LETTERS

Mutational robustness can facilitate adaptation

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Robustness seems to be the opposite of evolvability. If phenotypes are robust against mutation, we might expect that a population will have difficulty adapting to an environmental change, as several studies have suggested[1–4]. However, other studies contend that robust organisms are more adaptable[5–8]. A quantitative understanding of the relationship between robustness and evolvability will help resolve these conflicting reports and will clarify outstanding problems in molecular and experimental evolution, evolutionary developmental biology and protein engineering. Here we demonstrate, using a general population genetics model, that mutational robustness can either impede or facilitate adaptation, depending on the population size, the mutation rate and the structure of the fitness landscape. In particular, neutral diversity in a robust population can accelerate adaptation as long as the number of phenotypes accessible to an individual by mutation is smaller than the total number of phenotypes in the fitness landscape. These results provide a quantitative resolution to a significant ambiguity in evolutionary theory.

The relationship between robustness and evolvability is complex because robust populations harbour a large diversity of neutral genotypes that may be important in adaptation[9–11]. Although neutral mutations do not change an organism's phenotype, they may nevertheless have epistatic consequences for the phenotypic effects of subsequent mutations[12–18]. In particular, a neutral mutation can alter an individual's 'phenotypic neighbourhood', that is, the set of distinct phenotypes that the individual can access through a further mutation. Pioneering studies based on RNA folding and network dynamics suggest that genotypes expressing a particular phenotype are often linked by neutral mutations into a large neutral network, and that members of a neutral network differ widely in their phenotypic neighbourhoods[1,19–21]. Numerous studies have documented the importance of neutral variation in allowing a population to access adaptive phenotypes[5,17,18,22–24], and neutral networks have consequently been proposed to facilitate adaptation[9–11].

Here we analyse the relationship between robustness and evolvability using a population genetics model that specifies statistical properties of the fitness landscape. Our approach bypasses the tremendous complexity of explicit neutral networks[1,11,17,19,21,22] to focus instead on the essential evolutionary consequences of epistatic mutations. We consider a population of N individuals reproducing according to the discrete-time, infinite-sites Moran model. In each time step, a randomly chosen individual produces one offspring. Upon replication, a mutation occurs with probability μ, producing a novel genotype. With probability q, the mutation is neutral. The parameter q therefore quantifies robustness, which is assumed to be the same for all genotypes on a network. With probability $1 - q$, the mutation is non-neutral and changes the offspring's phenotype to one of K phenotypes accessible from a given genotype. Each genotype has a specific set of K accessible phenotypes that constitute its phenotypic neighbourhood; these K phenotypes are drawn uniformly from P possible alternatives. Phenotypic neighbourhoods are assumed to be independent, such that the K accessible phenotypes are redrawn whenever a mutation occurs (we

relax this and other assumptions below). When the number of phenotypes accessible to an individual, K, is significantly smaller the total number of alternative phenotypes in the landscape, P, neutral mutations can profoundly alter an individual's phenotypic neighbourhood. This genotype–phenotype map is illustrated in Fig. 1.

Our model implicitly represents a space of adjacent neutral networks. Neutral mutations produce other genotypes on the focal network, whereas non-neutral mutations produce genotypes on adjacent networks, each expressing one of P alternative phenotypes. To study evolution on the focal network, we assume that initially all of the P alternative phenotypes are lethal (our results hold more generally; see Supplementary Information, section 5). We analyse the relationship between robustness, q, and the time required to adapt to a novel environment; this analysis is outlined in Box 1 and detailed in Supplementary Information, section 1.

We find that a robust population may adapt either more slowly or more quickly than one that is less robust (Fig. 2). Starting from a steady-state population with robustness q, we consider an environmental shift that assigns one of the P alternative phenotypes the greatest fitness. We have derived an analytic expression for the mean waiting time before this fittest phenotype subsequently arises in the population (Supplementary Information, section 1.4). When all phenotypes are accessible from any genotype ($K = P$), neutral mutations have no epistatic consequences and we observe what is naively expected: more robust populations always adapt more slowly (Fig. 2). However, when the phenotypic neighbourhood size, K, is smaller than the total number of phenotypes, P, we find an unexpected pattern: the relationship between robustness and evolvability is non-monotonic. In particular, populations with an intermediate
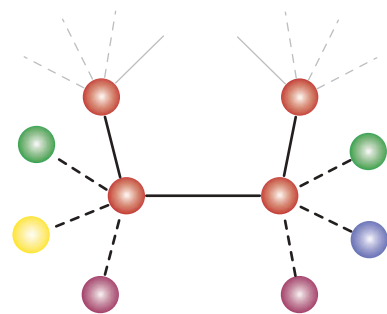

Figure 1 | The genotype–phenotype model. Schematic representation of the genotype–phenotype map used in our analysis. Each circle corresponds to a genotype; colours denote phenotypes. The model parameter q quantifies robustness: a proportion q of mutations are neutral (solid lines) and the remaining mutations are non-neutral (dashed lines). A non-neutral mutation changes an individual's phenotype to one of the K accessible alternatives that form the individual's phenotypic neighbourhood. When K is smaller than the total number of alternative phenotypes in the landscape, P, individuals may have different phenotypic neighbourhoods. The central pair of adjacent genotypes shown here express the same phenotype, but they have different phenotypic neighbourhoods.

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Box 1 | Analysis of adaptation time

We study the time, following an environmental change, until the newly beneficial phenotype arises in a population with robustness q. A genotype is said to be 'adaptable' if its phenotypic neighbourhood contains the beneficial phenotype; our analysis links the stochastic evolution of these adaptable types to the adaptation time. Let $p(t, y)$ denote the probability density of there being y adaptable individuals at time t, scaling space and time by the factor $\sqrt{N}$. Then $p(t, y)$ is well approximated by the solution to

$$\frac{\partial p}{\partial t} = \frac{\partial^2}{\partial y^2}[yp(t,y)] - \frac{\partial}{\partial y}\left[\frac{\beta Kq}{P}p(t,y)\right] - \frac{\beta(1-q)}{K}yp(t,y)$$

where $\beta = N\mu$. The first term in this expression quantifies genetic drift, the second term quantifies the increase in adaptable individuals through mutation and the third term describes the rate of mutations that produce the beneficial phenotype. The conflicting effects of robustness on adaptation are evident in this expression: an increase in robustness (q) increases the supply of adaptable individuals, but it also reduces the rate at which beneficial mutations arise in such individuals. Solving a boundary-value problem related to this equation produces an analytic expression for the expected arrival time of the beneficial phenotype (Supplementary Information, section 1), a graph of which is shown in Fig. 2.

amount of robustness adapt more quickly than populations with little or no robustness (Fig. 2).

There is a simple explanation for this counterintuitive result. In a population with little robustness (small q), most mutations are lethal and little genetic variation accumulates. As a result, the population may not contain any adaptable individuals, that is, those that are a single mutation away from the beneficial phenotype. Thus, when q is small the population may need to wait a long time before an adaptable individual arises, and then wait further for the adaptive phenotype to arise. However, slightly more robust populations contain a greater diversity of neutral genotypes, each of which has an independent chance (probability K/P) of being adaptable; thus, more robust populations may adapt more quickly.

Adaptation is most rapid when a population has an intermediate level of robustness. Moreover, this optimal level of robustness increases as the ratio K/P decreases (Fig. 2). This trend confirms the primary intuition behind our result: when phenotypic neighbourhoods are small, less robust populations contain few individuals who are 'prepared to adapt'. In this range (q is small and $K < P$), increasing robustness results in a larger repertoire of phenotypes accessible to the population, thereby accelerating adaptive evolution.
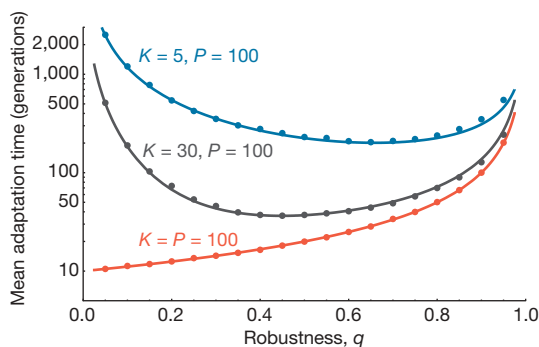
In addition to adaptation time, we have also studied another measure of evolvability, namely the diversity of phenotypes produced by mutations in a population in a steady state. Again, the naive expectation is that as robustness increases, fewer non-neutral mutants are produced each generation and, as a result, the diversity of mutant phenotypes should decrease. However, an increase in robustness also increases the neutral genetic diversity within a population, and when K is less than P, each additional neutral type may increase the number of phenotypes accessible to the population through mutation. Thus, as with adaptation time, an unexpected, non-monotonic, relationship is apparent when $K < P$: more robust populations can produce greater phenotypic diversity than their less robust equivalents (Fig. 3). We have derived an analytic expression to quantify the range of parameters for which this relationship is non-monotonic (Supplementary Information, section 2). Our analysis shows that when K is smaller than a threshold determined by P, N and μ, the diversity of mutant phenotypes is maximized at an intermediate level of robustness.

There is an interesting difference between adaptation times and phenotypic diversity: increasing the population size or mutation rate makes the relationship between robustness and adaptation time more like the naive monotonic prediction, whereas it makes the relationship between robustness and phenotypic diversity less like the naive monotonic prediction (Supplementary Information, section 4). Although these influences of population size and mutation rate have some intuitive basis, they demonstrate that even qualitative predictions about the robustness–adaptability relationship require an explicit population genetics model.

Our analysis relies on four strong assumptions: a neutral mutation completely redraws the phenotypic neighbourhood; the number of phenotypes, K, in a genotype's neighbourhood is independent of its robustness, q; the values of K and q do not vary across the neutral network; and alternative phenotypes are generally lethal. Relaxing each of these assumptions does not change our qualitative results (Supplementary Information, section 5). Briefly, we relax the first assumption by introducing a parameter, f, which is the fraction of K neighbours that are redrawn following a neutral mutation. Allowing correlations between the phenotypic neighbourhoods of neutral neighbours (that is, allowing $f < 1$) still preserves the non-monotonic relationship between robustness and evolvability. Furthermore, a strong linear correlation between K and q, or variation in either quantity across the network, does not change our results. When q varies across the network, the population evolves

Figure 2 | Robustness and adaptation time. The relationship between robustness, q, and the average waiting time before the arrival of a specific beneficial mutation, for three fitness landscapes. Points show the means of 10,000 replicate Monte Carlo simulations, and lines show our analytic predictions (Box 1 and Supplementary Information, section 1). When all possible phenotypes in the landscape are directly accessible by a mutation from any genotype (that is, when $K = P$), robustness always inhibits adaptation (red curve). However, when phenotypic neighbourhoods are small (that is, when $K < P$), neutral mutations have epistatic consequences and the resulting relationship between robustness and adaptation time is non-monotonic: adaptation is most rapid at intermediate levels of robustness. $N = 10,000$, $\mu = 0.001$.
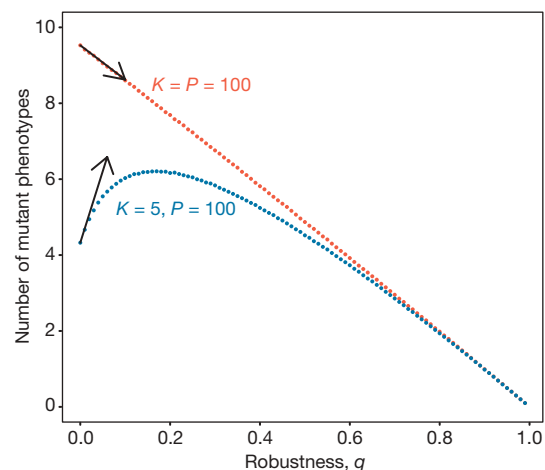

Figure 3 | Robustness and diversity. The relationship between robustness, q, and the diversity of phenotypes produced by mutation in each generation, for two fitness landscapes. Points show the means of 100,000 replicate simulations; arrows depict slopes calculated analytically (Supplementary Information, section 2). As these results demonstrate, an increase in robustness can increase phenotypic diversity, but only when the level of robustness, q, is small and the number of phenotypes accessible from a single genotype, K, is less than the total number of phenotypes in the landscape, P. $N = 10,000$, $\mu = 0.001$.

towards greater robustness as predicted by previous studies[25,26]. Nonetheless, the time required to acquire a new adaptive phenotype is still accurately described by our analytic formula, replacing the fixed value of q by the average q in the population. The same relationship between robustness and adaptation also holds when alternative phenotypes are moderately deleterious, as opposed to lethal. Therefore, our conclusions are not sensitive to any of the strong assumptions used to derive our analytical results.

Our results reveal a complex relationship between robustness and evolvability. In some situations, increasing robustness will decrease evolvability, whereas in other situations it will accelerate adaptation. The latter phenomenon can occur only when the number of phenotypes accessible to an individual, K, is smaller than the total number of alternative phenotypes in the landscape, P. To assess the plausibility of this condition, and to test the assumptions and predictions of our abstract model using an empirical, mechanistic genotype–phenotype map, we examined the folding and evolution of simulated RNA molecules, using the Vienna RNA Package (version 1.6.1) to estimate reasonable values of K, P, and f for RNA. Because these parameters vary among genotypes in an RNA neutral network, we determined appropriate averages of K, P, and f (Supplementary Information, section 6.1). For sequences of length 40 nucleotides, we estimated that $K \approx 19$ and that $P > 60{,}000$, confirming that $K < P$ for RNA. Furthermore, we found that $f \approx 0.3$, indicating that neutral mutations substantially alter phenotypic neighbourhoods. Finally, we evolved RNA populations *in silico* with varying levels of robustness, and observed a non-monotonic relation between evolvability and robustness, which was predicted accurately by our abstract model (Supplementary Information, section 6.2).

Recent studies have used theoretical[27,28] or biological[5,8] examples to argue that robustness increases evolvability. Another study has argued that robustness can either increase or decrease evolvability, depending upon the level at which robustness is described[11]. Although that study provided important intuition, it did not quantify the effects of robustness on adaptation in an evolving population. By contrast, our analysis describes the population genetics connecting these important properties. This perspective allows a quantitative resolution to opposing informal arguments, and highlights the complex interplay of influences shaping mutational robustness[29,30].

Our analysis also reveals general patterns that may guide future experimental studies. First, the relationship between robustness and evolvability can be non-monotonic. In light of this complexity, empirical studies must go beyond pairwise comparisons of high- and low-robustness strains[8], to measure evolvability over a broad range of robustness values. Second, the population size and mutation rate in part determine whether robustness increases or decreases adaptation time. This insight was not apparent from informal arguments linking robustness and evolvability[9–11], and has not yet been considered in any empirical work. Finally, the parameters K, P, and f provide a new way to quantify epistasis beyond the conventional framework of synergistic and antagonistic interactions among selected sites.

Even though most standing genetic variation is neutral, the epistatic consequences of neutral mutations have received little experimental study. Our results demonstrate that conditionally neutral mutations strongly influence a population's capacity to adapt; this form of 'neutral epistasis' therefore deserves direct experimental interrogation.

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