

Networking networks

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A major challenge for evolutionary biology is the reconstruction of the temporal sequences of evolutionary changes that have created particular phenotypes. The last decade has witnessed spectacular progress on the molecular front of this research program. For several years, ancient genes have been routinely reconstructed, synthesized, expressed, and functionally characterized (Thornton 2004). More recently, advances have been made in reconstructing the evolution of interactions between molecules, such as steroid hormones and their receptors (Bridgham et al. 2006), suggesting that data on larger networks of genetic interactions underlying complex traits cannot be far behind. Andreas Wagner (2005) has introduced a concept—the metagraph of gene regulatory networks—that holds much promise to illuminate such analyses.

The pattern of mutational connectivity among genotypes could also be described as a “network,” but we will instead refer to it here as a mutational graph to avoid confusion (“graph” is a technical name for a network). Two genotypes are connected in a mutational graph if one genotype can be obtained from the other through a single mutation. Until recently, mutational graphs have largely been studied in the context of sequence evolution (reviewed in Cowperthwaite and Meyers 2007). However, Wagner and collaborators have now begun to consider a mutational graph where the nodes are, themselves, regulatory networks (Wagner 2005; Ciliberti et al. 2007; for a related analysis see MacCarthy et al. 2003). They have called this special kind of mutational graph a metagraph, or graph of graphs. Mutational steps (connections) in the metagraph consist of single qualitative changes to an interaction between two genes (e.g., from present to absent, from positive to negative), that is, to the wiring of the regulatory network. Figure 1 shows a portion of the metagraph of all possible three-gene regulatory networks.

How does evolution operate in the world of metagraphs? Imagine a population of asexually reproducing, haploid organisms. Each node of the metagraph represents a gene-network genotype. Individuals distribute themselves over the nodes of the metagraph based on their network genotypes. When an individual is born, it will either appear in the node occupied by its parent or move to a node some distance away from the parental node, depending on how many mutations it

has acquired. Evolution, then, consists of changes through time in the proportions of individuals at each node of the metagraph. Two network genotypes may be connected by many potential evolutionary trajectories. For example, the lines highlighted in magenta and green display two possible trajectories connecting the feedback and feedforward networks. Other things being equal, evolution is more likely to follow trajectories with fewer steps like the magenta one.

The metagraph becomes more compelling when we consider the phenotypes associated with each gene network, such as the temporal patterns of gene expression the network can sustain. For example, Wagner (2005) constructed a metagraph of 378 gene networks. Each network was an interlocked Goodwin oscillator (Goodwin 1965), containing six gene products encoded by two genes: one RNA (R), protein (P), and modified protein (P^*) per gene. There were 10 possible molecular interactions between the six gene products: four were fixed ($R \rightarrow P \rightarrow P^*$, for each gene), and the other six were allowed to evolve ($P_1^* \rightarrow R_1$, $P_1^* \rightarrow R_2$, $P_1^* \rightarrow P_2^*$, and their reciprocals). Wagner used the metagraph to investigate the evolution of a gene network’s ability to produce circadian limit-cycle oscillations. He found that the 378 gene networks differed extensively in the fraction P of the space of parameters, such as the rates of synthesis and decay of different gene products, over which circadian oscillations were supported. Although most networks (201/378) produced oscillations for at least 0.02% of random parameter combinations ($P > 0.02\%$), a small number (47) oscillated for $P > 1\%$, and two networks were actually quite good at it ($P > 10\%$). For a given gene network, P was positively correlated with the robustness of its circadian oscillations to changes in parameter values (Wagner 2005).

Are good circadian oscillators easily evolvable from poorly oscillating ancestors? Wagner’s insight was that this is essentially a question about the pattern of connections between those networks capable of circadian oscillations. Two extreme scenarios are conceivable. First, oscillating networks may occupy many disconnected “islands” in the metagraph. This would mean that the best oscillators cannot evolve gradually from mediocre ones. Rather, populations would tend to get stuck in the local optima of individual islands. Second,

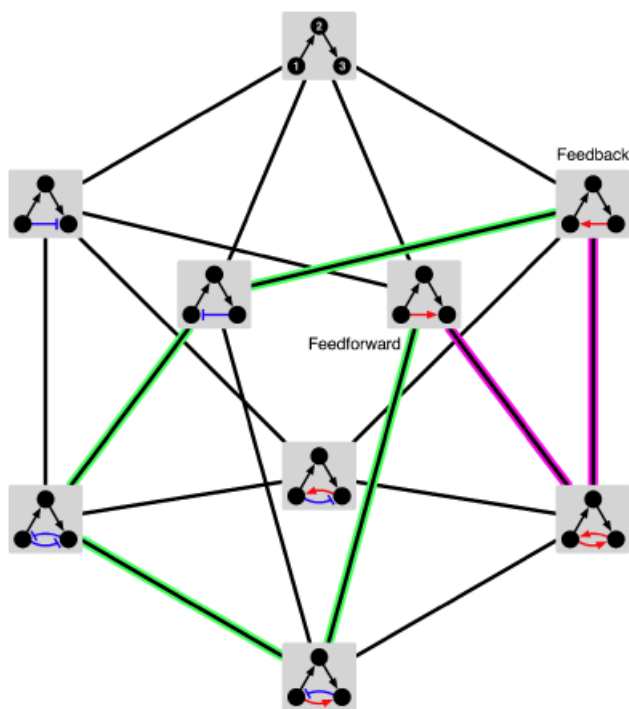


Fig. 1. Example of a metagraph of gene networks. Each box contains a three-gene regulatory network. Connections between genes in each network indicate that the product of one gene regulates the expression of another; arrows and bars denote activation and repression, respectively. Lines between boxes indicate that the connected networks differ in a single interaction between two genes—positive, negative, or nonexistent. A linear pathway, $1 \rightarrow 2 \rightarrow 3$, is shown in the top network. The remaining networks illustrate the addition of all possible types of interaction between genes 1 and 3 (in red or blue) to this pathway. Magenta and green lines show two of many hypothetical evolutionary trajectories connecting the positive feedback and positive feedforward networks. The complete metagraph of all possible three-gene networks (including self-interactions) would contain 3411 gene networks with different wiring patterns and 29,610 connections between them.

oscillating networks may form a completely connected submetagraph, such that any two gene networks are connected by many alternative paths. This scenario would imply that a good oscillator is accessible by “numerous, successive, slight modifications” (Darwin 1859) from the gene network of a mediocre oscillator. Wagner’s metagraph conformed to the latter evolvability scenario. When he deleted all nodes and connections from the metagraph apart from the subset of 47 networks (nodes) capable of producing oscillations for $P > 1\%$, and the direct connections between them, he was left with a completely connected submetagraph. This result indicates that once a network capable of driving circadian oscillations is discovered, a highly robust oscillator can evolve easily by gradual changes in genetic interactions (Wagner 2005). The topology of the metagraph suggests that this result

was not inevitable. When Wagner surveyed subsets of 47 nodes picked at random from the metagraph (i.e., independently of their ability to drive oscillations), he found that they tended to be split into approximately 20 disconnected islands. In a follow-up article, Wagner has shown that robust gene expression patterns can evolve gradually in other types of gene regulatory networks as well (Ciliberti et al. 2007).

Although the metagraph approach has been developed in the context of the evolution of robustness, we believe that it can be applied to many other aspects of gene-network evolution. The metagraph concept provides a straightforward way to model the evolution of architectural features of gene networks such as connectivity, clustering, and modularity (Fig. 1; Barabási and Oltvai 2004). By overlaying fitnesses on the metagraph, Wagner’s approach brings decades of theoretical work on adaptive landscapes to bear on the evolution of gene networks. Further integration of the mutational graph and adaptive landscape approaches to evolution promises to strengthen evolutionary theory (Cowperthwaite and Meyers 2007). For example, mutational, developmental, and selective constraints or biases (Arthur 2004) can be expressed naturally in terms of variation in connectivity across the metagraph as selection acts during development. As comparative data on the wiring of gene networks become available, we can begin to estimate real evolutionary trajectories through metagraphs (Weinreich et al. 2006), thus bringing this concept to life.

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