Suppression of Beneficial Mutations in Dynamic Microbial Populations

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Quantitative predictions for the spread of mutations in bacterial populations are essential to interpret evolution experiments and to improve the stability of synthetic gene circuits. We derive analytical expressions for the suppression factor for beneficial mutations in populations that undergo periodic dilutions, covering arbitrary population sizes, dilution factors, and growth advantages in a single stochastic model. We find that the suppression factor grows with the dilution factor and depends nontrivially on the growth advantage, resulting in the preferential elimination of mutations with certain growth advantages. We confirm our results by extensive numerical simulations.

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The fixation of random mutations is the driving force of evolutionary adaptation. Mutations can also be problematic, e.g., in synthetic biology, where long-term stability is required to reliably and safely translate more than a decade of circuit design to *in vivo* or industrial settings, but disabling a synthetic gene network is often beneficial to the cell [1]. Maintaining a bacterial strain over long times in a lab setting to study its evolution [2,3] requires enforcing certain population dynamics. To be able to interpret the results and build quantitative models, it is therefore essential to understand how these dynamics themselves alter the impact of beneficial mutations.

We consider the most widely used protocol, serial passage [3], which is characterized by phases of exponential growth alternating with strong reductions in population size ("bottlenecks"). A constant population size maintained, e.g., in a turbidostat [4] or in microfluidic traps [5] serves as the reference scenario for which theoretical results are well known [6–9]. These established results were recently extended to include populations that vary in size [10,11], transmission phases [12], or clonal interference [13]. While repeated pruning of an exponentially growing population was considered before [14–17], closed-form predictions currently only exist for certain limiting cases and, as we show below, their range of applicability is even more limited than previously thought. Therefore, a complete and consistent picture of the fixation process during serial passage is still lacking. Using two complementary approaches for a single evolutionary model, we derive closed-form analytical expressions which provide a quantitative characterization of mutant fixation for arbitrary dilution factors, population sizes, and selective advantages that agrees with direct numerical simulations.

We use a stochastic model of division by binary fission:

$$X \xrightarrow{\alpha(1-\mu)} 2X; \qquad X \xrightarrow{\alpha\mu} X + Y; \qquad Y \xrightarrow{(1+s)\alpha} 2Y. \tag{1}$$

X and Y represent wild-type and mutant cells, respectively. To obtain analytical results, memoryless reactions are assumed, resulting in exponentially distributed division times. However, we will later extend some of our results to more realistic distributions. In Eq. (1), α is the wild-type division rate, $\mu \leq 1$ is the mutation probability upon division, and $s \geq 0$ is the growth rate change of the mutant. For a *constant* population with N_c individuals, a random individual is removed after each division (Moran process). In *dynamic* populations, cells divide freely for some time T, then the population is pruned ("diluted") to a fixed number of survivors N_s and the cycle repeats. The latter resembles subculturing in fresh growth medium in the serial passage protocol. The survival probability is assumed to be equal for all cells. On average, the population size before dilution is fN_s, where $f = \exp(\alpha T)$ is the dilution factor. We use $N_c = N_s(f-1)/\log f$, rounded to the nearest integer, to achieve approximately the same time-averaged population size in both cases (for $\mu = 0$).

Typical trajectories of the model are depicted in Figs. 1(a) and 1(b). The fixation time τ is defined as the time until the population consists of only mutants. We numerically computed the average fixation times τ_c and τ_d for a constant population and the dynamic protocol, respectively [see Fig. 1(c)]. Figure 1(d) shows that $\tau_d > \tau_c$ across all s, meaning that the dynamic population can withstand the evolutionary pressure of beneficial mutations longer.

Below, we will calculate the fixation probability p of a single mutation under the influence of the above population dynamics. If μ is sufficiently small, there is a direct correspondence between τ and p: Based on the idea of the slow-scale stochastic simulation algorithm [19], the mutation rate can be approximated by $\alpha\mu\overline{n_X}$ [20], where $\overline{n_X}$ is the time-averaged number of wild-type cells for $\mu = 0$. Since only a fraction p of mutations becomes fixed

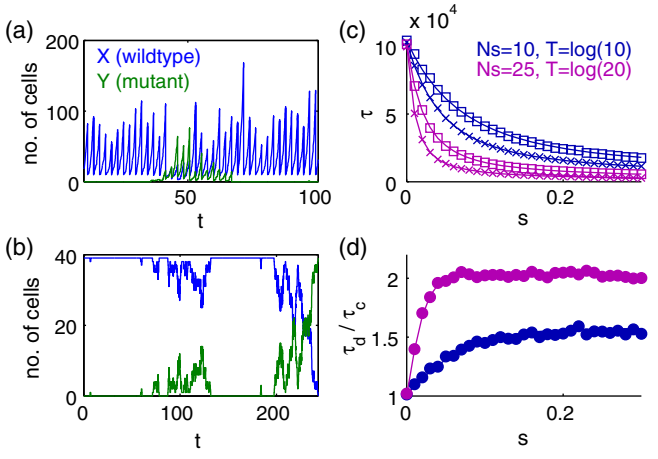

FIG. 1. Typical trajectories of the model Eq. (1) for (a) a dynamic population undergoing repeated pruning and (b) a constant population. Parameters are $\alpha = 1$, $N_s = 10$, $T = \log 10$, $\mu = 10^{-3}$, resulting in $f = 10$, $N_c = 39$. (c) Fixation times in constant (crosses) and dynamic (squares) populations from 10 000 stochastic simulations using an accelerated algorithm [18]. (d) Fixation time ratio. Solid lines in (c) and (d) indicate exact numerical values from Markov models for a constant population and a population pruned when reaching a fixed size fN_s.

eventually, the average fixation time is $\tau = (\alpha\mu\overline{n_X}p)^{-1}$, implying $\tau_d/\tau_c = p_c/p_d$.

We will first use a diffusion approach to characterize p for s close to zero and then employ a recently developed branching process approach for larger s.

Diffusion approximation.—This approach was initially developed by Kimura [9] and is valid for weakly beneficial mutations when the fixation process is dominated by genetic drift. In contrast to Wahl and co-workers [14,15], we will consider all contributions to stochastic fluctuations, including cell division, and model random selection upon dilution with the exact hypergeometric distribution. Let $M_{\delta y}(y)$ and $V_{\delta y}(y)$ be the mean and variance, respectively, of the change of the fraction of mutants from the current generation to the next, given that the current fraction of mutants is $y = n_Y/(n_X + n_Y)$. Then, with the definition $G(y) = \exp[-2\int_0^y M_{\delta y}(y')/V_{\delta y}(y')dy']$, the probability of fixation $u(y)$ is given by $u(y) = \int_0^y G(y')dy' / \int_0^1 G(y')dy'$. Let Λ be the Taylor expansion of $M_{\delta y}(y)/V_{\delta y}(y)$ near $s = 0$ up to the order of s chosen such that Λ is independent of y. Then, the fixation probability for an initial fraction of mutants y is

$$u(y) = \frac{1 - \exp(-2\Lambda y)}{1 - \exp(-2\Lambda)}. \tag{2}$$

For dynamic populations, we define y as the fraction of mutants at the beginning of each cycle. Therefore, $M_{\delta y}(y)/V_{\delta y}(y)$ describes the effect of one growth cycle and subsequent pruning. Mutations occur at any time during the growth phase, implying that the initial fraction of mutants y for Eq. (2) (i.e., at the beginning of the cycle following the mutation's introduction) is a random variable. We accommodate this by approximating $p_d \approx u_d(\bar{y}_d)$, where $\bar{y}_d$ is the average initial fraction of mutants. Hence, estimating p_d amounts to calculating Λ_d and $\bar{y}_d$.

To obtain $M_{\delta y}(y)/V_{\delta y}(y)$ for dynamic populations and subsequently Λ_d, we note that, for the growth phase, the wild-type and mutant subpopulations are described by simple birth processes which start with $(1 - y_0)N_s$ and y_0N_s individuals, respectively. At the end of a cycle, $t = T$, the mean and variance for a population starting with N_0 individuals and a division rate λ are

$$M_\lambda(N_0) = N_0 \exp(\lambda T), \tag{3a}$$

$$V_\lambda(N_0) = \xi^2 N_0 \exp(\lambda T)[\exp(\lambda T) - 1]. \tag{3b}$$

ξ^2 will allow us later to scale the stochastic fluctuations during growth, but we will initially evaluate only the case $\xi^2 = 1$, which corresponds to the model Eq. (1).

As dilution does not, on average, alter the fraction of mutants, Eq. (3) can be used directly to obtain $M_{\delta y}$, whereas for $V_{\delta y}$, Eq. (3) is combined with the variance of the hypergeometric distribution for the dilution event [20]. A first-order expansion of $M_{\delta y}/V_{\delta y}$ around $s = 0$ then leads to $\Lambda_d \approx 2s(N_s - \xi^2)f \log f/[(f-1)(1+\xi^2)]$. To estimate $\bar{y}_d$, we consider a mutant subpopulation that first appears at time θ into a cycle and initially consists of m_s individuals. For the model Eq. (1), $m_s = 1$, since the second reaction produces a single mutant cell. By the end of the initial cycle, the mutant subpopulation will have grown for a time $T - \theta$ to a size larger than m_s. As might be intuitive (and can be shown explicitly [20]), the probability distribution of θ is $p_{\text{mut}}(\theta) = \exp(\alpha\theta)/\int_0^T \exp(\alpha\theta')d\theta'$, i.e., proportional to the average rate of division events at a given time θ within a cycle. Using $p_{\text{mut}}(\theta)$, we obtain the average sizes of the wild-type and mutant subpopulations for random θ as weighted averages of Eq. (3b) and estimate the average initial fraction of mutants as $\lim_{N_s\to\infty} N_s\bar{y}_d = [m_s(f^s - 1)/s(f-1)]$, independent of ξ^2 [20].

Substituting $\bar{y}_d$ and Λ_d into Eq. (2), we obtain

$$p_d = \frac{1 - \exp\left(-\frac{2m_s}{1+\xi^2}\frac{(N_s-\xi^2)f\log f\,(f^s-1)}{N_s(f-1)^2}\right)}{1 - \exp\left(-\frac{2}{1+\xi^2}\frac{(N_s-\xi^2)f\log f}{f-1}s\right)}. \tag{4}$$

Equation (4) can also be used for the constant population case by replacing $N_s \to N_c$ and taking the limit $f \to 1$, so it reduces to

$$p_c = \frac{1 - \exp[-2m_s s/(1+\xi^2)]}{1 - \exp[-2N_c s/(1+\xi^2)]}. \tag{5}$$

Note that a more accurate approximation of p_c can be obtained by calculating Λ_c directly [20]. For $\xi^2 = 1$ and

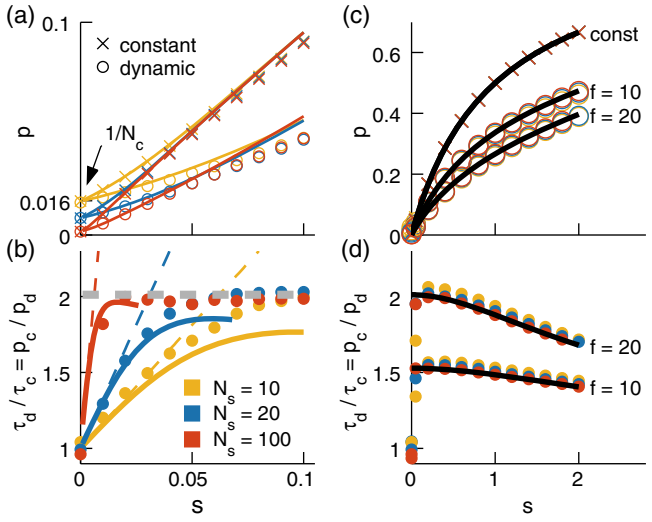

FIG. 2. (a) Fixation probabilities from numerical simulations (symbols) compared to diffusion approximation, Eqs. (5) and (4) (lines). (b) Numerical τ_d/τ_c (symbols) and diffusion approximation (lines). Colored dashed lines show initial slope at $s = 0$ according to Eq. (6); gray dashed line is the asymptotic ratio Δ, Eq. (7). For all data in (a) and (b) $f = 20$, $\xi^2 = 1$, $m_s = 1$. (c),(d) Numerical p and τ_d/τ_c (symbols) compared to branching process approximation, Eqs. (9) and (11) (lines). The y intercept in (d) is also Δ.

$m_s = 1$, the theoretical estimates Eqs. (4) and (5) are plotted in Fig. 2(a). As expected, the theory matches the numerical data towards $s = 0$. The accuracy is remarkable considering it being a continuous approximation of a discrete process in small populations and the usage of a large-N_s approximation for $\bar{y}_d$. A Taylor expansion of $\tau_d/\tau_c = p_c/p_d$ around $s = 0$ yields (for large N_s)

$$\tau_d/\tau_c \approx 1 + sN_s \frac{(f-1)[1-\Delta^{-1}(f)]}{(1+\xi^2)\log f} + \mathcal{O}(s^2), \quad (6)$$

with $\Delta(f) = [(f-1)/\log f]^2/f$. For any $f > 1$, Δ is larger than 1, so the slope of τ_d/τ_c at $s = 0$ is positive. Thus, periodic dilutions do not impact neutral mutations as expected, while beneficial mutations are suppressed by a factor that grows with s in the vicinity of $s = 0$. The slope of τ_d/τ_c increases with N_s, in agreement with Fig. 2(b).

First taking the limit $N_s \to \infty$ and subsequently considering small s, we obtain $p_d \approx [2m_s/(1+\xi^2)]\Delta^{-1}s$ and $p_c \approx [2m_s/(1+\xi^2)]s$ from Eqs. (4) and (5), respectively, which corresponds to the only limit for which analytical results were previously available [15]. The factor by which mutations are suppressed by serial dilutions in this limit is, therefore,

$$\lim_{s\to 0}\lim_{N_s\to\infty}\frac{\tau_d}{\tau_c} = \Delta(f), \quad (7)$$

which is shown as a gray dashed line in Fig. 2(b). For any finite population size, there is a smooth transition of τ_d/τ_c towards Δ with the rate indicated by Eq. (6).

Branching process approximation.—As a complementary approach, we employ the framework developed by Uecker and Hermisson [21]. Based on an inhomogeneous branching processes, they derived the following expression for the fixation probability:

$$p = 2\left[1 + \int_0^\infty (\lambda+\delta)(t)\exp\left(-\int_0^t (\lambda-\delta)(t')dt'\right)dt\right]^{-1}, \quad (8)$$

where $\lambda(t)$ and $\delta(t)$ are the per capita birth and death rates, respectively, of the mutant subpopulation in the "branching limit." It implicitly assumes that stochastic fluctuations of the wild-type population size can be ignored and, thus, we do not expect this approximation to capture finite population size effects present for small s.

For a constant population, we have the per capita birth rate $\lambda_c(t) = (1+s)\alpha$ for the mutant subpopulation. Mutant individuals are replaced by wild-type individuals when a wild-type individual is born with rate αn_X and a mutant is chosen for removal with probability $[n_Y/(n_X+n_Y+1)] \approx (n_Y/n_X)$. The per capita death rate is therefore $\delta_c(t) = \alpha$. Substitution into Eq. (8) yields

$$p_c = \frac{s}{1+s}. \quad (9)$$

For the population undergoing serial dilutions, we assume small time intervals of length $\sigma \ll T$ during which cells die with rate $\sigma^{-1}\log f$, reducing the population size from fN_s to N_s exactly in the branching limit. Assuming the mutation is introduced at time θ into a cycle, these "windows of death" occur at times $t_i = iT - \theta$, $i = 1, 2, \ldots$. Therefore, we have

$$\lambda_d(t) = (1+s)\alpha, \quad (10a)$$

$$\delta_d(t) = \begin{cases} \frac{\log f}{\sigma} & t_i < t < t_i + \sigma,\ i = 1, 2, \ldots \\ 0 & \text{otherwise.} \end{cases} \quad (10b)$$

Substituting these rates into Eq. (8) and taking the limit $\sigma \to 0$ yields the fixation probability $p_d(\theta)$ conditioned on the time of appearance θ [20]. By averaging over $p_{\text{mut}}(\theta)$, we obtain the unconditional fixation probability

$$p_d = \frac{1}{f-1}\left[fF\left(\frac{f-1}{1-f^{-s}}\right) - F\left(\frac{f-1}{f^{1+s}-f}\right)\right], \quad (11)$$

where $F(\cdot)$ is defined using the hypergeometric function ${}_2F_1(a,b;c;z)$ as $F(x) = {}_2F_1(1,1/(1+s);1+1/(1+s);-x)$. Figures 2(c) and 2(d) show a comparison of this theory with numerical simulations. As $s \to 0$, p_d converges to 0, but $\tau_d/\tau_c = p_c/p_d$ approaches the finite value Δ, which is identical to the result derived earlier from the diffusion approach, Eq. (7), and therefore consistent with

the implicit assumption of large populations. In contrast, for finite population sizes, only the diffusion approximation correctly captures the immediate vicinity of $s = 0$, where $\tau_d/\tau_c \to 1$ [compare Figs. 2(b) and 2(d)].

Exponential division time distributions, which have zero mode, are unrealistic, because cells need time to mature before the next division. In reality, the division time distribution has a clear peak with a Fano factor smaller than 1 [22]. According to Eqs. (6) and (7), the initial increase of τ_d/τ_c should be faster for a less stochastic division process (i.e., smaller ξ^2), while the plateau value Δ should not depend on ξ^2.

To test these predictions, we consider an extension of Eq. (1), where the simple memoryless division is replaced by a process with k stages, which has been characterized in detail by Kendall [23]: The total division time d of, e.g., a wild-type cell is distributed according to $2k\alpha d \sim \mathcal{X}^2_{2k}$. After individuals have established an equilibrium distribution across the k different stages, the population grows like $\exp[\alpha k(2^{1/k} - 1)t]$, leading to deterministic growth $\propto 2^{\alpha t}$ as $k \to \infty$. We use an adjusted growth rate of $\alpha[k(2^{1/k} - 1)]^{-1}$ in numerical simulations to maintain an effective population growth according to $\exp(\alpha t)$, resulting in the same average population size for unchanged cycle lengths T. For a population of individuals starting in the first stage, the initial population growth is delayed, reducing the effective initial size of the mutant subpopulation from 1 to $m_s = 1/[2k(1 - 2^{-1/k})]$. The variance of the population size is different from that of a memoryless division process by a factor of $\xi^2 \approx [2(\log 2)^2/k]$.

Figure 3(a) shows numerical simulations for different k, along with the approximation of Eq. (6), substituting the changed value for ξ^2. Note that this neglects the fact that division events in the mutant subpopulation are initially correlated. Nevertheless, there is good quantitative agreement with Eq. (6). Figure 3(b) shows that there are some quantitative differences for larger s, but, according to Fig. 3(d), for small s beyond the initial region of increase for finite population sizes [cf. Fig. 2(b)], τ_d/τ_c is indeed at most weakly dependent on k, as predicted by Eq. (7).

In this study, we have developed a complete analytical characterization of the fixation probability of beneficial mutations in exponentially growing populations with repeated bottlenecks, akin to serial passage. The most intriguing result is that the impact of serial passage on the fixation probability depends nontrivially on the growth rate change s, a novel effect not seen in the previously considered large-population, low-s limit, where all fixation probabilities were found to be proportional to s and, therefore, the ratio p_c/p_d is a constant [15,17]. Therefore, the experimental protocol acts as a filter which biases the distribution of selective advantages of fixed mutations with respect to a constant population or serial passage with different f. Our results provide quantitative

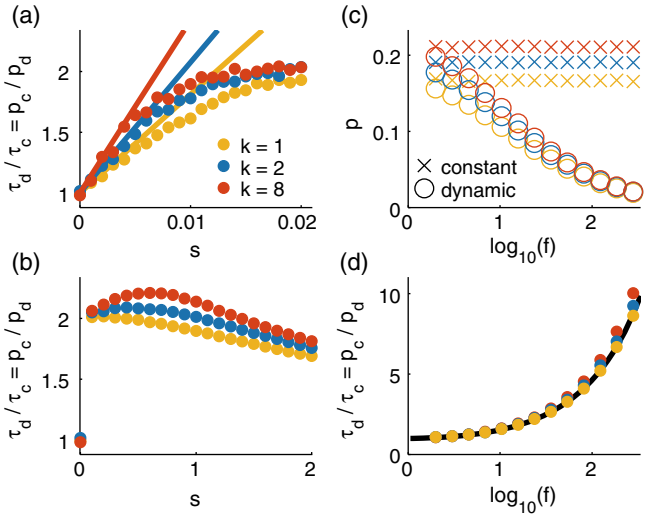

FIG. 3. Fixation probabilities and ratios in the multistage model. (a) τ_d/τ_c for small s for different k in numerical simulations (symbols). Lines indicate the slope predicted by Eq. (6) with $\xi^2 = 2(\log 2)^2/k$. (b) τ_d/τ_c from numerical simulations for larger s. (c) p_c and p_d as functions of the dilution factor f. (d) τ_d/τ_c for the data shown in (c) compared to the analytical approximation, Eq. (7). Parameters are $N_s = 50$, $f = 20$ (a), (b) and $N_s = 20$, $s = 0.2$ (c), (d).

predictions for three distinct regimes: Firstly, starting from $p_c/p_d = 1$ at $s = 0$ (no suppression of neutral mutations), the suppression factor increases gradually (and more quickly for larger N_s) as s increases, which is captured by the diffusion approach, Eqs. (4)–(6). Secondly, in an intermediate regime, p_c/p_d reaches a plateau value Δ, Eq. (7), which only depends on the dilution factor f. Thirdly, towards large s, the fixation probability for the serial passage protocol slowly returns to that for a constant population, as described by the branching process approximation, Eq. (11).

To our knowledge, no previous analytical results existed for the first and third regime. For the plateau, we find Δ to be monotonic with respect to f [cf. Fig. 3(d)] and to approach 1 for $f \to 1$, which is in contrast to the prediction of an optimal dilution factor f from the previously derived formula $\Delta' = f/(\log f)^2$ [15]. However, this earlier result used a binomial distribution for the dilution process, which is only a good approximation for large f [20], and, indeed, in this regime, $\Delta' \approx \Delta$. Our prediction is not only confirmed by full numerical simulations, but also intuitive as frequent but mild dilutions are experimentally indistinguishable from a constant population. Furthermore, as can be shown explicitly [20], it is consistent with Ref. [17].

Equation (6) reaches Δ at a selective advantage $\delta s = \{[(f - 1)(1 + \xi^2)]/[fN_s \log f]\}$, providing an order-of-magnitude estimate for regimes of validity of Eq. (6) ($s \lesssim \delta s$) versus Eq. (11) ($s \gtrsim \delta s$), which is particularly important for very small population sizes, when δs is large. For larger populations, the value of s at which Δ is

attained is negligible compared to the region in which $\tau_d/\tau_c \approx \Delta$ [cf. Fig. 2(b), $N_s = 100$], which indicates equal suppression of mutations conferring arbitrary moderate growth rate changes.

While, in reality, cell division and mutation are far more complex than described by the model Eq. (1), our results establish a baseline that can be used to gauge the influence of other effects. We confirmed that they hold qualitatively for more realistic division statistics and even quantitatively through the proxy parameters ξ^2 and m_s in the low-s regime (cf. Fig. 3). Generalizing the branching process approximation to take these statistics into account for larger s presents an interesting direction of future research. We also found that the exact periodicity of dilutions is not essential, as pruning at a fixed number of cells fN_s leads to almost identical numerical results [20]. Another possible extension is to consider nonexponential growth, although a previous study found little effect for the specific case considered there [15].

Our quantitative analytical results provide a framework for the interpretation of evolution experiments involving serial passage by predicting how the experimental protocol itself can facilitate or suppress the fixation of mutations with certain selective advantages, which is a prerequisite for investigating the relation between population level adaptation and its molecular basis for other than neutral mutations [24]. They may also provide guidance for limiting the impact of undesired mutations in engineered bacteria by adjusting the experimental protocol or employing synthetic ecologies to shape their inherent population dynamics.

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Suppression of beneficial mutations in dynamic microbial populations
Supplemental Information

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Figures and equations in the main text are referenced using a superscript letter "M". For example, Eq. (1) in the main text would be referenced as Eq. $(1)^M$.

I. COARSE-GRAINED MUTATION DYNAMICS

Here, we will give a formal argument to show that, for sufficiently small μ, mutations in the model

$$X \xrightarrow{\alpha(1-\mu)} 2X \tag{1a}$$

$$X \xrightarrow{\alpha\mu} X + Y \tag{1b}$$

$$Y \xrightarrow{(1+s)\alpha} 2Y \tag{1c}$$

occur according to a Poisson process with rate $\alpha\mu\overline{n_X}$, where $\overline{n_X}$ is the time-averaged number of wild-type cells for $\mu = 0$. The argument is based on the separation of time scales between the fast fluctuations in population size and rare mutations, similar to the derivation of the slow-scale stochastic simulation algorithm in Ref. [1]. However, in our case, it is necessary to consider the properties of the population dynamics not described by the reactions of Eq. (1) themselves. For a constant population, the statement above is clearly true since $n_X = \overline{n_X}$ for all times and therefore the rate of Eq. (1b) is $\alpha\mu\overline{n_X}$ by definition. However, as we will show below, the same formula is also a good approximation for the rate of mutations in the periodic dilution scenario if μ is small.

For simplicity, the number of wild-type individuals n_X will be denoted as x in this section.

Assume that there are currently no mutant cells in the population. What is the probability density $p_{\text{next}}(t)$ for the next mutation to occur at some time $t > 0$? If we split up the interval from 0 to t into $M \in \mathbb{N}$ equally spaced intervals $\Delta t = t/M$, the probability of no mutation occuring in the ith subinterval is $1 - \alpha\mu x(i\Delta t)\Delta t$. As the probability for a mutation occuring between t and $t + \mathrm{d}t$ is $\alpha\mu x(t)\,\mathrm{d}t$,

$$p_{\text{next}}(t)\,\mathrm{d}t = \alpha\mu x(t)\,\mathrm{d}t \cdot \lim_{M\to\infty} \prod_{i=1}^{M} \left[1 - \alpha\mu x\left(i\frac{t}{M}\right)\frac{t}{M}\right]. \tag{2}$$

Taking the log of the second factor allows us to convert it to the integral

$$\begin{aligned}
\lim_{M\to\infty} \log \prod_{i=1}^{M} \left[1 - \alpha\mu x\left(i\frac{t}{M}\right)\frac{t}{M}\right] &= \lim_{M\to\infty} \sum_{i=1}^{M} \log\left[1 - \alpha\mu x\left(i\frac{t}{M}\right)\frac{t}{M}\right] \\
&= -\alpha\mu \lim_{M\to\infty} \sum_{i=1}^{M} x\left(i\frac{t}{M}\right)\frac{t}{M} \\
&= -\alpha\mu \int_0^t x(\tau)\,\mathrm{d}\tau.
\end{aligned}$$

Substituting this into Eq. (2) yields

$$p_{\text{next}}(t) = \alpha\mu x(t) \cdot \exp\left(-\alpha\mu \int_0^t x(\tau)\,\mathrm{d}\tau\right) \tag{3}$$

This is the exact probability density for the time of the next mutation. The following

argument holds if the dynamics of x is made up of short time intervals (called "cycles" hereafter according to the model in the main text, but the logic applies more generally) that are all statistically identical, which is the case for the periodic dilution scenario. Then, let Δt_{meso} be some mesoscopic time period, where mesoscopic means that it is large compared to the length of the cycles and small compared to the time scale on which the second (exponential) factor of Eq. (3) changes. Choosing Δt_{meso} is always possible if μ is sufficiently small. This choice implies that x averaged over Δt_{meso} is close to $\overline{n_X}$ and that there are close to $\Delta t_{\text{meso}}/T$ cycles within that time period, where T is the average length of the cycles (in the case of periodic dilutions, the length of all cycles is exactly T). The probability that the next mutation will occur anywhere within $[t, t + \Delta t_{\text{meso}}]$ is

$$\tilde{p}_{\text{next}}(t)\Delta t_{\text{meso}} = \int_t^{t+\Delta t_{\text{meso}}} \alpha\mu x(t') \cdot \exp\left(-\alpha\mu \int_0^{t'} x(\tau)\,\mathrm{d}\tau\right) \mathrm{d}t' \tag{4}$$

$$= \left[-\exp\left(-\alpha\mu \int_0^{t'} x(\tau)\,\mathrm{d}\tau\right)\right]_t^{t+\Delta t_{\text{meso}}} \tag{5}$$

$$= \exp\left(-\alpha\mu \int_0^{t} x(\tau)\,\mathrm{d}\tau\right)\left[1 - \exp\left(-\alpha\mu \int_t^{t+\Delta t_{\text{meso}}} x(\tau)\,\mathrm{d}\tau\right)\right] \tag{6}$$

$$\approx \alpha\mu\overline{n_X} \exp\left(-\alpha\mu \int_0^{t} x(\tau)\,\mathrm{d}\tau\right)\Delta t_{\text{meso}}, \tag{7}$$

where the last step was possible due to the mesoscopic scale of Δt_{meso}. Because $\alpha\mu$ is assumed to be sufficiently small, the integral will already be close to $\overline{n_X}t$ when the argument to the exponential deviates substantially from zero. Replacing this term yields:

$$\tilde{p}_{\text{next}}(t)\Delta t_{\text{meso}} = \alpha\mu\overline{n_X} \exp(-\alpha\mu\overline{n_X}t)\Delta t_{\text{meso}}. \tag{8}$$

This confirms the intuitive fact that, on a coarse-grained time scale (where the fluctuations of the population size are averaged out), mutations occur according to a Poisson process with rate $\alpha\mu\overline{n_X}$ and, therefore, the average period of mutations is $T_{\text{mut}} = (\alpha\mu\overline{n_X})^{-1}$.

After a mutation has occurred, the population consists of both Xs and Ys, but only for a transitional homogenization period τ_{hom}, until the mutation has either been fixed or eliminated. For each single mutation τ_{hom} will depend on the state the population was in when the mutation was introduced. However, if the typical τ_{hom} is much smaller than the typical period τ_{mut} at which mutations occur (i.e. for small enough μ), then the transient coexistence of wild-type and mutant individuals will not significantly alter the statistics and the fixation time is

$$\tau = \frac{\tau_{\text{mut}}}{p} + \tau_{\text{hom}}^{+} \approx \frac{\tau_{\text{mut}}}{p} = (\alpha\mu\overline{n_X}p)^{-1}, \tag{9}$$

where p is the probability that a mutation occurring at unknown time and population state will eventually become fixed. τ_{hom}^{+} is the homogenization time conditioned on the fixation of the mutation. However, the contribution to τ is small if τ_{mut} is large and hence we ignore it after the first step in Eq. (9).

II. PROBABILITY OF MUTATION WITHIN A CYCLE

In general, the fixation probability of a mutation can depend on the time it is introduced and the state of the population at that time. To calculate the unconditional fixation probability p for an arbitrary mutation, it is therefore necessary to know the probability distribution of the introduction of a mutation across different times and states.

Assume that no mutation has occurred up to time t. As shown above, for sufficiently small μ, there exists a time scale Δt_{meso} spanning several cycles, on which the exponential factor in Eq. (3) does not change significantly. Therefore, the probability distribution for the time to the next mutation (i.e. when reaction (1b) fires next) is proportional to $\alpha\mu x(t)$ for several cycles, without the dampening factor. Because $T < \Delta t_{\text{meso}}$, the same is certainly true for the next complete cycle. In addition, all the cycles are assumed to be statistically identical. Therefore, the timing of the next mutation within the cycle in which it occurs does not depend on t (the time before which no mutation has occurred). This means that there is a universal probability $p_{\text{mut}}(x^*, \theta)$ that the next mutation happens at time θ into a cycle (in our case, the time since the last dilution event), when there are x^* wild-type individuals. Since $p_{\text{mut}}(x^*, \theta) \propto \alpha\mu x^*$, the probability is given by

$$p_{\text{mut}}(x^*, \theta)\,\mathrm{d}\theta = \frac{p_{\text{x}}(x^*, \theta) \cdot x^*\,\mathrm{d}\theta}{\int_0^\infty \sum_{k=0}^\infty p_{\text{x}}(k, \theta') \cdot k\,\mathrm{d}\theta'}, \tag{10}$$

where $p_x(x^*, \theta)$ is the probability that the population has size x^* at time θ into the cycle. For our specific case in the main text, only $p_{\text{mut}}(\theta)$ is required, i.e. the probability that the next mutation occurs at time θ after the last dilution event. We obtain this probability by summing over all possible x^*:

$$p_{\text{mut}}(\theta) = \sum_{k=0}^\infty p_{\text{mut}}(x^*, \theta) = \frac{\sum_{k=0}^\infty p_{\text{x}}(k, \theta) \cdot k\,\mathrm{d}\theta}{\int_0^\infty \sum_{k=0}^\infty p_{\text{x}}(k, \theta') \cdot k\,\mathrm{d}\theta'} = \frac{m_1(N_s; \alpha; \theta)}{\int_0^\infty m_1(N_s; \alpha; \theta')\,\mathrm{d}\theta'}, \tag{11}$$

where m_1 is the first moment of the population size. Since every cycle starts with N_s individuals which are constantly dividing with rate α, the first moment is simply $m_1(N_s; \alpha; \theta) = N_s \exp(\alpha t)$, leading to

$$p_{\text{mut}}(\theta) = \frac{\exp(\alpha\theta)}{\int_0^T \exp(\alpha\theta')\,\mathrm{d}\theta'}. \tag{12}$$

III. DIFFUSION APPROXIMATION FOR CONSTANT POPULATION

Although the fixation probability for a constant population was successfully derived in Eq. $(5)^M$ as a limit of the dynamic case, a more accurate approximation—without taking into account the effect of ξ^2—can be obtained directly by treating each division event as a "generation" in the context of Kimura's diffusion theory:
The probability that a non-mutant will divide next is $N_c(1-y)\alpha/[N_c(1-y)\alpha + N_c y\alpha(1+s)] = (1-y)/[(1-y)+y(1+s)]$, where y is the fraction of mutants in the population. Similarly,

the probability of a mutant dividing next is $y(1+s)/[(1-y)+y(1+s)]$. For the number of mutants in the population to change, either a mutant has to divide and then a non-mutant is chosen to be removed, or vice versa. The probability for the removal of a non-mutant after a mutant has divided is $(1-y)/(1+1/N_c)$, whereas the probability for the removal of a mutant after a non-mutant has divided is $y/(1+1/N_c)$. Therefore, the mean change in one cell division is:

$$M_{\delta y,c}(y) = \frac{1}{N_c}\left[\frac{y(1+s)}{1-y+y(1+s)} \cdot \frac{1-y}{1+1/N_c} - \frac{1-y}{1-y+y(1+s)} \cdot \frac{y}{1+1/N_c}\right] \tag{13}$$

$$= \frac{sy(1-y)}{(1+sy)(N_c+1)} \tag{14}$$

Similarly, the second moment is $\frac{(s+2)y(1-y)}{N_c(1+sy)(N_c+1)}$ and therefore, the variance evaluates to:

$$V_{\delta y,c}(y) = \frac{(s+2)y(1-y)}{N_c(1+sy)(N_c+1)} - \left(\frac{sy(1-y)}{(1+sy)(N_c+1)}\right)^2 \tag{15}$$

$$= \frac{(s+2)y(1-y)(1+sy)(N_c+1) - N_c s^2 y^2 (1-y)^2}{N_c(1+sy)^2(N_c+1)^2}. \tag{16}$$

Dividing the two equations yields

$$\frac{M_{\delta y,c}(y)}{V_{\delta y,c}(y)} = \frac{sN_c(1+sy)(N_c+1)}{(s+2)(1+sy)(N_c+1) - N_c s^2 y(1-y)}. \tag{17}$$

Expanding about $s=0$ shows that to second order, $\frac{M_{\delta y,c}(y)}{V_{\delta y,c}(y)}$ is independent of y:

$$\frac{M_{\delta y,c}(y)}{V_{\delta y,c}(y)} = \frac{N_c}{4}s(2-s) + \mathcal{O}(s^3) \tag{18}$$

We can therefore use $\Lambda_c = N_c s(2-s)/4$ in Eq. $(2)^M$ to calculate $u_c(y)$. In this case, every mutation starts with a single mutant cell in a population of N_c+1 cells. To be fixed, the mutation has to survive the removal step of the Moran process and then become fixed starting from a frequency $1/N_c$, finally leading to

$$p_c = \frac{N_c}{N_c+1}u_c(1/N_c) = \frac{N_c}{N_c+1} \cdot \frac{1-\exp(-\frac{1}{2}s(2-s))}{1-\exp(-\frac{N_c}{2}s(2-s))}. \tag{19}$$

We can read off Eq. (19) that $\bar{y}_c = 1/(N_c+1)$ if we wanted to use $p_c \approx u_c(\bar{y}_c)$ in analogy with the dynamic case. Note that Eq. $(5)^M$ (for $\xi^2 = 1$) is just a less accurate version of Eq. (19), which assumes $N_c/(N_c+1) \approx 1$ and uses only a first-order approximation for Λ_c. The two formulas converge for large N_c and small s, and therefore, Eq. (19) would still lead to the same result for the asymptotic fixation time ratio Δ in Eq. $(7)^M$.

IV. DIFFUSION APPROXIMATION FOR PERIODIC DILUTION: Λ_d

To calculate Λ_d, we note that the first and second moment a population of cells that starts out with N_0 cells and then grows by dividing with a rate λ are given by

$$m_1(N_0,\lambda,t) = N_0\exp(\lambda t) \tag{20a}$$

$$m_2(N_0,\lambda,t) = N_0\exp(\lambda t)[(\xi^2+N_0)\exp(\lambda t) - \xi^2] \tag{20b}$$

for $\xi^2 = 1$. These moments can be evaluated at $t = T$ to obtain the mean and variance contributed by the growth cycle for each sub-population, i.e. non-mutants and mutants, which start out with $(1-y)N_s$ and yN_s cells. In general, for a population starting with N_0 individuals and dividing with rate λ, the mean $M_\lambda(N_0)$ and the variance $V_\lambda(N_0)$ evaluate to:

$$M_\lambda(N_0) = N_0 \exp(\lambda T) \tag{21a}$$

$$\begin{aligned} V_\lambda(N_0) &= N_0 \exp(\lambda T)[(\xi^2 + N_0)\exp(\lambda T) - \xi^2] - N_0^2 \exp^2(\lambda T) \\ &= \xi^2 N_0 \exp(\lambda T)[\exp(\lambda T) - 1] \end{aligned} \tag{21b}$$

Note that ξ^2 in Eq. (20b) effectively scales the variance here. Assume the fraction of mutants at the beginning of a cycle is y. What we are first interested in are the mean and variance of the fraction of mutants at the end of the growth phase as a function of y. Since $y = n_Y/(n_X + n_Y)$, we use second and first-order approximations for the mean and variance of a ratio, respectively:

$$\left\langle \frac{a}{b} \right\rangle \approx \frac{\langle a \rangle}{\langle b \rangle} - \frac{\mathrm{cov}(a,b)}{\langle b \rangle^2} + \frac{\mathrm{var}(b)\langle a \rangle}{\langle b \rangle^3} \tag{22a}$$

$$\mathrm{var}\left(\frac{a}{b}\right) \approx \frac{\langle a \rangle^2}{\langle b \rangle^2}\left[\frac{\mathrm{var}(a)}{\langle a \rangle^2} - 2\frac{\mathrm{cov}(a,b)}{\langle a \rangle \langle b \rangle} + \frac{\mathrm{var}(b)}{\langle b \rangle^2}\right] \tag{22b}$$

In our case, $a = n_Y$ and $b = n_X + n_Y$, where n_X and n_Y are uncorrelated and so $\mathrm{cov}(a,b) = \mathrm{var}(n_Y)$. Therefore, all means and variances necessary for the above approximation can be calculated exactly from Eq. (21), giving approximations for the mean fraction of mutants $M_{\mathrm{end}}(y)$ at the end of the growth cycle and its variance $V_{\mathrm{end}}(y)$ introduced by the growth process:

$$\begin{aligned} M_{\mathrm{end}}(y) = {} & \frac{M_{\alpha(1+s)}(yN_s)}{M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s)} - \frac{V_{\alpha(1+s)}(yN_s)}{[M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s)]^2} \\ & + \frac{[V_\alpha((1-y)N_s) + V_{\alpha(1+s)}(yN_s)]M_{\alpha(1+s)}(yN_s)}{[M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s)]^3} \\ V_{\mathrm{end}}(y) = {} & \left(\frac{M_{\alpha(1+s)}(yN_s)}{M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s)}\right)^2 \cdot \\ & \left[\frac{V_{\alpha(1+s)}(yN_s)}{[M_{\alpha(1+s)}(yN_s)]^2} - 2\frac{V_{\alpha(1+s)}(yN_s)}{[M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s)] \cdot M_{\alpha(1+s)}(yN_s)}\right. \\ & \left. + \frac{V_\alpha((1-y)N_s) + V_{\alpha(1+s)}(yN_s)}{[M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s)]^2}\right] \end{aligned}$$

The dilution itself is the selection of N_s cells according to a hypergeometric distribution. For a mean growth cycle with a fraction of $z = M_{\mathrm{end}}(y)$ mutants at the end, this selection process will have a mean $N_s z$ and variance $N_s z(1-z)\frac{N_{\mathrm{end}}^{\mathrm{total}} - N_s}{N_{\mathrm{end}}^{\mathrm{total}} - 1}$, where $N_{\mathrm{end}}^{\mathrm{total}} = M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s)$ is the total number of cells at the end of the cycle, before the dilution happens. Dividing by N_s and N_s^2, respectively, yields the mean fraction of mutants after selection and the variance due to the selection process alone:

$$M_{\mathrm{select}}(y) = M_{\mathrm{end}}(y) \tag{23}$$

$$V_{\mathrm{select}}(y) = \frac{1}{N_s} M_{\mathrm{end}}(y)(1 - M_{\mathrm{end}}(y)) \frac{M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s) - N_s}{M_{\alpha(1+s)}(yN_s) + M_\alpha((1-y)N_s) - 1} \tag{24}$$

However, in reality, z is itself a random variable with variance $V_{\mathrm{end}}(y)$. If this variance is small, such that the variance of the selection process is approximately constant for different possible z around $z = M_{\mathrm{end}}(y)$, then the variances just add up. Thus, we have for the periodic dilution case (subscript d)

$$M_{\delta y,d}(y) = M_{\mathrm{end}}(y) - y \tag{25}$$

$$V_{\delta y,d}(y) = V_{\mathrm{end}}(y) + V_{\mathrm{select}}(y). \tag{26}$$

Expanding $M_{\delta y,d}/V_{\delta y,d}$ around $s = 0$ reveals that it is independent of y up to first order:

$$\frac{M_{\delta y,d}}{V_{\delta y,d}} = \frac{(N_s - \xi^2)(fN_s - 1)f\log(f)}{(f-1)(fN_s(1+\xi^2) - \xi^2)}s + \mathcal{O}(s^2), \tag{27}$$

where $f = \exp(\alpha T)$ as in the main text. Thus we obtain

$$\Lambda_d = \frac{(N_s - \xi^2)(fN_s - 1)f\log(f)}{(f-1)(fN_s(1+\xi^2) - \xi^2)}s \tag{28}$$

$$\approx \frac{(N_s - \xi^2)f\log(f)}{(f-1)(1+\xi^2)}s. \tag{29}$$

In the last step, we neglected the small additive corrections to fN_s terms in the enumerator and the denominator.

V. DIFFUSION APPROXIMATION FOR PERIODIC DILUTION: $\bar{y}_d$

To estimate the average initial fraction of mutants $\bar{y}_d$, we consider the growth cycle in which the mutation first occurs: The probability of a mutation due to reaction (1b) in a time interval $\mathrm{d}\theta$ is $\mathrm{d}\theta \cdot p_x(x,\theta)\cdot x / \int_0^T \sum_{x=N_s}^{\infty} p_x(x,\theta')\cdot x\,\mathrm{d}\theta'$ according to Eq. (10) (for small μ). If the mutation happens at time θ into the cycle, the expected number of mutants at the end of the cycle is given by $m_1(m_s;\alpha(1+s);T-\theta) = m_s\exp[(1+s)\alpha(T-\theta)]$, where $m_1(N_0;\lambda;t)$ is defined as in Eq. (20) and m_s is the initial size of the mutant subpopulation. Therefore, the first moment for the number of $m$utants at the end of the $i$nitial cycle (subscript mi) can be calculated as

$$m_{1,mi} = \int_0^T \sum_{x=N_s}^{\infty} m_1(m_s;\alpha(1+s);T-\theta)\frac{p_x(x,\theta)\cdot x}{\int_0^T m_1(N_s;\alpha;\theta')\,\mathrm{d}\theta'}\,\mathrm{d}\theta \tag{30}$$

$$= \frac{m_s f(f^s - 1)}{s(f-1)} \tag{31}$$

Similarly, the expected number of $w$ildtype individuals at the end of the $i$nitial cycle (subscript wi) is $m_1(x,\alpha;T-\theta) = x\exp[\alpha(T-\theta)]$, yielding

$$m_{1,\mathrm{wi}} = \int_0^T \sum_{x=N_s}^{\infty} x\exp[\alpha(T-\theta)]\frac{p_x(x,\theta)\cdot x}{\int_0^T m_1(N_s;\alpha;\theta')\,\mathrm{d}\theta'}\,\mathrm{d}\theta$$

$$= f\left[N_s + \xi^2\left(1 - \frac{\log(f)}{f-1}\right)\right] \tag{32}$$

by realizing that the sum over all the x-dependent terms under the integral simply evaluates to $m_2(N_s;\alpha;\theta)$. Setting $\xi^2 = 1$ and $m_s = 1$, it is noteworthy that, even in the limit of $s \to 0$ (i.e. mutants having the same growth rate as non-mutants), the sum of the two terms (31) and (32) becomes $(1+N_s)f$, which means that the population on average has a

larger size at the end of a cycle, if a mutation is introduced, than it has otherwise. In fact, even the number of non-mutants is larger than usual at the end of these cycles, because $\log(f)/(f-1)$ is smaller than 1 for all $f > 1$ in Eq. (32). This is due to the fact that the cases we select from the set of *all* cycles to calculate this average are conditioned on the introduction of a mutation, which is more likely to happen at larger population sizes and therefore introduces a bias. Consistently, Eq. (32) converges to exactly fN_s if there are no population size fluctuations during growth, corresponding to $\xi^2 = 0$.
Eqs. (31) and (32) lead to the estimate

$$\bar{y}_d \approx \frac{m_{1,\mathrm{mi}}}{m_{1,\mathrm{wi}} + m_{1,\mathrm{mi}}} \approx \frac{(f^s - 1)m_s}{s(f-1)N_s}, \tag{33}$$

where the last step is valid for large N_s. Since $\bar{y}_d$ is in fact a ratio of two random quantities, we also calculated higher moments and the covariance of the two subpopulations in the initial cycle to obtain a more accurate estimate of $\bar{y}_d$ with the help of Eq. (22), as done in section IV for Λ_d. However, we found that the more accurate value of $\bar{y}_d$ does not lead to appreciable changes in the predictions, in particular in Fig. 2^M a and b. Moreover, $\bar{y}_d$ still becomes independent of ξ^2 for large N_s. Therefore, we chose the approximation with the simpler formula, which is Eq. (33).

VI. RANGE OF s-DEPENDENCE FOR SMALL POPULATION SIZES

Equation $(7)^M$ specifies the the asymptotic ratio of fixation probabilities Δ for large populations and small s. Combined with the slope at $s = 0$ from Eq. $(6)^M$, this allows for an order-of-magnitude estimation of the range δs of (small) selective advantages where the fixation probability ratio actually depends on s. By assuming that the linear relationship of Eq. $(6)^M$ holds until p_c/p_d reaches $\Delta(f)$, we get

$$\delta s \sim \frac{(f-1)(1+\xi^2)}{fN_s \log(f)}. \tag{34}$$

While this only gives a rough estimate, it can be useful in determining whether mutations in a given range of selective advantages are in danger of unequal suppression by the experimental protocol, which might be undesirable for experimental evolution experiments. In accordance with Fig. 2^M b, Eq. (34) shows that δs is reduced for increasing population sizes.

VII. BRANCHING PROCESS APPROXIMATION FOR PERIODIC DILUTIONS

Conceptually, the calculation of p_d in the branching process limit simply requires substituting the rates of Eq. $(10)^M$ into Eq. $(8)^M$, taking the limit $\sigma \to 0$ and finally weighting the result appropriately for different θ. In practice, however, the calculation is rather non-trivial, so it is carried out explicitly in this section.
First, consider the integral

$$I = \int_0^\infty (\lambda + \delta)(t) \exp\left(- \int_0^t (\lambda - \delta)(\tau)\, \mathrm{d}\tau \right) \mathrm{d}t, \tag{35}$$

which can be split up into several integrals I_i, each from $t = t_i = i \cdot (\log f)/\alpha - \theta$ to $t = t_{i+1}$. We take I_0 to mean the first integral from $t = 0$ to $t = t_1$, which is the only integral that does not contain a period σ of pruning and has a length of $T - \theta$ instead of T. For $i = 0$,

the integrands therefore reduce to

$$(\lambda + \delta)(t) = \alpha(1+s) \tag{36}$$

$$-\int_0^t (\lambda - \delta)(\tau)\,\mathrm{d}\tau = -\alpha(1+s)t. \tag{37}$$

In contrast, for $i = 1, 2, \ldots$, they evaluate to

$$(\lambda + \delta)(t) = \alpha(1+s) + \log f \cdot \begin{cases} \frac{1}{\sigma} & \text{for } 0 < t - t_i < \sigma \\ 0 & \text{otherwise} \end{cases} \tag{38}$$

$$-\int_0^t (\lambda - \delta)(\tau)\,\mathrm{d}\tau = -\alpha(1+s)t + \log f \cdot \begin{cases} i - 1 + \frac{t-t_i}{\sigma} & \text{for } 0 < t - t_i < \sigma \\ i & \text{otherwise} \end{cases} \tag{39}$$

The integrals can then be calculated as

$$I_0 = 1 - \exp(\alpha(1+s)\theta) f^{-(1+s)} \tag{40}$$

$$I_i = f^{-is} \cdot \frac{f^{-(s+2)} \exp(\alpha(1+s)\theta)[(f^{1+s} - f)\alpha(1+s)\sigma + (f + f^{1+s} - 2\exp(-\alpha(1+s)\sigma) f^{2+s}) \log f]}{\alpha(1+s)\sigma - \log f}. \tag{41}$$

The only i-dependent term in I_i is f^{-is}, so $I = \sum_{i=0}^{\infty} I_i$ can be calculated using the geometric series. Using Eq. $(8)^M$, the fixation probability for finite σ is then given by

$$p_d(\theta, \sigma) = \frac{2}{1+I} = \left[1 + \frac{\exp(\alpha(1+s)\theta)(1 - \exp(-\alpha(1+s)\sigma) f) \log f}{(f^{1+s} - f)(\alpha(1+s)\sigma - \log f)}\right]^{-1} \tag{42}$$

We obtain the fixation probability for instantaneous selection and mutations occurring at time θ into a cycle by taking the limit $\sigma \to 0$:

$$p_d(\theta) = \left[1 + \frac{\exp(\alpha(1+s)\theta)(f-1)}{(f^{1+s} - f)}\right]^{-1} \tag{43}$$

With the probability that a mutation actually occurs at time θ into the cycle from Eq. (12), the unconditional fixation probability p_d therefore is

$$p_d = \frac{\int_0^T \exp(\alpha\theta) p_d(\theta)\,\mathrm{d}\theta}{\int_0^T \exp(\alpha\theta)\,\mathrm{d}\theta} = \frac{\alpha}{f-1} \int_0^T \exp(\alpha\theta) \left[1 + \frac{\exp(\alpha(1+s)\theta)(f-1)}{(f^{1+s} - f)}\right]^{-1} \mathrm{d}\theta, \tag{44}$$

where $T = (\log f)/\alpha$. The integrand is of the form $A^x(a + bB^x)^{-m}$, which has the indefinite integral

$$\int A^x (a + bB^x)^{-m}\,\mathrm{d}x = (\log A)^{-1} a^{-m} A^x {}_2F_1\left(m, \frac{\log A}{\log B}; \frac{\log A}{\log B} + 1; -\frac{bB^x}{a}\right) \tag{45}$$

In our case, $A = \exp(\alpha)$, $B = \exp(\alpha(1+s))$, $a = 1$, $b = \frac{f-1}{f^{1+s} - f}$, and $m = 1$, so the indefinite integral of the integrand in Eq. (44) is

$$\alpha^{-1} \exp(\alpha\theta) {}_2F_1\left(1, 1/(1+s), 1 + 1/(1+s), -\frac{f-1}{f^{1+s} - f} \exp(\alpha(1+s)\theta)\right).$$

Subtracting the values at the integral limits 0 and $T = (\log f)/\alpha$ then yields the result shown in Eq. $(11)^M$:

$$p_d = \frac{1}{f-1}\left[f\,{}_2F_1\left(1, \frac{1}{1+s}; 1+\frac{1}{1+s}; -\frac{f-1}{f^{1+s}-f}f^{1+s}\right) - {}_2F_1\left(1, \frac{1}{1+s}; 1+\frac{1}{1+s}; -\frac{f-1}{f^{1+s}-f}\right)\right] \tag{46}$$

VIII. RATIO OF FIXATION PROBABILITIES IN THE BRANCHING PROCESS APPROXIMATION

The fixation probability $p_c = s/(1+s)$ can easily be calculated directly from Eq. $(8)^M$ as described in the main text. However, it is worth noting that, as for the diffusion theory, p_d can be used for the constant population case by letting f tend to 1. In this case, it is particularly simple, since the limit $f \to 1$ also implies that $\theta \to 0$, because the length of growth phase tends to zero as $f \to 1$. Therefore, we can use the intermediate result of Eq. (43) to obtain

$$p_c = \lim_{f\to 1}\lim_{\theta\to 0} p_d(\theta) = \lim_{f\to 1}\left[1 + \frac{(f-1)}{(f^{1+s}-f)}\right]^{-1} = \left[1+\frac{1}{s}\right]^{-1} \tag{47}$$

$$= \frac{s}{1+s} \tag{48}$$

To determine the asymptotic ratio p_c/p_d for $s \to 0$ from the branching process approximation, it is actually easier to use again the intermediate result of Eq. (43) instead of carrying out the limit directly based on Eq. (46). As p_c does not depend on θ, we can first determine

$$\lim_{s\to 0}\frac{p_d(\theta)}{p_c} = \exp(-\alpha\theta)\frac{f\log f}{f-1}. \tag{49}$$

Integration with the probability $p_{\mathrm{mut}}(\theta)$ for the occurrence of a mutation at time θ into the cycle from Eq. (12) then leads to

$$\lim_{s\to 0}\frac{p_d}{p_c} = \int_0^T p_{\mathrm{mut}}(\theta)\lim_{s\to 0}\frac{p_d(\theta)}{p_c}\,\mathrm{d}\theta = \frac{\int_0^T \frac{f\log f}{f-1}\,\mathrm{d}\theta}{\int_0^T \exp(\alpha\theta)\,\mathrm{d}\theta} \tag{50}$$

$$= f\left(\frac{\log f}{f-1}\right)^2, \tag{51}$$

which is the inverse of the desired quantity Δ.

IX. COMPARISON WITH LIMITING CASES FOUND IN PREVIOUS STUDIES

To obtain analytical results, previous studies of the fixation probability in bottlenecked populations have focused mainly on the regime of large population sizes and small selective advantages [2, 3]. While our analytical approximations in the main text cover finite population sizes as well as larger selective advantages, they include the large population limit for small selective advantages, and we showed that both the diffusion approximation and the branching process approximation yield the same result in this particular limit, namely

Eq. (7)^M, according to which the fixation probability in this limit is reduced by a factor of

$$\frac{p_d}{p_c} = f\left(\frac{\log f}{f-1}\right)^2 \tag{52}$$

for serial passage with a dilution factor f. The result seems to contradict the fact that an optimal dilution factor was found in previous studies, because it converges monotonically to 1 for $f \to 1$.

First, in Ref. [2], the reduction factor for the fixation probability was calculated to be

$$D(\log D)^2 = (\log f)^2/f, \tag{53}$$

which has an optimum. This result is based on an earlier calculation by the same group according to which the fixation probability for a mutation occurring at time θ into a cycle for a cycle length T and wildtype growth rate α is (equation 12 in Ref. [4])

$$2s\frac{\alpha T}{\exp(\alpha\theta)}. \tag{54}$$

As we will show below, this approximation is only valid for large f (small D), in which case Eq. (52) and Eq. (53) indeed agree. Intuitively, the inapplicability of Eq. (54) for dilution factors close to 1 can be seen as follows: For a constant wild-type growth rate, we expect the fixation probability to converge to that of a constant population as $T \to 0$ (and $f \to 1$), because diluting very often by a factor close to 1 is experimentally indistinguishable from keeping the population constant. However, Eq. (54) converges to 0 for $T \to 0$. The reason for this discrepancy lies in authors' use of a binomial distribution to approximate the sampling process. The variance of the binomial distribution for selecting N_s individuals when the mutant ratio (i.e. the probability of selecting a mutant) is $y = n_Y/(n_X + n_Y)$ is

$$N_s y(1-y). \tag{55}$$

In reality, however, the sampling process is from a finite population, and so the hypergeometric distribution is appropriate. In this case, the variance for selecting N_s out of fN_s individuals when the mutant ratio is $y = n_Y/(n_X + n_Y)$ is

$$N_s y(1-y)\frac{fN_s - N_s}{fN_s - 1}, \tag{56}$$

which converges to Eq. (55) only for large f. For f closer to one, however, Eq. (55) greatly overestimates the variance, because Eq. (56) converges to zero in this limit. The gravity of this difference can be seen in the extreme case $f = 1$, when the whole population is selected for the next cycle. According to the binomial distribution, the number of mutants at the beginning of the next cycle would be a random quantity, whereas in reality (and according to the hypergeometric distribution), the number of mutants should be exactly the same as just before the dilution event. The overestimated stochasticity upon dilution leads to an underestimation of the fixation probability in Eq. (54) for f close to 1 (i.e. short T): $M_{\delta y}$ approaches 0 as $f \to 1$ (and therefore $T \to 0$), because there is no time for the fraction of mutants to change during the growth phase. If the binomial distribution is used, $V_{\delta y}$ stays finite according to Eq. (55) and therefore $M_{\delta y}/V_{\delta y} \to 0$ as $f \to 1$. In contrast, both $M_{\delta y}$ and $V_{\delta y}$ approach 0 as $f \to 1$ if the more realistic hypergeometric distribution is used, which leads to a consistent behavior of the fixation probability as $f \to 1$, as shown in the main text. Therefore, we conclude that Eq. (54) [4] and the factor (53) derived from it in Ref. [2] are only valid for large f and the optimum is an artifact stemming from the unphysical

behavior of the approximation towards $f = 1$.

The situation is different for the optimum found in Ref. [3]. The optimal dilution ratio for large populations and small s there is not found for the fixation *probability* (the quantity calculated in Ref. [2] and the present study), but for the *fixation rate per cycle*, derived as

$$\mu f N_s \frac{D(\log D)^2}{1-D} s = \mu N_s \frac{(\log f)^2}{1-1/f} s = \mu N_s \frac{f(\log f)^2}{f-1} s \tag{57}$$

in equation 12 of Ref. [3]. However, if the organism in question has a constant division rate and the only parameter that is varied experimentally is the dilution factor (and hence the dilution interval), then the fixation rate *per unit time* is a more appropriate measure of the adaptation rate of the population (the fixation rate per cycle might get smaller as the dilution factor approaches 1, but more cycles fit in a given time span). Using the authors' convention of a division rate equal to 1, the cycle length is $T = \log(f)$, and so Eq. (57) can be converted to a the *fixation rate per unit time* by dividing by T:

$$\mu N_s \frac{f \log f}{f-1} s \tag{58}$$

As expected, the above equation converges to $\mu N_s s$ for $f \to 1$, i.e. the fixation rate in a constant population of N_s individuals dividing with rate 1. Furthermore, it increases monotonically with f, such that there is no optimum, and therefore diluting the population more severely always leads to the loss of more beneficial mutations, if all other parameters are kept constant. In fact, our analysis yields the same fixation rate as in Eq. (58): The fixation rate per time is simply the number of mutations occurring per time multiplied by the fixation probability. The number of mutations per unit time for a growth rate of $\alpha = 1$ is $N_s(f-1)/\log(f)\mu$, and the fixation probability for large populations and small s is

$$f \left(\frac{\log f}{f-1} \right)^2 s \tag{59}$$

which is implied by Eq. $(7)^M$ or can be seen directly from Eq. $(4)^M$ by first taking the limit $N_s \to \infty$ and subsequently considering small s. Multiplying the two yields

$$N_s \mu f \frac{\log f}{f-1} s, \tag{60}$$

which is identical to Eq. (58). Our results are therefore consistent with Ref. [3]. However, the optimal dilution ratio found therein maximizes the fixation rate *per cycle*, which might be important for specific scenarios but is not the relevant quantity for the experimental situation we are considering: a population of cells dividing with fixed rate where only the dilution factor (and thus the dilution interval) can be chosen.

X. DENSITY-TRIGGERED DILUTION

In the main text, the population was pruned to N_s individuals periodically with period T. In this way, the population on average reaches a size of fN_s individuals before it is diluted, but the final size in individual cycles fluctuates due to the stochasticity of division times. We tested whether our results depend on the exact periodicity of the pruning and carried out numerical simulations where the population is reduced to N_s individuals once it reaches a fixed size fN_s. Now, instead of the final size, the period is a random quantity. Figures 1

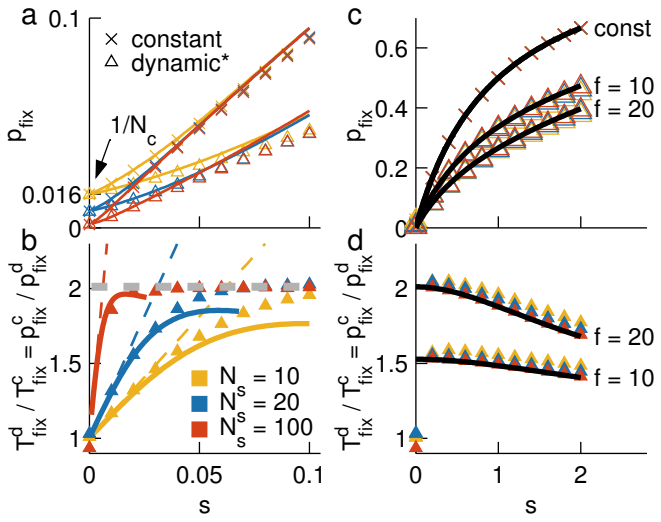

FIG. 1. Reproduction of Figure 2^M with numerical results for density-triggered instead of periodic dilutions. **(a)** Fixation probabilities from numerical simulations (symbols) compared to diffusion approximation, Eqs. $(5)^M$, $(4)^M$ (lines). **(b)** τ_d/τ_c from numerical simulations (symbols) and diffusion approximation (lines). Colored dashed lines: initial slope at $s = 0$ according to Eq. $(6)^M$; gray dashed line: asymptotic ratio, Eq. $(7)^M$. For all data in (a) and (b) $f = 20$, $\xi^2 = 1$, $m_s = 1$. **(c)**, **(d)** p and τ_d/τ_c from numerical simulations (symbols) compared to branching process approximation, Eqs. $(9)^M$, (46) (lines).

and 2 are identical to figures 2^M and 3^M, except that all data from numerical simulations was replaced with the alternative dynamic scenario, labeled "dynamic*". They show that the results are nearly identical, which might be important if the pruning is inherent to the cells, e.g., via an engineered density-triggered lysis mechanism.

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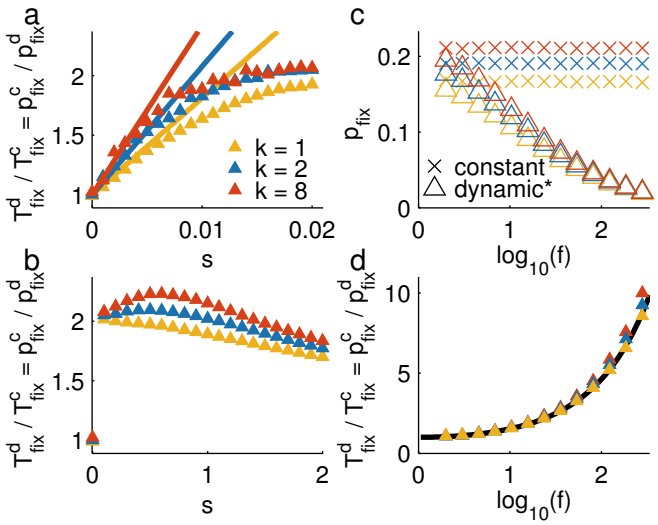

FIG. 2. Fixation probabilities and ratios in the multi-stage model. Reproduction of Figure 3^M with numerical results for density-triggered instead of periodic dilutions. **(a)** τ_d/τ_c for small s for different k in numerical simulations (symbols). Lines indicate the slope predicted by Eq. $(6)^M$ with $\xi^2 = \frac{2\log(2)^2}{k}$. **(b)** τ_d/τ_c from numerical simulations for larger s. **(c)** p as a function of the dilution factor f for a constant population and the two dynamic scenarios and different numbers of stages k. **(d)** τ_d/τ_c for the data shown in (c) compared to the analytical approximation, Eq. $(7)^M$. Parameters: $N_s = 50$, $f = 20$ (a,b) and $N_s = 20$, $s = 0.2$ (c,d).