Transient evolutionary epidemiology of viral adaptation and lethal mutagenesis

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Abstract: Beneficial mutations drive the within-host adaptation of viral populations and can prolong the duration of host infection. Yet, most mutations are not adaptive and the increase of the mean fitness of viral populations is hampered by deleterious and lethal mutations. Because of this ambivalent role of mutations, it is unclear if a higher mutation rate boosts or slows down viral adaptation. Here we study the interplay between selection, mutation, genetic drift and within-host dynamics of viral populations. We obtain good approximations for the transient evolutionary epidemiology of viral adaptation under the assumption that the mutation rate is high and the effects of non-lethal mutations remains small. We use this theoretical framework to discuss the feasibility of lethal mutagenesis to treat viral infections.

Introduction

The within-host dynamics of viral infections depends both on the availability of susceptible host cells and the ability of the virus to infect and exploit these cells. Mathematical epidemiology provides a theoretical framework to model how these life-history traits affect the dynamics of viral populations [1–3]. Yet these traits are not constant but may evolve and change during the course of the host infection. Indeed, many viruses undergo high mutation rates [4], yielding large amount of genetic and phenotypic diversity of within-host viral populations. This influx of mutations challenges the simplicity of classical models of viral dynamics and has led to the concept of *quasispecies* to describe the dynamics of viruses with high mutation rates [5, 6]. The effects of high mutation rates can also be captured within the classical population genetics framework [7–9]. As most mutations have deleterious effects, the constant influx of mutations generates a *mutation load* which measures the difference between fitness of the fittest strain and the mean fitness of the population [10]. In fact, some mutations can prevent viral replication and can be considered as *lethal mutations* [11]. The massive impact of deleterious mutations on viral fitness led to the "lethal mutagenesis hypothesis" which states that there is a mutation rate above which a viral population cannot grow and is driven to extinction [8, 12]. Drugs increasing mutation rates may thus constitute a broadly applicable therapeutic strategy against many viruses [13, 14], including SARS-CoV-2 [15–19]. A better evaluation of the balance between the therapeutic potential and the risk associated with these drugs relies on a better understanding of the within-host viral dynamics with increased mutation rates.

First, it is important to realise that mutation is a double-edged sword: even if most mutations are deleterious, some may be beneficial and could speed up within-host evolution. Some concern has emerged regarding the potential risk of "sublethal mutagenesis" that may result in immune escape or higher transmission [20–23]. Experimental evolution of bacteriophage T7 under high mutation rates showed that the exposition to a mutagen may boost adaptation and increase the mean fitness of the viral population [12, 24]. Several theoretical models explored the balance between the effects of deleterious and beneficial mutations [9, 25–27]. Yet, another aspect which is often overlooked in previous models is the epidemiological dynamics taking place within the infected host. The drop of the mean fitness of the virus consecutive to accumulation of deleterious mutations is expected to reduce the within-host viral load. This may trigger a rebound in the density of susceptible host cells, higher viral replication and, eventually, treatment failure. Hence, the critical mutation rate above which the virus population is expected to go extinct needs to account for this demographic feedback [9].

Second, even if lethal mutagenesis is a deterministic process occurring when the mutation load becomes overwhelmingly high, the effect of higher mutation rates may be amplified by demographic stochasticity. In finite populations, the most fit, least-loaded genotype will be lost as genetic drift overwhelms the effect of natural selection. This will result in a lower

population size, thus increasing the magnitude of genetic drift and the drop of mean fitness. There is thus a synergy between the demographic and evolutionary dynamics [28–30]. However, even a small influx of compensatory mutations can halt the meltdown of small populations and prevent their extinction [31]. But demographic stochasticity could increase the risk of extinction when the viral population becomes transiently very low after the start of a mutagenic treatment [27]. Yet, the theory of lethal mutagenesis is based on the analysis of the equilibrium of the deterministic dynamics of viral populations and the identification of the critical mutation rate beyond which the virus is driven to extinction. Yet, we currently lack a good understanding of the effects of higher mutation rates on the transient within-host dynamics of viral adaptation.

In the present work, we study the joint epidemiological and evolutionary within-host dynamics of a viral population exposed to high mutation rates. We use Fisher's Geometric Model (FGM) to link the phenotype of the virus to its within-host transmission rate [9]. This geometric model of adaptation generates distributions of fitness effects of mutations and allows us to account for deleterious, neutral or beneficial effects of mutations [32, 33]. Because these fitness distributions depend on the parental genotype, the model accounts also for pervasive epistasis in fitness between mutations [34]. In addition, we account for a distinct type of strictly lethal mutations. The analysis of the long-term equilibrium of the epidemiology and evolution of viral dynamics allows us to identify the critical mutation rate leading to the within-host eradication of the virus [9]. Crucially, we derive approximations for the transient within-host evolutionary epidemiology of the virus. The analysis of these transient dynamics allows us to identify the pros and cons of higher mutation rates under different scenarios. In particular, we discuss the potential risks of lethal mutagenesis for the treatment of viral infections. Finally, we check the validity of our deterministic predictions with stochastic simulations.

1 Model

To model the joint epidemiological and evolutionary within-host dynamics of a virus population we assume that the viral pathogen has access to a density S of susceptible host cells. We focus on the dynamics of the density I of infected cells and we do not explicitly model the dynamics of the free virus stage because we assume the lifetime of an infected cell is much larger than a free virus particle [3, 9]. Host cells are produced at a constant rate b and die at a constant rate d. We assume that different strains of the virus (i.e. different phenotypes $\mathbf{x}$) may circulate within the host and $I_{\mathbf{x}}(t)$ refers to the density of host cells infected by strain $\mathbf{x}$. An infected cell of phenotype $\mathbf{x}$ infects susceptible cells with a transmission rate $\beta_{\mathbf{x}}$ and dies at a rate $(d+\alpha+Uf)$ where α is the virulence and Uf is the rate of lethal mutations. These lethal mutations produce cells that are infected with a non-transmissible form of the virus. As there is no density-dependent reproduction of susceptible host cells in our model, the cells

infected by viruses with lethal mutations do not affect the dynamics of the system. Consequently, we do not monitor the density of the cells infected by non-transmissible pathogens, and we simply treat lethal mutations as an additional death term. We assume that each host cell can only be infected with a single viral strain (i.e. no multiple infections). The overall death rate of the infected cells is not affected by the phenotype of the virus and the above life cycle yields the following system of ordinary differential equations (the upper dot represents time derivation):

$$\begin{aligned} \dot{S}(t) &= b - S(t)\ \left(\overline{\beta}(t)\, I(t) + d\right) \\ \dot{I}(t) &= \overline{\beta}(t)\, S(t)\, I(t) - (\alpha + d + Uf)\, I(t) \end{aligned} \tag{1}$$

where $I(t) = \int I_{\mathbf{x}}(t) d\mathbf{x}$ is the total density of infected cells (i.e. cells infected by a transmissible virus), $\overline{\beta}(t) = \int p_{\mathbf{x}}(t)\beta_{\mathbf{x}} d\mathbf{x}$ is the mean transmission rate and $p_{\mathbf{x}}(t) = I_{\mathbf{x}}(t)/I(t)$ is the frequency of the phenotype $\mathbf{x}$ in the infected population. We can now introduce the per capita growth rate (i.e. malthusian fitness) of the phenotype $\mathbf{x}$ and the mean growth rate of the pathogen (i.e. the mean fitness) :

$$\begin{aligned} r_{\mathbf{x}}(t) &= \beta_{\mathbf{x}} S(t) - (d + \alpha + Uf) \\ \overline{r}(t) &= \int p_{\mathbf{x}}(t) r_{\mathbf{x}} d\mathbf{x} = \overline{\beta}(t) S(t) - (d + \alpha + Uf) \end{aligned} \tag{2}$$

The sign of the mean fitness indicates whether a population of infected cells can invade a population of hosts of size $S(t)$ (i.e., $\overline{r}(t) > 0$) or if it drops and eventually goes extinct (i.e., $\overline{r}(t) < 0$).

We define a phenotype $\mathbf{x}$ as a vector of size n, where each dimension refers to an independent continuous trait. To model the dependence of the transmission rate $\beta_{\mathbf{x}}$ on $\mathbf{x}$, we use a fitness landscape based on a quadratic Fisher's geometric model where the transmission rate depends on the euclidian distance of the vector $\mathbf{x}$ to the optimum (at the origin i.e. at $\mathbf{x} = (0, 0, .., 0)$) such that :

$$\beta_{\mathbf{x}} = \beta_0 - \frac{s_\beta \|\mathbf{x}\|^2}{2} \tag{3}$$

where $\|\mathbf{x}\|^2$ is the squared norm of the phenotypic vector $\mathbf{x}$. The term s_β governs the curvature of the fitness landscape and represents the strength of the directional selection towards the optimum where the transmission rate is β_0. Note that we use this quadratic form to approximate a Gaussian shape of the fitness landscape [9]. Indeed, equation (3) can yield a good approximation when the virus is not too far from the optimum. However, note that this approximation breaks down when the phenotype is very far from the optimum as transmission rate can become negative. We show how this fitness landscape links the n underlying phenotypes with the pathogens' life-history trait (i.e. transmission rate β) in **Figure 1**. In the following, we will track the evolution of the distribution of the life-history trait β itself. When the distributions of the phenotypic traits x_i are independent, identically distributed and

Gaussian, we can fully describe the evolutionary dynamics of the virus with one phenotypic dimension where $p_x(t)$ refers to the proportion of the virus population with phenotypic trait x, $\overline{x}(t) = \int p_x(t)x dx$ is the mean value of the phenotypic trait and $V_x(t) = \int p_x(t)(x-\overline{x}(t))^2 dx$ is the phenotypic variance. This framework allows us to explicitly write the mean transmission rate and the variance in transmission rate V_β:

$$\begin{aligned}
\overline{\beta}(t) &= \beta_0 - L_L(t) - L_M(t) \\
V_\beta(t) &= V_x(t) s_\beta \left(2L_L(t) + L_M(t)\right) \\
L_L(t) &= \frac{n s_\beta}{2} \overline{x}(t)^2 \\
L_M(t) &= \frac{n s_\beta}{2} V_x(t)
\end{aligned} \tag{4}$$

where $L_L(t)$ is the "lag load" and $L_M(t)$ is the "mutation load" [35]. The lag load depends on the distance to the optimum, and represents the difference between the maximum transmission rate and the transmission rate of the mean phenotype: $L_L(t) = \beta_0 - \beta_{\overline{x}(t)}$. The mutation load depends on the phenotypic variance, and represents the difference between the transmission rate of the mean phenotype and the mean transmission rate: $L_M(t) = \beta_{\overline{x}(t)} - \overline{\beta}(t)$. These load terms depend on the number of dimensions n because of the so called "cost of complexity" [32, 33]. A lower strength of selection s_β (a measure of the steepness of the landscape around the optimum) decreases both the lag load and the mutation load, as it makes the fitness landscape flatter. The variance in transmission rate depends on both the lag load and the mutation load, scaled again by the phenotypic variance $V_x(t)$ and the strength of selection s_β.

Next we need to describe the dynamics of the density $I_{\mathbf{x}}(t)$ of cells infected by each phenotype where U is the rate of mutation of the virus. This mutation process is assumed to be constant and unconditional on a transmission event. With probability f this mutation is lethal and we model this effect as an additional rate of mortality Uf for the infected cells. With probability $1-f$ this mutation is non-lethal and the new phenotype becomes $\mathbf{x}+\mathbf{y}$, where the mutation effect $\mathbf{y}$ is sampled in an isotropic multivariate normal distribution with mean 0 and variance λ. This mutation process yields the following integro-differential equation:

$$\dot{I}_{\mathbf{x}}(t) = \beta_{\mathbf{x}} S(t) I_{\mathbf{x}}(t) - (d + \alpha + Uf) I_{\mathbf{x}}(t) - U(1-f) I_{\mathbf{x}}(t) + U(1-f) \int I_{\mathbf{x}-\mathbf{u}}(t) \rho(\mathbf{u})\, d\mathbf{u}\,. \tag{5}$$

where ρ is the multivariate Gaussian kernel of mutational effects on phenotypes. The mutational variance λ is easily interpreted as it directly relates to the the mean effect of random mutations on transmission rate μ_β:

$$\mu_\beta = \int \rho(\mathbf{u}) \left(\beta_{\mathbf{x}+\mathbf{u}} - \beta_{\mathbf{x}}\right) d\mathbf{u} = -\frac{s_\beta\, n\, \lambda}{2} \tag{6}$$

We can directly relate this to the mean effect of mutations on fitness $\mu_r(t) = \mu_\beta S(t)$ which is also dependent on the density of infected cells at time t. Note that this quantity is always

negative and independent of the phenotype $\mathbf{x}$, meaning that mutated strains are always, on average, worse than their parental strain.

In the following, we use different approaches to monitor the epidemiological and evolutionary dynamics described in (5). First, for the sake of simplicity, we use a Weak Selection Strong Mutation (WSSM) approximation which implies that adaptation is the result of many mutations of small effects. In this regime, the distribution of phenotypes remains Gaussian [36]. Second, we relax this assumption and we study how mutations of larger effects can influence the evolutionary dynamics of the virus using a moment closure approximation. Third, we check the robustness of our approximations using numerical simulations, where we study the dynamics of discrete phenotypes on a 2D grid, with or without the assumption of small mutational effects. Finally, we use our theoretical framework to discuss the feasibility and the risks associated with lethal mutagenesis therapy under different scenarios. In particular, we use stochastic simulations to explore the effect of finite host and viral population sizes on our theoretical predictions on the probability of treatment failure.

2 Results

We use equation (5) to obtain dynamical equations for the cumulants of the distribution of the phenotypes $\mathbf{x}$. Under the WSSM assumptions, we can neglect higher order terms of the mutational variance (i.e., λ^2, λ^3 etc.), the phenotypic distribution is Gaussian and the evolutionary dynamics can be described with the first two moments of the phenotypic distribution [37]. These derivations yield the following dynamical system:

$$\begin{aligned} \dot{\overline{x}}(t) &= \overbrace{-V_x(t)\,S(t)\,s_\beta \overline{x}(t)}^{\text{selection}} \\ \dot{V}_x(t) &= \underbrace{-s_\beta\,V_x(t)^2\,S(t)}_{\text{selection}} + \underbrace{U(1-f)\lambda}_{\text{mutation}} \end{aligned} \tag{7}$$

Note how the epidemiological dynamics (1) feeds back on the evolution of the phenotypic distribution. The first equation shows how the mean phenotype moves towards the optimum at a speed governed by (i) the amount of susceptible cells $S(t)$, (ii) the phenotype variance $V_x(t)$ and (iii) the mean distance to the optimum $\overline{x}(t)$. The dynamics of $V_x(t)$ results from the balance between the effect of natural selection which consumes this variation, and the effect of mutation which introduces more genetic variation. Interestingly, the number of dimensions n only appears in the epidemiological equations through $\overline{\beta}(t)$ in (4) and does not directly influence the dynamics of the transmission rate.

Using the assumption that the distribution $\mathbf{x}$ is Gaussian, we obtain a dynamical equation for the mean transmission rate $\overline{\beta}$:

$$\dot{\overline{\beta}}(t) = \overbrace{V_\beta(t)S(t)}^{\text{selection}} + \overbrace{U(1-f)\mu_\beta}^{\text{mutation}} \tag{8}$$

The mean transmission rate is increased by natural selection with a speed controlled by the variance in transmission rate, scaled by the density of susceptible cells. In contrast, the direct effect of the mutation process is negative and equals to the rate of non-lethal mutations times the mean effect of these mutations on transmission rate. Indeed, as discussed above, the expected effect on the life history-trait is deleterious (see equation (6)).

2.1 Evolutionary equilibrium and critical mutation rate

Next, we focus on the long-term dynamics of the viral population to determine what is the critical mutation rate U_c above which natural selection is overwhelmed by the accumulation of deleterious mutations and the viral population is driven to extinction. At equilibrium, the mean phenotype is at the optimum and $\overline{x}^\star = 0$ (where the $\star$ indicates that the system is at equilibrium). The phenotypic distribution of this long-term equilibrium depends both on the epidemiology (equation (1)) and the evolution of the virus (equation (7) and (8)) yielding :

$$\begin{aligned}
V_x^\star &= \sqrt{\frac{U(1-f)\lambda}{s_\beta\, S^\star}} \\
S^\star &= S_0^\star + \Delta \\
I^\star &= I_0^\star - \frac{d}{\alpha + d + Uf}\Delta \\
\Delta &= \frac{n\, s_\beta \sqrt{U(1-f)\lambda}\,(2\gamma + n\sqrt{U(1-f)\lambda})}{8\,\beta_0^2} \\
\gamma &= \sqrt{\frac{4\,(\alpha + d + Uf)\beta_0}{s_\beta} + \frac{n^2 U(1-f)\lambda}{4}}
\end{aligned} \tag{9}$$

where $S_0^\star = (\alpha + d + Uf)/\beta_0$ and $I_0^\star = b/(\alpha + d + Uf) - d/\beta_0$ are the equilibrium densities of the susceptible and infected cells, respectively, when the virus population is monomorphic and the virus is at the optimum (i.e. $\beta = \beta_0$ is maximal). As expected, equation (9) shows that the constant influx of non-lethal mutations introduces a mutation load $L_M^\star$ that leads to a reduced density of infected cells at equilibrium (and an increased density of susceptible cells) because $\Delta > 0$. Using (4) we can compute the mean transmission rate $\overline{\beta}^\star = \beta_0 - L_M^\star$.

The critical mutation rate U_c is reached when $\overline{r} = 0$ and can be obtained from (9) by finding the value of the mutation rate where $I^\star = 0$ which yields (see also [9]):

$$U_c = \frac{s_\beta}{8 f^2 d}\left(A + B - \sqrt{A(A+2B)}\right) \tag{10}$$

with $A = (1-f)\lambda\, b\, n^2$ and $B = 8f(\beta_0\, b - d(d+\alpha))/s_\beta$. As expected, the fraction f of lethal mutations has a massive influence on the critical mutation rate:

$$U_c = \begin{cases} \frac{4(\beta_0\, b - d(d+\alpha))^2}{s_\beta\, b\, d\, \lambda\, n^2} & \text{if } f = 0 \\ \beta_0 \frac{b}{d} - (d+\alpha) & \text{if } f = 1 \end{cases} \tag{11}$$

To understand the expression of the critical mutation rate it is useful to note that U_c is also the mutation rate above which the virus population cannot grow when the density of susceptible cells is b/d (i.e., the equilibrium density of the host cells in the absence of the virus). Using equation (2), (4) and (9) to obtain the mean fitness of the population at equilibrium when $S(t) = b/d$,yields the following condition:

$$\overline{r} = \frac{b}{d}\Big(\beta_0 - \underbrace{\frac{n}{2}\sqrt{\frac{U(1-f)\lambda\, s_\beta}{b/d}}}_{L_M}\Big) - (d + \alpha + Uf) = 0 \tag{12}$$

The final term in (12) accounts for the direct effect of lethal mutations: increasing the proportion of lethal mutations increases the *death rate* of infections, and consequently decreases the critical mutation rate leading to viral extinctions. Yet, lethal mutations have an additional effect on the *birth rate* of infections. Indeed, the first term in equation (12) refers to the rate of new infections and this rate drops with higher mutation load L_M (see equation (4)). This mutation load drops when most mutations are lethal because only viable mutations are accounted for in this load. Hence, increasing the proportion of lethal mutations decreases the mutation load. This effect is relatively small when the number n of phenotypic dimensions is low, but it can counteract the direct effect of lethal mutations when n becomes large. In other words, we find that the effect of the cost of complexity, which is expected to decrease the critical mutation rate for larger values of n, is actually dependent on the proportion of lethal mutations f (**Figure 2**).

2.2 Critical mutation rate when selection is stronger

Under the above WSSM assumption, we neglected higher order terms of the mutational variance (i.e., λ^2, see Supplementary Information). In the following we relax the weak selection assumption and these higher order terms cannot be neglected anymore. In this situation, the phenotypic distribution is no longer multivariate Gaussian. We thus have to account for the effects of K_3 and K_4, the third and fourth cumulants of the phenotypic distribution at equilibrium (where the $\circledast$ indicates this new equilibrium):

$$V_x^{\circledast} = \sqrt{\frac{U\,(1-f)\,\lambda}{s_\beta\, S^{\circledast}} - K_4^{\circledast}/2} \tag{13}$$

The fourth cumulant builds up with the second moment of the distribution of mutational effects λ^2 and is always positive (see Supplementary Information, **Figure S1**), and the equilibrium variance is smaller than expected in the WSSM approximation (**Figure S2**). Consequently, when the effects of the mutation are stronger we need to account for K_4 to compute the critical mutation rat (orange dashed line in **Figure 3**). The critical mutation rate drops with higher values of λ (see **Figure S3**) but the above analysis breaks down when λ becomes too high relative to the standing variance (see Supplementary Information).

For larger values of λ we can use another approximation to describe the viral dynamics under a regime of mutation where the variance of mutation overwhelms the effect of the parental strain: the classical "House-of-Cards" (HC) approximation has been used to derive the equilibrium mutation load [36, 37]. After incorporating the influence of epidemiological feedbacks we obtain the following expectation for the phenotypic variance at equilibrium:

$$V_x^{HC} = \frac{2U(1-f)}{n\, s_\beta\, S^{HC}} \tag{14}$$

where S^{HC} is the equilibrium density of susceptible cells under the HC approximation. Note that the mutation load given by (4) becomes $L_M = U(1-f)/S^{HC}$ and, as discussed in [36], the mutation load on *fitness* (see equation (2) to express $\overline{r}$ as a function of $\overline{\beta}$) is simply equal to $U(1-f)$, the non-lethal mutation rate. Another striking feature of the HC approximation is the independence of equilibrium phenotypic variance to the mutational variance λ. Note, however, that higher order HC approximations for the equilibrium phenotypic variance do depend on λ^2 [37]. Another interesting feature of the equilibrium distribution of fitness under this regime is the emergence of a spike in this distribution (i.e. a Dirac delta function) when $n > 2$, which is due to the proportion of the population having exactly optimal fitness value [36, 38].

Numerical computations of the equilibrium phenotypic variance confirm the validity of the above approximations (**Figure S2**). As expected, the equilibrium variance predicted with the WSSM approximation is very accurate when the strength of selection is low, but this approximation breaks down when the effect of mutations becomes high. The HC approximation becomes better than the WSSM approximation when:

$$s_\beta\, \lambda > \frac{4U(1-f)}{n^2\, S^\star}. \tag{15}$$

We show how the critical mutation rate changes with the strength of selection in **Figure 3**. The critical mutation rate predicted by the WSSM approximation is accurate until the condition (15) is verified. For higher values of s_β, the mutation rate required to achieve lethal mutagenesis is higher under the HC approximation than under the WSSM approximation (**Figure 3**). Interestingly, the critical mutation rate predicted under the HC approximation is the same as the one obtained under the WSSM approximation if all mutations were lethal (see (11)):

$$U_c^{HC} = \beta_0 \frac{b}{d} - (d+\alpha) \tag{16}$$

Note that this critical mutation rate depends on the demographic parameters but does not depend on parameters governing the fitness landscape (and in particular on s_β, **Figure 3**).

2.3 Transient dynamics of adaptation

Next, we jointly use equations (1), (4), (7) and (8) to study the interplay between the transient within-host dynamics of the virus and the adaptation of the viral populations in the WSSM

regime. In **Figure 4** we explore the dynamics of the mean transmission rate $\overline{\beta}(t)$ for different initial conditions. We vary the initial distance of the mean phenotype to the the optimal phenotype, and the standing variance. We contrast a scenario where the virus population is monomorphic ($V_x(0) = 0$, full line) and a scenario with some standing genetic variance ($V_x(0) = 0.1$, dashed line). As expected, regardless of these initial conditions, the dynamics converge to the same equilibrium, which is given by (4) and (9). However, the initial conditions govern the speed at which this equilibrium is reached. First, the standing genetic variance induces a mutation load, which explains the lower value of $\overline{\beta}$ at $t = 0$ in **Figure 4**. Second, the absence of a genetic variation in the clonal population implies that the speed of adaptation is initially very slow. In fact, the mean transmission rate initially drops because of the effect of deleterious mutations (see equation (8)). Genetic variation first needs to build up before selection can act on the mean transmission rate. In contrast, the speed of adaptation is faster with standing genetic variance. This faster adaptation allows to rapidly overcome the initial mutation load and the mean transmission rate $\overline{\beta}$ becomes rapidly higher than in initially clonal populations. Finally, the rate of change in mean transmission rate is increased when the initial distance to the optimum (or initial lag load) is higher (see equation (7)). This is due to a faster decrease in the lag load but note that it does not affect the dynamics of the phenotypic variance and thus mutation load.

The transient dynamics is generally well described by (7) but, when selection s_β is strong and/or the effect of mutation λ is large, the phenotypic distribution does not remain Gaussian during adaptation. We show in the Supplementary Information (see equations (38) and (39)) that K_3 and K_4 are expected to build up during the transient phase of adaptation. Crucially, these higher moments increase the phenotypic variance transiently, which speeds up the adaptation of the virus population (see **Figure S1**). In the long-term, however, we expect $K_3 \to 0$ and $K_4 \to \frac{3U(1-f)\lambda^2}{s_\beta V_x S}$. Consequently, when these higher moments of the distribution are accounted for, the phenotypic variance is lower at the endemic equilibrium (**Figure S2**). Hence, as pointed out in the previous section, the critical mutation rate is higher when K_4 is accounted for (compare equations (10) and (13)).

Yet the epidemiological dynamics of the virus population is driven by $\overline{r}(t)$ and not by $\overline{\beta}(t)$. To better understand the dynamics of viral adaptation, it is useful to decompose the dynamics of viral mean fitness $\overline{r}(t)$ into separate effects following the framework of Gandon & Day [39] (**Figure S4**):

$$\dot{\overline{r}}(t) = \Delta r_{ns} + \Delta r_m + \Delta r_{ec} \tag{17}$$

where Δr_{ns}, Δr_m and Δr_{ec} refer to the changes in mean fitness due to natural selection, mutation and environmental change, respectively.

$$\begin{aligned} \Delta r_{ns} &= V_r(t) = S^2(t)V_\beta = s_\beta V_x(t)S(t)^2\left(2L_L(t) + L_M(t)\right) \\ \Delta r_m &= U(1-f)\mu_r(t) \\ \Delta r_{ec} &= \overline{\beta}(t)\dot{S}(t) \end{aligned} \tag{18}$$

First, as expected from Fisher's fundamental theorem, the change of mean fitness from natural selection is always positive and equal to the variance in fitness $V_r(t)$. This variance increases with the lag load $L_L(t)$ (the farther a phenotype is from the optimum, the larger the strength of selection towards this optimum) and the mutation load $L_M(t)$ (even if this load has a negative impact on mean fitness, it has a positive influence on the speed of adaptation). Selection is also fueled by the population of susceptible cells and the phenotypic variance, scaled by the shape of the fitness landscape, s_β. Second, the effect of mutations on mean fitness is simply equal to the influx of non-lethal mutation $U(1-f)$ multiplied by the mean effect of mutations on fitness μ_r (recall that $\mu_r(t) = \mu_\beta S(t)$). Because $\mu_r < 0$ (see equation (6)), the direct effect of the mutation process is always negative on the dynamics of adaptation in our model. Note that this quantity is exactly equal to the drop in mean fitness in mutation accumulation experiments where the radical bottlenecking at each passage ensures that natural selection does not operate (because the variance in fitness $V_r = 0$). Finally, the third term accounts for the environmental change consecutive to a drop in the density of susceptible cells. This final term can be either positive or negative, depending on the change in the density of susceptible host cells. During the initial phase of an infection, the density of susceptible cells is expected to drop and to have a negative impact on the growth rate of the epidemic (density-dependent regulation). In contrast, during the initial phase of therapy, drugs are expected to reduce the density of infected cells and, consequently, the density of susceptible cells may increase. We illustrate below how this within-host epidemiological feedback may affect the feasibility of lethal mutagenesis to treat viral infections.

2.4 Feasibility of lethal mutagenesis as an antiviral therapeutic strategy

A therapeutic drug may act in, at least, two different ways in our model. First, a drug may have a mutagenic effect and act via an increase of the mutation load L_M of the virus population. Second, a drug may move the fitness optimum away from the virus population and increase the phenotypic distance $\overline{x}(0)$. In the following we explore how these two effects act on the transient within-host dynamics of the virus in the WSSM regime. For simplicity, we assume that the other parameters of the model are unaffected by the use of the drug.

Figure 5 shows virus dynamics during and after the start of drug therapy using the approximation which accounts for the moments K_3 and K_4 (see equations (36)-(39)). When, the mutation rate induced by the drug is sufficiently high above U_c, the drug can drive the virus population to extinction before the end of treatment. But when the mutagenic effect is lower, the initial stress level $x(0)$ is low and/or the duration of the treatment is short, the drug may fail to clear the infection and could even lead to higher rates of viral replication and higher viral loads after the end of the treatment. This rebound is due to the increased rate of viral adaptation induced by the mutagen and to the increase of the density of the susceptible host cells during treatment. It would thus be particularly important to monitor

the patients before deciding to end the treatment to avoid the risk of recrudescence of the infection if it was not cleared. The risk of viral evolutionary rescue is less likely if we account for the build up of an effective immune response of the host against the virus as in the case of an acute infection where the death rate of the infected cells will increase after some time. For instance, we show in **Figure S5** how host immunity may reduce the risk of recrudescence. In this scenario, the use of a mutagen may be used as a way to buy some time and reduce the viral load before the immune response kicks in and effectively controls the infection.

The above numerical exploration of the effect of a mutagenic drug on within-host viral dynamics ignored the influence of demographic stochasticity. Demographic stochasticity is expected to be particularly high when the viral load drops to very low levels. We explore the influence of demographic stochasticity in **Figure 6** by computing the probability of evolutionary rescue after the start of the mutagenic treatment as a function of the viral mutation rate U induced by the drug and $x(0)$ the initial stress. As expected, viral extinction occurs when $U > U_c$ and larger values of $x(0)$ always promote viral extinction. Note, however, that when $x(0)$ is sufficiently large, the virus population may only survive when the mutation rate is above a minimal mutation rate U_{min}. Indeed, intermediate mutation rates (i.e. $U_{min} < U < U_c$) can speed up viral adaptation and allow the viral population to avoid extinction at the beginning of the treatment without inducing an unbearable mutation load at equilibrium. The influence of the mutation rate U on the probability of extinction has been analysed in [40] without a demographic feedback. Our simulation models accounts for the within-host epidemiological dynamics and show that larger fraction of lethal mutants can have a massive impact on the interaction between the effect of U and the initial stress level $x(0)$. As expected, the effects of demographic stochasticity are amplified by smaller host and pathogen population size (**see Figure S6**). To appreciate the effects of the environmental feedback of the density if susceptible cells, we show in **Figure S7** scenarios of evolutionary rescue with the same parameters but a constant susceptible density of b/d that show increased probabilities of rescue overall.

Discussion

To explore the validity of the lethal mutagenesis hypothesis we need to understand the within-host dynamics of a viral population exposed to a high rate of mutation. We developed a model to track both the epidemiological and evolutionary dynamics of the virus. Viral adaptation is assumed to take place in a fitness landscape with a single fitness optimum. Mutations allow the virus phenotype to move in this landscape on n independent dimensions. Each dimension is under stabilizing selection and contributes to the within-host viral transmission rate $\beta_{\mathbf{x}}$. We couple this model of adaptation with an epidemiological model, in which the availability of susceptible host cells feeds back on the growth rate of the virus. We model the dynamics of the phenotypic distribution through the derivation of the cumulants of the distribution [37].

Our model accounts for the occurrence of both lethal and non-lethal mutations as observed in empirical measures of distribution of fitness effects in viruses [4, 11]. Lethal mutations have a strong but instantaneous effect and they can be viewed as an extra source of viral mortality in our model. Non-lethal deleterious mutations, in contrast, can accumulate over time and bring down the mean transmission rate of the virus.

Critical mutation rate: Our model can be used to predict the long-term evolutionary equilibrium of the virus population. In particular, we can predict the critical mutation rate U_c above which the mutation load is so high that virus population is driven to extinction. We derive different approximations for U_c under different regimes of selection. First, when the effect of mutation λ is small and selection s_β is relatively weak (WSSM regime), the equilibrium mutation load L_M (combine equations (4) and (9)) is driven by the effects of non-lethal mutations which depends on the number n of phenotypic dimensions (i.e. complexity). Indeed when phenotypic complexity is high, the proportion of beneficial mutations decreases in favor of deleterious mutations. There has been several attempts to estimate this value of complexity for different organisms (reviewed in [34]) and estimates vary between 0.21 and 26 for bacteria [33, 41–44], and between 1.07 and 90 for viruses [33, 45]. The mutation load depends also on λs_β which measures the product of the phenotypic effects of mutations λ and the strength s_β of stabilizing selection. As expected, larger values of λs_β reduce U_c (**Figure S3**). Second, when selection is relatively strong (condition (15)), the phenotypic variance of the viral population is lower than in the WSSM regime and we can use other approximations to predict the critical mutation rate. First, we can account for the fourth cumulant of the phenotypic distribution to improve our prediction. Second, when selection is much stronger, we can use the classical "House of Cards" (HC) approximation. In this regime, the effects of non-lethal mutations is so strong that they can be viewed as effectively lethal in the WSSM regime (compare equations (11) and (16)).

The analysis of the long-term equilibrium of the system is key to discuss the feasibility of lethal mutagenesis. In principle, viral eradication is expected to occur when $U > U_c$. But the time needed to eradicate the virus population could be longer than the duration of the treatment. Hence, in practice, viral recrudescence may occur after the end of a treatment with a strong mutagen (**Figure 5**). Besides, we also showed scenarios where the virus population can go extinct even when $U < U_c$ (**Figure 6**). In other words, the condition $U > U_c$ is neither necessary nor sufficient to eradicate the virus. To increase our ability to predict the viability of the virus population we need to understand the transient within-host dynamics of the virus.

Transient within-host dynamics: We studied the transient within-host dynamics of the virus in the WSSM regime where the phenotypic distribution remains Gaussian during the adaptation of the virus population. When selection is stronger, however, the distribution can

move away from the Gaussian distribution (equations (36)-(39)) [37, 46–48]. The transient build up of Skewness increases the variance in fitness and tends to speed up the rate of adaptation (**Figure S1**). The analysis of the transient dynamics under the HC regime (when selection is very strong) remains to be carried out.

This framework allowed us to explore the effect of a mutagenic drug on viral dynamics. The drug is expected to affect the epidemiological dynamics (both the density of infected and uninfected cells) and the evolutionary potential of the virus. We showed that if the treatment is stopped before the eradication of the virus, the infection could rebound and reach very high viral loads (even if the drug is an effective mutagen and $U > U_c$). The risk of this viral rebound, however, is limited in hosts that are able to mount an effective immune response (see **Figure S5**)). Yet, the risk of rebound could be high for immunodepressed hosts and chronic infections. Hence, it is particularly important to monitor the patients treated with a mutagen after the end of the treatment.

When we explored the robustness of our predictions in a finite population of host cells we found that the virus could go extinct even when $U < U_c$. These extinctions occur when the viral population is initially far away from its fitness optimum. The lower the initial level of adaptation, the more likely the virus population may be driven to extinction by the drug. This could occur if a mutagenic treatment is combined with a direct reduction in fitness by another drug. This effect is particularly strong when the fraction f of lethal mutations is high because the evolutionary potential is limited in this case (**Figure 6**).

Concluding remarks: In our model, mutations can only affect the within-host transmission rate of the virus. However, other life-history traits could be involved in viral fitness and it is necessary to describe the joint distribution of mutation effects on these different life-history traits. For instance, we could study the joint evolution of transmission β and virulence α. Interestingly, the effect of transmission on fitness is scaled by the density of infected cells, but not virulence (see equation (2)). We may thus expect epidemiological feedbacks to have different effects on the evolution of transmission and virulence [39, 49]. Crucially, the optimum phenotype may differ when selection acts on transmission or on virulence which may lead to a trade-off between the two traits (i.e. impossible to maximize transmission and minimize virulence). It will be interesting to test the robustness lethal mutagenesis when multiple life-history traits are jointly evolving, but we leave this for future studies.

Our model focuses on within-host dynamics but if a patient treated with a mutagenic drug does not fully clear the infection, drug-mutated viruses can be transmitted to a new hosts. For example, the use of molnupiravir, an antiviral mutagen used against SARS-CoV-2 in some countries is associated with the rise of specific G-to-A mutations in the virus in these countries [50]. In principle, some of these mutations may affect the between-host fitness of the virus (e.g. higher between-host transmission, ability to evade host immunity and infect

vaccinated hosts). These mutations may not necessarily affect within-host dynamics but they could have dramatic consequences on the emergence of new variants. Hence, a more comprehensive measure of evolutionary safety of lethal mutagenesis therapy should also consider the cumulated number of mutations that may be transmitted to new hosts [51]. An in depth analysis of the within-host and between-host consequences of mutagenic drugs remains to be explored.

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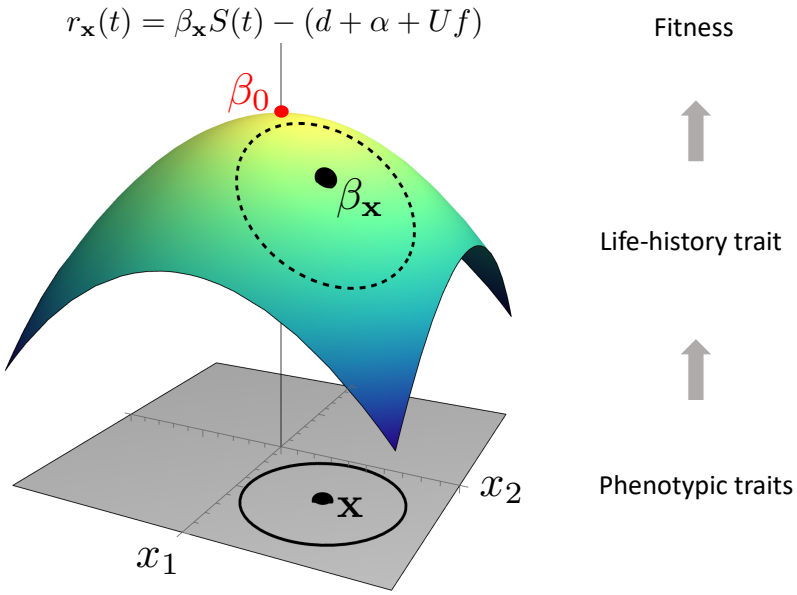

Figure 1: The fitness landscape links the underlying phenotypic traits with life-history traits and malthusian fitness. We represent our phenotype to life-history trait landscape in two dimensions ($n = 2$). A phenotype $\mathbf{x}$ is translated to a transmission rate $\beta_{\mathbf{x}}$ using equation (3). This life-history trait is translated to a fitness value with equation (2), which depends on time through the number of susceptible cells $S(t)$. A black circle is shown around phenotype $\mathbf{x}$, which is translated to a dashed red circle of transmission rates, showing how the FGM distorts the distributions.

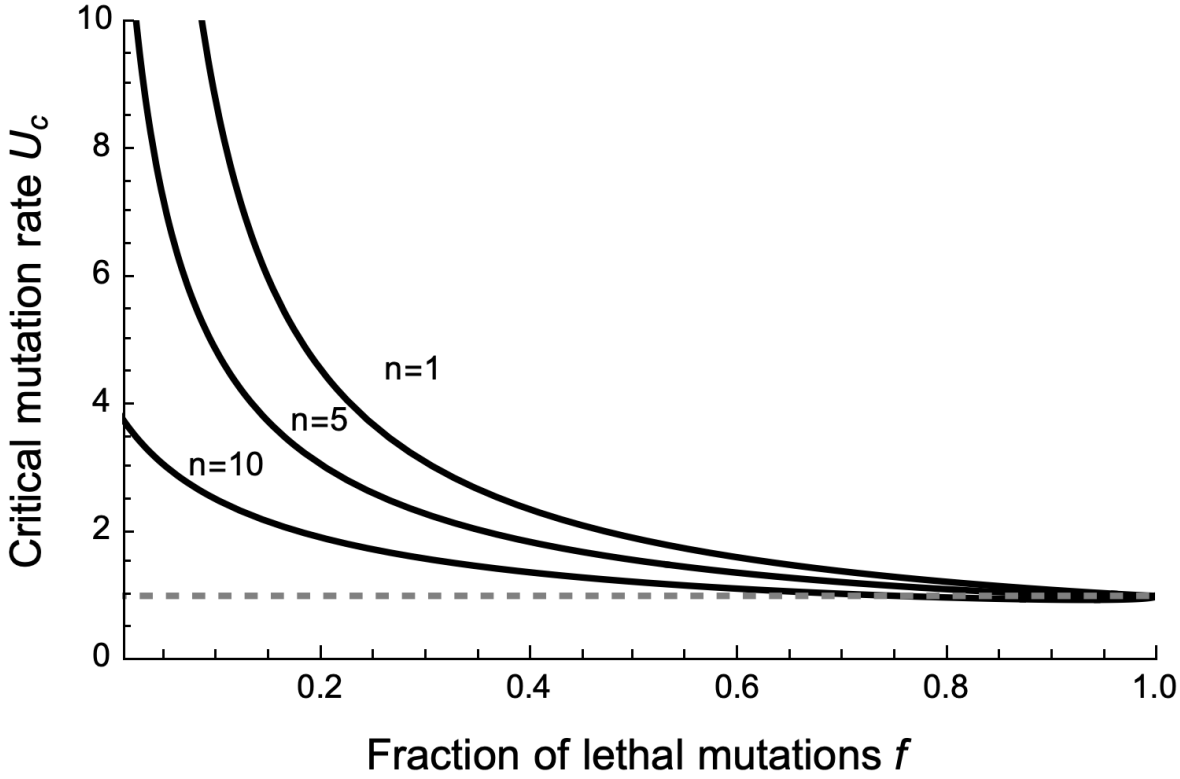

Figure 2: Effect of the proportion of lethal mutations and the phenotypic dimension of the fitness landscape on the critical mutation rate. The plot shows the effect of both fraction of lethal mutations f and number of dimensions n on the critical mutation rate required for lethal mutagenesis. The solid lines correspond to the critical mutation rate computed for the WSSM regime. In comparison, the dashed grey line shows the critical mutation rate predicted by the House of Cards (HC) regime, which does not vary with f. Parameters used: $b = 2, d = 1, \beta_0 = 2, \alpha = 2, s_\beta = 1, \lambda = 0.005$.

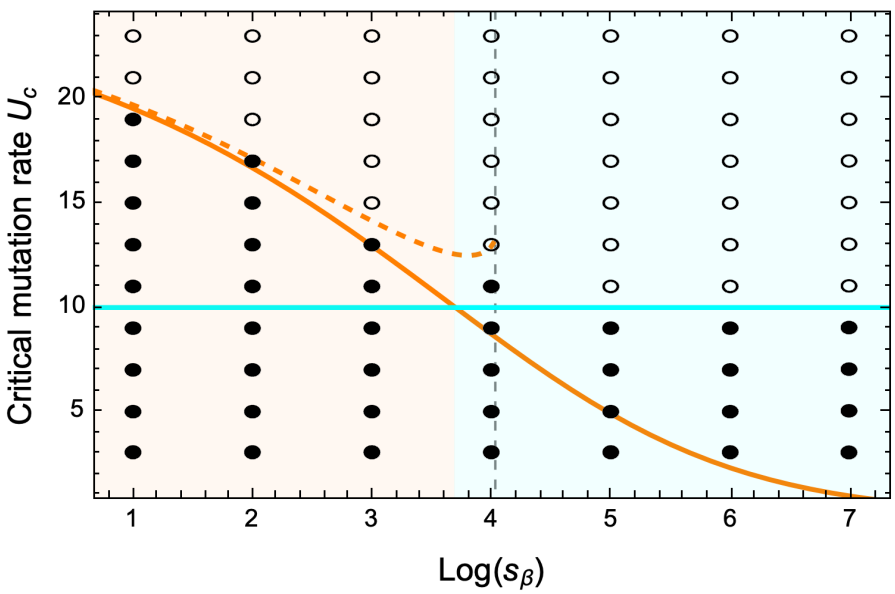

Figure 3: Lethal mutagenesis is feasible for lower mutation rates with steeper fitness landscapes. The critical mutation rate U_c is shown as a function of the selection parameter s_β. The results are shown under three approximation: the WSSM approximation (orange), the approximation accounting for K_3 and K_4 cumulants (dashed orange), the House of Cards (HC) approximation (cyan). Data from numerical simulations are shown as filled circles when the infected population survives, and empty circles when the infected population goes to extinction. The shaded orange and cyan areas indicate the validity conditions of the WSSM and the House of Cards regimes, respectively (see equation (15)). The vertical dashed line shows the maximum value of s_β for which the WSSM+cumulant approach is valid. Parameters used: $b = 3, d = 1, \beta_0 = 4, \alpha = 1, f = 0.4, \lambda = 0.05, n = 2$. For the stochastic simulations, we used $\tau = 0.05, t_{max} = 50$

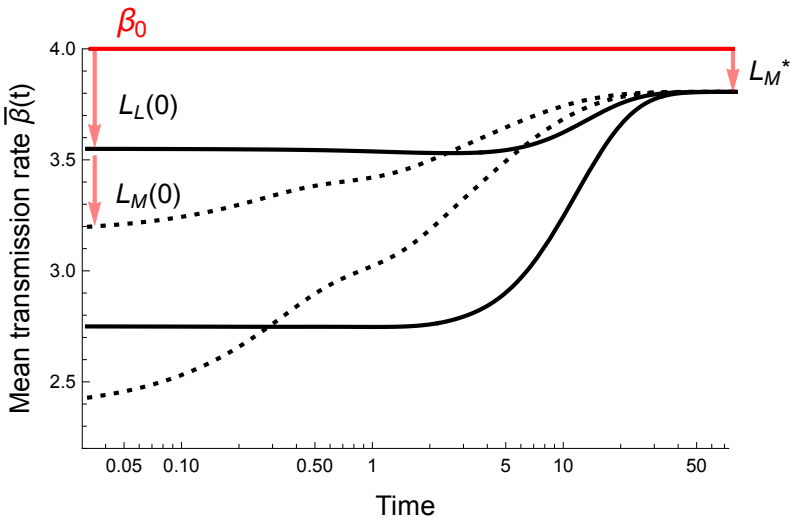

Figure 4: The transient evolutionary dynamics depends on the distance to the optimum and the initial phenotypic variance. We show the dynamics of mean transmission rate $\overline{\beta}(t)$ through time starting from a clonal populations at different initial values of $\overline{x}$. Represented in solid black the infected population is initially clonal with $V_x(0) = 0$ while in dashed black the population is initially diverse with $V_x(0) = 0.15$. We show two staring values for the mean phenotypic trait: $\overline{x}(0) = 0.43$ and $\overline{x}(0) = 0.75$. L_M^* represents the mutation load at equilibrium, which is the difference between β_0 and the equilibrium mean transmission rate $\overline{\beta}^\star$. $L_L(0)$ and $L_M(0)$ respectively show the initial lag load and mutation load for the scenario $\overline{x}(0) = 0.3$. The parameters were $b = 4, d = 1, \beta_0 = 4, \alpha = 2, s_\beta = 1, U = 2, f = 0.4, \lambda = 0.005, n = 5$. Initial conditions were $I(0) = 0.1, S(0) = b/d$

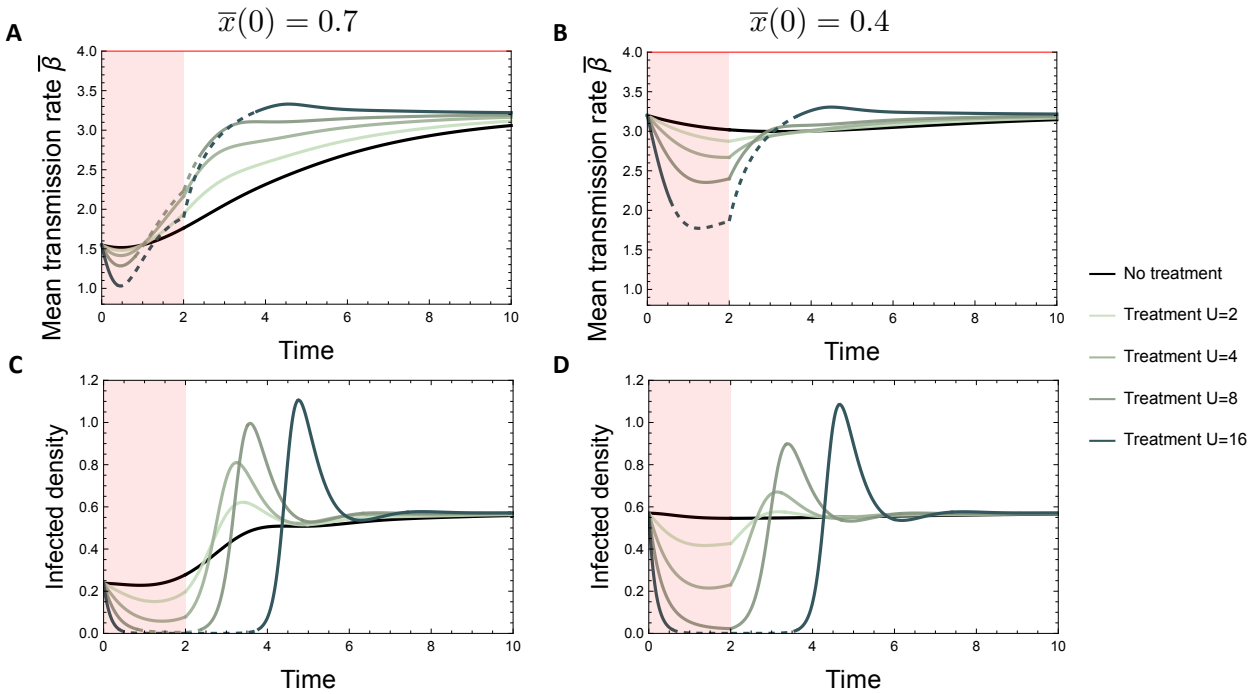

Figure 5: Effect of a mutagenic treatment on chronic infections. The mean transmission rate (A,B) and infected density (C,D) are shown through time depending on an initial distance to the optimum of the pathogen of $\overline{x}(0) = 0.7$ (A,C) or $\overline{x}(0) = 0.4$ (B,D) infection, for the WSSM+cumulant model. Simulations are initialized with clones (null variance) and the densities of susceptible and infected cells are initialized with the equilibrium values obtained with $U = 1$. During the treatment (red shaded window), the mutation rate is increased from 1 to the value of U specified on the right of the plots. The computed deterministic critical mutation rate is $U_c = 10.4$. The treatment stops at time $t = 3$ where the mutation rate goes back to 1. When the Infected density drops below a value of $\epsilon = 0.02$, the corresponding section of the Infected density and mean transmission rate curves become dashed. The horizontal red line in (A,B) shows the maximum transmission rate β_0. The parameters used were: $b = 3, d = 1, \beta_0 = 4, \alpha = 2, f = 0.4, \lambda = 0.05, n = 5$.

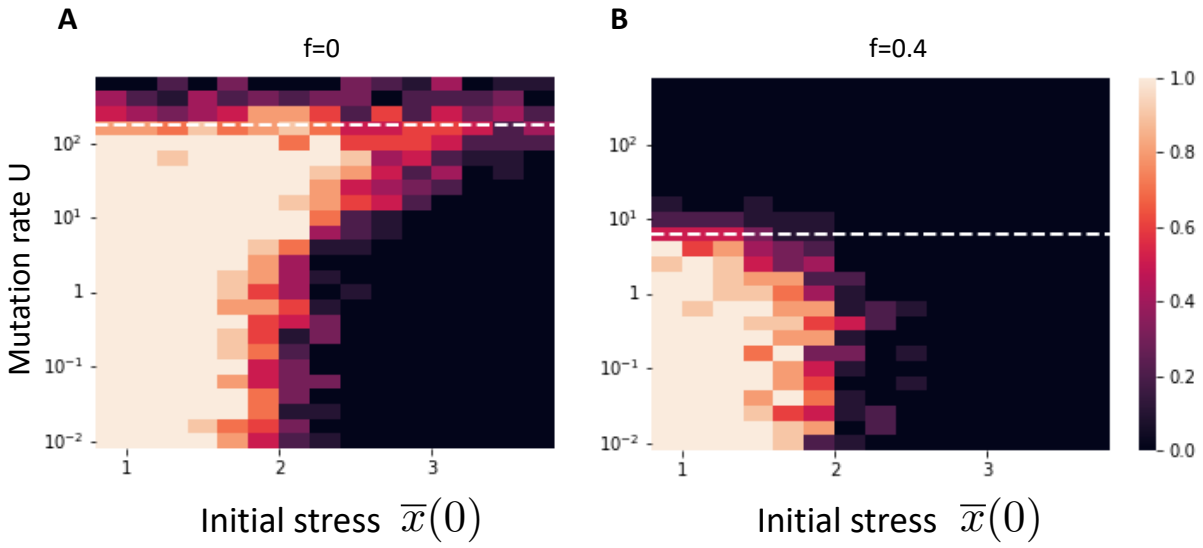

Figure 6: Probability of evolutionary rescue with (A) and without (B) lethal mutations. The simulations are initialized with the equilibrium values of S,I and variance σ for a mutation rate of $U = 10^{-2}$. The color scale represent the proportion of simulations in which the infected populations survived. There are 10 simulations per parameter combination. The horizontal dashed white line represents the value of critical mutation rate U_c above which the infected population goes to extinction in the deterministic model. The parameters used were: $b = 2, d = 1, \beta_0 = 4, \alpha = 4, \lambda = 0.05, n = 2, s_\beta = 0.5, \mathcal{A} = 1000, \tau = 0.05, t_{max} = 50$.

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