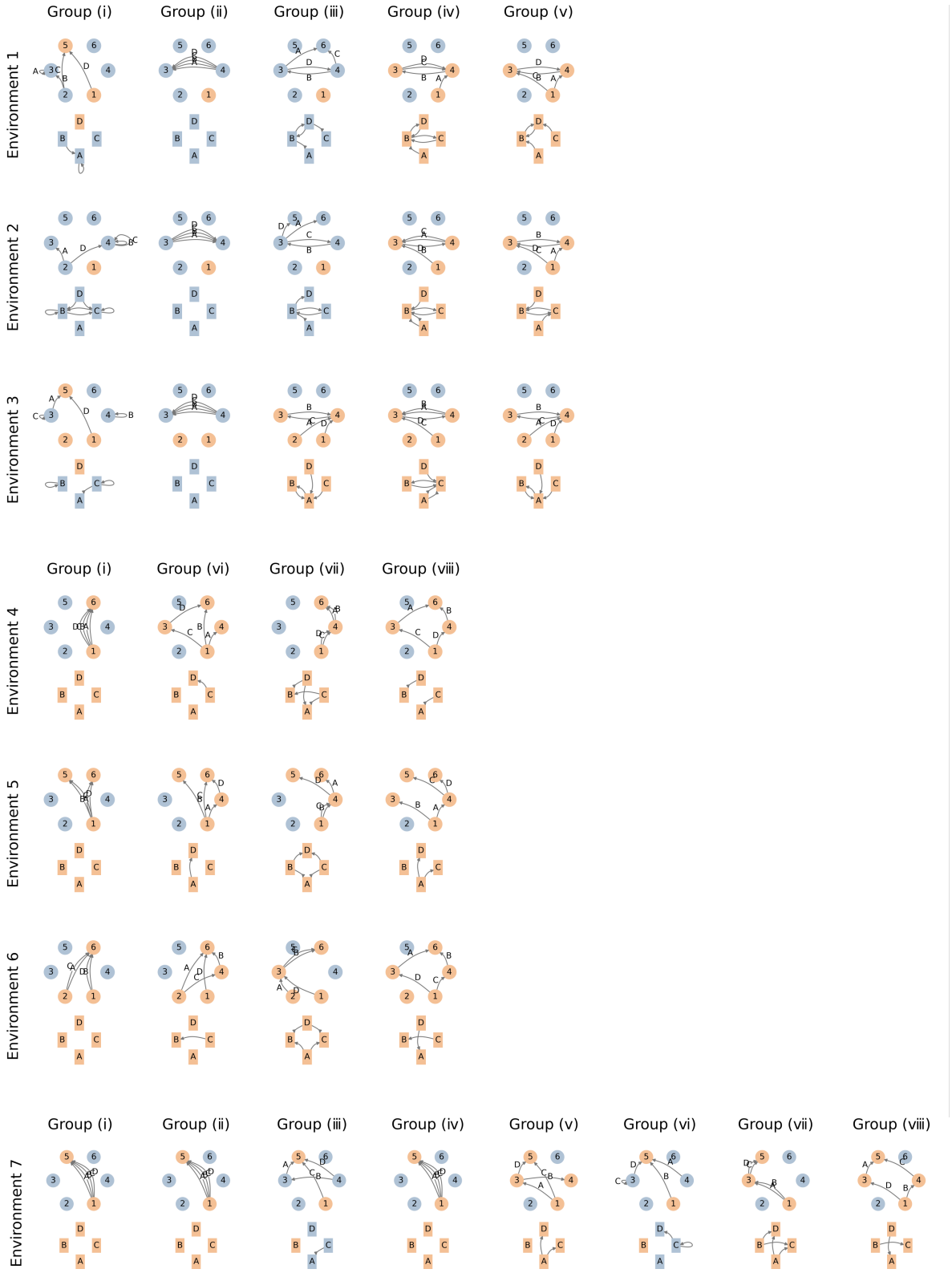

Figure 4: GRN that has the largest eigenvector centrality in the neutral network for different environmental conditions (Table 1) and among different constrained groups of GRNs (Table 2). For each prevalent GRN, its pathway framework representation is plotted by the circles and the labeled arrows, while 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.

if multiple essential targets or stimuli exist (group (i)). When redundant genes are artificially excluded (group (vi)), the prevalent GRNs turn into a structure that manifests multiple pathways between the stimuli and the essential proteins. While constrained by no direct gene expression activated by a stimulus and producing an essential target (group (vii)), the prevalent GRNs similarly show multiple pathways yet each of which involves at least two genes, and these pathways share the same intermediate protein that serves as the product of one and the activator of another. Jointly imposing the two constraints mentioned above (group (viii)) results in the prevalent GRN structure that maintains multiple regulatory pathways and simultaneously triggers the presence state of the underlying proteins, if plausible. Notice that for these environmental condition, the prevalent GRNs take advantages of the functionality of every gene, and all the genes are activated.

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

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

4 Discussion

In this work, we analyze the evolutionary dynamics of GRNs under a population genetic model with selection, mutation, and asexual reproduction. Integrating with the pathway framework of GRNs that abstracts the alleles of genes through their expression behavior, we analytically show that the population dynamics always converges to a stationary distribution of GRNs given any arbitrary viability function and stochastic mutational transition as long as no mutation is prohibited. This stationary distribution characterizes the ensemble of regulatory circuits under mutation-selection balance, and it implicates the structural features of GRNs to be predicted favorable under long-term evolution. Next, we investigate a case study assuming binary viability, identical reproductivity and rare mutation, and find that the stationary distribution of GRNs can be derived from the topology of the genotype network. Specifically, the probability to observe a GRN under mutation-selection balance is proportional to the GRN's eigenvector centrality in the neutral network, which is a subgraph of the genotype network consisting of all viable GRNs.

Our derivation sheds light on how we may interpret the prevalence of GRNs under rare mutation and strong selection on the resulting phenotypic functionality. When first introduced [49], the eigenvector centrality was designed to capture an individual's global "importance" as measured by their social ties in a communication network. In particular, the eigenvector centrality is computed based on the idea that a node's importance is proportional to the sum of its neighbors' importance scores. This interpretation is nicely translated to the content of the neutral network of regulatory circuits: Under mutation-selection balance, our derivation predicts that the probability to observe a GRN is proportional to the total likelihood to find its viable, mutational neighbors in the population. Intriguingly, the interpretation of eigenvector centrality leads to some emerging concept of robustness [52], where the prevalence of a GRN is not only due to its selective advantage but also the overall prevalence of its mutational neighboring GRNs.

Moreover, the observed prevalent structures of GRNs in our analyses also provides a possible alternative explanation for evolutionary robustness. We inductively find that these prevalent regulatory structures follow the same pattern to achieve a minimal number of plausible inviable mutants (see section 3.3). Since the genotype network is a regular graph under the pathway framework of GRNs (recalling from section 2.3), i.e., every GRN has the same amount of mutational neighbors, minimizing the number of inviable mutants optimally increases the viable mutants for a GRN. In other words, the observed prevalent GRNs under various environmental conditions appear to show the regulatory structures with the maximal number of neighbors in the neutral network, and indeed the degree of a node is known to be strongly correlated with its eigenvector centrality in the network science literature [50]. We emphasize that these concepts of robustness naturally emerge from the mechanistic, population genetic model of GRN evolution rather than an *a prior* assumption about prevalent regulatory circuits.

Previous work often focused on relating the topological features of genotype networks to evolutionary processes of interest. For example, evolvability has been approximated by the size of the genotype network of a given phenotype [53], as well as the number of "neighboring" phenotypes inferred from the genotype network [54]. Robustness has been modeled as the node degree in the genotype network [54], and [55] adopted the average path length in the genotype network as a proxy for genetic heterogeneity. To our best knowledge, Van Nimwegen et al. was the first to bridge between the asymptotic abundance of different genotypes under a population genetic model and their eigenvector centrality in the neutral network [52]. Recently, Aguirre et al. consolidated a framework that models the evolutionary dynamics of a growing population as a diffusion process on the genotype network with a mean-field fashion [46].

The current scope of the work presented here is not without a few noteworthy limitations. First, we assume a constant, static surrounding in which the population evolves, whereas populations certainly experience shifting or alternating environmental conditions [56, 57, 58]. Second, our model mainly focused on the joint forces of selection and mutation. Although this simple model can indeed be extended through more sophisticated mechanisms known to play a role in evolutionary dynamics such as recombination [59, 60], gene duplication [61, 62], and demographic information [63], we leave such extensions–along with their possible implications–to future work. Third, when the time scale of environmental changes is much faster than that of the evolutionary dynamics (see Appendix E), the transient constitution of GRNs in a population shall acquire more attention than their stationary distribution at mutation-selection balance [64, 65, 66]. Put simply, it remains an open question whether real-world populations should ever be conceptualized as at equilibrium (even dyanmic) as opposed to existing in some far-from equilibrium state [67]. As a result, further investigation should focus on the transient distributions and/or trajectories of GRNs under various population genetic models. Finally, despite confirmation between the derived stationary distribution of GRNs in an infinitely large population and the long-term numerical simulations, we also find that a finite population size moderately influences the transient evolutionary dynamics. Developing a richer understanding of the role drift plays in structuring the evolution of GRNs is an important extension of our work.

The observed structure of molecular interaction networks is a result of myriad evolutionary forces. By analyzing such topologies using a network-science approach, it may be possible to construct a mechanistic

theory for how evolution shapes and is constrained by higher-order interactions. Across a broad scope of genotype/neutral networks–with applications ranging from RNA sequences to metabolic reactions–our work rigorously shows that the neutral network of GRNs must be connected (in agreement with existing computational work [68]) and that the relative frequency at equilibrium of various GRNs can be predicted from first principles. Therefore, our work connects the evolutionary forces/mechanisms embedded in a population genetic model with the accordingly favorable GRN structure through the topology of the neutral network. Clearly, our predicted prevalent regulatory structures under mutation-selection balance may not capture all the features in empirical GRNs [69, 70]; however, we establish a null expectation for how GRNs are shaped by mutations and selection [26, 27]. Critically, this null expectation appears to recapitulate many of the topological features of molecular interaction networks currently associated with evolvability and robustness. Perhaps, more broadly speaking, the emergence of complex fitness landscapes can result from simple evolutionary rules.

References

[1] Hopi E Hoekstra and Jerry A Coyne. The locus of evolution: evo devo and the genetics of adaptation. *Evolution: International Journal of Organic Evolution*, 61(5):995–1016, 2007.

[2] Sean B Carroll. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell*, 134(1):25–36, 2008.

[3] Sam Yeaman and Michael C Whitlock. The genetic architecture of adaptation under migration–selection balance. *Evolution: International Journal of Organic Evolution*, 65(7):1897–1911, 2011.

[4] Alan A Cohen, Lynn B Martin, John C Wingfield, Scott R McWilliams, and Jennifer A Dunne. Physiological regulatory networks: ecological roles and evolutionary constraints. *Trends in ecology & evolution*, 27(8):428–435, 2012.

[5] Thomas C Nelson, Matthew R Jones, Jonathan P Velotta, Abhilesh S Dhawanjewar, and Rena M Schweizer. Unveiling connections between genotype, phenotype, and fitness in natural populations. *Molecular ecology*, 28(8):1866–1876, 2019.

[6] Bryan C Daniels, Hyunju Kim, Douglas Moore, Siyu Zhou, Harrison B Smith, Bradley Karas, Stuart A Kauffman, and Sara I Walker. Criticality distinguishes the ensemble of biological regulatory networks. *Physical Review Letters*, 121(13):138102, 2018.

[7] Naama Barkai and Stan Leibler. Robustness in simple biochemical networks. *Nature*, 387(6636):913, 1997.

[8] Raymond Cheong, Alex Rhee, Chiaochun Joanne Wang, Ilya Nemenman, and Andre Levchenko. Information transduction capacity of noisy biochemical signaling networks. *Science*, page 1204553, 2011.

[9] Richard E Lenski, Jeffrey E Barrick, and Charles Ofria. Balancing robustness and evolvability. *PLoS biology*, 4(12):e428, 2006.

[10] Andreas Wagner and David A Fell. The small world inside large metabolic networks. *Proceedings of the Royal Society of London B: Biological Sciences*, 268(1478):1803–1810, 2001.

[11] TC Per Bak and Kurt Wiesenfeld. Self-organized criticality: and explanation of 1/f noise. *Phys. Rev. Let*, 59:381–384, 1987.

[12] Sergey N Dorogovtsev and Jose FF Mendes. Evolution of networks. *Advances in physics*, 51(4): 1079–1187, 2002.

[13] David L Des Marais, Rafael F Guerrero, Jesse R Lasky, and Samuel V Scarpino. Topological features of a gene co-expression network predict patterns of natural diversity in environmental response. *Proceedings of the Royal Society B: Biological Sciences*, 284(1856):20170914, 2017.

[14] Katie E Lotterhos, Sam Yeaman, Jon Degner, Sally Aitken, and Kathryn A Hodgins. Modularity of genes involved in local adaptation to climate despite physical linkage. *Genome Biology*, 19(1):157, 2018.

[15] José Luis Blanco-Pastor, Philippe Barre, Thomas Keep, Thomas Ledauphin, Abraham Escobar-Gutiérrez, Anna Maria Roschanski, Evelyn Willner, Klaus J Dehmer, Matthew Hegarty, Hilde Muylle, et al. Canonical correlations reveal adaptive loci and phenotypic responses to climate in perennial ryegrass. *Molecular Ecology Resources*, 2020.

[16] William Andres Lopez-Arboleda, Stephan Reinert, Magnus Nordborg, and Arthur Korte. Global genetic heterogeneity in adaptive traits. *bioRxiv*, 2021.

[17] M Madan Babu and Sarah A Teichmann. Evolution of transcription factors and the gene regulatory network in escherichia coli. *Nucleic acids research*, 31(4):1234–1244, 2003.

[18] Sarah A Teichmann and M Madan Babu. Gene regulatory network growth by duplication. *Nature genetics*, 36(5):492–496, 2004.

[19] Dawn A Thompson, Sushmita Roy, Michelle Chan, Mark P Styczynsky, Jenna Pfiffner, Courtney French, Amanda Socha, Anne Thielke, Sara Napolitano, Paul Muller, et al. Evolutionary principles of modular gene regulation in yeasts. *Elife*, 2:e00603, 2013.

[20] Josep Martí-Solans, Olga V Belyaeva, Nuria P Torres-Aguila, Natalia Y Kedishvili, Ricard Albalat, and Cristian Cañestro. Coelimination and survival in gene network evolution: Dismantling the ra-signaling in a chordate. *Molecular biology and evolution*, 33(9):2401–2416, 2016.

[21] Yoram Louzoun, Lev Muchnik, and Sorin Solomon. Copying nodes versus editing links: the source of the difference between genetic regulatory networks and the www. *Bioinformatics*, 22(5):581–588, 2006.

[22] Yung-Keun Kwon and Kwang-Hyun Cho. Analysis of feedback loops and robustness in network evolution based on boolean models. *BMC bioinformatics*, 8(1):1–9, 2007.

[23] Sergei Maslov, Sandeep Krishna, Tin Yau Pang, and Kim Sneppen. Toolbox model of evolution of prokaryotic metabolic networks and their regulation. *Proceedings of the National Academy of Sciences*, 106(24):9743–9748, 2009.

[24] Feixiong Cheng, Chuang Liu, Chen-Ching Lin, Junfei Zhao, Peilin Jia, Wen-Hsiung Li, and Zhongming Zhao. A gene gravity model for the evolution of cancer genomes: a study of 3,000 cancer genomes across 9 cancer types. *PLoS Comput Biol*, 11(9):e1004497, 2015.

[25] Andreas Odorico, Estelle Rünneburger, and Arnaud Le Rouzic. Modelling the influence of parental effects on gene-network evolution. *Journal of evolutionary biology*, 31(5):687–700, 2018.

[26] Allan Force, William A Cresko, F Bryan Pickett, Steven R Proulx, Chris Amemiya, and Michael Lynch. The origin of subfunctions and modular gene regulation. *Genetics*, 170(1):433–446, 2005.

[27] Carlos Espinosa-Soto and Andreas Wagner. Specialization can drive the evolution of modularity. *PLoS Comput Biol*, 6(3):e1000719, 2010.

[28] Weilin Peng, Ping Liu, Yuan Xue, and Murat Acar. Evolution of gene network activity by tuning the strength of negative-feedback regulation. *Nature communications*, 6(1):1–9, 2015.

[29] Rishi R Masalia, Adam J Bewick, and John M Burke. Connectivity in gene coexpression networks negatively correlates with rates of molecular evolution in flowering plants. *PLoS One*, 12(7):e0182289, 2017.

[30] Bram A. Siebert, Cameron L. Hall, James P. Gleeson, and Malbor Asllani. Role of modularity in self-organization dynamics in biological networks. *Phys. Rev. E*, 102:052306, 2020.

[31] Evan A Boyle, Yang I Li, and Jonathan K Pritchard. An expanded view of complex traits: from polygenic to omnigenic. *Cell*, 169(7):1177–1186, 2017.

[32] Thomas F Hansen. The evolution of genetic architecture. *Annu. Rev. Ecol. Evol. Syst.*, 37:123–157, 2006.

[33] Andreas Wagner. Evolution of gene networks by gene duplications: a mathematical model and its implications on genome organization. *Proceedings of the National Academy of Sciences*, 91(10):4387–4391, 1994.

[34] Mark L Siegal and Aviv Bergman. Waddington's canalization revisited: developmental stability and evolution. *Proceedings of the National Academy of Sciences*, 99(16):10528–10532, 2002.

[35] Mauricio O Carneiro, Clifford H Taubes, and Daniel L Hartl. Model transcriptional networks with continuously varying expression levels. *BMC evolutionary biology*, 11(1):363, 2011.

[36] Josh S Schiffman and Peter L Ralph. System drift and speciation. *bioRxiv*, page 231209, 2018.

[37] Chia-Hung Yang and Samuel V Scarpino. Reproductive barriers as a byproduct of gene network evolution. *bioRxiv*, 2020.

[38] Andreas Wagner. Does evolutionary plasticity evolve? *Evolution*, 50(3):1008–1023, 1996.

[39] Michael E Palmer and Marcus W Feldman. Dynamics of hybrid incompatibility in gene networks in a constant environment. *Evolution: International Journal of Organic Evolution*, 63(2):418–431, 2009.

[40] Natasa Puzovic. *Effect of gene network topology on the evolution of gene-specific expression noise*. PhD thesis, Christian-Albrechts-Universität Kiel, 2020.

[41] Z Burda, A Krzywicki, OC Martin, and M Zagorski. Distribution of essential interactions in model gene regulatory networks under mutation-selection balance. *Physical Review E*, 82(1):011908, 2010.

[42] Andreas Wagner. *The Origins of Evolutionary Innovations: A Theory of Transformative Change in Living Systems*. Oxford University Press, 2011.

[43] John Maynard Smith. Natural selection and the concept of a protein space. *Nature*, 225(5232): 563–564, 1970.

[44] Peter Schuster, Walter Fontana, Peter F Stadler, and Ivo L Hofacker. From sequences to shapes and back: a case study in rna secondary structures. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 255(1344):279–284, 1994.

[45] Matthew C Cowperthwaite and Lauren Ancel Meyers. How mutational networks shape evolution: lessons from rna models. *Annu. Rev. Ecol. Evol. Syst.*, 38:203–230, 2007.

[46] Jacobo Aguirre, Pablo Catalán, José A Cuesta, and Susanna Manrubia. On the networked architecture of genotype spaces and its critical effects on molecular evolution. *Open biology*, 8(7):180069, 2018.

[47] Alan L Andrew, K-W Eric Chu, and Peter Lancaster. Derivatives of eigenvalues and eigenvectors of matrix functions. *SIAM journal on matrix analysis and applications*, 14(4):903–926, 1993.

[48] Anne Greenbaum, Ren-cang Li, and Michael L Overton. First-order perturbation theory for eigenvalues and eigenvectors. *SIAM review*, 62(2):463–482, 2020.

[49] Phillip Bonacich. Power and centrality: A family of measures. *American journal of sociology*, 92(5): 1170–1182, 1987.

[50] Mark Newman. *Networks*. Oxford university press, 2018.

[51] Richard R Hudson. Generating samples under a wright–fisher neutral model of genetic variation. *Bioinformatics*, 18(2):337–338, 2002.

[52] Erik Van Nimwegen, James P Crutchfield, and Martijn Huynen. Neutral evolution of mutational robustness. *Proceedings of the National Academy of Sciences*, 96(17):9716–9720, 1999.

[53] Matthew C Cowperthwaite, Evan P Economo, William R Harcombe, Eric L Miller, and Lauren Ancel Meyers. The ascent of the abundant: how mutational networks constrain evolution. *PLoS Computational Biology*, 4(7):e1000110, 2008.

[54] Andreas Wagner. Robustness and evolvability: a paradox resolved. *Proceedings of the Royal Society B: Biological Sciences*, 275(1630):91–100, 2007.

[55] Giovanni Marco Dall'Olio, Jaume Bertranpetit, Andreas Wagner, and Hafid Laayouni. Human genome variation and the concept of genotype networks. *PLoS One*, 9(6):e99424, 2014.

[56] Ryan T Hietpas, Claudia Bank, Jeffrey D Jensen, and Daniel NA Bolon. Shifting fitness landscapes in response to altered environments. *Evolution*, 67(12):3512–3522, 2013.

[57] M Szűcs, ML Vahsen, BA Melbourne, C Hoover, C Weiss-Lehman, and RA Hufbauer. Rapid adaptive evolution in novel environments acts as an architect of population range expansion. *Proceedings of the National Academy of Sciences*, 114(51):13501–13506, 2017.

[58] Rebecca K French and Edward C Holmes. An ecosystems perspective on virus evolution and emergence. *Trends in microbiology*, 28(3):165–175, 2020.

[59] Marcus W Feldman, Sarah P Otto, and Freddy B Christiansen. Population genetic perspectives on the evolution of recombination. *Annual review of genetics*, 30(1):261–295, 1996.

[60] Daniel Ortiz-Barrientos, Jan Engelstädter, and Loren H Rieseberg. Recombination rate evolution and the origin of species. *Trends in ecology & evolution*, 31(3):226–236, 2016.

[61] Jianzhi Zhang. Evolution by gene duplication: an update. *Trends in ecology & evolution*, 18(6): 292–298, 2003.

[62] Anke Konrad, Ashley I Teufel, Johan A Grahnen, and David A Liberles. Toward a general model for the evolutionary dynamics of gene duplicates. *Genome biology and evolution*, 3:1197–1209, 2011.

[63] Junrui Li, Haipeng Li, Mattias Jakobsson, SEN Li, PER SjÖDin, and Martin Lascoux. Joint analysis of demography and selection in population genetics: where do we stand and where could we go? *Molecular ecology*, 21(1):28–44, 2012.

[64] Takeo Maruyama and Paul A Fuerst. Population bottlenecks and nonequilibrium models in population genetics. ii. number of alleles in a small population that was formed by a recent bottleneck. *Genetics*, 111(3):675–689, 1985.

[65] Marlène Gamelon, Olivier Gimenez, Eric Baubet, Tim Coulson, Shripad Tuljapurkar, and Jean-Michel Gaillard. Influence of life-history tactics on transient dynamics: a comparative analysis across mammalian populations. *The American Naturalist*, 184(5):673–683, 2014.

[66] J-B Burie, R Djidjou-Demasse, and Arnaud Ducrot. Asymptotic and transient behaviour for a nonlocal problem arising in population genetics. *European Journal of Applied Mathematics*, 31(1):84–110, 2020.

[67] Nigel Goldenfeld and Carl Woese. Life is physics: evolution as a collective phenomenon far from equilibrium. *Annu. Rev. Condens. Matter Phys.*, 2(1):375–399, 2011.

[68] Stefano Ciliberti, Olivier C Martin, and Andreas Wagner. Robustness can evolve gradually in complex regulatory gene networks with varying topology. *PLoS Comput Biol*, 3(2):e15, 2007.

[69] Shai S Shen-Orr, Ron Milo, Shmoolik Mangan, and Uri Alon. Network motifs in the transcriptional regulation network of escherichia coli. *Nature genetics*, 31(1):64–68, 2002.

[70] Nabil Guelzim, Samuele Bottani, Paul Bourgine, and François Képès. Topological and causal structure of the yeast transcriptional regulatory network. *Nature genetics*, 31(1):60–63, 2002.

[71] Jeffrey S Rosenthal. Convergence rates for markov chains. *SIAM Review*, 37(3):387–405, 1995.

Appendices

A Mathematical Formulation of the Pathway Framework of GRNs

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

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

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

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

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

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

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

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

Definition A.3. The **activator projection** and the **product projection** of a gene regulatory network g are 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 Ω_s and Ω_t respectively.

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

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

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

$$\Delta(g_1, g_2) \;=\; \{\gamma \in \Gamma \mid g_1(\gamma) \neq g_2(\gamma)\} \tag{15}$$

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

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

$$N(g) \;=\; \left\{g' \in \mathcal{G} \mid d(g, g') = 1\right\} \tag{16}$$

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

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

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

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

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 proteins. The **phenotype** of g is the function $\chi_g : \mathcal{P}(\Omega - \Omega_t) \to \mathcal{P}(\Omega)$[4], where for any protein $\omega \in \Omega$, $\omega \in \chi_g(\Omega_0)$ if 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 $i = 1, 2, \ldots, k-1$, and $s_g(\gamma_1) \in \Omega_0$.

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

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 environmental condition respectively. The selective environment, or simply **selection**, is the triplet $\mathbb{S} = (\Omega_0, \Omega_+, \Omega_-)$. We define the set of **viable** gene regulatory networks under selection $\mathbb{S}$ by

$$\mathcal{G}_{\mathbb{S}} \;=\; \{g \in \mathcal{G} \mid \Omega_+ \subset \chi_g(\Omega_0),\ \Omega_- \subset \Omega - \chi_g(\Omega_0)\} \tag{17}$$

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

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

B Formal Definition of the Genotype Network and the Neutral Network of GRNs

With the constant sets of activators Ω_s, products Ω_t, and genes Γ, and the pre-determined sets of stimulated proteins Ω_0, essential proteins Ω_+, and fatal proteins Ω_-, we have the following definitions:

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

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 gene regulatory networks under $\mathbb{S}$. The **neutral network** subjected to $\mathbb{S}$ is a graph $G_{\mathbb{S}}$ whose nodes $V(G_{\mathbb{S}}) = \mathcal{G}_{\mathbb{S}}$ 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 network G on nodes $\mathcal{G}_{\mathbb{S}}$.

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 relavent 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 Ω_s, products Ω_t, and genes Γ, 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 γ. The number of mutatnts is

$$|N(g)| \ = \ |\Gamma| \left(|\Omega_s \times \Omega_t| - 1\right) \tag{18}$$

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

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 $g_s = g_0, g_1, \ldots, g_{k-1}, g_k = g_t \in \mathcal{G}_{\mathbb{S}}$ 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 $i = 1, 2, \ldots, k$. 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 Ω_0 to each of the essential proteins Ω_+ in the GRNs.

(II) There is no path from the stimulated proteins Ω_0 to any of the fatal proteins Ω_- 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 Ω_+ or Ω_-. In 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 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 in pathways from Ω_0 to Ω_+ in g_s and g_t respectively.

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 $\Omega_s \times \Omega_t$. We will denote such a tuple as α. If $\Omega_s \cap \Omega_t$ is non-empty, then there is a protein ω' that can serve both an 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 $\Omega_s \setminus \Omega_0$, 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 $\alpha = (u', v')$ for some $v' \in \Omega_-$. Note that the allele α is said "safe" in the sense that introducing α will never break invariant (II).

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

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 $g_1'(\gamma) = g_s(\gamma)$ for $\gamma \notin \Gamma_s^{(-)}$. During this rewiring process, invariant (I) holds since the alleles of $\Pi_s \subset \Gamma \setminus \Gamma_s^{(-)}$ remain unchanged, and invariant (II) holds because this step simply introduces the safe allele α.

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 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 Ω_0 and Ω_+, both invariant (I) and (II) are guaranteed.

3. Rewire edges in g_2' to generate another viable GRN g_3' such that $g_3'(\gamma) = g_t(\gamma)$ for any gene $\gamma \notin \Gamma_s^{(+)} \cup \Gamma_t^{(-)}$ 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 remain unchanged. Since in g_1' (and thus g_2') proteins Ω_- have no incoming incident edges, and no rewiring leads to an incoming edge of Ω_- in this step, invariant (II) is also ensured.

4. Rewire edges in g_3' to generate another viable GRN g_4' such that $g_4'(\gamma) = g_t(\gamma)$ for any gene $\gamma \in \Gamma_s^{(+)} \setminus \Gamma_t^{(-)}$ 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 achieved via an intermediate gene $\gamma' \in \Gamma$ due to the pre-condition that $|\Gamma| > |\Omega_+|$[5]. Since for each $\gamma \in \Pi_t \setminus \Gamma_s^{(+)}$, Step 3 has properly rewire its edge/allele to $g_3'(\gamma) = g_t(\gamma)$, the essential pathways formed by Π_t are gradually completed throughout the process of rewiring edges corresponding to $\Gamma_s^{(+)} \setminus \Gamma_t^{(-)}$. And similar to Step 3, no edge is rewired to be an incoming edge of Ω_- in this step, and thus invariant (II) holds as well.

5. Rewiring edges corresponding to $\Gamma_t^{(-)}$ in g_4' to generate g_t completes the viable mutational trajectory. Because edges of $\Pi_t \subset \Gamma \setminus \Gamma_t^{(-)}$ remain unchanged in this step, invariant (I) still holds. Furthermore, since g_t is viable, rewiring edges of $\Gamma_t^{(-)}$ also preserves invariant (II).

□

Note that since for a GRN to satisfy the selection criterion, every protein in Ω_+ must be produced by a gene, 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 the GRNs form $|\Omega_+|!$ components in the neutral network, each of size $|\Omega_0|^{|\Omega_+|}$. Moreover, the proof we provide here is general enough such that Lemma C.2 holds even if one adapts additional constraints and defines a mutation as changing either the protein activator or the protein product of a gene but not both.

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

Here we show a general, analogous proof for (8) in the case that the semi-transition matrix $\mathbf{T}$ is non-symmetric. Any square matrix can be factored by its general eigenvectors and its Jordan normal form. In particular, we have $\mathbf{T} = \mathbf{P}\mathbf{J}\mathbf{P}^{-1}$ (or equivalently $\mathbf{T}\mathbf{P} = \mathbf{P}\mathbf{J}$), where $\mathbf{P}$ is a matrix consisting of linearly independent column vectors, and $\mathbf{J}$ is a block diagonal matrix such that

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

$$\mathbf{J}_k = \begin{bmatrix} \lambda_k & 1 & & \\ & \lambda_k & \ddots & \\ & & \ddots & 1 \\ & & & \lambda_k \end{bmatrix}, \quad \text{for } k = 1, 2, \ldots, m\,. \tag{19}$$

[5]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 γ' whose allele does not produce $t_{g_s}(\gamma)$ or $t_{g_t}(\gamma)$ in g_3', where the existence of γ' 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 τ back to $g_3'(\gamma')$ avoids the potential break of the essential pathways.

The diagonal entries of $\mathbf{J}$ are the eigenvalues of $\mathbf{T}$ with multiplicities. The matrix $\mathbf{J}$ is called the **Jordan normal form** of $\mathbf{T}$, and the column vectors of $\mathbf{P}$ are called the **generalized eigenvectors** of $\mathbf{T}$. Similar to the derivation 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.

There are a few noteworthy points about factoring the matrix $\mathbf{T}$ by its generalized eigenvectors and its Jordan normal form. First, note that the generalized eigenvecotrs are linearly independent and form a basis for n-dimensional 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 $\left\{\mathbf{v}_i^{(k)}\right\}_{i=1}^{n_k}$ be the generalized eigenvectors corresponding to the eigenvalues in $\mathbf{J}_k$. Recalling from the notation in section 3.1, the initial distribution 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 combination of the generalized eigenvectors

$$\mathbf{p}^{(0)} \;=\; \sum_{k=1}^{m}\sum_{i=1}^{n_k} a_i^{(k)}\mathbf{v}_i^{(k)}\,. \tag{20}$$

Second, since $\mathbf{T}$ is a positive matrix (see section 3.1), by the Perron-Frobenius theorem, the size of the first Jordan block n_1 equals to 1. Specifically, the only entry in $\mathbf{J}_1$ is the leading eigenvalue λ_1, and $|\lambda_1| > |\lambda_k|$ for any $k = 2, 3, \ldots, m$. For convenience, we abuse the notation and write $\mathbf{v}_1 = \mathbf{v}_1^{(1)}$.

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

$$\begin{aligned}
\mathbf{p}^{(t-1)} \;&\propto\; \mathbf{T}^{t-1}\ \mathbf{p}^{(0)} \\
&=\; \mathbf{T}^{t-2}\ \cdot\ \sum_{k=1}^{m}\sum_{i=1}^{n_k} a_i^{(k)}\mathbf{T}\mathbf{v}_i^{(k)} \\
&=\; \mathbf{T}^{t-2}\ \cdot\ \sum_{k=1}^{m}\sum_{i=1}^{n_k} a_i^{(k)}\left[\lambda_k\mathbf{v}_i^{(k)} + (1-\delta_{i,1})\,\mathbf{v}_{i-1}^{(k)}\right] \\
&=\; \mathbf{T}^{t-2}\ \cdot\ \sum_{k=1}^{m}\sum_{i=1}^{n_k} \left[\lambda_k a_i^{(k)} + (1-\delta_{i,n_k})\,a_{i+1}^{(k)}\right]\mathbf{v}_i^{(k)} \\
&\;\;\vdots \\
&=\; \sum_{k=1}^{m}\sum_{i=1}^{n_k} \left[\lambda_k^{t-1}a_i^{(k)} + (1-\delta_{i,n_k})\left(\sum_{s=0}^{t-2}\lambda_k^s\right)a_{i+1}^{(k)}\right]\mathbf{v}_i^{(k)}\,.
\end{aligned} \tag{21}$$

Plugging (21) into the master equation (5), we have

$$\begin{aligned}
\mathbf{p}^{(t)} \;&=\; \frac{\mathbf{T}\cdot\sum_{k=1}^{m}\sum_{i=1}^{n_k}\left[\lambda_k^{t-1}a_i^{(k)} + (1-\delta_{i,n_k})\left(\sum_{s=0}^{t-2}\lambda_k^s\right)a_{i+1}^{(k)}\right]\mathbf{v}_i^{(k)}}{\mathbf{1}^\top(\mathbf{T}+\mathbf{R})\cdot\sum_{k=1}^{m}\sum_{i=1}^{n_k}\left[\lambda_k^{t-1}a_i^{(k)} + (1-\delta_{i,n_k})\left(\sum_{s=0}^{t-2}\lambda_k^s\right)a_{i+1}^{(k)}\right]\mathbf{v}_i^{(k)}} \\
&=\; \frac{\sum_{k=1}^{m}\sum_{i=1}^{n_k}\left[\lambda_k^{t}a_i^{(k)} + \frac{(\lambda_k^t-1)(1-\delta_{i,n_k})}{\lambda_k-1}a_{i+1}^{(k)}\right]\mathbf{v}_i^{(k)}}{\sum_{k=1}^{m}\sum_{i=1}^{n_k}\left\{\left[\lambda_k^{t}a_i^{(k)} + \frac{(\lambda_k^t-1)(1-\delta_{i,n_k})}{\lambda_k-1}a_{i+1}^{(k)}\right]\left(\mathbf{1}^\top\mathbf{v}_i^{(k)}\right) \;+\; \left[\lambda_k^{t-1}a_i^{(k)} + \frac{(\lambda_k^{t-1}-1)(1-\delta_{i,n_k})}{\lambda_k-1}a_{i+1}^{(k)}\right]\left(\mathbf{1}^\top\mathbf{R}\mathbf{v}_i^{(k)}\right)\right\}} \\
&=\; \frac{f_1^{(1)}(t)\ \mathbf{v}_1 \;+\; \sum_{k=2}^{m}\sum_{i=1}^{n_k} f_i^{(k)}(t)\ \mathbf{v}_i^k}{\left(f_1^{(1)}(t)\ \mathbf{1}^\top\mathbf{v}_1 \;+\; f_1^{(1)}(t-1)\ \frac{\mathbf{1}^\top\mathbf{R}\mathbf{v}_1}{\lambda_1}\right) \;+\; \sum_{k=2}^{m}\sum_{i=1}^{n_k}\left(f_i^{(k)}(t)\ \mathbf{1}^\top\mathbf{v}_i^{(k)} \;+\; f_i^{(k)}(t-1)\ \frac{\mathbf{1}^\top\mathbf{R}\mathbf{v}_i^{(k)}}{\lambda_1}\right)}\,,
\end{aligned} \tag{22}$$

where

$$f_i^{(k)}(t) \;=\; \left(\frac{\lambda_k}{\lambda_1}\right)^t a_i^{(k)} + \frac{(\lambda_k^t-1)(1-\delta_{i,n_k})}{\lambda_1^t(\lambda_k-1)}a_{i+1}^{(k)}\,. \tag{23}$$

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

E Convergence Rate to the Stationary Distribution of GRNs

In this section, we provide an estimate of the rate that the master equation (4) converges to its stationary distribution, using the technique known as the uniform minorization condition of Markov chains [71]. Specifically, given a sequence of probability distribution $\{p_t\}_{t=1}^{\infty}$ over a finite discrete space $\mathbb{X}$ which converges to $\pi = \lim_{t\to\infty} p_t$, we will find an upper bound of the variation distance

$$\|p_t - \pi\| \;=\; \sup_{A\subset\mathbb{X}} |p_t(A) - \pi(A)| \,. \tag{24}$$

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 tolerance ϵ.

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

$$F\left(g', g, t\right) \;=\; \frac{1}{\nu_t}\, \rho_{g'}\; \mu_{g'g}\; \nu_g \,. \tag{25}$$

Since $\nu_t = \mathbb{P}\left[\Psi\left(I_t\right)\right]$ is always less than or equal to 1, observe that

$$F\left(g', g, t\right) \;\geq\; \beta\; \zeta(g)\,, \tag{26}$$

where

$$\beta \;=\; \sum_{g\in\mathcal{G}} \min_{g'\in\mathcal{G}}\; \rho_{g'}\mu_{g'g}\nu_g \tag{27}$$

and ζ is a probability distribution over $\mathcal{G}$ such that $\zeta(g) = \frac{1}{\beta}\min_{g'\in\mathcal{G}} \rho_{g'}\mu_{g'g}\nu_g$ for any $g \in \mathcal{G}$. The inequality (26) is a uniform minorization condition, which has been recognized to elegantly estimate the convergence rate of Markov chains. We will adapt the derivation for Markov chains as reviewed by [71] and only summarize the key steps in what follows.

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

$$p\left(X_1\right) \;=\; \left\{\mathbb{P}\left[g\left(I_1\right) = g \mid \Psi\left(I_1\right)\right]\right\}_{g\in\mathcal{G}}\,, \tag{28}$$

$$p\left(Y_1\right) \;=\; \left\{\lim_{t\to\infty} \mathbb{P}\left[g\left(I_t\right) = g \mid \Psi\left(I_t\right)\right]\right\}_{g\in\mathcal{G}} \tag{29}$$

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

(i) With probability β, set $X_{t+1} = Y_{t+1}$ which follows the probability distribution ζ;

(ii) Otherwise, X_{t+1} and Y_{t+1} are independent random variables such that

$$\mathbb{P}\left[X_{t+1} = g \mid X_t = g'\right] \;=\; \frac{F(g', g, t) - \beta\zeta(g)}{1-\beta}\,, \tag{30}$$

$$\mathbb{P}\left[Y_{t+1} = g \mid Y_t = g'\right] \;=\; \frac{F(g', g, t) - \beta\zeta(g)}{1-\beta} \tag{31}$$

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

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

Suppose random variable T to be the first time step that scenario (i) occurs so $X_T = Y_T$. By construction, 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 $t \leq T$ 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 distribution of (4). It is not hard to see that the variation distance between two probability distributions is bounded from above by the probability that the two corresponding random variables are not equal (for details, see [...]), and

$$\begin{aligned} \|p_t - \pi\| &= \|p(X_t) - p(Z_t)\| \\ &\leq \mathbb{P}[X_t \neq Z_t] \\ &\leq \mathbb{P}[T > t] \\ &= (1-\beta)^t \,. \end{aligned} \tag{32}$$

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

$$t \;>\; \frac{\log(\epsilon)}{\log(1-\beta)} \,. \tag{33}$$