Supplementary Material: Model and test in a fungus of the probability that beneficial mutations survive drift.

1 Estimating escape probabilities

We assume the plate is inoculated with $N_i$ wildtype spores, and a single mutant spore, in a droplet inoculum with radius $r_i$.

We derive a lower bound for the probability that the mutant escapes the inoculum. To do this, imagine that the mutant is seeded at distance $d$ from the closest point to the mutant along the edge of the inoculum, point $E$, as illustrated in Figure 1. In the time that the mutant colony grows distance $d$, the wildtype grows distance $dw/m$. Thus any wildtype spore which is seeded within distance $dw/m$ of point $E$ will reach $E$ first, and the mutant colony will be blocked from reaching $E$.

Clearly, the mutant colony might also be blocked before reaching $E$ (or in fact after reaching $E$, just outside of the inoculum edge). For every point along the path between the mutant spore and $E$, there is a circle in which no wildtype may occur, or the mutant would be blocked. The union of these circles gives an area which must be free of wildtype individuals for the mutant to escape at $E$. Since $N_i$ is large, only mutant spores which are quite close to the edge of the inoculum are likely to escape, that is, $d$ will be small relative to $r_i$. Thus the curvature of the inoculum boundary will be negligible and the area will be very close to a triangle with height $d$ and base $2dw/\sqrt{m^2 - w^2}$. Introducing $f_i = \sqrt{m^2 - w^2}/w$, the area of the triangle is $d^2/f_i$.

The probability that a single wildtype nucleus from the inoculum is found within this triangle is simply the area of the triangle divided by the inoculum area. Then the probability that no wildtype
spore exists within the triangle is the complement of this ratio raised to the \( N_i \)'th power:

\[
\text{prob(no wt in triangle)} = \left(1 - \frac{d^2}{f_i \pi r_i^2}\right)^{N_i} \\
\approx \exp\left(-\frac{N_i d^2}{f_i \pi r_i^2}\right)
\]

The approximation holds assuming once again that only spores which begin relatively close to the edge of the inoculum have a chance to escape, thus \( d^2 << r_i^2 \).

To compute the escape probability for the mutant, we integrate the probability that the triangle at distance \( d \) contains no wildtype spores, times the probability that the mutant spore from the inoculum lies at distance \( d \) from the edge of the inoculum. The probability that a spore is at radius \( r = r_i - d \) is proportional to \( r \). Using \( K \) to denote \( \frac{N_i}{f_i \pi r_i^2} \), and normalizing the probability densities appropriately we arrive at the escape probability:

\[
p_e \approx \frac{2}{r_i} \int_{d=0}^{r_i} (r_i - d) e^{-Kd^2} \, dd \\
= \frac{2}{r_i} \int_{d=0}^{r_i} e^{-Kd^2} \, dd - \frac{\pi f_i}{N_i} \\
= \frac{2}{r_i \sqrt{K}} \frac{\sqrt{\pi}}{2} - \frac{\pi f_i}{N_i} \\
= \pi \left(\sqrt{\frac{f_i}{N_i}} - \frac{f_i}{N_i}\right).
\]

Here we have used the fact that \( N_i \) and thus \( K \) is very large, and that the integral of the error function \( e^{x^2} \) from 0 to \( \infty \) is \( \sqrt{\pi}/2 \).

This approximation for \( p_e \) gives an approximate lower bound because we have assumed that the mutant hyphae escape the inoculum along the shortest path, through point \( E \). More accurate estimates can be computed numerically by considering points to the left and right of point \( E \), through which the mutant colony might also escape given that the wildtype is absent in circles of appropriate radius along the mutant path. The appropriate integrals are approximated numerically for all points along the circumference of the inoculum. This approach is also an underestimate of the escape probability because we still assume that the mutant subcolony has reached the inoculum boundary by colonizing at least one straight path to the edge. We are able to relax this assumption in the simulations; in the simulations, mutants can take circuitous paths to the edge of the inoculum.

The analytical approximation of \( p_e \) suggests that the escape probability \( p_e \) depends only on the
mutant and wildtype growth rates, and the number of wildtype in the inoculum, but not on the inoculum radius, which has been cancelled out in each term of the integration. Although this may seem counter-intuitive, it should be interpreted carefully: if $r_i$ changes but $N_i$ does not change, the escape probability should stay the same. However if inocula of increasing size were used in an experiment, with a constant density of wildtype nuclei in the inoculate, $N_i$ would increase accordingly, and we predict that the escape probability would decrease for larger radii.

2 Effects of serial transfer

Given $p_e$, the probability that a single mutant spore in the inoculum successfully escapes and creates a sector, we would like to know the ultimate fixation (or extinction) probability of the mutant lineage after serial transfer.

We assume that if a sector is formed, it accounts for fraction $A$ of the total area of the final colony, and that $n = N_iA$ mutant spores are transferred as part of the next inoculum. We further assume that each of these spores can make a new sector with probability $p_e$. Thus the probability generating function which describes the branching process of sectors producing further sectors can be written:

$$S(x) = (1 - p_e + p_e x)^n,$$

and the extinction probability, given that we start with a mutant sector, is the fixed point $S(X_s) = X_s$. Intuitively we expect that the extinction probability, once a sector has been formed, is small. We thus solve the following

$$0 = (1 - p_e + p_e X_s)^n - X_s$$

by a second-order Taylor (Maclaurin) series around $X_s = 0$.

Solving the resulting quadratic equation, we also employ a third order Maclaurin series approximation of the discriminant:

$$\sqrt{1 - 2\alpha - \alpha^2} \approx 1 - \alpha - \alpha^2 - \alpha^3$$
where \( \alpha = np_e(1 - p_e)^{n-1} \) is small since \( n \) is fairly large.

Using \( q_e = 1 - p_e \), this yields:

\[
X_s \approx \frac{n}{n-1} \left( q_e^n + np_eq_e^{2n-1} \right)
\]

Recall that \( X_s \) is the extinction probability of the lineage when starting from a single sector. To get the extinction probability starting from a single spore, we need

\[
X = \text{prob(no sector)} + \text{prob(sector)} X_s = q_e + p_e q_e^n + np_e^2 q_e^{2n-1}
\]

when \( n \) is sufficiently large that \( n/(n-1) \approx 1 \). However \( q_e \) is the probability that the initial mutant did not successfully make a sector. For practicality from the experimental point of view, we cannot have \( q_e \) close to 1. Thus when \( n \) is large, \( q_e^n \) approaches zero, and we have:

\[
X \approx 1 - p_e
\]

This means that for all practical experiments (whenever \( n = N_iA \) is large enough that \( n/(n-1) \approx 1 \) and \( (1 - p_e)^n \approx 0 \)) the ultimate fixation probability of the mutant lineage is very well-approximated by \( p_e \), the probability that the mutant successfully makes a sector before the first transfer. This purely mathematical result co-incides with our experimental intuitions about the process as well.

### 3 Effects of Poisson sampling

The experimental data in Figure 2 in the main paper show the proportion of colonies in which the beneficial mutation escaped the inoculum. However, note that this proportion is computed as a fraction of those colonies which received at least one mutant spore. We diluted the mutant spore suspensions such that the inoculum volume should contain on average 1 mutant spore per inoculum. The average number of spores in each inoculum was determined by counting the number of germinated spores under a microscope in \( n = 142 \) inocula after 1 hour of growth at 37 °C. The
distribution of spores in inocula was indistinguishable from a Poisson distribution with \( \lambda = 1 \) (mean = 1.02, variance = 1.00, likelihood-ratio test: \( \chi^2 = 3.77, P = 0.44 \), Supplemental figure 2). Thus, to compute theoretical predictions of the overall escape proportion, we take a weighted average of the probability that at least one mutant subcolony escapes the inoculum, conditioned on the number of mutant spores in the inoculum. Letting \( P(i) \) denote the probability that \( N_i \) mutant spores were seeded in the inoculum (a Poisson distribution with mean one), we find:

\[
\text{proportion escaping inoculum} = P(1)p_e + P(2)(1 - (1 - p_e)^2) + P(3)(1 - (1 - p_e)^3) + ...
\]

To be thorough, we include all terms in this sum up to \( P(10) \), although in reality the chance that a single inoculum had 10 mutant spores is less than \( 10^{-6} \).

### 4 Classical Prediction

To compare our results with classical predictions for fixation probabilities, we use the prediction of Kimura’s diffusion approach, assuming a haploid population of size \( N \) and a mutation initially occurring in a single copy with selective advantage \( s \):

\[
p_c = \frac{1 - e^{-2s}}{1 - e^{-2Ns}}
\]

where \( p_c \) is the classically-predicted fixation probability. When \( Ns \) is sufficiently large that the denominator is close to unity, and \( s \) is sufficiently small that a Taylor series approximation suffices in the numerator, this yields Haldane’s well-known \( 2s \) approximation. Using the approach described in the previous section, to account for the possibility of multiple mutant spores per inoculum, we compute

\[
\text{proportion predicted to fix} = P(1)p_c + P(2)(1 - (1 - p_c)^2) + P(3)(1 - (1 - p_c)^3) + ...
\]

This predicted proportion is plotted by the solid grey line in the Results figure in the main text.
Supplementary Figure 1: If a wildtype spore is in a triangle with height $d$ and base $2d/f$, the mutant will not escape the inoculum.

Supplementary Figure 2: Distribution of spores in the inoculum droplet had mean = 1.02 and variance = 1.00 (bars). Crosses are expected counts from a Poisson distribution with $\lambda = 1$ and $n = 142$ draws.