ORIGINAL ARTICLE

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# Robustness to noise in gene expression evolves despite epistatic constraints in a model of gene networks

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**Stochastic noise in gene expression causes variation in the development of phenotypes, making such noise a potential target of stabilizing selection. Here, we develop a new simulation model of gene networks to study the adaptive landscape underlying the evolution of robustness to noise. We find that epistatic interactions between the determinants of the expression of a gene and its downstream effect impose significant constraints on evolution, but these interactions do allow the gradual evolution of increased robustness. Despite strong sign epistasis, adaptation rarely proceeds via deleterious intermediate steps, but instead occurs primarily through small beneficial mutations. A simple mathematical model captures the relevant features of the single-gene fitness landscape and explains counterintuitive patterns, such as a correlation between the mean and standard deviation of phenotypes. In more complex networks, mutations in regulatory regions provide evolutionary pathways to increased robustness. These results chart the constraints and possibilities of adaptation to reduce expression noise and demonstrate the potential of a novel modeling framework for gene networks.**

**KEY WORDS:** **Adaptation, epistasis, models/simulations.**

Developmental noise—the phenotypic variation caused by intrinsic sources of stochasticity or microenvironment variation—has interested evolutionary biologists since Waddington and others first articulated the idea that development was canalized (Waddington 1942; Gibson and Wagner 2000). Experiments have shown that the magnitude of developmental noise is heritable and therefore can evolve (Clarke and McKenzie 1987). The possibility of evolution of developmental noise leads to a range of theoretical predictions. Selection for a specific optimal phenotype is expected to favor a reduction in developmental noise, at least until the costs or pleiotropic effects of such adaptations outweigh the benefits of further reduction (Gavrilets and Hastings 1994; Wagner et al. 1997). However, greater variability may be favored when populations are poorly adapted (Tănase-Nicola and ten Wolde 2008) or when the environment is fluctuating, particularly when such changes are infrequent or costly to predict (Kussell and Leibler 2005). By contributing variation in reproductive success, noise can reduce the effective population size, potentially slowing the overall rate of adaptation (Wang and Zhang 2011). Studies have also suggested that evolutionary change in developmental noise may cause parallel changes in mutational robustness (de Visser et al. 2003). Evolutionary responses to noise may therefore shape the adaptive potential of populations through the link between robustness to mutation and evolvability (Ancel and Fontana 2000; Wagner 2005; Bloom et al. 2006; Elena and Sanjuán 2008; McBride et al. 2008; Cuevas et al. 2009; Draghi et al. 2010; Masel and Trotter 2010; Lauring et al. 2012; Stewart et al. 2012; Goldhill et al. 2014). Understanding how species have adapted to the ubiquitous challenge of developmental noise may therefore explain the variational properties of organisms (Wagner and Altenberg 1996) as well as the structure of gene networks (Alon 2006; Chalancon et al. 2012).

Although early interest in developmental noise arose from embryology, recent work on stochastic bet-hedging in microbes (Veening et al. 2008) and advances in single-cell measurements have focused attention on the extent to which

protein abundances vary among clonal cells due to chance differences. This specific category of phenotypic noise may arise from common cellular mechanisms and help explain phenotypic variation at larger scales. In particular, new experiments that control for differences in cellular state show that much of this noise in protein expression is intrinsic to the processes of regulation, transcription, and translation (Elowitz et al. 2002; Swain et al. 2002; but see Raser and O'Shea 2005; Raj and van Oudenaarden 2008). A dominant cause of this intrinsic noise is the low abundances of mRNA molecules in a cell (Kærn et al. 2005; Bar-Even et al. 2006; Cai et al. 2006; Newman et al. 2006; Yu et al. 2006).

Noise in expression can be beneficial in specific scenarios (Silva-Rocha and de Lorenzo 2010), but may often impose a cost, and selection may act to reduce expression noise in critical genes (Fraser et al. 2004; Batada and Hurst 2007; Lehner 2008). Intuitively, negative autoregulation is an obvious solution to intrinsic noise: if the expression of a gene is regulated by its own products, then this autoregulation could compensate for stochastic over- or underproduction. However, recent theoretical work has challenged this intuition, suggesting that negative feedback can reduce expression noise only in limited circumstances (Stekel and Jenkins 2008; Marquez-Lago and Stelling 2010; Lestas et al. 2010; Stewart et al. 2013). A different way to reduce noise stems from the idea that protein concentrations are expected to be less noisy for genes with higher levels of transcription and lower rates of translation (Thattai and van Oudenaarden 2001; Ozbudak et al. 2002; Fraser et al. 2004). Organisms might therefore reduce expression noise for critical genes by producing more transcripts and reducing the downstream effect of each one, via changes in the decay rate or translational efficiency of the mRNA, or in the decay rate or activity of the protein product. Costs of mRNA production might pose one limit to this mechanism of adapting to intrinsic noise, but this scenario also faces an evolutionary problem: any single change in transcription rate, translation efficiency, or protein effect will perturb the means of downstream phenotypes as well as their variances. Without a concomitant change in some other parameter, an increase in transcription might therefore be deleterious. If adaptation via the increased expression of critical genes requires the substitutions of mutations that are typically deleterious, then the evolution of robustness to intrinsic noise might therefore be a widespread example of evolutionary constraints creating epistasis for fitness.

Gene network models have been used to investigate how epistasis shapes adaptation, particularly with regard to mutational robustness (Wagner 1996; Siegal and Bergman 2002; Azevedo et al. 2006; Kaneko 2007; Leclerc 2008; van Dijk et al. 2012; Pujato et al. 2013) and evolvability (Crombach and Hogeweg 2008; Draghi and Wagner 2009; Fierst 2011; Wagner 2011; Draghi and Whitlock 2012; Macía et al. 2012; Pujato et al. 2013). However, few evolutionary models have considered noise, and most of the exceptions (Ciliberti et al. 2007; Kaneko 2007; Braunewell and Bornholdt 2008; Sevim and Rikvold 2008; Rohlf and Winkler 2009; Espinosa-Soto et al. 2011) have revealed interesting links between adaptation and noise but have not modeled expression with sufficient detail to permit the study of robustness to the stochastic production of transcripts. Biophysicists have devised sophisticated models of stochastic gene expression and have begun to explore the evolution of such modeled networks (Kratz et al. 2008; Krishnan et al. 2008; Jenkins and Stekel 2010). However, in comparison to the importance of the topic, very few studies have modeled gene networks with equal weight given to the biophysical mechanisms of stochastic expression and the evolutionary mechanisms of inheritance, mutation, and selection.

We developed a new simulation model of transcriptionally regulated gene networks. This approach simulates the mechanisms behind intrinsic noise in gene expression. The model tracks discrete abundances of both mRNAs and proteins for several genes and models production and decay of both types of molecules as stochastic processes. Regulation is simulated in much more detail than in previous evolutionary models (e.g., Wagner 1996), but our model is still sufficiently simplified to allow for relatively rapid computation of the phenotypes of large populations over thousands of generations. The model includes several categories of mutations affecting the transcription rates of genes, as well as parameters for the downstream effects of proteins. It is therefore well suited to study the epistatic constraints on the evolution of robustness to intrinsic noise.

We begin this article with a short section describing some simple analytic predictions about the coevolution of gene expression and effect. This model makes some straightforward predictions about how the mean phenotype and developmental noise should evolve in the face of stochastic expression of mRNA and proteins. The bulk of the paper describes the new simulation model. Results from these simulations confirm the simple predictions of the mathematical approximations: genes can evolve higher expression and lower per protein effects through a sequence of beneficial changes. These results also demonstrate how feedback can evolve alongside these changes to further reduce harmful developmental noise. Epistatic constraints do limit the rate of adaption to less noisy genotypes, but adaptation can proceed without a significant role for deleterious substitutions or adaptive valley crossing. Finally, we explore more complex networks and document how mutations with network-wide effects can further circumvent evolutionary constraints on the evolution of noise.

## A Simple Approximate Model of Gene Expression

We developed a simple mathematical model of stochasticity in genes expressed without feedback to help articulate one hypothesis for how robustness to intrinsic noise might evolve. Focusing only on the stochasticity inherent in the production of transcripts, we model protein production (at rate $\psi$ per minute per mRNA) and decay of both proteins (at rate $\lambda_P$) and mRNAs (at rate $\lambda_R$) as deterministic processes. This equates to assuming that each protein or mRNA molecule exists for $1/\gamma_P$ or $1/\gamma_R$ minutes, respectively, before decaying, and that exactly $\psi$ proteins are produced by each mRNA per minute. We also ignore the complication of lags between the production of a molecule and its phenotypic effect. We therefore focus entirely on the effects of two evolvable traits: $Y$, the total number of transcripts produced during development, and $\beta$, the phenotypic effect of each molecule of the protein. The phenotype is a random variable $z_i$ defined by

$$z_i \sim c\beta \,\text{Poisson}\,(Y)\,, \tag{1}$$

where

$$c = \frac{\psi}{\gamma_R \gamma_P}.$$

$z_i$ has moments derived from the standard Poisson:

$$\mu_z = c\beta Y, \tag{2}$$

$$\sigma_z = c\beta\sqrt{Y}. \tag{3}$$

We assume that the trait experiences stabilizing selection with a Gaussian function:

$$w_z = \exp\left(-\frac{(z - z_{opt})^2}{2{\sigma_{opt}}^2}\right), \tag{4}$$

where $z_{opt}$ is the optimum phenotype and $\sigma^2_{opt}$ represents the width of the Gaussian selection function, by analogy with the variance of a Gaussian distribution.

If we allow negative values of $z$ instead of truncating at zero and make the further assumption that the Poisson distribution is well approximated by the Gaussian over the range of $Y$, then we can express mean fitness as the integral of the product of two Gaussian functions:

$$\bar{w}\,(Y, \beta) = \frac{1}{\sigma_z\sqrt{2\pi}} \int_{-\infty}^{\infty} e^{-\frac{(z\,-\,\mu_{opt})^2}{2\sigma^2_{opt}}} e^{-\frac{(z\,-\,\mu_z)^2}{2\sigma^2_z}} dz$$

$$\bar{w}\,(Y, \beta) = \exp\left(-\frac{(\mu_{opt} - \mu_z)^2}{2\left(\sigma^2_z + \sigma^2_{opt}\right)}\right) \frac{\sigma_{opt}}{\sqrt{\sigma^2_z + \sigma^2_{opt}}}$$

$$\bar{w}\,(Y, \beta) = \exp\left(-\frac{\left(\mu_{opt} - c\beta Y\right)^2}{2\left(c^2\beta^2 Y + \sigma^2_{opt}\right)}\right) \frac{\sigma_{opt}}{\sqrt{c^2\beta^2 Y + \sigma^2_{opt}}}\,. \tag{5}$$

Figure 1 shows two views of the fitness landscape calculated from the above equation. To investigate the fundamental evolutionary properties of this landscape, we used numerical optimization to find the best expression level given a specific phenotypic effect, and the converse. The thick line in Figure 1A depicts the changes as each variable is alternatingly optimized; this process is equivalent to a population evolving through sequential, optimally beneficial mutations. Note that the optimal trait combinations are always below the dashed line, which represents combinations which produce the optimal mean. Figure 1B explains why this fitness landscape favors suboptimal means by showing the same mutations translated into changes in the mean phenotype and its standard deviation. Mutations in either expression ($Y$) or effect ($\beta$) produce correlated changes in both the mean and noise; however, the slope of these correlations differs, as predicted by equations (2) and (3). Therefore, a reduction in phenotypic effect can worsen the mean while improving noise, with a net positive effect on fitness. Subsequently, an increase in expression can improve the mean while increasing noise, again with a beneficial net effect. The combined effect of both mutations is a small net improvement in both traits. As illustrated in Figure 1B, repetitions of this cycle can effectively cause the population to "tack" up the gradient of the fitness landscape. Although both types of mutations are constrained to positively correlated pleiotropic effects on the mean and standard deviation, selection achieves a gradual increase in the mean and a decrease in noise.

Conceptually, this landscape is defined by a rising "ridge" leading from low-expression, high-effect genes with noisy phenotypes to high-expression, low-effect genes with narrow distributions. Mutations in either phenotypic effect or expression move at acute angles to this ridge, such that each beneficial mutation moves both up the ridge and perpendicular to it. Stronger selection (smaller $\sigma_{opt}$) therefore has two effects on this process—the ridge ascends more quickly, but also drops off more steeply on its sides. Using adaptive walks, we explored whether stronger selection accelerates or impedes adaptation to noise. Figure S1A shows that stronger selection leads to a faster response to selection. Stronger selection does reduce the size of the average substitution in terms of the change in the log parameter values ($0.05 \pm 0.002$ for $\sigma_{opt} = 200$; $0.065 \pm 0.002$ for $\sigma_{opt} = 500$; $0.075 \pm 0.003$ for $\sigma_{opt} = 800$), but also increases the number of mutations that substitute (out of one million proposed mutations, $102 \pm 0.33$ substitute for $\sigma_{opt} = 200$; $70 \pm 0.27$ for $\sigma_{opt} = 500$; $54 \pm 0.23$ for $\sigma_{opt} = 800$). Figure S1B shows that the selection coefficients of optimal changes in phenotypic effect (corresponding to the dotted lines

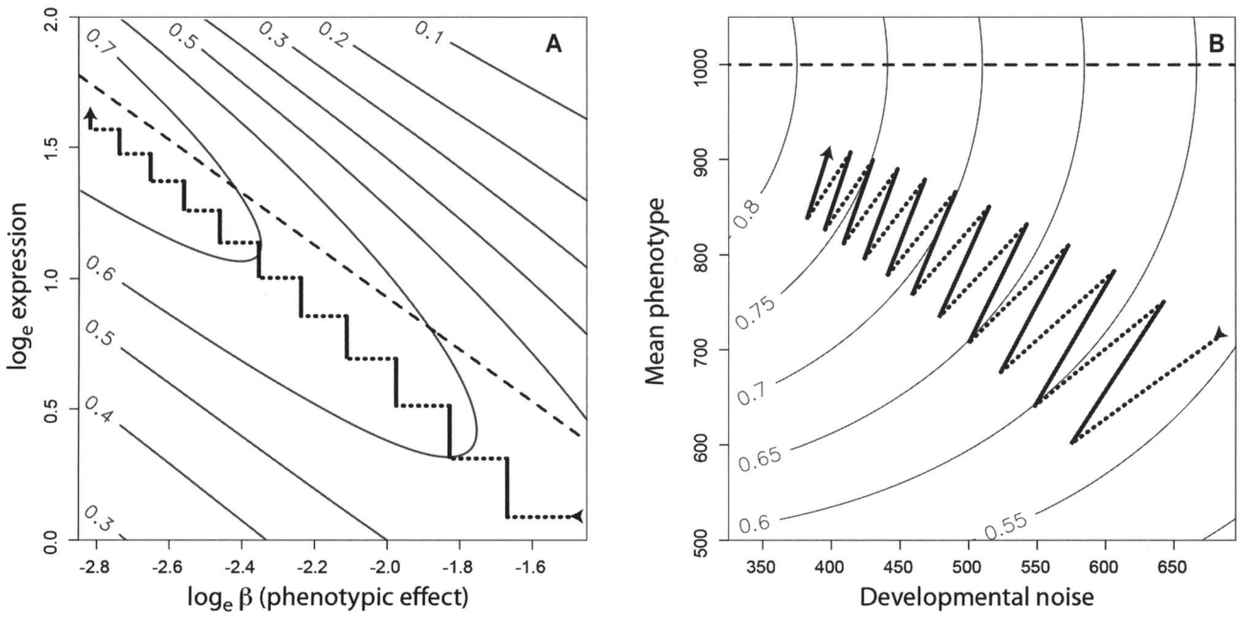


**Figure 1.** **Fitness landscapes of the Gaussian one-gene model. Contour lines are drawn according to equation (5) based on a phenotypic optimum of 1000 and Gaussian selection with $\sigma_{opt} = 500$. The dashed line depicts the values of phenotypic effect and mean lifetime expression that produce the optimal mean. The thick lines depict the effects of optimal beneficial mutations that change either phenotypic effect (dotted) or expression (solid). (A) Optimal adaptive steps favor alternating decreases in β and increases in expression. (B) The same series of mutations either decrease both the phenotypic mean and noise (dotted), or increase both (solid). Alternating changes in both expression and phenotypic effect achieve a net improvement in both mean and noise.**

in Fig. 1) shrink as noise decreases, but are overall larger when stabilizing selection is stronger. Noisy genotypes do suffer a reduced rate of adaptation in stochastic simulations of invasion, particularly when selection is strong (dotted lines in Fig. S1). This additional constraint is caused by the excess variation in reproductive success that results from high phenotypic variance, which reduces the effective population size (Wang and Zhang 2011); the effect, however, is quite small.

## *Simulation Methods*

### OVERVIEW: ENTWINE MODEL

Our goal is to build a model of gene regulation and development which is sufficiently abstract to be general and computationally tractable, but which also allows developmental noise, epistasis, pleiotropy, and plasticity to arise by realistic mechanisms. We named this model Evolving NeTworks WIth NoisE (ENTWINE). We focus on a small gene network expressed in a single eukaryotic cell over a period of hours (Fig. 2). Genes belong to one of two essential types—*regulatory* genes and those that directly construct the *phenotype*. Regulatory genes produce protein products that can regulate both regulatory and phenotype genes. Phenotype genes may also regulate other genes and determine the growth rate of one or more phenotypic traits. Neither type of gene can mutate to the other, but genes of each class may be deleted, or may duplicate and diverge. Each gene expresses a unique transcript from which proteins are translated. By implementing a model of regulation grounded in biophysical mechanisms, we allow genes to respond to external and internal signals and affect each others' expression by *cis*-regulatory interactions. To focus on *cis*-regulation, we fix the rate of translation, and the rates of decay of both mRNA and proteins, for all genes. Therefore, mutations may only affect regulation by altering the rate of transcription of a gene; proteins are produced at a uniform rate per mRNA molecule, regardless of the specific gene.

Protein levels in a cell are typically subject to substantial intrinsic noise because mRNA transcripts are often present in small numbers (Bar-Even et al. 2006; Cai et al. 2006; Newman et al. 2006; Yu et al. 2006). Stochasticity in rates of transcription and mRNA lifetimes cause random noise in the abundances of even highly expressed proteins; proteins that are present in small numbers are subject to additional noise from the stochasticity of translation and protein decay. In our model, both transcripts and proteins are produced as Poisson processes scaled by propensities, which represent the sums of regulatory effects, and both are removed by stochastic exponential decay. Although this approach incorporates several important sources of biological noise, it does not span all potentially important mechanisms of random

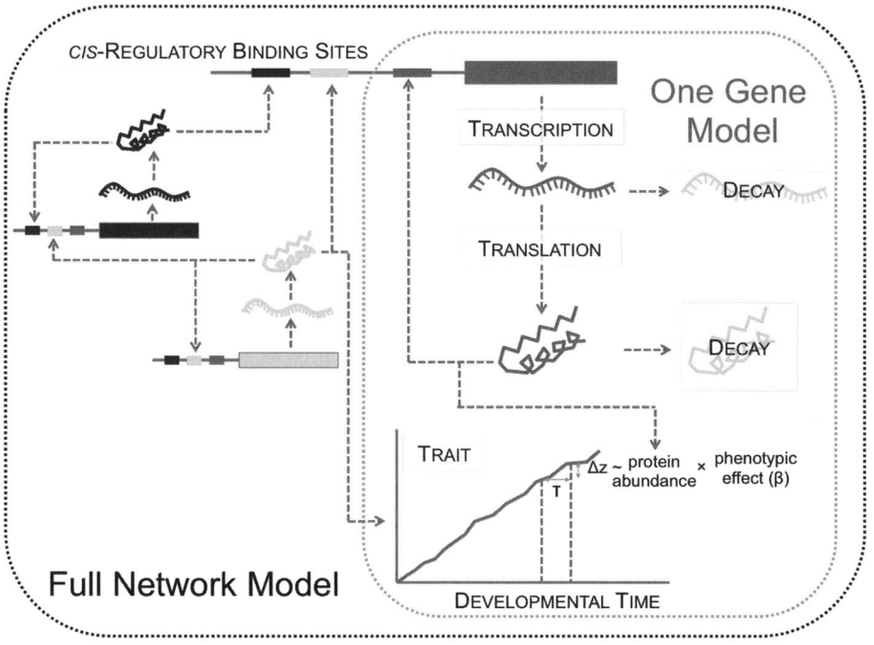


**Figure 2.** **Schematic diagram of the model. Inner box: A model of a single gene with both regulatory and phenotypic effects. Production and decay of both mRNAs and proteins are modeled discretely and stochastically. Protein abundances have regulatory effects on the rate of transcription by interactions with binding sites, located in *cis* with each gene. A trait develops by incremental change over periods of time, τ, which are calculated dynamically. The rate of trait increase over each interval τ is proportional to the abundance of each protein times its phenotypic effect. Outer box: A schematic of a network of multiple genes, some of which have only regulatory functionality.**

variation in gene expression. We assume that transcription factor binding turns over quickly and that transcription events are uncorrelated, which may be approximately true for some organisms such as *Saccharomyces cerevisiae* (Zenklusen et al. 2008, but see Pedraza and Paulsson 2008). Some other mechanisms of noise, such as transcription reinitiation or slow changes in gene accessibility, are clearly important sources of noise in some systems (Dar et al. 2012) but would require a more complex framework to model them.

Some parameters of our model are assigned fixed values from the literature; however, many aspects of a gene can evolve. As detailed below and in the supplement, *cis*- and *trans*-factors determining the expression of a gene are mutable, as are the targets and effects of regulatory proteins and the trait effects of proteins with phenotypic effects. For example, autoregulatory feedback involves four mutable aspects of the genotype: the *cis*-regulatory target motif of the protein, the protein's inherent regulatory effect, the binding affinity of the targeted binding site, and the *cis*-regulatory effect associated with that binding site. Mutations in any of these variables can change autoregulation, but each has different mutational rules and different implications for further evolutionary change.

Below, we briefly describe the three main levels of our model. Additional details and references are provided in the Supporting Information.

## GENOTYPE-TO-PHENOTYPE MAP

### *Simulating developmental dynamics*

Development is modeled as a stochastic chemical system with integer numbers of proteins and mRNA transcripts and discrete transcription, translation, and decay events. A modified version of Gillespie's τ-leaping algorithm (Gillespie 2007) is used to follow the dynamics for a predetermined number of time units $t_{\max}$. This method assumes that, within the interval τ, the productions of proteins and transcripts can be treated as Poisson processes with constant rates. We choose τ with regard to the expected change in rates, aiming to keep the relative change in any rate over the interval τ below the threshold ε. Using the observed change in each rate over the previous interval τ', we calculate τ based on the current value of each rate, $r_i$, and its previous value, $r_i'$.

$$\tau = \min \frac{\varepsilon \tau' r_i'}{r_i' - r_i}. \tag{6}$$

The Supporting Information derives this equation in more detail, describes minor additional heuristics for minimizing error, and documents the relative insensitivity of our results to the choice of ε.

Once τ is determined, the time $t$ is advanced to $t + \tau$ and the change in each integer number of proteins or transcripts is calculated from stochastic production and decay processes. The production of molecular species $i$ is Poisson distributed with mean

$r_i$. Although decay is most accurately described as a binomial process, we use computationally faster Poisson random numbers to model decay under certain conditions; specifically, when the expected decay over the interval $\tau$ is less than 10% of the total molecular abundance. Let $x_i$ be the number of copies of protein $i$ in the cell and $y_i$ the number of transcripts corresponding to protein $i$. Equation (7) defines the distribution of the change in $x_i$; changes in $y_i$ are defined in exactly the same way.

$$\Delta x_i \sim \begin{cases} \text{Poisson}\left(r_j\tau\right) - \text{Binomial}\left(x_i,\ 1 - e^{-\gamma_P\tau}\right) \\ \quad \text{if } \gamma_P\tau > 0.1x_i \\ \text{Poisson}\left(r_j\tau\right) - \text{Poisson}\left(\gamma_P\tau x_i\right) \\ \quad \text{if } \gamma_P\tau \leq 0.1x_i \end{cases}. \quad (7)$$

The trait $z$ is initialized at zero and changes in proportion to the quantity and trait effects, $\beta_j$, of each of the $P$ phenotype genes in $\{1...P\}$. This calculation is essentially taking a numerical integral with a variable step size $\tau$. We therefore use a simple midpoint approximation to reduce the error caused by large time steps.

$$\Delta Z = \sum_j^P \left(x_{j,t} + \Delta x_j/2\right)\beta_j\tau. \quad (8)$$

### *Modeling cis-regulation*

Our model of gene regulation sums regulatory inputs to determine the accessibility of the basal transcription apparatus to the RNA polymerase; this formulation is inspired by Bintu et al. (2005) and Sherman and Cohen (2012), but simplified to serve an evolutionary modeling approach. Recall that $x_i$ is the number of copies of protein $i$ in the cell and $y_i$ is the number of transcripts corresponding to protein $i$. Let $\phi_i(\boldsymbol{x})$ be the rate of transcription of gene $i$, $\psi$ the rate of translation, and $\gamma_P$ and $\gamma_R$ the per-copy rates of protein and mRNA decay. The deterministic rates of change in $x_i$ and $y_i$ are

$$\frac{dy_i}{dt} = \phi_i(\boldsymbol{x}) - \gamma_R y_i, \quad (9)$$

$$\frac{dx_i}{dt} = \psi y_i - \gamma_P x_i. \quad (10)$$

This formulation involves several biological assumptions. First, we assume that gene expression is regulated primarily by differences in transcription and that initiation is the rate-limiting step in transcription. These assumptions are certainly not universally true, but are consistent with basic eukaryotic gene expression. Although many post-transcriptional levels of regulation are recognized, transcription is typically considered the most important level and regulatory changes often modify initiation (Latchman 2010). We also assume that the time between the initiation of transcription or translation and the appearance of the product is small enough to be ignored. This simplification greatly reduces the complexity of our simulations but may inflate the utility of feedback mechanisms by reducing lag.

We derived an equation for a simplified model of regulation based on a few assumptions. We assume that the effects of transcription factors are proportional to how often they are bound and quantify this by the Michaelis–Menten like function, $\frac{x}{K+x}$, where $K$ is the half-saturation constant. This approach follows common thermodynamics approaches to modeling gene regulation through a separation of time scales, which have been shown to be accurate approximations (e.g., Gertz et al. 2009). We assume that the rate of transcription ranges from zero, in the absence of activation, to $\phi_{\max}$. Finally, we assume that activation has diminishing returns when multiple activators act at once, as does repression. From these principles, we derive equation (11), below, for the transcription rate of gene $i$, $\phi_i$, as a function of the vector of protein concentrations $\boldsymbol{x}$.

$$\phi_i(\boldsymbol{x}) = \phi_{\max}\left(1 - \prod_{j\in A}\left(1 - \frac{p_{ij}x_j}{K_{ij}+x_j}\right)\right) \prod_{j\in R}\left(1 - \frac{p_{ij}x_j}{K_{ij}+x_j}\right). \quad (11)$$

Here, $A$ is the set of proteins with activating effects on gene $i$ and $R$ is the set with repressing effects. As described below, each protein $j$ has a *cis*-effect $c_{ij}$ when specifically regulating gene $i$ and a *trans*-effect, $t_j$, that captures the inherent effects of that protein. If $c_{ij}t_j$ is positive, protein $j$ is an activator of gene $i$; if the product is negative, then protein $j$ represses gene $i$. We calculate these combined effects as:

$$p_{ij} = 1 - e^{-|c_{ij}t_j|}. \quad (12)$$

Intuitively, $p_{ij}$ is the probability that a bound transcription factor is acting over an infinitesimal interval $dt$. In that interval, transcription occurs at the rate $\phi_{\max}$ if at least one activator and no repressors are acting; if any repressors are acting during that interval $dt$, then transcription is zero. This form does allow for a biologically realistic asymmetry between activation and repression: a very strong repressor can effectively block transcription, regardless of the strength of activation.

The half-saturation parameters $K_{ij}$ are derived from their genomic representation—the number of mismatches $k_{ij}$ from an ideal binding sequence—by simple thermodynamic arguments (Gerland et al. 2002).

$$K = c_k e^{\alpha k}. \quad (13)$$

Here, $\alpha$ scales the effect of each mismatch and $c_K$ quantifies the affinity of a perfect binding sequence. For speed and

simplicity, we ignore any transcription factor site interaction with a number of mismatches greater than four.

Equation (11) implicitly assumes that each binding site interacts with at most one protein. In practice, we extend these expressions to model multiple, distinct protein species that bind to the same site: their abundances are summed and the effects described by equation (12) are weighted by relative abundances. Let $X_{ij}$ be the sum of all protein abundances with the same binding site for gene $i$ as protein $j$; then this weighting is accomplished by replacing $x_j$ with $X_{ij}$ in each denominator of equation (11).

Values for rates of transcription, translation, and decay, as well as other parameters, are drawn from the literature as described in the Supporting Information (*parameter values for the genotype–phenotype map*).

### REPRESENTATION OF GENOMES

Genes are regulated by proteins binding to *cis*-regulatory sites. We assume that there are $B$ distinct types of protein–DNA binding motifs. Any gene can have a defined *cis*-regulatory site for any of the $B$ possible DNA-binding motifs, whether any protein in the organism actually produces a protein with that regulatory target.

During reproduction, genes can experience several classes of mutations, including deletions and duplications. Any gene can be deleted at a per-gene rate $\mu_{del}$; genes are duplicated at a per-genome rate of $\mu_{dup}$. Other mutational processes are based on a per-nucleotide mutation rate $\mu_{nuc}$, as described in the Supporting Information.

### EVOLUTIONARY SIMULATIONS

Our simulated organisms are haploid asexuals. We use the Wright–Fisher model: constant population size $N$, selection on fertility, and nonoverlapping generations. Selection is Gaussian (as in eq. (4)) with an optimal value of 1000 and a strength of selection inversely proportional to the parameter $\sigma_{opt}$, which is set to 500 unless otherwise noted. Populations are initially genetically uniform and start with fixed numbers of regulatory and phenotype genes. A candidate initial genotype is constructed by random choices of gene properties, as described in the Supporting Information. For the full-network simulations, the fitness of this genotype is then tested with 100 replicate simulations. If its mean fitness falls in the range (0.15, 0.25), this genotype becomes the founder of a population; otherwise, it is discarded and a new candidate is generated. This filter assures that all starting genotypes produced a nonzero trait value with ample opportunity for further adaptation. A similar approach was used for other simulations as described in the Results.

We recorded the nature and genetic background of every mutation that arose in every population; in addition, we saved a complete population record every 5000 generations. To reconstruct the line of descent, we first determined which haplotype was most common at 100,000 generations. We could then unambiguously trace the chain of parental haplotypes back to the founder. We measured phenotypic moments, selective coefficients, and other properties by reconstructing each haplotype and performing independent simulations of its development with high replication.

## Simulation Results

Robustness to intrinsic noise evolved through multiple mechanisms in our gene network model. To unravel this complexity, we developed a simple analysis of one significant mechanism: a shift to higher expression and lower per protein phenotypic effect, as modeled in the analytical theory above. After confirming that the mathematical approach predicts the evolution of a reduced version of our model, we return to the full version of our model to explore the role of feedback mechanisms. Finally, we end by considering how mutations with compound effects can also contribute to the evolutionary reduction of noise.

### EVOLUTION OF ROBUSTNESS IN A ONE GENE MODEL WITHOUT FEEDBACK

We performed simulations with a reduced version of our gene network model to test the accuracy of our simple mathematical analysis of expression noise. These simulations used a single gene without any possibility of feedback (i.e., the gene was not allowed to be sensitive to the concentration of its protein). Despite this simplicity, these simulations violated the assumptions used to derive equation (5) in several ways: phenotypes were limited to positive values and their distributions were not constrained to Gaussian shapes, and all four central processes (transcription, translation, and decay of mRNA and proteins) were stochastically modeled. Figure S2 shows that these more realistic simulations evolved as predicted, with alternations between decreases in effect and increases in expression producing small net improvements in developmental noise (phenotypic standard deviation).

### CHARACTERIZING THE EVOLUTION OF ROBUSTNESS TO NOISE IN COMPLEX SINGLE-GENE SIMULATIONS

Starting from initial genotypes with high noise, we evolved replicate populations of our stochastic gene network model (Fig. 2) under stabilizing selection for a single phenotypic trait. Populations consisted of 10,000 haploid asexual individuals evolving for 100,000 generations with an overall mutation rate of 0.15 mutations per population per generation (mutation details are given in the Supporting Information, Table S1). To focus on the evolution of noise, phenotypic effect was set to be initially high ($\beta = 0.3$) and genotypes were chosen to have starting means within a hundred units of the optimum. We first examine populations with a single gene with both phenotypic and regulatory effect (one-gene model); more complex networks are considered in the

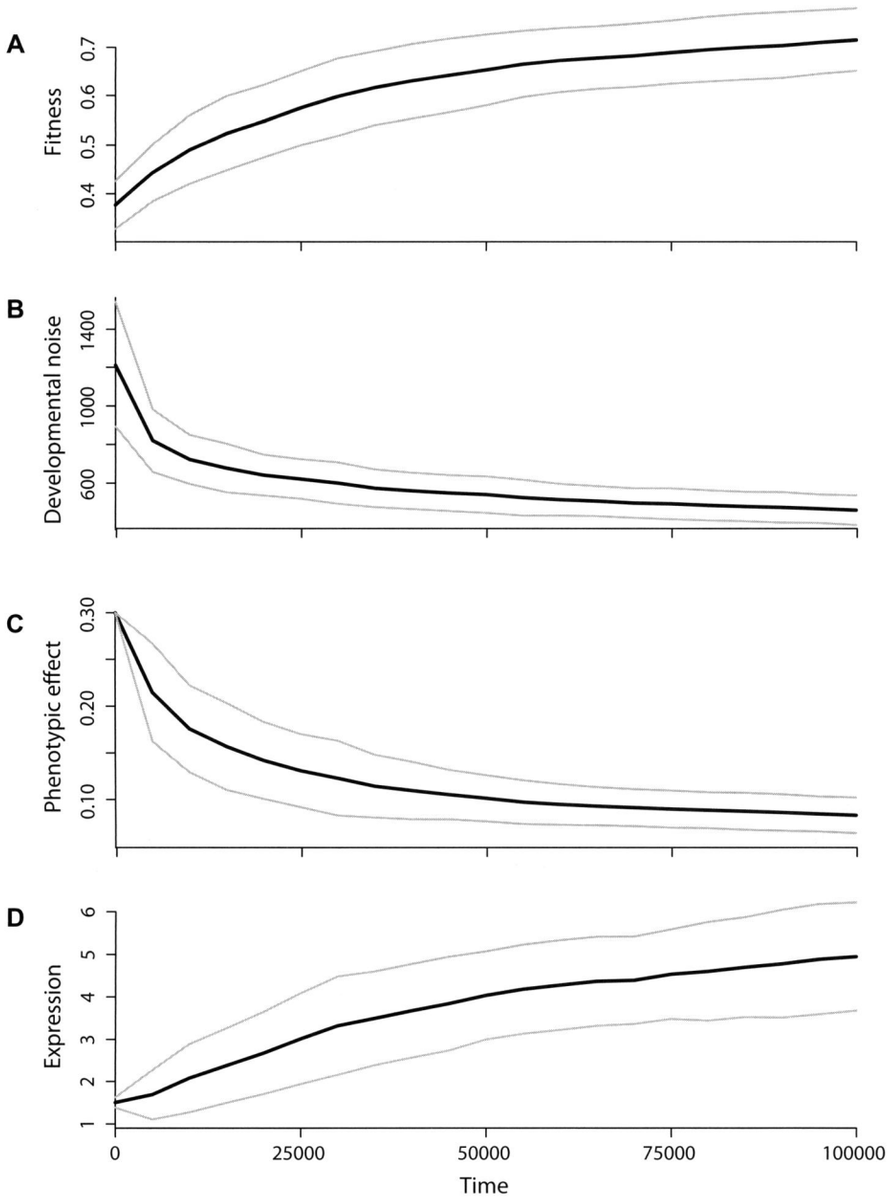


**Figure 3.** **Changes in the fitness (A), phenotypic standard deviation (B), phenotypic effect per protein (C), and lifetime expression (D) of the most common genotype in each of 100 replicate simulations. Phenotypic effects per protein are read directly from the genotypes, whereas the other three measures are based on 100,000 replicate measurements of each genotype. Black lines represent the means across replicates, whereas gray lines indicate the mean plus and minus one standard deviation. Genotypes have one gene with potential feedback (one gene model). $N = 10{,}000$ and populations experience an average mutation rate of 0.15 mutations per generation (mutation details are given in the Supporting Information, Table S1).**

following section. Our primary goal is to understand the mechanisms by which mutations can decrease the effects of intrinsic noise. In particular, we want to quantify the role of autoregulation and verify if phenotypic effects evolve to smaller values and expression evolve to larger values as predicted in the previous section.

Across all populations, fitness increases and developmental noise decreases throughout the durations of the simulations (Fig. 3A and B). Although most substitutions are beneficial (96.3% selection coefficients are positive when measured with 10,000 replicates), only a little more than half of these beneficial substitutions (59%) decrease noise. This pattern is consistent with the picture of evolution in Figure 1, in which many mutations are selected to increase both the mean phenotype and noise. Overall, the phenotypic effects of each gene decrease and their expression increases, again as predicted from our simple analytic approach.

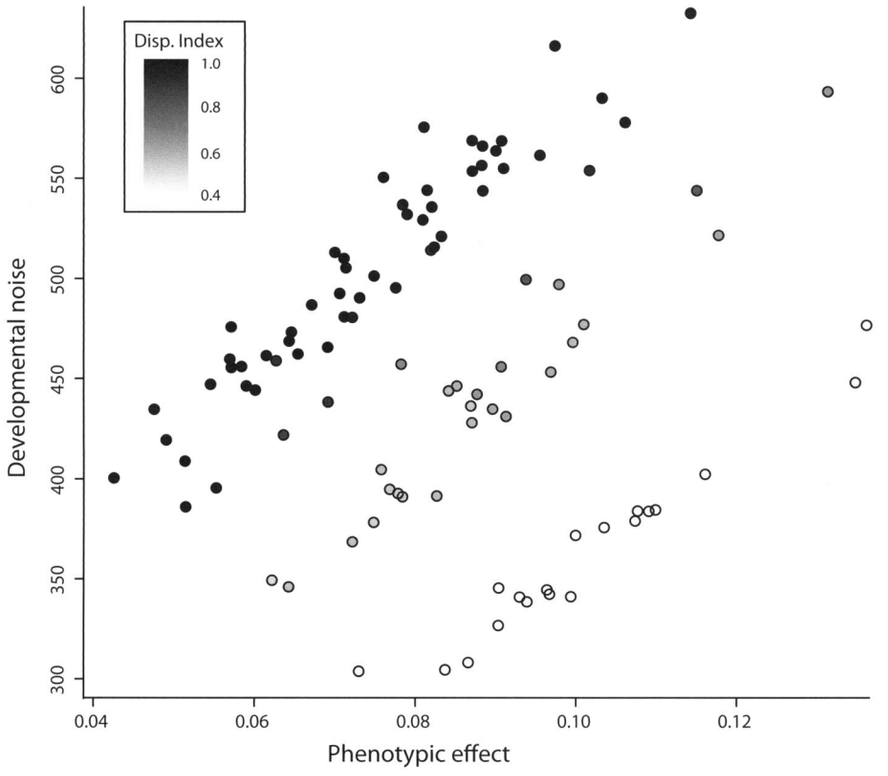


**Figure 4. The relationship between the phenotypic effect of a gene and developmental noise for the most common genotypes after 100,000 generations of evolution. Shading indicates the index of dispersion of the expression of each genotype. Simulations are from the one-gene model; dispersion index and developmental noise are means estimated from 100,000 replicate trials for each genotype.**

Our analytic model predicts that phenotypic effect will correlate positively with developmental noise; however, little correlation is evident over all the evolved gene networks (Fig. 4; $R^2 = 0.014$). One possible explanation is that some genotypes may not be producing Poisson-distributed numbers of transcripts, as assumed in our analytical model. In particular, genotypes with undispersed expression may produce less developmental noise than we would predict from their phenotypic effects. Adding this additional dimension of expression—the dispersion index, measured as the variance of lifetime expression over its mean, as shown by gray scale on the plotted points in Figure 4—allows the model to explain the majority of variation in developmental noise ($R^2 = 0.926$ for a linear model with both centered variables and their interaction; all terms are significant with $P$-values less than $1 \times 10^{-8}$). The explanation for this statistical result is clear from Figure 4; phenotypic effect predicts noise when dispersion index is held constant, but genotypes with indices of dispersion substantially below one show less noise than expected for their value of $\beta$.

We hypothesized that the dispersion index was measuring feedback from protein concentrations to transcription rates and that genotypes with dispersion indices below one had evolved negative feedback to mitigate intrinsic noise. We tested this by simulating the addition of protein to developing organisms and measuring the change in total expression; Figure S3 confirms that genotypes with smaller dispersion indices show negative feedback (Spearman's $\rho = 0.95$). This tendency toward underdispersion is not present in the original genotypes, but evolves over time (Fig. S4). Overdispersion in the ancestral genotypes is rapidly lost while the fraction of populations with significantly underdispersed genotypes rises steadily.

Figure 5 shows an example population to illustrate the interaction of changes in feedback with the reductions in phenotypic effect and increases in expression predicted from the analytical model. The plotted population changed substantially in dispersion index (it is represented by the lower-left point in Fig. 4); however, its evolutionary dynamics closely resemble the predictions of the previous section. Figure 5B in particular shows that long-term decreases in developmental noise can be accomplished by alternating changes whose individual effects on both the mean and standard deviation sum to much smaller net effects. This predicts that the average rate of genetic change in fitness-related traits is much higher than would be expected from the relatively slow rate of improvement in developmental noise.

We tested this prediction by comparing the net evolved change in both the mean and in developmental noise to the sum of

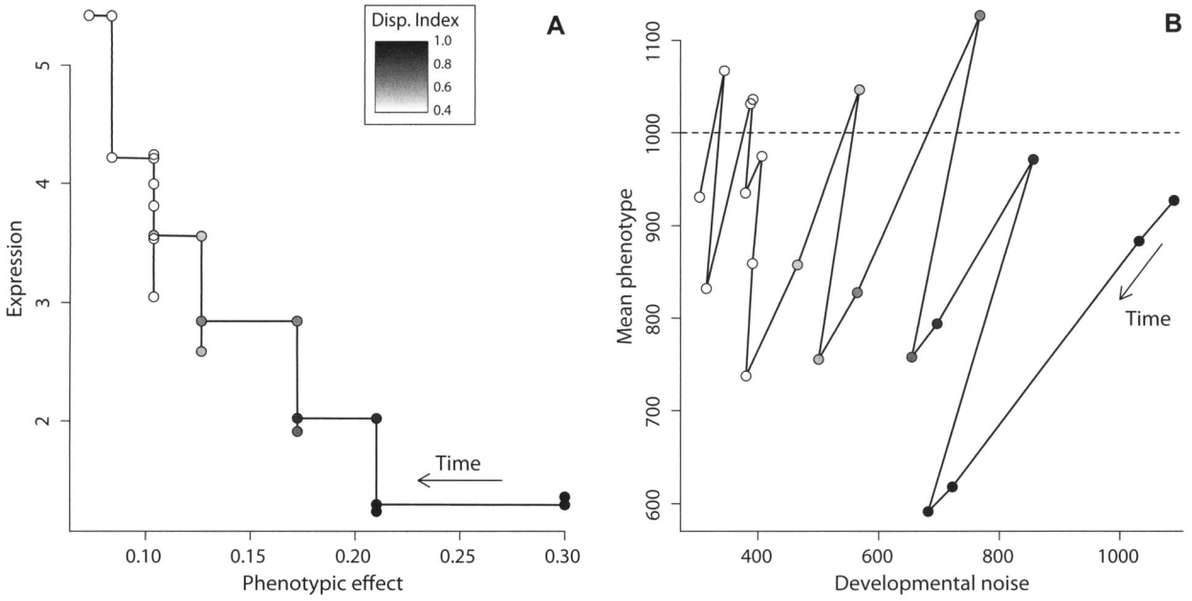


**Figure 5. Substitutions in an exceptional single-gene population (single-gene model) show stepwise, adaptive changes in both effect and expression (A), which are mirrored by zigzag changes in the mean and standard deviation of a genotype's phenotype distribution (B). The ordering of substitutions is shown by the labeled arrows. Shading indicates the index of dispersion, on a relative scale from the maximum of 1 (black) to 0.42 (white).**

the unsigned magnitude of all changes in each lineage. On average, substitutions in a population led to a net change in the mean phenotype of 120 units, with a total evolved change of nearly 2500 units. The disparity in changes to developmental noise is much smaller: populations decreased in standard deviation by an average of 750 units and experienced an average total of 1643 units of evolved change. Overall, an average of 16 substitutions occurred per population. These numbers indicate that the short-term rate of change in the mean and, to a lesser extent, in the noise around that mean is much faster than the net change in both aspects of the phenotype.

The fitness landscape defined by this simple model could also be traversed by groups of mutations that include deleterious steps; by moving off of, then back to, the high-fitness ridge, these substitutions would cross an adaptive valley. Initial measurements showed that most substitutions in the single-gene simulations of the complex model are beneficial, but a fraction are potentially deleterious (60 substitutions out of 1599 have negative selective coefficients based on 10,000 replicates simulations of each genotype). However, only two substitutions were statistically significantly deleterious in a more precise test (one million replicate fitness measurements; Mann–Whitney test corrected for multiple comparisons with a Bonferroni correction). Deleterious intermediate steps can play a role in adaptation on this fitness landscape, but their contribution is relatively small.

## ADAPTATION TO NOISE IN NETWORKS OF GENES

The preceding sections outline two pathways by which evolution can reduce phenotypic noise: increases in expression complemented by decreases in per protein effects and negative autoregulation. We explored simulations of more complicated networks with a focused goal: to identify mechanisms of the evolution of intrinsic noise that differed qualitatively from those discovered in simpler simulations. To identify any such mechanisms, we ran a simulation set with three regulatory and three phenotype genes as well as duplication and deletion mutations (labeled *full network simulations*). We then examined the evolution of phenotypic effect and expression in these replicates, focusing on populations that showed a particularly large improvement in fitness between 10,000 and 100,000 generations. Figure 6 shows the evolution of developmental noise in one such exceptional population. Two substitutions with large, beneficial effects on noise are highlighted by diagraming their direct effects on the regulation of phenotype genes. In both cases, the substitutions were changes in the target DNA-binding motif of a protein. By changing which class of *cis*-regulatory sites the proteins bound to, these two substitutions changed the regulatory effect of a single protein on multiple target genes in a single mutational step. As a result, the expression of a protein with large phenotypic effect was reduced, whereas the expressions of several proteins with small effects were simultaneously increased.

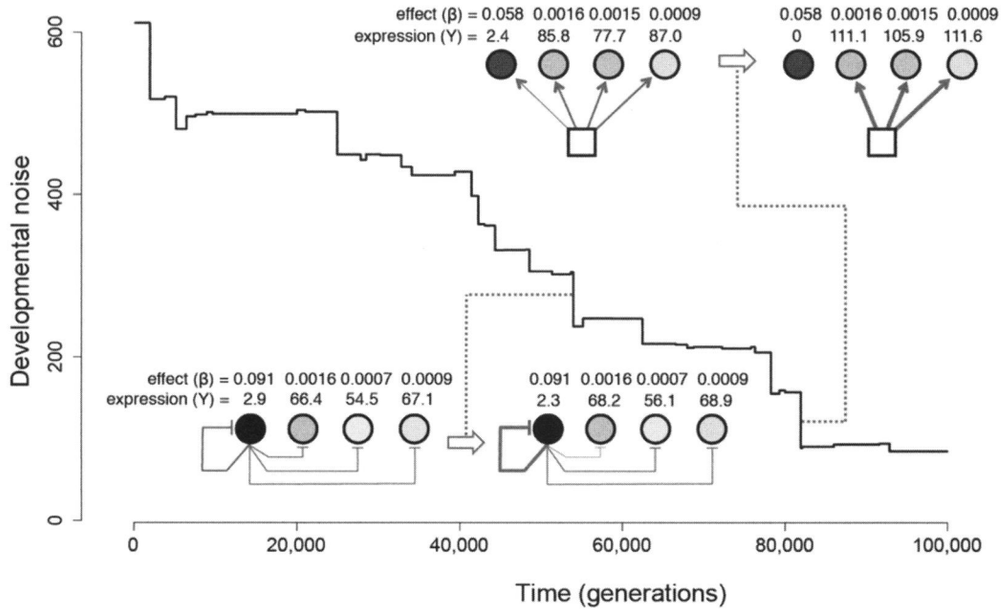


**Figure 6.** **Changes in the magnitude of noise through evolution in an example population with a complex gene network (full network simulations). Two substitutions, both changing the *cis*-regulatory binding site target of a protein with regulatory action, are diagrammed. Circles indicate phenotype genes, whereas squares depict regulatory genes (some of the latter are omitted for clarity). Arrows indicate the regulatory connections that change as a direct result of each substitution, with their thicknesses indicative of the relative strengths of their effect. Pointed arrowheads indicate positive regulation, whereas flat arrowheads indicate negative regulation. In each case, a single mutational change to the regulatory target of a protein directly downregulates a gene with large phenotypic effect and upregulates genes with smaller phenotypic effects. The phenotypic effect and the measured expression (mean total transcription, measured over 10,000 replicates) are labeled for each phenotype gene; for both substitutions, all phenotype genes change significantly in expression. Developmental noise is replicated over 10,000 measurements for each genotype. The two earliest substitutions on the line of descent are omitted for scale.**

A single mutation that turns down genes of large effect and turns up genes of small effect could have a potent benefit: in principle, a wholesale change in expression profile could reduce developmental noise while conserving mean phenotype. We examined all substitutions that changed the DNA-binding motif in the 500 populations with multiple regulatory and phenotype genes (full network simulations), testing for coordinated changes in expression that match the pattern in Figure 6. Specifically, we looked for pairs of phenotype genes in which the gene with the larger trait effect decreased significantly in expression, whereas the gene with the smaller effect simultaneously increased. To account for nonnormality of expression data and multiple comparisons, we used the Wilcoxon rank-sum test with $\alpha = 0.005$. Among the 1173 substituted changes in DNA-binding motifs, 246 significantly matched this pattern; of these, about half (129) occurred in regulatory genes. The median selection coefficient of these coordinated DNA-binding motif changes is larger than all other substitution classes (Table 1). Shifts in regulatory patterns from one target binding site to another are therefore a significant source of adaptive mutations in this model.

## Discussion

We have shown that in a stochastic model of gene expression, evolution under stabilizing selection leads to a gradual reduction in developmental noise. The main mechanism of this noise reduction is by an increase in expression concomitant with lower functional effects per molecule, which together reduce stochastic variation in the downstream effects of the protein pool. A simple mathematical model linking stochastic expression noise to phenotypic noise successfully explains how the adaptive substitution of small, alternating changes in both expression and effect can achieve adaptation to noise. Two notable predictions of this evolutionary model are that the mean phenotype often evolves away from its optimum to smaller values and that the evolutionary trajectory of the mean changes direction frequently. Also, the small and positive selection coefficients of the mutations involved predicts that adaptation to noise will proceed more quickly in larger populations.

For this work, we have developed a novel simulation model of evolution of a developmental system. This model is intended to offer a new framework for asking questions about the evolution

of gene networks. This simulation tracks the evolution of populations consisting of individuals who each develop according to rules determined by the basic rules of gene regulation, accounting for the finite and stochastic number of mRNA transcripts and protein molecules extant in the organism at any given time point. The model allows for site-specific binding of promoters and repressors of transcription, using simple biochemical relationships to determine the patterns of gene expression. Although not intended to capture the full range of biological complexity, this model is designed to mimic many basic biological processes and in so doing create patterns of mutational effects, epistasis, and gene interaction in a more natural way than can be captured by more abstract models of evolution.

Although other models of stochastic gene expression are available (e.g., Kratz et al. 2008), our approach is unique in its focus on evolutionary applications. Genotype–phenotype models for evolutionary simulation face two main challenges: determination of a stochastic phenotype for a genotype must be fast, because this calculation must be repeated for every individual in a large population, for each generation and for each replicate, and a mutational model for each mutable parameter must be specified. This model is one attempt to capture realistic mechanisms of expression and regulation while satisfying these constraints. Although the model is far from a complete description of the mechanics of gene expression, it does allow insights that could not have been gained by the use of simpler frameworks such as the Wagner model (Wagner 1996) or quantitative genetic models with assumed Gaussian mutational effects. By separating out quantities such as the expression and effect of a gene, or the binding affinity of a protein binding site interaction from the effect of the protein when bound, our model accounts for a greater proportion of the degeneracy underlying biological systems, allowing new evolutionary dynamics—such as the replacement of low-expression, high-effect genes with high-expression, low-effect genes—to emerge. Other recent explorations following the same philosophy, such as the deterministic model of gene expression developed by Pujato et al. (2013), also demonstrate the value of mechanistic models that capture multiple aspects of gene effects. We expect that our ENTWINE model will allow the investigation of many novel questions about the evolution of robustness and phenotypic variability.

The major pattern we document—that genotypes evolve to use high-expression, low-effect genes to minimize noise—aligns with the empirical finding that important genes in yeast show high expression and low translational efficiency (Fraser et al. 2004). Although translational efficiency is not mutable in our model, our phenotypic effect parameter plays the same role by scaling the effect on the phenotype of each individual transcript, as mediated through the phenotypic products created by the protein coded by that transcript. Other properties of gene expression and protein dynamics, such as mRNA and protein decay rates, could potentially play analogous roles; expanding our model to allow these properties to evolve would be a productive next step. Costs of the production of mRNA or proteins may limit the observed mechanism of robustness to intrinsic noise to genes whose effects are particularly sensitive to stochastic variation. However, for such genes, the "brute-force" strategy of high expression may be the most effective means of buffering against expression noise (see also Lestas et al. 2010).

**Table 1. Median selection coefficients for the full network simulations.**

| Class | Median $s$ | $N$ |
|---|---|---|
| DNA-binding motif (coordinated) | 0.032 | 246 |
| DNA-binding motif (other) | 0.014 | 927 |
| Phenotypic effect | 0.016 | 1439 |
| *Cis*-site effect | 0.0006 | 3372 |
| *Cis*-site $K$ | 0.0005 | 531 |
| Protein *trans*-effect | 0.006 | 1241 |
| Duplication | 0.02 | 713 |
| Deletion | 0.008 | 1640 |

The role of negative autoregulation in reducing noise in gene expression is controversial (Stekel and Jenkins 2008; Marquez-Lago and Stelling 2010; Lestas et al. 2010). In our model, the evolution of novel negative autoregulation plays a significant role in the evolution of robustness to noise and coevolves with a transition to lower protein effect and higher expression. The value of negative feedback may depend on details of our model; the design of our model of trait development places limits on the potential usefulness of feedback. Our model only allows feedback from the availability of a protein to the regulatory process, and no mechanism is available for feedback from the phenotypic trait itself. Without feedback from the trait, autoregulation can only act on the information contained in the current protein concentrations. These concentrations change rapidly and are not likely to convey much information about the long-term history of expression. Future work will extend the model to allow for feedback from the trait value, either directly or as an internal measure of performance, permitting a more thorough evaluation of the role of feedback in robustness to noise.

Our results illustrate the benefits to combining a mechanistic understanding of gene expression with an evolutionary framework. Molecular and systems biologists have begun to link the structure of networks with the dynamics of their responses to noise (e.g., Thattai and van Oudenaarden 2001, Alon 2006); here, we have begun to explore how evolutionary forces act on the

expression strategies of genes in networks. Our initial hypotheses suggested that clusters of mutations crossing "fitness valleys" —intermediate genotypes with fitnesses below their immediate ancestors and descendants—would be required to avoid constraints on the evolution of noise. By helping to disprove this hypothesis, and elucidate the alternative, this complex model contributes to our evolutionary understanding of genes as regulatory elements in dynamic networks.

## DATA ARCHIVING

C and R code for all simulations and analyses have been archived at Dryad: 10.5061/dryad.8c81j.

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## *Supporting Information*

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Table S1.** Mutation rates.

**Figure S1.** (A) Improvement in phenotypic standard deviation in weak mutation adaptive walk simulations, plotted against the number of proposed mutations.

**Figure S2.** Substitutions in representative replicate simulations.

**Figure S3.** Relationship between the dispersion index of expression and a measure of feedback in the evolved genotypes from one-gene simulations.

**Figure S4.** Significant over- and underdispersion in one-gene populations (set *1P*).