Supplementary Methods: Evolution of microbial growth traits under serial dilution

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I. DETERMINISTIC POPULATION DYNAMICS OVER SERIAL DILUTIONS

At the beginning of the nth growth cycle, let the total population size be $N_0(n)$ and frequency of each strain k be $x_k(n)$. To determine the strain frequencies $\{x_k(n+1)\}$ and the initial population size $N_0(n+1)$ for cycle $n+1$, we first note that the selection coefficients relate the frequencies between consecutive cycles according to

$$s_{ij}(n) = \ln\left(\frac{x_i(n+1)}{x_j(n+1)}\right) - \ln\left(\frac{x_i(n)}{x_j(n)}\right), \tag{S1}$$

which follows from the definition in Eq. 2 and the condition that dilution preserves frequencies, i.e., the frequencies at the end of cycle n equal the frequencies at the beginning of cycle $n+1$ (neglecting stochastic effects of sampling). We can rearrange Eq. S1 to determine the frequencies in cycle $n+1$ as functions of the frequencies in cycle n and the selection coefficients:

$$x_i(n+1) = \frac{x_i(n)}{\sum_{\text{strain } k} x_k(n) e^{s_{ki}(n)}}. \tag{S2}$$

The population size $N_0(n+1)$ for the beginning of cycle $n+1$ is the population size at the end of the nth cycle diluted by D. The total population size at the end of the nth cycle is

$$\begin{aligned}
\sum_{\text{strain } k} N_0(n) x_k(n) e^{r_k(t_c(n)-L_k)} &= \left(\frac{R}{N_0(n)\sum_{\text{strain } \ell} \frac{x_\ell(n)}{Y_\ell} e^{r_\ell(t_c(n)-L_\ell)}}\right) \sum_{\text{strain } k} N_0(n) x_k(n) e^{r_k(t_c(n)-L_k)} \\
&= R\left(\sum_{\text{strain } \ell} \frac{1}{Y_\ell} \frac{x_\ell(n) e^{r_\ell(t_c(n)-L_\ell)}}{\sum_{\text{strain } k} x_k(n) e^{r_k(t_c(n)-L_k)}}\right)^{-1} \\
&= R\left(\sum_{\text{strain } \ell} \frac{1}{Y_\ell} \frac{x_\ell(n)}{\sum_{\text{strain } k} x_k(n) e^{s_{k\ell}(n)}}\right)^{-1} \\
&= R\left(\sum_{\text{strain } \ell} \frac{x_\ell(n+1)}{Y_\ell}\right)^{-1},
\end{aligned} \tag{S3}$$

where we have inserted the quantity in parentheses on the right-hand side of the first line because it equals 1 according to the saturation equation (Eq. 1), and we invoke Eq. S2 to obtain the last line. Therefore the initial population size in cycle $n+1$ equals this quantity diluted by D:

$$N_0(n+1) = \frac{R}{D}\left(\sum_{\text{strain } \ell} \frac{x_\ell(n+1)}{Y_\ell}\right)^{-1}. \tag{S4}$$

Equation S4 shows that the ratio R/D controls the overall magnitude of the bottleneck population size $N_0(n)$, and hence the effective population size for evolutionary dynamics. Furthermore, Eq. S4 indicates that for $n \geq 1$, the effective population yield and initial population size are constrained such that

$$\frac{R\bar{Y}(n)}{N_0(n)} = D, \tag{S5}$$

where we define the effective population yield as

$$\bar{Y}(n) = \left(\sum_{\text{strain } k} \frac{x_k(n)}{Y_k} \right)^{-1}. \tag{S6}$$

II. EQUATIONS FOR SELECTION COEFFICIENTS

Equation 1 in the main text defines the time t_c at which the population exhausts the resource and growth stops; Eq. 2 then defines the selection coefficients s_{ij} in terms of t_c. To determine how all s_{ij} depend explicitly on the parameters of the model, we first rewrite Eq. 2 to get t_c in terms of each s_{ij}:

$$t_c = \frac{s_{ij} + r_i L_i - r_j L_j}{r_i - r_j}. \tag{S7}$$

We then substitute this for t_c in Eq. 1 and rearrange to obtain an implicit nonlinear equation for the selection coefficients s_{ij}:

$$s_{ij} = -\frac{\Delta r_{ij}}{r_i} \ln \left(\frac{N_0}{R} \sum_{\text{strain } k} \frac{x_k}{Y_k} e^{s_{ki}} \right) - \Delta L_{ij} r_j, \tag{S8}$$

where $\Delta r_{ij} = r_i - r_j$ is the difference in growth rates and $\Delta L_{ij} = L_i - L_j$ is the difference in lag times. We can obtain an approximate analytical solution in the limit of weak selection $|s_{ij}| \ll 1$, as shown in previous work [1, 2]:

$$s_{ij} \approx s_{ij}^{\text{lag}} + s_{ij}^{\text{growth}} + \sum_{\text{strain } k} s_{ijk}^{\text{coupling}}, \tag{S9a}$$

where

$$s_{ij}^{\text{lag}} = -\Delta L_{ij} \frac{r_i r_j}{\bar{r}}, \tag{S9b}$$

$$s_{ij}^{\text{growth}} = \frac{\Delta r_{ij}}{\bar{r}} \ln \left(\frac{R\bar{Y}}{N_0} \right), \tag{S9c}$$

$$s_{ijk}^{\text{coupling}} = -\frac{x_k \bar{Y}}{\bar{r} Y_k} (r_i \Delta L_{ik} \Delta r_{kj} - r_j \Delta r_{ik} \Delta L_{kj}), \tag{S9d}$$

are the components of selection on the lag phase, on the growth phase, and on the coupling between lag and growth, and where $\bar{r} = \sum_{\text{strain } k} r_k x_k \frac{\bar{Y}}{Y_k}$ is the effective population growth rate.

III. FREQUENCY-DEPENDENT SELECTION AND COEXISTENCE

In general the selection coefficients are frequency-dependent, meaning they depend not only on the traits of the individual strains (lag times $\{L_k\}$, growth rate $\{r_k\}$, and yields $\{Y_k\}$) but also on their frequencies $\{x_k\}$ at the beginning of the growth cycle. To find the condition for coexistence of all the strains, we set $s_{ij} = 0$ for all pairs of strains i and j in Eq. S8 to obtain

$$0 = -\frac{\Delta r_{ij}}{r_i} \ln \left(\frac{N_0}{R\bar{Y}} \right) - \Delta L_{ij} r_j, \tag{S10}$$

using the definition for the effective population yield $\bar{Y}$ in Eq. S6. Furthermore, since $R\bar{Y}(n)/N_0(n) = D$ for $n \geq 1$ (Eq. S5), the dependence on the frequencies $\{x_k\}$ drops out and we obtain

$$\frac{r_i r_j \Delta L_{ij}}{\Delta r_{ij}} = \ln D. \tag{S11}$$

Geometrically, this means that the lag times $\{L_k\}$ and the reciprocal growth rates $\{1/r_k\}$ for all strains must lie on a straight line, with slope $-\ln D$ (implying a tradeoff between lag and growth) [2]. If this condition is satisfied by all strains, then the population dynamics are neutral at all frequencies $\{x_k\}$. Conversely, if Eq. S11 is not satisfied, the selection coefficients must be nonzero and because Eq. S11 is independent of the frequencies, the selection coefficient can never change sign. Furthermore, previous work showed that the variation in selection coefficients over the range of frequencies tends to be small [1]. Therefore we can approximate the selection on a strain as its selection coefficient at a low mutant frequency, which we do in the next section.

IV. APPROXIMATE SELECTION COEFFICIENT FOR TWO STRAINS

In the two-strain case, we can rewrite the selection coefficient equation (Eq. S8) as

$$s = -\frac{\gamma}{1+\gamma} \ln\left(\frac{N_0}{R}\left[\frac{1-x}{Y_1}e^{-s} + \frac{x}{Y_2}\right]\right) - \omega, \tag{S12}$$

where $s = s_{21}$ is the selection coefficient of the mutant over the wild-type, $\gamma = (r_2 - r_1)/r_1$ is the relative mutant growth rate, $\omega = (L_2 - L_1)r_1$ is the relative mutant lag time, and $x = x_2$ is the mutant frequency. We approximate the selection coefficient by considering the case of mutant being very rare ($x \to 0$), which is the relevant case for the calculation of the fixation probability [3]. In this case we can exactly solve Eq. S12 to obtain

$$\lim_{x\to 0} s = \gamma \ln\left(\frac{RY_1}{N_0}\right) - \omega(1+\gamma). \tag{S13}$$

We invoke the relation $RY_1/N_0 = D$ over serial dilutions (Eq. S5) and drop higher-order terms in γ and ω to finally obtain (Eq. 3)

$$s \approx \gamma \ln D - \omega. \tag{S14}$$

Alternatively, if we assume the selection coefficient s is small in magnitude, we can expand Eq. S12 in s, which yields an identical solution to leading order in γ and ω [1, 2].

V. DISTRIBUTIONS OF MUTATIONAL EFFECTS

When a mutation arises on a background strain with traits r_1, L_1 and Y_1, we randomly generate the new traits r_2, L_2, and Y_2 from a distribution. We assume the changes in traits scale with the values of the background strain's traits, so that the distribution of mutational effects only depends on the relative changes $\gamma = (r_2 - r_1)/r_1$, $\omega = (L_2 - L_1)r_1$, and $\delta = (Y_2 - Y_1)/Y_1$. We ignore epistasis so that mutational effects are additive. In the main text we use a uniform distribution for simplicity:

$$p_{\text{mut}}(\gamma, \omega, \delta) = \begin{cases} (8\gamma_{\max}\omega_{\max}\delta_{\max})^{-1} & \text{for } -\gamma_{\max} < \gamma < \gamma_{\max},\ -\omega_{\max} < \omega < \omega_{\max}, \text{ and } -\delta_{\max} < \delta < \delta_{\max}, \\ 0 & \text{otherwise.} \end{cases} \tag{S15}$$

We use $\gamma_{\max} = 0.02$, $\omega_{\max} = 0.05$, and $\delta_{\max} = 0.02$. In Fig. S6d we generalize this uniform distribution by shifting the mean of γ and ω to nonzero values, so that γ and ω satisfy $-\gamma_{\max} + \mu_\gamma < \gamma < \gamma_{\max} + \mu_\gamma$ and $-\omega_{\max} + \mu_\omega < \omega < \omega_{\max} + \mu_\omega$, where μ_γ and μ_ω are the respective means.

We also consider a Gaussian distribution (Fig. S1 and Fig. S6e), with a potentially nonzero Pearson correlation coefficient ρ_{mut} between growth effects γ and lag effects ω:

$$p_{\text{mut}}(\gamma, \omega, \delta) = \frac{1}{(2\pi)^{3/2}\sigma_\gamma\sigma_\omega\sigma_\delta\sqrt{1-\rho_{\text{mut}}^2}} \exp\left(-\frac{1}{2(1-\rho_{\text{mut}}^2)}\left[\frac{\gamma^2}{\sigma_\gamma^2} + \frac{\omega^2}{\sigma_\omega^2} - \frac{2\rho_{\text{mut}}\gamma\omega}{\sigma_\gamma\sigma_\omega}\right] - \frac{\delta^2}{2\sigma_\delta^2}\right). \tag{S16}$$

VI. EMPIRICAL DISTRIBUTION OF MUTATIONAL EFFECTS IN *E. COLI*

To empirically estimate the distribution of γ, ω, and δ arising from spontaneous mutations, we use data from the Keio collection of single-gene knockouts in *E. coli* [4] as a proxy. Campos et al. [5] measured a population growth curve for each strain in this collection in minimal media with glucose (for example, see Fig. S3a). For each of these mutant growth curves, we infer the growth rate r by fitting the data in the exponential growth phase and then calculate the lag time as $L = t - \ln(N(t)/N(0))/r$, where t is an arbitrary time in the exponential growth phase and $N(t)$ is a proxy for population size (optical density at 600 nm). We also calculate the ratio between the final OD in the stationary phase and the average cell size for each strain; this should be proportional to the total number of cells, and hence also proportional to the yield for a fixed amount of resources. We then determine the mutation's growth rate change $\gamma = (r - r_{\rm wt})/r_{\rm wt}$, lag time change $\omega = (L - L_{\rm wt})r_{\rm wt}$, and yield change $\delta = (Y - Y_{\rm wt})/Y_{\rm wt}$ relative to the wild-type, which has growth rate $r_{\rm wt}$, lag time $L_{\rm wt}$, and yield $Y_{\rm wt}$ averaged over replicates. To correct for plate-dependent effects on these measurements, we follow a prescription determined by the original authors of this data set [5]: we shift all traits in a plate-dependent manner such that the median value of the trait on each plate matches the median value of the trait across all wild-type replicates. Combining this data for all single-gene knockout mutants, we obtain an empirical version of the distribution $p_{\rm mut}(\gamma, \omega, \delta)$ (Fig. S3b,c). For the evolutionary simulations, we restrict $|\gamma| < 0.2$ and $|\omega| < 0.2$ to avoid very large growth rates and negative lag times.

These growth traits are affected by uncertainties due to instrument noise, biological variation across initial inocula, stochastic variation of the growth dynamics, and environmental variation. To estimate the magnitude of this uncertainty, we use 240 growth curves of wild-type replicates from this same data set. Figure S3d,e shows the distributions of growth rates, lag times, and yield proxies of these wild-type replicates along with all mutant strains. The standard deviations of growth rates, lag times, and yield proxies across wild-type replicates are, respectively, 0.0007 min^{-1}, 46 min, and 0.0177 $\text{OD}/\mu\text{m}^3$; for the mutants, they are 0.001 min^{-1}, 62 min, and 0.0279 $\text{OD}/\mu\text{m}^3$. This suggests that many mutant traits are not statistically distinguishable from the wild-type, since they fall within the variation of the wild-type replicates. We can also translate these numbers into rough estimates of minimum values of $|\gamma|$, $|\omega|$, and $|\delta|$ by normalizing by the mean wild-type growth rate (0.009 min^{-1}) and mean wild-type yield proxy (0.2363 $\text{OD}/\mu\text{m}^3$). This indicates that minimum distinguishable $|\gamma|$, $|\omega|$, and $|\delta|$ are approximately 0.08, 0.4 and 0.08, respectively.

VII. ESTIMATING OF THE FIXATION PROBABILITY FROM SIMULATIONS

To calculate the fixation probabilities as functions of γ and ω, we first discretize the space of relative growth rates γ and relative lag times ω (e.g., Fig. 2a). In each bin we calculate the fixation probability as the ratio between the total number of fixed mutations and the total number of mutations that arose in that bin, across 1000 independent populations. We run each simulation for 5000 growth cycles. To ensure the results are independent of the initial conditions, we collect fixation statistics based only on the last 2500 cycles; the results remain the same if we instead only use the last 1250 cycles.

For the uniform distribution of mutational effects (Eq. S15), we use bin sizes of 0.004 for γ and 0.01 for ω (Fig. 2). For the Gaussian distribution (Eq. S16), we use bins of 0.02 for both γ and ω (Fig. S1). Because the ranges of γ and ω of fixed mutations in the Gaussian case are broader than they are in the uniform case, the resulting fixation probabilities are noisier. However, we do not see any systematic effect on the fixation probability from varying the correlation coefficient between γ and ω (Fig. S1).

In Fig. S4a, we further verify the robustness of the fixation probability dependence on the selection coefficient $s = \gamma \ln D - \omega$ by coloring each point according to its neutral phenotype $t = \gamma/\ln D + \omega$ (orthogonal to the selection coefficient s); this quantifies the range of trait combinations that nevertheless have the same selection coefficient and fixation probability. We also plot the fixation probability against the partial selection coefficient $s = \gamma \ln D$ (component of selection on growth alone) in Fig. S4b, which shows that this component of selection alone is insufficient to determine fixation probability.

VIII. FIXATION PROBABILITY UNDER SERIAL DILUTION IN THE SSWM REGIME

Here we calculate the fixation probability of a mutation in the strong-selection weak-mutation (SSWM) regime — where mutations arise and either fix or go extinct one at a time — accounting for serial dilution dynamics (Eq. 5) [6, 7]. The wild-type population has lag time L_1 and growth rate r_1, while the mutant has lag time L_2 and growth rate r_2; the relative growth rate and lag time are therefore $\gamma = (r_2 - r_1)/r_1$ and $\omega = (L_2 - L_1)r_1$, respectively. A single mutant present at the beginning of the growth cycle has fixation probability $2s \approx 2(\gamma \ln D - \omega)$ (Eq. 3), since the dynamics of the mutant and wild-type across growth cycles is mathematically equivalent to a Wright-Fisher process [1, 8].

However, in general mutants will arise sometime in the middle of the growth cycle since they are tied to cell division events. In that case, the fixation probability of a mutation acquires a correction due to the time it arises during that first growth cycle.

Let the total time of the growth cycle be t_c; we assume the saturation time for the first cycle in which the mutant appears is dictated entirely by the wild-type, so that $t_c = L_1 + r_1^{-1} \ln D$. Suppose the mutant arises at time t such that $L_1 < t < L_1 + r_1^{-1} \ln D$. Therefore the number of mutant cells at the end of this first cycle is $e^{r_2(L_1 + r_1^{-1} \ln D - t)}$. The average number of mutant cells at the beginning of the next cycle is simply the number at the end of the previous cycle divided by the dilution factor D. The fixation probability of each of these mutants at the beginning of the next cycle is then given by $2(\gamma \ln D - \omega)$. Assuming $2(\gamma \ln D - \omega)$ is small, the total fixation probability of the original mutant arising at time t is

$$\begin{aligned} \phi_{\mathrm{SSWM}}(\gamma, \omega | t) &= 2(\gamma \ln D - \omega) \frac{e^{r_2(L_1 + r_1^{-1} \ln D - t)}}{D} \\ &= 2(\gamma \ln D - \omega) D^{\gamma} e^{-r_2(t - L_1)}. \end{aligned} \tag{S17}$$

The fixation probability of a mutant therefore decreases exponentially as it occurs later in the growth cycle, since it takes less advantage of that first cycle. Note that if $t = L_1$, i.e., the mutation arises immediately at the beginning of growth, then the fixation probability should be exactly $2(\gamma \ln D - \omega)$ but is off by a factor of D^{γ} due to the approximations during the first cycle; however, this contributes only terms higher-order in γ.

We now average this quantity over all times during the growth cycle. The probability density $p_{\mathrm{arise}}(t)$ of a mutation arising at time t is the rate at which the wild-type population produces mutants per unit time, $\mu r_1 N(t) = \mu r_1 N_0 e^{r_1(t - L_1)}$, divided by the total number of mutants in the growth cycle:

$$\begin{aligned} p_{\mathrm{arise}}(t) &= \frac{\mu r_1 N_0 e^{r_1(t - L_1)}}{\int_{L_1}^{L_1 + r_1^{-1} \ln D} dt \; \mu r_1 N_0 e^{r_1(t - L_1)}} \\ &= \frac{r_1 e^{r_1(t - L_1)}}{D - 1}. \end{aligned} \tag{S18}$$

Therefore the average fixation probability is

$$\begin{aligned} \phi_{\mathrm{SSWM}}(\gamma, \omega) &= \int_{L_1}^{L_1 + r_1^{-1} \ln D} dt \; \phi_{\mathrm{SSWM}}(\gamma, \omega | t) p_{\mathrm{arise}}(t) \\ &= 2(\gamma \ln D - \omega) \frac{D^{\gamma} - 1}{\gamma (D - 1)} \\ &\approx \frac{2 \ln D}{D - 1} (\gamma \ln D - \omega) \left(1 + \gamma \frac{\ln D}{2} \right), \end{aligned} \tag{S19}$$

where on the last line we have kept terms only to second order in γ. The leading-order component is the SSWM fixation probability used in the main text (Eq. 5), where $\ln D/(D-1)$ is the overall correction factor due to the distribution of mutation occurrence times during the growth cycle. Equation S19 furthermore shows that mutations affecting growth rate have an additional benefit over mutations affecting just lag time, since they gain an advantage even in the first cycle (lag time mutations do not have an effect until the next growth cycle) [7, 9]. We note that this calculation is merely an estimate of this effect, since we neglect other corrections second-order in γ and ω, but it nevertheless shows that this effect is at most of order $O(s^2)$.

IX. DISTRIBUTION OF FIXED MUTATIONAL EFFECTS IN THE SSWM REGIME

In the SSWM regime, the probability of fixing a mutation with effects γ and ω conditioned on the event of some mutation fixing is

$$P_{\mathrm{fixed}}(\gamma, \omega) = \frac{1}{Z} p_{\mathrm{mut}}(\gamma, \omega) \phi_{\mathrm{SSWM}}(\gamma \ln D - \omega), \tag{S20}$$

where $p_{\mathrm{mut}}(\gamma,\omega)$ is the probability of a mutation with effects γ and ω arising, and the probability of the mutation fixing is (Eq. 5)

$$\phi_{\mathrm{SSWM}}(s) = \frac{2\ln D}{D-1} s\Theta(s), \tag{S21}$$

where $\Theta(s)$ is the Heaviside theta function. We approximate the selection coefficient of the mutation as $s = \gamma \ln D - \omega$ (Eq. 3 or Eq. S14). The normalization factor is Z, the probability that a randomly chosen mutation fixes:

$$Z = \int d\gamma \int d\omega \; p_{\mathrm{mut}}(\gamma,\omega)\phi_{\mathrm{SSWM}}(\gamma \ln D - \omega). \tag{S22}$$

To calculate moments of the growth rate effect γ and lag time effect ω of fixed mutations, we must take averages over this distribution. That is, we can calculate the mean value of a function $f(\gamma,\omega)$ as

$$\langle f(\gamma,\omega)\rangle_{\mathrm{fixed}} = \int d\gamma \int d\omega \; P_{\mathrm{fixed}}(\gamma,\omega) f(\gamma,\omega). \tag{S23}$$

A. Uniform distribution of mutations

We first consider the case where mutational effects have a uniform distribution (Eq. S15). The normalization factor is

$$\begin{aligned} Z &= \int_{-\gamma_{\max}}^{\gamma_{\max}} d\gamma \int_{-\omega_{\max}}^{\omega_{\max}} d\omega \left(\frac{1}{4\gamma_{\max}\omega_{\max}}\right)\left(\frac{2\ln D}{D-1}\right)(\gamma \ln D - \omega)\Theta(\gamma \ln D - \omega) \\ &= \begin{cases} \dfrac{3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2}{6\gamma_{\max}(D-1)} & \text{if } \gamma_{\max}\ln D > \omega_{\max}, \\ \dfrac{(\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2)\ln D}{6\omega_{\max}(D-1)} & \text{if } \gamma_{\max}\ln D < \omega_{\max}. \end{cases} \end{aligned} \tag{S24}$$

Therefore the moments of γ and ω are (carrying out integrals in a manner similar to Eq. S24)

$$\langle\gamma\rangle_{\text{fixed}} = \begin{cases} \dfrac{2\gamma_{\max}^3 \ln^2 D}{3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2} & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ \dfrac{2\gamma_{\max}^2 \omega_{\max} \ln D}{\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2} & \text{if } \gamma_{\max} \ln D < \omega_{\max}, \end{cases} \tag{S25a}$$

$$\langle\omega\rangle_{\text{fixed}} = \begin{cases} -\dfrac{2\gamma_{\max}\omega_{\max}^2 \ln D}{3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2} & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ -\dfrac{2\omega_{\max}^3}{\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2} & \text{if } \gamma_{\max} \ln D < \omega_{\max}. \end{cases} \tag{S25b}$$

$$\langle\gamma^2\rangle_{\text{fixed}} = \begin{cases} \dfrac{15\gamma_{\max}^4 \ln^4 D + \omega_{\max}^4}{10(\ln^2 D)(3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2)} & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ \dfrac{1}{5}\gamma_{\max}^2 \left(3 - \dfrac{4\omega_{\max}^2}{\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2}\right) & \text{if } \gamma_{\max} \ln D < \omega_{\max}, \end{cases} \tag{S25c}$$

$$\langle\omega^2\rangle_{\text{fixed}} = \begin{cases} \dfrac{1}{15}\omega_{\max}^2 \left(5 + \dfrac{4\omega_{\max}^2}{3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2}\right) & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ \dfrac{\gamma_{\max}^4 \ln^4 D + 15\omega_{\max}^4}{10\left(\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2\right)} & \text{if } \gamma_{\max} \ln D < \omega_{\max}, \end{cases} \tag{S25d}$$

$$\langle\gamma\omega\rangle_{\text{fixed}} = \begin{cases} -\dfrac{\omega_{\max}^2(5\gamma_{\max}^2 \ln^2 D - \omega_{\max}^2)}{5(\ln D)(3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2)} & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ -\dfrac{1}{5}\gamma_{\max}^2 (\ln D) \left(\dfrac{8\omega_{\max}^2}{\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2} - 1\right) & \text{if } \gamma_{\max} \ln D < \omega_{\max}. \end{cases} \tag{S25e}$$

We can also calculate the variances and covariances:

$$\langle\gamma^2\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}^2 = \begin{cases} \dfrac{5\gamma_{\max}^6 \ln^6 D + 15\gamma_{\max}^4\omega_{\max}^2 \ln^4 D + 3\gamma_{\max}^2\omega_{\max}^4 \ln^2 D + \omega_{\max}^6}{10(\ln^2 D)\left(3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2\right)^2} & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ \dfrac{3\left(\gamma_{\max}^6 \ln^4 D - 2\gamma_{\max}^4\omega_{\max}^2 \ln^2 D + 5\gamma_{\max}^2\omega_{\max}^4\right)}{5\left(\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2\right)^2} & \text{if } \gamma_{\max} \ln D < \omega_{\max}, \end{cases} \tag{S26a}$$

$$\langle\omega^2\rangle_{\text{fixed}} - \langle\omega\rangle_{\text{fixed}}^2 = \begin{cases} \dfrac{3\left(5\gamma_{\max}^4\omega_{\max}^2 \ln^4 D - 2\gamma_{\max}^2\omega_{\max}^4 \ln^2 D + \omega_{\max}^6\right)}{5\left(3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2\right)^2} & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ \dfrac{1}{5}\gamma_{\max}^2 (\ln D) \left(1 - \dfrac{8\omega_{\max}^2}{\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2}\right) - \dfrac{4\omega_{\max}^6}{\left(\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2\right)^2} & \text{if } \gamma_{\max} \ln D < \omega_{\max}, \end{cases} \tag{S26b}$$

$$\langle\gamma\omega\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}\langle\omega\rangle_{\text{fixed}} = \begin{cases} \dfrac{\omega_{\max}^2\left(5\gamma_{\max}^4 \ln^4 D - 2\gamma_{\max}^2\omega_{\max}^2 \ln^2 D + \omega_{\max}^4\right)}{5(\ln D)\left(3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2\right)^2} & \text{if } \gamma_{\max} \ln D > \omega_{\max}, \\ \dfrac{4\gamma_{\max}^2\omega_{\max}^4 \ln D}{\left(\gamma_{\max}^2 \ln^2 D + 3\omega_{\max}^2\right)^2} + \dfrac{\omega_{\max}^2\left(\omega_{\max}^2 - 5\gamma_{\max}^2 \ln^2 D\right)}{5(\ln D)\left(3\gamma_{\max}^2 \ln^2 D + \omega_{\max}^2\right)} & \text{if } \gamma_{\max} \ln D < \omega_{\max}. \end{cases} \tag{S26c}$$

B. Gaussian distribution of mutations

We now repeat the calculation for a Gaussian distribution of mutational effects (Eq. S16). The normalization factor is

$$\begin{aligned} Z &= \int_{-\infty}^{\infty} d\gamma \int_{-\infty}^{\infty} d\omega \left(\frac{1}{2\pi\sigma_\gamma\sigma_\omega}\exp\left(-\frac{\gamma^2}{2\sigma_\gamma^2}-\frac{\omega^2}{2\sigma_\omega^2}\right)\right)\left(\frac{2\ln D}{D-1}\right)(\gamma\ln D-\omega)\Theta(\gamma\ln D-\omega) \\ &= \int_{-\infty}^{\infty} d\gamma \int_{-\infty}^{\gamma\ln D} d\omega \left(\frac{1}{2\pi\sigma_\gamma\sigma_\omega}\exp\left(-\frac{\gamma^2}{2\sigma_\gamma^2}-\frac{\omega^2}{2\sigma_\omega^2}\right)\right)\left(\frac{2\ln D}{D-1}\right)(\gamma\ln D-\omega) \\ &= \frac{2\ln D}{D-1}\sqrt{\frac{\sigma_\gamma^2\ln^2 D+\sigma_\omega^2}{2\pi}}. \end{aligned} \tag{S27}$$

Therefore the moments of γ and ω are (carrying out integrals in a manner similar to Eq. S27)

$$\langle\gamma\rangle_{\text{fixed}} = \frac{\sigma_\gamma^2\ln D}{2}\sqrt{\frac{2\pi}{\sigma_\gamma^2\ln^2 D+\sigma_\omega^2}}, \tag{S28a}$$

$$\langle\omega\rangle_{\text{fixed}} = -\frac{\sigma_\omega^2}{2}\sqrt{\frac{2\pi}{\sigma_\gamma^2\ln^2 D+\sigma_\omega^2}}, \tag{S28b}$$

$$\langle\gamma^2\rangle_{\text{fixed}} = \sigma_\gamma^2\left(2-\frac{\sigma_\omega^2}{\sigma_\gamma^2\ln^2 D+\sigma_\omega^2}\right), \tag{S28c}$$

$$\langle\omega^2\rangle_{\text{fixed}} = \sigma_\omega^2\left(1+\frac{\sigma_\omega^2}{\sigma_\gamma^2\ln^2 D+\sigma_\omega^2}\right), \tag{S28d}$$

$$\langle\gamma\omega\rangle_{\text{fixed}} = -\frac{\sigma_\gamma^2\sigma_\omega^2\ln D}{\sigma_\gamma^2\ln^2 D+\sigma_\omega^2}. \tag{S28e}$$

The variances and covariances are

$$\langle\gamma^2\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}^2 = \sigma_\gamma^2\left(1-\frac{(\pi-2)\sigma_\gamma^2\ln^2 D)}{2(\sigma_\gamma^2\ln^2 D+\sigma_\omega^2)}\right), \tag{S29a}$$

$$\langle\omega^2\rangle_{\text{fixed}} - \langle\omega\rangle_{\text{fixed}}^2 = \sigma_\omega^2\left(1-\frac{(\pi-2)\sigma_\omega^2}{2(\sigma_\gamma^2\ln^2 D+\sigma_\omega^2)}\right), \tag{S29b}$$

$$\langle\gamma\omega\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}\langle\omega\rangle_{\text{fixed}} = \frac{(\pi-2)\sigma_\gamma^2\sigma_\omega^2\ln D}{2(\sigma_\gamma^2\ln^2 D+\sigma_\omega^2)}. \tag{S29c}$$

C. A general proof on the sign of $\langle\gamma\omega\rangle_{\text{fixed}}$

The quantity $\langle\gamma\omega\rangle_{\text{fixed}}$ is negative for both the uniform (Eq. S25e) and Gaussian (Eq. S28e) cases shown above. We now present a general argument (shared by Yipei Guo) that it must be negative for any distribution of mutational effects $p_{\text{mut}}(\gamma,\omega)$ such that γ and ω are independent ($p_{\text{mut}}(\gamma,\omega) = p_{\text{mut,growth}}(\gamma)p_{\text{mut,lag}}(\omega)$) and the distribution is symmetric around zero ($p_{\text{mut,growth}}(\gamma) = p_{\text{mut,growth}}(-\gamma)$ and $p_{\text{mut,lag}}(\omega) = p_{\text{mut,lag}}(-\omega)$). We want to find the sign of the following integral:

$$I = \int_\gamma d\gamma \int_\omega d\omega\ \gamma\omega(\gamma\ln D-\omega)\Theta(\gamma\ln D-\omega)p_{\text{mut,growth}}(\gamma)p_{\text{mut,lag}}(\omega). \tag{S30}$$

Using the symmetry of $p_{\text{mut,growth}}(\gamma)$ and $p_{\text{mut,lag}}(\omega)$, the above integral must be equal to

$$I' = \int_\gamma d\gamma \int_\omega d\omega\ \gamma\omega|\gamma \ln D - \omega|\Theta(\omega - \gamma \ln D)p_{\mathrm{mut,growth}}(\gamma)p_{\mathrm{mut,lag}}(\omega). \tag{S31}$$

Therefore we can rewrite I and remove the Θ function:

$$I = \frac{1}{2}\int_\gamma d\gamma \int_\omega d\omega\ \gamma\omega|\gamma \ln D - \omega|p_{\mathrm{mut,growth}}(\gamma)p_{\mathrm{mut,lag}}(\omega). \tag{S32}$$

Given any point in the above integral such that $\gamma\omega > 0$, we can find a corresponding point with $\gamma\omega < 0$ with equal or higher $|\gamma \ln(D) - \omega|$ that occurs with the same probability. Therefore the total integral, and hence $\langle\gamma\omega\rangle_{\mathrm{fixed}}$, must be negative.

X. ADAPTATION RATES OF THE GROWTH RATE AND LAG TIME IN THE SSWM REGIME

In this section, we calculate the average adaptation speeds of growth rate and lag time using the average changes in these traits determined in Sec. IX. First, the total number of cell divisions in a growth cycle is the population size at the end of the cycle, $N_{\mathrm{final}} = \sum_{\mathrm{strain}\ i} N_i(t_c)$, minus the population size at the beginning, N_0. We can approximate the final population size as RY_0, which assumes that the yields of the evolved strains do not vary significantly from the ancestral yield Y_0 (as confirmed by simulations, e.g., Fig. 3e); we also assume $D \gg 1$ so that $N_{\mathrm{final}} - N_0 \approx N_{\mathrm{final}}$. Therefore the total number of mutation events per growth cycle is approximately μRY_0. For each mutation, the average probability that it fixes is Z (Eq. S22). The expected change in growth rate for a mutation is approximately $\langle\gamma\rangle_{\mathrm{fixed}} r_0$, assuming a small number of fixed mutations so that the growth rate has not changed significantly from the ancestral growth rate r_0; similarly, the expected change in lag time is approximately $\langle\omega\rangle_{\mathrm{fixed}}/r_0$.

We find that for both the uniform and Gaussian distributions of mutations, the expected changes in growth rate and lag time per cycle are (Eq. 8)

$$\begin{aligned} W_{\mathrm{growth}} &= \mu RY_0 Z\langle\gamma\rangle_{\mathrm{fixed}} r_0 \\ &= \sigma_\gamma^2 r_0(\ln D)\left(\frac{\mu RY_0 \ln D}{D-1}\right), \end{aligned} \tag{S33}$$

$$\begin{aligned} W_{\mathrm{lag}} &= \mu RY_0 Z\frac{\langle\omega\rangle_{\mathrm{fixed}}}{r_0} \\ &= -\frac{\sigma_\omega^2}{r_0}\left(\frac{\mu RY_0 \ln D}{D-1}\right). \end{aligned} \tag{S34}$$

where $\sigma_\gamma^2 = \gamma_{\max}^2/3$ and $\sigma_\omega^2 = \omega_{\max}^2/3$ are the variances of γ and ω in the case of a uniform distribution (Eq. S15). The ratio between the growth and lag adaptation speeds defines the average direction of evolution in growth-lag trait space (Eq. 9):

$$\frac{W_{\mathrm{growth}}}{W_{\mathrm{lag}}} = -r_0^2\frac{\sigma_\gamma^2}{\sigma_\omega^2}\ln D. \tag{S35}$$

We can use this relation to predict the average trajectory of the population growth rate r_{pop} and lag time L_{pop} over evolution. In the SSWM regime, we can approximate the average population growth rate and lag time as

$$\langle r_{\mathrm{pop}}\rangle = r_0 + nW_{\mathrm{growth}}, \tag{S36}$$

$$\langle L_{\mathrm{pop}}\rangle = L_0 + nW_{\mathrm{lag}}, \tag{S37}$$

where n is the total number of cycles. Therefore,

$$\frac{\langle r_{\mathrm{pop}}\rangle - r_0}{\langle L_{\mathrm{pop}}\rangle - L_0} = -r_0^2\frac{\sigma_\gamma^2}{\sigma_\omega^2}\ln D. \tag{S38}$$

In Fig. S5 we compare this equation with the trajectories obtained from simulations for three values of the dilution factor D. The prediction matches best for large D (Fig. S5a), since that produces smaller population sizes (through Eq. 4) and therefore better approximates the SSWM limit. The prediction becomes less accurate for small D (Fig. S5b,c) when clonal interference plays a larger role, but still matches well at early times.

XI. CORRELATION BETWEEN GROWTH RATES AND LAG TIMES

In this section we calculate the evolved correlations between growth rates and lag times. For a single fixed mutation, the correlation coefficient between the relative change in growth rate γ and relative change in lag time ω is

$$\rho_{\text{fixed}} = \frac{\langle\gamma\omega\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}\langle\omega\rangle_{\text{fixed}}}{\sqrt{\left(\langle\gamma^2\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}^2\right)\left(\langle\omega^2\rangle_{\text{fixed}} - \langle\omega\rangle_{\text{fixed}}^2\right)}}. \tag{S39}$$

However, the quantity more relevant to experimental data is the correlation between absolute growth rate r and lag time L between replicate populations at a given time when multiple mutations have fixed. To calculate this, we focus on the SSWM regime and assume that each mutation has small effects on the growth rate and lag time, so that the total growth rate r and lag time L can be approximated as sums of these effects:

$$\begin{aligned} r &\approx r_0 + r_0 \sum_{i=1}^{m} \gamma_i, \\ L &\approx L_0 + \frac{1}{r_0} \sum_{i=1}^{m} \omega_i, \end{aligned} \tag{S40}$$

where r_0 and L_0 are the ancestral growth rate and lag time, and the sums are over all fixed mutations (indexed by i) up to the total number m.

We can now calculate moments of the evolved growth rate and lag time by averaging over both the distribution of fixed mutations (Eq. S23) and across populations with different numbers m of fixed mutations:

$$\begin{aligned} \overline{\langle Lr\rangle_{\text{fixed}}} &\approx \frac{1}{M} \sum_{\text{population } \alpha} \left\langle \left(r_0 + r_0 \sum_{i=1}^{m_\alpha} \gamma_i\right)\left(L_0 + \frac{1}{r_0}\sum_{i=1}^{m_\alpha}\omega_i\right)\right\rangle_{\text{fixed}} \\ &= \frac{1}{M} \sum_{\text{population } \alpha} \left(r_0 L_0 + m_\alpha \langle\omega\rangle_{\text{fixed}} + L_0 r_0 m_\alpha \langle\gamma\rangle_{\text{fixed}} + m_\alpha \langle\gamma\omega\rangle_{\text{fixed}} + (m_\alpha^2 - m_\alpha)\langle\gamma\rangle_{\text{fixed}}\langle\omega\rangle_{\text{fixed}}\right) \\ &= r_0 L_0 + \overline{m}\langle\omega\rangle_{\text{fixed}} + L_0 r_0 \overline{m}\langle\gamma\rangle_{\text{fixed}} + \overline{m}\langle\gamma\omega\rangle_{\text{fixed}} + (\overline{m^2} - \overline{m})\langle\gamma\rangle_{\text{fixed}}\langle\omega\rangle_{\text{fixed}}. \end{aligned} \tag{S41}$$

Here the bar indicates an average over all independent populations (total number M). Similar calculations yield the (co)variances:

$$\overline{\langle Lr\rangle_{\text{fixed}}} - \overline{\langle L\rangle_{\text{fixed}}}\,\overline{\langle r\rangle_{\text{fixed}}} = \overline{m}\left(\langle\gamma\omega\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}\langle\omega\rangle_{\text{fixed}}\right) + \left(\overline{m^2} - \overline{m}^2\right)\langle\gamma\rangle_{\text{fixed}}\langle\omega\rangle_{\text{fixed}}, \tag{S42}$$

$$\overline{\langle r^2\rangle_{\text{fixed}}} - \left(\overline{\langle r\rangle_{\text{fixed}}}\right)^2 = r_0^2\overline{m}\left(\langle\gamma^2\rangle_{\text{fixed}} - \langle\gamma\rangle_{\text{fixed}}^2\right) + r_0^2\left(\overline{m^2} - \overline{m}^2\right)\langle\gamma\rangle_{\text{fixed}}^2, \tag{S43}$$

$$\overline{\langle L^2\rangle_{\text{fixed}}} - \left(\overline{\langle L\rangle_{\text{fixed}}}\right)^2 = \frac{1}{r_0^2}\overline{m}\left(\langle\omega^2\rangle_{\text{fixed}} - \langle\omega\rangle_{\text{fixed}}^2\right) + \frac{1}{r_0^2}\left(\overline{m^2} - \overline{m}^2\right)\langle\omega\rangle_{\text{fixed}}^2. \tag{S44}$$

That is, the (co)variances of the growth rate and lag time are sums of the (co)variance in the traits for a single fixed mutation and the variance of number of mutations ($\overline{m^2} - \overline{m}^2$). In the SSWM regime, different fixed mutations are independent of each other and the probability of any mutation fixing (Z, Eqs. S24 and S27) is small ($Z \sim D^{-1}$ with $D \gg 1$); therefore the number of fixed mutations over a finite time will be approximately Poisson-distributed, so that the variance approximately equals the mean:

$$\overline{m^2} - \overline{m}^2 \approx \overline{m}. \tag{S45}$$

The Pearson correlation coefficient of the evolved growth rate and lag time is therefore (Eq. 11)

$$\begin{aligned}\rho_{\text{evo}} &= \frac{\overline{\langle Lr\rangle_{\text{fixed}}} - \overline{\langle L\rangle_{\text{fixed}}}\,\overline{\langle r\rangle_{\text{fixed}}}}{\sqrt{\left(\overline{\langle r^2\rangle_{\text{fixed}}} - \left(\overline{\langle r\rangle_{\text{fixed}}}\right)^2\right)\left(\overline{\langle L^2\rangle_{\text{fixed}}} - \left(\overline{\langle L\rangle_{\text{fixed}}}\right)^2\right)}} \\ &\approx \frac{\langle\gamma\omega\rangle_{\text{fixed}}}{\sqrt{\langle\gamma^2\rangle_{\text{fixed}}\langle\omega^2\rangle_{\text{fixed}}}}.\end{aligned} \tag{S46}$$

That is, the correlation between evolved growth and lag depends entirely on the moments of growth and lag for a single fixed mutation, but is not identical to the correlation coefficient for a single fixed mutation (Eq. S39).

For the uniform distribution of mutations (Eq. S15), these two correlations equal:

$$\rho_{\text{fixed}} = \begin{cases} \sqrt{\dfrac{2\omega_{\max}^2(5\gamma_{\max}^4\ln^4 D - 2\gamma_{\max}^2\omega_{\max}^2\ln^2 D + \omega_{\max}^4)}{3(5\gamma_{\max}^6\ln^6 D + 15\gamma_{\max}^4\omega_{\max}^2\ln^4 D + 3\gamma_{\max}^2\omega_{\max}^4\ln^2 D + \omega_{\max}^6)}} & \text{if } \gamma_{\max}\ln D > \omega_{\max}, \\ \dfrac{\left(-5\gamma_{\max}^6\omega_{\max}^2\ln^6 D + 31\gamma_{\max}^4\omega_{\max}^4\ln^4 D - 19\gamma_{\max}^2\omega_{\max}^6\ln^2 D + 9\omega_{\max}^8\right)\left(\sqrt{3}\gamma_{\max}(\ln D)\left(3\gamma_{\max}^2\ln^2 D + \omega_{\max}^2\right)\right)^{-1}}{\sqrt{(\gamma_{\max}^4\ln^4 D - 2\gamma_{\max}^2\omega_{\max}^2\ln^2 D + 5\omega_{\max}^4)(\gamma_{\max}^6\ln^5 D - 2\gamma_{\max}^4\omega_{\max}^2\ln^3 D - 15\gamma_{\max}^2\omega_{\max}^4\ln D - 20\omega_{\max}^6)}} & \text{if } \gamma_{\max}\ln D < \omega_{\max}, \end{cases} \tag{S47a}$$

$$\rho_{\text{evo}} = \begin{cases} -\dfrac{\sqrt{2}\omega_{\max}\left(5\gamma_{\max}^2\ln^2 D - \omega_{\max}^2\right)}{\sqrt{\left(5\gamma_{\max}^2\ln^2 D + 3\omega_{\max}^2\right)\left(15\gamma_{\max}^4\ln^4 D + \omega_{\max}^4\right)}} & \text{if } \gamma_{\max}\ln D > \omega_{\max}, \\ -\dfrac{\omega_{\max}^2\left(\gamma_{\max}^2\ln^2 D + 3\omega_{\max}^2\right)\left(5\gamma_{\max}^2\ln^2 D - \omega_{\max}^2\right)}{\gamma_{\max}^2(\ln D)\left(3\gamma_{\max}^2\ln^2 D + \omega_{\max}^2\right)\sqrt{(\ln D)\left(\gamma_{\max}^2\ln^2 D - 5\omega_{\max}^2\right)\left(3\gamma_{\max}^2\ln^2 D + 5\omega_{\max}^2\right)}} & \text{if } \gamma_{\max}\ln D < \omega_{\max}, \end{cases} \tag{S47b}$$

while for the Gaussian distribution of mutations (Eq. S16) they are

$$\rho_{\text{fixed}} = \frac{(\pi-2)\sigma_\gamma\sigma_\omega\ln D}{\sqrt{\left[(4-\pi)\sigma_\gamma^2\ln^2 D + 2\sigma_\omega^2\right]\left[2\sigma_\gamma^2\ln^2 D + (4-\pi)\sigma_\omega^2\right]}}, \tag{S48a}$$

$$\rho_{\text{evo}} = -\frac{\sigma_\gamma\sigma_\omega\ln D}{\sqrt{2\sigma_\gamma^4\ln^4 D + 5\sigma_\gamma^2\sigma_\omega^2\ln^2 D + 2\sigma_\omega^4}}. \tag{S48b}$$

Note that the correlation ρ_{fixed} for a single fixed mutation is positive in the uniform and Gaussian cases, while the correlation ρ_{evo} between evolved traits is negative (cf. Fig. 5). The latter is true for any independent, symmetric distributions of γ and ω as proved in Sec. IX C.

[1] M. Manhart, B. V. Adkar, and E. I. Shakhnovich, Proc R Soc B **285**, 20172459 (2018).
[2] M. Manhart and E. I. Shakhnovich, Nat Commun **9**, 3214 (2018).
[3] Y. Guo, M. Vucelja, and A. Amir, Sci Adv **5**, eaav3842 (2019).
[4] T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsenko, M. Tomita, B. L. Wanner, and H. Mori, Mol Syst Biol **2**, 2006.0008 (2006).
[5] M. Campos, S. K. Govers, I. Irnov, G. S. Dobihal, F. Cornet, and C. Jacobs-Wagner, Mol Syst Biol **14**, e7573 (2018).
[6] L. M. Wahl and P. J. Gerrish, Evolution **55**, 2606 (2001).
[7] L. M. Wahl and A. D. Zhu, Genetics **200**, 309 (2015).
[8] J. F. Crow and M. Kimura, An Introduction to Population Genetics Theory (Harper and Row, New York, 1970).
[9] Y. Li, S. Venkataram, A. Agarwala, B. Dunn, D. A. Petrov, G. Sherlock, and D. S. Fisher, Current Biology **28**, 515 (2018).

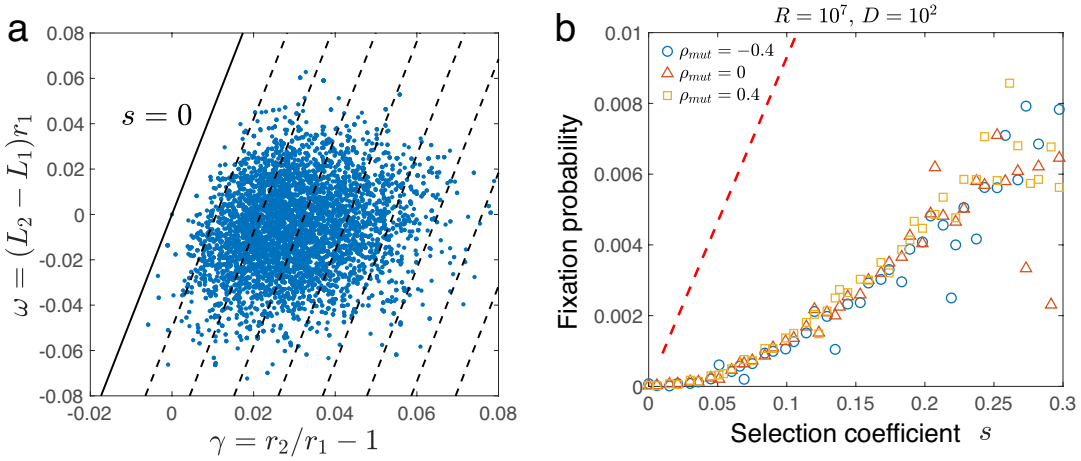

FIG. S1. **Fixation probabilities of mutations with Gaussian-distributed mutational effects** (a) The relative growth rates γ and the relative lag times ω of fixed mutations against their background strain. Dashed lines mark contours of constant selection coefficient with interval $\Delta s = 0.05$ while the solid line marks $s = 0$. The standard deviations of the Gaussian distribution (Eq. S16) are $\sigma_\gamma = \sigma_\omega = \sigma_\delta = 0.02$, with zero correlation $\rho_{\rm mut}$ between γ and ω. The parameters of the population dynamics are $D = 10^2$ and $R = 10^7$. (b) We bin mutations according to their effects γ and ω, and for each bin we calculate the fixation probability and the selection coefficient according to Eq. 3. Different colors represent different growth-lag correlation coefficients $\rho_{\rm mut}$. The red dashed line shows the fixation probability predicted in the SSWM regime (Eq. 5 in the main text).

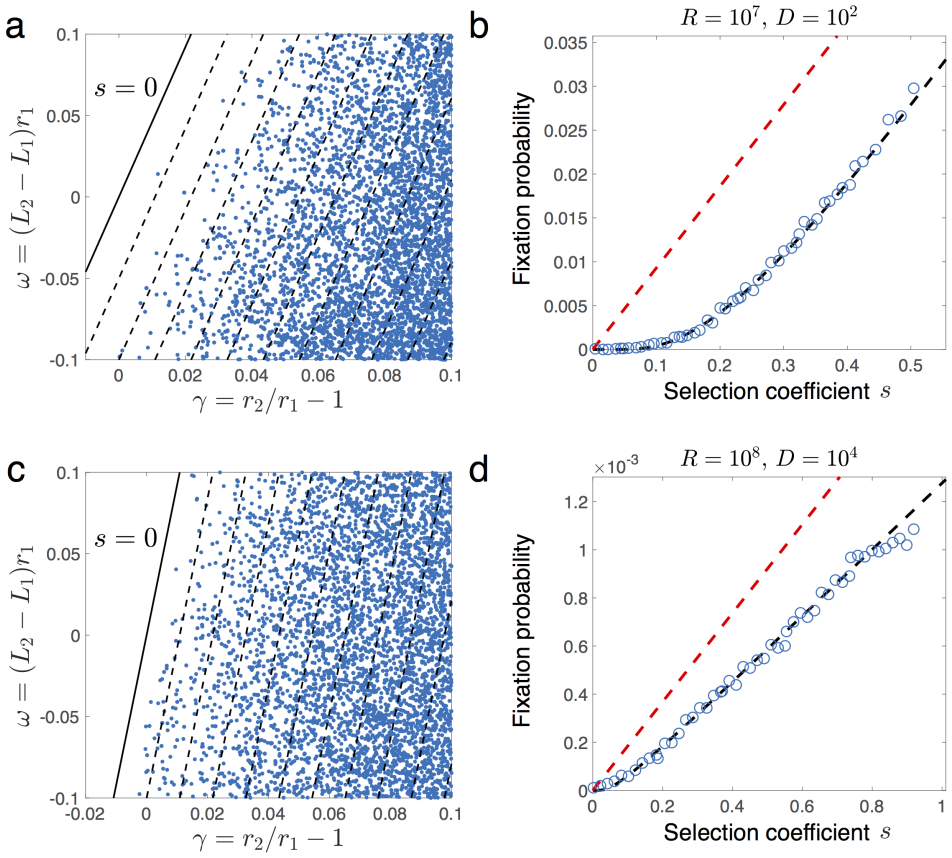

FIG. S2. **Fixation probabilities for large-effect mutations.** (a) The relative growth rate γ and lag time ω of fixed mutations. Dashed lines mark contours of constant selection coefficient with interval $\Delta s = 0.05$, while the solid line marks $s = 0$. The parameters of the population dynamics are $D = 10^2$ and $R = 10^7$. (b) Fixation probability of mutations against their selection coefficient, using fixed mutations from panel (a). The red dashed line shows the fixation probability predicted in the SSWM regime (Eq. 5 in the main text), while the black line shows a numerical fit of the data points to the fixation probability under clonal interference (Eq. 6 in the main text; $A = 0.1072$ and $B = 0.3261$). (c) Same as (a) but for $D = 10^4$ $R = 10^8$ and with $\Delta s = 0.1$. (d) Same as (b) but for fixed mutations in panel (c). Numerical fit of Eq. 6 produces parameters $A = 0.0014$ and $B = 0.0820$. In all panels mutations randomly arise from a uniform distribution $p_{\rm mut}$ where $-0.1 < \gamma < 0.1$ and $-0.1 < \omega < 0.1$, with the mutation rate $\mu = 10^{-6}$ and the distributions of the relative yield δ the same as Fig. 3.

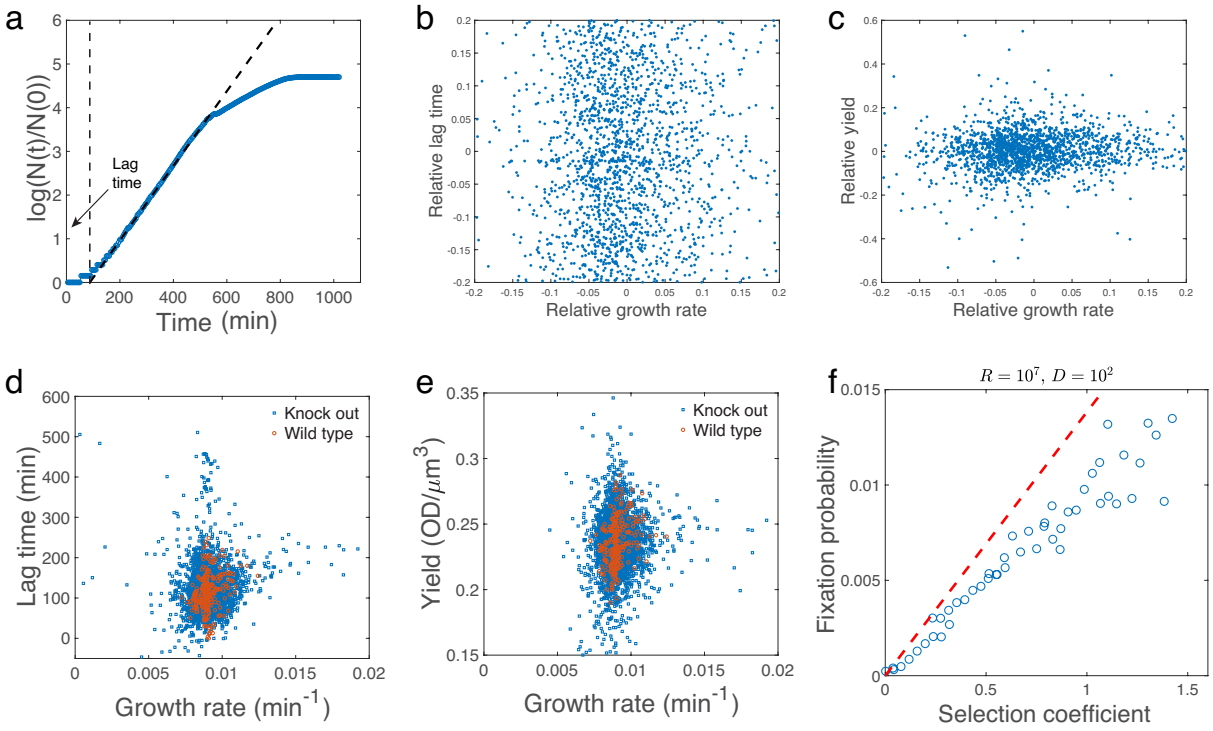

FIG. S3. **Empirical estimate of mutational effects from single-gene knockout collection of** *E. coli*. (a) Example growth curve (optical density at 600 nm) from a single-gene knockout strain (cyoC deleted) from which we estimate the growth rate (slope of diagonal dashed line) and the lag time (vertical dashed line). We obtain a proxy for the yield by taking the maximum optical density and normalizing by the average cell size. (b) Relative growth rates γ and relative lag times ω of all knockout mutants compared to the wild-type. The Pearson correlation coefficient between γ and ω is 0.02 ± 0.05. (c) Relative growth rates γ and relative yields δ of all knockout mutants compared to the wild-type. The Pearson correlation coefficient between γ and δ is 0.09 ± 0.05. (d) Growth rates and lag times for all knockout mutants as well as wild-type replicates in the data set. (e) Growth rates and yields for all knockout mutants as well as wild-type replicates in the data set. (f) Fixation probabilities of mutations as functions of the selection coefficient, using the knockout mutant data as the distribution of mutational effects; other parameters are $R = 10^7$, $D = 10^3$, and $\mu = 5 \times 10^{-7}$. The red dashed line shows the fixation probability predicted in the SSWM regime (Eq. 5). Raw growth curve data is from Campos et al. [5].

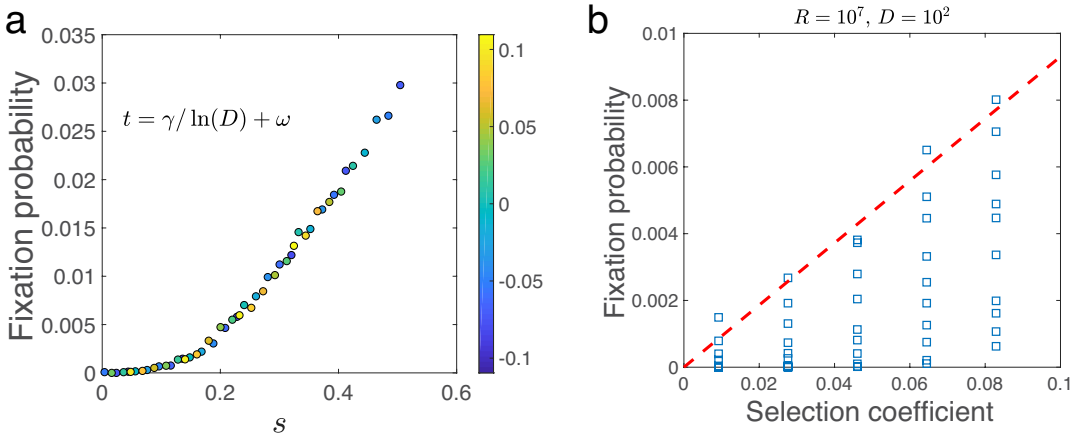

FIG. S4. **Robustness of fixation probability dependence on the selection coefficient.** (a) Fixation probability of mutations as a function of their selection coefficients, but with each data point colored by its neutral phenotype $t = \gamma \ln D + \omega$. The simulation data is the same as in Fig. S2b. (b) We replot Fig. 2e in the main text with the partial selection coefficient $s = \gamma \ln D$ (component of selection of growth alone), which does not lead to a collapse of data.

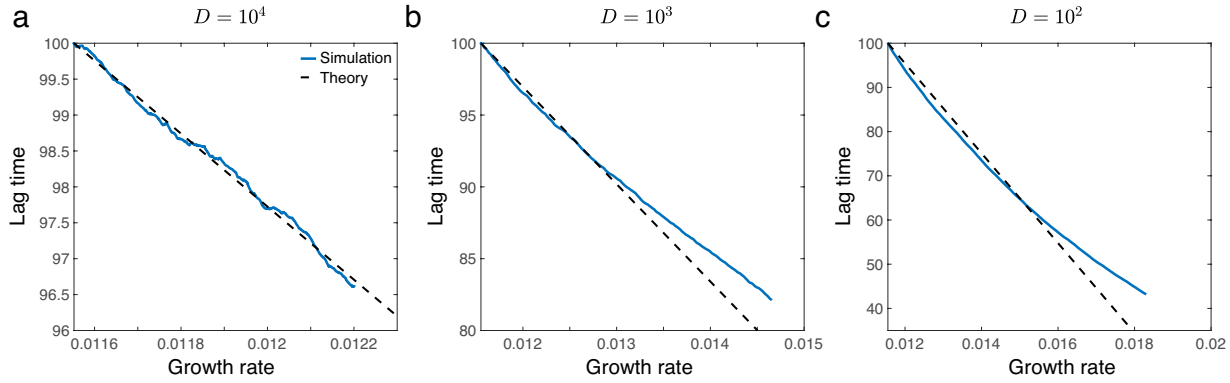

FIG. S5. **Average evolutionary trajectories in growth-lag trait space.** We plot the average population growth rate r_{pop} and lag time L_{pop} from simulations (solid blue lines) with (a) $D = 10^4$, (b) $D = 10^3$, and (c) $D = 10^2$, along with the predicted trajectories in the SSWM limit (Eq. S38; dashed black line). In all panels $R = 10^7$.

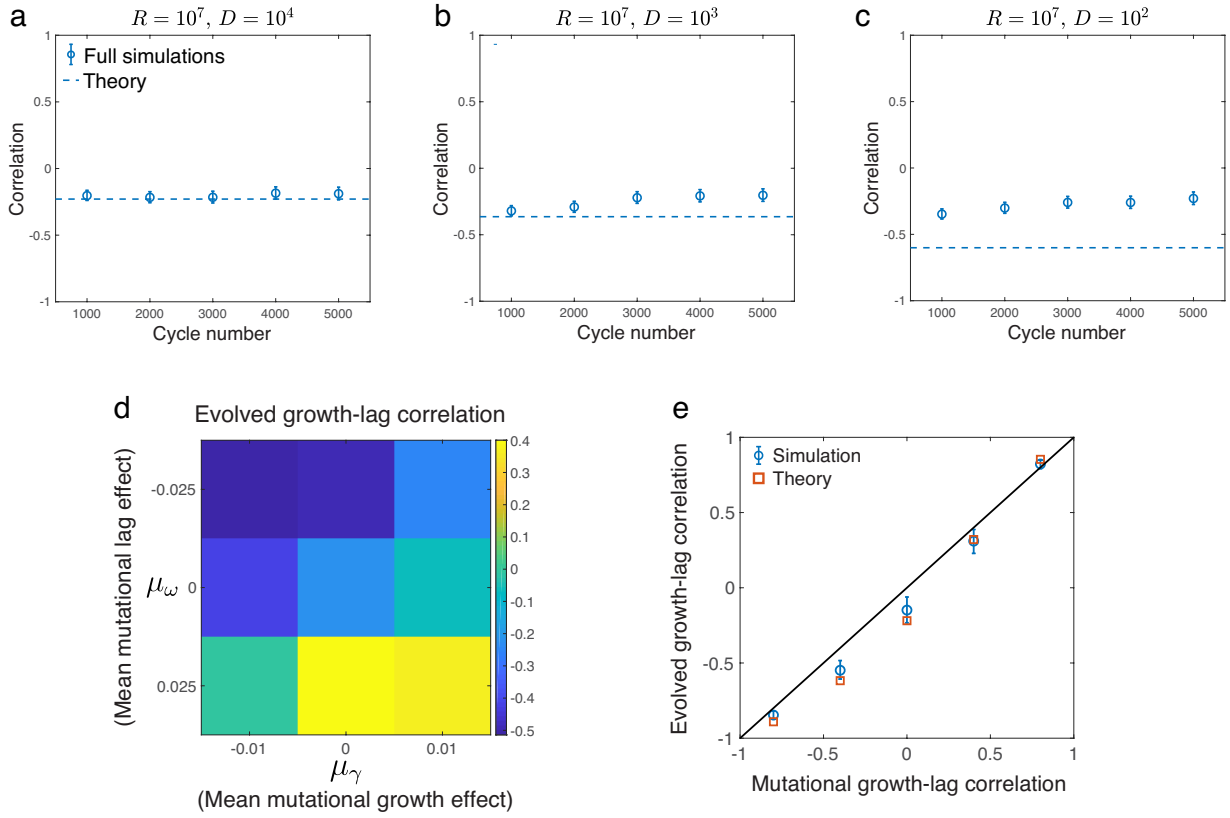

FIG. S6. **Evolved patterns of covaration among growth traits.** (a-c) Pearson correlation coefficients of the population-averaged growth rates and lag times versus the cycle number from simulations. The blue circles are the measured values from the full simulations and the dashed lines are the predictions for the SSWM regime (Eq. 11). The error bars represent 95% confidence intervals. (d) Evolved correlation coefficient ρ_{evo} of growth rate and lag time (after 50000 mutational trials) as a function of the mean mutational effects on growth rate and lag time (Eq. S15). (e) Evolved correlation coefficient ρ_{evo} of growth rate and lag time (after 50000 mutational trials) as a function of the mutational correlation ρ_{mut} of these two traits (Eq. S16). The blue points show simulation results, while the red points show the prediction from Eq. 11. The black line shows the line of identity. In both (d) and (e), we simulate the SSWM regime by introducing random mutations one-by-one and determining their fixation from Eq. 5 with $D = 10^3$.