Mutation accumulation in exponentially growing populations

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Abstract

Stochastic models of mutation accumulation in exponentially growing cellular populations are widely used to quantify cancer and bacterial evolution. Across manifold scenarios, recurrent research questions are: how many cells exist with a given set of alterations, and how long will it take for these cells to appear. These questions have been tackled in special cases, often within a branching processes framework. However, the general situation of cells sequentially acquiring an arbitrary number of mutations which may be selectively advantageous, neutral, or disadvantageous remains unaddressed. Here, we consider this setting in the biologically relevant limiting regimes of large times and small mutation rates. We provide analytic expressions for the number, and arrival time, of cells with \( n \) mutations. Universal probability distributions for both quantities are presented, and the consequences of our results on cancer driver mutation accumulation and bacterial fluctuation assays are highlighted.

1 Introduction

To quantitatively characterise diseases, in settings such as cancer, and bacterial and viral infections, a concerted effort has been made to study evolutionary dynamics in exponentially expanding populations. Understanding the timescale of evolution is a key aspect of this research program which has proven useful in a diverse range of areas such as: measuring

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mutation rates [1], assessing the likelihood of therapy resistance developing [2, 3], inferring the selective advantage of cancer driver events [4, 5], and exploring the necessary steps in the metastatic process [6, 7]. The common theme within these works is that we use information about when a particular cell type arises within the population of interest. For a concrete example, whose roots lie in the celebrated work of Luria and Delbrück [1], if we imagine a growing colony of bacteria, we might wish to know how quickly a mutant bacterium will develop with a specific mutation that confers resistance to an antibiotic therapy.

The time until a cell type emerges, and expands to a detectable population size, depends on a variety of factors. Most obvious are the relevant mutation rates, however selection also plays an important role. For instance, if we start an experiment with an unmutated cell and wait for a cell with 2 mutations, a low division rate of cells with one mutation slows down this process. In the scenario of the sequential acquisition of driver alterations in cancer, with each mutation providing a selective advantage, Durrett and Moseley characterised the time to acquire \( n \) driver mutations [8]. We recently examined the setting of drug resistance conferring mutations, which often have a deleterious effect, so that the original cell type grew the fastest [9]. However, in general, the effects of mutation and selection on evolutionary timescales within exponentially growing populations remains unclear.

In this study we build upon the mathematical machinery developed in Refs. [8, 9] to investigate this question in extensive scope. We focus on the biologically relevant setting of the small mutation rate limiting regime. Broad-ranging features of the waiting time distribution are highlighted - including a universal simple distribution - and explicit expressions make the impact of mutation and selection clear. En route, we present results on the number of cells of a given type as a function of time.

2 Model and summary of results

Model: We consider a population of cells, where each cell can be associated with a given ‘type’ (for example ‘type 3’ might be cells with 3 particular mutations). Cells of type \( n \) divide, die, and mutate to a cell of type \( n + 1 \), at rates \( \alpha_n \), \( \beta_n \) and \( \nu_n \); if \((n)\) represents a type \( n \) cell and \( \emptyset \) symbolises a dead cell then with all cells behaving independently, our cell level dynamics can be represented as (see also Fig. 1 A):

\[
(n) \rightarrow \begin{cases} 
(n), (n) & \text{at rate } \alpha_n \\
\emptyset & \text{at rate } \beta_n \\
(n), (n+1) & \text{at rate } \nu_n.
\end{cases}
\]

The process starts with a single cell of type 1 at time \( t = 0 \), and we suppose the type 1 population survives (does not undergo stochastic extinction). We focus on two quantities; the number of cells of type \( n \) at \( t \) - denoted \( Z_n(t) \), and the arrival time of the first type \( n \) cell - termed \( \tau_n \) (see Fig. 1 B).
To describe the growth of the cellular populations, let the net growth rate of the type \(n\) cells be \(\lambda_n = \alpha_n - \beta_n\), and \(\delta_n\) be the ‘running-max’ fitness which is the largest growth rate of the cell types among \(1, \ldots, n\), i.e. \(\delta_n = \max_{i=1,\ldots,n} \lambda_i\). Further, we introduce \(r_n\) as the number of times the running-max has been obtained over the cell types up to \(n\), that is \(r_n = \#\{i = 1, \ldots, n : \lambda_i = \delta_n\}\).

Our model considers a linear evolutionary path of cells mutating from type 1 to 2 to 3, and so on. This could represent cells accumulating \(n\) cancer driver mutations or acquiring resistance to \(n\) drugs. Instead, one could consider a more general model of competing evolutionary paths, where the space of types is a directed graph (special versions of this have been seen in, e.g., [9, 10, 11, 12]). In such a setting, to determine the fastest path to a target evolutionary type, one may focus on each evolutionary path to the target type separately as a single linear path - which we treat in this paper - and then compare the median time to traverse each evolutionary path using the results presented below.
Results summary: Understanding the distribution of the number of cells of type \( n \) at a fixed time \( t \) (e.g. the probability that 5 cells exist of type 2 at \( t = 2 \)) can be complex [13], however a surprising level of simplicity emerges at large times with small mutation rates; the number of cells of type \( n \) can be decomposed into the product of a time-independent random variable and a simple time-dependent deterministic function controlled by the running-max fitness:

\[
Z_n(t) \approx V_n t^{r_n-1} e^{\delta_n t}.
\]  

Thus, on a logarithmic scale (as in Fig. 1C), at large times the number of cells approximately follows a straight line with gradient that increases only when the running-max fitness increases. Notably, if the type \( n \) cells have net growth rate smaller than the running-max fitness (\( \lambda_n < \delta_{n-1} \)), then as the large time behaviour is controlled by \( \delta_n = \delta_{n-1} \), we see the flux from the type \( n - 1 \) population eventually drives the cell growth. One can observe this behaviour in Fig. 1: Although the type 2 cells have lower fitness than type 1, the population sizes eventually both grow at the same rate of \( \lambda_1 \). However, the type 3 cells have the largest fitness so far, hence the cell number grows at its own rate \( \lambda_3 \).

The fluctuations in cell number are controlled by the random variable \( V_n \) which has a Mittag-Leffler distribution with tail parameter \( \lambda_1 / \delta_n \), and scale parameter \( \omega_n \). Its density has a particularly simple Laplace transform \( \mathbb{E} e^{-\theta V_n} = (1 + (\omega_n \theta)^{\lambda_1 / \delta_n})^{-1} \). The parameter \( \omega_n \) may be computed by the following recurrence relations: setting \( \omega_1 = \alpha_1 / \lambda_1 \), then for \( n > 1 \),

\[
\omega_{n+1} = \begin{cases}
\frac{\nu_n}{\delta_n - \delta_{n+1}} \omega_n & \delta_n > \lambda_{n+1} \text{ ‘stay below max fitness’} \\
\frac{\nu_n}{\delta_n} \omega_n & \delta_n = \lambda_{n+1} \text{ ‘equal to max fitness’} \\
\left[c_n \nu_n (\log \nu_n)^{-1} r_n^{-1} \omega_n \right]^{\lambda_{n+1} / \delta_n} & \delta_n < \lambda_{n+1} \text{ ‘increase max fitness’}
\end{cases}
\]  

where \( c_n = \pi \left( \frac{n+1}{\lambda_{n+1}} \right)^{\delta_n / \lambda_{n+1}} \left( \alpha_{n+1} \delta_{n+1}^{-1} \sin \frac{\pi \delta_n}{\lambda_{n+1}} \right)^{-1} \). Notably, when type 1 has the maximal growth rate of all types up to type \( n \), that is \( \delta_1 = \lambda_1 \), the Mittag-Leffler distribution collapses to an exponential distribution. Stochastic simulations of the scaled number of type \( n \) cells for large times, \( e^{-\delta_n t} (r_n^{-1}) Z_n(t) \approx V_n \), which according to Eq. (1) is Mittag-Leffler distributed, are compared with theory in Fig. 2. The variable \( V_n / \omega_n \) is a single parameter Mittag-Leffler random variable with scale parameter one, and tail parameter \( \gamma = \lambda_1 / \delta_n \). For \( \gamma < 1 \) the density has a \( x^{\gamma-1} \) singularity at the origin and a \( x^{-\gamma-1} \) tail.

One might ask, for example, whether the number of cells of type \( n \) is greater than a given size \( k \) and how the growth rates and mutation rates in the system influence this (say for example we wish to know whether cells carrying \( n - 1 \) deleterious variants, such as immunogenic antigens, are detectable [14]); this problem can be approached using

\[
P(Z_n(t) > k) \approx P(V_n > kt^{1-r_n} e^{-\delta_n t}).
\]

Numerically evaluating the resulting distribution function is standard in scientific software (e.g. using the Mittag-Leffler package in R [15]).

Similarly to the population sizes, the exact distribution of the arrival time is analytically intractable outside of the simplest settings. However, when the mutation rates are small
Figure 2: Comparison of limiting Mittag-Leffler distribution for the number of type \( n \) cells with stochastic simulations. Eq. (1), states that for large times and small mutation rates, the scaled number of type \( n \) cells, \( e^{-\delta_n t}(r_n-1)Z_n(t) \approx V_n \), is approximately Mittag-Leffler distributed with scale \( \omega_n \) and tail \( \lambda_1/\delta_n \). Here, we compare simulations of the scaled number of type \( n \) divided by \( \omega_n \), to the density of \( V_n/\omega_n \) which is Mittag-Leffler with scale parameter 1, and tail parameter \( \lambda_1/\delta_n \in (0,1] \). We chose three tail parameter values \( \lambda_1/\delta_n = 0.25, 0.5, 1.0 \), and these curves are depicted with solid lines. The simulation parameter were always \( \alpha_1 = 1.2, \beta_1 = 0.2, \nu_1 = 0.01, \beta_2 = 0.3 \) and for \( n = 2 \) types: sim 1: \( \alpha_2 = 4.3, t = 5 \); sim 2: \( \alpha_2 = 2.3, t = 7 \); sim 3: \( \alpha_2 = 1.0, t = 12 \). Then for \( n = 3 \) types sim 4: as in sim 3 plus \( \alpha_3 = 2.4, \beta_3 = 0.4, \nu_3 = 0.001, t = 12 \). Density lines were created in Mathematica using \( x^{-1}\text{MittagLefflerE}[\gamma, \gamma, -x] \).

Simplicity again emerges; the time until a cell of type \((n+1)\) exists approximately has a logistic distribution with scale given by \( \lambda_1^{-1} \) and median given by

\[
\tilde{t}_{1/2}^{(n+1)} = \frac{1}{\delta_n} \log \frac{\delta_n}{\omega_n}\nu_n [\delta_n^{-1}\log(\nu_n^{-1})]^{r_n-1}
\]

(3)

where \( \omega_n \) is the scale parameter defined in (2). The distribution can therefore be neatly presented as

\[
\mathbb{P}(\tau_{n+1} > t) \approx \left[1 + \exp \left(\lambda_1(t - \tilde{t}_{1/2}^{(n+1)})\right)\right]^{-1}.
\]

(4)

If we assume each running-max fitness is attained only by one type (\( r_i = 1 \) for each \( i \)) then
the medians satisfy the following recursion: with
\[ t_{1/2}^{(2)} = \frac{1}{\lambda_1} \log \frac{\lambda_1^2}{\alpha_1 \nu_1}, \] (5)
then for \( n > 1 \)
\[ t_{1/2}^{(n+1)} = t_{1/2}^{(n)} + \left\{ \begin{array}{ll}
\frac{1}{\delta_n} \log \frac{\delta_n - \lambda_n}{\nu_n} & \delta_n > \lambda_n \\
\frac{1}{\delta_n} \log \frac{\delta_n - 1}{\delta_{n-1}} & \delta_n - 1 < \lambda_n.
\end{array} \right. \] (6)

If the running-max fitness may be obtained multiple times, then a more detailed recursion also exists, given as Lemma 4.16 in Methods. Note that since the distribution in Eq. (4) is symmetric, the median and the mean coincide.

The arrival time density has a general shape centred at \( t_{1/2}^{(n)} \) (Fig. 3). We can immediately point out common properties, for example the median arrival time increases with \( n \) or as the mutation rates decreases, and the recursion of Eq. 6 explicitly details how these parameters interact. Similarly, the variance of the arrival time is \( \approx \pi^2/(3\lambda_1^2) \). In fact, the entire shape of the distribution, modulo a shift given by \( t_{1/2}^{(n)} \), is determined by \( \lambda_1 \). Thus, for \( t_{1/2}^{(n+1)} \gg \pi^2/(3\lambda_1^2) \), modellers may safely ignore the stochastic nature of waiting times and treat the arrival time of the type \( n \) cells as deterministic. However, this raises questions for statistical identifiability; aiming to distinguish between models, e.g. does a phenotype of interest require 2 or 3 mutations, based on fluctuations may be difficult due to the common logistic distribution.

The formulas for the arrival times (6) are valid for small mutation rates, and an intuitive understanding of their dominant behaviour can be given as follows. Momentarily assume that: (i) the arrival time for the type \( n + 1 \) cells approximately occurs when the type \( n \) population size reaches \( 1/\nu_n \) and (ii) we can ignore fluctuations in population size such that the type \( n \) population follows the deterministic part of Eq. (1). Then, solving for \( t_{1/2}^{(2)} \) in \( e^{\lambda_1 t_{1/2}^{(2)}} = 1/\nu_1 \), produces the leading order as \( \nu_1 \to 0 \) (to be compared with Eq. (5)). For the arrival times for type \( n + 1 \), suppose we start an exponential function at \( t_{1/2}^{(n)} \) with net growth rate \( \delta_n \); this growth will take \( \delta_n^{-1} \log(\nu_n) \) time to reach the threshold of \( \nu_n^{-1} \). To leading order in small mutation rates, this reproduces the recursion of Eq. (6).

Our approximation (1) for the cell number of the type \( n \) cells is valid for large times. Additionally small mutation rates are required when the running-max fitness increases, so \( \delta_n > \lambda_1 \). Heuristically, we expect the approximation to be valid at large enough times such that the type \( n \) cells have been seeded with high probability, that is for \( t \gg t_{1/2}^{(n)} \). Around the arrival time for the type \( n \) cells, \( t \approx t_{1/2}^{(n)} \), fluctuations in the cell number can be greater, which can be seen even in the two-type setting: in the neutral case (\( \lambda_1 = \lambda_2 \)), when \( t \gg t_{1/2}^{(2)} \), then from Eq. (1), indeed \( Z_2(t) \approx V_2 e^{\lambda_1 t} \) where \( V_2 \) is exponentially distributed, and therefore has an exponentially decaying tail. However, for \( t \approx t_{1/2}^{(2)} \) (or \( e^{\lambda_1 t} \approx \nu_1^{-1} \)), it is known that \( Z_2(t) \) has a heavy-tailed distribution, commonly known as the Luria-Delbrück distribution [16, 17, 18].
On the other hand, for $\lambda_1 < \lambda_2$, we found that $V_2$ is a Mittag-Leffler and does have a power-law heavy-tail as for the Luria-Delbrück distribution. Therefore, at times around the arrival time for type $n$ cells, the fluctuations in cell number may exceed the characterisation given in Eq. (1), but at larger times they are described by the Mittag-Leffler random variable $V_n$. We also note that, in the scale parameter recursion of Eq. 2, when mutations are mildly deleterious ($\delta_n - \lambda_{n-1} \ll 1$), the scale parameter can take large values. Therefore, caution should be adopted when using our approximation in this case.

**Illustrative examples:** (i) Modelling whether cells exist with alterations imbuing therapy resistance has provided insight in a range of bacterial and cancer studies such as, examining strategies for combination therapies in cancer [3], and providing the statistical machinery for mutation rate inference [1, 19]. For example, combining the probability of no therapy resistant cells with the fraction of repeated cultures unable to survive therapy-exposure enables measurement of the mutation rate in the classic and widely used ‘p0-method’ for Luria-Delbrück fluctuation assays [20]. Originally, only therapy sensitive and resistant cells were considered, however including multiple types is required when assessing multidrug resistance, or investigating resistant-intermediates such as persistor cells [21]. Typically, resistant mutations are modelled as neutral, ($\lambda_1 = \ldots = \lambda_n$); hence, $\delta_n = \lambda_1$, $r_n = n - 1$, the tail parameter of $V_n$ is 1, and using the recursion Eq. (2), we see

$$\omega_n = \frac{\alpha_1 \prod_{i=1}^{n-1} \nu_i}{\lambda_1 (n - 1)!}.$$  

Therefore, by Eq. (3), the median arrival time of the $(n+1)$th type

$$t_{1/2}^{(n+1)} = \frac{1}{\lambda_1} \log \frac{\lambda_1^2 (n - 1)!}{\alpha_1 [\lambda_1^{-1} \log(\nu_n^{-1})]^{n-1} \prod_{i=1}^n \nu_i}. \tag{7}$$
In a multidrug resistance setting, the probability of therapy failure is then immediately given by Eq. (4). As the product of mutation rates appears in Eq. (7), independently inferring these rates in a multidrug extended ‘p0-method’ is likely challenging; however, the product may be inferred, and compared to the mutation rates inferred in single therapy fluctuation assays.

(ii) Formulas for the time until the \( n \)th driver mutation is acquired in cancer, starting from a cell with a single driver mutation are given in Refs. [4, 8]. In this setting \( \lambda_i < \lambda_{i+1} \) and if we assume a constant driver mutation rate \( (\nu_1 = \ldots \nu_n) \) then notably the waiting time between the \( n \)th and \((n+1)\)th driver mutation decreases as \( n \) increases; this can be seen directly from Eq. (6) as

\[
\frac{t_{1/2}^{(n+1)}}{t_{1/2}^{(n)}} = \frac{1}{\lambda_n} \log \frac{\lambda_n}{\nu_n} - \frac{1}{\lambda_{n-1}} \log c_{n-1} \lambda_{n-1}
\]

which decreases as a function of \( n \). Hence, under this model, evolution accelerates later in a cancer’s growth.

(iii) There has been increasing attention paid to the role deleterious variants (such as immunogenic neoantigens) play in tumour evolution; potentially created due to the genomic instability that can be induced by mutations in driver genes, such as \( TP53 \) [22]. With the same formulation as (ii) above, suppose that the first cell with the \((n+1)\)th driver mutation, had acquired \( k \) deleterious variants in its lineage after the \( n \)th driver was acquired. How much do these deleterious variants affect the conclusion from (ii)? Let each deleterious variant be acquired at rate \( \nu_d \) and assume each variant reduced the cells’ net growth by \( s_d \); so cells with \( n \) drivers and with 1 deleterious variant have net growth \( \lambda_n - s_d \), with 2 variants \( \lambda_n - 2s_d \), etc. Then the waiting time between acquiring the \( n \)th and \((n+1)\)th driver is

\[
\frac{t_{1/2}^{(n+1)}}{t_{1/2}^{(n)}} = \frac{1}{\lambda_n} \log \frac{\lambda_n}{\nu_n} - \frac{1}{\lambda_{n-1}} \log c_{n-1} \lambda_{n-1} + \frac{1}{\lambda_n} \log \frac{s_d k!}{\nu_d}
\]

Hence there is a larger effect on the waiting times if deleterious variants occur earlier (smaller \( n \), due to \( \lambda_i < \lambda_{i+1} \).

(iv) Combining the above cases, the effects of advantageous, neutral, and deleterious mutations on the arrival and detection time, for different cell types is displayed in Fig. 4. The type 1 cells divide at rate \( 1 + s_a \), and die at rate 1; motivated by cancer where a small selective advantage is thought to push cells out of homeostasis (the units of time can therefore be given as the average time until normal cell death). For illustrative purposes we take a detection mark of 20,000 cells which is approximately the threshold for acute myeloid leukemia (diagnosis occurs when 20% [23] of the \( 10^5 \) hematopoetic stem cells [24] are cancerous blast cells). We highlight the curvature of the arrival time line in the advantageous mutation setting of Fig. 4B, displaying the acceleration of evolution, as discussed above in (ii). Also, the arrival time for the type 4 cells in Fig. 4B is similar to the detection time (Fig. 4A) for the advantageous mutation case, but far less for the other cases; this is due to the lag between the arrival time and the detection time being far diminished due to the rapid growth of the type 4 population in the advantageous case.
3 Discussion

Due to their appealing simplicity, and ability to model fundamental biology such as cell division, death, and mutation, multitype branching processes have become a standard tool for quantitative researchers investigating evolutionary dynamics in exponentially growing populations. Further, these models are able to link detailed microscopic molecular processes to explain macroscopic experimental, clinical, and epidemiological data [25, 26]. Despite the importance of this framework, even simple questions are often challenging to examine. Whilst numerical and simulation based methods have proven powerful for both model exploration and statistical inference, the computational expense of simulating to plausible scales can lead to challenges; e.g. simulating to tumour sizes orders of magnitude smaller than reality, which provides obstacles for biological interpretation of inferred parameters. Moreover, it is often unclear how to precisely summarise the manner in which a large number of parameters interact to influence quantities of interest, such as the the time until a triply resistant cell emerges. In this study, we analysed the regimes of large times, and small mutation rates, in order to develop limiting formulas that can be used to quickly gain intuition or for approximate statistical inference.

We have focused on the number, and arrival time, of cells with \( n \) mutations. While this problem dates back at least to the work of Luria and Delbrück - where a mutation resulted in phage resistant bacteria - specific instances of the problem are commonly used to study a variety of biological phenomena [20, 27, 6, 7, 14, 3, 10, 4, 28, 2]. The time of first mutation is well known, however the arrival time of cells with \( n \) alterations is unclear outside of specific fitness landscapes [8, 9]. Here, we developed approximations for the cell number and arrival time regardless of whether mutations increase, decrease, or have no effect on the growth rate of the cells carrying the alterations. We showed that, within relevant limiting regimes, the
number of type \( n \) cells can be decoupled into the product of a deterministic time-dependent function and a time-independent Mittag-Leffler random variable; meanwhile the arrival time of type \( n \) cells follows a logistic distribution with a shape that depends only on the net growth of the type 1 cells. The features of these distributions, such as median arrival time, can be exactly mapped to the underlying model parameters, that is the division, death, and mutation rates. These results illuminate the effects of mutation and selection, and can be readily numerically evaluated to explore particular biological hypotheses.

As the biological processes studied become increasingly complex, so too will the mathematical models constructed to describe such processes. We hope that the results of the present paper will enable researchers to find simplicity in an arbitrarily complex parameter landscape for a fundamental class of mathematical models.
4 Methods

In this section we provide detailed results and proofs in their general form.

4.1 Branching process: population growth

We first look to understand the number of cells of type $n$ at time $t$, that is $Z_n(t)$, at large times.

**Proposition 4.1.** For each $n \in \mathbb{N}$, there exists a $(0, \infty)$-valued random variable $W_n$ such that
\[
\lim_{t \to \infty} t^{-r_n+1} e^{-\delta_n t} Z_n(t) = W_n
\]
almost surely.

As our branching process is not irreducible this result would not be considered classical [29]. Heuristically, the result says that for large $t$, $Z_n(t) \approx W_n t^{r_n-1} e^{-\delta_n t}$ and so at large times all the stochasticity of $Z_n(t)$ is bundled into the variable $W_n$.

Towards proving Proposition 4.1, we consider a model of a deterministically growing population which seeds mutants as a Poisson process, the mutants growing as a branching process. The next result defines the model and describes the large-time number of mutants, generalising a result of [30].

**Lemma 4.2.** Let $(f(t))_{t \geq 0}$ be a non-negative cadlag function, $x, \delta > 0$, and $r \geq 0$, with
\[
\lim_{t \to \infty} t^{-r} e^{-\delta t} f(t) = x.
\]
Suppose that $(T_i)_{i \in \mathbb{N}}$ come from a Poisson process on $[0, \infty)$ with intensity $f(\cdot)$. Suppose that $(Y_i(t))_{i \geq 0}$, $i \in \mathbb{N}$, are i.i.d. birth-death branching processes, with birth and death rates $\alpha$ and $\beta$. Let $\lambda = \alpha - \beta > 0$. Define
\[
Z(t) = \sum_{i: T_i \leq t} Y_i(t - T_i).
\]

Then
\[
\begin{aligned}
\lim_{t \to \infty} t^{-r} e^{-\delta t} Z(t) &= \frac{x}{\delta - \lambda}, & \text{for } \delta > \lambda; \\
\lim_{t \to \infty} t^{-r-1} e^{-\delta t} Z(t) &= \frac{x}{r+1}, & \text{for } \delta = \lambda; \\
\lim_{t \to \infty} e^{-\lambda t} Z(t) &= V, & \text{for } \delta < \lambda;
\end{aligned}
\]
almost surely. Here $V$ is some positive random variable with mean $\int_0^\infty e^{-\lambda s} f(s) ds$.

**Proof.** First we claim that
\[
M(t) = e^{-\lambda t} Z(t) - \int_0^t e^{-\lambda s} f(s) ds, \quad t \geq 0;
\]
is a martingale with respect to the natural filtration. Indeed,

\[
\mathbb{E}[M(t)|\mathcal{F}_s] = e^{-\lambda t} \mathbb{E}[Z(t)|\mathcal{F}_s] - \int_0^t e^{-\lambda u} f(u) du
\]

\[
e^{-\lambda t} \left( Z(s)e^{\lambda(t-s)} + \int_s^t f(u)e^{\lambda(t-u)} du \right) - \int_0^t e^{-\lambda u} f(u) du
\]

\[= M(s),\]

as required.

Next we look to bound the second moment of \(M(t)\). To this end, observe that \(Z(t) = \sum_{i:T_i \leq t} Y_i(t - T_i)\) is a compound Poisson distribution which is a Poisson \(\left( \int_0^t f(s) ds \right)\) sum of i.i.d. random variables distributed as \(Y_1(t - \xi)\), where \(\xi\) is a \([0,t]\)-valued random variable with density proportional to \(f\). Using the already-known second moment for a birth-death branching process [31],

\[
\mathbb{E}[Y_1(t)^2] = \frac{2\alpha}{\lambda} e^{2\lambda t} - \frac{\alpha + \beta}{\lambda} e^{\lambda t},
\]

we have that

\[
\mathbb{E}[Y_1(t - \xi)^2] = \int_0^t f(s) \left( \frac{2\alpha}{\lambda} e^{2\lambda(t-s)} - \frac{\alpha + \beta}{\lambda} e^{\lambda(t-s)} \right) ds.
\]

It follows that

\[
\text{Var}Z(t) = \mathbb{E}[Y_1(t - \xi)^2] \int_0^t f(s) ds
\]

\[
= \int_0^t f(s) \left( \frac{2\alpha}{\lambda} e^{2\lambda(t-s)} - \frac{\alpha + \beta}{\lambda} e^{\lambda(t-s)} \right) ds,
\]

and since \(\mathbb{E}M(t) = 0\), we find that

\[
\mathbb{E}[M(t)^2] = \text{Var}[M(t)] = e^{-2\lambda t} \text{Var}Z(t)
\]

\[
= e^{-2\lambda t} \int_0^t f(s) \left( \frac{2\alpha}{\lambda} e^{2\lambda(t-s)} - \frac{\alpha + \beta}{\lambda} e^{\lambda(t-s)} \right) ds
\]

\[
\leq \frac{2\alpha}{\lambda} \int_0^t e^{-2\lambda s} f(s) ds
\]

\[
= \frac{2\alpha}{\lambda} \int_0^t (s \lor 1)^{\delta} e^{(\delta-2\lambda)s} f(s) ds
\]

\[
\leq \frac{2\alpha}{\lambda} \sup_{s \geq 0} [(s \lor 1)^{-\delta} e^{-\delta s} f(s)] \int_0^t (s \lor 1)^{\delta} e^{(\delta-2\lambda)s} ds.
\]

Therefore

\[
\mathbb{E}[M(t)^2] \leq \begin{cases} 
 Ct^{\delta/(\delta-2\lambda)}, & \text{for } \delta > 2\lambda; \\
 Dt^{\delta+1}, & \text{for } \delta = 2\lambda; \\
 E, & \text{for } \delta < 2\lambda,
\end{cases}
\]

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where \( C, D \) and \( E \) are positive constants.

To conclude the proof, we will separately consider the three cases listed in the Lemma’s statement: \( \delta < \lambda \), \( \delta = \lambda \), and \( \delta > \lambda \) (which are different from the three cases in (8)).

The first case that is \( \delta < \lambda \) is the easiest. Here the martingale \( M(t) \) has a bounded second moment. By the martingale convergence theorem, \( M(t) \) converges to some random variable \( V' \) with mean zero. Rearranging the limit of \( M(t) \),

\[
\lim_{t \to \infty} e^{-\lambda t} Z(t) = \int_0^\infty e^{-\lambda s} f(s) ds + V',
\]

almost surely, where the integral converges because the integrand has an exponentially decaying tail. This gives the result for \( \delta < \lambda \).

The second case is \( \delta = \lambda \). Here the second moment of \( M(t) \) is still bounded and so we can again apply the martingale convergence theorem to see that \( M(t) \) converges almost surely. It follows that

\[
t^{-r-1} M(t) = t^{-r-1} e^{-\delta t} Z(t) - t^{-r-1} \int_0^t e^{-\delta s} f(s) ds
\]

converges to zero almost surely. Thus, using dominated convergence,

\[
\lim_{t \to \infty} t^{-r-1} \int_0^t e^{-\delta s} f(s) ds = \lim_{t \to \infty} \int_0^1 u^r(tu)^{-r} e^{-\delta tu} f(tu) du
= x \int_0^1 u^r du
= \frac{x}{r + 1}
\]

is the almost sure limit of \( t^{-r-1} e^{-\delta t} Z(t) \).

The third and final case is \( \delta > \lambda \). This case requires a new perspective because the second moment of \( M(t) \) may not be bounded, disallowing the martingale convergence theorem. Instead we appeal to Borel-Cantelli. For \( \epsilon > 0 \) and \( n \in \mathbb{N} \), consider the events

\[
B^\epsilon_n := \left\{ \sup_{t \in [n, n+1]} \left( t^{-r} e^{(\lambda - \delta)t} M(t) \right)^2 > \epsilon \right\}.
\]

Then

\[
P[B^\epsilon_n] \leq P \left[ \sup_{t \in [n, n+1]} M(t)^2 > \epsilon n^{2r} e^{2(\delta - \lambda)n} \right]
\leq \frac{\mathbb{E}[M(n + 1)^2]}{\epsilon n^{2r} e^{2(\delta - \lambda)n}}
\leq Ge^{-\gamma n},
\]
by Doob’s martingale inequality and then (8); here $G$ and $\gamma$ are positive numbers which do not depend on $n$. By Borel-Cantelli, the probability that only finitely many of $(B^t_n)_{n \in \mathbb{N}}$ occur is one. Equivalently,

$$t^{-r}e^{(\lambda - \delta)t}M(t) = t^{-r}e^{-\delta t}Z(t) - t^{-r}e^{(\lambda - \delta)t}\int_0^t e^{-\lambda s}f(s)ds$$

converges to zero almost surely. Thus, using dominated convergence,

$$\lim_{t \to \infty} t^{-r}e^{(\lambda - \delta)t}\int_0^t e^{-\lambda s}f(s)ds = \lim_{t \to \infty} \int_0^t (t^{-r}(t-s)r(t-s)-r^{-\delta(t-s)}f(t-s)) e^{(\lambda - \delta)s}ds$$

$$= \int_0^\infty xe^{(\lambda - \delta)s}ds = \frac{x}{\delta - \lambda}$$

is the almost sure limit of $t^{-r}e^{-\delta t}Z(t)$. \hfill \Box

We can now give the proof of Proposition 4.1 on the convergence of cell numbers.

**Proof of Proposition 4.1.** We prove the result by induction. Clearly it is true for $n = 1$. Now suppose that

$$\lim_{t \to \infty} t^{-r_n}e^{-\delta_n t}Z_n(t) = W_n \in (0, \infty)$$

almost surely. Condition on the trajectory of $Z_n(\cdot)$, and apply Lemma 4.2 to see that

$$\lim_{t \to \infty} t^{-r_{n+1}}e^{-\delta_{n+1} t}Z_{n+1}(t) = W_{n+1} \in (0, \infty)$$

almost surely. \hfill \Box

Having proven that the cell numbers grow asymptotically as a deterministic function of time multiplied by a time-independent random amplitude $W_n$, our next aim is to determine the distribution of this random amplitude. We shall proceed via induction. To establish the base case we restate a classic result [29, 32]:

**Lemma 4.3.** The random variable $W_1$ from Proposition 4.1 has exponential distribution with parameter $1 - \beta_1/\alpha_1$.

Since the type $n$ population seeds the type $n + 1$ population, one might expect that the random amplitudes $W_n$ and $W_{n+1}$ of the two populations are related. The next result says that this is indeed the case for a part of parameter space - when the type $n + 1$ fitness is no greater than the fitnesses of previous types.

**Proposition 4.4.** Let $n \geq 1$. If $\delta_n > \lambda_{n+1}$

$$W_{n+1} = \frac{\nu_n W_n}{\delta_n - \lambda_{n+1}} \text{ a.s.},$$

while for $\delta_n = \lambda_{n+1}$

$$W_{n+1} = \frac{\nu_n W_n}{r_n} \text{ a.s.}$$
Proof. Immediate from Lemma 4.2.

Proposition 4.4 focuses on the case that the fitness of type \( n+1 \) does not dominate the fitnesses of types 1 to \( n \); here it says that the random amplitude \( W_{n+1} \) is simply a constant multiple of \( W_n \), meaning that the large-time stochasticity of the type \( n+1 \) population size is perfectly inherited from the type \( n \) population. A special example is that type 1 has a larger fitness than all subsequent types, in which case \( W_n \) is a constant multiple of \( W_1 \) and thus all random amplitudes are exponentially distributed, recovering a result of [9]. Proposition 4.4 is also a generalisation of Theorem 3.2 parts 1 and 2 of [18] which provided the distribution of \( W_2 \) in terms of \( W_1 \).

The remaining region of parameter space - where a new type may have a fitness greater than the fitness of all previous types is our next focus. Here, contrasting with the region considered in Proposition 4.4, the random amplitudes seem to be rather complex. The distribution of \( W_2 \) takes an intricate form, which is calculated in [13] (Eq. 56) and we do not restate it here for brevity. The distribution of \( W_n \) for \( n > 2 \) apparently are unknown. We aim to find simple approximations for the \( W_n \) in Sections 4.2 and 4.3.

### 4.2 Approximate model introduction

The exact distribution of the random amplitude \( W_n \) for a generic sequence of birth and death rates appears to be analytically intractable. Thus we look to approximate \( W_n \) in the limit of small mutation rates. Towards such an approximation, we choose to follow a method inspired by Durrett and Moseley [8] which simplifies calculations by introducing an approximate model. The approximate model is motivated by the following heuristic argument: mutations to create cells of type \((n+1)\) occur at rate \( \nu_n Z_n(t) \); when the mutation rates are small it will take some time for the first cell of type \((n+1)\) to appear; at large times \( Z_n(t) \sim W_n e^{\delta_n t/r_n-1} \) (Proposition 4.1); therefore for small mutation rates, mutations to create cells of type \((n+1)\) should occur at rate \( \approx \nu_n W_n e^{\delta_n t/r_n-1} \). We carefully define the approximate model momentarily, but briefly it arises by assuming the type \((n+1)\) arrive at rate \( \nu_n W_n e^{\delta_n t/r_n-1} \) and then letting the type \((n+1)\) cells follow the dynamics we’ve already been assuming.

Formally, we define the approximate model iteratively. We let \( Z_n^*(t) \) be the size of the type \( n \) population at time \( t \), set \( Z_1^*(t) = W_1 e^{\lambda_1 t} \) for \( t \geq 0 \), and fix \( W_1^* = W_1 \). Then, given \( W_n^* \), let \((T_{n+1,i}^*, i)\) be the times from a Cox process with rate \( t^{r_n-1} e^{\delta_n t/r_n-1} \nu_n W_n^* \).

Then, we set

\[
Z_{n+1}^*(t) = \sum_{i:T_{n+1,i}^* \leq t} Y_{n+1,i}^*(t-T_{n+1,i}^*)
\]

where the \( Y_{n,i}(\cdot) \) are independent birth-death processes with birth and death rates \( \alpha_n \) and
We hypothesise but do not prove that the distribution of the random amplitudes $W^*_n$ and $W_n$ for the approximate and original models respectively coincide in the limit of small mutation rates; this is known to be true in the two-type setting (Section 4.4 of [13]).

4.3 Approximate model: population growth

First we have the counterpart to Proposition 4.1, clarifying that the approximate model is well defined.

**Proposition 4.5.** For $n \geq 1$, there exists a $(0, \infty)$-valued random variable $W^*_n$ such that

$$
\lim_{t \to \infty} t^{r_n-1} e^{-\delta_n t} Z^*_n(t) = W^*_n
$$

almost surely.

**Proof.** Identical to the proof of Proposition 4.1.

Analogously to Proposition 4.4 we can relate the random amplitudes of type $n+1$ with that of type $n$ for the approximate process - now we include also the case where type $n$ has a larger growth rate than the type $(n-1)$ cells. We give the results at the level of the Laplace transform, as it turns out this function will dictate the distribution of the arrival times, to be seen in Section 4.4

**Corollary 4.6.** Let $n \geq 1$. Then

$$
\mathbb{E}[\exp(-\theta W^*_{n+1})] = \mathbb{E}[\exp(-h_n(\theta)W^*_n)],
$$

where $h_n(\theta)$ is defined by

$$
h_n(\theta) = \begin{cases} 
\frac{\nu_n \theta}{\delta_n - \lambda_{n+1}} & \delta_n > \lambda_{n+1} \\
\frac{\nu_n \theta}{r_n} & \delta_n = \lambda_{n+1} \\
\nu_n \frac{\theta \Gamma(r_n)}{\lambda_{n+1}^{r_n}} \Phi(-\theta \alpha_{n+1}/\lambda_{n+1}, r_n, 1 - \delta_n/\lambda_{n+1}) & \delta_n < \lambda_{n+1},
\end{cases}
$$

$\Gamma$ is the gamma function, and $\Phi$ is the Lerch transcendent function.

**Proof.** For the cases of $\delta_n > \lambda_{n+1}$ or $\delta_n = \lambda_{n+1}$ we can appeal directly to Lemma 4.2.

For $\delta_n < \lambda_{n+1}$, let $\zeta_{n+1}(t, z) = \mathbb{E} e^{-z Y_{n+1}(t)}$ which is the Laplace transform for a linear birth-death process initiated with a single cell, at time $t$ with division and death rates $\alpha_n, \beta_n$. If we fix $W^*_n$, then the arrivals to the type $n+1$ population occur as a Poisson process, so by
Applying this to \( Z^{i.i.d.} \) random variables \( X \). Generally, if we have a compound Poisson variable, defined by the sum of \( N \sim \text{Poisson}(\lambda) \) i.i.d. random variables \( X_i \), then its Laplace transform follows

\[
\mathbb{E}\exp\left(-\theta \sum_{i=1}^{N} X_i\right) = \exp[-\lambda(1 - \mathbb{E}e^{-\theta X_1})].
\]

Applying this to \( Z_{n+1}^*(t) \) we have

\[
\mathbb{E}[\exp(-e^{-\lambda_{n+1}t}Z_{n+1}^*(t)\theta)|W_n^*] = \exp \left(-\nu_n W_n^* \int_0^t s^{r_n-1} e^{\delta_n s}[1 - \zeta_{n+1}(t - s, \theta e^{-\lambda_{n+1}t})] ds \right).
\]

To obtain the limit of the integrand we use the well known result (see Ref. [8] Section 2) that if \( Y(\cdot) \) is a linear birth-death process with division, and death rates \( \alpha_{n+1}, \beta_{n+1} \), and with \( \phi_{n+1} = \gamma_{n+1}/\alpha_{n+1} \), then as \( t \to \infty \), \( e^{-\lambda_{n+1}t}Y(t) \overset{d}{\to} B \times E \) where \( B \sim \text{Bernoulli}(\phi_{n+1}) \), \( E \sim \text{Expo}(\phi_{n+1}) \), and both random variables are independent from each other. Hence its Laplace transform converges to

\[
\mathbb{E}\exp \left(-\theta Y(t)e^{-\lambda_{n+1}t}\right) \to 1 - \phi_{n+1} + \phi_{n+1} \int_0^\infty e^{-\theta x} \theta e^{-\phi_{n+1}x} dx = 1 - \phi_{n+1} \left(1 - \frac{1}{1 + \theta/\phi_{n+1}}\right).
\]

Then

\[
1 - \zeta(t - s, \theta e^{-\lambda_{n+1}t}) = 1 - \mathbb{E}\exp \left(-\theta e^{-\lambda s} e^{-\lambda(t-s)}Y(t - s)\right) \to \phi_{n+1} \left(1 - \frac{1}{1 + \theta e^{-\lambda s}/\phi_{n+1}}\right)
\]
as \( t \to \infty \). Using this and taking the \( t \to \infty \) limit over Eq. 10 results in

\[
\lim_{t \to \infty} \mathbb{E}[\exp(-e^{-\lambda_{n+1}t}Z_{n+1}^*(t)\theta)|W_n^*] = \exp \left(-\nu_n W_n^* \frac{\delta_n}{\phi_{n+1}} \int_0^\infty s^{r_n-1} e^{\delta_n s}\left(1 - \frac{1}{1 + e^{-\lambda_{n+1}s/\phi_{n+1}}}\right) ds\right).
\]

Let \( \gamma_n = \delta_n/\delta_{n+1} \) and recall the Lerch transcendent has integral representation

\[
\Phi(z, s, a) = \frac{1}{\Gamma(s)} \int_0^\infty \frac{t^{s-1} e^{-at}}{1 - ze^{-t}} dt
\]

which converges for \( z \in \mathbb{C} \setminus [1, \infty) \). Upon the substitution \( t = \lambda_{n+1}s \) we see

\[
h_n(\theta) = \nu_n \frac{\phi_{n+1}}{\lambda_{n+1}} \int_0^\infty s^{r_n-1} e^{\delta_n s}\left(1 - \frac{1}{1 + e^{-\lambda_{n+1}s/\phi_{n+1}}}\right) ds
\]

\[
= \nu_n \frac{\theta}{\lambda_{n+1}} \int_0^\infty \frac{t^{r_n-1} e^{-(1-\gamma_n)t}}{1 + \theta e^{-t/\phi_{n+1}}} dt
\]

\[
= \nu_n \frac{\theta \Gamma(r_n)}{\lambda_{n+1}} \Phi(-\theta/\phi_{n+1}, r_n, 1 - \gamma_n).
\]
Corollary 4.6 implies that
\[
\mathbb{E} \left[ \exp (-W_n^* \theta) \right] = \mathbb{E} \left[ \exp (-W_1^* h_1 \circ \ldots \circ h_{n-1}(\theta)) \right] = (1 + h_1 \circ \ldots \circ h_{n-1}(\theta) \alpha_1 / \lambda_1)^{-1},
\] (11)
which means that the distribution of the random amplitude $W_n^*$ is possible to numerically evaluate. Such numerical computation for the approximate model is already a step beyond what we could do for the original model.

Recall that it was heuristically argued that the random amplitudes of the approximate and original models coincide in the limit of small mutation rates. Therefore the exact distribution of $W_n^*$ seen in (11) is not so much our interest as is its limit for small mutation rates. Our task for the remainder of this section is thus to take the small mutation rate limit of (11).

To state the limit we now introduce some notation.

Let
\[
f_i(\nu_i) = \begin{cases} 
\nu_i^{-1} & \lambda_{i+1} \leq \delta_i \\
\nu_i^{-1} \log(\nu_i^{-1})^{-(r_i-1)} & \lambda_{i+1} > \delta_i
\end{cases}
\] (12)
Then, writing $\mathbf{\nu} = (\nu_1, \nu_2, \ldots)$, we define
\[
\mathcal{F}_n(\mathbf{\nu}) = \prod_{i=1}^{n} f_i(\nu_i)^{\delta_{n+1}/\delta_i}.
\] (13)
This function satisfies
\[
\mathcal{F}_n(\mathbf{\nu}) = (f_n(\nu_n)\mathcal{F}_{n-1}(\mathbf{\nu}))^{\delta_{n+1}/\delta_n}
\]
Further let $\gamma_n = \delta_n / \delta_{n+1}$, and
\[
\kappa_n = \begin{cases} 
(\delta_n - \lambda_{n+1})^{-1} & \delta_n > \lambda_{n+1} \\
r_n^{-1} & \delta_n = \lambda_{n+1} \\
\lambda_{n+1}^{-1} \frac{\sin \gamma_n \pi}{\sin \gamma_n \pi} & \delta_n < \lambda_{n+1}.
\end{cases}
\]
Note that $c_n$ from Section 2 is $\kappa_n$ when $\delta_n < \lambda_{n+1}$. Then, for small mutation rates, the distribution of $W_n^*$ may be related to $W_1^*$:

**Proposition 4.7.**
\[
\lim_{\nu_1 \to 0} \ldots \lim_{\nu_n \to 0} \mathbb{E} \left[ \exp (-W_{n+1}^* \theta \mathcal{F}_n(\mathbf{\nu})) \right] = \mathbb{E} \left[ \exp \left( -W_1^* \theta \prod_{i=1}^{n} \kappa_i^{\delta_i/\delta_i} \right) \right] = \left( 1 + (1 - \beta_1 / \alpha_1)^{-1} \prod_{i=1}^{n} \kappa_i^{\delta_i/\delta_i} \right)^{-1}
\]
Before proving this proposition we give two required lemmas in order to understand the limit behaviour of the function $h_n(\theta)$.

**Lemma 4.8.** With $\Phi$ as the Lerch transcendent function with $0 < a < 1$, as $z \to -\infty$

$$\Phi(z, s, a) \sim \frac{\pi}{\sin a\pi} \frac{1}{(-z)^a} \frac{(\log -z)^{s-1}}{(s-1)!}.$$  

**Proof.** We first rewrite $\Phi$ in terms of the generalised hypergeometric function (see 16.2.1 in [33]) for integer $s$

$$\Phi(z, s, a) = a^{-s} \frac{\Gamma(1 + x) \Gamma(-x)}{(a + x)^s} (-z)^x,$$

which identity can be easily checked by using the definitions of these special functions. Then we use its integral representation (Eq. 16.5.1 at [33])

$$\Phi(z, s, a) = \frac{1}{2\pi i} \int_{-i\infty}^{i\infty} \frac{\Gamma(1 + x) \Gamma(-x)}{(a + x)^s} (-z)^x \, dx.$$

The integrand has poles at $-a$ (where $0 < a < 1$) and at all real integers due to the Gamma functions. The contour of integration separates the poles at $-a$ and 0. From the residue theorem for $z < 0$ we can rewrite the integral as the sum of the residues coming from all poles on the left of the contour

$$\Phi(z, s, a) = \text{Res}_{x=-a} \left( \frac{\Gamma(1 + x) \Gamma(-x)}{(a + x)^s} (-z)^x \right) + (-1)^s \sum_{n=1}^{\infty} \frac{z^{-n}}{(n-a)^s}.$$

The first term on the right hand side is the contribution from the pole at $-a$, while the sum goes over the contributions from all other poles at $-n = -1, -2, \ldots$. The leading order term comes from the residue of closest pole to the origin at $x = -a$, which can be written as a finite sum of terms including powers of $\log -z$. The leading order of these terms is

$$\Phi(z, s, a) \sim \frac{\pi}{\sin a\pi} \frac{1}{(s-1)!(-z)^a} \frac{(\log -z)^{s-1}}{(s-1)!} + O \left( \frac{(\log -z)^{s-2}}{(-z)^a} \right)$$

□

Before giving the next lemma we recall $h_n$ for convenience

$$h_n(\theta) = \begin{cases} \frac{\nu_n\theta}{\delta_n - \lambda_{n+1}} & \delta_n > \lambda_{n+1} \\ \frac{\nu_n\theta}{\delta_n} & \delta_n = \lambda_{n+1} \\ \frac{\nu_n\theta}{\delta_n}(\theta - \alpha_n) & \delta_n < \lambda_{n+1}. \end{cases}$$

Then the following lemma will be of use.
Lemma 4.9. For $\delta_n > \lambda_{n+1}$

$$\lim_{\nu_n \to 0} h_n(\nu_n^{-1}\theta) = \frac{\theta}{\delta_n - \lambda_{n+1}},$$

for $\lambda_{n+1} = \delta_n$,

$$\lim_{\nu_n \to 0} h_n(\nu_n^{-1}\theta) = \frac{\theta}{\tau_n},$$

while for $\delta_n < \lambda_{n+1}$

$$\lim_{\nu_n \to 0} h_n(\nu_n^{-1/\alpha_n} \log(\nu_n^{-1})^{(r_n-1)/\alpha_n \theta}) = \frac{\phi_n^{1-\alpha_n}}{\lambda_n^{\alpha_n} \gamma_n^{r_n-1}} \frac{\pi}{\sin \gamma_n \pi} \theta^\alpha_n$$

Note the right hand side of the limits in Lemma 4.9 can be written as $\theta \kappa_n$, where $\kappa_n$ is defined in Eq. (4.3).

Proof. Recall $\gamma_n = \delta_n / \lambda_{n+1}$, $\phi_n + 1 = \lambda_{n+1} / \alpha_{n+1}$. The lemma is clearly true by the definition of $h_n(\theta)$ for $\delta_n > \lambda_{n+1}$ and $\delta_n = \lambda_{n+1}$.

We turn to the case of $\delta_n < \lambda_{n+1}$. For ease of notation we drop ‘$n$’ subscripts and introduce $l_\nu = \log(\nu^{-1})$. From the definition of $h(\theta)$ in this case we see we require the limit of the Lerch transcendent for large first argument given in Lemma 4.9. Further, observe that for $a \in [0, 1]$, $\sin a \pi = \sin (1 - a) \pi$. Hence, as $\nu \to 0$,

$$\Phi(-\theta \nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma \phi^{-1}}, r, 1 - \gamma) \sim \frac{\pi}{\sin \gamma \pi} \frac{1}{(\theta \nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma \phi^{-1}})^{1-\gamma}} \frac{(\log[\theta \nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma \phi^{-1}}])^{r-1}}{(r-1)!}$$

and so

$$h(\nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma \theta}) \sim \nu^{1-1/\gamma} l_\nu^{-(r-1)/\gamma \theta} \Gamma(r) \frac{\phi^{1-\gamma \theta}}{\lambda^r} \frac{\pi}{\sin \gamma \pi} (\theta \nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma \phi^{-1}})^{1-\gamma} \frac{(\log[\theta \nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma \phi^{-1}}])^{r-1}}{(r-1)!}.$$ 

The $\nu$ terms outside of the logarithms immediately cancel, leaving the logarithmic terms. Collecting the logarithmic terms together, and recalling that $\Gamma(r_n) = (r_n - 1)!$, we have

$$h(\nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma \theta}) \sim \frac{\phi^{1-\gamma \theta}}{\lambda^r} \frac{\pi}{\sin \gamma \pi} \times l_\nu^{-(r-1)/\gamma} \frac{1}{l_\nu^{-(r-1)/\gamma}} \frac{[\log(\theta \nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma})]^{r-1}}{(r-1)!}.$$ 

Notice that

$$[\log(\theta \nu^{-1/\gamma} l_\nu^{-(r-1)/\gamma})]^{r-1} = (\log(\nu^{-1/\gamma}) + \log(l_\nu^{-(r-1)/\gamma \theta}))^{r-1} \sim [\gamma^{-1} l_\nu]^{r-1}.$$
Hence
\[ l_{\nu}^{-1/(r-1)/\gamma} \frac{1}{\nu} \log(\theta \nu^{-1/\gamma} l_{\nu}^{-(r-1)/\gamma})^{r-1} \rightarrow \gamma^{-(r-1)}. \]

This leaves
\[ h(\nu^{-1/\gamma} l_{\nu}^{-(r-1)/\gamma}) \rightarrow \phi^{1-\gamma} \gamma^{\gamma} \frac{\pi}{\lambda^{\gamma} r^{1-\gamma} \phi} \sin \gamma \pi \]
as required. \( \square \)

We can now give the proof of Proposition 4.7:

**Proof of Proposition 4.7.** The base case is clear, we now argue by induction. We recall that
\[ \mathbb{E} [\exp (-W_{n+1}^* \theta)] = \mathbb{E} [\exp (-W_n^* \theta)]. \]
Hence
\[ \mathbb{E} [\exp (-W_{n+1}^* \theta \mathcal{F}_n(\nu))] = \mathbb{E} [\exp (-W_n^* \theta \mathcal{F}_n(\nu))] = \mathbb{E} [\exp (-W_n^* \theta f_n(\nu_{n+1}) \mathcal{F}_{n-1}(\nu) \theta)] . \]
Thus
\[ \lim_{\nu_1 \to 0} \ldots \lim_{\nu_n \to 0} \mathbb{E} [\exp (-W_{n+1}^* \theta \mathcal{F}_n(\nu))] = \lim_{\nu_1 \to 0} \ldots \lim_{\nu_{n-1} \to 0} \mathbb{E} [\exp (-W_n^* \theta f_n(\nu_{n+1}) \mathcal{F}_{n-1}(\nu) \theta)] . \]
Using Lemma 4.9, we have
\[ \lim_{\nu_1 \to 0} \ldots \lim_{\nu_{n-1} \to 0} \mathbb{E} [\exp (-W_n^* \kappa_n \mathcal{F}_{n-1}(\nu) \theta)] = \lim_{\nu_1 \to 0} \ldots \lim_{\nu_{n-1} \to 0} \mathbb{E} [\exp (-W_n^* \kappa_n \mathcal{F}_{n-1}(\nu) \theta)]. \]
Using the induction hypothesis
\[ \lim_{\nu_1 \to 0} \ldots \lim_{\nu_{n-1} \to 0} \mathbb{E} [\exp (-W_n^* \kappa_n \mathcal{F}_{n-1}(\nu) \theta)] = \mathbb{E} \left[ \exp \left( -W_1^* (\kappa_1 \theta) \delta_1 / \delta_n \prod_{i=1}^{n-1} \kappa_i^{\delta_i / \delta_i} \right) \right] \]
\[ = \mathbb{E} \left[ \exp \left( -W_1^* \theta \delta_{n+1} \prod_{i=1}^{n} \kappa_i^{\delta_i / \delta_i} \right) \right]. \]
\( \square \)

We remark that when \( \lambda_{i+1} \leq \delta_i \) (a fitness increase does not occur), we are not required to take the limit above on \( \nu_i \) - that is the statement of Proposition 4.7 is true without applying these limits.

Summarising thus far, we see
\[ \lim_{\nu_1 \to 0} \ldots \lim_{\nu_{n-1} \to 0} \lim_{t \to \infty} \mathcal{F}_n(\nu) e^{-\delta_{n+1} t} \mathcal{F}_{n-1}(\nu) Z_{n+1}(t) \]
has a Mittag-Leffler distribution with tail parameter \( \delta_1/\delta_{n+1} \) and scale parameter
\[
\left(1 - \beta_1/\alpha_1\right)^{-\delta_{n+1}/\delta_1} \prod_{i=1}^{n} \kappa_i^{\delta_i/\delta_1} = (1 - \beta_1/\alpha_1)^{-\delta_{n+1}/\delta_1} \prod_{i=1}^{n} \kappa_i^{\delta_{n+1}/\delta_i}.
\]

Separating into a time-dependent component this implies that
\[
Z_{n+1}(t) \approx V_{n+1} \exp^{\delta_{n+1}t^{n+1}}
\]
with \( V_{n+1} \) being Mittag-Leffler with tail parameter \( \delta_1/\delta_{n+1} \) and scale parameter
\[
\omega_{n+1} = (1 - \beta_1/\alpha_1)^{-\delta_{n+1}/\delta_1} \mathcal{F}_n(\nu)^{-1} \prod_{i=1}^{n} \kappa_i^{\delta_{n+1}/\delta_i}.
\]

If we consider the family of random variables \( V_{n+1} \) then the scale parameters \( \omega_{n+1} \) satisfy the following recursion

**Lemma 4.10.** Set \( \omega_1 = (1 - \beta_1/\alpha_1)^{-1} \), then for \( n > 1 \),
\[
\omega_{n+1} = \begin{cases} \frac{\nu_n}{\delta_n - \lambda_{n+1}} \omega_n & \delta_n > \lambda_{n+1} \\ \frac{\nu_n}{\lambda_{n+1}} \omega_n & \delta_n = \lambda_{n+1} \\ (\omega_n \nu_n \log(\nu_n^{-1})^{r_n-1} \kappa_n)^{\lambda_{n+1}/\delta_n} & \delta_n < \lambda_{n+1} \end{cases}
\]  
(14)

**Proof.** Take
\[
\omega_n = (1 - \beta_1/\alpha_1)^{-\delta_n/\lambda_1} \mathcal{F}_{n-1}(\nu)^{-1} \prod_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_1},
\]

If \( \delta_n \geq \lambda_{n+1} \) then \( \delta_n = \delta_{n+1}, f_n(\nu_n) = \nu_n^{-1} \), and so
\[
\omega_{n+1} = (1 - \beta_1/\alpha_1)^{-\delta_n/\lambda_1} \nu_n \mathcal{F}_{n-1}(\nu)^{-1} \prod_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_1}
= (1 - \beta_1/\alpha_1)^{-\delta_n/\lambda_1} \mathcal{F}_n(\nu)^{-1} \prod_{i=1}^{n} \kappa_i^{\delta_i/\delta_1}
\]
where \( \kappa_n \) is either \( (\delta_n - \lambda_{n+1})^{-1} \) for \( \delta_n > \lambda_{n+1} \) or \( r_n^{-1} \) for \( \delta_n = \lambda_{n+1} \).

In the case of \( \delta_n < \lambda_{n+1} \), and so \( f_n(\nu_n)^{-1} = \nu_n \log(\nu_n^{-1})^{r_n-1} \),
\[
\omega_{n+1} = \left[ (1 - \beta_1/\alpha_1)^{-\delta_n/\lambda_1} \mathcal{F}_{n-1}(\nu)^{-1} \prod_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_1} f_n(\nu_n) \kappa_n \right]^{\lambda_{n+1}/\delta_n}
= (1 - \beta_1/\alpha_1)^{-\delta_{n+1}/\lambda_1} \mathcal{F}_n(\nu)^{-1} \prod_{i=1}^{n} \kappa_i^{\delta_{n+1}/\delta_i}
\]
where we used
\[
\mathcal{F}_n(\nu) = (f_n(\nu_n) \mathcal{F}_{n-1}(\nu))^{\lambda_{n+1}/\delta_n}.
\]
We summarise this approximate form of $Z_{n+1}(t)$ as a theorem, to emphasise that it is the culmination of the results in this section.

**Theorem 4.11.** For $t$ large, and all $\nu_i$ small

$$Z_{n+1}(t) \approx V_{n+1} e^{\delta_{n+1} t (r_{n+1} - 1)}$$

where $V_{n+1}$ is Mittag-Leffler distributed with tail parameter $\delta_1/\delta_{n+1}$ and scale parameter $\omega_{n+1}$ which satisfies the recurrence of Lemma 4.10.

The tail of $Z_{n+1}(t)$ can be understood with use of the following lemma:

**Lemma 4.12.** For $c > 0, 0 < \alpha < 1$, let $X$ have Laplace transform

$$E[\exp(-\theta X)] = (1 + (c\theta)^\alpha)^{-1}$$

then

$$P(X > x) \sim \frac{(c/x)^\alpha}{\Gamma(1 - \alpha)}$$

**Proof.** See Durrett and Moseley [8], proof of Theorem 3.

Hence

**Corollary 4.13.** For $t$ large, and all $\nu_i$ small

$$P(Z_{n+1}(t) > x) \approx P(V_{n+1} > xe^{-\delta_{n+1} t (r_{n+1} - 1)}) \sim \frac{(\omega_{n+1}/x)^{\delta_1/\delta_{n+1}} e^{\delta_{n+1} t (r_{n+1} - 1) \delta_1/\delta_{n+1}}}{\Gamma(1 - \delta_1/\delta_{n+1})}$$

as $xe^{-\delta_{n+1} t (r_{n+1} - 1)} \to \infty$.

### 4.4 Hitting times

We now turn to the time at which the type $n$ population arrives. Our limit results concerning this question are identical for both the original and approximate model, with only the parameters in the limit expressions changing. To avoid repeating results we introduce the superscript $\circ$, such that statements with variables with $\circ$ superscript are true for both models. Here, the first time a cell arrives of type $n+1$ is

$$\tau_{n+1}^\circ = \min\{t \geq 0 : Z_{n+1}^\circ(t) > 0\}.$$ 

It turns out $\tau_{n+1}^\circ$ can be appropriately centered using the following variables

$$\sigma_n = \delta_n^{-1} \log(\nu_n^{-1}), \quad m_n = \delta_n^{-1} \log(\nu_n^{-1} \sigma_n^{-1} \sigma_n^{-1} r_n)$$

such that its distribution simplifies for small final seeding rates.
Proposition 4.14. As $\nu_n \to 0$, 
$$
P(\tau_{n+1}^\circ - m_n > t) \to \mathbb{E}[\exp(-W_n^\circ e^{\delta_1 t} / \delta_1)].$$

Proof of Proposition 4.14. We introduce $\rho_n = \delta_n^{-1} \log(\sigma_n^{-1})$ so that $m_n = \sigma_n - \rho_n$. First let’s condition on $Z_n = (Z_n^\circ(s))_{s \in \mathbb{R}}$
$$
P(\tau_{n+1}^\circ - (\sigma_n - \rho_n) > t | Z_n) = \exp \left(-\nu_n \int_0^{t+\sigma_n-\rho_n} Z_n^\circ(s) \, ds \right)$$
$$= \exp \left(-\nu_n \int_{-(\sigma_n-\rho_n)}^{t} Z_n^\circ(u + \sigma_n - \rho_n) \, du \right)$$

Observe that $\nu_n Z_n^\circ(u + \sigma_n - \rho_n)$ can be expressed as
$$\frac{Z_n^\circ(u + \sigma_n - \rho_n)}{\exp(\delta_n(u + \sigma_n - \rho_n)) (u + \sigma_n - \rho_n)^{r_n}} \times \nu_n \exp(\delta_n(u + \sigma_n - \rho_n)) (u + \sigma_n - \rho_n)^{r_n-1}.$$ 

As $\nu_n \to 0$ the first factor above converges to $W_n^\circ$. The second factor may be expressed as 
$$e^{\delta_n u (u + \sigma_n - \rho_n)^{r_n-1}}$$
which converges to $e^{\delta_n u}$ as $\nu_n \to 0$. Hence $\nu_n Z_n^\circ(u + \sigma_n - \rho_n) \to W_n^\circ e^{\delta_n u}$.

Propositions 4.1 and 4.5 imply that for any realisation we may find small enough $x$ such that for $\nu_n \leq x$
$$Z_n^\circ(u + \sigma_n - \rho_n) \leq 2 W_n^\circ e^{\delta_n u} u^{r_n-1}$$
which is integrable over $(-\infty, t]$. Using dominated convergence we have the claimed result.

We know that with $\delta_n = \lambda_1$, $W_n^\circ$ has an exponential distribution, and so the limit distribution for $\tau_{n+1}^\circ$ may be immediately obtained [9]. If there are fitness increases, we turn to our small mutation results for the approximate model.

For the remainder of this section we discuss only results for the approximate model. The below results also hold for the original branching processes if the running-max fitness does not increase, i.e. $\delta_n = \lambda_1$.

Thus with $\mathcal{F}_{n-1}(\nu)$ as in Eq. 13, and using Proposition 4.7, we see that:

Corollary 4.15.

$$\lim_{\nu_1 \to 0} \ldots \lim_{\nu_n \to 0} \mathbb{P}(\tau_{n+1}^* - m_n - \delta_n^{-1} \log \mathcal{F}_{n-1}(\nu) > t) = \mathbb{E} \left[ \exp \left( -W_1^* e^{\delta_1 t} \frac{\delta_n}{\delta_1} \prod_{i=1}^{n-1} \frac{\delta_1 / \delta_i}{\delta_i} \right) \right]$$
$$= \left( 1 + \left[ (1 - \beta_1 / \alpha_1) \delta_n / \delta_1 \right]^{-1} e^{\delta_1 t} \prod_{i=1}^{n-1} \frac{\delta_1 / \delta_i}{\delta_i} \right)^{-1}$$

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Proof. From Proposition 4.14
\[
\lim_{\nu_1 \to 0} \ldots \lim_{\nu_n \to 0} \mathbb{P}(\tau_{n+1}^* - m_n - \delta_n^{-1} \log \mathcal{F}_{n-1}(\nu) > t) = \lim_{\nu_1 \to 0} \ldots \lim_{\nu_{n-1} \to 0} \mathbb{E}[\exp(-W_n^* \mathcal{F}_{n-1}(\nu)e^{\delta_n t}/\delta_n)].
\]
While from Proposition 4.7,
\[
\lim_{\nu_1 \to 0} \ldots \lim_{\nu_n \to 0} \mathbb{E} \left[ \exp \left( -W_n^* \mathcal{F}_{n-1}(\nu)e^{\delta_n t}/\delta_n \right) \right] = \mathbb{E} \left[ \exp \left( -W_n^* (e^{\delta_n t}/\delta_n)\sum_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_n} \right) \right] = \left( 1 + [(1 - \beta_1/\alpha_1)\delta_n^{\delta_i/\delta_n} - 1]e^{\delta_n t}\prod_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_n} \right)^{-1}.
\]
This implies that for small mutation rates
\[
\mathbb{P}(\tau_{n+1}^* > t) = \mathbb{P}(\tau_{n+1}^* - m_n - \delta_n^{-1} \log \mathcal{F}_{n-1}(\nu) > t - m_n - \delta_n^{-1} \log \mathcal{F}_{n-1}(\nu)) \\
\approx \mathbb{E} \left[ \exp \left( -W_n^* \delta_n^{\delta_i/\delta_n} e^{\delta_n t} \mathcal{F}_{n-1}(\nu)^{-\delta_i/\delta_n} e^{-\delta_n m_n} \prod_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_n} \right) \right] \\
= \left( 1 + \delta_n^{\delta_i/\delta_n} e^{\delta_n t} (1 - \beta_1/\alpha_1)^{-1} \mathcal{F}_{n-1}(\nu)^{-\delta_i/\delta_n} e^{-\delta_n m_n} \prod_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_n} \right)^{-1}.
\]
Recall that
\[
\omega_n = (1 - \beta_1/\alpha_1)^{-\delta_n/\delta_i} \mathcal{F}_{n-1}(\nu)^{-1} \prod_{i=1}^{n-1} \kappa_i^{\delta_i/\delta_n},
\]
and that by the definition of \(m_n\),
\[
e^{-\delta_n m_n} = \exp \left[ -\frac{\delta_i}{\delta_n} \log[\nu_n^{-1}(\delta_n^{-1} \log(\nu_n^{-1}))^{(r_n-1)}] \right] = \nu_n^{\delta_i/\delta_n} (\delta_n^{-1} \log(\nu_n^{-1}))^{(r_n-1)}\delta_i/\delta_n.
\]
Hence
\[
\mathbb{P}(\tau_{n+1}^* > t) \approx \left[ 1 + e^{\delta_n t} \left( \frac{\omega_n \nu_n (\delta_n^{-1} \log(\nu_n^{-1}))^{(r_n-1)}}{\delta_n} \right)^{\delta_i/\delta_n} \right]^{-1}.
\]
Defining
\[
t_{1/2}^{(n+1)} = \frac{1}{\delta_n} \log \frac{\delta_n}{\omega_n \nu_n (\delta_n^{-1} \log(\nu_n^{-1}))^{r_n-1}}
\]
we see that \(\tau_{n+1}^*\) has a logistic distribution with scale parameter \(\delta_1^{-1}\) and median \(t_{1/2}^{(n+1)}\)
\[
\mathbb{P}(\tau_{n+1}^* > t) \approx \left[ 1 + e^{\delta_1(t-t_{1/2}^{(n+1)})} \right]^{-1} \quad (16)
\]
The median times satisfy the following recurrence:
Lemma 4.16. Set

\[ t^{(2)}_{1/2} = \frac{1}{\delta_1} \log \frac{\delta_1^2}{\alpha_1 \nu_1}. \]

Then for \( n \geq 2 \)

\[ t^{(n+1)}_{1/2} = t^{(n)}_{1/2} + \begin{cases} 
\frac{1}{\delta_n} \log \frac{(\delta_{n-1}-\lambda_n)}{\nu_n} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} & \text{if } \delta_{n-1} > \lambda_n \\
\frac{1}{\delta_n} \log \frac{\nu_n}{\nu_{n-1}} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} & \text{if } \delta_{n-1} = \lambda_n \\
\frac{1}{\delta_n} \log \frac{\nu_n}{\nu_{n-1}} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} - \frac{1}{\delta_{n-1}} \log(\delta_{n-1}^{\nu_n^{-1}} \kappa_{n-1}) & \text{if } \delta_{n-1} < \lambda_n 
\end{cases} \] (17)

Proof. We start with \( \lambda_n < \delta_{n-1} \), in which case \( \omega_n = \frac{\nu_{n-1}}{\delta_{n-1}-\lambda_n} \omega_{n-1} \), and \( \delta_{n-1} = \delta_n, r_n = r_{n-1} \), thus

\[ t^{(n+1)}_{1/2} = \frac{1}{\delta_n} \log \frac{\delta_n (\delta_{n-1} - \lambda_n)}{\nu_n \left[ \log(\nu_n^{-1}) \right]^{r_{n-1}} \nu_{n-1} \omega_{n-1}} \\
= \frac{1}{\delta_n} \log \frac{(\delta_{n-1} - \lambda_n)}{\nu_n \left[ \log(\nu_n^{-1}) \right]^{r_{n-1}}} + \frac{1}{\delta_n} \log \frac{\delta_n}{\nu_{n-1} \omega_{n-1}} \\
= \frac{1}{\delta_n} \log \frac{(\delta_{n-1} - \lambda_n)}{\nu_n} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} + \frac{1}{\delta_n} \log \frac{\delta_n}{\nu_{n-1} \omega_{n-1} \left[ \log(\nu_n^{-1}) \right]^{r_{n-1}}} \\
= \frac{1}{\delta_n} \log \frac{(\delta_{n-1} - \lambda_n)}{\nu_n} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} + t^{(n)}_{1/2}. \]

For the case of \( \lambda_n = \delta_{n-1} \), then \( \omega_n = \nu_{n-1} \omega_{n-1} / r_{n-1} \) and \( \delta_n = \delta_{n-1}, r_n = r_{n-1} + 1 \), thus

\[ t^{(n+1)}_{1/2} = \frac{1}{\delta_n} \log \frac{\delta_n r_{n-1}}{\nu_n \left[ \log(\nu_n^{-1}) \right]^{r_{n-1}} \nu_{n-1} \omega_{n-1}} \\
= \frac{1}{\delta_n} \log \frac{r_{n-1}}{\nu_n} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} + \frac{1}{\delta_n} \log \frac{\delta_n}{\nu_{n-1} \omega_{n-1} \left[ \log(\nu_n^{-1}) \right]^{r_{n-1}}} \\
= \frac{1}{\delta_n} \log \frac{r_{n-1} \delta_{n-1} - \lambda_n}{\nu_n} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} + \frac{1}{\delta_n} \log \frac{\delta_n}{\nu_{n-1} \omega_{n-1} \left[ \log(\nu_n^{-1}) \right]^{r_{n-1}}} \\
= \frac{1}{\delta_n} \log \frac{r_{n-1} \delta_{n-1} - \lambda_n}{\nu_n} \left[ \frac{\log(\nu_n^{-1})}{\log(\nu_n^{-1})} \right]^{r_{n-1}} + t^{(n)}_{1/2}. \]

Turning to the case of \( \lambda_n > \delta_{n-1} \), we have \( \omega_n = (\omega_{n-1} \nu_{n-1} \log(\nu_n^{-1})^{r_{n-1}} \kappa_{n-1})^{\lambda_n/\delta_{n-1}} \), or alternatively

\[ \omega_n \delta_{n-1}^{-\lambda_n/\delta_{n-1}} = \left[ \omega_{n-1} \nu_{n-1} \delta_{n-1}^{-1} \log(\nu_n^{-1})^{r_{n-1} \kappa_{n-1}} \right]^{\lambda_n/\delta_{n-1}}. \]
and we also have $\delta_n = \lambda_n$ and $r_n = r_{n-1}$. Similarly to before

$$t_{1/2}^{(n+1)} = \frac{1}{\delta_n} \log \frac{\delta_n}{\nu_n[\delta_n^{-1} \log(\nu_n^{-1})]^{r_n-1}} + \frac{1}{\delta_n} \log \frac{\delta_n^{-(r_n-1)} \lambda_n/\delta_{n-1}}{\omega_n^{(r_n-1)} \lambda_n/\delta_{n-1}}$$

$$= \frac{1}{\delta_n} \log \frac{\delta_n}{\nu_n[\delta_n^{-1} \log(\nu_n^{-1})]^{r_n-1}} + \frac{1}{\delta_n} \log \delta_n^{-(r_n-1)} \delta_n/\delta_{n-1}$$

$$+ \frac{1}{\delta_n} \log \frac{1}{(\delta_n^{-1} \kappa_{n-1}) \delta_n/\delta_{n-1}}$$

$$= \frac{1}{\delta_n} \log \frac{\delta_n}{\nu_n[\delta_n^{-1} \log(\nu_n^{-1})]^{r_n-1}} + \frac{1}{\delta_n} \log \delta_n^{r_n-1} \kappa_{n-1} + t_{1/2}^{(n)}$$

\[\square\]

We summarise this approximate distribution of $\tau_{n+1}^*$ as a theorem, to emphasise that it is the culmination of the results in this section.

**Theorem 4.17.** For $t$ all $\nu_i$ small

$$\mathbb{P}(\tau_{n+1}^* > t) \approx \left[1 + e^{\delta_1(t-t_{1/2}^{(n+1)})}\right]^{-1}.$$  

where the median times $t_{1/2}^{(n+1)}$ which satisfies the recurrence of Lemma 4.16.

We have stated our result for the hitting time $\tau_{n+1}$. However, the population initiated by the first cell of type $n + 1$ could go extinct, and so we might wish to instead consider the waiting time until the first type $n + 1$ cell whose lineage survives. All lineages of type $n + 1$ will eventually go extinct unless $\lambda_n > 0$. If $\lambda_n > 0$ then the results presented in this section for this altered arrival time if we replace $m_n$ by $m'_n = m_n + \delta_n^{-1} \log(1 - \beta_{n+1}/\alpha_{n+1})^{-1}$.

**Remark 4.18.** In the above results we take the ordered limit $\lim_{\nu_{i_1} \to 0} \ldots \lim_{\nu_{i_n} \to 0}$ for two technical reasons:

(i) In the proof of Proposition 4.14 we used the almost sure convergence of the scaled type $n$ cell number, that is Proposition 4.5. As the type $n$ populations’ growth is unaffected by the value of $\nu_n$, no issues arise. However, the type $n$’s growth is affected by $\nu_1, \ldots, \nu_{n-1}$, and so almost sure convergence of cell numbers would not hold when simultaneously sending these mutation rates to 0, thus invalidating our proof strategy.

(ii) We build our understanding of the limit random variable $W_{n+1}^*$ from the distribution of $W_n^*$, as seen in Corollary 4.6. Small mutation rate limits were required to circumvent the complexity introduced by the Lerch transcendent in $h_n(\theta)$, and then ultimately in the composite function $\mathcal{H}_n(\theta)$. In $\mathcal{H}_n(\theta)$, the function $h_{i+1}$ is applied before $h_i$, hence the mutation rate ordering.
This specific ordering may have consequences on higher order details; for example in Eq. (16), the final mutation rate $\nu_n$ is privileged, appearing in the $\log(\nu_n^{-1})$ term. In other limits, e.g. all mutation rates are equal, this term may alter. On the other hand, when considering $\tau_{n+1}$, we wait for the first mutation of type $n+1$, whereas multiple mutations may occur from type $i \to i+1$ for $i = \ldots, n-1$; so the $\log(\nu_n^{-1})$ might remain in alternative limit orders. However, for practical scenarios we do not expect this feature to considerably impact results; this may be seen by the considering the median time $t^{(n)}_{1/2}$, where it’s clear that the privileged term acts as a higher order log log correction to the leading behaviour.

4.5 Illustrative examples derivations

In the fitness increasing case we show that the time between the type $n$ arising and the type $n+1$ arising decreases as a function of $n$. Take $\nu_i = \nu$, and recall $\gamma_{n-1} = \lambda_{n-1}/\lambda_n$, then

$$t_{1/2}^{(n+1)} - t_{1/2}^{(n)} = \frac{1}{\lambda_n} \log \frac{\lambda_n}{\nu} + \frac{1}{\lambda_{n-1}} \log \frac{\sin \pi \gamma_{n-1}}{\gamma_{n-1} (\lambda_n/\alpha_n)^{1-\gamma_{n-1}} \pi}.$$ 

Thus for $\nu \to 0$,

$$t_{1/2}^{(n+1)} - t_{1/2}^{(n)} \sim \frac{1}{\lambda_n} \log \frac{\lambda_n}{\nu}.$$

As

$$\frac{d}{dx} x^{-1} \log(x/c) = \frac{1 - \log(x/c)}{x^2}$$

we see that $\frac{1}{\lambda_n} \log \frac{\lambda_n}{\nu}$ decreases as $\lambda_n$ increases, so long as $\lambda_n > e\nu$. Hence for $\nu$ small $t_{1/2}^{(n+1)} - t_{1/2}^{(n)}$ decreases as a function of $n$; it is also likely $\nu$ small is not required here but we opt to refrain from giving detailed inequalities.

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