Ergodicity: Hidden Bias and the Growthrate Gain

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Abstract

The identification and separation of intrinsic variability from age-related phenomena has been the subject of a growing body of literature, supporting renewed interest in traditional analytic methodologies including use of the “von Foerster equation” for predicting population growth and cell age distributions. Here we discuss how some of the most popular implementations of this machinery assume a strong condition on the ergodicity of the cell cycle duration ensemble and that two common definitions for the term, ergodicity, while identical in an ensemble with a fixed number of individuals, are in fact unique constraints when the ensemble is growing. We further explore the impact on growthrate of generational correlations between cell cycle durations. Finally, we explore the “growthrate gain” - the phenomenon that noise in the cell cycle duration leads to an improved bulk growthrate - in this context. We highlight that, fundamentally, this effect is due to asymmetric division.

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I. INTRODUCTION

A species is generally considered “fit” within its environment when it experiences exponential growth. If that environment changes, the species must adapt to maintain this growth. When that environmental change is due to increasing population density of the species itself, it may be unable to sufficiently adapt to maintain its expanse. If E. coli is grown in LB media, just a few cells can expand to number in the billions within twelve hours; but once the culture is turbid, the population will stagnate and eventually collapse. In 1960, von Foerster et. al considered the fate of humanity with the popular “doomsday” paper[11], predicting that in as soon as 2026, the global population will approach infinity. While a hyperbolic work, the predicted growthrate actually slightly underestimated the population for 30 years[10] before global rates finally did begin to curb in the 1990’s[2]. Unlike E. coli, humans are able to adapt to our own modifications of the environment as discussed in the “doomsday” work which attributes this adaptability to communication and cooperation. While E. coli may not be capable of adapting to the rapid environmental changes initiated by their own confined growth, they do exhibit the adaptability necessary to survive in a wide variety of environments. Here we will take a look at how bulk growthrate relates to certain aspects of heterogeneity and adaptability. Let us begin by discussing the framework developed in the 1960’s to model population age and growthrate dynamics.

Consider the following conservation of population where \( n(a,t) \) is the population per unit age at time \( t \), \( n(a + \Delta a, t + \Delta t)\Delta a = n(a, t)\Delta a - \lambda(a)n(a, t)\Delta a\Delta t \). This states the population of cells at age \( a + \Delta a \) and time \( t + \Delta t \), is equal to the population of cells at age \( a \) and time \( t \) after removing those that have left the ensemble (due to mitosis, etc.). The loss rate, in units of \( 1/\{t\} \) is notated as \( \lambda(a) \). For the purposes of this paper, we may consider “chronological age”, \( da/dt = 1 \), though in the case where the “age” of interest is cell phase or another non-chronological age, this introduces some added complexity. In the simpler case, \( da/dt = 1 \), we find the relatively simple form:

\[
\frac{\partial n(a,t)}{\partial t} + \frac{\partial n(a,t)}{\partial a} = -\lambda(a)n(a,t)
\]  \quad (1)

To solve this equation, we will first assume the species is undergoing exponential growth, \( n(a, t) = n(0, t)\exp(bt)g(a) \) where \( b \) is the bulk growthrate and \( g(a) \) is the steady state age distribution. Using this assumed form, we may solve for \( g(a) = g(0)\exp[-ba - \int_0^a \lambda(a')da'] \).
To move forward, we need some information about $\lambda(a)$. We are focusing on the case where the species multiplies through mitosis. In this case, when the death rate is negligible, $\lambda(a)$ may be obtained directly from the cell cycle duration distribution, $w(a)$. We note that $\lambda(a)$ becomes the division rate, $\lambda(a) = \frac{w(a)}{1 - \int_0^a w(a')da'}$: the probability of undergoing mitosis per unit time at age $a$ is the ratio of the population of cells observed to divide at age $a$ over the population of cells which have matured to age $a$ without yet dividing. We note that this too is only valid in the chronological case (this is clear from just the units: here $\lambda(a)$, which as defined has units of $1/[a]$, takes the units of $w(a)$, $1/[a]$). This form of $\lambda(a)$ also gives us a boundary condition: the number of cells at age zero and time $t$ is just twice the number of cells that divided at time $t$: $n(0,t) = 2 \int_0^\infty k(a)n(a,t)da$. We may now rewrite this boundary condition in terms of $w(a)$ explicitly:

$$1 = 2 \int_0^\infty \exp(-ba)w(a)da$$  \hspace{1cm} (2)

and similarly rewrite the steady state age distribution to yield:

$$g(a) = 2b \exp(-ba) \left[ \int_a^\infty w(a)da' \right]$$  \hspace{1cm} (3)

This framework (Equs. 2 and 3) provides a way to calculate the bulk growth rate, $b$, and the age distribution, $g(a)$, given only the cell cycle duration distribution, $w(a)$; however, it is built on some strong assumptions owing to the interpretation of Equ. 1. We have already discussed that this result is only valid for the case of chronological aging, and noted that we assume cell death is negligible. Beyond this, there is a third assumption which leads to some important consequences we want to discuss. We rewrote the division rate in terms of the cell cycle duration distribution, $\lambda(a) = \frac{w(a)}{1 - \int_0^a w(a')da'}$ and in doing so, assumed that $w(a)$ is the same for every cell, at all times. In other words, consider the transition probability between successive cell cycle durations, $P(a_n \rightarrow a_{n+1})$ where $a_n$ represents the cell cycle duration for the cell of interest during generation $n$. In general, this function may have some dependence on $a_n$, $a_{n+1}$, and even explicit dependence on time; however, we have strictly assumed:

$$P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a)$$  \hspace{1cm} (4)

To see how this assumption impacts the growth rate, let us first take a look at how $w(a)$ maps to $g(a)$ in exponentially growing ensembles as well as ensembles containing a fixed
number of individuals where only one daughter cell remains in the ensemble after division.

II. AGE DISTRIBUTION PROPERTIES

We will illustrate that, under the condition \( P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a) \), the age distribution is often “mean-scalable”. To illustrate this property, let us first take another look at \( w(a) \). Under many circumstances, \( w(a) \) belongs to a class of functions which are mean-scalable\(^9\). In other words, let us label the mean of \( w(a) \), \( \mu \), and introduce the variable \( x = \frac{a}{\mu} \); we will say \( w(a) \) is mean-scalable when there exists a scaled function \( \Omega(x) \) which is conserved across all \( \mu \) (and this function satisfies normalization):

\[
\Omega(x) = \mu w(a); \int_0^\infty \Omega(x) \, dx = 1
\]  

(5)

We can show that the mean-scalability of \( w(a) \) confers the same property to \( g(a) \). Rewriting Equ. 2 in terms of \( x, \Omega(x) \), and \( B = \mu b \), the scaled bulk growth rate, we find:

\[
1 = 2 \int_0^\infty \exp(-Bx) \Omega(x) \, dx.
\]

Note that this uniquely defines \( B \) given \( \Omega(x) \) and that for fixed \( B \), increasing \( \mu \) decreases \( b \). This inverse relationship expresses that cells which take longer to divide result in a slower growing ensemble. Similarly, we may now consider the function \( G(x) = \mu w(a) \) where the factor of \( \mu \) is introduced again to maintain normalization. Rewriting Equ. 3 yields:

\[
G(x) = 2B \exp(-Bx) \left[ \int_x^\infty \Omega(x) \, dx \right].
\]

We just saw that \( B \) is completely determined by \( \Omega(x) \) which implies that \( G(x) \) is also completely determined by \( \Omega(x) \). In other words, whenever \( w(a) \) is mean-scalable, \( g(a) \) is also mean-scalable. This property is useful because it has been observed that \( w(a) \) is often mean-scalable. More specifically, \( w(a) \) is well represented by a gamma distribution:

\[
w(a) = \frac{\beta^\alpha}{\Gamma(\alpha)} a^{\alpha-1} e^{-\beta a} \quad a \geq 0; \quad \beta > 0, \quad \mu = \frac{\alpha}{\beta} \quad \text{and} \quad CV = \frac{1}{\sqrt{\alpha}},
\]

across widely differing cell types. In this case \( \Omega(x) = \frac{\alpha}{\Gamma(\alpha)} x^{\alpha-1} e^{-\alpha x} \) and we see that the ensemble is no longer mean-scalable when the \( CV \) (a function of alpha) changes. We note, as shown in Fig. 1, that even when the \( CV \) for the cell cycle duration distribution does vary and \( w(a) \) is not mean-scalable, the differences in the resultant scaled age distributions, \( G(x) \), are still much smaller than the original cell cycle duration distributions.

As mentioned in the introduction, the von Foerster equation is usually solved after making some important assumptions. One straightforward assumption is that the species studied is undergoing exponential growth \( n(a,t) = \exp(bt)\bar{g}(a) \); but as clear as this may seem, it is not valid for some experimentally relevant ensembles. A wide variety of microfluidic devices and
Figure 1. Example $w(a)$ and their corresponding $g(a)$ and $G(x)$. First Row: The mean is varied $[25, 50, 75]$ while the CV is kept constant $[0.2]$. Second Row: The CV is varied $[0.1, 0.5, 0.9]$ while the mean is kept constant $[50]$. We see that while the scaled age distribution is not conserved, the differences between populations are still much smaller than that between the original cell cycle duration distributions. The cartoon is meant to illustrate how the age distribution, $g(a)$, is generated and that it is weighted towards young cells.

extracellular matrix patterns are now available to study bacterial, yeast, and mammalian cell populations at constant density, following a single cell for multiple cycles. In these devices, only one of two daughter cells is maintained in the ensemble after each division. This keeps the total number of cells constant over time and modifies the age distribution. Let us take a moment to look at the age distributions that result from these ensembles.

We begin again with the cell cycle duration distribution $w(a)$. Unlike an exponentially growing ensemble, an ensemble with a fixed number of individuals has very simple lineages: $a_1 \rightarrow a_2 \rightarrow \ldots \rightarrow a_N$ where $a_N$ is the cell cycle duration of generation $N$. Since we are still considering the condition $P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a)$, we may also note that the behavior of each lineage recapitulates that of the entire ensemble (this idea is discussed in detail in the following section on Ergodicity). We can see that after $N$ generations, the probability of having observed a cell cycle of duration $a$ is simply the duration of the cycle
multiplied by the number of times it occurred \( P_{\text{obs}}(a_m) = \frac{n_m a_m}{n_1 a_1 + n_2 a_2 + \ldots + n_m a_m + \ldots + n_N a_N} \). This generalizes to the probability density function:

\[
w_{\text{obs}}(a) = \frac{aw(a)}{\int_0^\infty aw(a) \, da} = \frac{a}{\mu}w(a)
\]

for observing a cycle of duration \( a \). Now we may consider that the probability of observing a cell within the age range \( a' + \Delta a' \), during a cycle of duration \( a \) is simply \( \frac{\Delta a'}{a} \) if \( a \geq a' + \Delta a' \) and 0 if \( a' \) is smaller. Thus the probability of observing a cell of age \( a \) from any cycle is:

\[
g_{\text{Fixed}}(a) = \int_a^\infty \frac{da'}{a}w_{\text{obs}}(a') = \int_a^\infty \frac{1}{a'} \frac{a'}{\mu}w(a') \, da' = \frac{1}{\mu} \int_a^\infty w(a') \, da'
\]

We may note using the same method as above, that when \( w(a) \) is mean scalable, this ensemble is mean scalable too: \( G_{\text{Fixed}}(x) = \int_x^\infty \Omega(x) \, dx \). Another simple and straightforward, but useful observation is that the mean age of the fixed ensemble can be calculated with a single integral. Written in the usual way, it is a bit cumbersome to calculate directly:

\[
\bar{a} = \int_0^\infty ag(a) \, da = \int_0^\infty a \left( \frac{1}{\mu} \int_0^\infty w(a') \, da' \right) \, da.
\]

However, we may more easily write down an expression for the mean age of any lineage, which will be the same as the mean age of the entire ensemble. The mean age of each lineage is simply the average of the mean age of each cycle (the mean age of a cycle of length \( a \) is simply \( \frac{a^2}{2} \)):

\[
\bar{a}_{\text{Fixed}} = \int_0^\infty \frac{a' a'}{2} \frac{w(a')}{\mu} \, da' = \frac{1}{2\mu} \int_0^\infty a^2 w(a') \, da'
\]

In the case where \( w(a) \) is gamma distributed, this yields a closed form expression:

\[
\bar{a}_{\text{Fixed}} = \frac{\alpha}{2\beta} \left[ 1 + \frac{1}{\alpha} \right] = \frac{\mu}{2} \left[ 1 + CV^2 \right]
\]

We see that the mean age remains close to \( \frac{\mu}{2} \) until the \( CV \) gets quite large (since the \( CV \) is rarely greater than 1). We may also note that in the delta function limit for the cell cycle duration distribution, \( g(a)_{\text{Fixed}} = \frac{1}{\mu} \int_0^\infty \delta (\mu - a') \, da' = \frac{1}{\mu} \) is simply uniform. In contrast for the exponentially growing case, in the limit where the \([CV]\) tends to zero and \( w(a) \) is a delta function at \( a = \mu \), we retrieve \( g(a) = \frac{2 \ln(2)}{\mu} \exp \left( -\frac{\ln(2)}{\mu} a \right) \). We see in this case, the mean age is:

\[
\bar{a} = \int_0^\infty a \frac{2 \ln(2)}{\mu} \exp \left( -\frac{\ln(2)}{\mu} a \right) \, da = \mu \left( \frac{1}{\ln(2)} - 1 \right)
\]
This is about $0.44 \mu$. In Fig. 2 we show that the fixed population is older than the exponentially growing population when $w(a)$ is gamma distributed for all $CV$. We also want to emphasize this is true in general, independent of the form of $w(a)$. This can be observed from a comparison of the age distributions: 

$$g(a) = 2b \exp(-ba) \left[ \int_a^\infty w(a') da' \right]$$

and

$$g_{\text{Fixed}}(a) = \frac{1}{\mu} \int_a^\infty w(a') da'. $$

The two expressions differ only by the factor $2b \exp(-ba)$ which monotonically decrease with age. This means if you examine a snapshot of cells which have been maintained in a population of fixed number (e.g. mother cells in a microfluidic device), the ensemble will be older than a group of unconstrained cells. In Fig. 2, we examine how $g(a)_{\text{Fixed}}$ changes with respect to changing $CV$ of the cell cycle duration distribution, assuming gamma-distributed $w(a)$, and compare $g(a)$ with $g_{\text{Fixed}}(a)$. We see that there is a stronger dependence on the $CV$ of $w(a)$ for the fixed distributions.

### III. ERGODICITY

The condition, $P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a)$, which led to the nice properties of the age distributions discussed above is really a statement about the expected ergodicity of the cell cycle duration distribution. The following definition is usually used to describe an ergodic system: consider an ensemble of measurements $x(t,y)$ made at time $t$ of individual $y$. This ensemble is considered to be ergodic if $P(x(t_i,y_i)) = P(x(t_j,y_j)$; that is, the probability of observing state $x$ is the same at anytime and from any individual. Alternatively, this can be written:

$$P(x(t_i,y_i)) = P(x) \tag{11}$$

where $P(x)$ is the probability of observing state $x$ conserved across all individuals at all times. We want to illustrate that, for some ensembles, this condition implies that over time the same behavior is recapitulated as that over space. Let us consider a discrete probability distribution with $M$ bins, indexed with $j$, and call the probability associated with each bin $P(x) \rightarrow P(j)$. Further let us consider the case where the number of individuals does not change. Following a single individual, after $N$ observations, each bin may be filled with up to $N$ occupants from that individual. The probability of bin $i$ containing $j$ occupants after the $Nth$ observation is given by the binomial distribution, $P(i,j,N) = \binom{n}{j} P(i)^j [1 - P(i)]^{N-j}$
Figure 2. Example $w(a)$ and their corresponding $g_{Fixed}(a)$ and $G_{Fixed}(x)$. First Row: the mean is kept constant [50] and the CV is varied [0.1, 0.5, 0.9]. Second Row: a sample cell cycle distribution $w(a)$, mean [50] and CV [0.1]; a comparison of $g(a)$ and $g_{Fixed}(a)$ for the sample $w(a)$; and the mean of and $g(a)$ and $g_{Fixed}(a)$ resulting from cell cycle duration distributions of fixed mean [50] and varying CV [0.1, 0.9]. Note that the inflection point displayed in the mean of $g(a)$, in the bottom right plot, may be due to numerical error stemming from highly skewed $g(a)$ when $w(a)$ has a large CV (the curve for $g_{Fixed}(a)$ is analytic). The cartoon is meant to illustrate that following multiple generations of one cell in/on a single channel/pattern is equivalent to observing a single generation from multiple cells.

which is well approximated by a Gaussian distribution with mean $NP(i)$ and variance $NP(i)(1 - P(i))$. The error in the mean of this distribution with respect to the original distribution $P(i)$ is $Err = \frac{\sum_{n=1}^{M} Bin Error * Bin Width}{N * M * Bin Width}$ where $Bin Error$ is the root of the square of the expected value of the difference, $y$, between the actual bin occupation and the ideal distribution. When the Gaussian approximation is used, we may write

$$Bin Error = \sqrt{\int_{-\infty}^{\infty} \frac{y^2}{\sigma^2} \exp\left(-\frac{y^2}{2\sigma^2}\right) dy} \leq \sqrt{\int_{-\infty}^{\infty} \frac{y^2}{\sigma^2} \exp\left(-\frac{y^2}{2\sigma^2}\right) dy} = 2^{1/4} = 2^{1/4} \sqrt{NP(i)(1 - P(i))}. $$

Thus we have $Err = \frac{\sum_{n=1}^{M} 2^{1/4} \sqrt{NP(i)(1 - P(i))}}{N * M} = \frac{\sum_{n=1}^{M} 2^{1/4} \sqrt{P(i)(1 - P(i))}}{N * M}$. We may note that $\sqrt{P(i)(1 - P(i))} \leq \frac{1}{2}$, and so (this is basically just a restricted statement of the central limit theorem):
Thus we see that if we observe a single individual at many times, it recapitulates the behavior of the entire ensemble at a single time: the ensemble behaves the same way over time and space. We have shown that Equ. 12 is implied by Equ. 11; however, we have only shown this is true for the case where the number of individuals in the ensemble does not vary. We will show that in general, Equs. 11 and 12 are unique conditions and will refer to them as follows:

### Ergodicity Type I

We will define an ensemble to be of ergodicity type I if:

\[ P(x(t_i, y_i)) = P(x(t_j, y_j)) = P(x) \]  

(13)

In other words, the ensemble has an unbiased selection of states which does not depend on time or the individual observed. Our condition of interest, \( P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a) \) is a statement of ergodicity type I.

### Ergodicity Type II

We will define an ensemble to be of ergodicity type II if there exists some value, \( \varepsilon \), such that:

\[ Err(N) \leq \frac{\varepsilon}{\sqrt{N}} \]  

(14)

where \( Err(N) \), defined above, is the magnitude of the difference between the ensemble of \( N \) observations made from a single individual and that of the entire group. In other words, each individual in the ensemble will, over time, recapitulate the behavior of the entire ensemble.

We have just seen when the number of individuals is fixed, ergodicity type I implies ergodicity type II; however the converse is not true. Consider an ensemble composed of two individuals \( x_1 \) and \( x_2 \) which may occupy two possible states \( a \) and \( b \). Suppose that at odd observations \( x_1 \) occupies \( a \) and \( x_2 \) occupies \( b \) and vice versa. The probability of observing
Figure 3. Illustration of the two types of ergodicity: Ergodicity I where the ensemble has an unbiased selection of states which does not depend on time or the individual observed and Ergodicity II where each individual in the ensemble will, over time, recapitulate the behavior of the entire ensemble.

either state in the entire ensemble is $\frac{1}{2}$ at any time and $Err(N) < \frac{a+b}{N}$ for both individuals; however $P(x_1(t_i, [a,b])) \neq P(x_2(t_j, [a,b]))$ for all times. Thus this ensemble is ergodic in the second sense but not ergodic in the first sense.

When the number of individuals varies, as it does in the exponentially growing ensemble, ergodicity I does not imply ergodicity II. Let us turn to our ensemble of interest, the cell cycle duration distribution. We may utilize the von-Foerster equation as we did earlier and note that using the same formalism assumes ergodicity I through the condition $P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a)$ as we discuss above. We may return to the expression for the growthrate, $\nu$: $1 = 2 \int_0^\infty \exp(-\nu a) w(a) da$ and note that $\exp(-\nu a)$ is a convex function. By Jensen’s inequality this implies $E(f(x)) = \int_0^\infty \exp(-\nu a) w(a) da \geq \exp(-\nu \int_0^\infty aw(a) da) = f(E(x))$. Further, $f \equiv \exp(-\nu a)$ is strictly convex, which means equality holds only when $w(a)$ approaches a delta function. Considering the case where $w(a) = \delta(a-\mu)$:

$$1 = 2 \int_0^\infty \exp(-\nu a) \delta(a-\mu) d\tau \Rightarrow \nu = \frac{\ln(2)}{\mu}$$

Considering the case where $w(a)$ is not a delta function but another distribution with the same mean:
Thus, for any non-delta function distribution, \( \nu > \frac{\ln(2)}{\mu} \). This means that \( P(i,j,N) \) never relaxes to the division distribution, because the mean is always lower, and there exists no constant \( \epsilon \) such that \( Err(N) \leq \frac{\epsilon}{\sqrt{N}} \). Thus ergodicity type I does not imply ergodicity type II when the number of individuals varies. We may further note that ergodicity II does not imply ergodicity I for this case either. Similar to the argument for a constant number of individuals, consider an ensemble initially composed of two individuals \( x_1 \) and \( x_2 \) which may occupy two possible states \( a \) and \( b \). Suppose that at observation one, \( x_1 \) occupies \( a \) and \( x_2 \) occupies \( b \). Further consider that at every subsequent observation, each individual becomes two new individuals of the alternative state (i.e. \( x_1 \) occupying \( a \) at observation one becomes two individuals occupying \( b \) at observation two). The probability of observing either state in the entire ensemble is \( \frac{1}{2} \) at any time and \( Err(N) < \frac{a+b}{N} \) for all lineages; however \( P(x_i(t_i,[a,b])) \neq P(x_j(t_j,[a,b])) \) whenever \( x_i \) stems from \( x_1 \) and \( x_j \) stands for \( x_2 \) or vice versa. Thus this ensemble is of ergodicity type II but not ergodicity type I. This brings us to a statement of the relative strengths of these conditions:

**Hierarchy of Ergodicities Types I and II**

*For an ensemble with a fixed number of individuals, ergodicity type I implies ergodicity type II; however, ergodicity type II does not imply ergodicity type I. Thus for these ensembles, ergodicity type I is the stronger condition. For an ensemble with a variable number of individuals, ergodicity types I and II are mutually independent. This distinction is of interest relative to the qualitative notion that an ergodic ensemble, recapitulates within a single individual over many observations the behavior of the entire ensemble at a single observation, or behaves the same way over time and space. These statements represent ergodicity type II and may not accurately describe an ensemble of ergodicity type I with a varying number of individuals.*
GROWTHRATE GAIN

We have discussed how the cell cycle duration ensemble is of ergodicity I under the condition $P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a)$ which is assumed in the traditional formalism used to solve the von Foerster equation. Thus we know unless $\rho(\tau) \equiv \delta(\tau - \mu)$, the growthrate is higher than the “naive” growthrate $\ln(2)\mu$ and the bulk doubling time is shorter than the mean division time. This phenomenon is commonly referred to as the “growthrate gain”. It was discussed first as far back as the 1950’s[6] and has been the object of renewed recent interest[1, 3]. At the center of the issue sits the finding that when the duration of mother-daughter cell cycles are positively correlated, the growthrate increases and when they are negatively correlated, the growthrate decreases. We have shown that even in the case without explicit correlation, ergodicity type I, the growthrate gain appears. This is due to the nature of the growing ensemble. Cells which divide quickly are equally likely to form daughter cells which divide quickly as they are to form cells which divide slowly; however, this leads to an unequally large number of short cell cycles represented in the ensemble (see Fig. 3). On the other hand, we will show if the the ensemble is of ergodicity II, then there is no growthrate gain: the bulk doubling time is equal to the mean division time.

Consider an exponentially growing ensemble which begins with a single individual such that all individuals in the ensemble will be of ergodicity II: $Err(N) \leq \frac{\epsilon}{\sqrt{N}}$. Suppose at any given time, the ensemble contains $M$ cell cycle durations structured into $L$ lineages each of length $N_l$. Let us consider the composite error of all the lineages within the ensemble labeling $max_L (\epsilon_l) = \epsilon$, where $\epsilon_l$ is the error associated with the $l^{th}$ lineage:

$$E = \sum_{l=1}^{L} \frac{\epsilon_l}{\sqrt{N_l}} \leq \epsilon \sum_{l=1}^{L} \frac{1}{\sqrt{N_l}}$$

We may now consider the lineage tree which maximizes error. Adding a division to the ensemble causes a change in the composite error associated with the addition of two more cycle durations and in effect generating two new lineages while removing one old lineage: $\Delta E \leq \epsilon \left[\frac{2}{\sqrt{N+1}} - \frac{1}{\sqrt{N}}\right]$. We may note that this function is positive for all positive integer values and that it decreases with respect to $N$ whenever $N > 2$. (There is only one possible ensemble for $N = 1, 2$.) Therefore the ensemble with the maximum error is obtained by adding onto the shortest lineage. Let us now focus on completely symmetric ensembles, $N_1 = \ldots = N_L = N$, which maximize error for a given $M$. Noting that for these completely
symmetric ensembles $M = 2^{N-1}$ and the composite error is subject to the inequality: $E \leq \frac{2^{N-1}}{\sqrt{N}}$. We can calculate $Err(N)$ for the entire ensemble by normalizing with respect to the total number of divisions observed during this period:

$$Err(N) \leq \epsilon \frac{2^{N-1}}{\sqrt{N}} \frac{1}{\sum_{i=1}^{N-1} 2^i} \leq \frac{\epsilon}{\sqrt{N}}$$

(18)

Thus if all lineages are of ergodicity type II, the ensemble must also be of ergodicity type II. We may recall that the bulk doubling time is defined to be $t_D$ such that $N(t) = \exp\left(\frac{\ln(2)}{t_D}\right) N(0)$. Thus $t_D$ is the mean of every cell cycle observed in the population (over some window of time observed) - which if the ensemble is of ergodicity II, is simply $\mu$.

V. EXPLICIT CORRELATION

We have shown when Ergodicity I is maintained in the case of the cell cycle distribution, $P(a_n \rightarrow a_{n+1}) = w(a_{n+1}) \equiv w(a)$, the ensemble growth rate is higher than $\ln(2) \mu$. This growth rate gain may be attributed to the prevalence of lineages comprising solely cell cycles shorter than the mean. While there is no explicit mother-daughter correlation within the selection of cell cycle durations, there is an effective correlation arising from this variable weighting of lineages. We sought to probe the degree of this effective correlation through the addition of explicitly negative mother-daughter correlation. In this way, we could find the degree of negative correlation which must be imposed to return an ensemble to the naive growth rate, $\ln(2) \mu$. We chose a form for $P(a_n \rightarrow a_{n+1})$ based on the model presented in [7]:

$$P(a_n \rightarrow a_{n+1}) = A \exp \left[ -\frac{1}{2\sigma_1^2} (a_{n+1} + a_n - 2\mu)^2 \right] \exp \left[ -\frac{1}{2\sigma_2^2} (a_{n+1} - a_n)^2 \right]$$

(19)

The autocorrelation function generated from $P(a_n \rightarrow a_{n+1})$ is:

$$C(n) = \left( \frac{\sigma_1^2 - \alpha \sigma_2^2}{\sigma_1^2 + \sigma_2^2} \right)^n$$

(20)

The object of interest here is $C(1)$, the mother-daughter correlation. We simulated lineage trees of cell cycle durations with each successive cycle duration drawn from $P(a_n \rightarrow a_{n+1})$. See Fig. 4. The first generation cycle duration was drawn from a Gaussian distribution with mean $\mu$ and the standard deviation corresponding to the stationary distribution. Without
Figure 4. A display of the growthrate gain as a function of ensemble CV and mother-daughter cell cycle duration correlation $C(1)$. A strong, negative mother-daughter correlation is required to return the ensemble to the naive bulk growthrate of $\frac{\ln(2)}{\mu}$. The cartoon illustrates that a cell which divided after a cycle of duration $\mu + x$ is most likely to form daughter cells which attain cycles of duration $\mu + C(1) x$.

loss of generality, $\mu$ was taken to be 20 (unitless) and $\sigma_1$ and $\sigma_2$ were chosen to obtain the desired $C(1)$ correlation. Each cell lineage simulation was continued until a generation was reached where the cell cycle duration distribution was sufficiently close to the steady-state distribution evolving from $P(a_n \rightarrow a_{n+1})$. The test distribution was considered to be sufficiently close to the steady-state when the relative $\ell_2 - norm$ difference was no more than 5%. Once the steady-state was reached, the growthrate of the population was calculated as the slope of the best-fit line to the logarithm of average cell number over 100 trials.

We found a very substantial degree of negative correlation ($C(1) \approx -0.75$) must be imposed to return an ensemble to the naive growthrate, $\frac{\ln(2)}{\mu}$; however, this phenomenon depends on the CV of the cell cycle duration distribution since the growthrate gain is higher when the CV is larger. In other words, for an ensemble to attain a bulk growthrate of $\frac{\ln(2)}{\mu}$ given only mother-daughter correlations (i.e. grandmother-granddaughter cell cycle
durations are uncorrelated), an anti-correlation of about 75% must be imposed. Under these conditions, a cell which divided after a cycle of duration $\mu + x$ is most likely to form daughter cells which attain cycles of duration $\mu - 0.75x$. Stated another way, this implies that ensembles which enforce ergodicity I are essentially approximately 75% correlated.

VI. DISCUSSION

We have seen that the concept of ergodicity bifurcates into two distinct properties within ensembles that have a variable number of individuals: ergodicity I, the unbiased selection of states and ergodicity II, the recapitulation of whole-ensemble behavior from any single individual. Many of the nice properties of the traditional formalism used to solve the von Foerster equation rely on the assertion that the cell cycle duration ensemble is of ergodicity I. Under these conditions, the bulk growthrate of the population is higher than $\frac{\ln(2)}{\mu}$ due to a disproportionately large number of short cell cycles represented in the ensemble. When the ensemble is of ergodicity II, similar to the condition where there is a weakly negative mother-daughter cell cycle duration correlation, the growthrate returns to $\frac{\ln(2)}{\mu}$. Since the “growthrate gain” observed within ensembles of ergodicity I stems from disparities between lineages composed of primarily short cell cycle durations and those of long cell cycle durations, the larger the CV of the cell cycle duration distribution, $w(a)$, the greater the effect. In the most basic sense, this growthrate gain comes from asymmetric divisions. When ergodicity II is not enforced, lineages of exclusively short cell cycles arise. Many sister cells of cells within these lineages have long cell cycle durations. This phenomenon may be purely due to stochasticity present after division has finished or it might be due to programmatic asymmetry in the allocation of resources to each daughter cell. Asymmetric division has been well established in a variety of pro- and eukaryotes[4]. It has been shown that even E. coli display complex polar protein localization[5] and that pathological polar aggregates can be asymmetrically inherited which may increase fitness by “rejuvenating” the daughter cell that accepts less damage[13]. Completely symmetric division requires cells to fix inherited damage; otherwise, all cells will eventually have accumulated critical amounts. There are likely to be costs associated with coordinating asymmetric division and inherited damage mitigation which are balanced in optimal growth strategies. It has been reported that under some conditions, E. coli age within ten generations[8] while under others, stable growth has
been observed for hundreds of generations\cite{12}. Perhaps in the later case, higher growth rates can be achieved through damage mitigation in all cells than the asymmetric inheritance of damage and subsequent loss of the damaged population.

**Author Contributions**

NR conducted the analysis in sections II, III, and IV. DP completed the simulations in section V. NR, DP, and SXS wrote the paper.

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