Natural selection can favor the evolution of ratchet robustness over evolution of mutational robustness

Yinghong Lan[1*], Aaron Trout[2], Daniel M Weinreich[1], C Scott Wylie[1]

1 Department of Ecology and Evolutionary Biology, and Center for Computational Molecular Biology, Brown University, Providence, RI, USA

2 Department of Mathematics, Chatham University, Pittsburgh, PA, USA

* yinghong_lan@brown.edu

Abstract

The vast majority of fitness-affecting mutations are deleterious. How natural populations evolve to cope is a question of fundamental interest. Previous studies have reported the evolution of mutational robustness, that is, natural selection favoring mutations that reduce effects of deleterious mutations elsewhere in the genome. Here, we demonstrate that when mutational robustness increases, finite, asexual populations' ability to purge recurrent deleterious mutations declines. Consequently, higher mutational robustness leads to higher risk of extinction by Muller's ratchet. We therefore hypothesize that in the long run, natural populations may instead evolve robustness against Muller's ratchet by increasing sensitivity to deleterious mutations, despite the short-term fitness consequences. We call this phenomenon "ratchet robustness". Using individual-based simulations, we first confirm that ratchet robustness is inversely correlated with mutational robustness on fitness landscapes without epistasis. Next, we demonstrate that negative epistasis increases ratchet robustness, precisely because on fitness landscapes with negative epistasis sensitivity to deleterious mutations increases as mutations accumulate. We also show that on a fitness landscape with adjacent fitness peaks exhibiting exclusively positive and negative epistasis, the populations will converge on the latter. On the other hand, introducing

even a vanishingly small region of positive epistasis on a single-peaked fitness landscape that otherwise exhibits negative epistasis is enough to entirely ablate ratchet robustness, exposing the population to Muller's ratchet. We conclude that while regions of the fitness landscape with higher mutational robustness could be favored in the short term, purely because of temporary fitness advantage, in the long run mutational robustness may render a population vulnerable to extinction. Some empirical support exists for this prediction.

Introduction

The fitness landscape maps genotype to fitness, and describes fitness effects of all possible mutations. In doing so, it determines the extent to which populations can sustain their fitness when perturbed by mutations, namely their mutational robustness. Mutational robustness has been viewed as equivalent to neutrality, that is, mutational robustness means that mutations have little to no deleterious effects ([1, 2]). In other words, mutational robustness is manifested as comparatively flat fitness landscapes. Previous studies have concluded that under the pressure of high mutation rate, natural selection will cause populations to evolve mutational robustness. Equivalently, it has been proposed that populations that experience high mutation rates will evolve to occupy flatter regions of the fitness landscape ([3, 4]). However, finite asexual populations on flatter landscapes are also more vulnerable to extinction by Muller's ratchet ([5]). This suggests the possibility that natural selection might also favor lineages that reside in regions of the fitness landscape that protect them from such extinction, a property we here designate "ratchet robustness". To our knowledge, this tension between the evolution of mutational robustness and ratchet robustness has never been explored ([3, 4, 6–9]).

In infinite populations, where drift can be neglected, mean fitness depends only on mutation rate and is independent of fitness landscape ([10, 11]). Consequently, equilibrium fitness of infinite populations is insensitive to mutations that affect mutational robustness. On the other hand, finite asexual populations risk Muller's ratchet ([12, 13]), because even the fittest genotype can be lost to genetic drift. Even in the presence of beneficial mutations, Muller's ratchet can still happen if deleterious

mutations are sufficiently common. Indeed, only if the proportion of beneficial mutations is high enough, can finite asexual populations halt the ratchet and reach Mutation-Selection-Drift Equilibrium (MSDE) ([5, 14–16]).

Here, we confirm first that other things being equal, populations on locally steeper regions of the fitness landscape exhibit more ratchet robustness. Next, we show that negative pairwise epistasis increases ratchet robustness (as previously seen in a model lacking beneficial mutations [17]), while positive epistasis reduces it and introduces a novel, accelerating form of Muller's ratchet. As a result, populations converge to regions of the landscape with pure negative epistasis. Finally, we demonstrate that even the smallest amount of positive epistasis on a fitness landscape otherwise dominated by negative epistasis is enough to ablate a population's ratchet robustness. We conclude that while in the short term mutational robustness can be selected for, in the long run natural selection sacrifices mutational robustness in favor of ratchet robustness.

Methods

Evolutionary model

For all simulations, we implemented discrete-time Wright-Fisher evolutionary model with custom Python code. Within populations of fixed size N, each individual's genotype is solely identified by the number of deleterious mutations it has, which is denoted by i, $i = 0, 1, ..., L$. L represents the genome length, which is also the maximum number of deleterious mutations possible. Populations "live in" vectors V of length $L + 1$, where each bucket V_i records the number of individuals with i deleterious mutations so that $\sum_i V_i = N$. Individuals with i mutations have fitness denoted by

$$W_i = e^{-si^{1-\epsilon}} \tag{1}$$

with selection coefficient s and epistasis parameter ϵ. $\epsilon = 0$ in the absence of epistasis (Fig 1), $\epsilon < 0$ with negative epistasis (Fig 2A & B: ϵ = -0.25), $\epsilon > 0$ with positive epistasis (Fig 2C & D: $\epsilon = 0.25$).

Each generation of evolution starts with reproduction with selection. Number of offspring with i mutations is a random variable proportional to V_i, i.e., number of

parents carrying i mutations, times W_i, i.e., fitness of these parents. Then, mutations are imposed by sampling from Poisson distribution with parameter U_{del} to generate number of deleterious mutations, and from Poisson distribution with parameter $U_{ben} = 0.01U_{del}$ to generate number of beneficial mutations. Eventually, a new vector V' is "born", where V'_i records number of individuals with i mutations in the new generation. Simulations are performed for a prespecified number of generations, usually 10,000, to ensure the result is unaffected by transient effects. At the end of simulations, we identify populations in which all individuals are carrying $i = L$ deleterious alleles as being at extinction. Simulations in Fig 1 and Fig 2 are performed on simple fitness landscapes with $L = 300$. These landscapes have only one peak (as opposed to two in Fig 3) and their only peak is isotropic (as opposed to hybrid peak in Fig 4), where the fitness of any genotype only depends on number of deleterious mutations.

In Fig 1B, we present variance of fitness across time for simulations conducted in Fig 1A. Notably, critical U_{del}, i.e., the highest U_{del} under which populations could resist Muller's ratchet, or the lowest U_{del} under which populations start to succumb to Muller's ratchet, seems to overlap with the U_{del} under which time variance of fitness reaches maximum. This is expected, as when U_{del} is below or above critical U_{del}, populations are either at MSDE or extinction, both showing low time variance of fitness. At critical U_{del}, the "tug-of-war" between the two possible states reasonably shows maximum stochasticity.

We utilized the above observation to locate critical in Fig 1C, across different population size N and selection coefficient s. For each N and s combination, we recorded time variance of fitness under different U_{del}, and reported the U_{del} associated with highest time variance as critical U_{del}. There are three sources of uncertainty during our analysis. First, it's impossible to conduct simulations under every possible U_{del} value. In practice, we sampled intervaled by 0.025, meaning that the "true" critical U_{del} could fall in between two sampled U_{del} values. Second, because of the intrinsic granularity, detected maximum time variance may not be the true maximum. We measured such uncertainty as the range of U_{del} values that show time variance above half of the detected maximum. Third, there is inevitable variation in recorded time variance across replicated simulations, due to the stochastic nature of the simulation. We found that the second source of variation dominates the other two by at least one

order of magnitude. Therefore, we portrayed only the second type of uncertainty as error bars in Fig 1C.

Multi-peak fitness landscape and hybrid peaks

For simulations in Fig 3, as shown in Fig 3A, we fused two isotropic peaks together: on the positive epistasis side of the intervening fitness valley, $\epsilon = 0.25$ and $L = 270$; on the negative epistasis side, $\epsilon = -0.25$ and $L = 29$. Mutation operations are applied with beneficial mutations always going towards the peak at the same side of the valley, and deleterious mutations always going towards the valley.

For simulations in Fig 4, as shown in Fig 4A, fraction p of the first mutations puts individuals in the positive epistasis domain, where $\epsilon = 0.25$ and $L = 300$, while the rest $1 - p$ puts individuals at the negative epistasis domain where $\epsilon = -0.25$ and $L = 300$. If an individual is brought back to the peak by beneficial mutations, the same p and $1 - p$ apply, i.e., individuals can "travel" between the two domains through the peak.

Results

In this study, we use individual-based simulations to evolve asexual finite populations on four kinds of fitness landscapes with increasing complexity, in order to study how fitness landscape affects mutational and ratchet robustness. On all fitness landscapes, we implement a constant ratio of beneficial to deleterious mutation rates ($U_{ben}/U_{del} = 0.01$, where mutation rate represents expected number of mutations per genome per duplication, see Methods). This is equivalent to assuming that the number of loci carrying deleterious alleles remains small compared to the total genome size. This assumption is reasonable because individuals in our simulations have large genome size ($L = 100$), and effectively reach zero fitness long before the effect of reversion mutations becomes prominent. (Of course, evolving U_{ben}/U_{del} also influences ratchet robustness, [5], a point to which we return in the Discussion.) We define a fitness peak as isotropic if the fitness of any genotype only depends on its Hamming distance from the peak, i.e., on number of deleterious mutations, but not their identity (Eqn 1). We hold population size constant in all our simulations, and regard populations in which all individuals are carrying L deleterious alleles as being at extinction (see Discussion). We

first examine evolutionary behavior on fitness landscapes with a single isotropic peak in absence of epistasis (Eqn 1 with $\epsilon = 0$, Fig 1A inset). Next, we introduce a pairwise epistatic term to the isotropic peak (Eqn 1, $\epsilon \neq 0$), distinguishing between negative ($\epsilon < 0$, Fig 2A) and positive ($\epsilon > 0$, Fig 2C) epistasis. We then examine evolutionary behavior on fitness landscapes consisting of mutationally adjacent isotropic peaks with negative and positive epistasis (Fig 3A). Finally, we relax our assumption of isotropic peaks and model populations evolving on hybrid fitness peaks where a fraction (p) of all first deleterious mutations leaving the peak lead to domains of positive epistasis, while the rest ($1 - p$) lead to domains of negative epistasis (Fig 4A).

Populations residing on flatter fitness landscapes exhibit reduced ratchet robustness

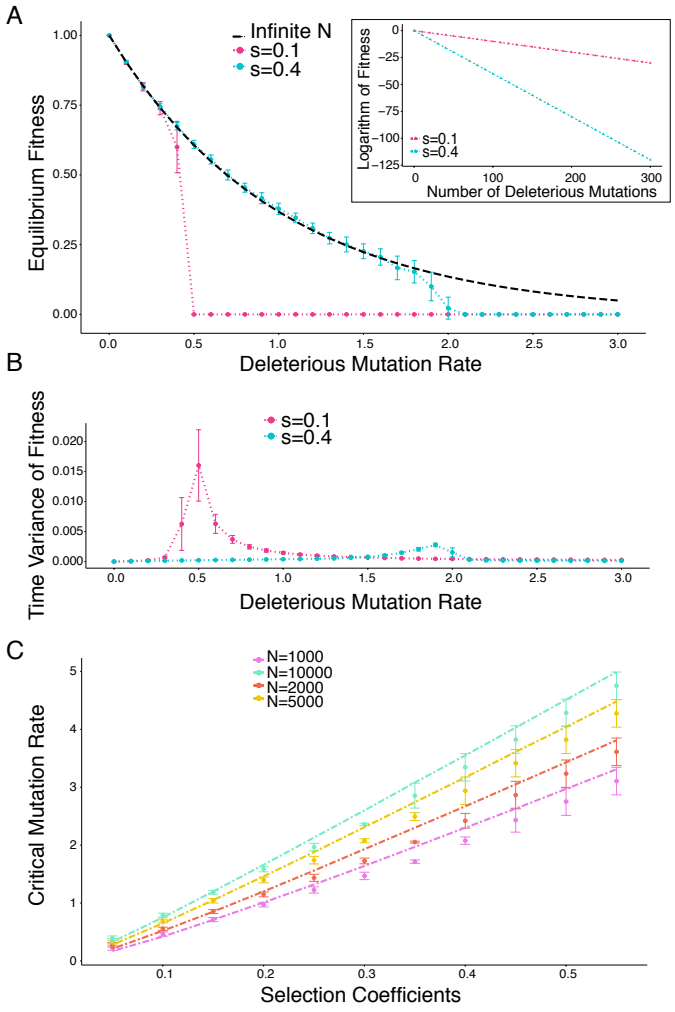


Fig 1. In the absence of epistasis, populations residing on flatter fitness landscapes exhibit reduced ratchet robustness. A. Equilibrium fitness of finite populations under different mutation rates (U_{del}) on fitness landscapes with different selection coefficients (s). Dashed black line: theoretical prediction for equilibrium fitness in infinite populations ($e^{-U_{del}}$). Steeper fitness landscapes (blue), i.e., ones with larger s, maintain MSDE under higher U_{del}. Beneficial mutation rate $U_{ben} = 0.01U_{del}$. Population size $N = 1000$. Average fitness is recorded after evolving for 10,000 generations (see Methods). Error bars: standard deviation over 50 replicates. Inset: comparison of the two fitness landscapes, each of which is composed of one isotropic peak without epistasis. B. Variance of fitness over time for the same populations as in panel A. Variance peaks around critical U_{del}, i.e., the largest U_{del} under which populations maintain MSDE. Based on this observation, we searched for U_{del} with maximum time variance when locating critical U_{del} for different fitness landscapes in panel C (see Methods). Populations approach their dynamic equilibrium so quickly that running the simulations longer does not significantly change their time variance (data not shown). Error bars: standard deviation over 50 replicates. All parameters as in panel A. C. Critical U_{del} values from simulations (points) and analysis (lines, derived numerically from Eqn 7 in [5]), for landscapes with different selection coefficients in the absence of epistasis. Error bars: uncertainty associated with locating critical U_{del} from simulation results (see Methods). Regardless of population size, steeper landscapes always protect populations from Muller's ratchet under higher U_{del}.

We start by studying whether populations on flatter fitness landscapes, i.e., those with greater mutational robustness ([4]), are more resilient to Muller's ratchet than ones on steeper landscapes. We constructed two fitness landscapes, each composed of one isotropic peak but with different steepness (selection coefficients s) in the absence of epistasis (Fig 1A inset). We simulated the evolution of finite asexual populations on such landscapes under different deleterious mutation rates (recall $U_{del} = 0.99U$), and recorded their fitness at equilibrium (Fig 1A).

Under low U_{del}, selection is more than sufficient to purify deleterious mutations, and drift (and thus, the ratchet) is negligible on both flat and steep landscapes. This is because loss of fittest class is extremely rare: purifying selection is strong relative to mutation. Moreover, the fittest class is quickly restored by beneficial mutations if lost. Consequently, equilibrium fitness lies close to the well-known infinite population expectation $e^{-U_{del}}$ ([10, 11], black dashed line in Fig 1A), which depends only on U_{del}. However, after U_{del} exceeds some critical value (Fig 1A: $U_{del} \approx 0.4$ for $s = 0.1$ and $U_{del} \approx 1.9$ for $s = 0.4$), rapidly accumulating deleterious mutations overwhelm selection, leading to extinction via Muller's ratchet. We name the highest U_{del} under which populations are able to resist Muller's ratchet "critical U_{del}". Given a fixed population size (N) and the proportion of beneficial mutations, populations on steeper landscapes

demonstrate higher critical U_{del} and thus are able to resist Muller's ratchet under higher U_{del} (Fig 1A&C). In other words, although populations on flatter landscapes have higher mutational robustness ([1,2]), they fail to maintain MSDE under higher U_{del} and thus exhibit lower ratchet robustness.

Populations on fitness landscapes with negative epistasis have higher ratchet robustness than ones with positive epistasis

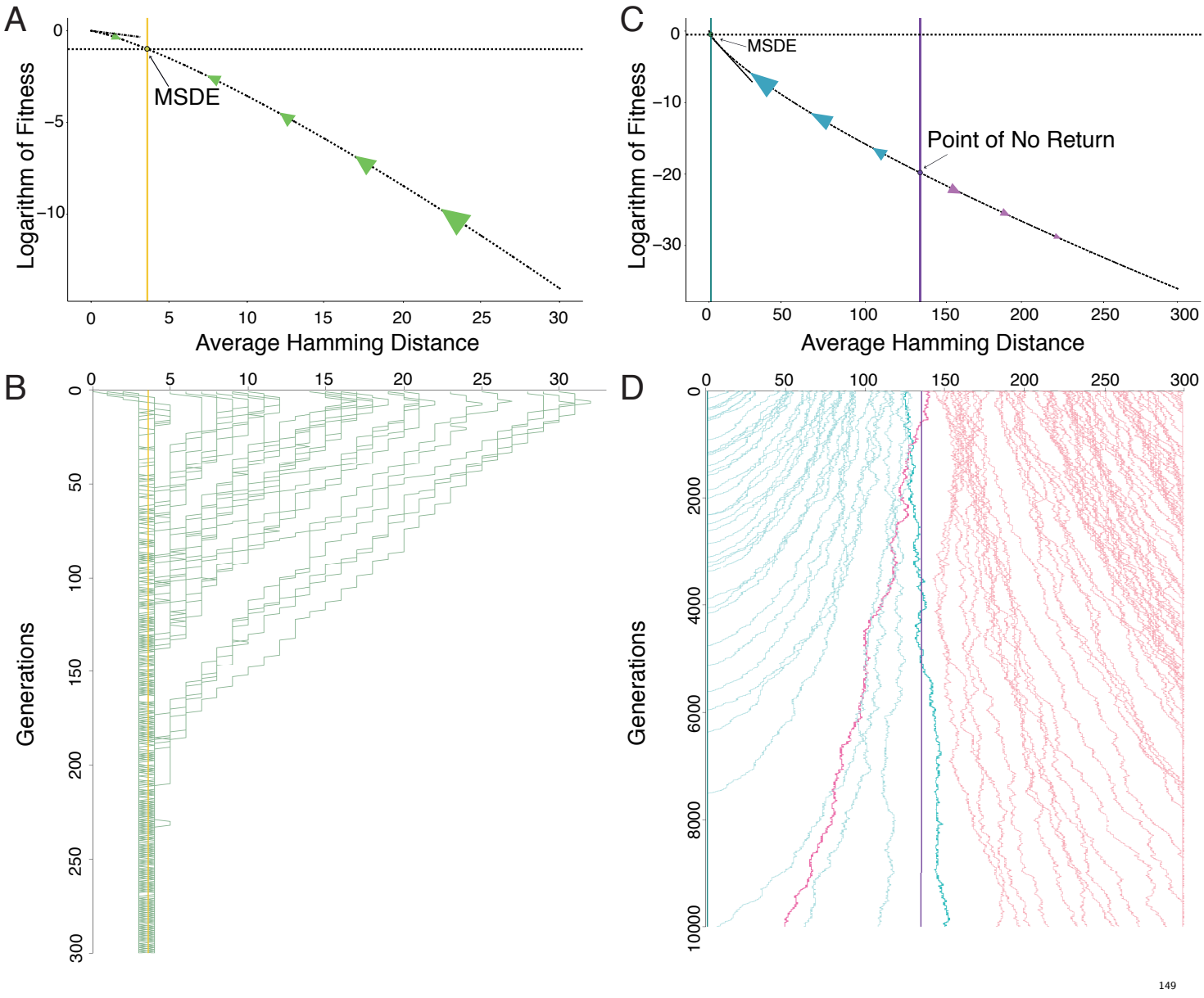


Fig 2. Fitness landscapes with negative epistasis have higher ratchet robustness than ones with positive epistasis. A. Cartoon of evolutionary dynamics of populations on fitness landscape composed of one isotropic peak with negative epistasis. Curved dashed line: fitness landscape. Straight tangent dashed line: fitness landscape without epistasis that shares the same selection coefficient at the peak. Note that selection coefficient (the slope of the fitness landscape) increases with Hamming distance from the peak. Golden vertical line and horizontal dashed line: $e^{-U_{del}}$. B. Time course of average Hamming distance in 35 populations initialized at random points on isotropic peak with negative epistasis during 10,000 generations ($N = 1000$, $s = 0.2$, $U_{del} = 1.0$, $U_{ben} = 0.01U_{del}$, $\epsilon = -0.25$). Golden vertical line: same as in panel A. All populations converge to the attractor wherever they are initiated (green traces). C. Cartoon of evolutionary dynamics of populations on fitness landscape composed of one isotropic peak with positive epistasis. Curved dashed line: fitness landscape. Straight tangent dashed line: fitness landscape without epistasis that shares the same selection coefficient at the peak. Note that selection coefficient (equal to the slope of the fitness landscape) decreases with Hamming distance from the peak. Cyan vertical line and horizontal dashed line: $e^{-U_{del}}$. Violet vertical line: predicted point of no return (derived numerically from Eqn 7 in [5]). D. Time course of average Hamming distance in 100 populations initialized at random points on isotropic peak with positive epistasis during 10,000 generations ($N = 1000$, $s = 0.5$, $U_{del} = 0.5$, $U_{ben} = 0.01U_{del}$, $\epsilon = 0.25$). Cyan and violet vertical lines: same as in panel C. Populations initiated above the point of no return tend to evolve to the peak (blue traces), whereas ones initialized below it tend to succumb to Muller's ratchet (pink traces). Realizations that fluctuate across the point of no return are colored in brighter blue and pink.

To understand how epistasis affects ratchet robustness, we added pairwise epistasis to a single isotropic peak and studied two simple cases: peaks with only negative epistasis (Fig 2A), and ones with only positive epistasis (Fig 2C). Note that when epistasis is negative, the local strength of purifying selection increases with Hamming distance to the peak, and consequently deleterious mutations are less likely to accumulate. On the other hand, when epistasis is positive, the local strength of purifying selection decreases with Hamming distance to the peak, and consequently deleterious mutations are more likely to accumulate. Since Hamming distance itself increases with mutation rate, this suggests that a population evolving on an isotropic fitness peak with negative epistasis might enjoy heightened ratchet robustness as a consequence of negative feedback between mutation rate and the tendency to accumulate deleterious mutations (Fig 2A). In other words, as mutation rate increases, deleterious mutations accumulate, but such accumulation is slowed by the increasing fitness cost of each subsequent deleterious mutation. Conversely, a population evolving on an isotropic fitness peak with positive epistasis might be particularly susceptible to Muller's ratchet as a consequence of positive feedback between mutation rate and the

tendency to accumulate deleterious mutations (Fig 2C).

Consistent with this intuition, we find that on isotropic peaks with negative epistasis, selection will drive the population back to the peak (Fig 2B) regardless of where on the landscape it is initialized, if U_{del} is below critical U_{del} at the peak (i.e., the highest U_{del} at which the peak could be sustained by the population). On the other hand, even if U_{del} is above the critical U_{del} at the peak, there exists a point on the landscape where selection exactly offsets such U_{del}, because purifying selection increases monotonically from the peak (see also S1 Text). If a population is initialized below this point, selection locally will be strong enough to push populations upward until this point is reached. If, instead, a population is initialized above this point, mutation will be strong enough to push the population downward to this point. This point thus represents an attractor ([5, 18]), a stable equilibrium that must be achieved regardless of starting point on the landscape.

Conversely, on isotropic peaks with positive epistasis, if U_{del} is below critical U_{del} at the peak, populations initiated at the peak can maintain MSDE. However, because purifying selection decreases monotonically from the peak, there exists a point on the landscape where selection exactly offsets such U_{del}. Populations initiated above this point would benefit from selection stronger than required and adapt to the peak, while ones initiated below this point suffer from selection weaker than needed, and succumb to Muller's ratchet (Fig 2D). Therefore, we refer to this point as "point of no return". Importantly, even if populations have equilibrated around the peak, stochastic fluctuations will eventually take them across this point. Moreover, as U_{del} increases, selection required to oppose mutation naturally increases as well. Correspondingly, as U_{del} increases, the point of no return migrates towards the peak. This imposes a greater danger of succumbing to Muller's ratchet for populations in the vicinity of the peak via stochastic fluctuations. Finally, once U_{del} is above the critical U_{del} at the peak, the point of no return overlaps with the peak and mutation overwhelms selection everywhere on the fitness landscape.

Populations converge to peak with negative epistasis on multi-peak fitness landscape

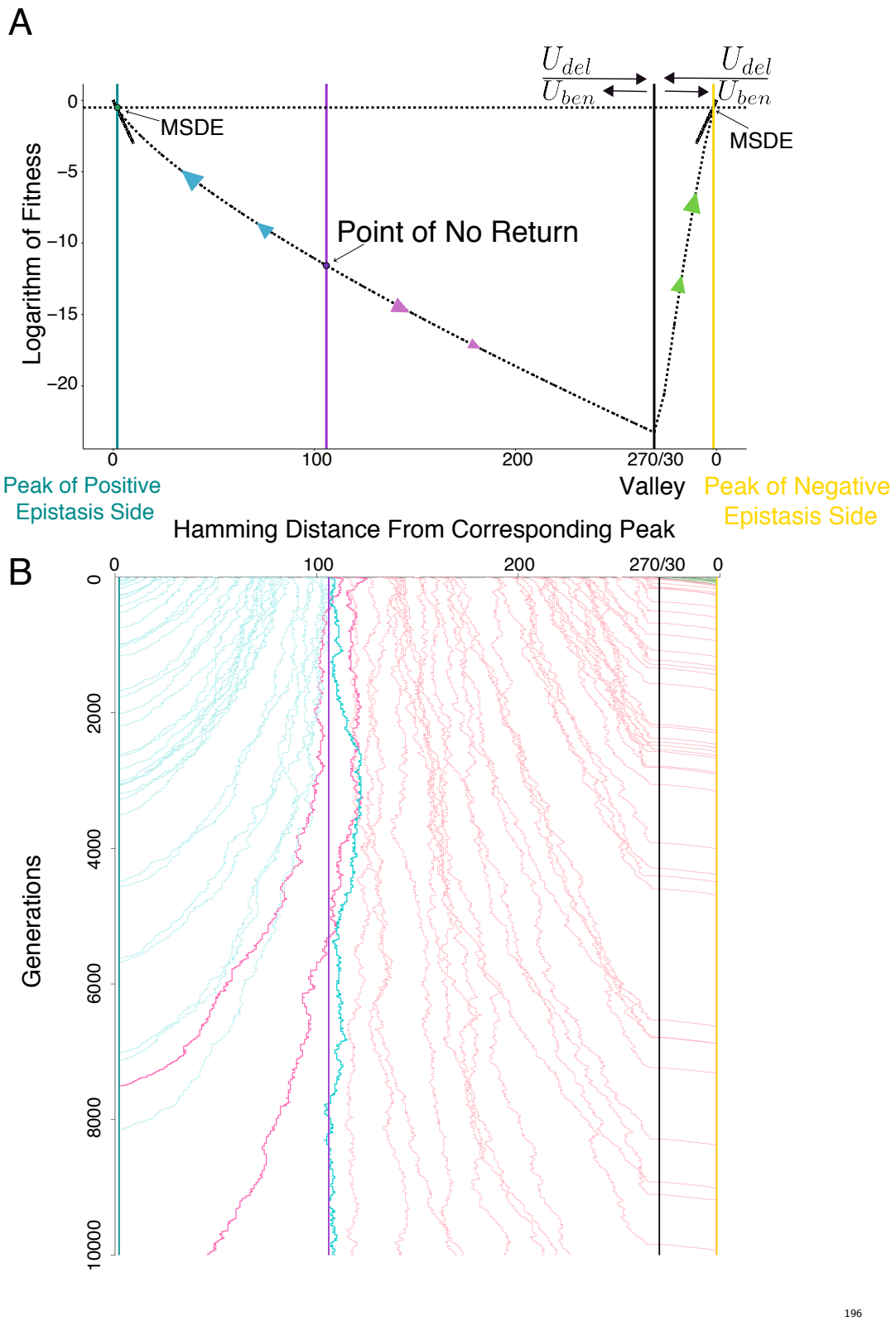


Fig 3. Populations converge to peak with negative epistasis on multi-peak fitness landscape. A. Cartoon of evolutionary dynamics of populations on multi-peak fitness landscape. Both peaks are isotropic, one with only positive epistasis (left) and the other with only negative epistasis (right). Curved dashed lines on both sides: fitness landscape. Straight tangent dashed line on both sides: slope corresponding to selection coefficient at peaks on both sides. Horizontal dashed line: $e^{-U_{del}}$ on both sides. Cyan vertical line: $e^{-U_{del}}$ on the positive epistasis side. Violet vertical line: numerically derived point of no return on the positive epistasis side (Eqn 7 in [5]). Black vertical line: valley of the landscape. Golden vertical line: $e^{-U_{del}}$ on the negative epistasis side. B. Time course of average Hamming distance in 100 populations initiated at random points on multi-peak landscape during 10,000 generations ($N = 10,000$, $s = 0.3$, $U_{del} = 0.5$, $U_{ben} = 0.01U_{del}$, positive epistasis side $\epsilon = 0.25$, negative epistasis side $\epsilon = -0.25$). Cyan, violet, black and golden vertical lines: same as in panel B. Populations are color coded based on their starting point (blue traces: above the point of no return on the positive epistasis side, pink traces: below the point of no return on the positive epistasis side, bright pink and blue traces: realizations that fluctuate across the point of no return on the positive epistasis side, green traces: populations initialized on the negative epistasis side). The vast majority of populations below the point of no return rapidly cross the valley and converge to the attractor on the negative epistasis side. Due to uniformly stronger selection on the negative epistasis side of the valley, populations there exhibit less stochasticity in their trajectories. Simulations in smaller population size ($N = 1000$) show qualitatively the same results, although it takes much longer to converge to the negative epistasis side (data not shown).

To validate the observation that regions of the landscape with negative epistasis have intrinsically higher ratchet robustness, and to demonstrate that populations could evolve ratchet robustness via occupying such regions, we constructed a fitness landscape composed of two mutationally adjacent isotropic peaks featuring opposite signs of epistasis (Fig 3A). Populations finding themselves below the point of no return on the positive epistasis side will initially decline in fitness and come to the valley, similar to populations declining to the bottom of the landscape in Fig 2D. However, at this point, strongly beneficial mutations become available, drawing populations onto the negative epistasis side of the valley, after which they quickly climb to the attractor (Fig 3B). (Note that $U_{ben} = 0.01U_{del}$ on both sides of the valley, and consequently this behavior is driven entirely by natural selection.) Moreover, populations above the point of no return on the positive epistasis side will nevertheless experience stochastic fluctuations (Fig 2D). Eventually, they will be carried over the point of no return, at which point they will experience selection lower than required to offset current mutation rate and decline to the bottom of the landscape due to Muller's ratchet, followed by convergence to the negative epistasis side. Note that the two peaks share identical selection at the

peak, meaning that the positive epistasis side has uniformly higher mutational robustness but uniformly lower ratchet robustness.

Mutational robustness and ratchet robustness on hybrid peaks

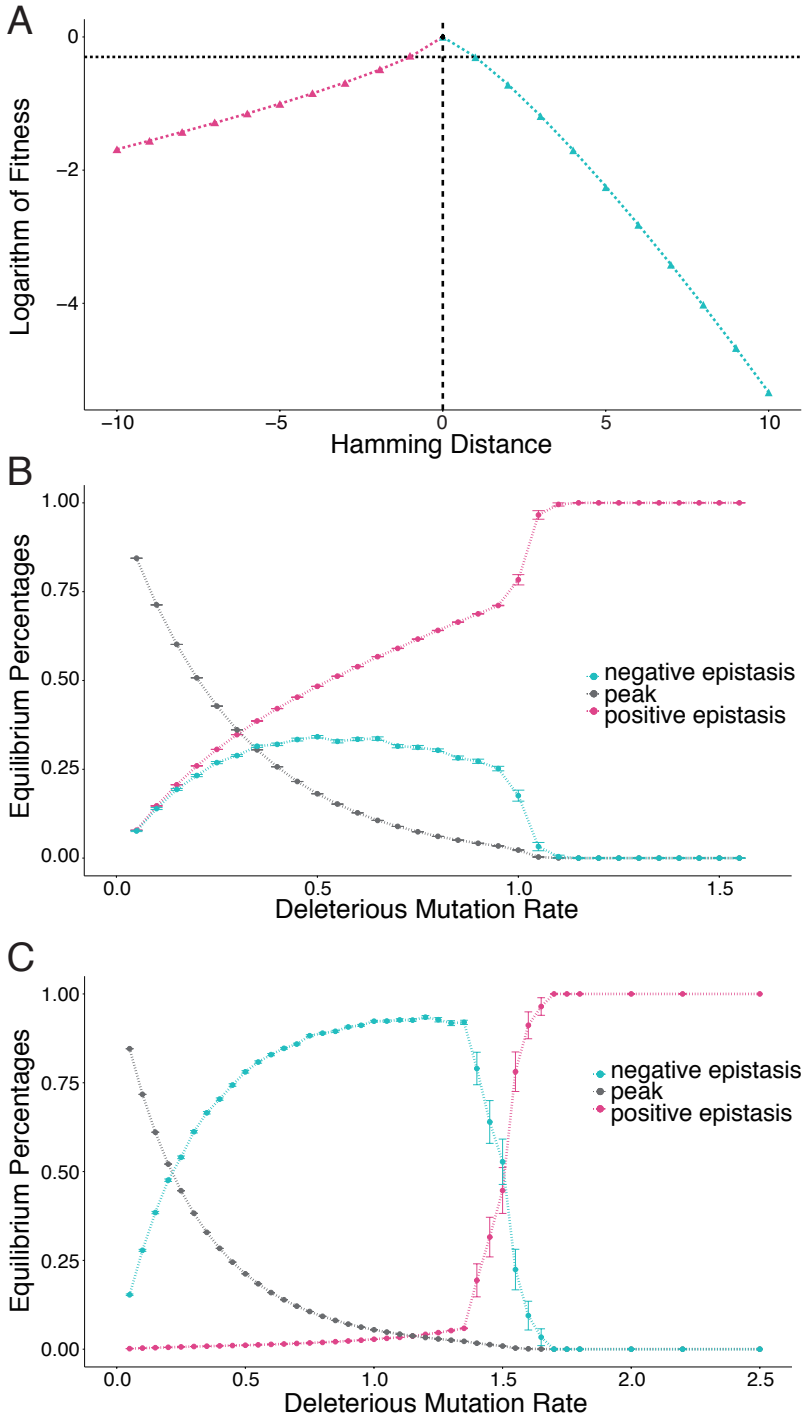


Fig 4. Mutational robustness and ratchet robustness on hybrid peaks. A. Hybrid peaks: while all first mutations leaving the peak share the same fitness effect, a fraction p of them cause subsequent mutations to exhibit positive pairwise epistasis, and the remaining $1-p$ cause subsequent mutations to exhibit negative epistasis. Populations are always initiated at the peak. Here $p = 0.5$. B. Equilibrium proportions of individuals at the peak (black), the negative epistasis region (blue), and the positive epistasis region (pink), under different U_{del} ($U_{ben} = 0.01U_{del}$, $p = 0.5$, $N = 1000$, $s = 0.35$, negative epistasis region: $\epsilon = -0.25$, positive epistasis region: $\epsilon = 0.25$, evolved for 10,000 generations, error bars: standard deviation across 50 replicates). Under U_{del} less than critical U_{del} at the peak (here ~ 1.1; see main text), the subpopulation on the negative epistasis region exists in mutation-selection balance and relies on continual mutational input from subpopulation on the peak. After U_{del} exceeds critical U_{del} at the peak, the subpopulation on the peak goes extinct, and with it, the subpopulation on the negative epistasis region. At this point, the remaining population finds itself beyond the point of no return on the positive epistasis region (see main text), and it succumbs to Muller's ratchet. C. Equilibrium proportions of individuals at the peak (black), the negative epistasis region (blue), and the positive epistasis region (pink), under different U_{del} ($U_{ben} = 0.01U_{del}$, $p = 0.01$, $N = 1000$, $s = 0.35$, negative epistasis region: $\epsilon = -0.25$, positive epistasis region: $\epsilon = 0.25$, evolved for 10,000 generations, error bars: standard deviation across 50 replicates). Population dynamics resemble $p = 0.5$ (see main text).

Biologically realistic landscape peaks are unlikely to be isotropic: fitness effects of mutations at any Hamming distance usually depend on current genome background and follow complex distributions. As a first attempt to capture part of the reality on our model landscape with one peak, we now allow the sign of epistatic effects of mutations to be dependent on the first mutation away from the peak. Specifically, a certain fraction (p) of the first mutations now place the evolving population on a region of the landscape exhibiting positive epistasis, while the rest ($1-p$) place the evolving population on a region of the landscape exhibiting negative epitasis (Fig 4A). Concretely, among all the paths leaving from the peak, p of them show positive epistasis, while the result $1-p$ show negative epistasis. We assume that all first mutations share identical fitness effects, so that there is no immediate fitness advantage in choosing the region with negative or positive epistasis. However, for all Hamming distances greater than one, fitness is necessarily higher in regions of the landscape with positive epistasis than in regions with negative epistasis (Eqn 1). In other words, regions of the landscape with positive epistasis have uniformly higher fitness and mutational robustness, but lower ratchet robustness compared with ones with negative epistasis. Note that the only mutational path between regions of this landscape exhibiting positive and negative epistasis is through the peak. We evolved populations on such hybrid peak with $p = 0.5$

and report the proportions of populations residing exactly at the peak, in the negative epistasis region, and in the positive epistasis region as a function of U_{del} (Fig 4B).

When U_{del} is so low that majority of the individuals carry zero or one mutation (here ∼0.2, Fig 4B, S2 FigA), the proportion of the population on the negative epistasis region is very close to that on the positive epistasis region. This merely reflects the fact that p equals 0.5, since the fitness cost of the first mutation is the same. However, as U_{del} rises to moderate level (here $\sim 0.2 < U_{del} <\sim 1.1$, Fig 4B, S2 FigB), the proportion of the population on the negative epistasis region begins to drop below $p = 0.5$. This reflects selective enrichment for the subpopulation experiencing positive epistasis: all mutations after the first are always less deleterious on the positive epistasis region than on the negative epistasis region. Nevertheless, in this intermediate range of values of U_{del}, a subpopulation on the negative epistasis region is still sustained despite lower fitness, thanks to net mutational inflow from the subpopulation at the peak (S1 Table). In essence, the subpopulation on the negative epistasis region is at mutation-selection balance within the population: constantly being purified by selection but being regenerated by mutation from the peak.

However, after U_{del} increases high enough that the peak can no longer be sustained (here, ∼1.1), the proportion of the population at the peak become negligible (Fig 4B, S2 FigC). As a result, the negative epistasis region is disconnected from mutation input from the peak and is quickly wiped out by selection. The remaining population now occupies only the positive epistasis region. However, since U_{del} has overwhelmed selection at the peak, it necessarily does so also at every other point on the positive epistasis region and the population quickly succumbs to Muller's ratchet. This threshold recapitulates results seen when the point of no return overlaps with the peak for the positive epistasis region (Fig 2C&D, although the numeric value of the critical mutation rate differs here, reflecting its dependence on the contours of the fitness landscape).

More importantly, the observed pattern does not depend on the particular value $p = 0.5$: even when there is only a very small fraction of paths leaving the peak with positive epistasis, subpopulations on the positive epistasis region of the landscape will always be favored due to short term fitness advantage. Such advantage is amplified by higher U_{del}, so long as it remains less than the critical U_{del} at the peak, i.e., the U_{del} under which the peak is lost. In this regime, the two subpopulations accumulate more

mutations, reach larger Hamming distances from the peak, and thus experience increased fitness differences. Eventually, populations on the positive epistasis region dominate. Indeed, this phenomenon apparently occurs with much smaller p ($p = 0.01$, Fig 4C). At low U_{del}, a fraction approximately equal to p of the population not on the peak resides in the positive epistasis region. But as U_{del} increases, this fraction increases, again because fitness is higher there. And as we observe when $p = 0.5$ (Fig 4B), once U_{del} exceeds the critical mutation rate at the peak, the mutational connection between subpopulations on the fitness landscape is extinguished. At this point both subpopulations are doomed. The (lower-fitness) subpopulation on the region of the landscape with negative epistasis will lose its mutational input and go extinct. At the same time, because U_{del} exceeds the critical U_{del} at the peak, it's certainly higher than critical U_{del} everywhere else on the positive epistasis region, meaning that the point of no return has reached the peak and the remaining (higher-fitness) subpopulation on the region with positive epistasis will necessarily succumb to Muller's ratchet.

Discussion

Our findings suggest that although mutational robustness may be favored in the short term, resilience against Muller's ratchet, i.e., ratchet robustness, can be selected for in the long term. We showed first that in the absence of epistasis, fitness landscapes with higher mutational robustness are more susceptible to Muller's ratchet, meaning that they offer lower ratchet robustness, and vice versa (Fig 1). We next demonstrated that landscapes with negative epistasis provide higher ratchet robustness, while landscapes with positive epistasis are intrinsically unstable (Figs 2 and 3). Finally, while mutational robustness may be selected for in the short term, this can lead to population extinction in the long term (Fig 4, see also [19]).

In all of our simulations, we implemented soft selection, where population size is held constant. However, results won't be qualitatively different had we instead implemented hard selection, thus allowing decreasing population size with Muller's ratchet (i.e., mutational meltdown, [20]). The reason is that decreasing population size renders the population more prone to mutation accumulation, thereby amplifying the rate at which the ratchet proceeds (see Fig 1C, where smaller populations have lower critical U_{del} on

the same landscapes).

Negative feedback in mutation or selection can confer ratchet robustness

In the presence of negative epistasis, decreasing fitness leads to increasing selection strength, which halts fitness decline (Fig 2A). Such negative feedback therefore protects the population from Muller's ratchet, thereby providing superior ratchet robustness relative to a landscape with no or positive epistasis (see Results). As previously noted ([5]), similar negative feedback could also be achieved through increasing the ratio of beneficial to deleterious mutation rates (U_{ben}/U_{del}) with decreasing fitness. While negative epistasis enables more effective purifying selection, increasing U_{ben}/U_{del} reduces occurrence of deleterious mutations. In other words, while negative epistasis operates at the selection level, increasing U_{ben}/U_{del} functions at the level of mutational input. For example, this quantity automatically increases during evolution because each successive deleterious fixation represents a new beneficial site in the genome. In simulations here, we have explicitly prevented this effect by fixing the ratio at 0.01, and suggest more generally that in large genomes this quantity may be unlikely to change appreciably before fitness declines to essentially zero.

Increasing U_{ben}/U_{del} can also be realized by compensatory mutations (for example, [21]), mutations that are neutral in the wild type but beneficial after certain deleterious mutations have occurred. Note that this is positive epistasis, but among beneficial mutations, which therefore doesn't incur the point of no return (Fig 2C), and instead contributes to higher ratchet robustness. However, compensatory mutations are outside the scope of the simple model examined here.

Natural selection favors mutational robustness in the short term despite long-term peril

Results in Fig 1 strongly suggest that natural populations cannot survive on isotropic fitness peaks with arbitrarily high mutational robustness, or equivalently, insufficient ratchet robustness. Furthermore, results in Fig 2 and 3 indicate that natural populations cannot permanently survive on isotropic peaks with positive epistasis, since

here ratchet robustness decreases with mutation rate. However, results in Fig 4 show that mutational robustness provides short term fitness advantage, since, by definition, increased mutational robustness means that mutations have less deleterious effect ([1]). This effect results in populations becoming more susceptible to Muller's ratchet. Importantly, even when mutational robustness is only available on small fraction of the fitness landscape, populations are still blind to the long term perils of mutational robustness (Fig 4C). For simplicity, in Fig 4, we constructed fitness landscape with two domains, one of which only has negative epistasis, the other positive epistasis. However, fitness landscapes in reality are highly unlikely to be composed of a few domains of distinct mutational robustness ([22]). Nevertheless, our conclusion that populations will favor the short-term advantage of mutational robustness in spite of the long-term hazard of Muller's ratchet may apply to the local mutational neighborhood in which a biological population finds itself on the landscape.

Importantly, a previous study ([23]) has shown that subdivision can protect populations from myopic selection for mutational robustness. Because selection is more effective at purging deleterious mutations in demes dominated by ratchet robust individuals, net dispersal rates were higher from those demes, and the population in total was thus enriched for such individuals in spite of the short-term disadvantage. We predict that any population structure capable of hindering rapid fixation of mutational robustness will help natural selection favor ratchet robustness. However, a detailed survey of possible mechanisms is outside the scope of this study.

Widespread empirical observations of mutational robustness do not necessarily demonstrate selection for mutational robustness

We find that mutational robustness is unlikely to be selected for in the long term at the expense of ratchet robustness. However, mutational robustness is seen at many levels of biological systems ([24]). For example, it has been observed that many proteins are tolerant of single mutations, a finding taken as evidence for selection for mutational robustness (e.g., [25]). We note however that the existence of mutational robustness need not imply selection for mutational robustness ([26–28]). Following others ([7]), we suggest instead that mutational robustness may often evolve as a correlated

consequence of selection for environmental robustness, i.e. an organism's ability to sustain fitness against environmental perturbations. These perturbations can be external, such as temperature or rainfall variation, or internal, such as thermal noises of microenvironments inside an organism. In the presence of environmental noise, expected reproductive success should be measured as average fitness under different environments weighted by the probabilities of each environments appearing, while also considering relative timescale between generation and environmental change ([29]). In most cases of fluctuating environments, lineages with higher environmental robustness have higher overall reproductive success, and environmental robustness will be selected for ([30]). In fact, diverse mechanisms of achieving environmental robustness have evolved in response to various forms of environmental perturbations. While an exhaustive survey of this work is outside the scope of this study, the interested reader is directed to [24].

Instead, our focus here is on the relationship between environmental and mutational robustness. Theoretically and empirically, environmental robustness has been shown to give rise to mutational robustness ([7, 31, 32]). For example, RNA molecules that can sustain their secondary structure despite thermal noises also show mutational robustness ([33–35]). Proteins that evolved to be robust against transcription errors can also tolerate deleterious mutations ([36]). Consequently, selection for environmental robustness can give rise to mutational robustness in nature, even if selection is unlikely to favor mutational robustness *per se*.

Why can natural selection favor the evolution of environmental but not mutational robustness? The key distinction is that mutational robustness requires tolerating heritable perturbations, which inevitably alters the "starting point" of future generations. Such heritable decay is intrinsic to Muller's ratchet. By contrast, selection for environmental robustness entails non-heritable environmental perturbation. Consequently, the short term advantage of environmental robustness is not offset by any long term cost, accounting for the absence of an "environmental ratchet". In summary, while mutational robustness may be widespread in nature, we suggest an alternative interpretation for its evolution: namely as a correlated consequence of selection for environmental robustness ([7]).

Previous theoretical studies on the evolution of mutational robustness

Interestingly, a few previous theoretical studies have uncovered the long-term cost of mutational robustness ([1,37,38]), using different methods from ours. However, while we interpret empirical evidence of mutational robustness as reflecting selection for environmental robustness, some theoretical studies have suggested the possibility of selection for mutational robustness. Among these, the most well-known are work by van Nimwegen et al. using neutral networks ([3]) and work by Wilke et al. using the computational model of evolution by natural selection called Avida ([4]). We reconcile our conclusions with these previous studies next.

In the neutral network study of van Nimwegen et al. ([3]), the fitness landscape consists of a subset of genotypes sharing identical non-zero fitness, while all other genotypes are inviable. The authors represent the viable subset of genotypes by a connected graph G, where each vertex corresponds to a genotype and two vertices are connected by an edge when they can be reached from each other via a single mutation. In other words, all edges within G represent neutral mutations; hence G is a neutral network. Mutational robustness of each genotype is proportional to the number of mutational neighbors on G (the degree of corresponding vertex), since higher degree means higher proportion of neutral mutations. van Nimwegen et al. ([3]) prove that populations where $N\mu \ll 1$ (N: population size, μ: total mutation rate) effectively take a random walk on G, experiencing the average mutational robustness over all genotypes in G. On the other hand, if $N\mu \gg 1$, populations evolving on G equilibrate on highly connected regions of G, i.e. in regions of high mutational robustness.

As previously noted ([39]), these findings are actually in agreement with our conclusions here. Specifically, by construction Muller's ratchet is impossible in van Nimwegen et al.'s model, since any mutation off of G is lethal and so is instantaneously eliminated. Put another way, under this model, evolving populations enjoy the benefits of increased mutational robustness observed in Fig 4, without the otherwise concomitant risk of succumbing to Muller's ratchet. Relaxing van Nimwegen et al.'s assumption of strict lethality for all genotypes off of G recovers exactly our predicted behavior: at equilibrium mutational robustness declines with mutation rate (see Fig S5

in [39]). The above argument also applies to other studies where all deleterious mutations are lethal (e.g., [40]).

Wilke et al. ([4]) examined the evolution of mutational robustness using Avida, a platform for conducting *in silico* evolutionary experiments. Digital organisms in Avida are computer programs capable of self-replicating, and they compete for the limiting resource – CPU cycles – to reproduce. Their genotypes are the instructions making up the program, and their phenotypes, evaluated via executing the program, determine their fitness, i.e., how many CPU cycles will be allocated to them and thus how fast they can replicate. Mutations influence their fitness by changing instructions inside the program, i.e., their genotypes, mimicking biological mutations.

Wilke et al. evolved 40 paired populations (each initialized from a common genotype) under low and high mutation rates for 1000 generations. Next, the most abundant genotype was extracted from the low- and high-mutation rate populations; these were designated as A and B in [4]. In each pair, A almost always had higher fitness than B, reflecting the lower mutational load, and Wilke et al. focused on 12 cases in which the fitness of A was at least 1.5-fold higher than that of B. Competitions were conducted for each such pair under different mutation rates.

Not surprisingly, at low mutation rate A eliminated B, since A always had higher fitness. However, the study's key result was that in each case, B outcompeted A when mutation rate is high. Representative results are shown in Fig 1 of [4], and these were taken to suggest that B has higher mutational robustness. To confirm this interpretation, Fig 2 in [4] presented fitness distributions of genotype in the mutational neighborhood of a representative A and B. The authors write that "the competitive reversal [at high mutation rate] reflects a shift toward less fit genotypes, which is more pronounced for A than B," implying that "A occupied a higher but narrower fitness peak, whereas B was on a lower but broader peak" (p. 322 in [4]).

However, to our mind Fig 2 is actually more consistent with the evolution of ratchet robustness than of mutational robustness. Specifically, the fitness distribution of genotypes surrounding B appears to contain more lethal mutations (Fig 2 in [4]: lower panels show larger fractions of black, which correspond to more mutants with zero fitness). Furthermore, A seems more prone to mutation accumulation than B (Fig 2 in [4]: right upper panel skews towards more mutations compared with right lower panel,

which is only plausible if A has more weakly deleterious mutations available). Thus we interpret Fig 2 in [4] to suggest that B actually has more lethal and deleterious mutations, i.e., lower mutational robustness, and consequently higher ratchet robustness, which is consistent with our results.

There also exist studies attempting to experimentally validate conclusions in Wilke et al.. Here, two viroid ([41]) or RNA virus ([42]) populations were evolved in regimes analogous to those for populations A and B in Wilke et al., and then competed against each other under different mutation rates. Unfortunately, these studies fail to accurately characterize A or B's fitness landscapes, thus cannot support the claim that population B has higher mutational robustness than A. Specifically, only nonlethal genotypes are considered, while lethal mutations are ignored. As noted above, lethal mutations can protect a population from Muller's ratchet.

Fitness landscapes with negative epistasis are common in nature

We note that many surveys of biological fitness landscapes find extensive evidence for negative epistasis, precisely the property that our results predict facilitate long-term survival. For example, a mutation accumulation study in TEM-1 β-latctamase revealed evidence for negative epistasis ([43]), a genome wide study of pairwise epistasis in *Saccharomyces cerevisiae* found twice as many cases of negative epistasis as positive epistasis ([44]), and another study on evolving *Escherichia coli* populations reported negative epistasis between beneficial mutations ([45]). Adaptation of β-latctamase alleles has also been found to lead to regions of the fitness landscape with decreased mutational robustness and increased negative epistasis ([46]). Relatedly, biophysical principles predict negative epistasis among mutations in protein-coding genes, mediated by their effect on protein folding stability ([39]). On the other hand, metabolic control theory ([47]) implies that whether or not negative epistasis is favored depends on metabolic pathway topology ([48]). It's worth noting that, although we predict selection for ratchet robustness favors landscapes with negative epistasis, selection for other evolutionary traits may have opposite effects.

Conclusion

Our simulations demonstrate that ubiquitous pairwise negative epistasis profoundly influences ratchet robustness (Fig 2, Fig 3), with the negative feedback between mutation and selection strength. However, epistasis present in real fitness landscapes are often more complex than simply pairwise interactions, and it has been previously proposed that higher order interactions could play an important role in evolutionary dynamics ([49, 50]). At this point, we are unable to even speculate on the characteristics of complex landscapes that promote high ratchet robustness.

Supporting information

S1 Text. Two different kinds of MSDE in the presence of negative epistasis

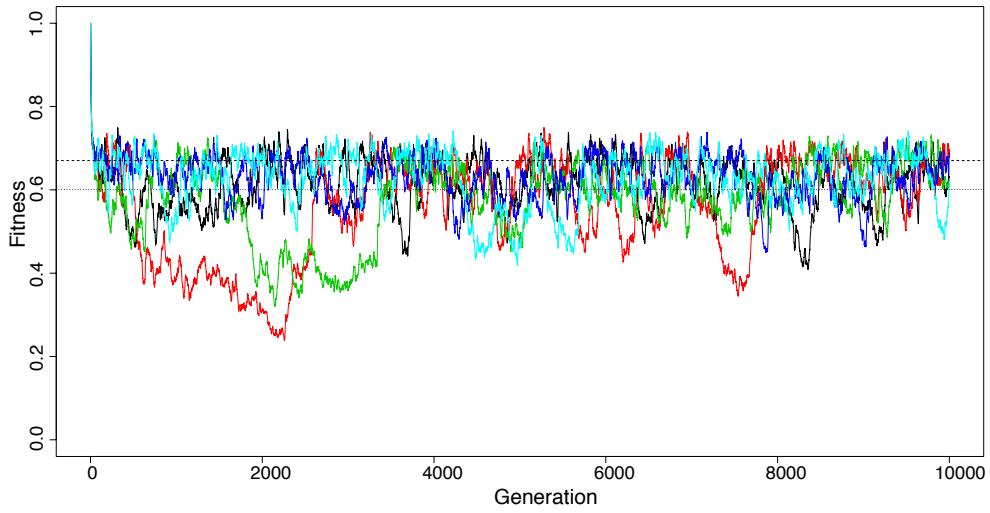
Note that two distinct kinds of MSDE exist on fitness landscapes with negative epistasis. When U_{del} is lower than critical U_{del} at the peak, the first kind of MSDE, which we call $\text{MSDE}_{@\text{p}}$ is sustained at the peak (i.e., at $\text{MSDE}_{@\text{p}}$ fitness equals $e^{-U_{del}}$). $\text{MSDE}_{@\text{p}}$ is characterized by selection greater than that required for offsetting current U_{del}. Populations reside at the $\text{MSDE}_{@\text{p}}$ merely because genotypes at the peak have exhausted their supply of beneficial mutations ([5]).

In contrast, when U_{del} grows larger than critical U_{del} at the peak, populations move downwards away from the peak. But importantly, because of the negative feedback between mutation and the strength of selection induced by negative epistasis (see Results), the population will again equilibrate, but now at the second kind of MSDE, which we call $\text{MSDE}_{\text{n@p}}$. Unlike $\text{MSDE}_{@\text{p}}$, $\text{MSDE}_{\text{n@p}}$ exists where purifying selection exactly offsets deleterious mutation and genetic drift. The position of this second equilibrium was recently solved quantitatively ([5]), although in that study the essential negative feedback was realized by increasing U_{ben}/U_{ben} with decreasing fitness, rather than increasing s locally, as on our landscape.

Importantly, $\text{MSDE}_{\text{n@p}}$ does not exist on fitness landscape with positive epistasis, because positive epistasis means positive feedback between mutation rate and the strength of purifying selection. Consequently, populations below the point of no return

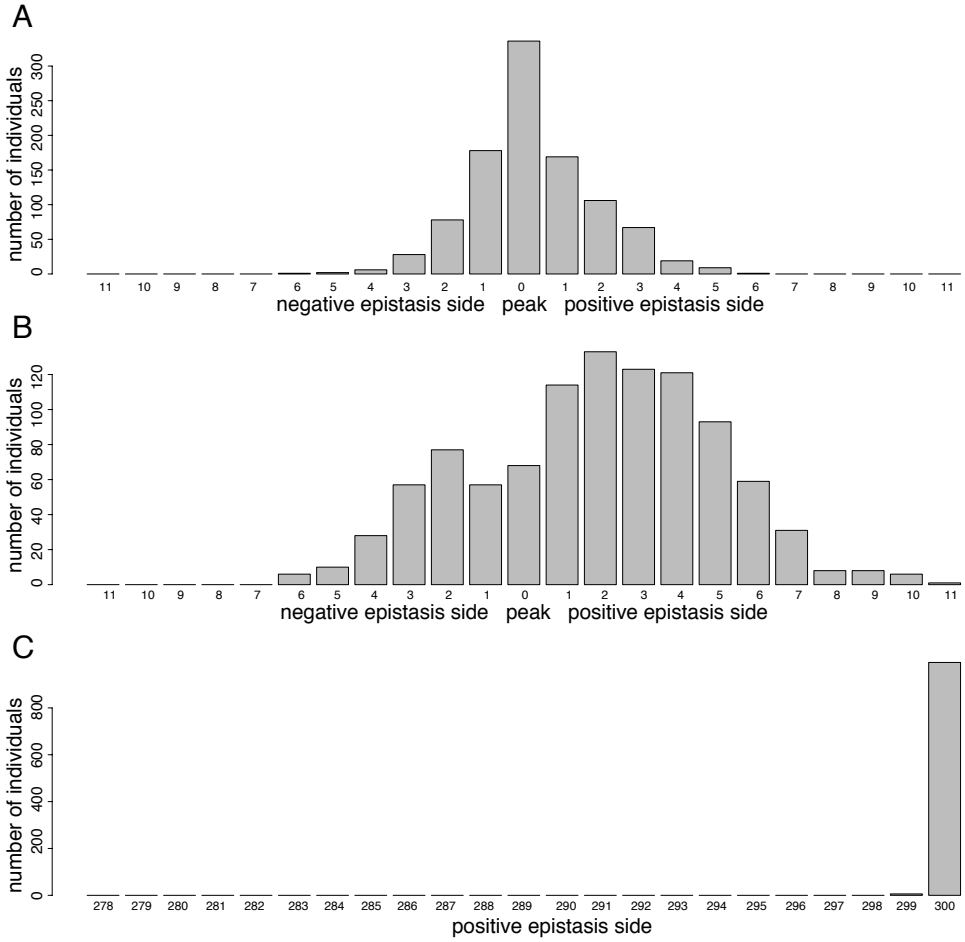
experience accumulation of deleterious mutations accelerated by weakening of selection, quickly succumbing to Muller's ratchet. Populations above the point of no return encounter stronger and stronger selection while driven upward by selection, until halted by the peak, and establish $\text{MSDE}_{@\text{p}}$ around the peak, where selection is the strongest.

Finally, the log-linear fitness landscape, or landscape with no epistasis, presents a still subtler scenario, since log-linear fitness landscapes have no feedback between U_{del} and s. Thus at first glance, it seems that since selection is identical everywhere on the landscape, critical U_{del} at the peak should be the same as critical U_{del} anywhere else. This suggests that no $\text{MSDE}_{\text{n@p}}$ should exist in the absence of epistasis, since once $\text{MSDE}_{@\text{p}}$ is no longer sustainable, mutation should overwhelm selection anywhere else as well. However, as noted above, the genotype at the fitness peak uniquely lacks beneficial mutations. Consequently critical U_{del} at the peak is actually slightly lower than critical U_{del} anywhere else, allowing for existence of $\text{MSDE}_{\text{n@p}}$ at U_{del} higher than critical U_{del} at the peak but lower than critical U_{del} elsewhere. This effect can be seen in Fig 1 A, where at intermediate values of U_{del} population equilibrates at fitness values between $e^{-U_{del}}$ and 0 (see also S1 Fig).



S1 Fig. Average fitness over time for populations evolving on fitness landscape in the absence of epistasis. Lines of different colors represent five independent replicates ($N = 1000$, $s = 0.1$, $U_{del} = 0.4$, $U_{ben} = 0.01U_{del}$). Upper dashed line denotes $w_{max}e^{-U_{del}}$ (w_{max} is fitness at the peak), whereas lower dashed line

denotes average equilibrium fitness at generation 10,000 over five replicates, which equals $w_s e^{-U_{del}}$ (w_s is fitness of genotype one mutation away from the peak). This shows that current U_{del} exceeds critical U_{del} at the peak, but is still lower than critical U_{del} everywhere else (see Discussion).



S2 Fig. Equilibrium distribution of individuals at negative epistasis region, peak and positive epistasis region on hybrid peaks under different mutation rates. Counts of individuals at different parts of the landscape for A: $U_{del} = 0.3$, B: $U_{del} = 0.7$, C: $U_{del} = 1.0$ ($U_{ben} = 0.01U_{del}$, $p = 0.5$, $N = 1000$, $s = 0.3$, negative epistasis region: $\epsilon = -0.25$, positive epistasis region: $\epsilon = 0.25$, evolved for 10,000 generations). There are no individuals present at omitted parts. Note that for panel C, every individual is at the "bottom" of the positive epistasis region after succumbing to Muller's ratchet.

Peak → Negative	Negative → Peak	Peak → Positive	Positive → Peak
57.9168	0.5154	57.8357	0.5256

S1 Table. Average number of individuals leaving or entering peak from either negative epistasis region or positive epistasis region. $U_{del} = 0.5$, $U_{ben} = 0.01U_{del}$, $p = 0.5$, $N = 1000$, $s = 0.35$, negative epistasis region: $\epsilon = -0.25$, positive epistasis region: $\epsilon = 0.25$, average over 10,000 generations. The mutational net effect results in increase in individuals at the negative epistasis region, which offsets decrease in individuals at the negative epistasis region due to lower fitness (see Results).

Acknowledgments

We want to thank the following people for useful comments on earlier versions of the manuscript: Dan S. Tawfick, Daniel Balick, Claus Wilke, Jeremy Draghi, Stephen Proulx, Arjan de Visser, Christina Burch.

References

1. Krakauer DC, Plotkin JB. Redundancy, antiredundancy, and the robustness of genomes. Proceedings of the National Academy of Sciences. 2002 Feb 5;99(3):1405-9.

2. Wilke CO, Adami C. Evolution of mutational robustness. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2003 Jan 28;522(1):3-11.

3. Van Nimwegen E, Crutchfield JP, Huynen M. Neutral evolution of mutational robustness. Proceedings of the National Academy of Sciences. 1999 Aug 17;96(17):9716-20.

4. 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 19;412(6844):331-3.

5. Goyal S, Balick DJ, Jerison ER, Neher RA, Shraiman BI, Desai MM. Dynamic mutation–selection balance as an evolutionary attractor. Genetics. 2012 Aug 1;191(4):1309-19.

6. Wilke CO. Selection for fitness versus selection for robustness in RNA secondary structure folding. Evolution. 2001 Dec;55(12):2412-20.

7. de Visser JA, Hermisson J, Wagner GP, Meyers LA, Bagheri-Chaichian H, Blanchard JL, Chao L, Cheverud JM, Elena SF, Fontana W, Gibson G. Perspective: evolution and detection of genetic robustness. Evolution. 2003 Sep;57(9):1959-72.

8. Wagner A. Robustness, evolvability, and neutrality. FEBS letters. 2005 Mar 21;579(8):1772-8.

9. Lauring AS, Frydman J, Andino R. The role of mutational robustness in RNA virus evolution. Nature Reviews Microbiology. 2013 May 1;11(5):327-36.

10. Haldane JB. The effect of variation of fitness. The American Naturalist. 1937 Jul 1;71(735):337-49.

11. Kimura M, Maruyama T. The mutational load with epistatic gene interactions in fitness. Genetics. 1966 Dec;54(6):1337.

12. Muller HJ. The relation of recombination to mutational advance. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1964 May 31;1(1):2-9.

13. Haigh J. The accumulation of deleterious genes in a population—Muller's ratchet. Theoretical population biology. 1978 Oct 1;14(2):251-67.

14. Wagner GP, Gabriel W. Quantitative variation in finite parthenogenetic populations: what stops Muller's ratchet in the absence of recombination?. Evolution. 1990 May 1:715-31.

15. Schultz ST, Lynch M. Mutation and extinction: the role of variable mutational effects, synergistic epistasis, beneficial mutations, and degree of outcrossing. Evolution. 1997 Oct 1:1363-71.

16. Poon A, Otto SP. Compensating for our load of mutations: freezing the meltdown of small populations. Evolution. 2000 Oct;54(5):1467-79.

17. Kondrashov AS. Muller's ratchet under epistatic selection. Genetics. 1994 Apr 1;136(4):1469-73.

18. Strogatz SH. Nonlinear dynamics and chaos: with applications to physics, biology, chemistry, and engineering. Westview press; 2014 Jul 29.

19. O'Dea EB, Keller TE, Wilke CO. Does mutational robustness inhibit extinction by lethal mutagenesis in viral populations?. PLoS Comput Biol. 2010 Jun 10;6(6):e1000811.

20. Lynch M, Bürger R, Butcher D, Gabriel W. The mutational meltdown in asexual populations. Journal of Heredity. 1993 Sep 1;84(5):339-44.

21. Bershtein S, Goldin K, Tawfik DS. Intense neutral drifts yield robust and evolvable consensus proteins. Journal of molecular biology. 2008 Jun 20;379(5):1029-44.

22. De Visser JA, Krug J. Empirical fitness landscapes and the predictability of evolution. Nature Reviews Genetics. 2014 Jul 1;15(7):480-90.

23. D O'Fallon B, Adler FR, Proulx SR. Quasi-species evolution in subdivided populations favours maximally deleterious mutations. Proceedings of the Royal Society of London B: Biological Sciences. 2007 Dec 22;274(1629):3159-64.

24. Wagner A. Robustness and evolvability in living systems. Princeton University Press; 2013 Oct 24.

25. Firnberg E, Labonte JW, Gray JJ, Ostermeier M. A comprehensive, high-resolution map of a gene's fitness landscape. Molecular biology and evolution. 2014 Jun 1;31(6):1581-92.

26. Hermisson J, Wagner GP. Evolution of phenotypic robustness. Robust design: a repertoire from biology, ecology, and engineering. Oxford University Press, New York. 2004:47-70.

27. Proulx SR, Phillips PC. The opportunity for canalization and the evolution of genetic networks. The American Naturalist. 2004 Dec 6;165(2):147-62.

28. Siegal ML, Leu JY. On the nature and evolutionary impact of phenotypic robustness mechanisms. Annual review of ecology, evolution, and systematics. 2014 Nov 23;45:495-517.

29. Orr HA. Fitness and its role in evolutionary genetics. Nature Reviews Genetics. 2009 Aug 1;10(8):531-9.

30. Cvijović I, Good BH, Jerison ER, Desai MM. Fate of a mutation in a fluctuating environment. Proceedings of the National Academy of Sciences. 2015 Sep 8;112(36):E5021-8.

31. Wagner GP, Booth G, Bagheri-Chaichian H. A population genetic theory of canalization. Evolution. 1997 Apr 1:329-47.

32. Meiklejohn CD, Hartl DL. A single mode of canalization. Trends in Ecology & Evolution. 2002 Oct 1;17(10):468-73.

33. Ancel LW, Fontana W. Plasticity, evolvability, and modularity in RNA. Journal of Experimental Zoology. 2000 Oct 15;288(3):242-83.

34. Szöllősi GJ, Derényi I. Congruent evolution of genetic and environmental robustness in micro-RNA. Molecular biology and evolution. 2009 Apr 1;26(4):867-74.

35. DOMINGO-CALAP P, PEREIRA-GÓMEZ M, Sanjuán R. Selection for thermostability can lead to the emergence of mutational robustness in an RNA virus. Journal of evolutionary biology. 2010 Nov 1;23(11):2453-60.

36. Goldsmith M, Tawfik DS. Potential role of phenotypic mutations in the evolution of protein expression and stability. Proceedings of the National Academy of Sciences. 2009 Apr 14;106(15):6197-202.

37. Gros PA, Tenaillon O. Selection for chaperone-like mediated genetic robustness at low mutation rate: impact of drift, epistasis and complexity. Genetics. 2009 Jun 1;182(2):555-64.

38. LaBar T, Adami C. Evolution of Drift Robustness in Small Populations of Digital Organisms. bioRxiv. 2016 Jan 1:071894.

39. Wylie CS, Shakhnovich EI. A biophysical protein folding model accounts for most mutational fitness effects in viruses. Proceedings of the National Academy of Sciences. 2011 Jun 14;108(24):9916-21.

40. Wilke CO. Adaptive evolution on neutral networks. Bulletin of mathematical biology. 2001 Jul 1;63(4):715-30.

41. Codoñer FM, Darós JA, Solé RV, Elena SF. The fittest versus the flattest: experimental confirmation of the quasispecies effect with subviral pathogens. PLoS Pathog. 2006 Dec 29;2(12):e136.

42. Sanjuán R, Cuevas JM, Furió V, Holmes EC, Moya A. Selection for robustness in mutagenized RNA viruses. PLoS Genet. 2007 Jun 15;3(6):e93.

43. Bershtein S, Segal M, Bekerman R, Tokuriki N, Tawfik DS. Robustness–epistasis link shapes the fitness landscape of a randomly drifting protein. Nature. 2006 Dec 14;444(7121):929-32.

44. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JL, Toufighi K, Mostafavi S, Prinz J. The genetic landscape of a cell. science. 2010 Jan 22;327(5964):425-31.

45. Khan AI, Dinh DM, Schneider D, Lenski RE, Cooper TF. Negative epistasis between beneficial mutations in an evolving bacterial population. Science. 2011 Jun 3;332(6034):1193-6.

46. Steinberg B, Ostermeier M. Shifting fitness and epistatic landscapes reflect trade-offs along an evolutionary pathway. Journal of molecular biology. 2016 Jul 3;428(13):2730-43.

47. Kacser HA, Burns J. The control of flux. InSymp. Soc. Exp. Biol. 1973 (Vol. 27, pp. 65-104).

48. Szathmary E. Do deleterious mutations act synergistically? Metabolic control theory provides a partial answer. Genetics. 1993 Jan 1;133(1):127-32.

49. Weinreich DM, Lan Y, Wylie CS, Heckendorn RB. Should evolutionary geneticists worry about higher-order epistasis?. Current opinion in genetics & development. 2013 Dec 31;23(6):700-7.

50. Poelwijk FJ, Krishna V, Ranganathan R. The context-dependence of mutations: a linkage of formalisms. PLoS Comput Biol. 2016 Jun 23;12(6):e1004771.