Unifying (simple) models of genetic robustness and evolvability

Short title: How robustness affects evolvability.

Nate B. Hardy

Current address: Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA.

Email address: n8@auburn.edu

Abstract

Simple models, in which genetic robustness is expressed as the probability that a mutation is neutral, appear to offer disparate views of the relationship between robustness and evolvability. If we assume robustness trades off with evolvability, but let environmental and mutational robustness vary across genotypes, we find that evolvability is maximized at intermediate rates of environmental change. On the other hand, if we assume that the robustness of a genotype is fixed, but let the mutational networks connecting such genotypes vary, we find that robustness and evolvability need not trade off, and in fact, evolvability is maximized at intermediate levels of robustness. Here, I reconcile and extend these models. First, I show that even without a hard genotype-level trade-off between robustness and evolvability, selection optimizes phenotypic robustness depending on the rate of environmental change. Moreover, when adaptive landscapes are complex, counter-intuitively, certain increases in environmental stability can increase the frequency of environmentally-robust but mutationally-sensitive genotypes; in other words, some environments favor evolvable generalists. Next, with minor adjustments and re-interpretations, I show that simple models connecting mutational robustness to evolvability can be readily applied to environmental (or developmental) robustness. Then, with further model extensions, I show that even if the probability of neutral mutation is fixed, populations can evolve along the spectrum of robustness and evolvability by evolving the genotype-determined neighborhood of mutationally-accessible phenotypes. Indeed, because it allows for the evolution of increased evolvability without a concomitant increase in genetic load, selection should favor changes in the phenotypic neighborhood over changes in mutational sensitivity.

1. Introduction

Phenotypes tend to be robust against much genetic and environmental variation (1,2). If the targets of selection are stable, such robustness can be adaptive, as it promotes homeostasis and decreases genetic load (3–6). On the other hand, robustness can reduce heritable genetic variation (7). So, if the adaptive landscape varies, selection may promote genotypes that are more sensitive to mutational or environmental variation, and are thus more evolvable (8–10).

Genetic and environment variation map to phenotypic variation via developmental systems that can be complex, and fittingly, many studies of the relationship between robustness and evolvability have used complex models of such mappings (11,12). Examples include empirically-based models of RNA or protein folding (7,10,13), or more theoretical models of self-assembling systems (14,15) or gene regulatory interactions (16–19). Although apropos, the interpretation of such models is fraught due to the fact that robustness is not the only thing affected by structural variation in a genotype-phenotype map. In particular, such variation can also affect how a developmental system (1) accumulates cryptic genetic diversity (20), (2) phenotypically responds to non-neutral mutation (21,22), (3) transitions between ordered and chaotic dynamics (19), and (4) “learns” from past selective environments (22–24). So, with complex genotype-phenotype models, when a change in evolvability is observed, it can be difficult to say that a change in robustness *per se* is the cause.

An alternative approach is to abstract away the details of a developmental system, and model robustness simply as the probability that a change in the genetic or external environment will

affect a change in phenotype (25,26). Although this has the advantage of conceptual clarity, at first blush, such models would seem to offer conflicting views of the relationship between robustness and evolvability. Here, I show how this apparent conflict can be resolved, and how that resolution can yield new insights into the relationship between robustness and evolvability.

The heart of the matter is that robustness is relative. We can talk about the robustness of a genotype, or a phenotype. And we can talk about robustness against variation in the genetic, or external environment. Depending on our frame of reference, the relationship between robustness and evolvability changes (10). At the genotype level, robustness and evolvability trade off; robustness decreases phenotypic variance (10). Meyers et al. (26) show that when genotypes vary in their robustness against environmental and mutational variation, populations evolve to maximize evolvability at intermediate rates of fluctuating selection. But they consider robustness strictly at the genotype level, and assume a trade-off between mutational and environmental robustness, despite evidence to the contrary (27,28). At the phenotype level, robustness and evolvability need not trade off, since robustness can increase cryptic genetic diversity (29,30). Draghi et al. (25) show that the rate at which a population adapts to a change in the environment is maximized at intermediate levels of phenotypic robustness to mutation. But they assume that, in a population, genotypes are invariant for mutational and environmental robustness. So, previous authors have made different simplifying assumptions, and consequently our view of the relationship between robustness and evolvability is only partial.

Here is the question. How does robustness relate to evolvability when it can vary across genotypes and phenotypes, against variation in both the genetic and external environment? By merging and extending their models, I show that the key inferences of Meyers et al. (26) and Draghi et al. (25) are robust. In fact, their application is broader than has been appreciated; both tell us as much about the evolution of environmental robustness as mutational robustness. I also make two additional key insights. First, counter-intuitively, in certain genetic and environmental contexts, reducing the rate of environmental change can lead to selection for more environmentally robust genotypes, that is, generalists. This is because generalists tend to evolve lower levels of mutational robustness. Second, although Meyers et al. (26) and Draghi et al. (25) focus on the probability of non-neutral mutation, their models also include a parameterization of the mutationally-accessible phenotypic neighborhood of a genotype, and in fact, selection for increased evolvability works preferentially on this neighborhood. This is because evolability via phenotypic neighborhood evolution comes without an increase in mutational load.

2. Simple models of robustness

2.a. Genotype robustness

Let us start by considering the model of Meyers et al. (26), which elegantly demonstrates that given a genotype-level trade-off between robustness and evolvability (10), the optimal level of genetic robustness is a function of the rate of environmental change. Imagine a pentagonal, bidirectional, mutational network, where each vertex is a genotype, each edge is a mutation, and each vertex has an out-degree of two (Fig. 1a.). A population of genotypes that can mutate along the edges of this network is exposed to fluctuating selection. Each genotype maps to one of three

possible phenotypes: (1) phenotype *A* is optimal when the environment is in state *a*, (2) phenotype *B* is optimal when the environment is in state *b*, and (3) phenotype *V* has intermediate fitness in both environmental states. Although several such networks are possible, Meyers et al. (26) focus on the one in Fig. 1a because the genotypes differ in mutational robustness and evolvability. Here, to clarify how this model relates to others, let us define a couple new terms. Let q_i denote the probability that a mutation of genoptype g_i has no effect on the phenotype. In Fig. 1a, q_i varies from one for genotype g_1, to zero for g_3 and g_4. Note that for the phenotype of fitness, these genotypes also vary by environmental robustness; genotype expressing phenotypes *A* and *B* are more sensitive to environmental variation than the genotype expressing phenotype *V*. If we let r_i stand for the environmental robustness of genotype g_i, in Fig. 1a, it is one for g_3 and less than one for the rest. Mutational and environmental robustness are both genotype-specific.

Meyers et al. (26) show that given this mutational network, the expected frequency of each genotype depends on a parameter λ, the number of generations between a change in the selective environment (Fig. 2): (1) When the environment changes infrequently ($\lambda > 1000$) the most mutationally robust genotype, g_1, occurs at the highest frequency. In other words, we observe the "survival of the flattest" (5). (2) When the environment changes rapidly ($\lambda < 10$), the most important thing is the environmental robustness of a genotype, r_i; the generalist genotype g_3 occurs at the highest frequency (8). Note that this is just a different type of "survival of the flattest;" where g_1 is the flattest with respect to mutational variation, g_3 is the flattest with respect to environmental variation. (3) When the environment changes neither too fast nor too slow ($10 < \lambda < 1000$), the population evolves to maximize evolvability; the highest frequency genotype is

g_0, which is the genotype that maps to phenotype *A* that has the lowest combination of mutational and environmental robustness. In sum, selection works not just on the observable phenotype {*A*, *B*, *V*} but also the robustness and evolvability of those phenotypes.

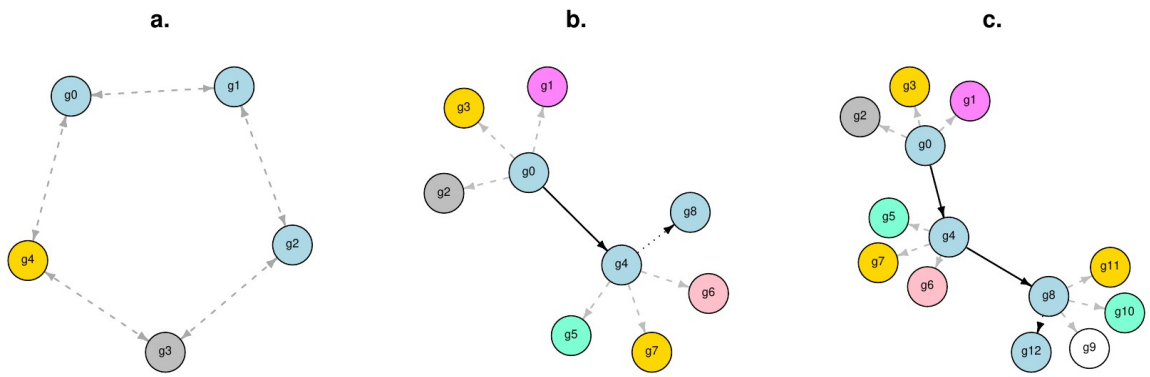

Figure 1. *Mutational networks with redundant mappings of genotypes to phenotypes. (**a**) The network of Meyers et al. (26). Five genotypes are possible, each of which maps to one of three phenotypes: A (light blue) is optimal in state a environments; B (gold) is optimal in state b environments; V (gray) has intermediate fitness across environmental states a and b. Back-mutation is possible and the graph forms a cycle. Robustness varies across genotypes, but the robustness and phenotypic neighborhood of each genotype does not change over time. (**b, c**) Example of the mutational network of Draghi et al. (25) at two time points. Different vertex colors denote different phenotypes, with the phenotype p_0 (light blue) being currently optimal. Potential phenotype-changing mutations are shows with dashed gray edges; the set of vertices connected by such edges to a genotype constitute that genotype's phenotypic neighborhood, $\mathbf{k}_i$. Potential neutral mutations are shown as dotted black edges, and realized neutral mutations are shows as solid black edges. Back-mutation is forbidden, thus, the graph is divergent. A*

mutational path can loop back to a particular phenotype, but in each step along the way, a new phenotypic neighborhood is selected for a genotype.

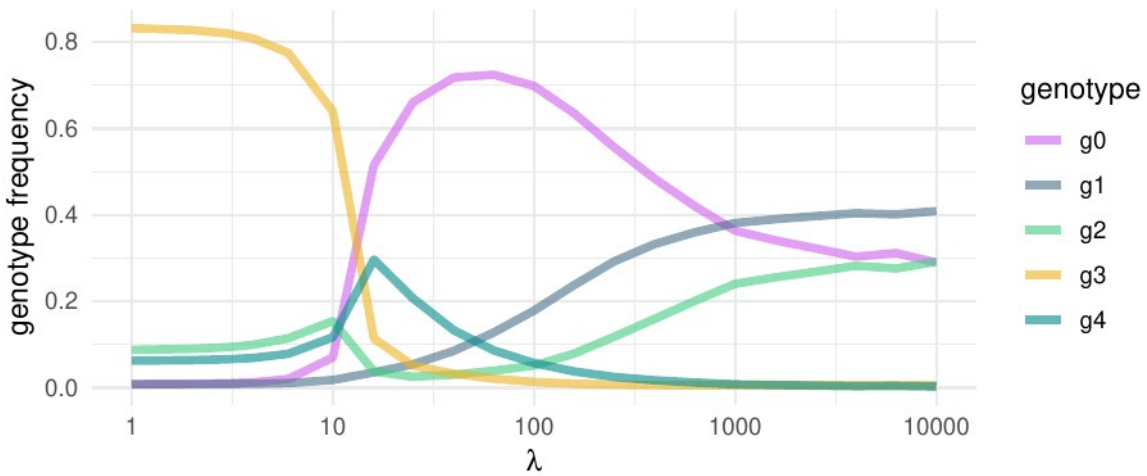

Figure 2. *Reproduction of the analysis of the Meyers et al. (26) with R code provided as Supplementary File S1. Environmental stability, λ, is given on a log scale on the x-axis. Curves show the mean frequency of each genotype when the environment is in state a.* Interpretation*: In volatile environments, generalists predominate. In stable environments mutationally robust genotypes rule. In environments that change at intermediate frequency, the population evolves to maximized evolvability; it becomes an evolvability specialist.*

2.b. Phenotype robustness

But robustness need not trade off with evolvability (31,32), an elegant demonstration of which is given by Draghi et al. (25). Like Meyers et al. (26), they consider an evolving population of

genotypes the relations of which can be visualized as a mutational network (Fig 1b,c). But in this case, instead of specifying a cyclical network *a priori*, they specify a mutational process, and allow a divergent network to emerge organically. They let each genotype g_i map to a phenotype p_i, and have a K-dimension set $\mathbf{k_i}$ of alternative phenotypes that can be reached by a single mutation. Thus, $\mathbf{k_i}$ is what has been referred to as the phenotypic 1-neighborhood of a genotype (10). (Note that in the Meyers et al. (26) model, each genotype also has a phenotypic neighborhood, but there is no requirement that adjacent genotypes have alternative phenotypes, and thus K varies across genotypes.) Phenotypes for p_i and $\mathbf{k_i}$ are chosen randomly from a set of possible phenotypes $\mathbf{P}$ of size P. Before time t_x, a population of genotypes is exposed to strong stabilizing selection about an initial phenotype p_0; all alternative phenotypes confer a fitness of zero. Mutations at rate μ are neutral with probability q; thus, as in the model of Meyers et al. (26), q affects mutational robustness, but in this case q does not vary across genotypes. At rate $\mu(1-q)$, mutation causes a change in the phenotype, with the new phenotype, p_i', selected from the phenotypic neighborhood $\mathbf{k_i}$. Whenever a mutation occurs, regardless of whether or not it is neutral, a new phenotypic neighborhood, $\mathbf{k_i}$, is randomly sampled from the phenotypic space $\mathbf{P}$. So, during this initial phase of evolution, while strong stabilizing selection prevents a population from diversifying phenotypically, the population can grow a network of phenotypically redundant genotypes, each of which has its own phenotypic neighborhood (Fig. 1b,c).

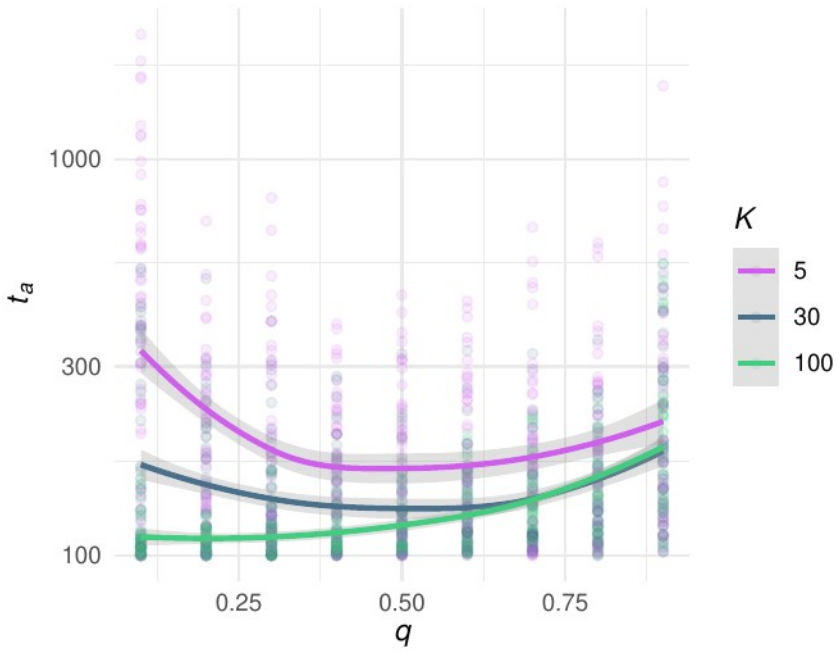

Figure 3. *The effect of mutational robustness, q, on evolvability,* t_a^{-1}*, depends on the difference between the number of possible phenotypes, K, and the size of the phenotypic neighborhood P. This reproduces the results of Draghi et al. (25) but with an individual-based simulation model (Supplementary File S2), with 50 replications for each combination of parameter values, P = 100, K = {5,30,100}, and q = (0.1, 0.2, …, 1.0). The y-axis is on a log scale. Curves are loess regressions, with a span of 1.0; shaded areas give 95% confidence intervals.* Interpretation*: Unless all phenotypes are accessible by one mutation, evolvability is maximized at intermediate levels of mutational robustness.*

At time t_x, a new optimal phenotype, p_x, is chosen from **P**. Draghi et al. (25) examine how, t_a, the time it takes for the population to evolve p_x depends on q, K, and P. They show that if $K = P$ – that is, every genotype can with one mutation reach every possible phenotype – increasing mutational robustness q monotonically increases the expected time for the population to evolve p_x (Fig. 3). As in Meyers et al. (26), robustness and evolvability are antithetical. But if $K < P$,

mutational robustness can increase the diversity of phenotypic neighborhoods, and the relationship between q and the expected time for the evolution of p_x is non-monotonic; evolvability is highest at intermediate values for q (Fig. 3).

3. Reconciling simple models of robustness

So, Meyers et al. (26) show that if evolvability trades off with robustness and there is fluctuating selection, then the optimal kind and level of robustness depends on the rate at which selection fluctuates. But Draghi et al. (25) show that evolvability and robustness might not trade off, and indeed, this is something that has been demonstrated repeatedly with analysis of more complex genotype-phenotype models (10,31). Does that invalidate the inferences of Meyers et al. (26)?

In fact, the two models are entirely compatible; so long as we recognize that they consider robustness from different frames of reference, as per Wagner (10). Meyers et al. (26) consider the mutational robustness of specific genotypes in a fixed mutational network; they let each genotype g_i have its own mutational and environmental robustness q_i, and r_i. At the genotype level, any increase in robustness causes a proportional decrease in evolvability. In contrast, in the Draghi et al. (25) model, mutational robustness q is a property of a generic mutational process that does not vary across genotypes; there is no variation in genotype-level robustness. But Draghi et al. (25) let the mutational network evolve, which allows the genotype-phenotype map to become more redundant, and the phenotypic neighborhood of a population to grow to approach P. They model robustness of phenotypes, and at that level, robustness and evolvability can vary independently.

Here, I reconcile these two views of robustness by extending each model. First, I extend the Draghi et al. (25) model so as to allow for variation in mutational and environmental robustness across genotypes, and to test if the optimality of robustness and evolvability depends in the same way as in Meyers et al. (26) on the rate of environmental change. Then, for a complementary view of how the rate of environmental change shapes genotype-phenotype maps, I extend the Meyers et al. (26) model so as to let the mutational network and genotype-phenotype mappings be freely evolvable.

3.a Divergent mutational networks of genotypes varying in robustness

3.a.i. Model description

Imagine a population of haploid individuals in an environment with a carrying capacity of N=500, and for which there is a P-dimensional set **P** of possible phenotypes. Each individual has three genetically-determined traits: (1) a phenotype p_i, (2) a phenotypic neighborhood $\mathbf{k_i}$ of size K, and (3) a mutational robustness q_i. The life cycle follows the Wright-Fisher model, with selection on reproductive success, clonal reproduction with mutation, and non-overlapping generations. At the start of each simulation, the population is monomorphic, with q_i = 0.1, and an initial phenotype p_0 and phenotypic neighborhood $\mathbf{k_0}$ selected randomly from **P**. Mutation happens at rate μ=2e-3 per individual per generation. Each mutation is neutral with probability q_i. Whenever a neutral mutation occurs, a new phenotypic neighborhood $\mathbf{k_i}$ is chosen from **P**. When a non-neutral mutation occurs, there is an equal chance that it affects either mutational robustness q_i, or the phenotype p_i. If the former, a new value for q_i is chosen from a random uniform

distribution. If the latter, a new phenotype p_i and phenotypic neighborhood $\mathbf{k_i}$ are randomly sampled from **P**.

This population is subjected to fluctuating selection at rate $\tilde{\lambda}^{-1}$. When the environment is in state *a*, phenotypes in *L*-dimensional set $\mathbf{O_a}$ are optimal, and when the environment is in state *b*, a non-overlapping *L*-dimensional set $\mathbf{O_b}$ of phenotypes are optimal. Phenotypes in *L*-dimensional set **V** have intermediate fitness in both environmental states. So,

$w_a(p_i) = \beta_0+s$ for $p_i \in \mathbf{O_a}$; β_0 for $p_i \in \mathbf{O_b}$; β_0+sh for $p_i \in \mathbf{V}$, and

$w_b(p_i) = \beta_0$ for $p_i \in \mathbf{O_a}$; β_0+s for $p_i \in \mathbf{O_b}$; β_0+sh for $p_i \in \mathbf{V}$

where, similar to Meyers et al. (26), w_j gives the fitness of phenotype p_i in environmental state *j*, $\beta_0 = 0.6$ is the base viability, $s = 0.4$ is the fitness advantage of expressing an appropriate specialist phenotype, and $h = 0.5$ determines the intermediacy of the generalist phenotype.

We simulate evolution by repeating this life cycle for 20K generations, keeping track of the following model variables: (1) *w-bar*, the mean population fitness, (2) q_i*-bar*, the mean population mutational robustness, (3) *D-bar*, the mean Simpson's Diversity of the population's phenotypic neighborhoods, and (4) f_V*-bar*, the mean frequency of individuals expressing a generalist phenotype, that is, $p_i \in \mathbf{V}$. Fifty replicated model simulations were run for every combination of model parameters (Table 1). Analyses were run using the SLiM framework (34); model codes are provided as Supplementary Document S3.

Table 1. Divergent network model parameters and variables.

Parameter	**Description**	**Values**
μ	Mutation rate	2e-3
N	Population size	500
P	Number of possible phenotypes, **P**	{6, 100}
K	Size of phenotypic neighborhood, $\mathbf{k_i}$	{1, 3, 6} | P=6
		{6, 30, 100} | P=100
L	Number of phenotypes in each set $\mathbf{O_a}$, $\mathbf{O_b}$, and **V**	1 | P=6
		$K/3$ | P=100
λ	Number of generations between change in environment	{1e+1… 1e+4}
β_0	Viability of specialist genotype in wrong environment	0.6
s	Viability advantage of specialist in right environment	0.4
h	Viability intermediacy of generalist phenotype	0.5
Variable	**Description**	**Range**
w-bar	Population mean fitness	$0 < w\text{-}bar < 1$
q_i-*bar*	Population mean mutational robustness	$0 < q_i\text{-}bar < 1$
f_V-*bar*	Frequency of generalist phenotypes ($p_i \in \mathbf{V}$)	$0 < f_V\text{-}bar < 1$
D-bar	Simpson's Diversity of phenotypic neighborhoods, $\mathbf{k_i}$	$0 < D\text{-}bar < 1$

3.a.ii Model Dynamics

Let us first consider what happens when the set of possible phenotypes is small (P=6) and only one phenotype is optimal in each environmental state (L=1) (Fig. 4). Regardless of phenotypic neighborhood size, K, when selection fluctuates rapidly (λ = 10) mean population fitness is ~ 0.8 (Fig. 4a), which is the viability of the generalist phenotype, and indeed, the generalist phenotype tends to occur at frequencies near one (Fig. 4c). As the rate of fluctuating selection decreases, mean population fitness rises, and the frequency of the generalist phenotype falls, so that when λ =10,000, *w-bar* is near one, and f_V*-bar* is near zero. (But note that there is a slight kink in the mapping of λ to f_V*-bar*, at ~ λ=100; we will return to this below.) In contrast, the relationships between λ and the other model statistics, q_i*-bar* and *D-bar*, are non-monotonic. Mutational robustness, q_i*-bar*, is lowest at intermediate values for λ, with the bend of the curve being more pronounced with larger phenotypic neighborhoods (Fig. 4b). We see a similar U-shaped mapping of λ to the mean diversity of phenotypic neighborhoods, *D-bar*, although when K=P, D equals one by definition, and the extent to which *D-bar* drops at intermediate values of λ increases with decreasing K. This is contrast to the observation by Draghi et al. (25) that when $K < P$, decreasing q increases the diversity of a population's phenotypic neighborhoods. This can be explained by the fact that instead of exposing a population that has evolved in a static environment to a one-time adaptive challenge, we expose a population to fluctuating selection. If populations are more mutationally sensitive and hence more evolvable, repeated bouts of positive selection will tend to sweep away much of the standing diversity in phenotypic neighborhoods (35). This effect is especially strong when K=1, in which case it takes longer for

D to rebound from a sweep. So, when $K \in \{3, 6\}$ and P=6, increased mutational sensitivity (1-q_i) clearly brings about increased evolvability. On the other hand, when K=1, this relationship weakens, as a relatively small dip in mutational robustness, q_i*-bar,* comes with a relatively large dip in the diversity of phenotypic neighborhoods, *D-bar;* though more mutations are non-neutral, they access a smaller part of the phenotypic space, *P*.

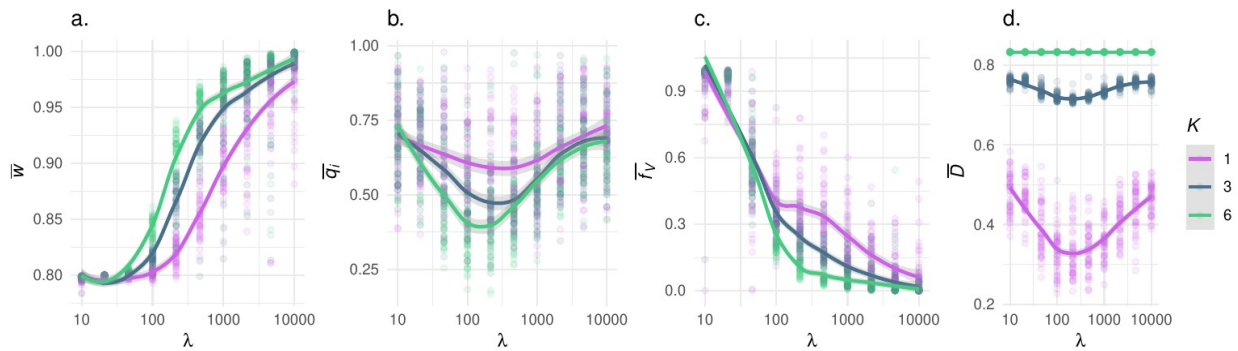

Figure 4. *Summary of models where K is fixed and* q_i *is freely evolvable. Panels show how the number of generations between environmental fluctuations, λ, affects (**a**) population mean fitness, w-bar, (**b**) mean mutational robustness,* q_i*-bar; (**c**) mean frequency of generalist genotypes* f_V*-bar, (**d**) mean population diversity of phenotypic neighborhoods, D-bar. Each point shows the result for one simulation. Lines show loess regressions. Point and line colors denote different values for phenotypic neighborhood size, K.* Interpretation*: As environments become increasingly stable, there is a succession of mutationally robust generalists, evolvable specialists, and mutationally robust specialists.*

When the set of possible phenotypes is comparatively large (P=100) and more than one phenotype is optimal in each environmental state (L=K/3), what we find is similar to when P=6 and L=1 (Fig. 5), but when K=6, the kink in the mapping of $\bar{\lambda}$ to f_V-*bar* is yet more striking. (Fig. 5c). In fact, polynomial regression analysis recovers support for a cubic functional relationship between f_V and $\bar{\lambda}$, and there is a region in which increasing environmental stability ($\bar{\lambda}$) increases the frequency of generalist genotypes, f_V. How do we explain this?

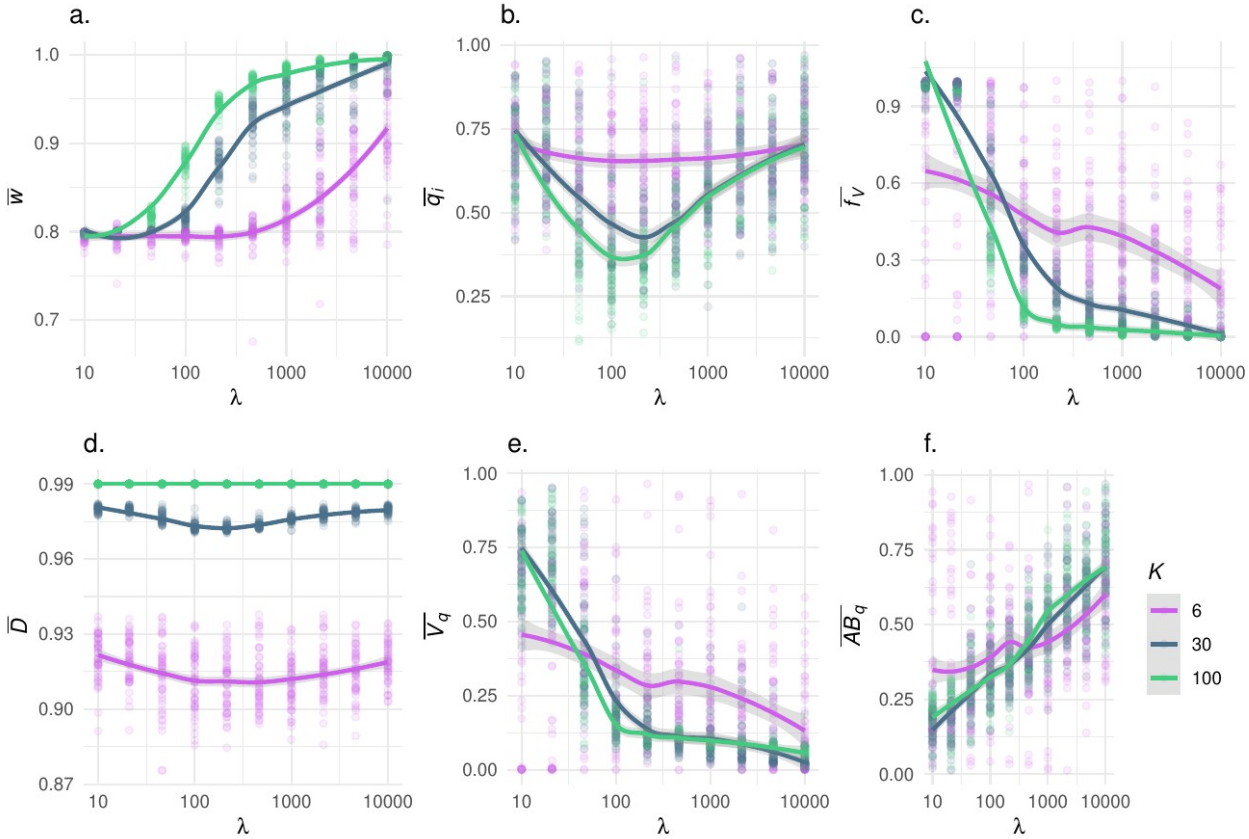

Figure 5. *How, when the phenotype space is large, P=100, the number of generations between environmental fluctuations, $\bar{\lambda}$, affects (**a**) the population mean fitness, w-bar, (**b**) the mean*

*genotype robustness against mutation, q_i-bar, (**c**) the mean frequency of generalist genotypes f_V-bar, (**d**) the mean population diversity of phenotypic neighborhoods D-bar, (**e**) the mean mutational robustness of genotypes expressing the generalist phenotype V_q-bar and (**f**) the mean mutational robustness of specialists, AB_q-bar. Each point shows the result for one simulation. Lines show loess regressions. Point and line colors denote different values for phenotypic neighborhood size, K.* Interpretation: *When P is large and K is small, it is unlikely that populations will evolve well-adapted specialists unless the environment is relatively stable. Counterintuitively, increasing environment stability can increase the frequency of mutationally sensitive generalists.*

Simply put, there is an interaction between environmental and mutational robustness. Mean population mutational robustness, q_i, changes little with λ. But this masks an underlying divergence in q_i between specialist and generalist genotypes (Fig 5e,f.). Specifically, as λ increases from 10 to 10,000, in generalists q_i-*bar* drops from 0.45 to 0.13, while in specialists it increases from 0.34 to 0.60. So, when populations with small phenotypic neighborhoods are challenged to adapt to a world in which there are many phenotypic possibilities, environmental stability selects for evolvability in generalists and mutational robustness in specialists. At rates of environmental change that most favor evolvability ($100 < \lambda < 1000$), generalists tends to be more evolvable. And so over this range of values for λ, we see an increase in the mean frequency of generalist phenotypes.

Why are generalists more evolvable in such environments? Could it be that low mutational robustness – and hence the production of diverse offspring – is part of a complex bet-hedging strategy against environmental uncertainty (36–38)? Expressing phenotype p_V is an example of what has been called conservative bet-hedging; p_V reduces the variance in fitness across generations by conferring the same intermediate fitness in all environments (39). Another bet-hedging strategy is to diversify (40,41); although this increases the variance in fitness across individuals, it also reduces the variance in fitness across generations. Previous theoretical work has shown that mixing conservative and diversifying bet-hedging strategies can out-perform either strategy on its own (36); in uncertain environments, genotypes can evolve to hedge their hedges. But here, it seems unlikely. The problem is that evolvable generalists self destruct; diversification entails genetic divergence, and so we lack the continuity across generations required of the units of selection (42).

An alternative hypothesis would be that generalists do not persist long enough for selection for mutational robustness to be effective. But closer analysis reveals evidence to the contrary. In fact, over the $\bar{\lambda}$ values at which f_V-*bar* increases, generalists tend to persist for several thousand generations (Fig. S1.c). Moreover, they tend to evolve lower mutational robustness than their specialist ancestors, and this dynamic becomes more pronounced as $\bar{\lambda}$ increases. If we divide the evolutionary history of a population into phases of generalism (f_V-*bar* > 0.9) and specialism (f_V-*bar* <= 0.9), and then calculate the mean change in q_i-*bar* over the course of a generalist phase, Δ_{qV}, the mean of this quantity decreases from -0.04 when $\bar{\lambda}$ = 10, to -0.18 when $\bar{\lambda}$ = 10,000 (Fig. S1.a). So, the high evolvability of generalists is not because generalists are ephemeral.

A third hypothesis is that selection against mutational sensitivity is less efficient in generalists, since the expected decrease in fitness from non-neutral mutation is less severe. Generalists tend to evolve evolvability via genetic drift. Sure enough, whereas increasing environmental stability, λ, causes the mean of Δ_{qV} to become more negative, the standard deviation of Δ_{qV} remains consistently positive, regardless of environmental stability (Fig. S1.b). And this helps us better understand the tendency for Δ_{qV} to be negative: Being a generalist does not increase evolvability. The evolution of increased evolvability presages the end of generalism.

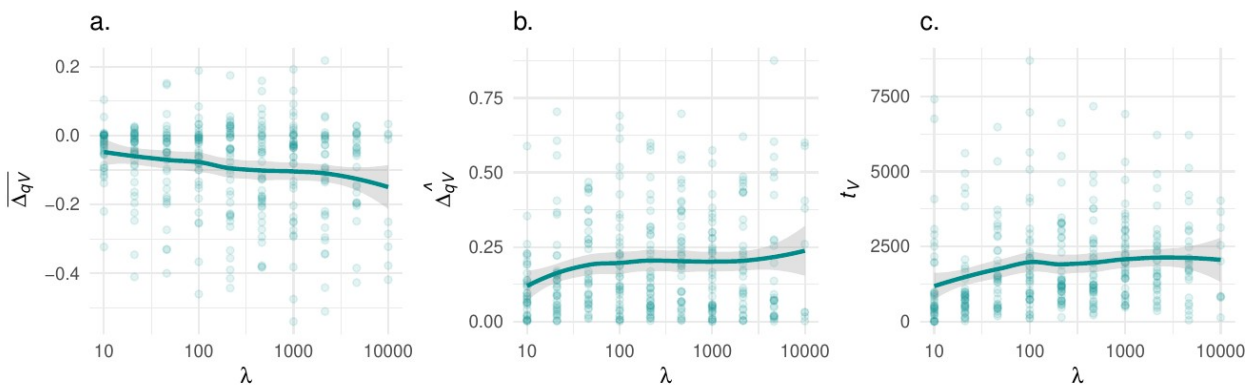

Figure S1. *The evolution of evolvable generalists. How the number of generations between environmental fluctuations, λ, affects (**a**) the mean change in mutational robustness over the course of an evolutionary phase of generalism, Δ_{qV}-bar, (**b**) the standard deviation of that same quantity, and (**c**) the mean duration of an evolutionary phase of generalism. Each point shows the value for one simulation. Lines show loess regressions.* Interpretation: *Generalists tend to persist for thousands of generations, and to evolve lower mutational robustness than their specialist ancestors. But the latter effect is likely due to neutral genetic processes.*

To sum up, in broad strokes, the inferences of Meyers et al. (26) are robust to relaxations of the their assumptions about genetic architecture. Even if we let mutational sensitivity and phenotypic neighborhood be freely-evolving genotype properties, over much of the explored parameter space, as environments become increasingly stable, we find the same succession of mutationally robust generalists, evolvable specialists, and mutationally robust specialists. On the other hand, we find some new patterns when phenotypic neighborhood sizes are a small fraction of the total number of possible phenotypes. In particular, there can be a negative feedback between mutational sensitivity ($1-q_i$) and phenotypic neighborhood diversity (D) such that more mutationally sensitive genotypes might not be more evolvable. And counter-intuitively, certain increases in environmental stability can cause an increase in the frequency of generalist phenotypes, provided that they are more mutationally sensitive than specialists.

3.b. Cyclical networks of genotypes varying in robustness

3.b.i. Model description

Let us now extend the Meyers et al. (26) model by letting the mutational network be freely evolvable individual-level property. We mostly follow the original and use the same set of possible phenotypes, $\mathbf{P} = \{A, B, V\}$, the same fitness functions, and the same regimes of fluctuating selection ($1 < \lambda < 10{,}000$). But we change the mutational process to allow for two kinds of effects. With probability $\rho=0.5$, a mutation transforms the genotype of an individual to one of the adjacent genotypes in their mutational network. With probability $1-\rho$, a mutation randomly re-assigns the phenotypic mapping of a randomly selected vertex in the individual's

mutational network. To clarify, in the divergent mutational network model of Draghi et al. (25), the network is a population-level property; an individual's position in that network is determined by their ancestry and the elements of their phenotypic 1-neighborhood. By contrast, with this extension of the Meyers et al. (26) cyclical network model, the network is essentially a higher-order, phenotypic 4-neighborhood, governing what phenotypes are accessible to a genotype via any number of mutations.

As for the divergent network model, we analyze the dynamics of this model with individual-based simulations, with 20K generations for each replicate, and 50 replicates per value of λ. Model codes are provided in Supplementary Document S4. In each generation, we record the following model statistics: (1) *w-bar*, the mean population fitness, (2) GP_{max}, the most common genotype-phenotype map, (3) S^{-1}*-bar*, the population mean of the inverse richness of mutationally-adjacent phenotypes, which is a measure of mutational robustness, (4) f_V*-bar*, the mean frequency of individuals expressing a generalist phenotype, and (5) f_{Vk}*-bar*, the mean frequency of phenotypic 1-neighborhoods containing the generalist phenotype p_V.

3.b.ii. Model dynamics

In broad strokes, when we recast the cyclical mutational network of Meyers et al. (26) as another kind of evolvable phenotypic neighborhood, we find the same λ-dependent succession of environmentally-robust generalists, evolvable specialists, and mutationally robust specialists (Fig. 6). But we also get a couple of new insights, and a surprise. The first new insight is that when environments turn over rapidly (λ=10), not only does the generalist phenotype p_V prevail, but mutational networks are enriched for p_V (Fig 7a.). In fact, the most common network,

occurring at an average frequency of ~0.43, is composed exclusively of p_V (Fig 7b.). Populations evolve to be both environmentally and mutationally robust. And the mutational robustness observed in this case is far more pronounced than anything we see in mutationally-robust specialists in stable environments.

A second new insight is that in stable environments (λ=10,000), mutational robustness is asymmetrical across specialist phenotypes, with higher average robustness for the phenotype that was optimal in the first environmental epoch, p_A. Historical contingency can have long-lasting effects on the evolution of the genotype-phenotype map.

The surprise is that – similar to what we saw for f_V with our extensions of Draghi et al. (25) model – there is a kink in the functional mapping of variation in λ to f_{Vk}', that is, the mean population frequency of genotypes containing the generalist phenotype p_V in their phenotypic 1-neighborhood. A dip in f_{Vk}' happens at λ ~ 100, where the balance between selection for evolvability and robustness tips most in favor on evolvability; at λ = 100, in evolved mutational networks, a mapping to p_V is rare, and there is relatively even mix of mappings to p_A and p_B (Fig 7a,c.). Beyond λ = 100, selection against p_V mappings weakens, as mutational robustness again becomes more valuable. In sum, it seems that selection for evolvable specialists more efficiently reduces the likelihood of generalism-causing mutations than does selection for robust specialists.

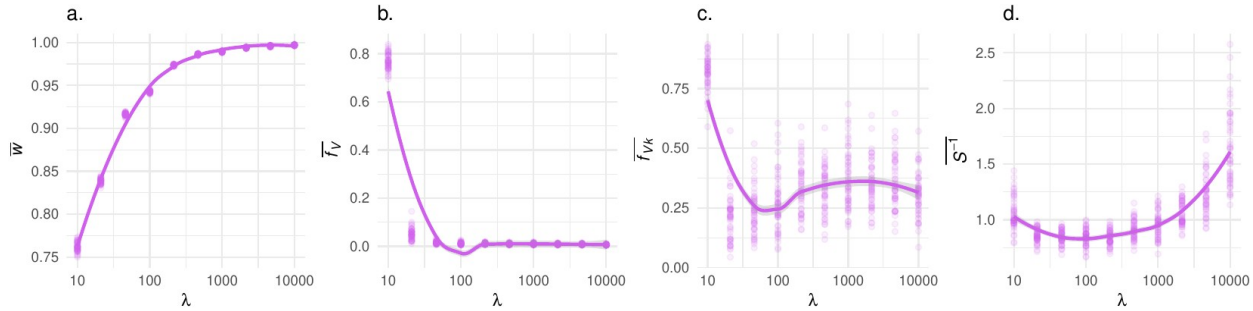

Figure 6. *Dynamics of evolving cyclical mutation networks. How the number of generations between environmental fluctuations, λ, affects (**a**) the population mean fitness, w-bar, (**b**) the mean frequency of a generalist genotypes f_V-bar, (**c**) the mean frequency of mutational networks containing the generalist phenotype f_{Vk}-bar, and (**d**) the mean phenotypic robustness against mutation, S^{-1}. Each point shows the result for one simulation. Lines show loess regressions. The mutation rate μ = 1e-2, and there is an equal probability that a mutation causes an individual to change to an adjacent genotype, or causes a change in the phenotype-assignment of one of the genotypes in the network.*

4. The evolution of environmental robustness

Next, let us briefly consider the transferability of the Draghi et al. (25) and Meyers et al. (26) models to questions about the relationship between environmental robustness and evolvability.

Imagine that, as in the Draghi et al. (25) model, there is a world with *P* possible phenotypes. But instead of a population of genotypes that explores this world via mutation, suppose there is a meta-population of genetically-invariant demes that explores it via migration. Phenotypes emerge from genotype-by-environment interactions, and environments vary such that each deme

has a K-dimensional phenotypic neighborhood, $\mathbf{k_i}$, accessible via migration. For a depiction of this model, Figure 1.b. should suffice; one need only reinterpret the nodes as demes, and the edges as migrations. Let q_m represent the probability that the environment of a new deme induces no change of phenotype; in other words, q_m determines the migrational robustness of the genotype to environmental variation. Since we assume the population is genetically monomorphic and have replaced mutation with migration, we can not assess the relationship between robustness and evolvability *per se*, at least not if we think about evolution only in terms of changes in genotype frequencies. But we can assess the relationship between robustness and plasticity, which is analogous. In the beginning, only phenotype p_0 is adaptive and only demes that express p_0 survive selection. But migration forms a neutral network of demes, and the diversity of phenotypic neighborhoods grows. If at some point a new phenotype, p_x, becomes optimal, as long as $K < P$, we know that the relationship between q_m and rate at which the population evolves via migration to express p_x will be non-monotonic, with the fastest rates occurring at intermediate values of q_m. This is because the logic of Draghi et al. (25) model of mutational robustness has not changed, only our interpretation of some of its variables and parameters. Neutral mutation can boost evolvability by changing the structure of a mutation network. Neutral migration can boost plasticity by changing the structure of a population in a heterogeneous environment.

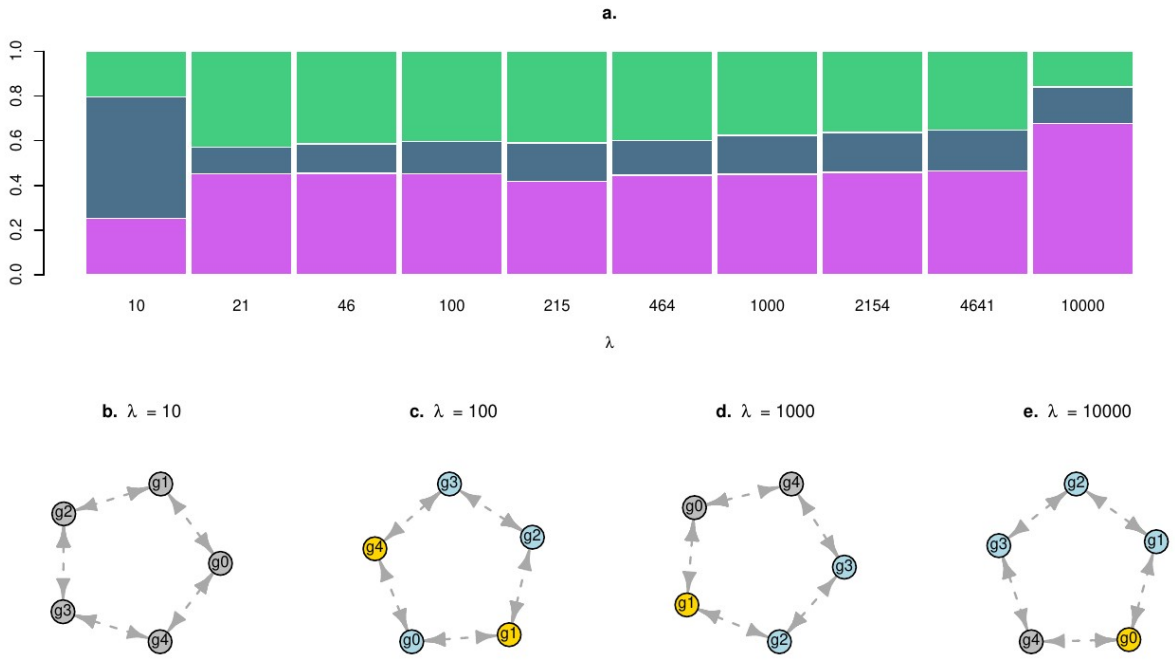

Fig 7. *Evolved mutational networks.* ***(a)*** *How the number of generations between environmental fluctuations, λ, affects the average frequency of each phenotype in the population of mutational networks, with the frequency of* p_A *shown in purple,* p_B *in green, and* p_V *in blue. Below,* ***(b-e)****, the most common mutational network is shown for a subset of λ values. Here genotypes mapping to phenotype* p_A *are in light blue,* p_B *in gold, and* p_V *in gray.* Interpretation: *When we let cyclical mutational networks evolve in volatile environments, populations evolve to be both mutationally and environmentally robust. Increases in evolvability are realized by evolving networks that are an even mix of specialist phenotypes. And in stable environments, historical contingency can cause long-lasting biases in phenotypic robustness.*

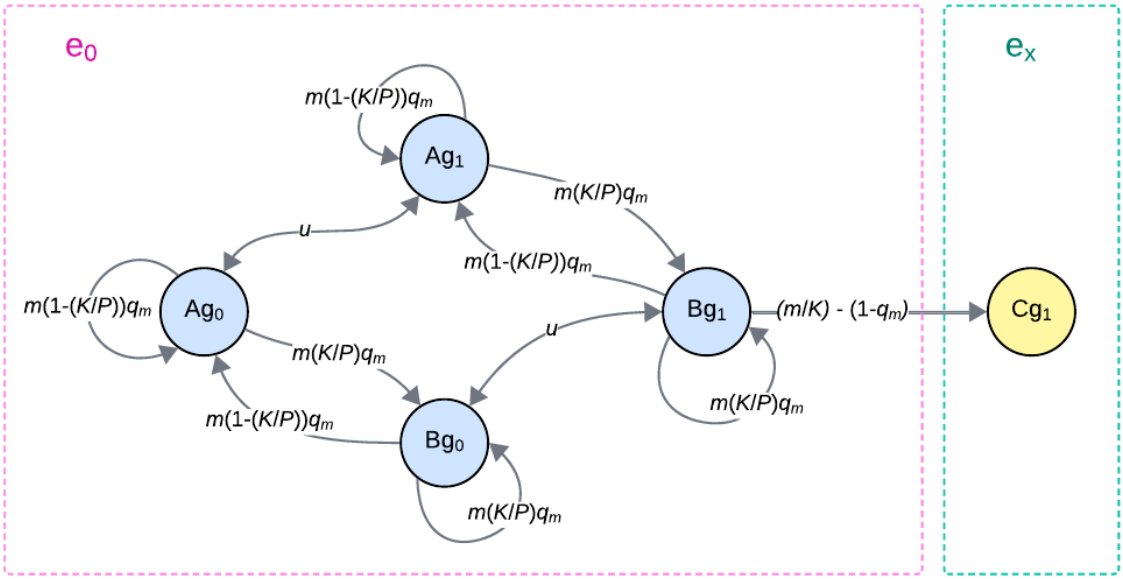

Figure S2. *Flow diagram of model relating environmental robustness to evolvability. Vertices represent model variables, and edges show how one variable affects another, or if an edge exits and enters the same vertex, how a variable affects itself. Extending the scheme of Draghi et al. (25), we divide the population of individuals into compartments, based on whether they, after* t_x*, (C) occupy and have high fitness in environment* e_x*, or (B) have* e_x *in their environmental neighborhood – a component of the decomposed phenotypic neighborhood* $\mathbf{k}_i$*, which combines environmental and genetic neighborhoods – or (A) do not have* e_x *in their environmental neighborhood, with compartments A and B further divided by genotype. So, each model variable is the number (or proportion) of a particular genotype in a particular environment type. The expression on each edge gives the rate at which individuals flow from one compartment to another. These expressions are based on basic probability theory. Take for an example the edge flowing from variable* B_{g1} *to* C_{g1}*. If an individual is in a B compartment, that means they have*

environment e_x in their environmental neighborhood. So the probability that an individual in a B compartment with the g_1 genotype migrates to environment e_x is equal to the migration rate m, times the probability that a non-neutral a migration lands them environment e_x, 1/K, minus the probability that a migration is not neutral (1-q_m).

To go beyond plasticity, and connect environmental robustness to evolvability, we need to allow for mutation as well as migration (Fig. S2). Above, we let phenotypes vary depending on the interaction between one genotype and many local environments. Suppose instead that each individual can have one of two genotypes, either g_0 or g_1. Initially, the population is monomorphic for g_0 but mutation between genotypes occurs at rate μ per individual per generation. In e_0 environments, genetic variation is cryptic; both genotypes express the optimal phenotype p_0. Initially, migration to any non-e_0 environment induces strongly deleterious changes in phenotype. Then at time t_x, a new phenotype p_x – which is expressed only by g_1 genotypes in e_x environments – becomes adaptive in e_x environments. Now we can ask the question that ties environmental robustness to evolvability: How does the time it takes a population to evolve phenotype p_x depend on environmental robustness, q_m? Analysis of this model recovers a similar non-monotonic relationship between robustness and and evolvability (Figure S3). Model codes are provided in Supplementary Document S5. Robustness is robustness; mutational and environmental robustness have similar effects on evolvability.

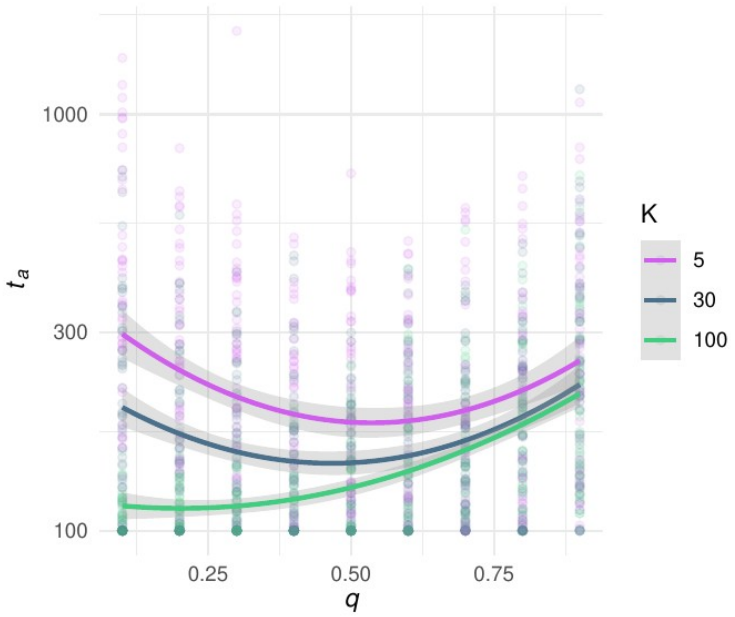

Figure S3. *The effect of migrational robustness, q_m, on evolvability, t_a, depends on the difference between the number of possible phenotypes, K, and the size of the phenotypic neighborhood P. Curves are loess regressions; shaded areas give 95% confidence intervals.* Interpretation*: Unless all phenotypes are accessible by one migrational step, evolvability is maximized at intermediate levels of migrational robustness.*

In a similar fashion, the model of Meyers et al. (26) can be transferred to the evolution of environmental robustness and evolvability; we need only change our interpretation of the edges and vertices in Fig. 1a. Instead of five genotypes, we have one phenotypically-plastic genotype, five spatial locations, and three different environment types, such that the genotype can expresses three different phenotypes. Instead of genotypes connected by mutation, we have demes connected by migration. If we apply the same kind of fluctuating selection – with the phenotype expresses in demes g_0, g_1, and g_3 being optimal in temporal state *a*, that expressed by deme g_4 being optimal in state *b*, and that in g_3 with intermediate fitness in both states – then the proportion of a meta-population in each deme will be a function of the rate at which selection fluctuates. When environmental change is slow, individuals will occur at the highest frequency in

deme g_1, since that minimizes migration load (43). When the world turns over rapidly, deme g_3 offers a safe harbor of stability. And at intermediate rates of change, the highest fitness can be realized by hopping back and forth between demes g_0 and g_4. Of course, when applied to the evolution of environmental robustness, some of the more liberal model assumptions stand out, in particular, just how small the model world is, and how strange it seems for there not to be any kind of negative density dependence on relative fitness. But such is the price of simplicity.

5. Robustness via phenotypic neighborhood evolution

In the previous sections, we saw that the evolution of mutational robustness, q_i, and how it affects evolvability depends on $\mathbf{k_i}$, the phenotypic neighborhood. In fact, selection on evolvability could affect q_i or $\mathbf{k_i}$. We can show this with another model variant (Supplementary Document S6). The model set-up is as described in Section 3.a, for P=6, except for three changes: (1) We fix the value of $q \in \{0.0, 0.1, 0.5\}$. (2) We let K evolve freely; hence, each genotype determines a phenotypic neighborhood size K_i. (3) We change the way that $\mathbf{k_i}$ evolves. Specifically, at the start of each simulation, the population is monomorphic for a three-dimensional $\mathbf{k_i}$, with elements randomly sampled from $\mathbf{P}$. Then, as the life cycle turns over, mutation can with equal probability add or subtract one element from $\mathbf{k_i}$, with the restriction that $0 < K_i \leq P$. We do not prevent redundancy of the elements of $\mathbf{k_i}$. This is to maximize flexibility for the design by selection of an optimal phenotypic neighborhood. To further resolve the model dynamics we also keep track of two additional model statistics, to wit, f_B-*bar* is the mean frequency of phenotypic neighborhoods that contain the optimal phenotype for the out-of-phase environmental state, and f_{Vk}-*bar* is the mean frequency of phenotypic 1-neighborhoods with the generalist phenotype.

With simulation analysis, we find that just as when we fix K and let q_i vary across genotypes, populations appear to evolve maximum evolvability at intermediate values for $\tilde{\lambda}$ (Fig 8). At intermediate values for $\tilde{\lambda}$, not only is the mean of K_i highest, but so is f_B, the probability that $\mathbf{k_i}$ contains the optimal phenotype of the out-of-phase environmental state, and in fact, the latter effect is more pronounced. Averaging across values of q, as $\tilde{\lambda}$ increases from 10 to 100, there is a 13% increase in K_i, and a concomitant 35% increase in f_B; simply put, evolvable populations stack their phenotypic neighborhoods with good options. With increasing $\tilde{\lambda}$ we also see a decline in the frequency of the generalist genotype along with f_{Vk}, that is, the frequency of genotypes that have the generalist phenotype in their neighborhood. These relations are robust to variation in mutational robustness q, although when q is closer to zero, we see more pronounced reductions of K_i and f_B at extreme values of $\tilde{\lambda}$.

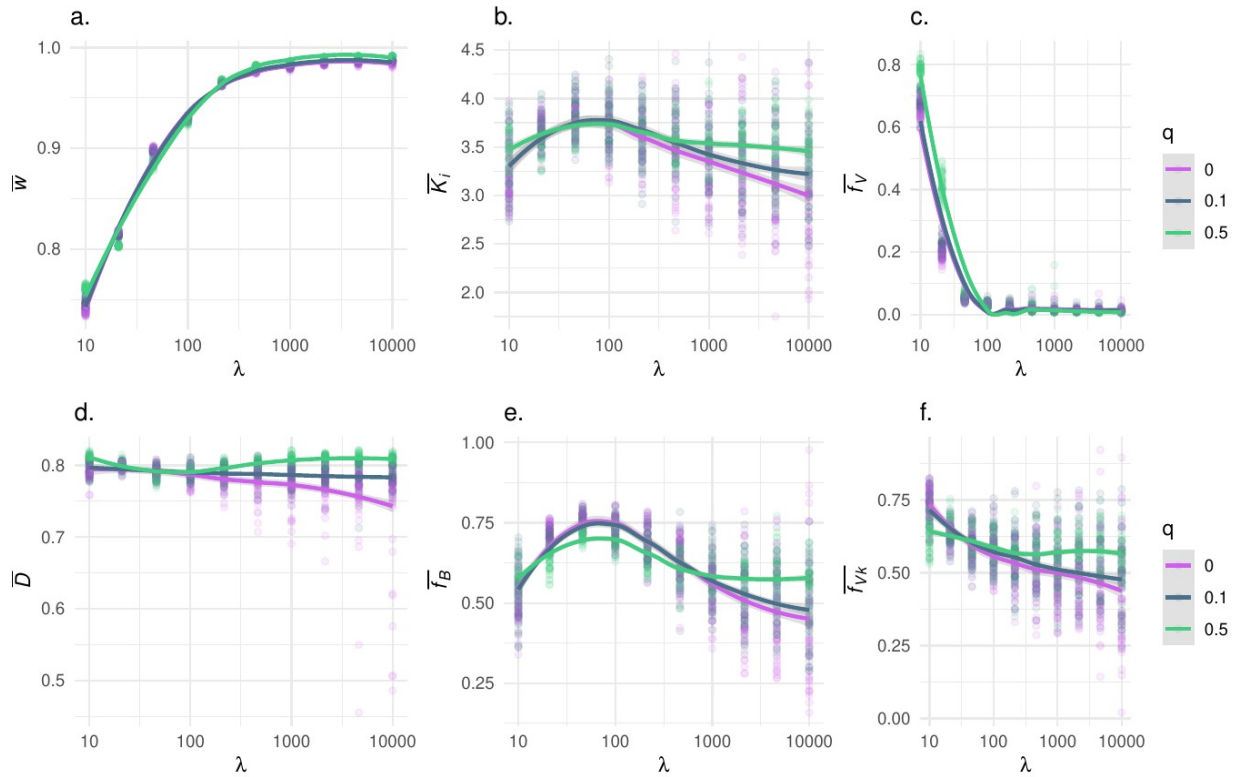

Figure 8. *Summary of models where q is fixed and K_i is freely evolvable. Panels show how changing the number of generations between environmental fluctuations, λ, affects (**a**) population mean fitness, w-bar, (**b**) mean phenotypic neighborhood size, K_i-bar; (**c**) mean frequency of generalist genotypes f_V-bar, (**d**) mean population diversity of phenotypic neighborhoods, D-bar; (**e**) mean frequency of phenotypic neighborhoods containing the optimal phenotype for the out-of-phase environmental state, f_B-bar; and (**f**) mean frequency of phenotypic neighborhoods containing the generalist phenotype, f_{Vk}-bar. Each point shows the result for one simulation. Lines show loess regressions. Point and line colors denote different values for q.*

Interpretation: *At intermediate rates of environmental change, populations evolve to maximize*

evolvability by enriching their phenotypic neighborhoods with phenotypes that are optimal in the out-of-phase environmental state.

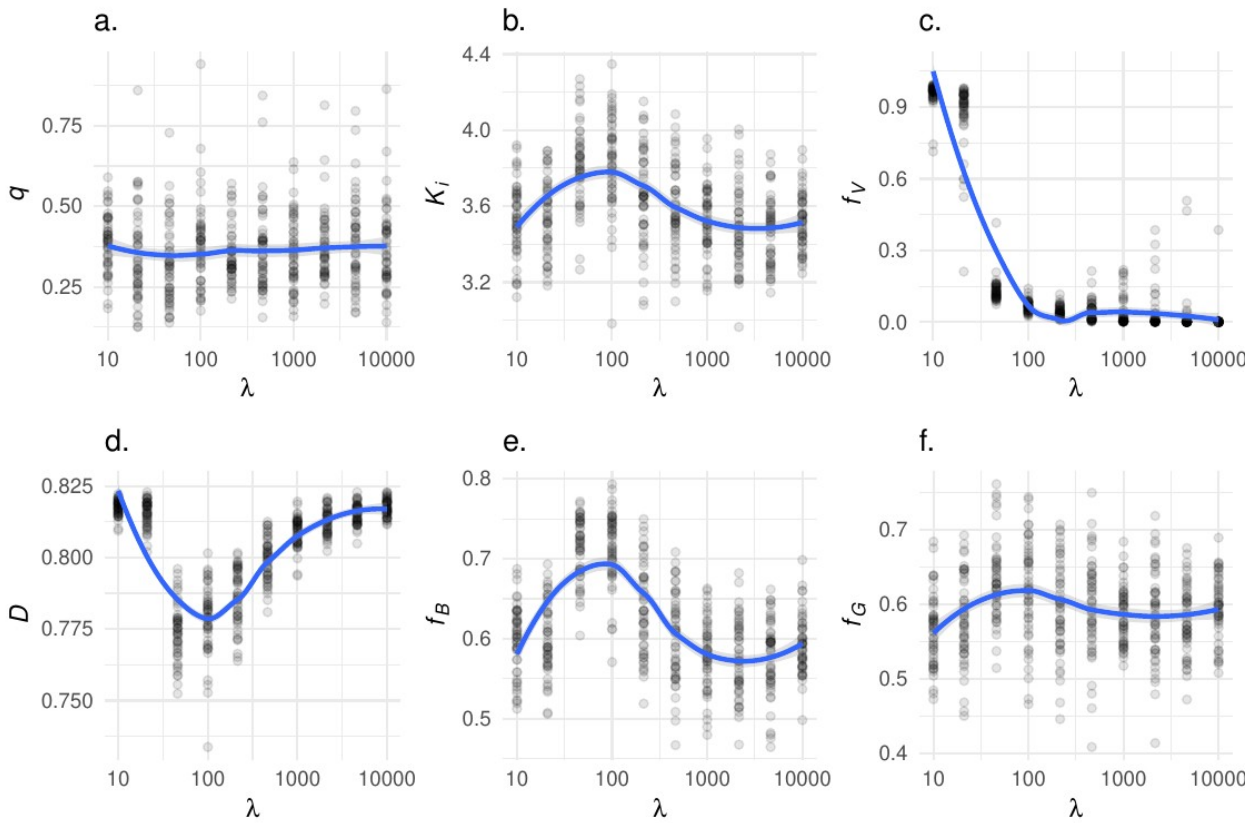

Figure S4. *Summary of models where qi and K_i are both freely evolvable. How changing the number of generations between environmental fluctuations, ƛ, affects (**a**) mean mutational robustness q_i-bar, (**b**) mean phenotypic neighborhood size, K_i-bar; (**c**) mean frequency of generalist genotypes f_V-bar, (**d**) mean population diversity of phenotypic neighborhoods, D-bar; (**e**) mean frequency of phenotypic neighborhoods containing the optimal phenotype for the out-of-phase environmental state, f_B-bar; and (**f**) mean frequency of phenotypic neighborhoods containing the generalist phenotype, f_{Vk}-bar. Each point shows the result for one simulation. Lines*

show loess regressions. Point and line colors denote different values for q. Interpretation*: Selection for evolvability favors changes in phenotypic neighborhood components over mutational robustness.*

To sum up, there is more than one way to evolve robustness and evolvability. Selection can shape the probability that a mutation will be neutral. Or it can shape the probabilities of the specific phenotypic effects of non-neutral mutation, as has been demonstrated previously with analyses of more complex genotype-phenotype maps (21–23). In fact, the latter would appear to be favored by selection, since tailoring the phenotypic neighborhood can increase the odds of beneficial mutations without also increasing the odds of deleterious ones. Sure enough, if we simulate the evolution of population in which mutational robustness q_i, phenotypic neighborhood size K_i, and phenotypic neighborhood components $\mathbf{k_i}$ are all free to vary, then environmental stability, $\tilde{\lambda}$, has little effect on the means of q_i (which tend to be ~ 0.4) and large effects on means of K_i and f_B , that is, the frequency of phenotypic neighborhood containing the phenotype that is optimal in the out-of-phase environment (Fig. S4). Something to stress here is that for selection on phenotypic neighborhood components to be effective, a genotype-phenotype map needs to have a memory of past selective environments. We made that possible, in an elementary fashion, by implementing a piece-wise mutational routine for adding and removing components of the phenotypic neighborhood, $\mathbf{k_i}$. But more complex genotype-phenotype maps can have better memories (22,24).

6. Discussion

Here we improved our intuition about how robustness affects evolvability by analyzing some simple models. In each, robustness is expressed as *q*, the probability that some perturbation does not affect the phenotype, and if a perturbation does affect the phenotype, such effects are mediated by **k**, the neighborhood of accessible phenotypes. For ease of discussion, let us call these *qk* (pronounced "cuke") models. Our premise was that by abstracting away the details of genotype-phenotype maps, *qk*-models could give us a clearer understanding of how robustness relates to evolvability. But even in the abstract, robustness is multi-faceted and can evolve in more than one way: Genotypes or phenotypes can be robust against variation in the genetic or external environment, and how such robustness affects evolvability depends not just on the level of biological organization (10), but also on the odds of specific phenotypic effects being induced by a genetic or environmental change (18,22,23). By merging and extending the *qk*-models of Meyers et al. (26) and Draghi et al. (25) we were able to demonstrate that their key insights are robust to relaxations of some of their key assumptions; we saw that when populations are free to evolve mutational and environmental robustness, as well as their phenotypic neighborhoods, they evolve to be least robust – and conversely most evolvable – at intermediate rates of fluctuating selection, and at intermediate levels of mutational sensitivity, unless all possible phenotypes can be accessed by one mutation.

We also gained some new insights. The first is that when there is selection for increased evolvability, it will preferentially act on the phenotypic neighborhood, rather than mutational sensitivity *per se*, since this can increase the odds of adaptive mutation without also increasing

the odds of deleterious mutation (44). That selection can affect the distribution of mutational effects has been shown before with analysis of more complex genotype-phenotype models (19,24,45,46). But to my knowledge, this is the clearest demonstration that increasing evolvability by evolving the phenotypic neighborhood evolution is a better option than evolving increased mutational sensitivity.

Another new insight is that *qk*-models of robustness against the genetic environment are readily applicable to robustness against the external environment. Just as a population's phenotypic neighborhood can increase via neutral mutation, so too can it increase via neutral migration (or, for that matter, neutral temporal changes in the environment), provided that we allow phenotypes to emerge from specific genotype-by-environment interactions in a heterogeneous environment. In that case, evolvability is maximized at intermediate levels of robustness to variation in the external environment, unless all possible plastic phenotypic responses can be accessed by one move through space or time in the environmental space. Although theorists often recognize robustness as a generic property of genetic and developmental systems (29), in practice, most work has focused on either mutation robustness (16,23) or phenotypic plasticity in response to variation in the external environment (47,48), and consequently two distinct and somewhat disconnected schools of thought have emerged. I suspect there are many other opportunities to transfer insights from one school to another.

A third new insight is that the interaction between different kinds of robustness can cause counter-intuitive evolutionary dynamics. Specifically, in complex environments, increasing

environmental stability can cause an increase in the frequency of environmentally-robust genotypes, that is, generalists. We saw that this corresponds to a divergence in the mutational sensitivities of generalists and specialists, and we traced this divergence to a neutral evolutionary process; the evolution of evolvability in generalists is accidental, but predictably so. This is yet another example of how robustness can increase evolvability by facilitating the accumulation of cryptic genetic diversity (44,49). Note how much this aligns with Sewell Wright's much-maligned "shifting balance" theory, where genetic drift helps populations move between peaks in a rugged fitness landscape (50) The particulars of that theory, especially about demography, made it contentious (51) but if we forgive some errant details, Wright was right; adaptation often takes neutral evolution.

There are many things these simple models of the evolution of robustness and evolvability can not do. To repeat a theme, robustness is just one of several properties of a genotype-phenotype map that can affect evolvability. Moreover, even the effects of robustness can depend on other facets of genetic architecture. Therefore, a richer understanding of the evolution of evolvability requires more complex models genotype-phenotype maps. Other germane properties include a developmental system's criticality, that is, the robustness of its robustness (19,52), as well as its capacity for plasticity and cryptically-neutral genetic diversity (18,20,22), and its capacity to adaptively remember and generalize from past selective environments (23,24,53). More complex genotype-phenotype map models can also yield insights into the specific design features that determine map properties such as robustness, evolvability and memory. A burgeoning body of research has already revealed several such network features (29), such as modularity (45,54),

excitation (17), and scale-free degree distribution (55,56). Researchers have also begun to detect important network motifs affecting robustness and evolvability, such as freed-back and feed-forward loops (57). So, the analysis of more complex genotype-phenotype map models promises to yield a more multi-faceted and mechanistic understanding of the evolution of evolvability. Nevertheless, by ignoring the genetic architectural details that might make one genotype-phenotype map more robust than another, *qk*-models throw into sharp relief the effects of robustness *per se* on evolvability.

Acknowledgments

Thanks to Jeremy Draghi, and Ben Normark for providing helpful comments on a draft of the manuscript.

Data accessibility

Supplementary Information files are available via a GitHub repository (https://github.com/n8-rd/qkModels.git) and will also be deposited in Zenodo (10.5281/zenodo.12206125.) [NB: The latter will be registered when published.]

References

1. de Visser JAGM, Hermisson J, Wagner GP, Meyers LA, Bagheri-Chaichian H, Blanchard JL, et al. Perspective: Evolution and Detection of Genetic Robustness. Evolution. 2003;57(9):1959–72.

2. Waddington CH. Genetic Assimilation of an Acquired Character. Evolution. 1953;7(2):118–26.

3. Siegal ML, Bergman A. Waddington's canalization revisited: Developmental stability and evolution. Proceedings of the National Academy of Sciences. 2002 Aug 6;99(16):10528–32.

4. Wagner GP, Booth G, Bagheri-Chaichian H. A population genetic theory or canalization. Evolution. 1997 Apr 1;51(2):329–47.

5. Wilke CO, Wang JL, Ofria C, Lenski RE, Adami C. Evolution of digital organisms at high mutation rates leads to survival of the flattest. Nature. 2001 Jul;412(6844):331–3.

6. Wright S. Fisher's Theory of Dominance. The American Naturalist. 1929 May;63(686):274–9.

7. Ancel LW, Fontana W. Plasticity, evolvability, and modularity in RNA. Journal of Experimental Zoology. 2000;288(3):242–83.

8. Kawecki TJ. The Evolution of Genetic Canalization Under Fluctuating Selection. Evolution. 2000;54(1):1–12.

9. Smith JM, Burian R, Kauffman S, Alberch P, Campbell J, Goodwin B, et al. Developmental Constraints and Evolution: A Perspective from the Mountain Lake Conference on Development and Evolution. The Quarterly Review of Biology. 1985 Sep;60(3):265–87.

10. Wagner A. Robustness and evolvability: a paradox resolved. Proceedings of the Royal Society B: Biological Sciences. 2007 Oct 31;275(1630):91–100.

11. Ahnert SE. Structural properties of genotype–phenotype maps. Journal of The Royal Society Interface. 2017 Jul 5;14(132):20170275.

12. Fierst JL, Phillips PC. Modeling the evolution of complex genetic systems: The gene network family tree. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution. 2015;324(1):1–12.

13. Bloom JD, Labthavikul ST, Otey CR, Arnold FH. Protein stability promotes evolvability. Proceedings of the National Academy of Sciences. 2006 Apr 11;103(15):5869–74.

14. Ahnert SE, Johnston IG, Fink TMA, Doye JPK, Louis AA. Self-assembly, modularity, and physical complexity. Phys Rev E. 2010 Aug 27;82(2):026117.

15. Greenbury SF, Johnston IG, Louis AA, Ahnert SE. A tractable genotype–phenotype map modelling the self-assembly of protein quaternary structure. Journal of The Royal Society Interface. 2014 Jun 6;11(95):20140249.

16. Ciliberti S, Martin OC, Wagner A. Robustness Can Evolve Gradually in Complex Regulatory Gene Networks with Varying Topology. PLOS Computational Biology. 2007 Feb 2;3(2):e15.

17. Draghi J, Wagner GP. The evolutionary dynamics of evolvability in a gene network model. Journal of Evolutionary Biology. 2009 Mar 1;22(3):599–611.

18. Fierst JL. A history of phenotypic plasticity accelerates adaptation to a new environment. Journal of Evolutionary Biology. 2011 Sep 1;24(9):1992–2001.

19. Torres-Sosa C, Huang S, Aldana M. Criticality Is an Emergent Property of Genetic Networks that Exhibit Evolvability. PLOS Computational Biology. 2012 Sep 6;8(9):e1002669.

20. Rutherford SL. From genotype to phenotype: buffering mechanisms and the storage of genetic information. BioEssays. 2000;22(12):1095–105.

21. Crombach A, Hogeweg P. Evolution of Evolvability in Gene Regulatory Networks. PLOS Computational Biology. 2008 Jul 11;4(7):e1000112.

22. Draghi JA, Whitlock MC. Phenotypic plasticity facilitqates mutational variance, genetic variance, and evolvability along the major axis of environemental variation. Evolution. 2012 Sep 1;66(9):2891–902.

23. Espinosa-Soto C, Wagner A. Specialization Can Drive the Evolution of Modularity. PLOS Computational Biology. 2010 Mar 26;6(3):e1000719.

24. Watson RA, Wagner GP, Pavlicev M, Weinreich DM, Mills R. The evolution of phenotypic correlations and “developmental memory.” Evolution. 2014 Apr 1;68(4):1124–38.

25. Draghi JA, Parsons TL, Wagner GP, Plotkin JB. Mutational robustness can facilitate adaptation. Nature. 2010 Jan;463(7279):353–5.

26. Meyers LA, Ancel FD, Lachmann M. Evolution of Genetic Potential. PLOS Computational Biology. 2005 Aug 26;1(3):e32.

27. Chevin LM, Leung C, Le Rouzic A, Uller T. Using phenotypic plasticity to understand the structure and evolution of the genotype–phenotype map. Genetica. 2022 Aug 1;150(3):209–21.

28. Hallgrímsson B, Willmore K, Hall BK. Canalization, developmental stability, and morphological integration in primate limbs. American Journal of Physical Anthropology. 2002;119(S35):131–58.

29. Kitano H. Biological robustness. Nat Rev Genet. 2004 Nov;5(11):826–37.

30. Wagner A. The role of robustness in phenotypic adaptation and innovation. Proceedings of the Royal Society B: Biological Sciences. 2012 Jan 4;279(1732):1249–58.

31. Fontana W, Schuster P. Continuity in Evolution: On the Nature of Transitions. Science. 1998 May 29;280(5368):1451–5.

32. Schuster P, Fontana W, Stadler PF, Hofacker IL. From sequences to shapes and back: a case study in RNA secondary structures. Proceedings of the Royal Society of London Series B: Biological Sciences. 1997 Jan;255(1344):279–84.

33. Beverton RJH, Holt SJ. On the Dynamics of Exploited Fish Populations. Springer Science & Business Media; 1957. 541 p.

34. Haller BC, Messer PW. SLiM 4: Multispecies Eco-Evolutionary Modeling. The American Naturalist. 2023 May;201(5):E127–39.

35. Smith JM, Haigh J. The hitch-hiking effect of a favourable gene. Genetics Research. 1974 Feb;23(1):23–35.

36. Olofsson H, Ripa J, Jonzén N. Bet-hedging as an evolutionary game: the trade-off between egg size and number. Proceedings of the Royal Society B: Biological Sciences. 2009 May 27;276(1669):2963–9.

37. Starrfelt J, Kokko H. Bet-hedging—a triple trade-off between means, variances and correlations. Biological Reviews. 2012;87(3):742–55.

38. Yasui Y. Evolutionary bet-hedging reconsidered: What is the mean–variance trade-off of fitness? Ecological Research. 2022;37(3):406–20.

39. Philippi, Tom, Seger, Jon. Hedging one's evolutionary bets, revisted. Trends in Ecology & Evolution. 1989;4(2):41–4.

40. Cohen D. Optimizing reproduction in a randomly varying environment. Journal of Theoretical Biology. 1966 Sep 1;12(1):119–29.

41. Simons AM. Fluctuating natural selection accounts for the evolution of diversification bet hedging. Proceedings of the Royal Society B: Biological Sciences [Internet]. 2009 Mar 4 [cited 2024 May 21]; Available from: https://royalsocietypublishing.org/doi/10.1098/rspb.2008.1920

42. Lewontin RC. The Units of Selection. Annual Review of Ecology and Systematics. 1970;1:1–18.

43. Bolnick DI, Nosil P. Natural selection in populations subject to migration load. Evolution. 2007 Sep 1;61(9):2229–43.

44. Masel J. Cryptic Genetic Variation Is Enriched for Potential Adaptations. Genetics. 2006 Mar 1;172(3):1985–91.

45. Hernández U, Posadas-Vidales L, Espinosa-Soto C. On the effects of the modularity of gene regulatory networks on phenotypic variability and its association with robustness. Biosystems. 2022 Feb 1;212:104586.

46. Oikonomou P, Cluzel P. Effects of topology on network evolution. Nature Phys. 2006 Aug;2(8):532–6.

47. Chevin L -M., Lande R. Adaptation to marginal habitats by evolution of increased phenotypic plasticity. Journal of Evolutionary Biology. 2011 Jul 1;24(7):1462–76.

48. Scheiner SM, Holt RD. The genetics of phenotypic plasticity. X. Variation versus uncertainty. Ecology and Evolution. 2012;2(4):751–67.

49. Paaby AB, Rockman MV. Cryptic genetic variation: evolution’s hidden substrate. Nat Rev Genet. 2014 Apr;15(4):247–58.

50. Wright S. Evolution in Mendelian Populations. Genetics. 1931 Mar;16(2):97–159.

51. Coyne JA, Barton NH, Turelli M. Perspective: A Critique of Sewall Wright’s Shifting Balance Theory of Evolution. Evolution. 1997;51(3):643–71.

52. Manicka S, Marques-Pita M, Rocha LM. Effective connectivity determines the critical dynamics of biochemical networks. Journal of The Royal Society Interface. 2022 Jan 19;19(186):20210659.

53. Aldana M, Balleza E, Kauffman S, Resendiz O. Robustness and evolvability in genetic regulatory networks. Journal of Theoretical Biology. 2007 Apr 7;245(3):433–48.

54. Verd B, Monk NA, Jaeger J. Modularity, criticality, and evolvability of a developmental gene regulatory network. Altenberg L, Wittkopp PJ, editors. eLife. 2019 Jun 6;8:e42832.

55. Greenbury SF, Johnston IG, Smith MA, Doye JPK, Louis AA. The effect of scale-free topology on the robustness and evolvability of genetic regulatory networks. Journal of Theoretical Biology. 2010 Nov 7;267(1):48–61.

56. Seo CH, Kim JR, Kim MS, Cho KH. Hub genes with positive feedbacks function as master switches in developmental gene regulatory networks. Bioinformatics. 2009 Aug 1;25(15):1898–904.

57. Kadelka C, Butrie TM, Hilton E, Kinseth J, Schmidt A, Serdarevic H. A meta-analysis of Boolean network models reveals design principles of gene regulatory networks. Science Advances. 2024 Jan 12;10(2):eadj0822.