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The influence of assortativity on the robustness and evolvability of gene regulatory networks upon gene birth

Dov A. Pechenick [a], Jason H. Moore [a,*], Joshua L. Payne [b]

[a] Computational Genetics Laboratory, Dartmouth College, Hanover, NH, USA
[b] Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

HIGHLIGHTS

- We study how topology affects the robustness and evolvability of GRNs.
- We examine the effects of varying assortativity in models of GRNs.
- As assortativity increases, robustness increases and evolvability decreases.
- Increased assortativity reduces attractor sizes, which leads to higher robustness.
- Increased assortativity reduces out-component sizes, which causes lower evolvability.

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ABSTRACT

Gene regulatory networks (GRNs) represent the interactions between genes and gene products, which drive the gene expression patterns that produce cellular phenotypes. GRNs display a number of characteristics that are beneficial for the development and evolution of organisms. For example, they are often robust to genetic perturbation, such as mutations in regulatory regions or loss of gene function. Simultaneously, GRNs are often evolvable as these genetic perturbations are occasionally exploited to innovate novel regulatory programs. Several topological properties, such as degree distribution, are known to influence the robustness and evolvability of GRNs. Assortativity, which measures the propensity of nodes of similar connectivity to connect to one another, is a separate topological property that has recently been shown to influence the robustness of GRNs to point mutations in *cis*-regulatory regions. However, it remains to be seen how assortativity may influence the robustness and evolvability of GRNs to other forms of genetic perturbation, such as gene birth via duplication or *de novo* origination. Here, we employ a computational model of genetic regulation to investigate whether the assortativity of a GRN influences its robustness and evolvability upon gene birth. We find that the robustness of a GRN generally increases with increasing assortativity, while its evolvability generally decreases. However, the rate of change in robustness outpaces that of evolvability, resulting in an increased proportion of assortative GRNs that are simultaneously robust and evolvable. By providing a mechanistic explanation for these observations, this work extends our understanding of how the assortativity of a GRN influences its robustness and evolvability upon gene birth.

1. Introduction

Gene expression determines cellular phenotype. The regulation of gene expression in turn governs the ability of a cell to respond to a new environment (Gasch et al., 2000; Causton et al., 2001) or differentiate along a particular lineage (Davidson et al., 2002; Huang et al., 2005). Understanding gene regulation at molecular resolution and how it results in stable, measurable phenotypes is one of the major ongoing challenges in evolutionary and developmental biology (Davidson, 2006).

The entirety of a cell's regulatory interactions can be conceptualized as a gene regulatory network (GRN), where genes are represented as nodes and regulatory interactions as edges. The gene expression patterns that produce cellular phenotypes are dictated by the dynamics of the GRN. Both experimental and theoretical studies have shown that GRNs possess certain attributes that contribute to the growth and perpetuation of organisms. For instance, GRNs can often maintain their function in the

* Correspondence to: 706 Rubin Cancer Center, HB 7922, One Medical Center Drive, Dartmouth Hitchcock Medical Center, Lebanon, NH 03756, USA.
Tel.: +1 603 653 9939.
E-mail addresses: dov.pechenick@dartmouth.edu (D.A. Pechenick), jason.h.moore@dartmouth.edu (J.H. Moore), joshua.payne@ieu.uzh.ch (J.L. Payne).

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face of genetic perturbation, a property known as robustness (Wagner, 2005). Illustrative examples include gene knockout in the yeast *Saccharomyces cerevisiae* (Jeong et al., 2001) and GRN rewiring in the bacterium *Escherichia coli* (Isalan et al., 2008); in both cases, genetic perturbations often fail to alter a growth phenotype. Theoretical models of GRNs have not only recapitulated this robustness (Wagner, 1994; Aldana et al., 2007), but have shown that robustness itself is an evolvable property (Ciliberti et al., 2007b).

Experimental (Guet et al., 2002; Hunziker et al., 2010) and theoretical studies (Aldana et al., 2007; Ciliberti et al., 2007a) have also shown that GRNs can respond to mutation by innovating phenotypes, and are therefore intrinsically evolvable (Wagner, 2011). For example, a diverse set of phenotypic responses to environmental conditions, akin to Boolean logic gates, was obtained by rewiring synthetic 3-gene regulatory circuits in *E. coli* (Guet et al., 2002). Adaptive evolution necessitates the innovation of such phenotypes, and the ability to generate new regulatory programs therefore confers a selective advantage (Levine and Tjian, 2003). And, like robustness, this ability has itself been shown to be an evolvable property in GRNs (Crombach and Hogeweg, 2008).

Extant GRNs are a product of mutation and selection, and a major mutational force that drives their evolution is the addition of new genes. New genes are often introduced via gene duplication (Ohno, 1970; Zhang, 2003; Conant and Wolfe, 2008), and the subsequent regulatory and biochemical divergence of the duplicate is thought to impact the growth and evolution of GRNs (Babu and Teichmann, 2003; Teichmann and Babu, 2004). New genes are also introduced via *de novo* origination (Tautz and Domazet-Lošo, 2011), which is now considered to be more important than previously appreciated (Carvunis et al., 2012). In either case, the introduction of a new gene is a perturbation that is most often detrimental (Lynch and Conery, 2000) and only rarely beneficial to the organism (Carvunis et al., 2012). Yet, the abundance of genetic material in living organisms that has been attributed to duplication (Lynch and Conery, 2000) and *de novo* origination (Carvunis et al., 2012) is a testament to the occasional success of these genetic perturbations. This occasional success is mirrored in theoretical models of GRNs, which not only find that the addition of new genes is sometimes tolerated, but also that it may permit the exploration of novel phenotypes (Aldana et al., 2007). However, it is not fully understood how the intrinsic properties of GRNs allow for the conservation of existing phenotypes (robustness) while simultaneously facilitating the exploration of novel phenotypes (evolvability).

The structural makeup of GRNs may help clarify this issue. Several theoretical analyses have demonstrated that the robustness and evolvability of GRNs are influenced by their underlying topological properties (Variano et al., 2004; Poblanno-Balp and Gershenson, 2011). For example, GRNs possess heavy-tailed distributions of the number of regulatory targets per gene (Babu et al., 2004), and qualitatively similar degree distributions have been shown to yield increased robustness to genetic perturbation (Aldana and Cluzel, 2003) and an enhanced capacity to evolve novel phenotypes (Oikonomou and Cluzel, 2006), as compared to homogeneous random degree distributions.

Assortativity is a separate topological property, which can be used to measure the tendency for pairs of connected nodes in a network to possess similar numbers of connections (Newman, 2002). This property can vary between networks, even if they possess identical degree distributions, and can affect their dynamical behavior (Pomerance et al., 2009; Pechenick et al., 2012). Assortativity is known to vary among real-world networks (Newman, 2002; Foster et al., 2010), and a recent study reported that the assortativity of GRNs tends to be positive (*i.e.*, assortative) (Piraveenan et al., 2012), whereas random networks with similarly heterogeneous degree distributions tend to be negative (*i.e.*, disassortative) (Johnson et al., 2010). In the context of a GRN composed primarily of transcription factors (TFs), this positive assortativity might reflect that TFs that regulate a large number of other TFs tend to mutually regulate each other more often than would be expected by chance. It could also reflect that those TFs tend not to fall under extensive regulation by TFs that only regulate a few other TFs. The reason for the purported assortativity of GRNs is unknown, but recent theoretical results suggest that assortative GRNs may have an advantage over disassortative GRNs due to an increased robustness to mutations in the *cis*-regulatory logic of their constituent genes (Pechenick et al., 2012).

While the robustness of a GRN influences its evolutionary success, the observed robustness to mutation in *cis*-regulatory regions (Pechenick et al., 2012) does not necessarily imply robustness to other perturbations. Given the apparent assortativity of GRNs (Piraveenan et al., 2012), and the evolutionary significance of gene duplication (Lynch and Conery, 2000) and *de novo* origination (Carvunis et al., 2012), it is important to understand whether the assortativity of a GRN influences its robustness to such genetic perturbations. Further, since gene birth may result in the advent of novel phenotypes, it is also important to understand how the assortativity of a GRN influences evolvability. Unfortunately, it is currently not possible to address such questions in an experimental system. While the construction of small synthetic regulatory circuits in cells is possible (Gardner et al., 2000; Elowitz and Leibler, 2000; Purnick and Weiss, 2009), the relatively large GRNs that are needed to vary assortativity at high resolution make the direct testing of these questions impractical. We therefore employ an abstract computational model of genetic regulation (Kauffman, 1969) to construct GRNs with different values of assortativity and then assess the rates at which they: (1) conserve their existing phenotypes following the introduction of a new gene, and (2) innovate new phenotypes as a result of the same perturbation. We thereby provide theoretical insight into how assortativity may affect the robustness and evolvability of GRNs upon gene birth.

2. Methods

2.1. Boolean networks

We used Boolean networks to model GRNs (Kauffman, 1969) (Fig. 1). In this model, genes are represented as nodes and regulatory interactions as directed edges. These edges emanate from nodes that are *regulators* and terminate at nodes that are

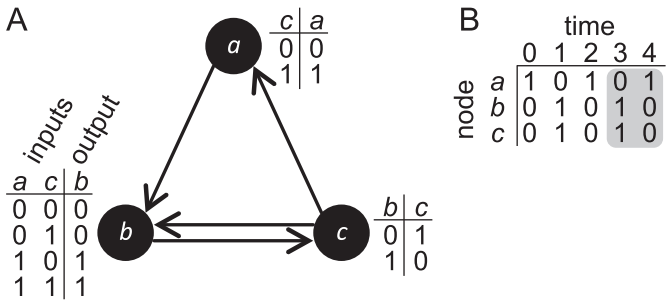

Fig. 1. A Boolean network example. (A) This Boolean network is composed of 3 nodes and 4 directed edges. Each node possesses a look-up table with the signal-integration logic that determines the dynamics of the Boolean network by defining the expression state of the node at time t+1 as a function of the states of its inputs at time t. For example, the signal-integration logic for node b shows how each possible combination of expression states $\sigma_a(t)$ and $\sigma_c(t)$ of the inputs at time t dictate the expression state $\sigma_b(t+1)$. (B) Starting with initial states at $t=0$, the states are updated according to the signal-integration logic until they repeat, forming an attractor (shaded region), which is analogous to a phenotype. In this example, the attractor length is two. For visual clarity, the size of the network depicted here is much smaller than those used in this study.