

# ENVIRONMENTAL ROBUSTNESS AND THE ADAPTABILITY OF POPULATIONS

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Recent work has shown that genetic robustness can either facilitate or impede adaptation. But the impact of environmental robustness on adaptation remains unclear. Environmental robustness helps ensure that organisms consistently develop the same phenotype in the face of “environmental noise” during development. Under purifying selection, those genotypes that express the optimal phenotype most reliably will be selectively favored. The resulting reduction in genetic variation tends to slow adaptation when the population is faced with a novel target phenotype. However, environmental noise sometimes induces the expression of an alternative advantageous phenotype, which may speed adaptation by genetic assimilation. Here, we use a population-genetic model to explore how these two opposing effects of environmental noise influence the capacity of a population to adapt. We analyze how the rate of adaptation depends on the frequency of environmental noise, the degree of environmental robustness in the population, the distribution of environmental robustness across genotypes, the population size, and the strength of selection for a newly adaptive phenotype. Over a broad regime, we find that environmental noise can either facilitate or impede adaptation. Our analysis uncovers several surprising insights about the relationship between environmental noise and adaptation, and it provides a general framework for interpreting empirical studies of both genetic and environmental robustness.

**KEY WORDS:** Adaptation, mutations, population genetics.

Robustness and adaptability are fundamental and seemingly conflicting properties of biological systems (de Visser et al. 2003; Lenski et al. 2006; Wagner 2008; Draghi et al. 2010). An organism is robust if it can reliably produce a given phenotype across a range of environmental and genetic perturbations. But adaptability requires that alternative phenotypes be expressed so that a population can adapt to new conditions. Naively, it seems that the more robust an organism is, the less frequently it expresses alternative phenotypes, and so the less adaptable (or evolvable) the population will be. However, recent work demonstrates that genetic robustness can either facilitate or impede a population’s capacity to adapt to a novel environment (Wagner 2008; Draghi et al. 2010). The relationship between adaptation and environmental robustness—that is, the robustness of an individual’s expressed phenotype against nonheritable perturbations—remains unclear.

There are reasons to believe that environmental noise may facilitate adaptation. It is well known that genetic and environmental perturbations often have similar phenotypic effects on an organism. As a result, occasionally expressing alternative phenotypes by environmental noise might help speed adaptation via “genetic assimilation”—that is, by promoting those individuals who will subsequently evolve the adaptive phenotype by mutation (Ancel and Fontana 2000; Rutherford 2003; Earl and Deem 2004; Gibson and Dworkin 2004; Masel 2005; Meyers et al. 2005). But there are also reasons to believe that environmental noise may impede adaptation: noise will select for the most environmentally robust genotypes, thus reducing neutral standing genetic variation in a given environment and thereby slowing adaptation to a new target phenotype (Hermisson and Wagner 2004; Wagner et al. 1997; van Nimwegen et al. 1999; Masel and Trotter 2010). Therefore,

we might expect a complex relationship between environmental noise, environmental robustness, and the rate at which a population adapts to a novel environment. It is this relationship that is the focus of this article.

### Phenocopy and Genetic Assimilation

Genetic and environmental perturbations often have similar phenotypic effects—a phenomenon known as “phenocopy.” Phenocopy was first discovered in the classic studies of Goldschmidt (1935) and Waddington (1953a), and it has since been observed at many different levels of biological organization, including protein-coding sequence, mRNA and protein structures, individual gene expression patterns, regulatory or metabolic network dynamics, and entire developmental pathways (Table 1).

The classic example of the phenocopy concept comes from studies of heat shock in *Drosophila* (Waddington 1942, 1953a,b, 1959). Waddington found that the type of phenotypic variation induced by heat shock could subsequently be produced by genetic changes accrued over several generations of artificial selection (Waddington 1953a). In other words, a phenotype that was initially produced as the result of an environmental stimulus was subsequently produced in the absence of that stimulus as the result of genetic changes—a process called “genetic assimilation.” The mechanistic basis for genetic assimilation in these heat shock experiments has been traced, in part, to the behavior of the molecular chaperone Hsp90 (Rutherford and Lindquist 1998; Chow and Chan 1999; Rutherford 2003; Sangster et al. 2004), which buffers the effects of standing genetic variation by aiding protein folding. When the function of Hsp90 is impaired, either through heat shock or through mutation, previously cryptic genetic variation is revealed as phenotypic variation. Any such phenotypic variation that is adaptive can quickly become fixed through genetic assimilation (Ancel and Fontana 2000; Gibson and Dworkin 2004; Jarosz and Lindquist 2010; Masel and Trotter 2010).

Environmental perturbations can also lead to phenocopy in a constant genetic background (i.e., even without standing cryptic genetic variation). One of the most important examples of this occurs in the secondary structures of RNA and protein molecules (Ancel and Fontana 2000; Bloom et al. 2006). Although the majority of genetically identical sequences fold to the same, minimum energy structure, some adopt higher energy structures. The alternative structures that a molecule can assume generally correspond to the minimum energy structures associated with the genotypes available by mutation to that molecule (Ancel and Fontana 2000; Bloom et al. 2006). The degree to which these alternative phenotypes are expressed depends on environmental factors, such as temperature. In this case, phenocopy occurs on a constant genetic background, without the need for cryptic genetic variation.

In this study, we will not specify a specific phenotype of interest (e.g., RNA secondary structure) or a specific mechanism

of phenocopy (e.g., a chaperone-mediated capacitor), but rather we will explore in greater generality how environmental robustness influences a population’s capacity to adapt, assuming that genetic and environmental perturbations tend to produce similar phenotypes.

### Variation in the Degree of Environmental Robustness among Genotypes

The degree to which an individual is robust to environmental noise may depend upon the individual’s genotype. When a population is under stabilizing selection for a given phenotype, and when genotypes vary in their environmental robustness, selection will tend to concentrate the population at the highly robust genotypes. The strength of this effect will increase as the frequency of environmental perturbations increases. As a result of this effect, the population will harbor less genetic diversity when environmental perturbations occur more frequently, which in turn will reduce the speed with which the population adapts to a new target phenotype. Thus, genotypic variation in environmental robustness can influence the adaptability of a population (Masel and Trotter 2010). (We use the term adaptability, which is more accurate in this context than the term evolvability). Standing genetic diversity increases the speed of adaptation both by increasing the probability that the population harbors adaptable individuals at the time of the environmental shift and by increasing the rate at which such individuals are produced following the environmental shift. Therefore, standing genetic diversity is important for adaptation when adaptable genotypes are rare.

### Presentation Outline

We will construct a general, population-genetic model to explore the effects of environmental robustness on adaptability. Our model assumes the concept of phenocopy and it also allows for variation in environmental robustness across genotypes. On the one hand, the phenocopy phenomenon has been shown to aid the adaptation of a population following an environmental shift in the presence of environmental noise (Ancel and Fontana 2000; Rutherford 2003; Earl and Deem 2004; Gibson and Dworkin 2004; Masel 2005; Meyers et al. 2005). But on the other hand, as discussed above, variation in environmental robustness across a neutral network tends to reduce genetic variation prior to an environmental shift (i.e., under stabilizing selection) when noise is present, and thus slows adaptation (Wagner et al. 1997; Hermisson and Wagner 2004; Masel and Trotter 2010). These two forces therefore have opposite influences on the capacity of a population to adapt to a new environment. Here, we resolve how these opposing forces interact to determine the adaptability of populations subject to environmental noise.

The article is structured as follows. We begin by introducing a general model for the evolution of a population on an

**Table 1.** Examples of phenocopy observed in different biological systems.

Phenotype	Genetic perturbation	Environmental perturbation	References
Protein misfolding	Mutations at protein-coding regions	Errors in transcription or translation	Goldsmith and Tawfik (2009), Meyerovich et al. (2010)
Incorrectly transcribed mRNA	Mutations at stop codons	Accidental read through of stop codons	Masel and Bergman (2003)
Protein/mRNA misfolding	Mutations to coding regions	Temperature induced noise	Ancel and Fontana (2000), Bloom et al. (2006)
Protein/mRNA misfolding	Knockout of molecular chaperone Hsp90	Heat shock	Rutherford and Lindquist (1998)
Incorrect protein expression level	Mutations to regulatory regions	Intrinsic/extrinsic noise	Raj and van Oudenaarden (2008)
Incorrect gene expression pattern	Gene knockout resulting in loss of buffering in gene network	Large environmental perturbation	Levy and Siegal (2008), Siegal and Bergman (2002), Lopez-Maury et al. (2008), Wagner and Wright (2007)
Incorrectly executed developmental/ metabolic pathway	Mutation resulting in loss of redundancy in pathways	Large environmental perturbation	McLoughlin and Copley (2008), Tawfik (2010)

arbitrary neutral network, with an arbitrary distribution of environmental robustness across the network. We consider a population initially adapted to one environment (one target phenotype) and then subsequently exposed to a new environment (a different target phenotype). We treat our model analytically to recover the full probability distribution of the time required to adapt to the new target phenotype. We next discuss a simplified neutral network with constant mutational robustness in which we separate genotypes into classes of high and low environmental robustness. This simple model is used to develop intuition for how environmental robustness affects adaptability. Finally, we summarize our results and discuss future directions, including how our framework might be used to interpret systematic studies in quantitative genetics.

## A Population-Genetic Model

Our general model is based on the Moran process from population genetics, in which each haploid individual in a population of constant size reproduces at a rate determined by its relative fitness, with its offspring replacing a randomly chosen individual. During reproduction, the offspring may acquire a mutation, which changes its genotype from that of its parent. Different genotypes may encode for different phenotypes; fitness is determined by comparing an individual's phenotype to the phenotype selected by the current environment, as described below.

In a given environment that selects for a specific phenotype, we will focus our analysis on the set of all genotypes that encode that phenotype. The set of such genotypes is called a "neutral network" because mutations among them do not change an individual's encoded phenotype. The structure of mutations among

the genotypes on the neutral network is defined by the adjacency matrix  $\mathbf{M}$ , which has entries  $m_{ij} = 1$ , if genotype  $i$  can mutate to genotype  $j$  without changing the phenotype, and  $m_{ij} = 0$  otherwise. We define the mutational robustness,  $q_i$ , of genotype  $i$  as the proportion of all mutations that do not change its phenotype. More specifically, we define

$$q_i = \frac{1}{L} \sum_j m_{ij}, \quad (1)$$

where  $L$  denotes the total number of different mutations that could arise in an individual (e.g., the length of its binary genome). The mutational robustness,  $q_i$ , therefore quantifies the chance that a mutant offspring of an individual with genotype  $i$  will remain on the neutral network (i.e., will still express the same phenotype), or not. Each genotype also has an associated environmental robustness,  $\phi_i$ , discussed in more detail below.

When an individual of genotype  $i$  is chosen to reproduce, a mutation occurs with probability  $\mu$ . The mutation rate  $\mu$  is therefore the genome wide mutation. We have typically chosen a mutation rate of  $\mu = 10^{-3}$  in our simulations, corresponding to a genome of about  $10^5$  nucleotides (see Supporting Information). If a mutation occurs, the resulting offspring remains on the neutral network (i.e., encodes the same phenotype) with probability  $q_i$ . In this case, the offspring nonetheless has a new genotype, which is drawn uniformly among  $i$ 's neutral neighbors (i.e., from the set of genotypes  $j$  with  $m_{ij} = 1$ ). With probability  $1 - q_i$ , on the other hand, the resulting offspring encodes a different phenotype and thus lies outside of the neutral network.

Following the work of Draghi et al. (2010), we assume that each genotype has a set of  $K$  alternative phenotypes that

constitute its phenotypic neighborhood—that is, the set of possible phenotypes that can be produced by a nonneutral mutation. These  $K$  phenotypes are drawn uniformly from a total of  $P$  possible alternative phenotypes. We make the simplifying assumption that phenotypic neighborhoods are independent, such that the  $K$  phenotypes accessible to a genotype are redrawn whenever a mutation occurs. When a mutation produces an individual lying outside the neutral network, its resulting phenotype is drawn from the  $K$  alternatives that belong to its phenotypic neighborhood. The form of genotype–phenotype map described above corresponds to evolution in a space of infinite genotypes, such that each mutation results in an entirely new genotype entering the population. Our model specifies the effects of such mutations on the phenotype, using summary statistics such as  $K$ ,  $q$ , and  $P$ . Such a statistical description of the genotype–phenotype map provides a very good approximation to evolution on an explicit, finite genotype–phenotype map, such as the RNA folding landscape (Draghi et al. 2010). Furthermore, we demonstrate in the Supporting Information that our results hold even when we relax the statistical assumptions described here and allow for correlations between the phenotypic neighborhoods of neighboring genotypes.

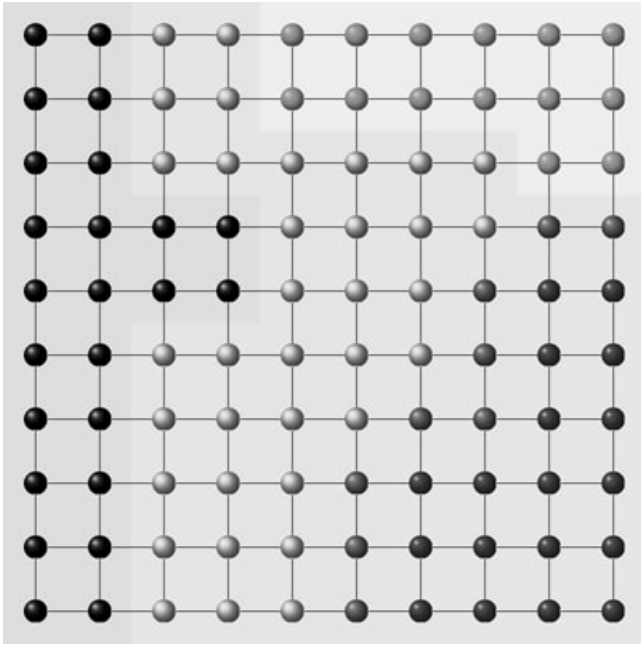
As described so far, our model is identical to the one explored by Draghi et al. (2010), and it can be used to study how mutational robustness influences the process of adaptation. To study environmental robustness, we will extend the model by assuming that when an individual produces an offspring, the offspring experiences an environmental perturbation with probability  $\epsilon$ . Such an environmental perturbation may result in the offspring developing a different phenotype from the “intended” phenotype. In other words, when a perturbation occurs, an offspring may express a different phenotype than the one encoded by its genotype. The perturbation is specific to the development of that offspring alone, and it does not affect other individuals in the population. The parameter  $\epsilon$  therefore measures the amount of noise that occurs during the development of an individual, which we call environmental noise. If such an environmental perturbation occurs during a reproduction event, the resulting individual adopts its intended phenotype with probability  $\phi_i$ . With probability  $1 - \phi_i$ , however, the offspring is not robust to the environmental perturbation and the offspring adopts an alternative phenotype. The parameter  $\phi_i$  therefore describes the robustness of genotype  $i$  against environmental noise.

We assume that the alternative phenotypes that result from mutation in an individual and those that result from environmental perturbation are the same—so that when an environmental perturbation produces an individual with an alternative phenotype, that alternative phenotype is drawn from the  $K$  phenotypes that constitute its phenotypic neighborhood (i.e., the same set of phenotypes the individual can produce by mutation). This assumption captures the concept of phenocopy—namely that ge-

netic and environmental perturbations tend to produce a similar set of phenotypes—whose longstanding empirical support we have described above.

We consider a haploid population of  $N$  asexuals that reproduce according to the Moran process and occupy a neutral network of the type described above. We assume that initially the phenotype associated with the focal neutral network is optimal for the environment, and we assign it fitness one without loss of generality. All alternative phenotypes are strongly deleterious, and assigned fitness zero (or, equivalently, any selective deficit  $\gg 1/N$  [van Nimwegen et al. 1999; Draghi et al. 2010]). After a long period of stabilizing selection, we assume that an environmental shift occurs, such that one of the  $P$  alternative phenotypes now has a selective advantage,  $s$ , over the initial phenotype. The newly adaptive phenotype is chosen uniformly from among the  $P$  alternative phenotypes. Once the environmental shift occurs, we study the adaptation time—defined as the time required for the population to produce an individual whose genotype encodes the newly adaptive phenotype. (Once one such adaptive genotype is produced, the subsequent probability of and time to its fixation are well understood for asexuals, according to classical population genetics [Ewens 2004]; however, explicit calculation of this quantity may be difficult or impossible in the presence of clonal interference or in sexual populations). The adaptation time is a random variable whose properties depend on how the population is distributed across the neutral network, and on the extent to which phenocopy speeds adaptation by occasionally producing individuals with the adaptive phenotype. As the rate of environmental perturbation,  $\epsilon$ , increases, the first of these two effects, increased selection for genotypes with high environmental robustness, will reduce genetic diversity and make it more and more difficult for the population to explore the neutral network, thus slowing adaptation. However, the second of these two effects, phenocopy, will result in more frequent expression of the adaptive phenotype, which will tend to speed adaptation by promoting those individuals who are a single mutation away from the adaptive phenotype. How strongly these two opposing effects influence adaptation time, and under what conditions, is the central problem we seek to resolve.

Following Draghi et al. (2010), we separate the neutral network into adaptable genotypes and nonadaptable genotypes. Adaptable genotypes are one mutation away from the newly adaptive phenotype, whereas nonadaptable genotypes do not contain the newly adaptive phenotype in their phenotypic neighborhoods (see Fig. 1). In the presence of environmental perturbations  $\epsilon > 0$  (and provided environmental robustness is not complete, i.e., provided  $\phi \neq 1$ ), adaptable genotypes gain a direct selective advantage in our model, compared to nonadaptable individuals with the same environmental robustness, because they occasionally express the newly adaptive phenotype.



**Figure 1.** Depiction of genotypes (dots) and phenotypes (colors). In this example, the initially adaptive phenotype is indicated by red, and the newly adaptive phenotype following the environmental shift is indicated by blue. Dark red indicates genotypes that are adaptable (i.e., those that can express the adaptive phenotype either by environmental noise or genetic mutation); light red indicates genotypes that are nonadaptable. All other phenotypes, shown here as black or green, are deleterious. Although some genotypes are shown as only neighboring other genotypes of the same color, in reality, all genotypes will always have neighbors that code for a different phenotype.

### Analysis

We focus on the situation in which adaptable individuals are rare at the time of the environmental shift, meaning that the number of adaptable individuals is  $O(\sqrt{N})$  or smaller. We further assume that the probability of environmental perturbation in an individual,  $\epsilon$ , is sufficiently small that the selective advantage of adaptable genotypes after the environmental shift is  $O(\frac{1}{\sqrt{N}})$  or smaller. The less realistic case in which adaptable individuals are common is analyzed in Supporting Information.

In our regime of interest, following the environmental shift, the frequencies of nonadaptable genotypes behave nearly deterministically, and so their dynamics can be accurately approximated by ordinary differential equations (ODEs). In particular, if we let  $X_i$  denote the frequency of nonadaptable genotype  $i$  in the population, then we can approximate the dynamics by the following system of ODEs:

$$\frac{dX_i}{dt} = \left( \sigma_i - \sum_{j \notin \mathcal{A}} \sigma_j X_j \right) X_i. \tag{2}$$

Here,  $\mathcal{A}$  denotes the set of adaptable genotypes, and  $\sigma_i$  denotes the selective coefficient of genotype  $i$ , which we define below. The sum  $\sum_{j \notin \mathcal{A}} \sigma_j X_j$  gives the mean fitness of genotypes that are not adaptable. Loosely speaking, the nonadaptable types behave in a deterministic manner because we have assumed they are common at the time of the environmental shift. We formally justify this deterministic approximation for nonadaptable genotypes in Supporting Information, and we demonstrate below that this approach provides a highly accurate approximation of our model by comparing it to Monte Carlo simulations of the exact Moran process.

Unlike the nonadaptable types, the adaptable types are assumed rare at the time of the environmental shift, and so we cannot use a deterministic approximation. Instead, we approximate the behavior of the adaptable genotypes by deriving a diffusion approximation of the Moran process (see Supporting Information). The resulting diffusion approximation can be described by a stochastic differential equation (SDE), which is similar to an ODE except that the instantaneous dynamics involve Brownian motion. In particular, we have derived the following SDE to approximate the (stochastic) dynamics of the frequencies of each adaptable genotype,  $j$ :

$$dY_j = \left[ \left( \sigma_j - \sum_{i \notin \mathcal{A}} \sigma_i X_i \right) Y_j + \theta \sum_{k \notin \mathcal{A}} m_{kj} X_k \right] dt + \sqrt{2Y_j} dB_j(t). \tag{3}$$

The notation used to express equation (3) is the standard notation for SDEs (Gardiner 2004), in which  $B_j(t)$  denotes independent Brownian motions. Intuitively, the SDE above indicates that the change in frequency of genotype  $j$ ,  $\Delta Y_j$ , in a small interval of time,  $\Delta t$ , is a Gaussian random variable with mean

$$\left( \sigma_j - \sum_{i \notin \mathcal{A}} \sigma_i X_i \right) Y_j + \theta \sum_{k \notin \mathcal{A}} m_{kj} X_k$$

and standard deviation

$$\sqrt{2Y_j}.$$

Here,  $Y_j$  denotes the number of individuals, rescaled by  $\frac{1}{\sqrt{N}}$ , at adaptable genotype  $j$  (see Supporting Information).  $\mathcal{A}$  denotes the set of adaptable genotypes,  $\theta = N\mu$  denotes the population-scaled mutation rate, and  $\sigma_j$  denotes selection coefficients defined below. The various terms in this SDE each have intuitive interpretations. As is typical in population-genetic models, the instantaneous mean change in the frequency of genotype  $j$  is determined by two terms, the first due to selection and the second due to mutation; whereas the instantaneous variance in the change of genotype  $j$ 's frequency is determined by its current frequency, which encodes the effect of genetic drift. The variance term arising from genetic drift has a somewhat unusual form and it involves a  $\sqrt{2Y_j}$  because we have scaled the population of

adaptable genotypes by  $\frac{1}{\sqrt{N}}$ , instead of the more typical  $\frac{1}{N}$  (see Supporting Information). We formally justify this diffusion approximation for adaptable genotypes in Supporting Information, and we demonstrate below that this approach provides a highly accurate approximation of our model by comparing it to Monte Carlo simulations of the exact Moran process.

The selection terms in equations (2) and (3) arise from environmental perturbations, which cause selective differences among genotypes on the neutral network. For a nonadaptable genotype  $i$ , such a perturbation will either have no effect (with probability  $\phi_i$ ), or it will produce a strongly deleterious phenotype with probability  $(1 - \phi_i)$ . Thus, the expected fitness of a nonadaptable genotype  $i$  is  $1 - (1 - \phi_i)\epsilon$ , and the selection coefficient on such individuals is:

$$\frac{\sigma_i}{\sqrt{N}} = -(1 - \phi_i)\epsilon, \quad i \notin \mathcal{A}. \quad (4)$$

Likewise, adaptable genotypes express a deleterious phenotype with probability  $\frac{K-1}{K}(1 - \phi_i)\epsilon$ , and they express the newly advantageous target phenotype, which has fitness  $1 + s$ , with probability  $\frac{1}{K}(1 - \phi_i)\epsilon$ . Therefore, the selection coefficient for an adaptable genotype is

$$\frac{\sigma_i}{\sqrt{N}} = \frac{1+s}{K}(1 - \phi_i)\epsilon - (1 - \phi_i)\epsilon, \quad i \in \mathcal{A}. \quad (5)$$

The factor  $1/\sqrt{N}$  appears the two equations above because we have assumed the selection coefficients are  $O\left(\frac{1}{\sqrt{N}}\right)$  (see Supporting Information).

Finally, we note that, following the environmental shift, adaptable genotypes mutate to the newly adaptive phenotype at rate  $\mu\left(\frac{1-q_i}{K}\right)$ . This flux into the newly adaptive phenotype, along with equations (2) and (3), can be used to solve analytically for the distribution of waiting times for a newly adaptive genotype to arise in the population. In particular, we can compute the mean time it takes for a population to adapt to a novel environment, for an arbitrary neutral network with arbitrary variation in environmental and genetic robustness across the neutral genotypes. The full analytical solution to this problem is given in the Supporting Information.

### A Simplified Model

We now consider a simplified version of the model above, which will allow us to develop intuition about the effects of environmental robustness on adaptability. We assume that all genotypes have the same mutational robustness,  $q$ . Each genotype on the neutral network is assigned either high or low environmental robustness, denoted by  $\phi_H$  and  $\phi_L$ . Following a neutral mutation, genotypes with high environmental robustness produce other genotypes with high environmental robustness with probability  $\pi_H$  (and there-

fore produce genotypes with low environmental robustness with probability  $1 - \pi_H$ ), whereas genotypes with low environmental robustness produce other genotypes with low environmental robustness with probability  $\pi_L$  (and therefore produce genotypes with low environmental robustness with probability  $1 - \pi_L$ ). The neutral network now consists of four distinct classes of genotypes: high environmental robustness and adaptable, high environmental robustness and nonadaptable, low environmental robustness and adaptable, and low environmental robustness and nonadaptable. The mutation rates among these four genotypes, and between the adaptable and the newly adaptive genotypes, are summarized in Figure 2. Henceforth, we analyze our model in terms of the population density at these four classes of genotypes, rather than in terms of the frequencies of each individual genotype. Each class represents a large number of distinct genotypes and therefore harbors much genetic diversity. As the rates of mutations into a particular class increase, this corresponds to increasing the number of distinct genotypes within that class, and thus increases the genetic diversity within the class. Figure 2 shows the rates of mutations between and within classes.

In our simplified model, the mutational robustness,  $q$ , is the same for all genotypes. Therefore, we henceforth refer to genotypes of high and low robustness, with the understanding that this means genotypes of high and low environmental robustness.

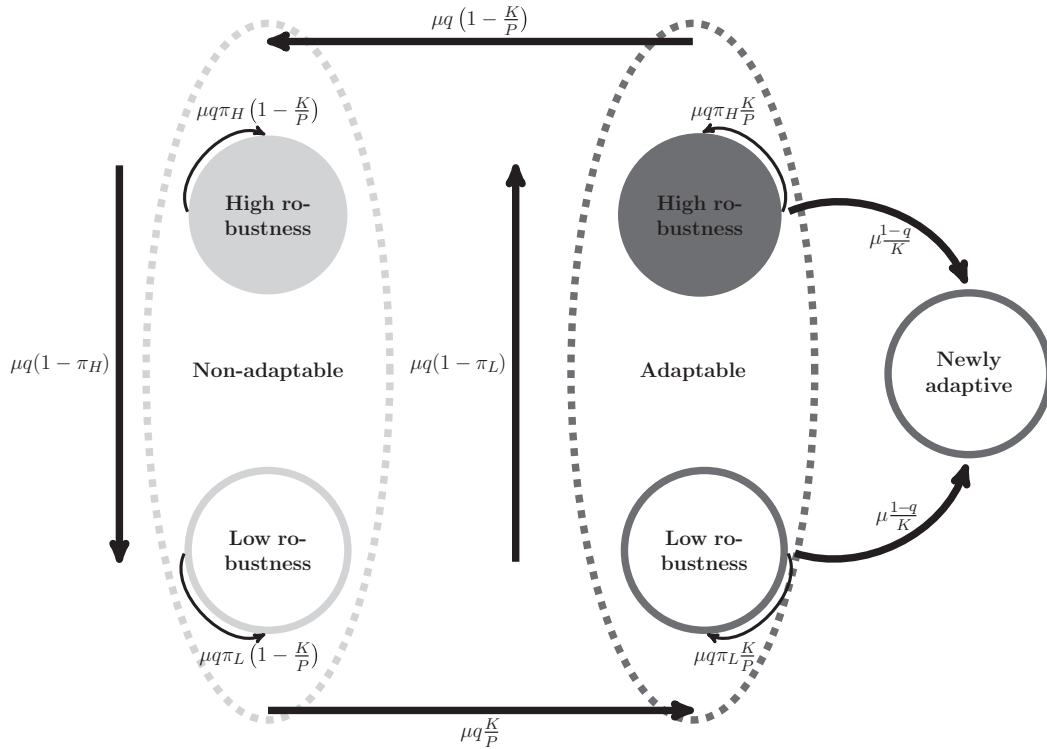
The parameters  $\pi_H$  and  $\pi_L$  provide a measure of how clustered the genotypes of high and low robustness are on the neutral network. In particular, an individual with a high-robustness genotype will undergo  $\frac{1}{1-\pi_H}$  neutral mutations, on average, before it encounters a genotype of low robustness. Similarly, an individual with a low-robustness genotype will undergo  $\frac{1}{1-\pi_L}$  mutations, on average, before it encounters a genotype of high robustness. Moreover, the overall fraction of genotypes in the neutral network that have high robustness is given by  $\frac{1-\pi_L}{2-(\pi_L+\pi_H)}$  (Fig. 3).

In the case of this simplified model, we can use equations (3)–(5) to write down the SDEs for the diffusion process associated with the two adaptable types (those of high and low environmental robustness):

$$dY_H = \left[ \sigma_H Y_H + q\theta \frac{K}{P} (\pi_H X_H + (1 - \pi_L)X_L) \right] dt + \sqrt{2Y_H} dB_H(t) \quad (6)$$

$$dY_L = \left[ \sigma_L Y_L + q\theta \frac{K}{P} ((1 - \pi_H)X_H + \pi_L X_L) \right] dt + \sqrt{2Y_L} dB_L(t), \quad (7)$$

where  $X_H$  and  $X_L$  denote the fraction of the population at high and low robustness nonadaptable genotypes, and  $Y_H$  and  $Y_L$



**Figure 2.** Rates of mutation among the four classes of genotypes, in our simplified model. Genotypes are either adaptable with high environmental robustness (top right), adaptable with low environmental robustness (bottom right), nonadaptable with high environmental robustness (top left), or nonadaptable with low environmental robustness (bottom left). Mutations change genotypes of high robustness (filled circles) to genotypes of low robustness (open circles) with probability  $q(1 - \pi_H)$ , and from low (pink) to high (red) robustness with probability  $q(1 - \pi_L)$ . Mutations change nonadaptable genotypes (left) to adaptable genotypes (right) with probability  $q(1 - \frac{K}{P})$  and adaptable to nonadaptable with probability  $q\frac{K}{P}$ . Adaptable genotypes mutate to a genotype encoding the newly adaptive phenotype with probability  $\frac{1-q}{K}$ . Mutations within classes are also indicated.

denote the number of individuals, rescaled by  $\frac{1}{\sqrt{N}}$  (see Supporting Information), at high- and low-robustness adaptable genotypes. As before, the deterministic part of the system consists of a selection term, and an influx term due to mutation from nonadaptable to adaptable types. We note that when the adaptable types are rare, the probability of back mutation is rare, and thus it does not appear in our limiting diffusions, as justified in the Supporting Information. Following the environmental shift, the selection terms  $\sigma_H$  and  $\sigma_L$ , for the high- and low-robustness adaptable genotypes, respectively, are given by

$$\frac{\sigma_H}{\sqrt{N}} = \epsilon \frac{1+s}{K} (1 - \phi_H) + \epsilon (\phi_H - \phi_L) X_L \tag{8}$$

$$\frac{\sigma_L}{\sqrt{N}} = \epsilon \frac{1+s}{K} (1 - \phi_L) - \epsilon (\phi_H - \phi_L) X_H. \tag{9}$$

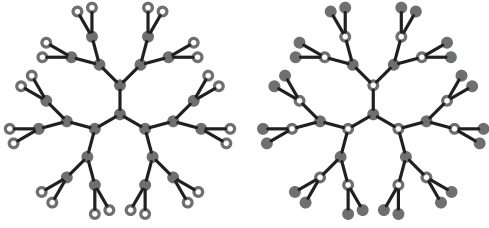
These selection coefficients have an intuitive interpretation. The first term in equation (8) and equation (9) represents the selective advantage of adaptable genotypes over the rest of the population that results from their occasionally expressing the newly adaptive phenotype. In particular, such individuals express an

alternative phenotype with probability  $\epsilon(1 - \phi)$ , and that alternative phenotype will be adaptive, with selective benefit  $s$ , with probability  $\frac{1}{K}$ . The second terms in equations (8) and (9) represent the strength of selection on genotypes of high and low robustness in the population. These can be understood as follows: high-robustness genotypes gain a selective advantage  $\epsilon(\phi_H - \phi_L)$  over low-robustness genotypes (because they express deleterious alternative phenotypes less frequently). This results in a selective advantage for high-robustness genotypes in equation (8) and a selective disadvantage for low-robustness genotypes in equation (9).

Since both terms in equation (8) favor high-robustness adaptable genotypes,  $\sigma_H$  is always positive. However, for low-robustness genotypes, one term gives a selective advantage and the other term a selective disadvantage; therefore,  $\sigma_L$  can be either positive or negative.

Finally, the influx terms in equation (6) and equation (7) depend on  $\pi_H$  and  $\pi_L$ —that is, on how genotypes of high and low robustness are clustered across the network.

In the Results section, we will use the analytical equations above to study how the various factors in our model influence the



**Figure 3.** Illustration of mutations within and between classes for different clusterings of individual genotypes. Left—genotypes with high environmental robustness (filled circles) are tightly clustered, and tend to neighbor one another rather than genotypes with low environmental robustness (empty circles). This corresponds to  $\pi_H \sim 1$ . Right—genotypes with high environmental robustness tend not to neighbor other genotypes of the same type. This corresponds to  $\pi_H \sim 0$ . When genotypes of the high-robustness type tend to neighbor each other, the population can spread to many different genotypes and the degree of genetic diversity in the population increases.

capacity of a population to adapt. We will also compare our analytic approximation for the mean adaptation time to exact Monte Carlo simulations of the Moran process. Simulations are carried out with populations of  $N = 10,000$  individuals. An individual is chosen to reproduce with a probability determined by its fitness relative the population as a whole. The chosen individual produces a viable offspring provided the offspring does not suffer a deleterious mutation or a deleterious environmental perturbation. Mutations occur at rate  $\mu = 10^{-3}$ , across the entire genome. A mutation will change the offspring's genotype, phenotypic neighborhood, and environmental robustness as described above (see Fig. 2). The offspring produced in such a reproduction event replaces an individual selected randomly from the population. Simulations are stopped whenever an individual arises whose genotype encodes the newly adaptive phenotype. The waiting time before such an adaptation arises is averaged over 10,000 replicate simulations, to produce the mean waiting time plotted in the figures below.

## Results

We focus on the case in which the population has evolved for a long time in response to selection for a given phenotype, and has reached its equilibrium distribution on the neutral network prior to the environmental shift. Since we are interested in the influence of environmental robustness on adaptation, we keep fixed the population-scaled mutation rate,  $\theta = N\mu$ , mutational robustness,  $q$ , number of phenotypic neighbors,  $K$ , and total number of alternative phenotypes,  $P$ ; and we vary the other parameters: the degree of environmental robustness,  $\phi_H$ ,  $\phi_L$ , the clustering of high- and low-robustness genotypes,  $\pi_H$ ,  $\pi_L$ , the selective advantage of the newly adaptive phenotype,  $s$ , and the probability that an individual experiences environmental noise  $\epsilon$ .

Prior to the environmental shift, approximately all nonadaptable individuals will have high robustness (i.e.,  $X_H = 1$  and  $X_L = 0$ ), since we have assumed high-robustness genotypes have a selective advantage over low-robustness genotypes  $O(\sqrt{N})$  (see Supporting Information). Following the environmental shift, the dynamics of the nonadaptable individuals are approximated by the ODE in equation (2). Substituting these values in equations (6)–(9), we see that the selection terms vary with the environmental robustness of the different genotypes,  $\phi_H$  and  $\phi_L$ , with the rate of environmental perturbations,  $\epsilon$ , and with the strength of selection for the newly adaptive type,  $s$ . However, the mutational influx of adaptable genotypes depends only on how genotypes of high robustness are distributed in the neutral network,  $\pi_H$ .

Our central question—how do opposing influences of environmental robustness resolve to affect adaptation time?—is most naturally addressed by considering how adaptation time varies with the probability that an individual experiences an environmental perturbation during development,  $\epsilon$ . However, this relationship is complex, and its form depends on the distribution of high-robustness genotypes,  $\pi_H$ , the degree of environmental robustness of different genotypes,  $\phi_H$  and  $\phi_L$ , and the strength of selection for the newly adaptive phenotype,  $s$ . Therefore, we begin by considering the relationship between these parameters and adaptation time, before considering the frequency of environmental perturbations.

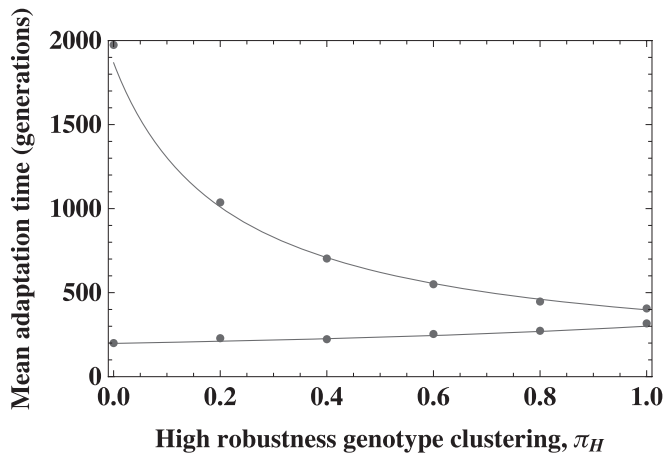
### DEPENDENCE OF ADAPTATION TIME ON THE CLUSTERING OF ROBUST GENOTYPES

We begin by considering how the clustering of high-robustness genotypes influences adaptation time. Varying  $\pi_H$  from 0 to 1 will increase the clustering of high-robustness genotypes. When  $\pi_H \sim 1$ , the neutral neighbors of high-robustness genotypes are almost always other high-robustness genotypes; whereas when  $\pi_H \sim 0$ , the neutral neighbors of high-robustness genotypes are almost always low-robustness genotypes (Fig. 3).

There are two distinct selective regimes that correspond to qualitatively different ways in which adaptation time varies with  $\pi_H$ . When  $\frac{1+s}{K} < 1$ , adaptable genotypes with high robustness are selected for more strongly than adaptable genotypes with low robustness (i.e.,  $\sigma_H > \sigma_L$ ), and adaptation time decreases as  $\pi_H$  increases (Fig. 4). In contrast, when  $\frac{1+s}{K} > 1$ , adaptable genotypes with low robustness are selected for more strongly than adaptable genotypes with high robustness ( $\sigma_H < \sigma_L$ ), and adaptation time increases as  $\pi_H$  increases (Fig. 4).

The first of these two regimes,  $\frac{1+s}{K} < 1$ , is more realistic because we expect the size of the phenotypic neighborhood,  $K$ , to be much greater than 1 and the selective advantage,  $s$ , of the newly adaptive phenotype to be less than 1, typically. In the most realistic scenario, therefore, adaptation time will decrease





**Figure 4.** Mean adaptation time as a function of the clustering of high-robustness genotypes,  $\pi_H$ . As  $\pi_H$  increases, the probability that a high-robustness genotype neighbors another high-robustness genotype increases (i.e., more clustering). When  $\frac{1+s}{K} < 1$  (red line), this causes a decrease in the mean adaptation time of the population. When  $\frac{1+s}{K} > 1$  (blue line), this causes an increase in mean adaptation time. Plots show populations of  $N = 10,000$  individuals, with  $\mu = 0.001$ ,  $\epsilon = 0.1$ ,  $P = 100$ ,  $K = 5$ ,  $q = 0.5$ ,  $\phi_H = 0.9$ , and  $\phi_L = 0.1$  with  $s = 1$  (red line) and  $s = 5$  (blue line). Lines indicate the analytic solution to our model, whereas dots indicate the means of 10,000 replicate Monte Carlo simulations.

with increasing  $\pi_H$ —that is, it takes longer for the population to adapt when high-robustness genotypes are not clustered. The intuition underlying this result is simple. Prior to the environmental shift, high-robustness genotypes are always selectively favored (because environmental perturbations are always deleterious). At the same time, after the environmental shift, high-robustness adaptable genotypes are more selectively favored than low-robustness adaptable genotypes, that is,  $\sigma_H > \sigma_L$  in equations 8–9. The more tightly clustered high-robustness genotypes are (i.e., the larger  $\pi_H$ ), the more often neutral mutations to genotypes of high robustness will produce other genotypes of high robustness. As a result, there is more standing neutral genetic variation in the population prior to the environmental shift, because there are a greater number of distinct genotypes within the class of high environmental robustness. This in turn causes adaptable genotypes to be produced at a higher rate after the environmental shift, speeding adaptation. In contrast, if  $\frac{1+s}{K} > 1$ , low-robustness adaptable genotypes are selectively favored over high-robustness adaptable genotypes, that is,  $\sigma_H < \sigma_L$  in equations (8)–(9). Therefore, increasing the clustering of high-robustness genotypes increases the neutral genetic variation in the population prior to the environmental shift, but decreases the rate at which advantageous adaptable genotypes are produced after the environmental shift. These two effects oppose each other, and we find that the overall effect is to slow adaptation. The adaptive dy-

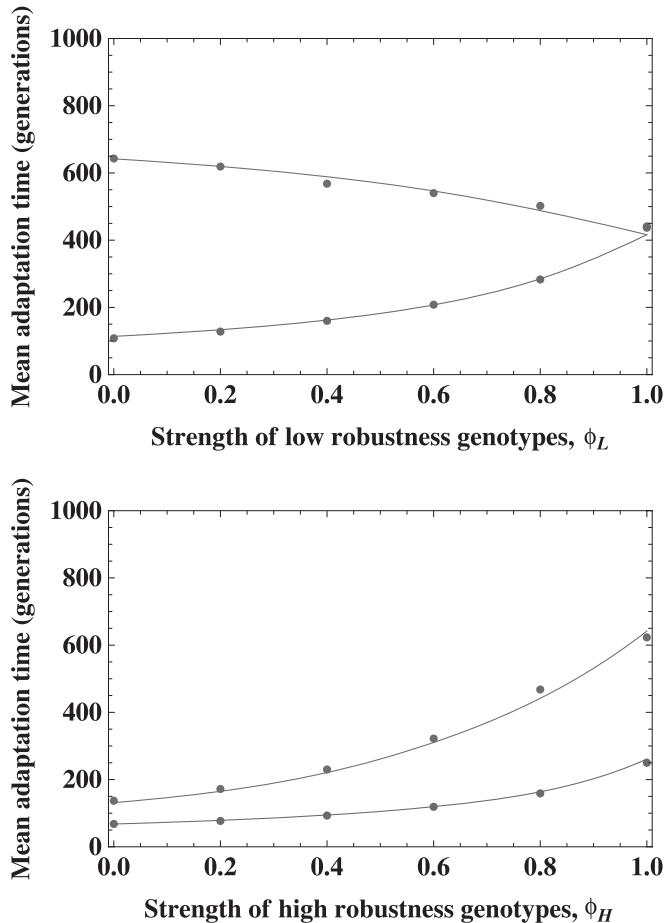
namics in these differing regimes can be illustrated through time series plots of the frequency of high- and low-robustness adaptable genotypes in the different selective regimes (see Supporting Information).

Here, we have focused on the distribution of high-robustness genotypes,  $\pi_H$ , assuming that the clustering of low-robustness genotypes,  $\pi_L$ , remains fixed. Varying the clustering of low-robustness genotypes does not affect adaptation time if, as we have assumed, the population is at equilibrium prior to the environmental shift (eqs. 6 and 7).

#### DEPENDENCE OF ADAPTATION TIME ON THE STRENGTH OF ENVIRONMENTAL ROBUSTNESS

We now consider how variation in the strength of environmental robustness (parameters  $\phi_H$  and  $\phi_L$ ) affects adaptation time. As Figure 5 shows adaptation time always increases with increasing  $\phi_H$  (i.e., when the robustness of the high-robust type is increased, adaptation is slower). There are two distinct reasons for this, associated with the selection terms in equation (8) and equation (9). First, as  $\phi_H$  increases, the (postshift) selection term for high-robustness adaptable genotypes decreases (this can be seen directly from eq. 8, setting  $X_H = 1$  and  $X_L = 0$ , but it is also intuitive: as  $\phi_H$  increases, the chance that a high-robust genotype expresses the adaptive phenotype decreases), so that the population is less likely to flow toward these adaptable genotypes, which slows adaptation. Second, as  $\phi_H$  increases, the selective term for low-robustness adaptable genotypes also decreases (this can be seen directly from eq. 9, with  $X_H = 1$  and  $X_L = 0$  but it is also intuitive: as  $\phi_H$  increases, the selective disadvantage of a low-robustness genotype compared to the rest of the population becomes greater), which again retards flow toward adaptable genotypes and slows adaptation.

Varying the environmental robustness of low-robustness genotypes,  $\phi_L$ , has a more complex effect on adaptation time. Increasing  $\phi_L$  has no effect on the selective advantage of high-robustness adaptable genotypes. However, it has conflicting effects on the selective advantage of low-robustness adaptable genotypes (as seen in eq. 9). On the one hand, increasing  $\phi_L$  decreases the probability that an environmental perturbation on a low-robustness adaptable genotype will produce the newly adaptive phenotype. However, increasing  $\phi_L$  also reduces the probability that an environmental perturbation on a low-robustness adaptable genotype will produce a deleterious phenotype. Whether the overall selection term  $\sigma_L$  is increasing or decreasing with  $\phi_L$  therefore depends on how advantageous it is to produce the newly adaptive phenotype. This depends on the ratio  $\frac{1+s}{K}$ . When  $\frac{1+s}{K} < 1$ , the selection term  $\sigma_L$  for the low-robustness adaptable genotypes increases as  $\phi_L$  increases (eq. 9); as a result, adaptation time decreases as  $\phi_L$  increases. When  $\frac{1+s}{K} > 1$ , the selection term for the low-robustness adaptable genotypes decreases as  $\phi_L$



**Figure 5.** Top—mean adaptation time as a function of the amount of environmental robustness,  $\phi_L$ , among low-robust genotypes. When selection for the newly adaptive phenotype is relatively weak ( $\frac{1+s}{K} < 1$ , red line), increasing  $\phi_L$  reduce the frequency of adaptable individuals, which retards adaptation. When selection is stronger ( $\frac{1+s}{K} > 1$ , blue line), increasing  $\phi_L$  promotes adaptable individuals and thus facilitates adaptation. Plots show populations of  $N = 10,000$  individuals, with  $\mu = 0.001$ ,  $\epsilon = 0.1$ ,  $P = 100$ ,  $K = 5$ ,  $q = 0.5$ ,  $\phi_H = 1$ , and  $\pi_H = 0.5$  with  $s = 2$  (red line) and  $s = 6$  (blue line). Bottom—mean adaptation time with  $\phi_H$ . As  $\phi_H$  increases, the selection term for both high- and low-robustness adaptable genotypes decreases. Plots show populations of  $N = 10,000$  individuals, with  $\mu = 0.001$ ,  $\epsilon = 0.1$ ,  $P = 100$ ,  $K = 5$ ,  $q = 0.5$ ,  $\phi_L = 0$ , and  $\pi_H = 0.5$  with  $s = 2$  (red line) and  $s = 5$  (blue line). Lines indicate the analytic solution to our model, whereas dots indicate the means of 10,000 replicate Monte Carlo simulations.

increases (eq. 9); as a result, adaptation time increases as  $\phi_L$  increases (Fig. 5). As in the previous section, the more realistic case occurs when  $\frac{1+s}{K} < 1$ . Therefore, we expect that increasing  $\phi_L$  will generally decrease adaptation time.

In summary, from the perspective of adaptation, the least environmentally robust genotypes in a population would generally prefer to increase their robustness; whereas the most environ-

mentally robust genotypes would always prefer to reduce their robustness, as we saw above. Thus, populations with less variation in environmental robustness will generally adapt faster than those with greater variation in environmental robustness.

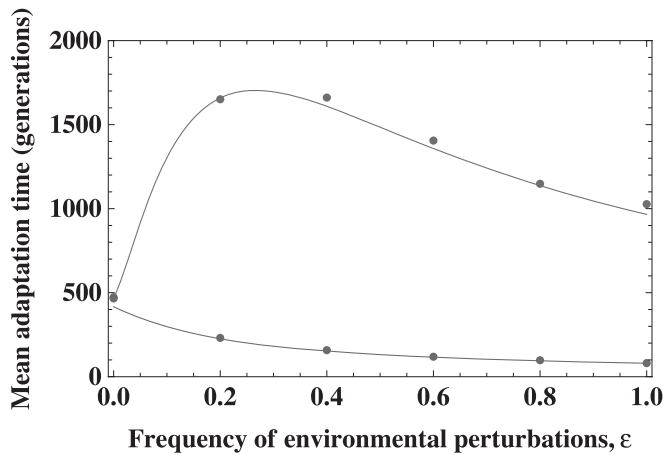
#### DEPENDENCE OF ADAPTATION TIME ON THE FREQUENCY OF ENVIRONMENTAL PERTURBATION, $\epsilon$

We now turn to the central question of this study—how does adaptation time vary with the frequency of environmental perturbations during development? Increasing the frequency of such perturbations,  $\epsilon$ , will increase the magnitude of the selection terms associated with both high- and low-robustness adaptable genotypes (eqs. 8 and 9). However, increasing  $\epsilon$  does not change the relative strength of these selection terms, the direction of selection, or the total rate of influx into the adaptable genotypes. These depend on the factors discussed in the previous two sections—the distribution of high-robustness genotypes, and the strength of environmental robustness associated with both high- and low-robustness genotypes.

There are two selective regimes of interest when considering how adaptation time depends on the frequency of environmental perturbations. The first regime occurs when low-robustness adaptable genotypes have lower fitness than the population as a whole, that is, when  $\sigma_L$  is negative. According to equation (9), this occurs when  $\frac{1+s}{K} < \frac{\phi_H - \phi_L}{1 - \phi_L}$ . The second regime occurs when low-robustness adaptable genotypes are selectively favored compared to the population as a whole, that is, when  $\sigma_L$  is positive:  $\frac{1+s}{K} > \frac{\phi_H - \phi_L}{1 - \phi_L}$ . High-robustness adaptable genotypes are always selectively favored compared to the population as a whole, that is,  $\sigma_H > 0$  (eq. 8).

In the first selective regime, increasing  $\epsilon$  increases the strength of selection for high-robustness adaptable genotypes, while increasing the strength of selection against low-robustness adaptable genotypes. As a result increasing  $\epsilon$  has conflicting effects on the adaptation time—it increases the adaptation time from low-robustness adaptable genotypes but decreases the adaptation time from high-robustness adaptable genotypes. Initially, as  $\epsilon$  increases from 0, adaptation time increases, as selection against low-robustness adaptable genotypes reduces the density of individuals at all adaptable genotypes. However, as  $\epsilon$  continues to increase, selection for high-robustness adaptable genotypes concentrates the population at these genotypes further, and causes the overall adaptation time to decrease. This results in a nonmonotonic relationship between adaptation time and  $\epsilon$ : increasing the frequency of environmental perturbation can either facilitate or impede adaptability (Fig. 6). In this regime, the population adapts most quickly when environmental noise is either very frequent or very infrequent.

In the second selective regime, increasing  $\epsilon$  increases the strength of selection for both high- and low-robustness adaptable



**Figure 6.** Mean adaptation time as a function of the probability of environmental perturbation,  $\epsilon$ . When selection for the newly adaptive phenotype is relatively strong ( $\frac{1+s}{K} > \frac{\phi_H - \phi_L}{1 - \phi_L}$ , blue line), increasing amounts of environmental perturbation promotes all types of adaptable genotypes, facilitating adaptation. When selection is weaker ( $\frac{1+s}{K} < \frac{\phi_H - \phi_L}{1 - \phi_L}$ , red line) increasing  $\epsilon$  has contrary effects on the low-robust and high-robust adaptable individuals; in this regime, the relationship between mean adaptation time and probability of environmental perturbation is non-monotonic (increasing and then decreasing). Plots show populations of  $N = 10,000$  individuals, with  $\mu = 0.001$ ,  $P = 100$ ,  $K = 5$ ,  $q = 0.5$ ,  $\phi_H = 0.9$ ,  $\phi_L = 0.1$ , and  $\pi_H = 0.1$  with  $s = 2$  (red line) and  $s = 5$  (blue line). Lines indicate the analytic solution to our model, whereas dots indicate the means of 10,000 replicate Monte Carlo simulations.

genotypes, and it therefore always decreases adaptation time. In this regime, more environmental noise always speeds adaptation.

In summary, when environmental fluctuations are rare ( $\epsilon \sim 0$ ), increasing their frequency can either impede or facilitate adaptation depending on the strength of selection for the newly adaptive phenotype. However, when environmental fluctuations are common ( $\epsilon \sim 1$ ), increasing their frequency will always speed adaptation.

We have also studied how the clustering of high-robustness genotypes influences the relationship between environmental perturbations and mean adaptation time (see Supporting Information).

#### DEPENDENCE OF ADAPTATION TIME ON MUTATIONAL ROBUSTNESS

We have also studied how environmental noise can influence the relationship between mutational robustness and adaptability. Previous work has shown that, in the absence of environmental noise, intermediate levels of mutational robustness produce the fastest rates of adaptation (Wagner 2008; Draghi et al. 2010). We find that the addition of environmental noise does not qualitatively change

the relationship between mutational robustness and adaptation (see Supporting Information).

### Discussion

The expression of a phenotype is inherently noisy. As a result, when a population experiences purifying selection, those genotypes that express the optimal phenotype more reliably will be selectively favored. This is the key insight behind Waddington's concept of environmental canalization, developed over half a century ago (Waddington 1942, 1953a,b, 1959). Nevertheless, the extent to which environmental robustness evolves in response to noise, and how this phenomenon impacts evolution over longer time scales, as the optimal phenotype itself changes, is not fully understood.

When canalization fails, environmental noise elicits the expression of an alternative phenotype in an individual. The resulting phenotype is often then a phenocopy—that is, a phenotype that mimics the effect of a mutation, but is not heritable. When a population is faced with a new environment, the phenocopy phenomenon may speed adaptation, because it allows some individuals to express adaptive phenotypes without waiting for mutations. However, when a population is adapted to the current environment, environmental noise tends to concentrate the population on the most robust genotypes, which will reduce genetic diversity and the capacity for adaptation. We have sought to elucidate how these two conflicting effects of environmental robustness resolve in determining the adaptability of populations.

We have employed a general population-genetic model that was previously used to study the interplay between mutational robustness and adaptability (Draghi et al. 2010). Our model specifies a fitness landscape in terms of the properties of a neutral network of genotypes. Whenever an individual develops an alternative phenotype as a result of environmental perturbation, it develops into one of the  $K$  phenotypes accessible by mutation; this assumption encodes the concept of phenocopy in our modeling framework.

We have studied the relationship between environmental noise and adaptation time—that is, the time required for a population, initially in equilibrium, to acquire a new target phenotype following an environmental shift. In our simplest model, the adaptation time depends on several parameters: the frequency of environmental perturbations ( $\epsilon$ ), the selective advantage of the novel target phenotype ( $s$ ), the levels of environmental robustness among genotypes on the neutral network ( $\phi_H$  and  $\phi_L$ ), and the clustering of genotypes with high environmental robustness ( $\pi_H$ ). The interplay of these parameters gives rise to a surprisingly rich set of behaviors, which are summarized in Table 2.

The relationship between environmental and mutational robustness may be further complicated if both are allowed to vary

**Table 2.** Summary of results.

Parameter varied	Selective regime	Mean adaptation time
High-robustness genotype clustering, $\pi_H$	$\frac{1+s}{K} < 1$	Decreasing
High-robustness genotype clustering, $\pi_H$	$\frac{1+s}{K} \geq 1$	Increasing
Low-robustness genotype clustering, $\pi_L$	All	Constant
High environmental robustness level, $\phi_H$	All	Increasing
Low environmental robustness level, $\phi_L$	$\frac{1+s}{K} < 1$	Decreasing
Low environmental robustness level, $\phi_L$	$\frac{1+s}{K} \geq 1$	Increasing
Rate of environmental perturbation, $\epsilon$	$\frac{1+s}{K} < \frac{\phi_H - \phi_L}{1 - \phi_L}$	Nonmonotonic (increasing then decreasing)
Rate of environmental perturbation, $\epsilon$	$\frac{1+s}{K} \geq \frac{\phi_H - \phi_L}{1 - \phi_L}$	Decreasing
Mutational robustness, $q$	All	Nonmonotonic (decreasing then increasing)

across the neutral network simultaneously. Our analysis has assumed the simplest case—when environmental and mutational robustness vary independently. Even in this simple case, we have found a rich diversity of possible behaviors (Table 2). The assumption of independence is partly supported by the few systematic empirical studies of mutational and environmental robustness. Such studies have found that polymorphisms affecting mutational robustness map to different loci than those influencing environmental robustness (Proulx et al. 2007; Fraser and Schadt 2010; Lehner 2010), suggesting that these traits may be largely independent. Nonetheless, the impact of covariation in these two types of robustness (Hermisson and Wagner 2004; Lehner 2010; Meiklejohn and Hartl 2002; Proulx et al. 2007; Wagner et al. 1997) remains an important topic for future research.

It is interesting to contrast our results on environmental noise with quantitative studies of the Baldwin effect, which also describe the genetic reinforcement of initially nonhereditary traits (Ancel 1999, 2000). Whereas we have focused here on the tension between costs and benefits of randomly expressing an alternative phenotype during development, studies of the Baldwin effect have been concerned with the tension between the adaptive advantage and inherent cost of phenotypic plasticity—that is, the ability of an individual to express a range of phenotypes and to deterministically choose among these the most fit phenotype in the current environment. In other words, in our study, the phenotype expressed by an individual is subject to random perturbation, whereas in studies of the Baldwin effect, the expressed phenotype is chosen deterministically: if an individual has the ability to express the optimal phenotype with its range of alternatives, it will. Models of the Baldwin effect have uncovered a complex interplay between the rate at which the environment changes, the degree of phenotypic plasticity in a population, and the rate of adaptation (Ancel 1999, 2000). We have studied a different phenomenon, but likewise found a complex relationship between the amount of development noise and the rate of adaptation. Both phenomena (phenotypic plasticity and environmental robustness) highlight the complex relationship between environmentally in-

duced phenotypic variation and the capacity for a population to adapt.

A phenomenon analogous to the Baldwin effect may occur in our system if the newly adapted individual that first fixes in the population has low robustness (analogous to high plasticity in the models of [Ancel 1999, 2000]); whereas subsequent stabilizing selection will then select for a high-robustness (adapted) individual. This situation is more likely to occur in the regime  $\sigma_L > \sigma_H$ .

In deriving our analytical results, we have relied on three strong assumptions: a neutral mutation completely redraws an individual's phenotypic neighborhood; the number of phenotypes,  $K$ , in a genotype's neighborhood does not vary across the neutral network; and alternative phenotypes are generally lethal. Relaxing each of these assumptions does not change our qualitative results (see Supporting Information). In particular, we relax the first assumption by introducing a parameter,  $f$ , which is the fraction of  $K$  neighbors that are redrawn following a neutral mutation. Allowing such correlations between the phenotypic neighborhoods of neutral neighbors affects the rate at which individuals arrive at adaptable genotypes through mutation, but it preserves the qualitative relationships between adaptability and environmental robustness reported above. Our qualitative results are also unchanged if the size of the phenotypic neighborhood,  $K$ , varies with genotype. In this case, however, the selective regimes described in Table 2 now depend on the average value of  $\frac{1+s}{K}$ . Finally, our qualitative results are unaltered if alternative phenotypes are moderately deleterious rather than lethal; however, the different selective regimes described in Table 2 would then depend on  $s$ ,  $K$ , and the size of the fitness penalty suffered by nonadaptive alternative phenotypes (see Supporting Information). We have limited our analysis to the regime in which the newly adaptive type is always favored over the initially fit phenotype, regardless of its environmental robustness. However, it is possible to imagine scenarios in which  $s$  is sufficiently small that newly adaptive genotypes with low robustness are deleterious compared to high-robustness individuals of the initially fit phenotype. This situation will occur when  $s$

is  $O(\epsilon)$  or smaller (see Supporting Information). To study adaptability in this case, we must determine the waiting time until a newly adaptive individual with high robustness is produced. However, the qualitative relationships we observe in this case are the same as those described in the main text (see Supporting Information).

In this article, we have considered adaptation to a single environmental shift. It would also be interesting to study repeated environmental shifts. To do so will require analyzing the adaptation time of a population which is not at equilibrium on its neutral network when the next environmental shift occurs. Accounting for such repeated transient dynamics should be possible in our modeling framework, but it will certainly be nontrivial. The relationship between the rate of environmental shifts and the evolution of the environmental robustness thus remains an interesting area for future research.

Our modeling framework has allowed for substantial variation in robustness among a large set of genotypes. This framework is appropriate if many sites across an organism's genome can independently influence its robustness, such as might be expected if networks of interacting genes jointly determine an organism's phenotype. In fact, the widespread contribution of many genomic sites to robustness has been directly observed in organisms ranging from yeast to plants and vertebrates (Fraser and Schadt 2010). The framework for our study is therefore rather different from models restricted to a specific locus (such as the HSP90 locus or a DNA repair locus) that alone determines variation in robustness.

Despite longstanding interest in phenotypic robustness, the role of robustness in adaptation remains a topic of active investigation. In contrast to most theoretical work, which has focused on how mutational robustness influences adaptation (de Visser et al. 2003; Lenski et al. 2006; Wagner 2008; Draghi et al. 2010), this study provides a framework for analyzing the effects of environmental robustness. Although we have uncovered a complex set of possible behaviors (summarized in Table 2), one important general observation emerges: environmental perturbations can either facilitate or impede adaptation. These results have been derived in a general theoretical framework, and so they should apply to a broad range of biological situations and systems.

Theoretical studies of environmental robustness must eventually be reconciled with empirical data, either from surveys of standing variation or, ideally, from evolution experiments. Fortunately, there is an extensive literature based on quantitative genetics that describes empirical patterns of environmental robustness in organisms, including yeast, plants, and mammals (Mackay and Lyman 2005; Hall et al. 2007; Nogami et al. 2007; Sangster et al. 2008; Fraser and Schadt 2010). Such studies typically find a broad array of traits (morphological, life-history, and gene expression levels) that are buffered against environmental noise and, furthermore, extensive standing genetic variation in the degree of

such buffering among individuals. Thus, environmental robustness is apparently selected for in many natural populations, in accordance with theoretical expectations for a population under stabilizing selection for a target phenotype (Wagner et al. 1997; Hermisson and Wagner 2004); but a natural population typically also contains variation in the degree of environmental robustness among genotypes. Our analysis suggests that the existence of robust variants in a population, and also the variation in robustness among individuals, will have a significant impact on the population's capacity to adapt to a novel environment. Thus, the concept of canalization, first considered by Waddington for populations under stabilizing selection, plays an important role in shaping the evolution of populations as they adapt to new environments over longer timescales.

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## Supporting Information

The following supporting information is available for this article:

**Figure S1.** Correlated phenotypic neighbourhood—Mean adaptation time as a function of the probability of environmental perturbation,  $\epsilon$ .

**Figure S2.** Variation in size of phenotypic neighbourhood—plots show populations of  $N = 10,000$  individuals, with  $\mu = 0.001$ ,  $P = 100$ ,  $\bar{K} = 5$ ,  $q = 0.5$ ,  $\phi_H = 0.9$ ,  $\phi_L = 0.1$  and  $\pi_H = 0.5$  with  $s = 2$  (red line) and  $s = 8$  (blue line).

**Figure S3.** Nonlethal deleterious mutations—plots show populations of  $N = 10,000$  individuals, with  $\mu = 0.001$ ,  $P = 100$ ,  $K = 10$ ,  $q = 0.2$ ,  $\phi_H = 0.9$ ,  $\phi_L = 0.1$  and  $\pi_H = 0.5$ ,  $d = 0.2$  with  $s = 0.5$  (red line) and  $s = 3$  (blue line).

**Figure S4.** Deleterious low robustness adaptive genotype—plots show populations of  $N = 10,000$  individuals, with  $\mu = 0.001$ ,  $P = 100$ ,  $K = 5$ ,  $q = 0.5$  and  $s = 0.05$ .

**Figure S5.** Mean adaptation time as a function of the probability of environmental perturbation,  $\epsilon$ , in the regime  $\frac{1+s}{K} < \frac{\phi_H - \phi_L}{1 - \phi_L}$ .

**Figure S6.** The mean adaptation time as a function of mutational robustness,  $q$ .

**Figure S7.** Most realistic selective regime with  $s/K = 0.2$ ,  $\pi_H = 0.5$  and  $\pi_L = 0.5$ .

**Figure S8.** Less realistic selective regime with  $s/K = 1.2$ ,  $\pi_H = 0.5$  and  $\pi_L = 0.5$ .

**Figure S9.** Most realistic selective regime with  $s/K = 0.2$ ,  $\pi_H = 0.5$  and  $\pi_L = 0.1$ .

**Table S1.** Model parameters.

Supporting Information may be found in the online version of this article.

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