Survival probability of beneficial mutations in bacterial batch culture

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Abstract

The survival of rare beneficial mutations can be extremely sensitive to the organism’s life history, and the trait affected by the mutation. Given the tremendous impact of bacteria in batch culture as a model system for the study of adaptation, it is important to understand the survival probability of beneficial mutations in these populations. Here we develop a life-history model for bacterial populations in batch culture, and predict the survival of mutations that increase fitness through their effects on specific traits: lag time, fission time, viability and the timing of stationary phase. We find that if beneficial mutations are present in the founding population at the beginning of culture growth, mutations that reduce the mortality of daughter cells are the most likely to survive drift. In contrast, of mutations that occur *de novo* during growth, those which delay the onset of stationary phase are the most likely to survive. Our model predicts that approximately five-fold population growth between bottlenecks will optimize the occurrence and survival of beneficial mutations of all four types. This prediction is relatively insensitive to other model parameters, such as the lag time, fission time or mortality rate of the population. We further estimate that bottlenecks that are more severe than this optimal prediction substantially reduce the occurrence and survival of adaptive mutations.

Keywords: fixation probability, serial passaging, adaptation, experimental evolution, life history
INTRODUCTION

It is well understood that most de novo mutations, even if they confer a substantial fitness advantage to the organism, do not survive the vicissitudes of genetic drift when initially rare (Fisher 1922; Haldane 1927; Wright 1929; Kimura 1964). The influences of population size, population structure, and environmental fluctuations on the fate of beneficial mutations have all been well studied, including cyclic (Otto and Whitlock 1997; Pollak 2000) or dynamically changing population sizes (Lambert 2006; Parsons and Quince 2007a; Parsons and Quince 2007b), population subdivision (Barton 1993; Cherry 2003; Whitlock 2003) and migration (Lundy and Possingham 1998; Shpak and Proulx 2007), fluctuating selection (Haccou and Iwasa 1996; Lande 2007), or several of these factors in combination (Uecker and Hermisson 2011; Waxman 2011).

A phenomenon that is perhaps less well appreciated is the sensitivity of a beneficial mutation’s fate – survival or extinction – to the details of the organism’s life history and the trait affected by the mutation. Studies of the extinction process in a population of changing size have demonstrated that extinction probabilities sensitively depend on whether mutations increase birth rates or reduce death rates (Parsons and Quince 2007a; Parsons and Quince 2007b), or if mutations affect other life history traits (Lambert 2006). Previous work has also compared the fate of mutations that reduce generation times with those that increase offspring survival (Wahl and DeHaan 2004). From these studies a clear picture is emerging: beneficial mutations that confer the same selective advantage, once established, can have quite different probabilities of surviving drift when rare.
While these effects have been well studied in viruses (Alexander and Wahl 2008; Patwa and Wahl 2008), the survival of de novo mutations in bacterial populations has received relatively little attention. Using a model specific to bacterial fission, Johnson and Gerrish demonstrated that if single strand DNA damage is incorrectly repaired immediately prior to replication, the fixation probability of the resulting mutation is $\approx 4s$, where $s$ is the selective advantage; in contrast, the familiar $2s$ was recovered for a mutation occurring through a copy error during DNA replication (Johnson and Gerrish 2002). The impact of the severe population bottlenecks inherent in bacterial serial passaging has also been examined in some detail (Wahl and Gerrish 2001; Wahl et al. 2002), but this work imposed the mathematically convenient assumptions that offspring distributions are Poisson-distributed, while generation times are either fixed, with a coefficient of variation (CV) of zero, or exponentially distributed, with a CV of one. Exponentially-distributed generation times have also been used in deriving the survival probability of beneficial mutations in the context of evolutionary rescue (Martin et al. 2013).

A Poisson distribution allows a single individual to have three or more offspring per generation; as pointed out by Gerrish and Lenski 1998, this is clearly an inaccurate model for bacterial fission. However it is unclear which evolutionary predictions are sensitive to this inaccuracy. Similarly, bacterial fission times are neither precisely fixed, nor exponentially distributed. For example, early observations of isolated cells under a light microscope demonstrated a unimodal distribution of fission times (Powell 1958). More recently, the development of microfluidic devices has allowed for detailed single-cell observations of bacterial fission on an unprecedented scale (Elfwing et al. 2004; Wakamoto et al. 2005; Siegal-Gaskins and Crosson 2008), demonstrating that fission times within the first few generations of a lineage remain correlated, with a CV of about 30%. 
Finally, bacterial populations in batch culture have become perhaps the most influential model system for the study of adaptation (Kawecki et al. 2012; Kussell 2012; Barrick and Lenski 2013), and growth in batch culture is characterized by its own “life history” traits. In particular, growth begins only after a well-documented delay, the lag phase, and continues until the population density is high and resources are depleted; when resources are sufficiently low, cell replication ceases and the relatively quiescent stationary phase begins.

Given the tremendous impact of bacterial serial passaging as an experimental system, particularly for the study of adaptation, it is important to understand how survival probability filters our view of the adaptive process. Are mutations that confer particular effects on life history more or less likely to survive when rare? How do population bottlenecks bias our observations of beneficial mutations? We sought to answer these questions by building a detailed life history model for bacteria in batch culture, and predicting the survival of mutations that increase fitness through their effects on specific traits: lag time, fission time, viability and the timing of stationary phase.

**LIFE HISTORY MODEL**

**Wildtype population:** We propose a model in which a population of wildtype bacteria, after lag time \( L \), grows by binary fission until entering the stationary phase at time \( T \). During the growth phase, the average fission time is \( F \); on division, each offspring is non-viable with probability \( M \). Thus the wildtype population size at time \( T \) can be approximated by:

\[
N(T) = N_0(2(1 - M))^{\frac{T - L}{F}} ,
\]
where $N_0$ is the wildtype population size at time zero. This model assumes that the wildtype population is sufficiently large that fission times, after the lag phase, are uncorrelated. In this case the distribution of fission times has no effect on the wildtype, and the wildtype population grows exponentially at rate:

$$r = \frac{\ln(2(1 - M))}{F},$$

and the number of wildtype doublings per growth phase is:

$$n = \log_2\left(\frac{N(T)}{N_0}\right) = \frac{T - L}{F} \left(1 + \log_2(1 - M)\right).$$

We assume that growth of the wildtype population stops completely at time $T$, and that cell death during stationary phase is negligible. Sometime after $T$, the population is diluted by a factor $D = \exp(-r(T - L))$ such that $DN(T) = N_0$, and the random sample of $N_0$ individuals thus obtained is used to found the subsequent population.

**Beneficial mutations:** We study the fate of *de novo* beneficial mutations that arise in the background of this wildtype population. Beneficial mutations may affect four possible traits, and are characterized by a reduction in either lag time $\lambda = L(1 - \delta_L)$, mean fission time $\phi = F(1 - \delta_F)$, or mortality $\mu = M(1 - \delta_M)$, or by a delay in the onset of stationary phase, $\tau = T(1 + \delta_T)$. It is clear that bacteria do not enter stationary phase at a fixed time, but rather in response to signals related to resource availability and population density. While the mutant subpopulation remains rare, however, it has negligible effect on the wildtype growth curve and so we use time as a proxy for these effects.

The distribution of fission times is an important feature of the mutant lineage when rare. To allow
for fission times that are neither fixed nor widely dispersed, we use a multitype, continuous time branching process to model the growth of the mutant subpopulation. Here, we assume that an individual cell must proceed through $k$ arbitrary stages before fission. This results in fission times that have mean $\phi$, but are gamma-distributed with shape parameter $k$. We can choose $k$ to explore fission times that are widely or narrowly dispersed; gamma distributions with the same mean but shape parameters $k = 1, 3, 5, 7$ and $9$ are shown in Figure 1 for illustration. At $k = 1$, we recover the exponential distribution with a CV of 1, while a CV of 33% (WAKAMOTO et al. 2005) corresponds to a value of $k \approx 9$.

The approach we use to estimate extinction probabilities is to derive probability generating functions (pgfs) describing the number of offspring in a mutant lineage. A powerful advantage of pgfs is that the extinction probability of a branching process is given by the fixed point of the pgf describing one “generation” of the process (ALLEN 2010). Overall, our strategy is to think of a single cycle of growth and sampling as a “generation”, and to thus compute the probability that the mutant lineage goes extinct after many such generations. We derive the necessary equations in the Appendix.

Finally, in order to compare mutations that affect different traits but have the same fitness, we estimate the Malthusian growth rate, $\rho$, of an established mutant population, assuming it has reached its equilibrium stage distribution. Numerically, we do this by following a successful mutant lineage for long times, until the population growth rate is nearly exponential. The selective advantage of the mutant, $s$, is then defined by:

$$e^{\rho(\tau-\lambda)-r(T-L)} = (1 + s)^n$$

where $n$ is the number of wildtype doublings per growth phase as defined previously.
**Parameter Values:** In the figures to follow, we base our parameters on values estimated for the long-term serial passaging of *E. coli* (Vasi et al. 1994). After a lag time of $L = 1.5$ hours, the population grows until $T = 8$ hours, for a net growth phase of 6.5 hours.

The proportion of bacterial daughter cells that are non-viable, during normal growth conditions in culture, has been estimated to be very low, on the order of one percent (Powell 1958). Thus a realistic value of our mortality parameter, $M$, is 0.01, and beneficial mutations that reduce mortality have little effect. In the figures to follow, however, we will use $M = 0.2$ for illustrative purposes, such that the effects of reduced mortality are visible. Higher mortality might be relevant when the bacterial population is cultured under stressful environmental conditions such as temperature stress (Bennett et al. 1990), UV irradiation (Alcántara-Díaz et al. 2004), or high ethanol (Goodarzi et al. 2010), and more generally in situations of evolutionary rescue (Alexander et al. 2014). We will later examine the sensitivity of our results to mortality in Figure 5.

The histogram in the second panel of Figure 1 illustrates the distribution of interdivision times recorded for single cells of *E. coli* followed for ten generations in a microchamber array (Wakamoto et al. 2005); the mean fission time was 52 minutes, with a CV of 0.33. This CV corresponds to a gamma distribution with shape parameter $k = 9$, also shown for comparison. Although clearly fission times and their dispersion about the mean will vary with experimental protocol, we take $k = 9$ and $F = 1$ hour as reasonable estimates in the quantitative work to follow. This implies that 6.5 doublings, or approximately 100-fold growth occurs before the onset of stationary phase.
RESULTS

Figure 2 illustrates survival probability versus the selective advantage, $s$, of mutations affecting different traits, assuming these mutations are initially present in the founding population before the growth phase begins. Note that lag time, fission time and mortality cannot be reduced below zero, and thus the selective advantage that can be realized by mutations of these types has an upper bound. Theoretically, the onset of stationary phase can be delayed indefinitely, and so the selective advantage of stationary phase mutations is unbounded in the model; in reality the depletion of resources would in fact limit this growth and impose some upper bound. We illustrate here the survival of mutations that occur in an individual in the first of $k$ maturation stages, that is, the mutation first occurs in a single copy immediately after binary fission.

Note that survival is influenced by two factors: the mutant lineage may go extinct due to the mortality inherent in the branching process ($M$); or the lineage may be eliminated through population bottlenecks ($D$). We can isolate these effects by noting that in the absence of bottlenecks, the extinction probability is the solution to:

$$X = (\mu + (1 - \mu)X)^2$$

which gives survival probability $\pi = 1 - (\frac{\mu}{1 - \mu})^2$. This value gives the survival probability if the population experienced infinite growth, and forms an upper bound on survival probability, as illustrated by the dotted lines in Figure 2. For mutations that do not change mortality, survival approaches the horizontal dotted line given by $\pi = 1 - (\frac{M}{1-M})^2$; for mortality mutations, survival approaches the curved dotted line since $\mu$ changes with $s$. Here we have used $M = 0.2$ for clarity; for many realistic experimental systems with $M \approx 0.01$, these upper bounds would in fact be close
to unity. We note that the difference between survival illustrated in Figure 2 and the appropriate upper bound is the contribution of the population bottlenecks.

Figure 2 predicts that mutations increasing the number of generations during the growth phase, that is, lag time, fission time and stationary phase mutations, all have equivalent survival probabilities for mutations of equivalent $s$. However, two interesting points emerge. First, we note that mutations that reduce mortality have a substantially higher survival probability than predicted by their fitness effect. Again, this phenomenon is markedly visible at $M = 0.2$; clearly when $M = 0.01$ the effect would be subtle. Nonetheless, in experimental protocols in which mortality is non-negligible, we predict that the survival of de novo mutations may be biased in favour of mutations that reduce mortality.

Secondly, we note the difference between the survival probabilities predicted here, and the classical prediction $1 - \exp(-2s)$ for a large population maintained at a constant size (grey line), or the well-known approximation $2s$ which is valid when $s$ is small (black line). As seen in similar models (Otto and Whitlock 1997; Wahl et al. 2002), the advantage to the mutant lineage of several generations of population growth outweighs the deleterious effects of the bottleneck, and the survival of beneficial mutations is increased relative to a constant-sized population.

These results change, however, if we consider mutations that occur de novo at some time $t_0$ during the growth phase. The top panel of Figure 3 illustrates that mutations occurring later during the growth phase have a reduced survival probability. While this is unsurprising, we note some interesting trait-specific differences in this effect. Most markedly, we see that mutations that reduce
lag time have reduced survival compared to the other types. This stems from the fact that these
mutant lineages are unable to realize any selective benefit during the growth phase in which they
first occur; only after surviving the first bottleneck does their reduced lag time come into effect.
(In contrast, Figure 2 shows survival for mutations that are present in a single copy in the founding
population, and realize this benefit immediately.) We also note in Figure 3 that when occurring
late, mutations that delay stationary phase have higher survival than mutations of other types,
again because these mutations are able to realize an advantage before the bottleneck.

Although this advantage to late-occurring stationary phase mutations appears subtle, beneficial
mutations are exponentially more likely to occur late in the growth phase due to the growth curve
of the wildtype. In the second panel of Figure 3 we show the product of de novo occurrence, which
we assume to be proportional to the number of fission events in the wildtype population at any
time, and survival probabilities. These values are thus proportional to the number of mutations
that are predicted to occur at time $t$, and ultimately survive. To allow comparison, the de novo
mutation rate to the various beneficial mutation types, $u_b$, is scaled out, and assumed equal across
types. Here we see fairly dramatic differences: given equivalent mutation rates, mutations that
delay the onset of stationary phase are predicted to emerge most frequently, while mutations that
reduce lag time are the least likely to emerge.

We can also integrate the curves in the lower panel of Figure 3 to predict the total influx of successful
new mutations per serial transfer, for growth phases of different lengths. To allow comparison, we
assume that the final population density is the limiting factor, and thus hold $N_f$ constant while
changing $N_0$ to produce growth phases of different lengths. Note that for longer transfer times,
more mutations occur, but fewer survive because the bottleneck becomes more severe. In Figure 4 we show the results of this calculation for wildtype growth phases, $T - L$, of various lengths. For mutations of all four types, we observe that adaptation rates are minimized by growth phases that are either very short (one generation time) or very long (6 or 7 generation times). In the top panel when $M = 0.2$, we find that bottleneck times that allow for three or four generations of growth maximize the rate at which successful new mutations emerge. The optimal bottleneck time, however, changes with the mortality rate. In the lower panel of Figure 4, when $M = 0.01$, the best growth phase is about 2.5 fission times. We also point out that this effect is not subtle; the $y$-axis units are proportional to the number of surviving mutations per transfer. Thus the model predicts that when $M = 0.01$, a growth phase of 6.5 generation times results in a 60-70% loss of adaptive mutations, compared to a growth phase of 2.5 generation times.

Figure 5 illustrates the dependence of the optimal length of the growth phase on mortality. The top panel illustrates that the growth phase should be longer when mortality is higher. However, when mortality is higher, the growth rate of the population decreases concomitantly. These effects precisely balance and, as shown in the top right panel of Figure 5, the optimal bottleneck fraction, $D$, is independent of mortality. These results predict that to optimize the number of successful beneficial mutations per serial transfer, the population should only grow about 5-fold between transfers.

An important question is whether this optimal bottleneck fraction is sensitive to the other parameter values we have assumed. We can non-dimensionalize time in this model relative to the mean fission time, and thus our predictions for the optimal number of generations per growth phase do not
depend on $L$ or $F$. However, these predictions do vary slightly for mutations affecting different traits, as seen in Figure 5, and may also vary for mutations of different magnitudes. We illustrate the latter effect in the lower left panel of Figure 5. Here we see that for very small effect mutations ($s \leq 0.02$), the optimal bottleneck fraction is larger, implying only 3-fold growth between transfers. However, our predictions reach a plateau, and for a range of $s$ values and mutation types, we predict that 5-fold growth between serial transfers is optimal for adaptation. The exception to this trend is mutations that delay the onset of stationary phase. The model predicts that these mutations have a greater survival advantage when the bottleneck fraction is smaller, i.e., for longer periods of growth between transfers. We will return to this observation in the Discussion.

We would also like to examine the degree to which these predictions are sensitive to our assumptions regarding the distribution of fission times. The lower right panel of Figure 5 shows how the optimal bottleneck fraction changes with the shape parameter for intermediate effect mutations, $s = 0.1$, of all four types. We find that the optimal growth of the population is quite insensitive to the dispersion of the fission times; as long as the shape parameter exceeds two or three, the optimal fraction is close to the asymptotic value. For mutations that reduce mortality or reduce fission times, the optimal bottleneck fraction corresponds once again to about 5-fold population growth; the optimal fraction is slightly larger for mutations that reduce the lag phase, and smaller for mutations that delay the onset of stationary phase.

Throughout this contribution, we have considered mutations that affect only one life history trait. The approach we describe can likewise be applied to mutations affecting several traits. In this case, given the four trait values for a mutant lineage, we can compute the mutant growth rate and thus
a net selection coefficient for the overall effect of the pleiotropic mutation. Although numerous functional trade-offs in pleiotropic effects are possible, in Figure 6 we examine the somewhat trivial case in which a single mutation has a randomly-distributed effect on each of the four life history traits. Thus we impose no particular trade-off function or correlation. In the top panel, we restrict our attention to purely beneficial mutations, that is, mutations that have a random beneficial effect on all four life history traits. Here we find that the survival probability of pleiotropic mutations lies within the bounds outlined by ‘pure’ mutations of each type. More realistically, in the lower panel, we allow both beneficial and deleterious effects on each life history trait, as long as the overall selective effect of the pleiotropic mutation is positive. In this case, we again see that mutations tend to have similar survival probabilities to ‘pure’ one-trait mutations, but are no longer bounded by these curves. A fuller examination of this phenomenon, including physiologically-motivated trade-off functions, would be an interesting path for future work.

DISCUSSION

Several of our results follow from the inherent effects of repeated bottlenecks, and thus echo similar results obtained for models of viral evolution (WAHL et al. 2002; PATWA and WAHL 2008). We predict that beneficial mutations are more likely to survive in a bottlenecked population than in a large population of constant size, because the benefits of population growth (OTTO and WHITLOCK 1997; POLLAK 2000) outweigh the impact of the bottleneck on survival.

The fact that mortality mutations have a higher chance of surviving, relative to other mutations with the same selective advantage, has also been previously predicted (ALEXANDER and WAHL 2008).
While mortality and fission time both affect the overall growth rate of the population, mortality has an incommensurate impact on survival, because loss during the first few critical generations of the lineage predisposes the lineage toward extinction. Thus if we compare the fate of beneficial mutations that are present in the founding population, mutations that reduce mortality are the most likely to survive drift.

In contrast, Figure 3 demonstrates that of mutations occurring *de novo* during the growth phase, mutations that delay the onset of stationary phase are somewhat more likely to survive, while mutations that reduce lag time are less likely. These results follow from the fact that a *de novo* mutation that delays stationary phase is able to realize an advantage immediately, during the growth phase in which it occurs, whereas a mutation that reduces lag time must first survive the population bottleneck before realizing any advantage. As shown in Figure 3, the effect is further amplified because the vast majority of *de novo* mutations occur late in the growth phase.

These predictions address the probability that a specific life history mutation survives drift. Clearly, the adaptive trajectory also depends on background mutation rates (e.g. *cis* versus *trans*, ROKYTA et al. 2005), epistasis (WEINREICH et al. 2005), and other factors affecting the availability of mutational pathways or ‘findability’ of genotypes (MCCANDLISH 2013). In addition, we compare mutations that confer equivalent effects on fitness, as measured by the long-term growth rate. However, if a relatively large change in trait value is necessary to confer this fitness effect, the mutational pathway may also be less accessible from a physiological perspective.

One way to address the latter issue is to compute the selection gradient for each trait, that is, to
measure the sensitivity of fitness to a relative change in trait value. For fitness $W$ and trait value $Y$, the selection gradient is given by $(\partial W/\partial Y)(Y/W)$ (Vasi et al. 1994). Table 1 shows the selection gradients computed for the four traits we consider, using the experimentally more relevant value of mortality, $M = 0.01$.

<table>
<thead>
<tr>
<th>Fitness Component</th>
<th>Selection Gradient</th>
<th>Fitness Component</th>
<th>Selection Gradient</th>
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<tbody>
<tr>
<td>$L$</td>
<td>-0.16</td>
<td>$L$</td>
<td>-0.25</td>
</tr>
<tr>
<td>$F$</td>
<td>-0.69</td>
<td>$V_m$</td>
<td>1.0</td>
</tr>
<tr>
<td>$T$</td>
<td>0.85</td>
<td>$K_s$</td>
<td>0.0066</td>
</tr>
<tr>
<td>$M$</td>
<td>-0.01</td>
<td>$D$</td>
<td>0</td>
</tr>
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Table 1: Selection gradients for each fitness component.

In a study of the long-term evolution of *E. coli* in batch culture, Vasi et al. 1994 also used a life-history model to examine changes in life-history traits. The model includes a lag time, $L$, after which growth is described by a Monod model of resource use. Thus the bacterial growth rate depends on resource concentration, and is described by two parameters: the maximum growth rate at high resource concentrations, $V_m$; and the resource concentration at which growth is half the maximum value, $K_s$. Once the resource is exhausted, growth halts and the model includes a death rate during stationary phase, $D$. Using parameter values measured in the ancestral population, Vasi et al. 1994 computed the selection gradients for these four traits; we provide these estimates in Table 1 for comparison.

We note that the selection gradients for lag time and growth rate (measured in the inverse as a fission time, $F$, in our model) are very similar between the two studies. As mentioned before, the death rate during stationary phase was not included in our model, but mortality during the growth
phase has a similarly low selection gradient when $M = 0.01$ (for comparison, when $M = 0.2$ the selection gradient increases in magnitude to -0.38).

The two approaches differ most clearly in their predictions regarding mutations that allow cell growth and division to continue after the wildtype has entered stationary phase. A simplification in our model is that we do not include resource concentration explicitly, and we therefore do not posit a specific mechanism by which the mutant lineage could continue growing after time $T$. From the selection gradient above, we see that mutations that could extend $T$ would be strongly selected for, and our previous results suggest these mutations would also have a high probability of surviving drift. However, the selection gradient computed by Vasi et al. 1994 suggests that such mutations, although they might involve only a small relative change in $T$, would involve a very large relative change in $K_s$, and may not be physiologically accessible. This insight is confirmed in the changes to life-history traits measured by Vasi and colleagues: lag time was reduced and growth rate was increased in the derived populations, but $K_s$ changed to a slightly higher value, which would have a negative effect on fitness. To address these issues in future work, it is clear that our model should be extended to explicitly include a finite carbon source and its depletion during population growth.

As seen in Figure 5, our model predicts that approximately five-fold population growth between bottlenecks will optimize the occurrence and survival of beneficial mutations in bacterial populations. This prediction is surprisingly robust: we found that it is not sensitive to the lag time, fission time or mortality rate of the population, and is most accurate for mutations with selective advantages within a ten-fold range, between about 0.02 and 0.2. The prediction is also not sensitive to the details of the fission time distribution, as long as this distribution is peaked (not exponential).
We further estimate that bottlenecks that are more severe than this optimal prediction can substantially reduce adaptation rates; for example, we expect that a ten-fold growth phase in bacteria can reduce adaptation by 60-70%. Although optimizing adaptation rates on theoretical grounds is unlikely to be a critical factor in most experimental design, this prediction may be weighed along with the numerous practical considerations in order to speed (or slow) the evolutionary trajectory under study.

Our approach to date has considered mutations affecting only four life history traits, each of which has a direct impact on fitness in bacterial serial passaging. It is clear however that a much wider range of adaptive strategies is possible in these populations. As mentioned above, the depletion of a finite resource is a clear avenue for future investigation. The influence of cell size in sequestering this resource (Vasi et al. 1994), in survival through stationary phase, or in survival through the bottleneck (Handel and Bennett 2008) could then also be addressed.

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APPENDIX

We begin with the probability generating functions (pgfs) describing the growth of an individual cell in the mutant lineage. Cells in stage $i < k$ proceed to the next stage at rate $k/\phi$, producing one cell in stage $i + 1$. Cells in the final stage $k$ likewise mature at rate $k/\phi$, producing two offspring; each of these offspring survives with probability $1 - \mu$. Thus, the pgfs for the offspring of each stage are:

$$P_i(\vec{s}) = \begin{cases} 
  s_{i+1}, & 1 \leq i < k \\
  (\mu + (1 - \mu)s_1)^2, & i = k 
\end{cases}$$

where $\vec{s} = [s_1 \ s_2 \ldots s_k]$.

We now let the pgf $G_i(t, \vec{s})$ describe the number of individuals in the mutant lineage at time $t$, starting with a single individual of type $i$ at time 0. For $k = 2$, this means

$$G_i(t, \vec{s}) = \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} p_{m,n}(t)s_1^m s_2^n$$

where $p_{m,n}(t)$ is the probability that there are $m$ type-1 mutants and $n$ type-2 mutants in the lineage at time $t$.

If there are $m$ type-1 mutants and $n$ type-2 mutants in the lineage at time $t$, then in timestep $\Delta t$, the probability that a type-1 mutant matures is given by $m\Delta t\frac{k}{\phi}$, and the probability mass $m\Delta t\frac{k}{\phi}p_{m,n}$ should be subtracted from the coefficient of $s_1^m s_2^n$ and added to the coefficient of $s_1^{m-1}s_2^{n+1}$. Taking into account the maturation of type-1 mutants only, this yields for example:

$$G_1(t + \Delta t, \vec{s}) = G_1(t, \vec{s}) - \Delta t\frac{k}{\phi} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} mp_{m,n}(t)s_1^m s_2^n + \Delta t\frac{k}{\phi} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} mp_{m,n}(t)s_1^{m-1}s_2^{n+1}.$$
Similarly, the probability that a type-2 mutant matures is given by $n \Delta t \frac{k}{\phi} p_{m,n}$, and the probability mass $n \Delta t \frac{k}{\phi} p_{m,n}$ should be likewise subtracted from the coefficient of $s_1^m s_2^n$. However in this case, zero offspring survive with probability $\mu^2$, and thus the probability mass $\mu^2 n \Delta t \frac{k}{\phi} p_{m,n}$ should be added to the coefficient of $s_1^m s_2^{n-1}$; corresponding probabilities are added to $s_1^{m+1} s_2^{n-1}$ and $s_1^{m+2} s_2^{n-1}$ for the cases when one or two offspring survive. Adding these terms to the expression above and taking the limit as $\Delta t \to 0$, we obtain

$$\frac{\partial}{\partial t} G_1(t, \vec{s}) = \frac{k}{\phi} \left[ \frac{\partial G_1}{\partial s_1} (P_1(\vec{s}) - s_1) + \frac{\partial G_1}{\partial s_2} (P_2(\vec{s}) - s_2) \right].$$

For higher $k$, this result generalizes in a straightforward way, yielding:

$$\frac{\partial}{\partial t} G_i(t, \vec{s}) = \frac{k}{\phi} \sum_j \frac{\partial G_i}{\partial s_j} (P_j(\vec{s}) - s_j) \quad (2)$$

with initial and boundary conditions

$$G_i(t = 0, \vec{s}) = s_i$$

$$G_i(t, s_1 = 1, \ldots, s_k = 1) = 1 .$$

The initial condition follows from the definition of the $G_i$, while the boundary condition applies to all pgfs by definition.

To obtain numerical results, we use the method of characteristics, writing the relation:

$$\frac{\partial G_i}{\partial t} \frac{dt}{dy} = \sum_{j=1}^{k} \frac{\partial G_i}{\partial s_j} \frac{ds_j}{dy} .$$

Comparison with Equation 2 implies the corresponding relations

$$\frac{dt}{dy} = \frac{\phi}{k}$$

$$\frac{ds_j}{dy} = P_j(\vec{s}) - s_j .$$
Hence for $j = 1, ..., k$, we have the following system of ordinary differential equations:

$$\frac{ds_j}{dt} = \frac{ds_j}{dy} \frac{dy}{dt} = \frac{k}{\phi_j} (P_j(s) - s_j)$$

(3)

and, usefully, points along a trajectory in $\vec{s}$ have the same values of $G_i$.

Integrating backward in time, Equation 3 allows us to associate any point at time $t$ with a point at time zero; for example with $k = 2$, we can associate $(s_1, s_2)$ at time $t$ with $(s'_1, s'_2)$ at time zero. In this case, $G_i(t, s_1, s_2)$ must be equal to $G_i(0, s'_1, s'_2)$, which is known from the initial conditions. We can thus use Equation 3 to build the surface $G_i(t, \vec{s})$ at any time.

In particular, for a mutation that exists in a single copy in the founding population, $G_i(\tau - \lambda, \vec{s})$ describes the mutant lineage after a single growth phase, at which time it will be diluted for serial transfer. Although the physiological effects of dilution and transfer to fresh medium are not well understood, at the population level it is clear that fission resumes only after the lag time, $\lambda$, has elapsed. It also seems reasonable to assume that cells that were closer to fission before the bottleneck (presumably larger cells) will also divide more quickly once growth resumes. Thus, we assume that cells retain their stage through the transfer process, and, if they survive the bottleneck, begin anew in that stage at time $\lambda$ in the subsequent culture. This assumption gives the very simple pgf, $b(x) = 1 - D + Dx$, for survival of each individual through the population bottleneck at serial transfer.

The vector of extinction probabilities $X_i(0)$ for mutant lineages starting in stage $i$ at time 0 is then given by the fixed point of the growth pgf composed with the bottleneck pgf:

$$\vec{X}(0) = \vec{G}(\tau - \lambda, \vec{b}(\vec{X}(0)))$$
where $\tilde{b}(\vec{X}) = [b(X_1) \ b(X_2) ... b(X_k)]$. The extinction probabilities $\vec{X}(0)$ can then be approximated by straightforward iteration, and the survival probability for a mutation that first appears in a single cell in the founding population is given by $1 - X_1(0)$ (for mutations that first occur in two copies (Johnson and Gerrish 2002), survival would be $1 - X_1(0)^2$.) As derived previously (Hubbarde et al. 2007), for a mutation that first appears at time $t_0$ during the growth phase, the extinction probabilities are given by $\vec{X}(t_0) = G(\tau - \lambda - t_0, \tilde{b}(\vec{X}(0)))$. 
Figure 1: Distribution of fission times. In the top panel, gamma distributions with the same mean but with shape parameter, $k = 1, 3, 5, 7$ and 9, are plotted. The peak in the distribution moves from left to right as $k$ increases. In the lower panel, the distribution of interdivision times recorded for single cells of *E. coli* followed for ten generations in a microchamber array (Wakamoto *et al.* 2005) are shown (grey bars), along with a gamma distribution with the same mean (52 min) and CV (33%), corresponding to $k=9$. 
Figure 2: Survival probability for beneficial mutations of different types. The survival probability for a mutation initially present in a single copy in the founding population, $1 - X_1(0)$, versus the selective advantage, $s$, for mutations that reduce lag time (green triangles), reduce fission time (blue crosses), reduce mortality (black x's) or delay stationary phase (red circles). In each case, the other three life history parameters are fixed at the wildtype values. Survival in an infinitely growing population (dotted lines) or in a large population of constant size (grey and black lines) are shown for comparison.
Figure 3: Fate of beneficial mutations occurring at different times. In the top panel, the survival probability of beneficial mutations is plotted versus the time, during the growth phase, at which the mutation first appears. All mutations have selective advantage $s = 0.1$, but affect different traits. In the lower panel, the relative numbers of mutations that occur at different times during growth and ultimately survive are plotted. Mutations are assumed to occur at rate $u_b$ per fission, such that the number of de novo mutations is proportional to the growth rate of the population at time $t_0$. This number is multiplied by the survival probability for mutations that emerge at time $t_0$. For consistency with figures to follow, the result is normalized by $u_b$ and the final population size, $N_f$. Thus $y$-axis units correspond to $(N_0/N_f) e^{r t_0} (1 - X_1(t_0))$. When multiplied by $N_f$ and $u_b$ for a particular experimental design, the rate per unit time at which surviving mutations appear is obtained. Colours and symbols are as described for Fig. 2.
Figure 4: Total successful mutations for growth phases of different lengths. The relative number of mutations that are expected to occur during the growth phase and ultimately survive is plotted versus the length of the growth phase, $T - L$. In comparing growth phases of different lengths, we assume that the final population size, $N_f$, is constant. In the top panel, parameter values are as described in the main text, with $s = 0.1$ but with varying $T$. Parameter values in the lower panel are the same but with lower mortality, $M = 0.01$; note that in this case a mortality mutation conferring $s = 0.1$ is not possible. Units on the $y$-axis are as described for Fig. 3, but have been integrated from $t_0 = L$ to $t_0 = T$. When multiplied by $N_f$ and $u_b$ for a particular experimental design, the rate per transfer at which surviving mutations appear is obtained. Colours and symbols are as described for Fig. 2.
Figure 5: Optimal bottleneck fraction. In the top left, the length of the growth phase, $T - L$, which yields the highest expected number of successful mutations is plotted versus mortality, $M$, for mutations affecting different traits with selective effect $s = 0.1$. The top right panel shows the same results, but plots the optimal bottleneck fraction, $D$. Note that $s = 0.1$ cannot be achieved by reducing mortality (black x’s) when $M$ is small. In the lower panels, the optimal bottleneck fraction is plotted versus the selective advantage of the beneficial mutation (left panel), and versus the shape parameter of the gamma distribution of fission times (right panel). Colours and symbols are as described for Fig. 2.
Figure 6: Survival probability versus overall selection coefficient for pleiotropic mutations. The survival probability for a mutation initially present in a single copy in the founding population, \(1 - X_1(0)\), versus the selective advantage, \(s\), for mutations that have effects on all four life history traits. In the top panel, mutational effects are drawn from uniform distributions such that \(0 < \lambda < L\), \(T < \tau < T + F\), \(0 < \phi < F\) and \(0 < \mu < M\). In the lower panel, \(0 < \lambda < 2L\), \(T - F < \tau < T + F\), \(0.5F < \phi < 1.5F\) and \(0 < \mu < 1.5M\). For both panels, after randomly drawing the four trait values, the overall \(s\) value of the mutation is computed and the mutation is included in the figure as a grey dot if \(0 < s < 1\). Other symbols show results for mutations affecting one trait only, as described in Fig. 2. In both panels, \(M = 0.2\).