Age distribution formulas for budding yeast

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Abstract

Yeast are an important eukaryotic model system in the study of aging. Replicative age in budding yeast can be quantitatively determined by visualizing chitanous bud scars. The dynamics of the process of growth and division effects the distribution of replicative age. How much physiological information is encoded in experimental age distributions is not fully understood. Formulas relating the stationary age distribution to the spectrum of generational and culture doubling times have been proposed by several authors over the past four decades. We discuss the assumptions upon which they rest and some natural extensions. We describe the replicative age distribution of a population growing exponentially in terms of generational flux residence times. We demonstrate the utility of this description and show that it produces excellent agreement with experimental data, and describe how it compares with previous work. We demonstrate that the age distribution in a variety of strains can be predicted by a realistic population model, and we indicate how the age distribution is altered by perturbations and control.

Keywords: Population Dynamics, Cell Cycle, Bioreactor

1 Introduction

Aging is perhaps the most compelling biological mystery to occupy human interest. Aging is currently a vigorous research area with several lines of converging evidence and enormous progress [1, 2, 12, **?**, 19]. Yeast are an ideal model organism central to this research effort. This is true in part because replicative age in budding yeast is straightforward to determine from the number of chitanuous bud scars apparent on the cell wall. Because age distribution data are easily accumulated it is of interest to understand the full extent of the information they carry about the process of growth and division, the eukaryotic cell cycle and its control. Quantitative information regarding the growth and division of older cells is especially valuable since many of the finer details are technically challenging to measure. What is to be gained from quantitative knowledge of the minute details of yeast growth and division? Beyond simple curiosity, profit currently lies in the ability to deconvolve population measurements. Measurements such as gene or protein expression routinely involve the isolation of biomolecules from a sample of 10^8 cells. We, and others [5, 20], have shown that experimentally annotated models of yeast growth and division can accurately stratify a population by age, by volume, by mass, by cell cycle progression, and through this attribution vastly expand the power and scope of a single measurement.

Replicative aging must in general be differentiated from chronological aging, lifespan and senescence. When yeast are growing exponentially in continuous culture it is likely that these notions agree. However, there is evidence that this is not the case for logistic growth in batch or in colonies grown on solid substrates [18]. The term *age* has been used with slightly different semantics in different contexts. For instance, in population balance models age is often used as a continuous variable of position to represent cell cycle progression [10, 13]. In the context of this work, age, is a discrete quantity that is determined from the number of bud scars and as such is in correspondence with the number of replicative divisions. We shall use the terms, age, replicative age, age class, generation, and P_k, interchangeably. We use the term age distribution to mean the fractional population density resident within each age class. For instance, if we remove a single yeast cell, uniformly at random, from a culture or community, what is the probability that the cell has exactly k bud scars? We denote this

quantity by a_k, and call the set of numbers $\{a_k\}_{k=0}^{\infty}$, for all k, an age distribution. It deserves mention that the in case $k = 0$, the cells contain no bud scars and are often refered to as daughters in the literature. Parents with $k > 0$ are often refered to as mothers.

Within an culture or community of yeast, the population density within each age class is not expected to be uniform, or stationary. From general considerations the only property expected of the distribution is that it monotonically decrease with increasing age. In the special case of synchronous and symmetric divisions the distribution of age is expected to follow the simple geometric series $\{(\frac{1}{2})^{k+1}\}_{k=0}^{\infty}$, where k denotes age class. Several authors have proposed formulas to describe the stationary age distribution of a population of yeast growing exponentially [8, 9, 17]. The interest has been to describe the age distribution in terms of the doubling times of the different age classes and the total population doubling time. It has been observed and appreciated for four decades that these doubling times can vary with age and often by considerable amounts. It has been perhaps less well appreciated that the rate at which cells traverse the cell cycle is not homogeneous [7].

Population models of yeast growth have been studied extensively in chemical engineering and biology. Excellent reviews can be found in []. We have been studying a discrete population model of yeast growth and division for the purpose of investigating phenomena related to cell cycle synchrony [4, 3, 5], quorum sensing [**?**] autonomous oscillations and model adaptive control [**?**]. The age distribution is computable and our interest in this subject began as we compared the the model predictions with the formulas described in [8, 9, 17]. The discrepancies aroused our curiosity and our attempts to understand them comprise this paper.

Our results can be summarized as follows: In general, there exist well defined flux residence times, τ_k, for each age class, k, that describe the length of time that it takes for influxing population density to efflux that age class. This notion will be made precise below. Under certain assumptions, the age distribution can be described in a simple way in terms of these times. The equality of the τ_k accross all generations implies that the age distribution is a simple geometric series. In general however, budding yeast of different age do not traverse their cell cycles with same average rate and thus the τ_k differ with k. Grover and Woldringh [8] correctly proposed that the flux residence times can be recovered from

an age distribution. However, their precise formulation is flawed. In a seminal work Lord and Wheals [17] presented a formula for the age distribution. Their work depends on the modeling assumptions made by Hartwell and Unger [10] that rest on the assumptions that parents and daughters are indistinguishable after a certain point in a common cell cycle, and that parents of all ages have the same uniform doubling time. These assumptions are violated in real yeast. These issues were addressed by Gyllenburg in [9] where the author derives a general formula for the age distribution. We implement the formula of Gyllenburg and show that it agrees with our population model predictions, our formula based on flux residence times, and experimental data. We describe a simple procedure to uniquely extract the flux residence times from age distribution data.

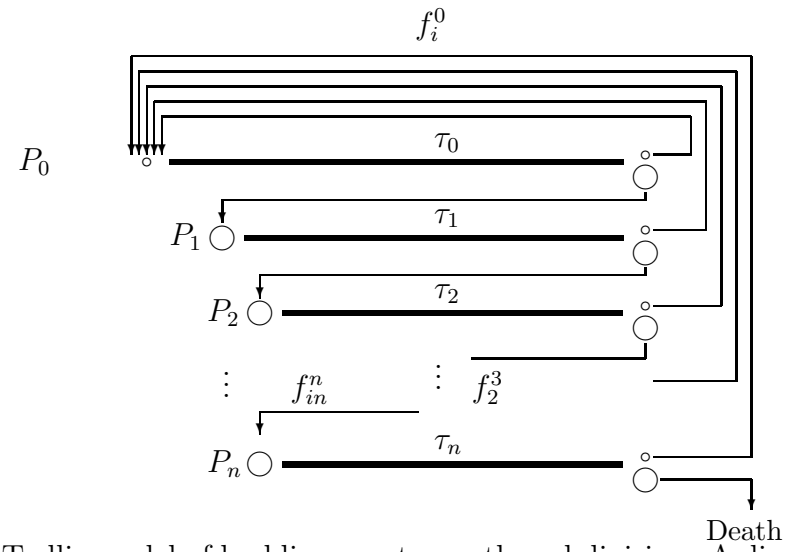

Figure 1: Trellis model of budding yeast growth and division. A discrete series of age classes are arranged vertically with daughter cells, P_0, at the top, that senesce downward with each division. The horizontal axes represents cell cycle progression within each age class. Newly divided cells begin a mitotic cell cycle at the far left, and division ocurs at the far right. The τ_k's represent the flux residence times that are formally defined through the conservation equations 2.

2 Notation and Models

Consider the diagram of Figure 1, to represent the process of yeast growth and division. At a logical level our trellis is identical to those in [20, figure 5] and [11, figure 3]. As described in the legend, each generation begins with the influx of newly divided cells entering at the beginning of the cell cycle into the G_1 phase on the left that eventually efflux at division ending the M phase on the right. The process of influx and efflux occurs within the cell cycles of each generation. The arrows in the trellis diagram indicate how the flux of cells connects the generations. There is an essential asymmetry in the flux graph because each division gives rise to a new daughter. Importantly, daughters born from parents of different ages are often statistically distinguishable based on volume [20, **?**], and are also thought to be statistically distinguishable metabolically [**?**]. One of our goals is to understand how these physiological details impact the dynamics and conversely how they can be recovered from dynamical measurements.

Given a population of yeast growing in a culture or in a community we might imagine using flow cytometry or an analagous experimental methodology to measure the population

distribution stratified according to the variables implied in Figure 1. In experimental practice this has been surprisingly hard to achieve because the chitin content of the cell wall remains nearly constant. Harder still are measurements of flux. However, given a population of yeast the notion of flux is well defined. By flux we mean the total number of cells entering or leaving an age class within a prescribed interval of time. In mathematical models of population growth this quantity is easily accessible.

$$\begin{cases} f_{in}^{i}(t) = \text{Total flux entering the ith generation at time } t \\ f_{i}^{0}(t) = \text{Daughter cells produced from a division in age class i at time } t \\ f_{out}^{i}(t) = f_{i}^{0}(t) \\ A_i(t) = \text{Total number of cells of generation i at time } t \\ a_i(t) = \text{Fractional number of cells of generation i at time } t \end{cases} \tag{1}$$

2.1 Age and Flux

Because the number of cells in a population can only increase through division, according to the routes depicted by the arrows in the trellis, the flux is related to the age distribution, through the following differential equation.

$$\dot{A}_k = f_{in}^{k} - f_{out}^{k} = \begin{cases} \sum_{i=0}^{n} f_i^0 - f_0^0, & \text{for } k = 0 \\ f_{out}^{k-1} - f_{out}^{k} = f_{k-1}^{0} - f_k^0 \end{cases}$$

Summing, we have the following.

$$\sum_{i=0}^{n} \dot{A}_i(t) = \sum_{i=0}^{n-1} f_i^0(t).$$

Since the total number of cells in the population is simply the sum over all the cells from all the generations we have by linearity:

$$\sum_{i=0}^{n} \dot{A}_i(t) = \frac{d}{dt} \sum_{i=0}^{n} A_i(t) = \frac{d}{dt} N(t)$$

We will use the term *stationary* exponential growth to denote a culture that is doubling at a rate that is invariant with respect to time. This hypothesis is satisfied by population models [**?**] and observed to be true for growth in a biorector. During stationary exponential growth, with growth rate α, the following conditions hold.

$$A_k(t) = \frac{1}{\alpha}\dot{A}_k(t) \quad \text{and} \quad N(t) = \frac{1}{\alpha}\dot{N}(t),$$

If we consider a finite number of age classes we have that the age distribution for n generations is:

$$a_k^n = \frac{A_k(t)}{N(t)} = \frac{\frac{1}{\alpha}\dot{A}_k(t)}{\frac{1}{\alpha}\sum_{i=0}^{n-1} f_i^0(t)} = \begin{cases} \frac{\sum_{i=0}^{n} f_i^0 - f_0^0}{\sum_{i=0}^{n-1} f_i^0(t)}, & \text{for } k = 0 \\ \frac{f_{k-1}^0 - f_k^0}{\sum_{i=0}^{n-1} f_i^0(t)}, & \text{for } k > 0 \end{cases}$$

And in the limit this becomes:

$$a_i^n \to a_i = \begin{cases} \frac{\sum_{i=0}^{\infty} f_i^0 - f_0^0}{\sum_{i=0}^{\infty} f_i^0(t)}, & \text{for } k = 0 \\ \frac{f_{k-1}^0 - f_k^0}{\sum_{i=0}^{\infty} f_i^0(t)}, & \text{for } k > 0 \end{cases} \quad \text{as } n \to \infty$$

This is simplified if we denote the total efflux by $\mathbf{F}(t)$, $\mathbf{F}(t) = \sum_{i=0}^{\infty} f_{out}^i(t) = \sum_{i=0}^{\infty} f_i^0(t)$, then

$$a_i = \begin{cases} \frac{\mathbf{F}(t) - f_0^0}{\mathbf{F}(t)}, & \text{for } k = 0 \\ \frac{f_{k-1}^0 - f_k^0}{\mathbf{F}(t)} & \text{for } k > 0 \end{cases}$$

2.2 Flux Residence Time

Under appropriate nutrient conditions, between birth and division the yeast progress through their cell cycle and grow in volume. Assuming that this process is reasonably uniform for all yeast of a given age class the growth implies the following conservation equations for the

flux.

$$\begin{cases} f_0^0(s+\tau_0) = \sum_{j=0} f_j^0(s) \\ f_i^0(s+\tau_i) = f_{(i-1)}^0(s) \qquad (i \geq 1) \end{cases} \tag{2}$$

The flux residence times τ_k, reflect the average time that cells of a given generation spend growing between successive rounds of replication. It has been well understood since the earliest work on yeast physiology that the rate of cell cycle progression depends on environmental variables. There has been enormous progress in modeling the interaction between metabolism and growth [**?**, 13] that currently includes efforts to integrate gene regulation, quorum sensing and control. These comments highlight the variable dependencies of the τ_k. These relationships were recognized by Grover and Woldringh [8], and it would be fair to call the flux residence times generational doubling times. The correspondence is sharp in the case of the parental generations. As noted above, in the case of daughters, the $\{f_i^0\}_{i=0}$ do not all enter at the same average place in the P_0 cell cycle. Hence, τ_0 is is an age weighted, ensemble average of residence times.

It is usefull to observe that the Equations (2) define a map with a Leslie-like matrix A [15, 16]:

$$\begin{bmatrix} f_0^0(s+\tau_0) \\ f_1^0(s+\tau_1) \\ f_2^0(s+\tau_2) \\ \vdots \\ f_n^0(s+\tau_n) \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 & \cdots & 1 & 1 \\ 1 & 0 & 0 & \cdots & 0 & 0 \\ 0 & 1 & 0 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & 1 & 0 \end{bmatrix} \begin{bmatrix} f_0^0(s) \\ f_1^0(s) \\ f_2^0(s) \\ \vdots \\ f_n^0(s) \end{bmatrix} = A \cdot \begin{bmatrix} f_0^0(s) \\ f_1^0(s) \\ f_2^0(s) \\ \vdots \\ f_n^0(s) \end{bmatrix} \tag{3}$$

There are two cases to consider:

- **(Case 1 : $\tau_i = T$ for all i)** In the absence of external forcing or feedback control to influence the flux, the asymptotic population distribution is determined by iterating the matrix A. The age distribution corresponds to the normalized eigenvector associated with the largest eigenvalue of the Leslie matrix []. This provides a simple proof that the age distribution converges to $\{(\frac{1}{2})^{(k+1)}\}_{k=0}$.

- **(Case 2 : $\tau_i \neq \tau_j$ for some $i \neq j$)** In [8, Equation] the limits of integration were

left unspecified, and assumed to be uniform. This error invalidates their formula. It is possible to devise a numerical algorithm to solve the dynamics in this case. The pseudocode for such an algorithm is presented in the appendix. It is far more interesting to observe that the matrix A is in fact invertible.

Under typical growth conditions yeast populations become stationary very rapidly. There are interesting and important exceptions related to autonomous oscillations []. Yeast that are grown asynchronously in a bioreactor typically double with a stationary rate that equals the dilution rate. Under the assumption of these conditions, we shall return to analyze the consequences of Equation (3) to derive a general formula for the stationary age distribition in terms of the flux residence times.

2.3 Recovery of flux residence times from age distribution data

Here we show that it is possible to recover the flux residence times uniquely from both stationary and non-stationary age distribution data. The stationary age distribution can be rewritten as the following matrix equation:

$$\begin{bmatrix} a_0^n \\ a_1^n \\ a_2^n \\ \vdots \\ a_n^n \end{bmatrix} = \begin{bmatrix} 0 & 1 & 1 & \cdots & 1 & 1 \\ 1 & -1 & 0 & \cdots & 0 & 0 \\ 0 & 1 & -1 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & -1 & 1 \end{bmatrix} \begin{bmatrix} f_0^0(t) \\ f_1^0(t) \\ f_2^0(t) \\ \vdots \\ f_n^0(t) \end{bmatrix} = B \cdot \frac{1}{\mathbf{F}^n(t)} \begin{bmatrix} f_0^0(t) \\ f_1^0(t) \\ f_2^0(t) \\ \vdots \\ f_n^0(t) \end{bmatrix},$$

The matrix B is invertible with inverse:

$$B^{-1} = \begin{bmatrix} \frac{1}{n} & 1 & \frac{n-1}{n} & \cdots & \frac{2}{n} & \frac{1}{n} \\ \frac{1}{n} & 0 & \frac{n-1}{n} & \cdots & \frac{2}{n} & \frac{1}{n} \\ \frac{1}{n} & 0 & -\frac{1}{n} & \cdots & \frac{2}{n} & \frac{1}{n} \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ \frac{1}{n} & 0 & -\frac{1}{n} & \cdots & -\frac{n-2}{n} & -\frac{n-1}{n} \end{bmatrix}.$$

So we have

$$\begin{bmatrix} f_0^0(t) \\ f_1^0(t) \\ f_2^0(t) \\ \vdots \\ f_n^0(t) \end{bmatrix} = \mathbf{F}^n(t)B^{-1} \cdot \begin{bmatrix} a_0^n \\ a_1^n \\ a_2^n \\ \vdots \\ a_n^n \end{bmatrix}$$

Let us denote $B^{-1}(a_i^n)_{0\leq i\leq n}^*$ by $(b_i^n)_{0\leq i\leq n}^*$ ($*$ is the transpose). Then

$$\begin{bmatrix} f_0^0(t) - b_0^n\mathbf{F}^n(t) \\ f_1^0(t) - b_1^n\mathbf{F}^n(t) \\ f_2^0(t) - b_2^n\mathbf{F}^n(t) \\ \vdots \\ f_n^0(t) - b_3^n\mathbf{F}^n(t) \end{bmatrix} = \begin{bmatrix} 1-b_0^n & -b_0^n & -b_0^n & \cdots & -b_0^n & 0 \\ -b_1^n & 1-b_1^n & -b_1^n & \cdots & -b_1^n & 0 \\ -b_2^n & -b_2^n & 1-b_2^n & \cdots & -b_2^n & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ -b_n^n & -b_n^n & -b_n^n & \cdots & -b_n^n & 1 \end{bmatrix} \cdot \begin{bmatrix} f_0^0(t) \\ f_1^0(t) \\ f_2^0(t) \\ \vdots \\ f_n^0(t) \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ \vdots \\ 0 \end{bmatrix}$$

Since the matrix in the middle term has zero determinent, there are infinitely many solutions. This makes sense because once the system is stationary, we can multiply the efflux vector by any constant and get the same age distribution. However, the flux constraints of Equations (2)

$$\begin{cases} f_0^0(t) = \sum_{i=0}^{n} f_i^0(t-\tau_0) \\ f_k^0(t) = f_{k-1}^0(t-\tau_k) \qquad (k \geq 1) \end{cases},$$

allow us to write the following equations to recover τ_i's :

$$\begin{cases} f_0^0(t) = b_0\mathbf{F}(t) = \sum_{i=0}^{n} b_i\mathbf{F}(t-\tau_0) = \sum_{i=0}^{n} f_i^0(t-\tau_0) \\ f_k^0(t) = b_k\mathbf{F}(t) = b_{k-1}\mathbf{F}(t-\tau_k) = f_{k-1}^0(t-\tau_k) \qquad (k \geq 1) \end{cases}, \tag{4}$$

and with $\mathbf{F}(t) = \alpha N(t)$

$$\begin{cases} f_0^0(t) = b_0N(t) = \sum_{i=0}^{n} b_iN(t-\tau_0) = \sum_{i=0}^{n} f_i^0(t-\tau_0) \\ f_k^0(t) = b_kN(t) = b_{k-1}N(t-\tau_k) = f_{k-1}^0(t-\tau_k) \qquad (k \geq 1) \end{cases}. \tag{5}$$

Since $\frac{N(t)}{N(t-T)}$ is monotonically increasing for $T > 0$, we have the existance of unique τ_i's that are the solution of the following equations :

$$\begin{cases} \frac{N(t)}{N(t-\tau_0)} = \frac{\sum_{i=0}^{n} b_i}{b_0} \\ \frac{N(t)}{N(t-\tau_k)} = \frac{b_{k-1}}{b_k} \qquad (k \geq 1) \end{cases} . \tag{6}$$

It turns out that more is possible. We can recover the flux residence times from an arbitrary age distribution that varies in time.

$$a_k^n(t) = \frac{A_k(t)}{N(t)} = \frac{\int_0^t (f_{in}^k(s) - f_{out}^k(s))ds}{\sum_{i=1}^{n} \int_0^t (f_{in}^i(s) - f_{out}^i(s))ds} = \begin{cases} \frac{\sum_{i=0}^{n} \int_0^t f_i^0(s)ds - \int_0^t f_0^0(s)ds}{\sum_{i=0}^{n-1} \int_0^t f_i^0(s)ds}, & \text{for } k = 0 \\ \frac{\int_0^t f_{k-1}^0(s)ds - \int_0^t f_k^0(s)ds}{\sum_{i=0}^{n-1} \int_0^t f_i^0(s)ds}, & \text{for } k > 0 \end{cases}$$

In fact, it can be expressed in a matrix form similar to the stationary case with the differences being the integration of effluxes and the added time dependencies $a_i^n(t)$ and $(b_i^n(t))_{0\leq i\leq n}^* = B^{-1}(a_i^n(t))_{0\leq i\leq n}^*$.

$$\begin{bmatrix} \int_0^t f_0^0(s)ds \\ \int_0^t f_1^0(s)ds \\ \int_0^t f_2^0(s)ds \\ \vdots \\ \int_0^t f_n^0(s)ds \end{bmatrix} = \left[\int_0^t \mathbf{F}^n(s)ds\right] B^{-1} \cdot \begin{bmatrix} a_0^n(t) \\ a_1^n(t) \\ a_2^n(t) \\ \vdots(t) \\ a_n^n(t) \end{bmatrix} = \begin{bmatrix} b_0^n(t) \int_0^t \mathbf{F}^n(s)ds \\ b_1^n(t) \int_0^t \mathbf{F}^n(s)ds \\ b_2^n(t) \int_0^t \mathbf{F}^n(s)ds \\ \vdots \\ b_n^n(t) \int_0^t \mathbf{F}^n(s)ds \end{bmatrix} = \begin{bmatrix} b_0^n(t)\, N(t) \\ b_1^n(t)\, N(t) \\ b_2^n(t)\, N(t) \\ \vdots \\ b_n^n(t)\, N(t) \end{bmatrix}$$

Differentiation of both sides gives us

$$\begin{bmatrix} f_0^0(s)ds \\ f_1^0(s)ds \\ f_2^0(s)ds \\ \vdots \\ f_n^0(s)ds \end{bmatrix} = \begin{bmatrix} \frac{d}{dt}[b_0^n(t)\, N(t)] + f_0^0(0) \\ \frac{d}{dt}[b_1^n(t)\, N(t)] + f_1^0(0) \\ \frac{d}{dt}[b_2^n(t)\, N(t)] + f_2^0(0) \\ \vdots \\ \frac{d}{dt}[b_n^n(t)\, N(t)] + f_n^0(0) \end{bmatrix} = \begin{bmatrix} \frac{d}{dt}[b_0^n(t)\, N(t)] \\ \frac{d}{dt}[b_1^n(t)\, N(t)] \\ \frac{d}{dt}[b_2^n(t)\, N(t)] \\ \vdots \\ \frac{d}{dt}[b_n^n(t)\, N(t)] \end{bmatrix}$$

with the assumption of no efflux at $t = 0$.

Arguing as we did in the stationary case using the efflux equality we have

$$\begin{cases} f_0^0(t) = \frac{d}{dt}[b_0(t)N(t)] = \sum_{i=0}^{n} \frac{d}{dt}[b_i(t-\tau_0)N(t-\tau_0)] = \sum_{i=0}^{n} f_i^0(t-\tau_0) \\ f_k^0(t) = \frac{d}{dt}[b_k(t)N(t)] = \frac{d}{dt}[b_{k-1}(t-\tau_k)N(t-\tau_k)] = f_{k-1}^0(t-\tau_k) \qquad (k \geq 1) \end{cases},$$

and thus

$$\begin{cases} \frac{d}{dt}[b_0(t)N(t)] = \sum_{i=0}^{n} \frac{d}{dt}[b_i(t-\tau_0)N(t-\tau_0)] \\ \frac{d}{dt}[b_k(t)N(t)] = \frac{d}{dt}[b_{k-1}(t-\tau_k)N(t-\tau_k)] \qquad (k \geq 1) \end{cases}. \tag{7}$$

Finally, the following generalized system of equations can be solved to recover the τ's.

$$\begin{cases} \frac{d}{dt}[b_0(t)N(t)]\big|_{t=T} = \sum_{i=0}^{n} \frac{d}{dt}[b_i(t-\tau_0)N(t-\tau_0)]\Big|_{t=T} \\ \frac{d}{dt}[b_k(t)N(t)]\big|_{t=T} = \frac{d}{dt}[b_{k-1}(t-\tau_k)N(t-\tau_k)]\big|_{t=T} \qquad (k \geq 1) \end{cases}. \tag{8}$$

The equation for the stationary state is seen to be a special case when the age distribution is constant. Figure 2 shows an example computation with Equation (7). It can be seen from this figure that the right hand side of Equation (7) lies above the left hand side, and that because both functions are increasing due to growth, there exists a unique value of τ that is easily computed. We show now that this is true in general, that the values of τ_k are either unique for each k, or that the efflux functions are periodic. In either case the analysis provides usefull information.

The a_i and the b_i are related through the linear isomorphism given above by the matrix B and its inverse. From this follow the relations:

$$\sum_{i=1}^{n} a_i(t) = \sum_{i=1}^{n-1} b_i(t) \qquad \text{and} \qquad b_i(t) = b_{i-1}(t) - a_i(t).$$

And from these:

$$\sum_{i=1}^{n} a_i(t) = 1, \qquad \text{it follows that} \qquad \sum_{i=1}^{n} b_i(t) = 1 + b_n(t).$$

$$b_n(t) = b_0(t) - \sum_{i=1}^{n} a_i(t) = b_0(t) - (1 - a_0(t)) = b_0(t) + a_0(t) - 1$$

Applying these relations we find

$$\begin{aligned}
\sum_{i=0}^{n} \frac{d}{dt}[b_i(t)N(t)] &= \frac{d}{dt}[N(t)\sum_{i=0}^{n} b_i(t)] \\
&= \frac{d}{dt}[N(t)(1 + b_n(t))] \\
&= \frac{d}{dt}[N(t)(1 + b_0(t) + a_0(t) - 1)] \\
&= \frac{d}{dt}[b_0(t)N(t)] + \frac{d}{dt}[a_0(t)N(t)] \\
&= \frac{d}{dt}[b_0(t)N(t)] + \frac{d}{dt}A_0(t) \\
&= \frac{d}{dt}[b_0(t)N(t)] + [f_{in}^0(t) - f_{out}^0(t)] \\
&= \frac{d}{dt}[b_0(t)N(t)] + [\sum_{i=0}^{n} f_i^0(t) - f_0^0(t)] \\
&= \frac{d}{dt}[b_0(t)N(t)] + [\sum_{i=0}^{n} f_i^0(t) - \sum_{i=0}^{n} f_i^0(t - \tau_0)]
\end{aligned}$$

For $k \geq 1$

$$\begin{aligned}
\frac{d}{dt}[b_{k-1}(t)N(t)] &= \frac{d}{dt}[(b_k(t) + a_k(t))N(t)] \\
&= \frac{d}{dt}[b_k(t)N(t)] + \frac{d}{dt}[a_k(t)N(t)] \\
&= \frac{d}{dt}[b_k(t)N(t)] + \frac{d}{dt}A_k(t) \\
&= \frac{d}{dt}[b_k(t)N(t)] + [f_{in}^k(t) - f_{out}^k(t)] \\
&= \frac{d}{dt}[b_k(t)N(t)] + [f_{k-1}^0(t) - f_k^0(t)] \\
&= \frac{d}{dt}[b_k(t)N(t)] + [f_{k-1}^0(t) - f_{k-1}^0(t - \tau_k)]
\end{aligned}$$

If population growth outpaces death then $\frac{d}{dt}A_0(t) = [\sum_{i=0}^{n} f_i^0(t) - \sum_{i=0}^{n} f_i^0(t - \tau_0)] \geq 0$

and $\frac{d}{dt}A_k(t) = [f^0_{k-1}(t) - f^0_{k-1}(t-\tau_k)] \geq 0$, are nonnegative for all k which implies

$$\sum_{i=0}^{n} \frac{d}{dt}[b_i(t)N(t)] \geq \frac{d}{dt}[b_0(t)N(t)] \tag{9}$$

$$\frac{d}{dt}[b_{k-1}(t)N(t)] \geq \frac{d}{dt}[b_k(t)N(t)], \tag{10}$$

Finally, suppose that the $f(t) = g(t-\tau)$ and also $f(t) = g(t-\sigma)$. Then, the change of variables $s = t - \tau$ reveals that $g(s) = g(s - (\sigma - \tau))$, hence g is periodic and f aswell. This implies that either the τ_k are unique or that the functions $b_k(t)N(t)$ are periodic.

3 Previous Formulations

In 1980 Lord and Wheals [17] derived a formula for the stationary age distribution of a population growing asynchronously.

$$a_k^{lw} = \begin{cases} e^{-\alpha P} & k = 0 \\ (e^{-\alpha P})^{k-1}(1 - e^{-\alpha P})^2 & k > 0 \end{cases} \tag{11}$$

The analysis assumed that all of the parental generations, $k > 0$, have the same doubling time, P, that is shorter than that of the daughters, D. Notice that Equation (11) depends on only two parameters, the population growth rate α, and P. We began our investigation into the representation of the age distribution after fitting Equation (11) to the output of our population model using P as the free parameter. Our model of the strain X2180 [**?**] contains at least 4 parental generations with independent growth parameters, see Table 5.8 and Appendix. The best fit value of P was uninformative. It did not correspond to any of the parental doubling times, nor to any reasonable function of them. Using the same logic employed to derive Equation (11), it is straightforward to extend this formula to take into account the τ_k spectrum.

3.1 An Extension

Assuming a population is growing with a stationary rate of α and that the population density function $\frac{dg}{dt}$ can be expressed by

$$\frac{dg}{dt} = Ce^{\alpha t}, \quad \text{for some fixed constant } C$$

The arguments of Lord and Wheals [17] produce

$$\begin{cases} a_0 = \int_0^{\tau_0} Ce^{\alpha t} dt \\ a_1 = e^{-\alpha\tau_1} \int_0^{\tau_1} Ce^{\alpha t} dt \\ \vdots \\ a_k = e^{-\sum_{i=1}^{k} \alpha\tau_i} \int_0^{\tau_k} Ce^{\alpha t} dt \\ \vdots \end{cases} \tag{12}$$

Since $\sum a_k(t) = 1$, we have

$$\int_0^{\tau_0} Ce^{\alpha t} dt + \sum_{k=1} e^{-\sum_{i=1}^{k} \alpha\tau_i} \int_0^{\tau_k} Ce^{\alpha t} dt = 1,$$

and this implies

$$C = \frac{\alpha}{(e^{\alpha\tau_0} - 1) + \sum\limits_{k=1} e^{-\sum_{i=1}^{k} \alpha\tau_i}(e^{\alpha\tau_k} - 1)}.$$

We observe that if the τ_k's are the same, then formula (12) produces the degenerate case $a_k = (\frac{1}{2})^{k+1}$.

Hartwell and Unger derived a simple relationship between the mother and daughter doubling times and the stationary culture growth rate. That formula can also be extended to a relationship between the τ_k and the stationary culture growth rate.

The matrix A defined in Equation (3) is invertible and that allows us to write:

$$\begin{bmatrix} f_0^0(s) \\ f_1^0(s) \\ f_2^0(s) \\ \vdots \\ f_n^0(s) \end{bmatrix} = A^{-1} \cdot \begin{bmatrix} f_0^0(s+\tau_0) \\ f_1^0(s+\tau_1) \\ f_2^0(s+\tau_2) \\ \vdots \\ f_n^0(s+\tau_n) \end{bmatrix} = \begin{bmatrix} 0 & 1 & 0 & \cdots & 0 & 0 \\ 0 & 0 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & 0 & 1 \\ 1 & -1 & -1 & \cdots & -1 & -1 \end{bmatrix} \begin{bmatrix} f_0^0(s+\tau_0) \\ f_1^0(s+\tau_1) \\ f_2^0(s+\tau_2) \\ \vdots \\ f_n^0(s+\tau_n) \end{bmatrix}.$$

Assuming the system is stationary the fluxes must grow at the same rate as the total population. Since the daughter cells are growing at the same rate as all other age classes, we can write:

$$A(t) + A(t - \tau_1) + A(t - \tau_1 - \tau_2) + \cdots + A(t - \sum_{i=1}^{n} \tau_i) = A(t + \tau_0).$$

Supposing that the growth rate is α, the relation implies:

$$e^{\alpha t} + e^{\alpha t} e^{-\alpha \tau_1} + e^{\alpha t} e^{-\alpha(\tau_1 + \tau_2)} + e^{\alpha t} e^{-\alpha(\sum_{i=1}^{n} \tau_i)} = e^{\alpha t} e^{\alpha \tau_0}$$

$$e^{\alpha \tau_0} - 1 = \sum_{k=1} e^{-\alpha \sum_{i=1}^{k} \tau_i}$$

This formula properly generalizes the relationship between the flux residence times and the culture growth rate. An analagous formula assuming only two independent times, a daughter doubling time and a parent doubling time was derived by Hartwell and Unger and has been extensively utilized in the literature. If we assume only two generations with flux residence times D and P, the formula reduces to

$$e^{\alpha D} - e^{-\alpha P} = 1.$$

This differs from that of Hartwell and Unger in that we assume a finite number of generations, and that the cells of the last generation die. This assumption is not essential, we enforce it only because it seems natural and reflects the assumptions of our population model.

3.2 The Formula of Gyllenburg

In 1986, M. Gyllenburg [9] in an elegant work, introduced an age distribution formula by reducing a population balance PDE problem to an integral equation through the Laplace transform. While the formula (13) appears accessible, some unraveling is required. Since, to the best of our knowledge, we have not seen this formula used in comparison to real data we briefly describe our implementation and a Matlab script appears in the appendix.

$$a_k^{mg} = \begin{cases} \frac{\beta - \beta_0}{\beta - \beta_n}, & k = 0 \\ \frac{\beta_{k-1} - \beta_k}{\beta - \beta_n}, & k \geq 1 \end{cases} \tag{13}$$

where x is a variable related to cell size and

$$\beta_j = e^{-\lambda_d[r_0+\cdots+r_j]} \int_{x_0}^{1} K_j(x) e^{-\lambda_d[G(x)-G(x_0)]} dx, \quad \text{and} \quad \beta = \sum_{j=0}^{n} \beta_j,$$

with

$$K_j(x) = \frac{b(x)}{g(x)} H_j(x, x_0) e^{-\int_{x_0}^{x} \frac{b(\xi)}{g(\xi)} d\xi},$$

and also r_j, x_0, $b(x)$, $g(x)$, $H_j(x)$, $G(x)$, and λ_d are defined by

j_k = the constant duration of the budded phase in the j^{th} scar class

x_0 = a size which must be reached by a cell before it can enter the budded phase

$b(x)$ =per capita rate at which cells of size x become budded

$g(x)$ = individual growth rate of unbudded cells

$H_0(x, x_0) = 1, \quad H_j(x, x_0) = \int_{x_0}^{x} \frac{b(\xi)}{g(\xi)} H_{j-1}(\xi, x_0) d\xi$

$G(x) = \int_a^x \frac{d\xi}{g(\xi)}$

λ_d = unique real root to

$$\sum_{j=0}^{n} e^{-\lambda[r_0+\cdots+r_j]} \int_{x_0}^{1} K_j(x) e^{-\lambda[G(x)-G(y_j(x))]} dx = 1, \tag{14}$$

where $y_j(x)$ is the size of the bud at time of bud separation.

To fit the formula to the data [6] we take the growth rate $g(x)$ to be the stationary exponential growth rate for each age class as described in Table 5.8. This choice implies that $G(x)$ is constant and $e^{-\lambda[G(x)-G(y_j(x))]} = e^{-\lambda_d[G(x)-G(x_0)]} = 1$. Moreover, $\int_{x_0}^{1} K_j(x) dx = 1$ for all j, that reduces the β_j and equation (14) to the following

$$\beta_j = e^{-\lambda_d[r_0+\cdots+r_j]} \qquad \text{and} \qquad \sum_{j=0}^{n} e^{-\lambda[r_0+\cdots+r_j]} = 1.$$

Identifying $r_k = \tau_k$ we solve equation (14) for λ. Then calculate β_k from λ_d. The entire procedure is given as a Matlab script in the Appendix.

3.3 Comparison of Results

Shown in Figure 3 is a comparison of the extended Lord and Wheals age distribution formula with that of Gyllenburg versus the experimental data of Beran et al. [6] and the compuational predictions of our population model [5]. The comparison is very favorable. The extended formula of Lord and Wheals and the population model simulations are virtually identical over the enire range of age classes. These former differ from the formula of Gyllenburg only slightly in the daughter generation, where all the theory overestimate the experimental data. The theories are in agreement for the first parental generation but slightly underestimate the experimental data.

Two points require mention. All of the theoretical formulas utilize τ_k computed using the X2180 milestones. The experimental data are for an unknown strain that is similar but not identitcal to the X2180 strain. We do not know the milestones for this strain, only the growth conditions and the dilution rate. The later is the sole variable we can adjust in theory to normalize the comparison. Finally, there is not enough information in the experimental data set to reasonably asses the errors and variation. Given these caveats the agreement is deemed very good. The theories all reproduce the non geometric gap in probability between the daughters and the P_1's, as well as the contraction of this gap between the P_1 and P_2's. The structure of these gaps are seen to be the salient features of the data.

In addition to the X2180 strain, we have measured milestones for the α-factor sensative strain LHY3865. These data are shown in Table 2 in the Appendix. The age distribution of LHY3865 was measured in our laboratory under controlled bioreactor conditions and independently in batch. A comparison of these data versus the various theoretical formulas is shown in Figure 4. This figure shows, as does the Beran data, that the logistic growth of a batch culture alters the age distribution. The various theoretical age distribution formula's compute the value's of τ_k, assuming continuous exponential growth. These formulas are able to distinguish bioreactor age distributions from those of a batch culture. It is possible to modify the population model to incorporate logistic or even power law growth. These results will be presented elesewhere.

4 Conclusions

We have presented a simple extension of the classical Lord and Wheals formula that fits real and simulated data. We have utilized a simple flux description to show that the flux residence times can be uniquely extracted from age distribution data, regardless of whether the state of the system is stationary or not. We assume only that the flux residence times, τ_k, depend only on environmental variables such as nutrient conditions, temperature, etc, and that for fixed conditions they are stationary in the sense that they do not depend on time.

We have described how to impliment the age distribution formula of Gyllenburg. The model of Gyllenburg allows for a growth rate that depends on cell cycle position, and can in principle capture aspects of non-homogeneous behavior. However that model, as described, only allows for a single cell cycle. That is, each age class cannot have its own cell cycle. We have learned from the data of Woldringh et al. [**?**], that indeed each age class has the possibility to traverse a very different cell cycle. Let us clarify. Typically, a continuous, monotone increasing property like volume or mass is used to define, measure, or monitor cell cycle progression. Each age class is observed to have very different minimal and maximal volumes, minimal and maximal masses etc. Gyllenburg mentions that the addition of age class dependent growth to the model is a simple extension and can be easily handled, at the expense of very complicated formulas.

Within budding yeast there exists a large degree of strain variation. These are extremely interesting and usefull for comparative studies, but also problematic for quantitative studies and modeling. For instance, it is likely that several years worth of work were required to produce the data that comprises the milestones table 5.8 in reference [**?**]. We, and many other labs [**?**, **?**] are interested in modeling the autonomous oxygen oscillations in the strain Cen.Pk strain. However, we have never seen a carefull study of the growth characteristics of this strain that could populate a table of parameters usefull for quantitative population modeling. One of the goals of this work was to investigate how much milestone information can be gleaned from the age distribution. While more work needs to be invested to fully understand the non-homogeneous case we are currently persuing the following conjecture.

Within the context of the population model whose equations are defined in the appendix, given $\{\bar{V}_k, \bar{V}_k\}$, and time series of age and bud index oscillation from an initially synchronous culture, there exists a contraction map that uniquely determines the remaining volume and growth milestones, namely $\lambda_k, BE_k, MDV_k, MEDV_k$. Such a mapping would allow us to parameterize the population model for a given strain very effectively, as the imputs are all relatively easily aquired, while the outputs are far harder to measure accurately.

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5 Appendix

Here we collect pseudocode to solve equation (3) described in the exposition, as well as a script to implement the formula derived by Gyllenburg [9]. We present the details of Leslie population model [5] of budding yeast growth and division that was used to predict the age distribution data using the LHY3865 and X2180 milestones. A matlab program for the population model is available from the authors upon request.

5.1 Homogeneous Flux Distribution

For computational purpose we assume that there are $n+1$ age classes, $0, 1, \cdots, n$, and parent cell after $n+1$ scars dies.

INPUT : budtime(budded time for each age class).
SET 'repeat' as the total number of iterations.
SET the $n \times n$ matrix $\mathrm{ADD^k} = (\mathrm{add^k_{ij}})$, $1 \le i, j \le (n+1)$ to check the influxes at k-th iteration
SET the $n \times n$ identity matrix $A = (a_{ij})$ for the age distribution.
SET the $(n+1)$ age population vector X^k at k-th iteration with the initial X^1
SET the initial ADD^1 with $(n+1) \times (n+1)$ diagonal matrix with
    i^{th} diagonal entry '1' if $X^1(i) \neq 0$, and othersie '0'.
FOR time=2 to the time we want to repeat
    $\mathrm{ADD^{time}} \leftarrow$ n $\times$ n zero matrix
    FOR index of class $j = n$ to 0
        IF (time-budtime(j)$>$ 0) THEN
            GO BACK to (time-budtime(j))-th step
            IF $\mathrm{add^{(time-budtime(j))}_{jk}} \neq 0$(nonzero influx from k^{th} class) THEN
                AND IF ($j \neq n$) THEN
                    $a_{0k} \leftarrow a_{0k} + \mathrm{add^{(time-budtime(j))}_{jk}}$
                    $a_{(j+1)k} \leftarrow a_{(j+1)k} + \mathrm{add^{(time-budtime(j))}_{jk}}$.
                    $\mathrm{add^{time}_{0k}} \leftarrow \mathrm{add^{(time-budtime(j))}_{jk}}$
                    $\mathrm{add^{time}_{(j+1)k}} \leftarrow \mathrm{add^{(time-budtime(j))}_{jk}}$
                ELSE IF (j $\neq$ n) THEN
                    $a_{0k} \leftarrow a_{0k} + \mathrm{add^{(time-budtime(j))}_{jk}}$
                    $\mathrm{add^{time}_{0k}} \leftarrow \mathrm{add^{(time-budtime(j))}_{jk}}$
            END IF
        END IF
    END FOR
    $X^{\mathrm{time}} = A \cdot X^{\mathrm{time}-1}$
END FOR

5.2 Implementing the Formula of Gyllenburg [9]

Below is a Matlab script that produces the age distribution formula derived in Gyllenburg [9], using the experimental milestones and derived quantities.

```
% V, V̄, λ, BE, MDV, and MEDV are given vector of size 14 from Table 1.
n=14 % the number of age classes
Begin=(BE+V)/2;
End=(MDV+V̄)/2;
for i=1:n
    tau(i)=log(End(i)/Begin(i))/GR(i);
end
tauC=zeros(1,n); % tauC=cumulative tau
tauC(1)=tau(1);
for i=2:n
    tauC(i)=tauC(i-1)+tau(i);
end
f=inline('exp(−λ*tauC(1))+exp(−λ*tauC(2))+exp(−λ*tauC(3))+exp(−λ*tauC(4))
        +exp(−λ*tauC(5))+exp(−λ*tauC(6))+exp(−λ*tauC(7))+exp(−λ*tauC(8))
        +exp(−λ*tauC(9))+exp(−λ*tauC(10))+exp(−λ*tauC(11))+exp(−λ*tauC(12))
        +exp(−λ*tauC(13))+exp(−λ*tauC(14))−1');
λ=fzero(f,0.0001) % finding the root to the equation (14)
λ_d = λ;
beta=zeros(1,n);
for i=1:n
    beta(i)=exp(−λ_d*tauC(i));
end
betaTOT=sum(beta);
AgeDist=zeros(1,n);
AgeDist(1)=(betaTOT-beta(1))/(betaTOT-beta(n));
for i=2:n
    AgeDist(i)=(beta(i-1)-beta(i))/(betaTOT-beta(n));
end
```

5.3 Leslie Population Models

5.3.1 Variables

The population model is organized around two variables: Discrete replicative cell age and total cell volume. Replicative age has been thoroughly described in the paper proper. Cell volume has been observed to increases monotonically with time until division, within a given age class, and thus is often used as a proxy for progression through the mitotic cell cycle. The volume of a budded cell is taken as the total volume of both mother cell and the bud, until division at which point they become distinct. Volume is consistently expressed in units of cubic microns throughout.

5.4 Volume Intervals and Time

Yeast cells of a given replicative age k, are observed to grow in volume between well defined limits. The minimum and maximum volumes naturally delimit and define intervals, $\mathcal{I}(k) := [\underline{V}_k, \overline{V}_k]$. We consider the temporal evolution of the system at a sequence of equally spaced times, $t_s := t_o + s\Delta t$. The volume intervals, $\mathcal{I}(k)$, are partitioned into subintervals $I(i,k) := [V(i,k), V(i+1,k)) \subset \mathcal{I}(k)$, with $\mathcal{I}(k) = \cup_i I(i,k)$, $i = 0, 1, ...n_k$, where $V(0,k) := \underline{V}_k$, and $V(n_k + 1, k) := \overline{V}_k$. The partitions are chosen according to the growth law, within each age class, such that any cell with volume in the interval $I(i,k)$ now, would have a volume in $I(i+1,k)$, precisely Δt later. The unit of time is minutes and we have taken $\Delta t = 1$ throughout. The state of the yeast population at time t_s is described by a vector,

$$\rho(i,k)(t_s) := \text{ number of cells of generation } k \text{ with volume } v \in I(i,k).$$

Each of the $\rho(i,k)(t_s)$ cells living in $I(i,k)$ at time t_s are faced with the following possibilities:

1. The cell dies
2. The volume of the cell increases
3. The cell divides

We describe the details of each of these possibilities in turn.

5.5 Cell Death

The probability that this happens is denoted by $d_{i,k}$. Mortality curves have been measured for several strains of yeast under a variety of conditions [**?**, **?**, **?**](In particular see Table 1 of the latter). These data can be used to determine an age class specific death rate. In [**?**] the authors observe that the death rate on average amounts to $10^{-10}/cell \cdot generation$.

5.6 Volume Growth

The probability that this happens is denoted by $g_{i,k}$, and the fraction of cells that survive and grow is $\kappa_{i,k} := g_{i,k}(1 - d_{i,k})$. Volume growth has been measured and is generally considered to increase exponentially with time. For all of the experiments and analysis in this paper we have considered exponential volume growth. Let λ_k denote the age class specific growth rate. Then, the volume intervals are conveniently described by

$$\begin{aligned} I(0,k) &= \left[\underline{V}_k, \underline{V}_k e^{\lambda_k \Delta t}\right) \\ &\vdots \\ I(i,k) &= \left[V(i,k), V(i,k) e^{\lambda_k \Delta t}\right) \\ &\vdots \\ I(n_k,k) &= \left[V(n_k,k), \overline{V}_k\right) \end{aligned}$$

5.7 Cell Division

All cells do not divide precisely at the same volume. The probability that division occurs is denoted $c_{i,k} := 1 - \kappa_{i,k}$. The importance of including *sloppy size control* in models of growth and division is discussed in [**?**]. We have implemented a variety of distributions. Two of the most natural are a Poisson process [**?**] to model division as time to failure, and second a Brownian process using a normal distribution. As will be described in the results section, qualitatively this choice makes little or no difference. The mean of $c_{i,k}$, for fixed k, is referred to as the mean division volume and denoted as k–MDV.

We assume that the division of a cell of volume v in age class P_k results in a cell of age class P_0 with volume v' and a cell of age class P_{k+1} with volume v''. Furthermore, $v = v' + v''$. We sometimes denote the division process as $P_k \rightarrow P_{k+1}$. It has been experimentally observed [**?**] that after a cell has budded, the ensuing volume growth is concentrated almost entirely in the bud. This implies that there is a conditional probability distribution for v' that depends on the size and age of the mother. Let $\mu_{i,j,k}$ be the probability that after a cell division, $P_k \rightarrow P_{k+1}$, we get a cell of age class P_0 with volume in $I(i,0)$ from a dividing cell in $I(j,k)$. The mean of $\mu_{i,j,k}$, for fixed k, is referred to as the mean emergent daughter volume and denoted k–MEDV. Let, $\nu_{i,j,k}$, represent the probability of parent cell of volume $I(j,k+1)$

emerges from a division in $I(i,k)$. The mean emergent parent volume is denoted k–MEPV. Generally, the distribution of division volumes has been observed to be normal [**?**, **?**].

Given these definitions we can present the projection formula that updates the population in time.

$$\rho(l,0)(t_{s+1}) = \kappa_{l-1,0}\rho(l-1,0)(t_s) + \sum_{k,i} \mu_{l,i,k}c_{i,k}\rho(i,k)(t_s) \quad (15)$$

$$\rho(l,m)(t_{s+1}) = \kappa_{l-1,m}\rho(l-1,m)(t_s) + \sum_{i} \nu_{l,i,m-1}c_{i,m-1}\rho(i,m-1)(t_s);\ m>0 \quad (16)$$

The first summand in each equation represents the volume growth contribution while the second summation term represents the density coming from division. The term $c_{i,k}\rho(i,k)(t_s)$ represents the fraction of dividing cells in volume interval $I(i,k)$ and $\mu_{l,i,k}c_{i,k}\rho(i,k)(t_s)$ is the fraction of those that end up in the volume interval $I(l,0)$. The first equation represents daughters and is distinguished because every division results in a daughter. In the higher age classes, $m>0$, density from division arrives from only one source, namely the age class P_{m-1}.

5.8 Milestones

Age(k)	$\underline{V}_k$	$\overline{V}_k$	λ_k	$\mathrm{BE_k}$	$\mathrm{MDV_k}$	$\mathrm{MEDV_k}$
0	14	75	0.0062	38.5	70.7	28.5
1	40	85	0.0061	46,8	75	24.4
2	48	87	0.0044	56.1	82.4	24.2
3	56	94	0.0047	63.9	88.9	22.3
4-13	64	125	0.0047	76.3	95	22.2

Table 1: Experimentally determined volume milestones and growth parameters for the strain X2180 [**?**] used in parameterize the population model described in the appendix. The quantities $\underline{V}$, $\overline{V}$, λ, BE, MDV, and MEDV denote for each generation a lower bound on the cell volume, an upper bound of cell volume, volume growth rate, mean volume at bud emergence, mean volume at division, and mean volume of the emergent daughter.

Age(k)	$\underline{V}_k$	$\overline{V}_k$	λ_k	BE_k	MDV_k	$MEDV_k$
0	30	105	0.0054	59.0	98	46.0
1	45	105	0.0049	69.5	97.5	43.0
2	53	104	0.0049	68.9	96.6	36.5
3	60	115	0.0049	78.8	110.4	36.5
4	73	140	0.0049	95.7	134.1	36.5
5	97	185	0.0049	123.0	179.1	36.5
6-13	129	190	0.0049	141.0	185.1	36.5

Table 2:

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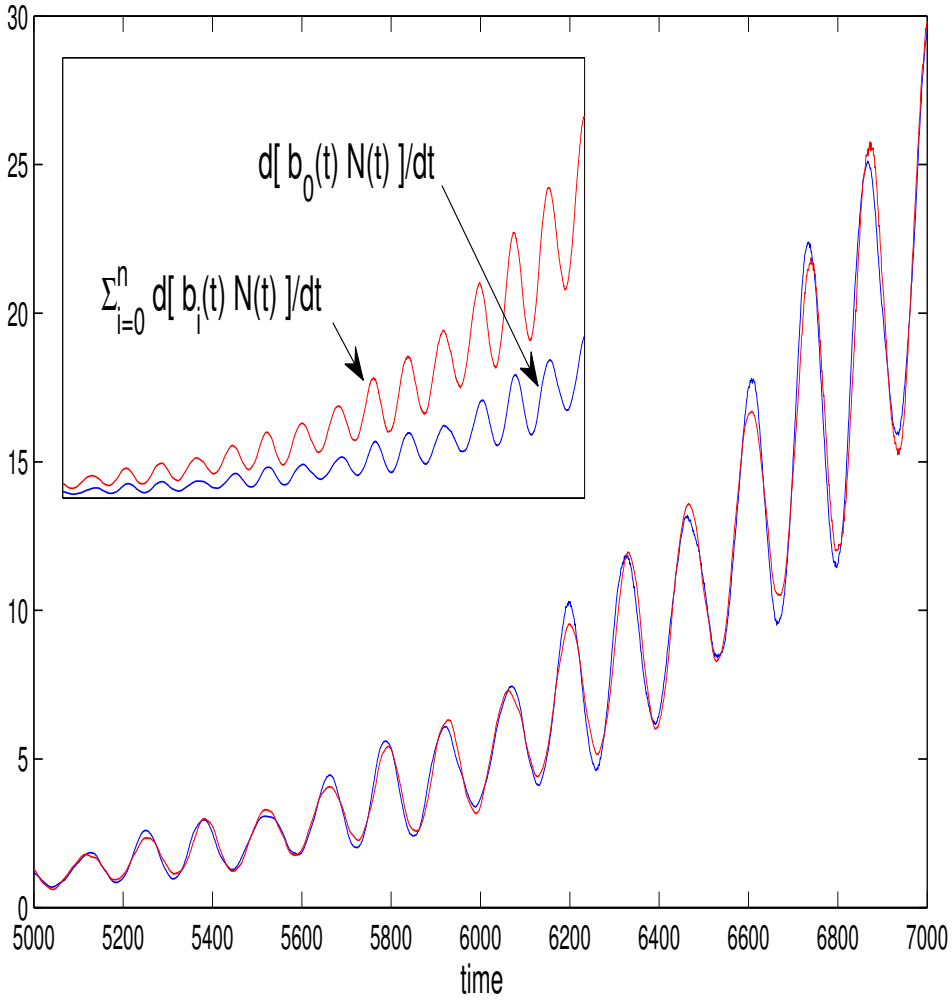

Figure 2: Uniqueness and computation of τ. The inset figure shows the graphs of the the left and right hand sides of Equation (7) from a simulation of the population model. It can be seen that there exists a unique value of $\tau = 550.31$ that shifts the upper function, the rhs, into alignment with the left hand side. The central figure displays the alignment. In practice, only a few oscillations worth of data are required to robustly calculate the value of τ using least squares.

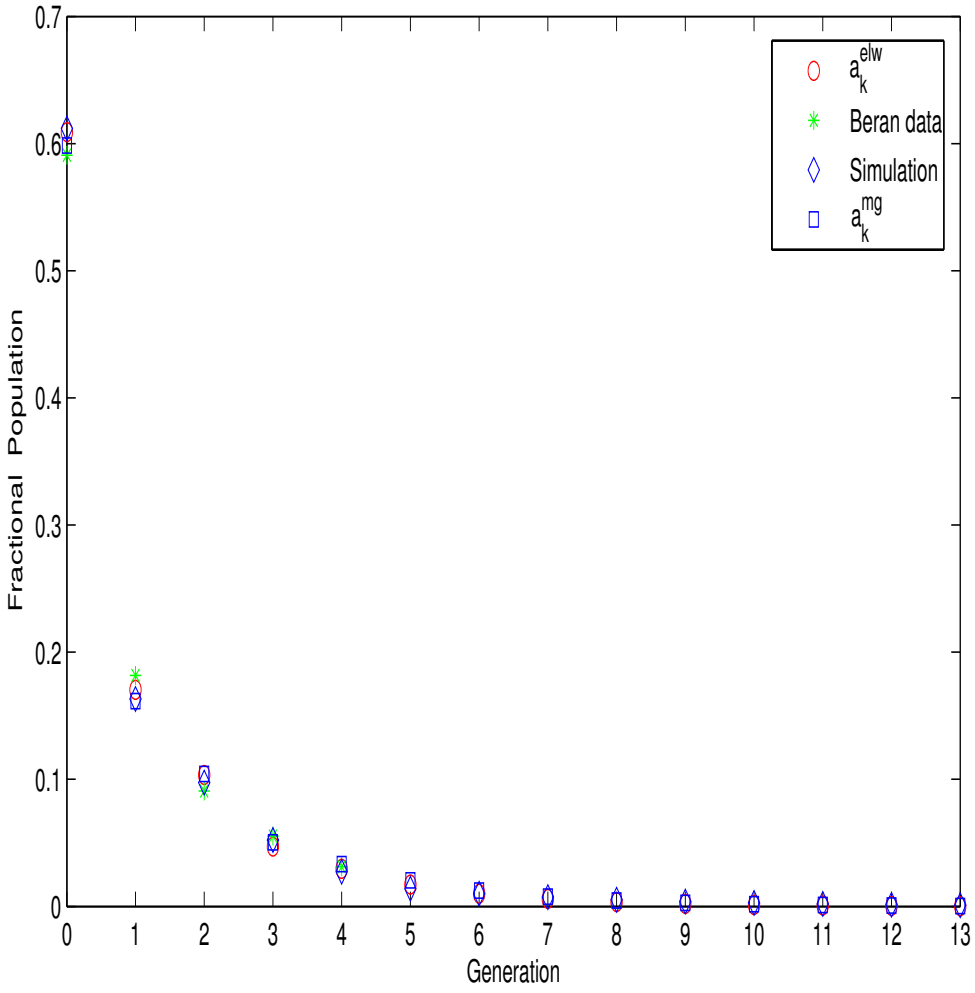

Figure 3: Comparison of age distribution formulas, simulation and experimental data. The theories are all parameterized using the X2180 strain milestones. The experimental data are for an unknown strain grown in a bioreactor at a comparable growth rate.

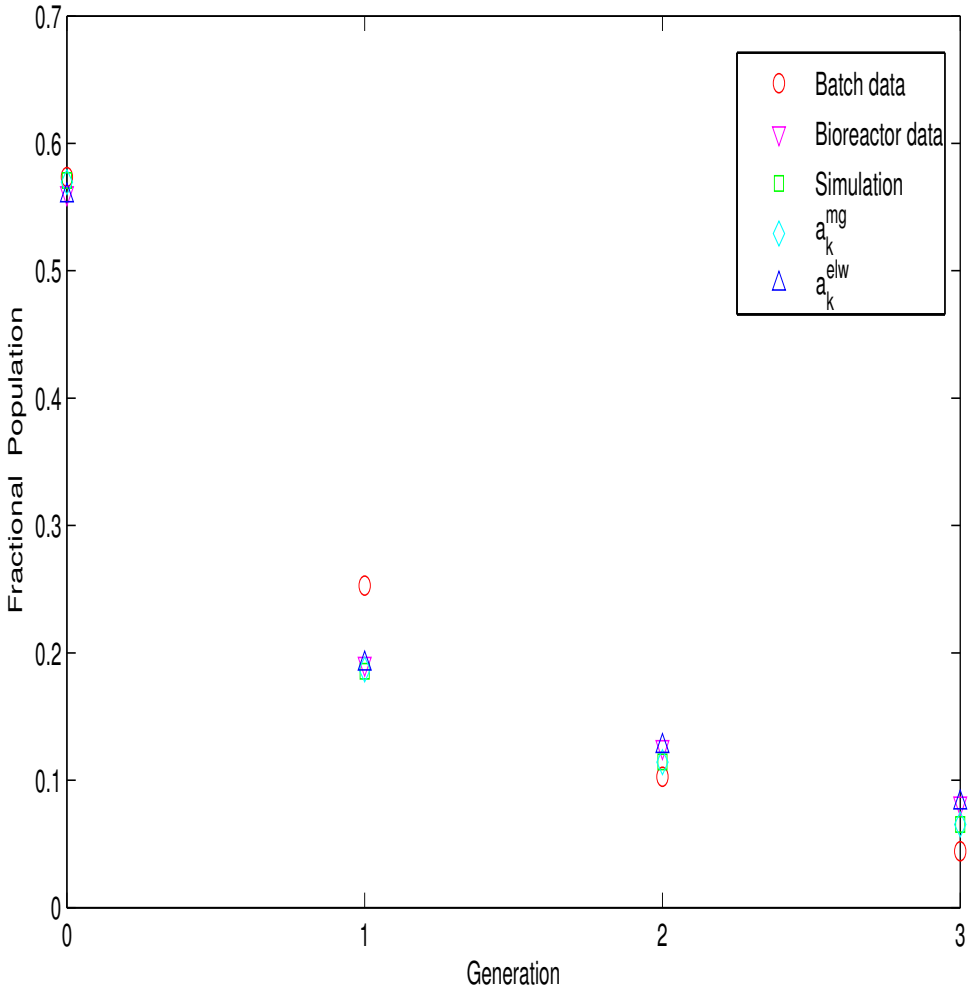

Figure 4: Comparison of age distribution formulas, simulation and experimental data for the strain LHY3865.