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A branching process model for flow cytometry and budding index measurements in cell synchrony experiments.

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Abstract

We present a flexible branching process model for cell population dynamics in synchrony/time-series experiments used to study important cellular processes. Its formulation is constructive, based on an accounting of the unique cohorts in the population as they arise and evolve over time, allowing it to be written in closed form. The model can attribute effects to subsets of the population, providing flexibility not available using the models historically applied to these populations. It provides a tool for *in silico* synchronization of the population and can be used to deconvolve population-level experimental measurements, such as temporal expression profiles. It also allows for the direct comparison of assay measurements made from multiple experiments. The model can be fit either to budding index or DNA content measurements, or both, and is easily adaptable to new forms of data. The ability to use DNA content data makes the model applicable to almost any organism. We describe the model and illustrate its utility and flexibility in a study of cell cycle progression in the yeast *Saccharomyces cerevisiae*.

Key words and phrases

Branching process; cell cycle; flow cytometry; budding index; synchrony experiment; Bayesian analysis

1. Introduction

In this paper we describe a novel branching process model that characterizes the temporal evolution of population heterogeneity in cell synchrony experiments. These experiments are

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designed to measure the dynamics of fundamental biological processes related to the cell's progression through the cell division cycle. Careful characterization of these dynamic processes requires experiments where quantitative measurements are made over time. In many cases, accurate measurements cannot be made on single cells because the quantitative methods lack the sensitivity to detect small numbers of biomolecules. For example, accurate quantitative measurements of genome-wide transcript levels by microarray require more mRNA than is physically available within a single cell. Thus, researchers are forced to work with populations of cells that have been synchronized to a discrete cell cycle state.

Two distinct problems arise in these synchrony/time-series experiments. First, synchronized populations are never completely synchronous to begin with, and tend to lose synchrony over time. The lack of perfect synchrony at any given time leads to a convolution of the measurements that reflects the distribution of cells over different cell cycle states. Second, multiple synchrony experiments are often needed to measure different aspects of a process, and it is often desirable to compare the temporal dynamics of these aspects. However, synchrony/time-series experiments, even in the best of experimental circumstances, exhibit considerable variability which make time-point to time-point, cross-experiment comparisons imprecise. Thus, a mechanism is required to accurately align the data collected from each of the synchrony/time-series experiments. The model we describe addresses both of these problems.

Most of the numerous models designed to measure cell population dynamics in synchrony/time-series experiments fall into two related classes: population balance (PB) and branching process (BP) models. PB models are usually formulated as partial-integro-differential equations and are often very difficult to work with except under special conditions [Liou, Srien and Fredrickson (1997), Sidoli, Mantalaris and Asprey (2004)]. BP models are stochastic models for population dynamics that have been used to study both the asymptotic [Alexandersson (2001)] and short term behaviors [Larsson et al. (2008), Orlando et al. (2007)] of populations; certain BP models have PB analogues [Arino and Kimmel (1993)]. Several models that do not explicitly account for reproduction, and hence are neither PB or BP models, have also been used to model data from asynchrony experiments [Bar-Joseph et al. (2004), Lu et al. (2004)].

The most critical distinction between models, however, is in the sources of synchrony loss the model includes. Most describe synchrony loss as the result of a single parameter, equivalent to a distribution over division times [Bar-Joseph et al. (2004), Chiorino et al. (2001), Larsson et al. (2008)]. In contrast, the model we describe here (the CLOCCS model, in reference to its ability to Characterize Loss of Cell Cycle Synchrony [Orlando et al. (2007)]) is the only model to account for variability in cell-division time, initial asynchrony in the starting population and variability due to asymmetric cell division [Chiorino et al. (2001)], all of which we will show to be important. The CLOCCS model is based on a novel branching process construction and can be written in closed form. Its formulation is constructive, based on an accounting of unique cohorts in the population at any given time. Hence, the model can attribute one-time effects to specific subsets of the population, demonstrating flexibility not available using the PB and BP models historically applied to these populations. Further, the model's construction allows full Bayesian inference without the use of approximations to the likelihood. The Bayesian approach to inference has the additional advantage that it sidesteps many of the difficulties encountered by frequentist inference for BP models [Guttorp (2001)].

In this paper we present a model which can utilize two forms of data that provide information regarding the cell cycle position of *Saccharomyces cerevisiae*, baker's yeast: DNA content data and budding index data. An overview of the yeast cell cycle and these

data types can be found in Section 2. While applied here to yeast, the ability to fit DNA content data, described in Section 3, is a critical advance that allows the CLOCCS model to be applied to an array of more complex organisms that do not undergo the kinds of morphological changes that yeast do (e.g., budding) during the cell division cycle. In Section 4 we apply the model to fit budding index and DNA content data from a synchrony/time-series experiment in yeast. Using these data, we compare the model to a collection of nested alternative parameterizations with subsets of the novel asynchrony sources removed. We conclude with a discussion of the model and of the results of this analysis in Section 5.

2. Yeast cell cycle

One organism commonly studied using synchrony/time-series experiments is the common baker's yeast, *S. cerevisiae*, because many features of its cell cycle are well characterized. Figure 1A depicts the landmark events that can be used to determine the cell cycle state of individual cells [Gordon and Elliott (1977), Hartwell (1974)]. The first, bud emergence, is a distinct morphological landmark easily detected by simple light microscopy. It first appears near the time that a cell transitions from G1 into S phase. Cells become unbudded after the completion of mitosis (M) when the cell and its bud separate. We refer to the progenitor cell as the “mother” and what had been the bud as the “daughter.” In *S. cerevisiae*, this division is often asymmetric: the mother cell is often larger and progresses more quickly through the cell cycle than the daughter [Hartwell and Unger (1977)]. Cell cycle position can also be determined by measuring genomic DNA content of the cell, which increases as cells progress through the S phase of the cell cycle [Haase and Reed (2002)]. Haploid yeast cells begin the cell cycle with one copy of genomic DNA (red bar in Figure 1). During the S phase, DNA is replicated such that, at the completion of the S phase, the cell has two copies of genomic DNA.

Counts of budded cells and cell-level DNA content are typically measured in independent samples, drawn at regular time points after the population's release from synchrony. The resulting time series of budded cell counts is referred to as a budding index. DNA content is measured by flow cytometry. Budding index and DNA content data can be used to fit accurate models of the underlying cell cycle position distributions.

3. Model

The model we describe is comprised of two components: an underlying model for the population dynamics of the cells in a synchrony/time-series experiment, and independent sampling models for the budding index and DNA content measurements made on samples drawn from the population. We refer to the population dynamics model component as CLOCCS. CLOCCS is a branching process model for position, P_b , of a randomly sampled cell in a linearized version of the cell cycle (Figure 1B)—which we refer to as a cell cycle lifeline—given the experimental time, t , at which the cell was sampled. The sampling models for the budding index and DNA content measurements are conditioned on the distribution of lifeline position and time. In what follows, we describe the model's components in greater detail.

3.1. Model for position given time

The CLOCCS model specifies the distribution of cell positions over an abstract cell cycle lifeline as a function of time. We define λ to be the amount of time, in minutes, required by a typical mother cell to undergo one full cell cycle. We divide the lifeline into λ units, thus the average cell will move one lifeline unit per minute. The advantage of using a lifeline characterization is that it allows for introduction of one-time effects, such as the recovery

period following release from synchrony or the delay in cell cycle progression of new daughter cells.

We model position as having three independent sources of variability: the velocity with which the cell traverses the cell cycle, the time it spends recovering from the synchronization procedure, and the additional time spent by a daughter cell as it traverses its first cell cycle [Hartwell and Unger (1977)]. It is well known that cells in synchrony experiments progress through the cell cycle with varying speeds. We assume that each cell moves at a constant velocity along the lifeline, and that this velocity is random, following a normal distribution. While this is technically inappropriate as velocities must be positive, in practice it is reasonable: fitted distributions give almost no mass to the negative half line. We measure velocity, V , in lifeline units per minute; by definition, the mean cell velocity is 1.0. The velocity distribution's variance, σ_v^2 , is unknown.

When released from synchrony, cells spend more time in their first G1 phase than they spend in G1 during subsequent cell cycles. The added time reflects a period of recovery from the synchronization process, whose length varies from cell to cell. We term this recovery period G_r as if it were a distinct cell cycle phase. We model this effect as a random offset, P_0 , in the starting position on the lifeline. While this offset should be strictly positive, we let P_0 be distributed $N(\mu_0, \sigma_0^2)$ for convenience. Later, we comment further on this choice. Daughter cells tend to be smaller and require additional time in G1 before they begin to divide. We term this daughter-specific period of growth G_d and model it by introducing a fixed offset, δ , to the cell's lifeline position.

With each wave of division, the population expands in size. If cells in the culture remained synchronous, the population would branch and double in size every λ minutes after an initial delay of μ_0 minutes. Because they do not, the dynamics of this expansion is more complex: at any point in time, the population may represent a number of distinct cohorts, each defined by its lineage. Cohorts are determined by g , their "generation"—the number of daughter stages in their lineage—and r , their "reproductive instance"—the wave of division that gave rise to the cohort. Figure 2A depicts the branching dynamics of this process and a snapshot in time projected onto a common lifeline (Figure 2B). In A, four distinct time periods are color coded with each cohort distribution labeled with its $\{g, r\}$ index. At time zero there is a single cohort, $\{0, 0\}$, depicted in black, whose position distribution is located in G_r and centered at $-\mu_0$. As time passes (red), this cohort enters its second cell cycle and spawns a daughter cohort, labeled $\{1, 1\}$, which begins on its own lifeline in G_d . Later (blue), cohort $\{0, 0\}$ gives rise at its second reproductive instance to another first generation cohort, $\{1, 2\}$. At the same time, cohort $\{1, 1\}$ cells are progressing through G2/M. At the last depicted time point (green), the population is comprised of four distinct cohorts, representing three generations of cells arising at three distinct reproductive instances. Figure 2B is a plot of the population at this time point on the common lifeline. The CLOCCS model is a distribution over position along this common lifeline as a function of time.

In what follows we use a description of the behavior of individual cells as a device for deriving population level cohort position distributions. Each such distribution is normal with parameters that depend on the starting position and velocity distributions, time t and the cohort's indices g and r . Since cells in the $\{0, 0\}$ cohort are unaffected by the daughter specific delay, δ , their positions, P_t , at time t are determined only by their starting positions, P_0 , and their velocity, V . For these cells, $P_t = P_0 + Vt$. Hence, $p(P_t|\Theta, R=0, G=0, t)$ is

normal with mean $-\mu_0 + t$ and standard deviation $\sqrt{\sigma_0^2 + t^2 \cdot \sigma_v^2}$. In contrast, cells in cohorts at generations greater than zero have their position distributions truncated at the beginning of G_d , $-\delta$ on the lifeline, and are set back by g daughter offsets of length δ and r cell cycle

offsets of length λ . The remaining contributions to such a cell's position are the velocity by time contributions of each of its ancestors and the initial position of its ancestor in cohort $\{0, 0\}$. For simplicity, we assume that daughter cells inherit their mother cell's velocity. With this, the velocity by time contribution to position simplifies to Vt , where V is the common velocity and t is total time since population release. For these cells, $P_t = P_0 + Vt - g\delta - r\lambda$, hence, position, $p(P_t|\Theta, G = 0, R = G, t)$, is normal with mean $-\mu_0 + t - g\delta - r\lambda$ and standard deviation $\sqrt{\sigma_0^2 + t^2 \cdot \sigma_v^2}$ truncated so that $P_t \geq -\delta$.

Thus, we write the model for position, P_t , given time, t , in closed form by enumerating the population's cohorts using the latent variables r and g . In particular,

$$p(P_t|\Theta, t) = \sum_C p(P_t|\Theta, r, g, t)p(g, r|\Theta, t), \quad (1)$$

where $\Theta = (\mu_0, \sigma_0^2, \sigma_v^2, \lambda, \delta)$ and where the sum is over possible cohorts, $\mathcal{C} = \{(g, r) : (g = 0 \wedge r = 0) \vee (0 < g \leq r \leq R)\}$. While the number of cohorts represented in the population could theoretically be large, in practice, their number is limited by the number of cell cycles that cohort $\{0, 0\}$ is able to undergo during the experimental period. In most cases, synchrony experiments are terminated after 2 or 3 cycles, so choosing $R = 4, 5$ or 6 is usually sufficient. For notational clarity, we use C to represent the sufficient number of cell cycles examined.

The marginal probability of drawing a representative of cohort $\{g, r\}$ from the population at time t is $p(g, r|\Theta, t)$. For example, in the scenario depicted in Figure 2B, $p(1, 1|\Theta, t)$ is the ratio of the mass under the cohort $\{1, 1\}$ density to the total mass under all of the cohort densities present on the lifeline. The mass under the cohort $\{1, 1\}$ density is the probability that a randomly drawn member of the $\{0, 0\}$ cohort has completed its first cell cycle and contributed a daughter cell to cohort $\{1, 1\}$. This probability is equal to

$(1 - \Phi(\frac{\mu_0 - t + \lambda}{\sqrt{\sigma_0^2 + t^2 \cdot \sigma_v^2}}))$. The mass under the cohort $\{2, 2\}$ density is the probability that a randomly drawn member of the $\{1, 1\}$ cohort has finished its first cell cycle; this, in turn, is the probability that a randomly chosen member of the $\{0, 0\}$ cohort has traveled δ units into its third cell cycle. The δ appears because the $\{1, 1\}$ cohort's progress through its first cell cycle is δ units longer than the $\{0, 0\}$ cohort's progress through its second cell cycle. In this way, the relative contribution of any cohort in the population can be determined by calculating the probability that the position of a randomly drawn member of the $\{0, 0\}$ cohort is past a threshold position that is a function of g and r . Let $M_\Theta(g, r, t)$ denote the mass under cohort $\{g, r\}$'s position distribution at time t ,

$$M_\Theta(g, r, t) = \begin{cases} 1, & g=0, r=0, \\ \left(1 - \Phi\left(\frac{\mu_0 - t + r \cdot \lambda + (g-1) \cdot \delta}{\sqrt{\sigma_0^2 + t^2 \cdot \sigma_v^2}}\right)\right) \cdot \binom{r-1}{g-1}, & g \geq 1, r \geq g, \\ 0, & \text{else,} \end{cases}$$

where $\Phi(\cdot)$ denotes the standard normal CDF. The combinatoric term arises from that fact that, for $r \geq g + 1$, multiple lineages may contribute members to a given cohort. For example, cohort $\{1, 1\}$ will contribute to cohort $\{2, 3\}$ as its members pass the point 2λ on its lifeline (rightmost point on the third branch from top in Figure 2A), while cohort $\{1, 2\}$ will contribute to the same cohort, $\{2, 3\}$, as its members pass the point λ on its lifeline (rightmost point on the second branch from top in Figure 2A). Finally, let $Q_\Theta(t)$ denote the mass under all cohort distributions in the population at that time,

$$Q_{\Theta}(t) = \sum_{\mathcal{C}} M_{\Theta}(g, r, t).$$

In general, $p(g, r|\Theta, t) = M_{\Theta}(g, r, t)/Q_{\Theta}(t)$.

3.2. Sampling models

To utilize the CLOCCS model, it is necessary to relate distributions over the artificial cell cycle lifeline to observable cell features. In the next two sections we present two sampling models which allow CLOCCS to utilize commonly collected landmark data, namely budding index and DNA content data. While time series of budding index and DNA content data are each sufficient to estimate the CLOCCS parameters, Θ , they provide complementary information on the cell cycle timing of distinct landmark events. Timing of these events is of independent interest, and estimates of the same may improve the utility of the model as a tool for deconvolution of transcription data and other types of downstream analysis.

3.3. Sampling model for budding index data

Presence or absence of a bud is an easily measured landmark tied to a cell's progression through the cell cycle (see Figure 1). Buds emerge and become detectable near the transition between G1 and S phases, at a fraction β of the way through the normal cell cycle and split off as daughter cells at cell cycle completion (Figure 3, dashed line).

Assume that budding index samples are drawn at T time points, t_i , $i = 1, \dots, T$, and that n_i cells are counted at time t_i . Let $b_{ji} = 1$ if the j th cell at time t_i is budded and $b_{ji} = 0$ otherwise. The event that $b_{ji} = 1$ implies that the position of the j th cell at time t_i , P_{ji} , falls into the lifeline interval $((c + \beta)\lambda, (c + 1)\lambda]$ for some cell cycle $c \geq 0$; the probability of this is dictated by the CLOCCS model.

Following the development of Section 3.1, we calculate $p(b_{ji} = 1|\beta, \Theta, t_i)$ by introducing cohorts and marginalizing over them. In particular, let

$$p(b_{ji} = 1|\beta, \Theta, t_i) = \sum_{\mathcal{C}} p(b_{ji} = 1|\beta, \Theta, g, r, t_i) p(g, r|\Theta, t_i),$$

where $p(b_{ji} = 1|\beta, \Theta, g, r, t_i)$ is the probability that a cell randomly sampled from cohort $\{g, r\}$ is budded at time t_i . For the progenitor cohort, $\{0, 0\}$,

$$p(b_{ji} = 1|\beta, \Theta, g, r, t_i) = \sum_{c=0}^{\infty} \left[\Phi \left(\frac{\lambda \cdot (c+1) - (-\mu_0 + t_i)}{\sqrt{\sigma_0^2 + t_i^2 \cdot \sigma_v^2}} \right) - \Phi \left(\frac{\lambda \cdot (c+\beta) - (-\mu_0 + t_i)}{\sqrt{\sigma_0^2 + t_i^2 \cdot \sigma_v^2}} \right) \right],$$

while, for subsequent cohorts, $0 < g \leq r$,

$$p(b_{ji}=1|\beta, \Theta, g, r, t_i) = \sum_{c=0}^C \left[\left(\Phi \left(\frac{\lambda \cdot (c+1) - (-\mu_0 + t_i - r \cdot \lambda - g \cdot \delta)}{\sqrt{\sigma_0^2 + t_i^2 \cdot \sigma_v^2}} \right) \right) - \Phi \left(\frac{\lambda \cdot (c+\beta) - (-\mu_0 + t_i - r \cdot \lambda - g \cdot \delta)}{\sqrt{\sigma_0^2 + t_i^2 \cdot \sigma_v^2}} \right) \right] / \left(1 - \Phi \left(\frac{-\delta - (-\mu_0 + t_i - r \cdot \lambda - g \cdot \delta)}{\sqrt{\sigma_0^2 + t_i^2 \cdot \sigma_v^2}} \right) \right)$$

We model bud presence as a Bernoulli random variable with success probability $p(b_{ji}=1|\beta, \Theta, t_j)$ and assume that samples drawn at the various time periods are independent conditional on the CLOCCS model.

3.4. Sampling model for DNA content data

DNA content data measured by flow cytometry provides an ordinal measurement of the DNA content of each cell in a sample: each cell appears in one of 1024 ordered channels on the basis of its fluorescence, which is proportional to its DNA content [Pierrez and Ronot (1992)]. In practice, channel number is often \log_2 transformed and treated as a continuous measurement.

Adapting the CLOCCS model to DNA content data requires that we annotate the lifeline with the positions, measured as fraction of cell cycle length, at which S phase begins and ends. We denote these locations γ_1 and γ_2 , respectively. As the population loses synchrony, the distribution of cells over channels will typically be bimodal, with one mode corresponding to cells in G1 (centered at α_1), and the another corresponding to G2/M (centered at $\alpha_1 + \alpha_2$). Cells transiting the S phase will fall between these points in expectation. Further, we assume that DNA content increases linearly over the course of the S phase. In particular, the expected DNA content of a cell is

$$\sum_{c=0}^C \begin{cases} \alpha_{1t}, & c\lambda \leq P_t < (c+\gamma_1)\lambda, & \text{G1,} \\ \omega_{1t}P_t + \omega_{0t}(c), & (c+\gamma_1)\lambda \leq P_t < (c+\gamma_2)\lambda, & \text{S-phase,} \\ \alpha_{2t} + \alpha_{1t}, & (c+\gamma_2)\lambda \leq P_t < (c+1)\lambda, & \text{G2/M,} \end{cases} \quad (2)$$

where $\omega_{1t} = \frac{\alpha_{2t}}{\lambda(\gamma_2 - \gamma_1)}$ and $\omega_{0t}(c) = \frac{\alpha_{1t}(\gamma_2 - \gamma_1) - \alpha_{2t}(\gamma_1 + c)}{\gamma_2 - \gamma_1}$. The black line in Figure 3 is a plot of this curve.

Measurement of DNA content by flow cytometry is imprecise. Machine noise, variation in the cell's orientation to the laser beam and variation in the performance of the fluorescent stain each contribute to measurement error [Pierrez and Ronot (1992)]. Hence, a flow cytometry measurement made on a sample of cells drawn at a particular time point will be a sample from the convolution of a noise distribution and the CLOCCS position distribution. In particular,

$$p(f_{ji}|\Psi, \Theta, t) = \int_{-\infty}^{\infty} p(f_{ji}|P_t, \Psi, \Theta, t)p(P_t|\Theta, t)dP_t, \quad (3)$$

where f_{ji} denotes the log fluorescence intensity of cell j at time t_i and where Ψ denotes the vector of parameters in the model for f_{ji} not in Θ . From above it follows that $p(f_{ji}|P_t, \Psi, \Theta, t)$ can be modeled as a normal with mean given in equation (2) and variance τ_i^2 . The log normal distribution is a common choice in this setting [Gray and Dean (1980)]. Additionally, the noise characteristics of the flow cytometer typically vary from one sample to the next, causing the locations of the G1 and G2/M modes, as well as the level of machine noise (τ) to vary. Hence, we allow the parameters of the DNA content sampling distribution, $p(f_{ji}|P_t, \Psi, \Theta, t)$, to vary across time periods.

Note that equation (3) can be written as

$$p(f_{ji}|\Psi, \Theta, t) = \sum_{g,r} I_{gr}(f_{ji})p(g, r|\Theta, t),$$

where

$$I_{gr}(f_{ji}) = \int_{-\infty}^{\infty} p(f_{ji}|P_t, \Psi, \Theta, t)p(P_t|\Theta, r, g, t)dP_t$$

is a convolution of two normals, one of which is truncated.

Let I_{gr} denote the left limit to the support of cohort $\{g, r\}$'s position distribution, where $I_{gr} = -\infty$ if $g = r = 0$ and $I_{gr} = -\delta$ otherwise. Further, let $G_{grt}(x)$ denote the normal cumulative distribution function with mean $-\mu_0 + t - r \cdot \lambda - g \cdot \delta$ and variance $\sigma_0^2 + t^2 \cdot \sigma_v^2$ evaluated at x and let $S_{cgrt}(x)$ denote the normal cumulative distribution function with mean

$$\left((\sigma_0^2 + t^2 \cdot \sigma_v^2) \left(\frac{f_{ji}}{\omega_{1t}} - \frac{\omega_{0t}(c)}{\omega_{1t}} \right) + \left(\frac{\tau^2}{\omega_{1t}^2} \right) (-\mu_0 + t - r \cdot \lambda - g \cdot \delta) \right) / \left(\sigma_0^2 + t^2 \cdot \sigma_v^2 + \frac{\tau^2}{\omega_{1t}^2} \right)$$

and variance

$$\left((\sigma_0^2 + t^2 \cdot \sigma_v^2) \frac{\tau^2}{\omega_{1t}^2} \right) / \left(\sigma_0^2 + t^2 \cdot \sigma_v^2 + \frac{\tau^2}{\omega_{1t}^2} \right)$$

evaluated at x . It can be shown that $I_{gr}(f_{ji}) = I_{gr}^*(f_{ji}) / (1 - G_{grt}(I_{gr}))$, where

$$\begin{aligned}
I_{gr}^*(f_{ji}) = & \frac{1}{\tau} \phi \left(\frac{f_{ji} - \alpha_{1t}}{\tau} \right) \\
& \times \left[(G_{grt}(\gamma_1 \lambda) - G_{grt}(l_{gr})) + \sum_{c=1}^C (G_{grt}((c+\gamma_1)\lambda) - G_{grt}(c\lambda)) \right] \\
& + \frac{1}{\tau} \phi \left(\frac{f_{ji} - \alpha_{1t} - \alpha_{2t}}{\tau} \right) \sum_{c=0}^C (G_{grt}((c+1)\lambda) - G_{grt}((c+\gamma_2)\lambda)) \\
& + \sum_{c=0}^C \left(\phi \left(\left(\frac{f_{ji}}{\omega_{1t}} - \frac{\omega_{0t}(c)}{\omega_{1t}} - (-\mu_0 + t - r \cdot \lambda - g \cdot \delta) \right) \right. \right. \\
& \quad \left. \left. / \sqrt{\sigma_0^2 + t^2 \cdot \sigma_v^2 + \frac{\tau^2}{\omega_{1t}^2}} \right) \right. \\
& \quad \left. / \sqrt{\omega_{1t}^2 (\sigma_0^2 + t^2 \cdot \sigma_v^2) + \tau^2} \right) \\
& \times (S_{cgrt}((c+\gamma_2)\lambda) - S_{cgrt}((c+\gamma_1)\lambda))
\end{aligned}$$

and where $\phi(\cdot)$ is the standard normal density function. In the equation above, the first line of the right-hand side corresponds to cells in G1, the second to cells in G2 or M, and the third to cells in S.

We assume that cell-level DNA content measurements are conditionally independent within and between samples drawn at the various time periods conditional on the CLOCCS model, Ψ and the sampling times. DNA content and budding index measurements are made on separate samples drawn from a population's culture, sometimes at the same points in time, sometimes not. Because they are distinct samples, we model the DNA content and budding index data as conditionally independent given the CLOCCS parameters Θ , the budding parameter β , the DNA content parameters Ψ and sampling times.

3.5. Prior distribution

What follows is a description of, and justification for, the prior choices used in our analysis. Columns 2 and 3 of Table 1 tabulate prior expected values and 95% equal-tailed intervals for each parameter as implied by these choices.

Lord and Wheals (1983) estimate *S. cerevisiae* cell cycle length in culture at 30 degrees Centigrade—the temperature employed by our lab—to be 78.2 minutes with a standard deviation of 9.1 minutes. To allow for differences in experimental protocol, we place a normal, mean 78.2, standard deviation 18.2 prior on cell cycle length, λ . In *S. cerevisiae*, duration of the S phase, $(\gamma_2 - \gamma_1)\lambda$, is about one quarter of the cell cycle; it begins a short time before buds can be visually detected and continues until mother and daughter cells separate [Vanoni, Vai and Frascotti (1984)]. Based on an analysis of 30 DNA content measurements made on an asynchronous population conducted using the same protocol as used in the synchrony experiment described in the next section, we estimate that γ_1 is approximately 0.1 and that β is approximately 0.12. Hence, we expect $\gamma_1 < \beta < \gamma_2$. With this in mind, we let $\gamma_1 \sim \text{Beta}(2, 18)$, $\beta \sim \text{Beta}(2.4, 17.6)$ and $\gamma_2 \sim \text{Beta}(7, 13)$, constrained as above. Bar-Joseph et al. (2004) estimates the standard deviation of the velocity distribution in *S. cerevisiae* to be 0.09 and observed a range of values 0.07 to 0.11 across 3 experiments. For this reason, we place an independent inverse-gamma(12, 1) prior distribution on σ_v .

Aspects of experimental protocol, most notably the method used to synchronize the population, have a strong influence on the parameters of the starting position distribution and on duration of the daughter-specific offset, δ . Centrifugal elutriation, the method used in the experiment we describe in the next section, selects for small unbudded cells, while other methods, such as α -factor arrest, do not. Because of their size, elutriated cells tend to spend more time in Gr and their daughters spend more time in Gd than their counterparts in α -factor experiments [Hartwell and Unger (1977)]. We have chosen to specify our prior distributions on these parameters to accommodate—not condition on—this source of protocol dependent uncertainty. In particular, we place an inverse-gamma distribution with shape parameter 2 and mean 78.2/3 on σ_0 and the minimally informative exponential, mean 78.2 prior distribution on μ_0 . The former reflects our belief that almost all cells will be in Gr at release; the latter places highest prior likelihood on a short Gr, as is expected in an α -factor experiment, but allows for the longer Gr that is expected in elutriation experiments. Similar reasoning was behind our choice of an exponential mean 55 prior distribution on δ : in α -factor experiments, δ can be very brief, while in elutriation experiments it can exceed 40% of the length of a typical cell cycle [Hartwell and Unger (1977), Lord and Wheals (1983)].

In the DNA content distributions, flow cytometer fluorescence noise, as measured by τ_i , and location of the G1 and G2/M modes, as measured by α_{1i} and α_{2i} respectively, vary randomly from assay to assay over time. We model this variability hierarchically: first placing independent normal prior distributions on $\log(\tau_i)$, α_{1i} and α_{2i} , $i = 1, \dots, T$, followed by independent conjugate normal-inverse-chi-square hyperprior distributions on the parameters of the normal distributions. The latter are parametrized as in Gelman et al. (1995). In particular,

$$\begin{aligned} \log(\tau_i) &\stackrel{\text{i.i.d.}}{\sim} N(\mu_\tau, \sigma_\tau^2), & \alpha_{1i} &\stackrel{\text{i.i.d.}}{\sim} N(\mu_{\alpha_1}, \sigma_{\alpha_1}^2), \\ \mu_\tau | \sigma_\tau^2 &\sim N(\eta_\tau, \sigma_\tau^2 / \kappa_\tau), & \mu_{\alpha_1} | \sigma_{\alpha_1}^2 &\sim N(\eta_{\alpha_1}, \sigma_{\alpha_1}^2 / \kappa_{\alpha_1}), \\ \sigma_\tau^2 &\sim \text{Inv} - \chi^2(\nu_\tau, \gamma_\tau^2), & \sigma_{\alpha_1}^2 &\sim \text{Inv} - \chi^2(\nu_{\alpha_1}, \gamma_{\alpha_1}^2), \\ \alpha_{2i} &\stackrel{\text{i.i.d.}}{\sim} N(\mu_{\alpha_2}, \sigma_{\alpha_2}^2), & & \\ \mu_{\alpha_2} | \sigma_{\alpha_2}^2 &\sim N(\eta_{\alpha_2}, \sigma_{\alpha_2}^2 / \kappa_{\alpha_2}), & & \\ \sigma_{\alpha_2}^2 &\sim \text{Inv} - \chi^2(\nu_{\alpha_2}, \gamma_{\alpha_2}^2), & & \end{aligned}$$

where $\text{Inv} - \chi^2(\nu, \gamma^2)$ denotes the scaled inverse χ^2 distribution with ν degrees of freedom and scale parameter γ . Given this specification, we define

$$\Psi = (\tau_1, \dots, \tau_T, \alpha_{11}, \dots, \alpha_{1T}, \alpha_{21}, \dots, \alpha_{2T}, \mu_\tau, \sigma_\tau^2, \mu_{\alpha_1}, \sigma_{\alpha_1}^2, \mu_{\alpha_2}, \sigma_{\alpha_2}^2).$$

We chose the hyperparameters of the above hierarchical model on the basis of an exploratory analysis of the same asynchronous DNA content data used above. We set $\eta_{\alpha_1} = 7.58$, $\eta_{\alpha_2} = 0.82$ and $\eta_\tau = -1.91$, the average of the observed estimates of α_1 , α_2 and τ , respectively. We set each of the prior sample size parameters, κ_τ , κ_{α_1} and κ_{α_2} , and each of the prior degrees of freedom parameters, ν_τ , ν_{α_1} and ν_{α_2} , equal to 2 to keep these margins of the prior distribution relatively diffuse. Finally, we set

$\gamma_\tau^2 = 0.13$, $\gamma_{\alpha_1}^2 = 0.065$ and $\gamma_{\alpha_2}^2 = 0.0089$ —in each case 16 times the observed variance in the asynchronous experiment.

4. Analysis

In what follows, we utilize the model to analyze budding index and DNA content data from a cell cycle synchrony experiment in *S. cerevisiae* using cells synchronized by centrifugal elutriation and cultured at 30°C. Details of the strain and growth conditions used can be found in Orlando et al. (2007). After synchronization, 32 samples were collected at 8 minute intervals starting 30 minutes after release. Two aliquots were taken from each sample, one for each type of measurement. Budding index was measured by microscopically assessing at least 200 cells for the presence of a bud and recording the number of budded and unbudded cells observed. The relative DNA content of 10,000 cells in each sample was measured by flow cytometry as described previously [Haase and Reed (2002)]. The observed fluorescence values for each measured cell in each sample were \log_2 transformed prior to analysis. The DNA content measurement of the 38 minute sample was not available due to a technical problem encountered during preparation of that sample.

We compare parameter estimates given both the budding index and DNA content data, given the DNA content data alone and given the budding index data alone. In addition, using only the budding index data, we estimate Bayes factors for the full CLOCCS model to submodels obtained by systematically removing each novel source of asynchrony, δ , μ_0 and σ_0 separately and in combination.

4.1. Estimates given the experimental data

We use a random walk Metropolis [Gilks, Richardson and Spiegelhalter (1996), Metropolis et al. (1953)] algorithm for each model fit. In each case, the algorithm was tuned to mix well and the chain was given a lengthy burn-in period. Subsequent to this, we ran the chain for 400,000 iterations and saved every fourth for inference. Plots of sampled values appear stationary, and the Raftery and Lewis diagnostic [Raftery and Lewis (1996)], implemented in the R package CODA, indicates that the sample is sufficient to estimate the 0.025th quantile of any marginal posterior to within 0.01 with probability 0.95. All coefficients and associated interval estimates are based on summary statistics of marginal sample distributions. We tested our implementation of the model and the Markov chain Monte Carlo sampler by analyzing simulated data sets. Parameter estimates derived from these analyses were consistent with their true values.

Table 1 provides marginal summaries of the prior (columns 1 and 2) and of the posterior distributions after fitting the model to both the DNA content and budding index data (columns 3 and 4), to the DNA content data only (columns 5 and 6) and to the budding index data only (columns 7 and 8). Note that point and interval estimates of common parameters derived using both the budding index and DNA content data are very close to their counterparts fit only to the DNA content data. This is not surprising given the information rich nature of the DNA content data: at each time period approximately 10,000 cells are assayed for DNA content, while only approximately 200 are assayed for presence of a bud. On average, point estimates of the common parameters differ by less than 1% and the associated posterior interval estimates are only about 2% narrower when the budding index data is added. The parameter β can only be estimated with budding index data, but it is estimated more accurately when DNA content data is included, owing to the fact that it is constrained by γ_1 and γ_2 .

Figure 4A is a plot of the observed budding index curve (black) overlaid with 95% pointwise interval estimates from the analysis of only the budding index data (green) and of both the budding index and DNA content data (red). The latter analysis estimates the recovery period (Gr) to be slightly shorter and more variable and estimates cell cycle length to be longer and less variable than estimated with the budding index data alone. This is

evident in the red confidence bands positioned to the left of the green between 70 and 100 minutes and to the right of the green between 190 and 225 minutes experimental time. Note that both curves increase more smoothly and sooner than the observed budding index following recovery from synchronization. This is likely due to our choice of the normal distribution to characterize time spent in Gr. It appears that a left skewed distribution may give a better fit to this feature in the data.

Figures 4B–F plot observed DNA content densities (gray) and their posterior mean estimates (red) at five experimental time points selected to highlight the population’s transition from G1 (B) through the S phase to G2/M (C and D) and the effect of its growing asynchrony (E and F). The corresponding time points are identified by labels on the budding index curve Figure 4A. The observed DNA content densities are discrete and unsmoothed. They are calculated by normalizing the raw DNA content channel counts and transforming them, via the change of variables formula, to the \log_2 scale. The estimates are extremely good: in all cases, the G1 and G2/M modes are accurately scaled and located and capture the shape of the distributions between the modes, suggesting that the model is accurately accounting for the cells transiting the S phase.

4.2. Model evaluation

In what follows, we estimate Bayes factors (BFs) [Kass and Raftery (1995)] for a series of pairs of models nested under the fully parametrized CLOCCS model using importance sampling. These quantities allow us to measure the weight of evidence in the budding index data in favor of alternate parametrizations of the model, including variants that drop the daughter offset and/or one or both parameters of the starting position distribution. The hierarchy of models we examine is not complete but accounts for all reasonable alternatives to the full model. The simplest model, where we set $\mu_0 = 0$, $\sigma_0^2 = 0$ and $\delta = 0$, corresponds to a branching process version of the Bar-Joseph et al. (2004) model. We employed a separate sampler to estimate each marginal likelihood and used 100 degrees-of-freedom multivariate t densities as the importance densities, each with mean and covariance matrix matching that estimated from a Markov chain Monte Carlo analysis of the associated model. For purposes of this calculation, we used only the budding index data to inform the model and drew 10,000 importance samples for each calculation. The variance of the normalized weights was less than 1.45 in all cases. Hence, the effective sample size [Liu (2001)] for estimating the marginal likelihood was never smaller than 4000.

Table 2 reports estimates of \log_e Bayes factors (IBFs) for various nested model comparisons given the budding index data. In these tables, the model indexed by an entry’s column is the larger of the models and is represented in the numerator of the IBFs in that column; the model indexed by an entry’s row is the smaller of the two. As a guide to interpreting these numbers, Kass and Raftery (1995) classify IBFs between 0 and 1 as “not worth more than a bare mention,” those from 1 to 3 “positive,” those from 3 to 5 “strong” and those greater than 5 “very strong.” Using this scale as a guide, the full CLOCCS model is very strongly preferred to all alternatives, including the model of Bar-Joseph et al. (2004). The worst alternative sets only $\mu_0 = 0$. When μ_0 is constrained to be zero, better fits to the data are achieved by setting one or the other, or preferably both, of δ and σ_0^2 to zero.

Figure 5 depicts posterior mean fits to the budding index data under each of the competing models. We estimated the posterior means using the MCMC output that was used to determine the importance distributions. Each MCMC analysis followed the same procedure, described in Section 4.1, used for the primary analyses. Note that the fits achieved by all model variants that set $\mu_0 = 0$ are visually indistinguishable and markedly inferior to any variant that allows $\mu_0 > 0$. The last two rows of Table 2 provide estimates of the root mean

squared error (RMSE) of the fits to the budding index data achieved by each model's posterior mean curve. These estimates reinforce what is evident from the marginal likelihood and graphical analyses, namely, that models that do not allow for a nonzero location in the distribution of initial cell position are markedly inferior to those that do and that accounting for a mother/daughter offset is particularly important, at least in the case where the cell population was arrested using centrifugal elutriation. Finally, these results demonstrate that the extremely good fits depicted in Figure 4 are the result of a parsimoniously parametrized model and not due to over-fitting.

5. Discussion

Synchrony/time-series experiments on populations of cells are essential for understanding the dynamic processes associated with the cell cycle. In this paper we have described the CLOCCS model, sampling models for fitting this model to both budding index and DNA content data, and a detailed model evaluation. We have demonstrated that accurate model fits can be obtained using budding index, DNA content data or both. While previous models only account for one source of asynchrony, namely, variation cell cycle length [Bar-Joseph et al. (2004), Chiorino et al. (2001), Larsson et al. (2008), Liou, Srienc and Fredrickson (1997)], the CLOCCS model adds two novel sources of asynchrony. These are variation in initial synchrony and variation due to asymmetric cell division. In Section 4.2 we showed that the CLOCCS model is very strongly preferred to all nested alternatives, including a branching process version of the model of Bar-Joseph et al. (2004).

The more accurate description of population dynamics achieved by the CLOCCS model will allow more accurate deconvolution of dynamic measurements such as transcript abundance. Additionally, because the model maps time-series data onto a common cell cycle lifeline, different data types (e.g., mRNA levels, protein levels, protein localization, etc.) from multiple synchrony/time-series experiments can be aligned such that the dynamics of multiple events can be temporally compared. Furthermore, DNA content measurements are commonly used to measure cell cycle position in organisms from yeast to mammals. Thus, the model permits the alignment and comparison of dynamics of cell cycle events across species, potentially providing an accurate view of evolutionary changes in cell cycle progression and regulation.

The model's parameter estimates are also interpretable in terms of biological quantities associated with the cell cycle, so their estimates are of independent interest. For example, the measure of initial synchrony, σ_0 , can be used to tune synchrony protocols for optimal results. When using budding index data, λ and β allow researchers to map temporal events to pre- or post-G1 cell cycle phases. When DNA content data is used, this resolution is increased and events can be placed accurately into the G1, S or G2/M phases of the cell cycle.

The CLOCCS model is unique, to our knowledge, for providing a closed form expression for the likelihood function in a complex branching process. This expression is written by enumerating and then marginalizing over the distinct cohorts present in the population at a given time. The explicit accounting of cohorts allows for extensions of the model that introduce cohort dependent effects such as one-time events and effects, such as the mother-daughter offset, that may diminish with generation. The approach we describe is very general and has the potential to provide a flexible and efficient alternative in a range of problems where population balance or branching process models are used to describe the short term dynamics of a branching population.

While CLOCCS is better than its nested alternatives, the model can be improved to better fit experimental data and to better reflect biological reality. First, our data suggest that a left skewed distribution with finite support may be more realistic a choice for the initial position. Second, while our data do not contradict a linear accumulation of DNA during the S phase, others have suggested alternative parametrizations [Larsson et al. (2008), Niemistö et al. (2007)]. We are currently exploring a flexibly parametrized S phase function that will allow inference on its functional form and, by doing so, address a question of fundamental interest to the greater biological community. Third, we plan to generalize the model to allow for an unspecified correlation between mother and daughter cell velocities; this parameter is currently set to one. Finally, we assume that the delay due to asymmetric cell division (δ) is constant over time. Evidence exists, however, that the magnitude of this effect may change as the experiment progresses. This issue can be addressed with a suitably parametrized cohort-specific delay term, although the duration of a typical time-course experiment may limit power to detect this effect.

The strength of the CLOCCS modeling framework lies in its flexibility. It is adaptable to new experimental measurements, and given its ability to use DNA content data, is already applicable to virtually all biological systems where synchronized populations are studied, most notably human cell-culture systems. Further integration of the model with deconvolution and alignment algorithms will provide researchers with a powerful new tool to aid in the study of dynamic processes during the cell division cycle. Software implementing the CLOCCS model can be found at <http://www.cs.duke.edu/~amink/software/cloccs>.

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REFERENCES

- Alexandersson M. On the existence of the stable birth-type distribution in a general branching process cell cycle model with unequal cell division. *J. Appl. Probab.* 2001; 38:685–695. MR1860206.
- Arino O, Kimmel M. Comparison of approaches to modeling of cell population dynamics. *SIAM J. App. Math.* 1993; 53:1480–1504. MR1239415.
- Bar-Joseph Z, Farkash S, Gifford D, Simon I, Rosenfeld R. Deconvolving cell cycle expression data with complementary information. *Bioinformatics.* 2004; 20:i23–i30. [PubMed: 15262777]
- Chiorino G, Metz J, Tomasoni D, Ubezio P. Desynchronization rate in cell populations: Mathematical modeling and experimental data. *J. Theoret. Biol.* 2001; 208:185–199. [PubMed: 11162063]
- Gelman, A.; Carlin, JB.; Stern, HS.; Rubin, DB. *Bayesian Data Analysis*. London: Chapman & Hall; 1995. MR1385925
- Gilks, WR.; Richardson, S.; Spiegelhalter, DJ. *Introducing Markov chain Monte Carlo*. In: Gilks, WR.; Richardson, S.; Spiegelhalter, DJ., editors. *Markov Chain Monte Carlo in Practice*. London: Chapman & Hall; 1996. MR1397966
- Gordon C, Elliott S. Fractionation of *Saccharomyces cerevisiae* cell populations by centrifugal elutriation. *J. Bacteriol.* 1977; 129:97–100. [PubMed: 318655]
- Gray J, Dean P. Display and analysis of flow cytometric data. *Annual Reviews in Biophysics and Bioengineering.* 1980; 9:509–539.
- Guttorp, P. *Statistical Inference for Branching Processes*. New York: Wiley; 2001. MR1254434
- Haase S, Reed S. Improved flow cytometric analysis of the budding yeast cell cycle. *Cell Cycle.* 2002; 1:132–136. [PubMed: 12429922]

- Hartwell L. *Saccharomyces cerevisiae* cell cycle. *Bacteriological Reviews*. 1974; 38:164–198. [PubMed: 4599449]
- Hartwell L, Unger M. Unequal division in *Saccharomyces cerevisiae* and its implications for the control of cell division. *The Journal of Cell Biology*. 1977; 75:422–435. [PubMed: 400873]
- Kass RE, Raftery AE. Bayes factors. *J. Amer. Statist. Assoc.* 1995; 90:773–795.
- Larsson S, Ryden T, Holst U, Oredsson S, Johansson M. Estimating the variation in S phase duration from flow cytometric histograms. *Math. Biosci.* 2008; 213:40–49. MR2414830. [PubMed: 18433802]
- Liou J, Srienc F, Fredrickson A. Solutions of population balance models based on a successive generations approach. *Chemical Engineering Science*. 1997; 52:1529–1540.
- Liu, J. *Monte Carlo Strategies in Scientific Computing*. New York: Springer; 2001. MR1842342
- Lord PG, Wheals AE. Rate of cell cycle initiation of yeast cells when cell size is not a rate-determining factor. *Journal of Cell Science*. 1983; 59:183–201. [PubMed: 6345558]
- Lu X, Zhang W, Qin ZS, Kwast KE, Liu JS. Statistical resynchronization and Bayesian detection of periodically expressed genes. *Nucleic Acids Research*. 2004; 32:447–455. [PubMed: 14739237]
- Metropolis N, Rosenbluth AW, Rosenbluth MN, Teller AH, Teller E. Equations of state calculated by fast computing machines. *Journal of Chemical Physics*. 1953; 21:1087–1092.
- Niemistö A, Nykter M, Aho T, Jalovaara H, Marjanen K, Ahdesmäki M, Ruusuvoori P, Tiainen M, Linne M, Yli-Harja O. Computational methods for estimation of cell cycle phase distributions of yeast cells. *EURASIP Journal on Bioinformatics and Systems Biology*. 2007; 2007:1–9.
- Orlando D, Lin C, Bernard A, Iversen E, Hartemink A, Haase S. A probabilistic model for cell cycle distributions in synchrony experiments. *Cell Cycle*. 2007; 6:478–488. [PubMed: 17329975]
- Pierrez J, Ronot X. Flow cytometric analysis of the cell cycle: Mathematical modeling and biological interpretation. *Acta Biotheoretica*. 1992; 40:131–137. [PubMed: 1462731]
- Raftery, AE.; Lewis, SM. Implementing MCMC. In: Gilks, WR.; Richardson, S.; Spiegelhalter, DJ., editors. *Markov Chain Monte Carlo in Practice*. London: Chapman & Hall; 1996. p. 115-127. MR1397966
- Sidoli FR, Mantalaris A, Asprey SP. Modelling of mammalian cells and cell culture processes. *Cytotechnology*. 2004; 44:27–46. [PubMed: 19003227]
- Vanoni M, Vai M, Frascotti G. Effects of temperature on the yeast cell cycle analysed by flow cytometry. *Cytometry*. 1984; 5:530–533. [PubMed: 6386390]

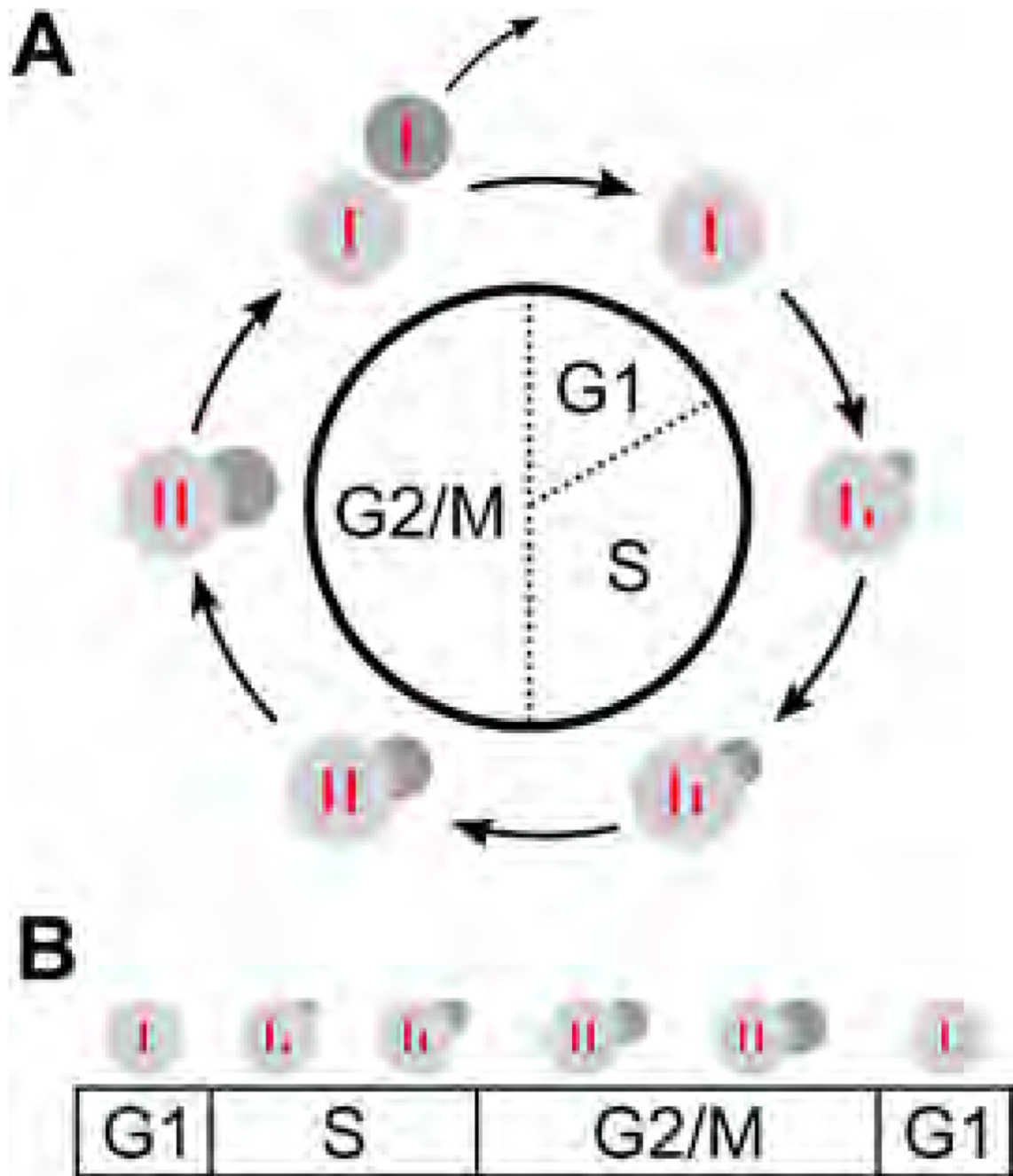


FIG. 1.

Over the course of its life, the cell repeatedly traverses the cell cycle, which is divided by landmark events associated with asexual reproduction into the G1, S and G2/M phases. In the figure, this corresponds to the cell in light gray traveling around the circle in A or from left to right in B. At each completion of G2/M it spawns a daughter cell. This process begins with development of a bud (dark gray) and the start of DNA replication (denoted by the appearance of a second red bar) and is completed when the daughter cell (dark gray) separates from the mother cell at the end of G2/M with a full complement of DNA.

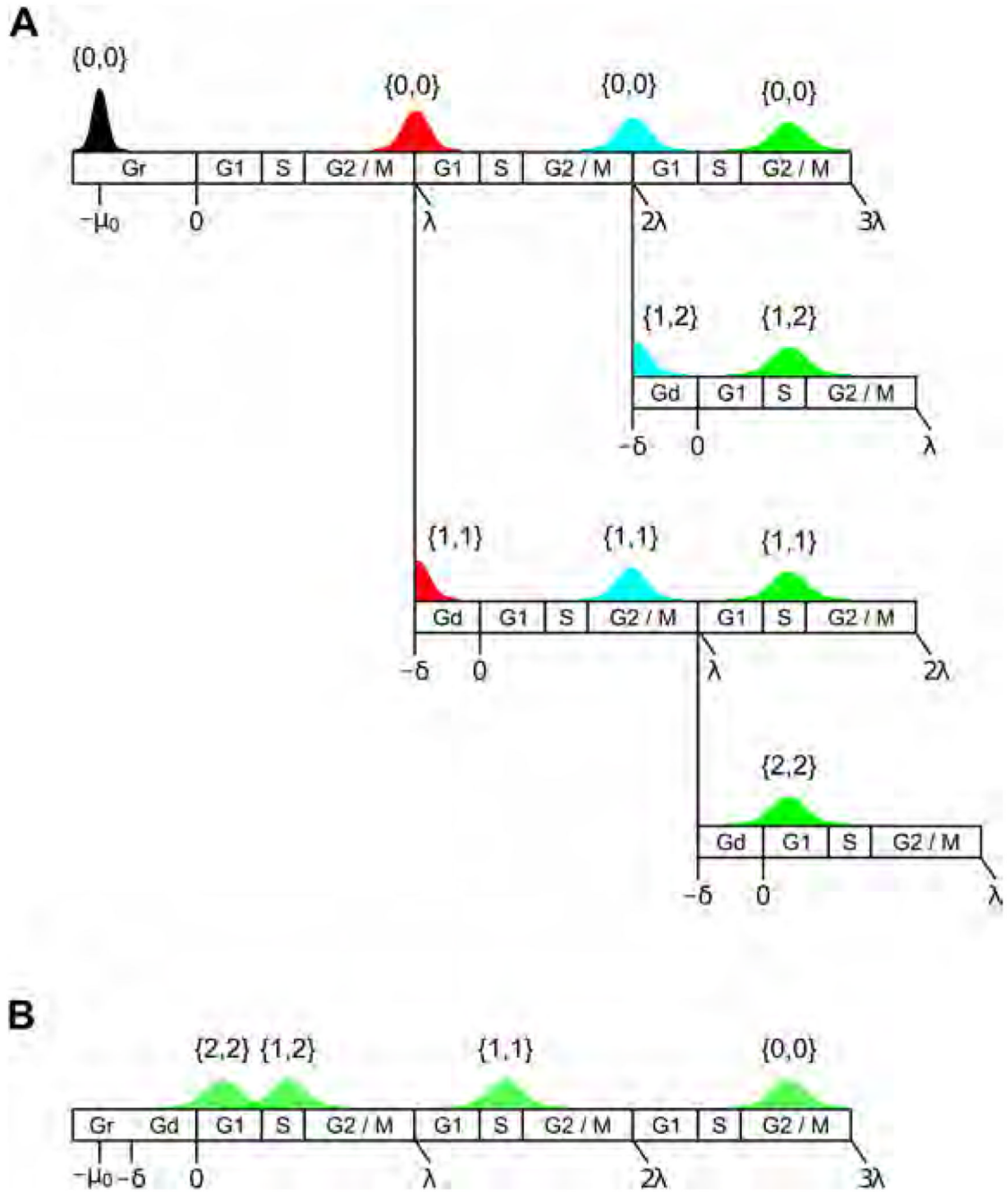


FIG. 2. Graphical representation of the branching dynamics of the cell population, A, and a snapshot in time plotted on a common lifeline, B. A: The position distributions of the cohorts, indexed by $\{g, r\}$, in the population at four points in time, each color-coded. Black: at release from synchrony, there is a single cohort, $\{0, 0\}$. Red: as it enters its second cell cycle, $\{0, 0\}$ spawns a daughter cohort, $\{1, 1\}$, located on its own lifeline in Gd. Blue: at cohort $\{0, 0\}$'s second reproductive instance, it gives rise to another first generation cohort, $\{1, 2\}$; meanwhile, most cells in cohort $\{1, 1\}$ are progressing through G2/M. Green: the population is comprised of four distinct cohorts; B: a plot of the population at this time point on a common lifeline.

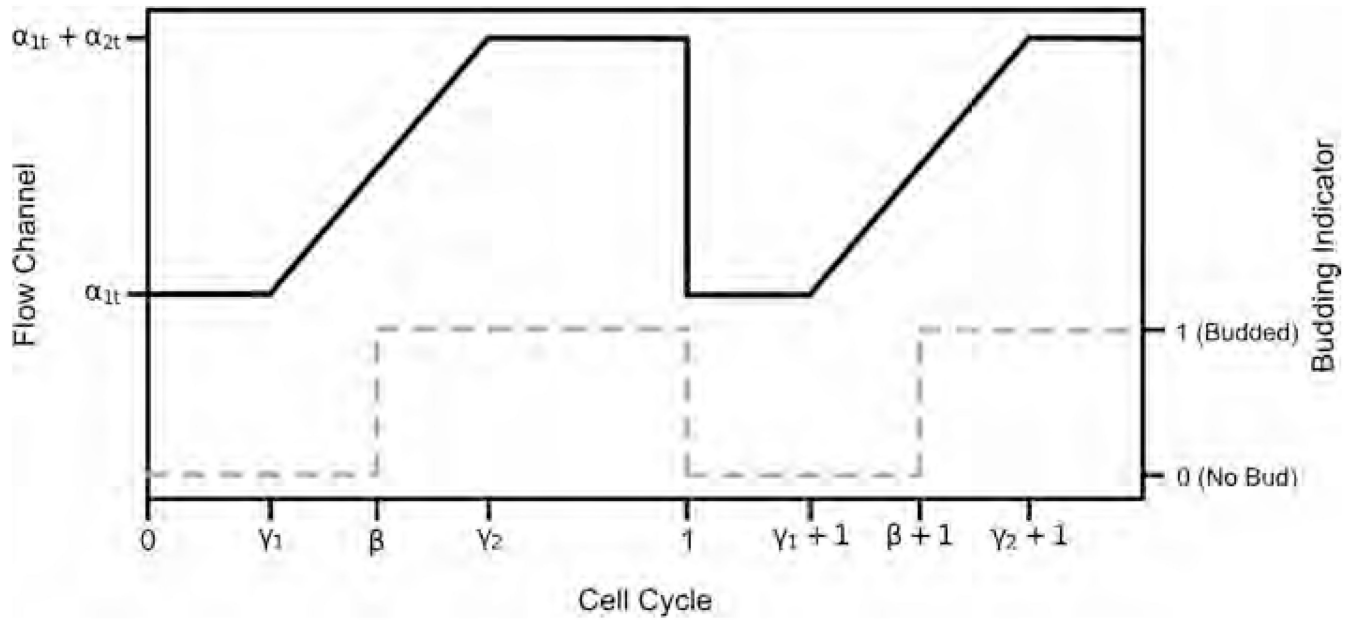
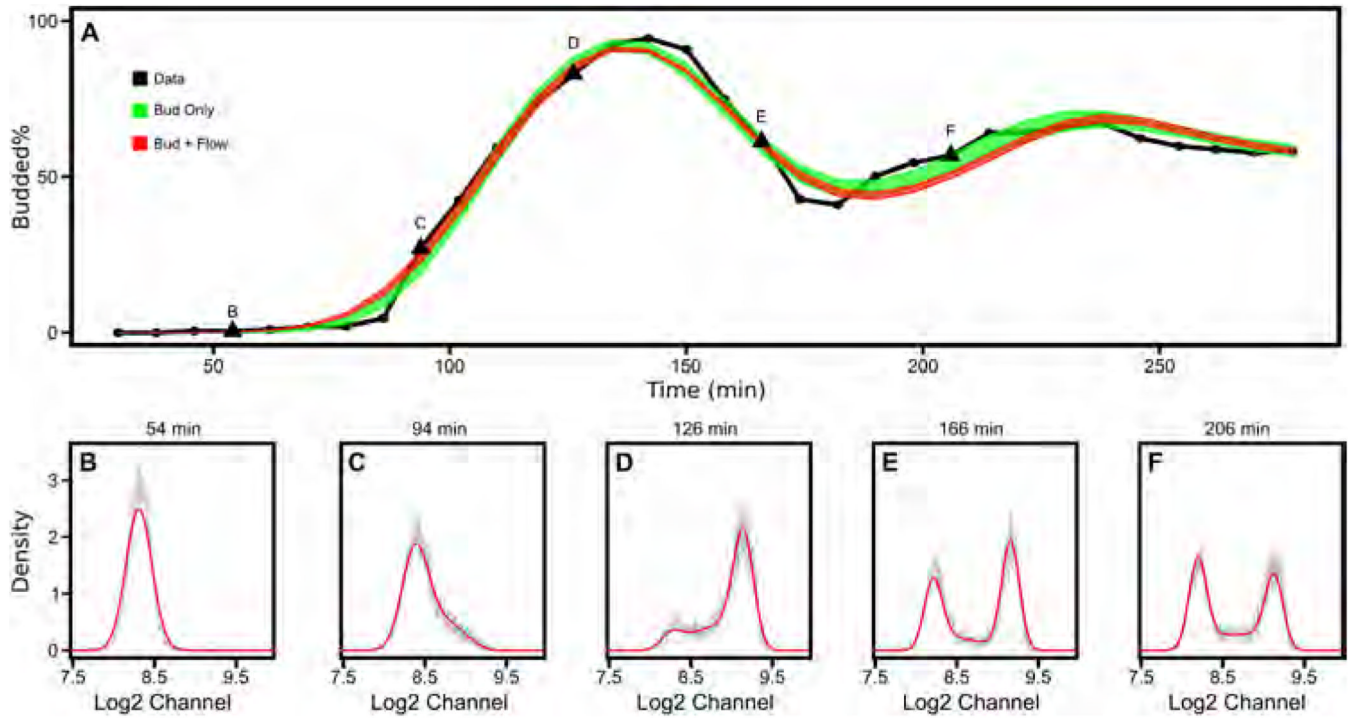


FIG. 3. Plot of expected flow cytometry channel for a cell given its lifeline position in units of λ (black curve, left vertical axis). An indicator function for the cell's budding status is also plotted (grey dashed curve, right vertical axis).

**FIG. 4.**

Estimated budding and DNA content curves accurately reflect complex, biologically relevant patterns in the data. A: plot of observed budding index curve (black) and 95% pointwise interval estimates from budding index only analysis (green) and budding index/DNA content analysis (red). B–F: DNA content densities (gray) and their posterior mean estimates (red) at five points in time, highlighting the population’s transition from G1 (B) through the S phase to G2/M (C and D) and the effect of its growing asynchrony (E and F). The corresponding time points are labeled above the budding index curve. In all cases, the G1 and G2/M modes are accurately scaled and located, as is the shape of the distributions between the modes.

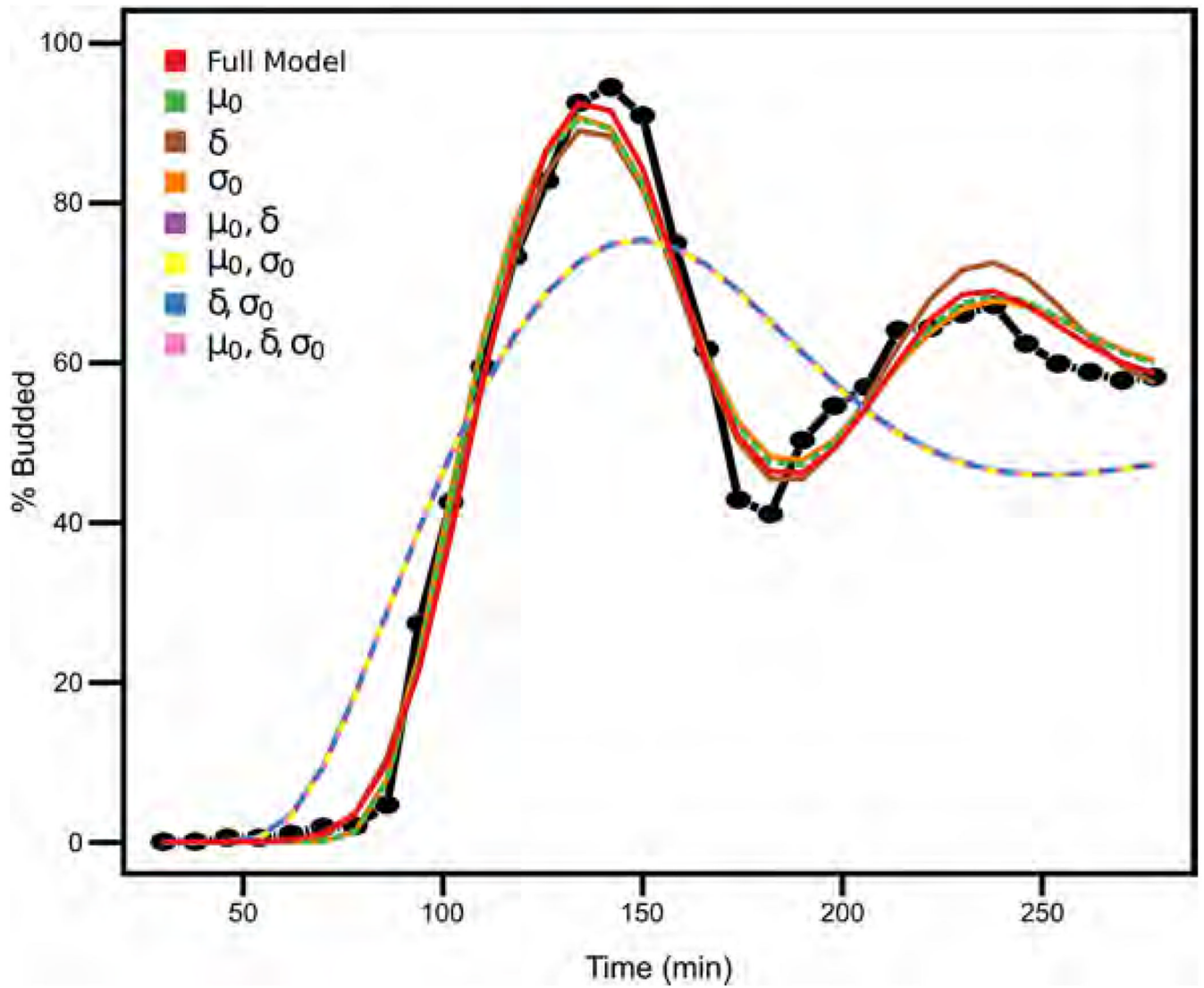


FIG. 5. Plot of observed budding index curve (black) and posterior mean fitted curves under each of the competing models for the budding index data. The full model is plotted in red; the competing models are obtained by constraining the parameter(s) indicated in the figure legend to be zero. Quantitative summaries of these fits can be found in Table 2.

TABLE 1

Prior (columns 1 and 2) and marginal posterior summaries given both the DNA content and budding index data (columns 3 and 4), given the DNA content data only (columns 5 and 6) and given the budding index data only (columns 7 and 8)

	Prior		Flow and budding		Flow only		Budding only	
	Mean	95% interval	Mean	95% interval	Mean	95% interval	Mean	95% interval
μ_0	78.200	(22.497, 288.470)	-94.387	(-94.778, -94.000)	-94.279	(-94.674, -93.878)	-95.967	(-98.770, -92.915)
δ	55.000	(15.823, 202.888)	44.318	(43.842, 44.790)	44.326	(43.845, 44.810)	41.748	(36.623, 46.424)
σ_0	26.066	(4.679, 107.150)	17.961	(17.765, 18.150)	18.015	(17.819, 18.207)	15.328	(13.992, 16.555)
σ_v	0.091	(0.051, 0.162)	0.025	(0.020, 0.029)	0.025	(0.020, 0.029)	0.058	(0.040, 0.079)
λ	78.200	(65.924, 113.871)	79.487	(78.974, 79.995)	79.647	(79.133, 80.169)	76.785	(72.536, 81.354)
β	0.144	(0.045, 0.289)	0.153	(0.141, 0.165)	0.050	(0.046, 0.054)	0.142	(0.114, 0.169)
γ_1	0.068	(0.010, 0.168)	0.049	(0.046, 0.053)	0.050	(0.046, 0.054)		
γ_2	0.358	(0.181, 0.568)	0.349	(0.345, 0.352)	0.349	(0.345, 0.353)		
μ_{a1}	7.576	(6.477, 8.675)	8.237	(8.170, 8.304)	8.237	(8.170, 8.304)		
σ_{a1}^2	0.065	(0.012, 0.269)	0.038	(0.023, 0.062)	0.038	(0.023, 0.062)		
μ_{a2}	0.818	(0.411, 1.224)	1.038	(0.841, 1.245)	1.035	(0.840, 1.237)		
σ_{a2}^2	0.009	(0.002, 0.037)	0.334	(0.174, 0.598)	0.326	(0.171, 0.583)		
μ_τ	-1.906	(-3.467, -0.346)	-2.094	(-2.154, -2.033)	-2.094	(-2.154, -2.033)		
σ_τ^2	0.132	(0.024, 0.543)	0.031	(0.019, 0.050)	0.031	(0.019, 0.050)		

TABLE 2

Estimates of log Bayes factors (IBFs) for various nested model comparisons given the budding index data alone. The model indexed by an entry's column is the larger of the models and is represented in the numerator of the IBFs in that column; the model indexed by an entry's row is the smaller of the two. The last two rows of the table provide the average and standard deviation of the RMSE of the model's fitted values to the observed budding index data over a sample of 1000 draws from the posterior

Submodel	Full model	$\mu_0 = 0$	$\delta = 0$	$\sigma_0^2 = 0$	$\mu_0 = 0$ $\delta = 0$	$\mu_0 = 0$ $\sigma_0^2 = 0$	$\delta = 0$ $\sigma_0^2 = 0$	$\mu_0 = 0$ $\delta = 0$ $\sigma_0^2 = 0$
$\mu_0 = 0$	368.62	0.00						
$\delta = 0$	18.75		0.00					
$\sigma_0^2 = 0$	31.00			0.00				
$\mu_0 = \delta = 0$	364.22	-4.40	345.47		0.00			
$\mu_0 = \sigma_0^2 = 0$	363.78	-4.84		332.78		0.00		
$\delta = \sigma_0^2 = 0$	31.04		12.28	0.04			0.00	
$\mu_0 = \delta = \sigma_0^2 = 0$	359.37	-9.25	340.62	328.37	-4.85	-4.41	328.33	0.00
E(RMSE)	3.70	13.69	4.35	3.99	13.67	13.65	3.92	13.63
SD(RMSE)	0.16	0.10	0.19	0.15	0.10	0.09	0.14	0.09