

MODELS AND APPROXIMATIONS FOR SYNCHRONOUS CELLULAR GROWTH

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1. Introduction

To recall the notion of a synchronous culture, it will suffice to reproduce one of the many graphs in the literature illustrating the results of experiments with such cultures. Figure 1 is from Mitchison and Vincent [6], reprinted with permission of Mitchison, and shows cell numbers in a synchronous culture prepared by a technique of density gradient centrifugation. Alongside this figure we may place an idealization (Figure 2).

In common with many other authors, in evaluating their technique for preparing synchronous cultures, Mitchison and Vincent refer to the "degree of synchrony" it produces. Leaving aside the question of numerical measurement of this degree, "perfect synchrony" would be represented by Figure 2, while "asyn-

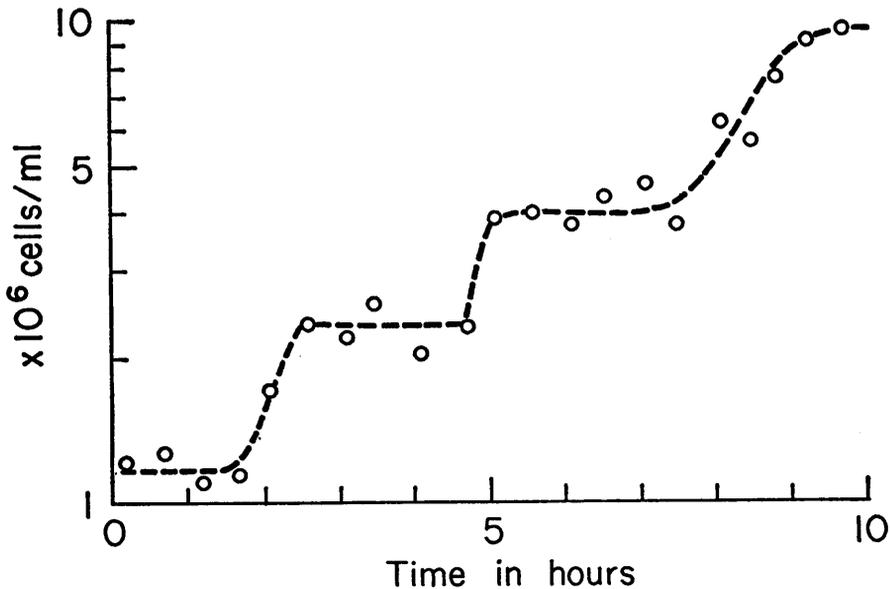


FIGURE 1

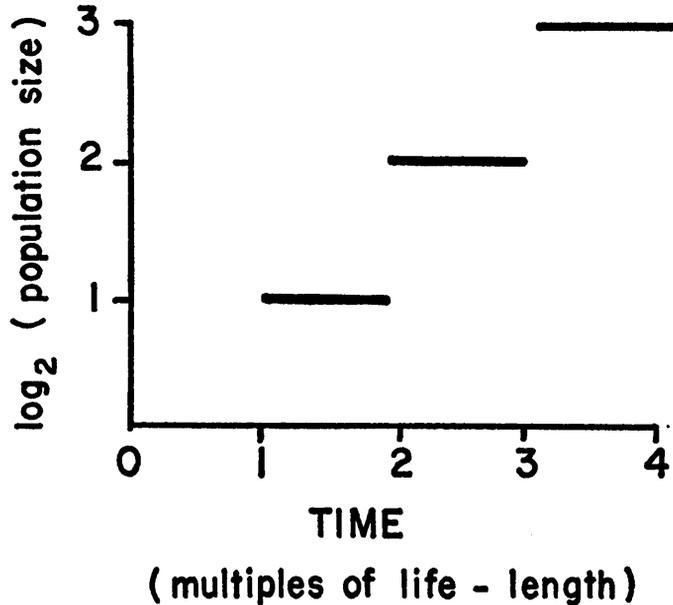


FIGURE 2

chronous growth" is linear growth of the logarithm of the population size. Growth such as is actually observed, as in Figure 1, is called "parasynchronous."

Stochastic effects ensure that experiments always lead to parasynchronous growth. Some of these may be termed "accidental" such as errors of observation and unavoidable fluctuations in conditions. These are not the subject of the present paper. We shall consider at least two stochastic effects as "essential": (i) stochastic fluctuations in the life lengths of individuals, (ii) random values of the ages of the individual or individuals present at the beginning of observation (time $t = 0$) which will lead to different probabilities for fission at later times.

A common situation is that a synchronizing event (for example, environmental shock or selection of newly born cells) is followed by a period during which the population grows parasynchronously according to some growth process.

A mathematical model of a synchronous culture must contain counterparts to these two features of the experiment. The first part of this paper contains a proposed classification of models according to the way they are specified. This classification is analogous to the well-known classification of queuing models according to the input process, service time distribution, and number of servers. It will be applied to models set up by various authors and will also be used to discuss the relationships of simpler models (as approximations) to more realistic but mathematically less tractable ones. Some examples of one type of approximation will be given.

2. Specification of the growth mechanism

Almost any mathematical model that has been proposed for the growth and reproduction of cellular populations could be used for this part of the complete model. General models would allow the reproduction probabilities to depend on the population size and the age of each individual, and would include correlations between mother and daughter or between sisters. More special models allow more complete solutions, and such models as branching and renewal processes are commonly considered. For later reference we shall call these B and R , respectively, and refer to the general model as G . The model may be Markovian M in which case the life lengths are negative exponential. An opposite extreme is provided by the "clock models" C in which the life length distributions are degenerate; they give probability one to a certain fixed life length.

In specifying the growth mechanism, a second classification is also useful. The process may be handled in terms of expectations, as in actuarial practice, perhaps because the population can be considered as large at all times. We shall refer to this as A . An alternative is a stochastic model S in which the population may be small (starting perhaps with 1 at $t = 0$), leading to calculations with distributions of ages and other variables.

3. Specification of the starting conditions

We shall suppose that a procedure to induce synchrony takes place up to or at time $t = 0$. This means that the growth mechanism already specified develops out of an initial state determined by a probability distribution $I(t)$ (the "initial state distribution" of Engelberg and Hirsch [4]). We have

$$(3.1) \quad P\{\text{individual alive at time 0 undergoes fission by time } t\} = I(t).$$

A general distribution G could be arbitrarily adopted. However, $I(t)$ may also be derived from more basic assumptions about the age distribution at $t = 0$ and about life lengths conditional upon ages.

The age distribution may be stable for the conditions prevailing before $t = 0$, or it may consist of such a distribution with some age class deleted or modified, and referred to as D . It is convenient again to consider degenerate distributions F , giving fixed ages at the start. An environmental shock may be modelled E by taking the age distribution resulting from one growth process as initial state for another.

The conditional life length distributions for the starting generation may involve age dependent fission probabilities. In this case they may be the same A as prevail throughout the whole subsequent growth scheme or they may be modified, referred to as A mod, say due to viability being changed by the induction. They may also be degenerate as in the clock model C .

4. Specification of time scale and structure

Time may be treated as discrete Δ or continuous Γ . Most models involve time homogeneous growth mechanisms h , but Engelberg and Hirsch, for example, postulate fission probabilities that fluctuate cyclically with time as a model for forced synchrony. This gives a nonhomogeneous growth model n .

5. Classification of models: examples

Summing up Sections 1 through 4, we shall classify models by means of a sequence of symbols representing: growth mechanism|“actuarial” or “stochastic”|initial state|time structure.

The proposed symbols for use in this sequence may be summarized under the corresponding four headings in Table I.

TABLE I
CLASSIFICATION OF MODELS

Growth mechanism	Nature of process	Initial state	Time structure
Branching B	“Actuarial”	General	G Discrete Δ
Renewal R	(mean	Stable	S Continuous Γ
General G	growth) A	Deletion of age class	D Homogeneous h
“Clock” C	“Stochastic” S	Fixed age at start	F Nonhomogenous n
		Environmental shock	E
		Age dependent life length distributions same for first and later generations	A
		Age dependent life length distributions in first generation modified relative to others	$A \text{ mod}$
		Fixed life length for first generation (“clock”)	C

Some examples follow.

EXAMPLE 5.1. Engelberg and Hirsch [4] study $G|A|G|\Delta, h$ and briefly $G|A|G|\Delta, n$, in an essay on general models of synchrony.

EXAMPLE 5.2. Burnett-Hall and Waugh [2] attempted to quantify the decay of synchrony in branching processes with age dependent birth rates. Synchrony was supposed to arise through the individuals at the start being of zero age, and the population was considered in terms of its expected growth with time, leading to a model $B|A|F, A|\Gamma, h$. Sankoff [7] considers the same problem and model.

EXAMPLE 5.3. Selection synchrony such as sizing methods or filtration will lead to models of the form $-|D|$ - (here a dash indicates an unspecified compo-

ment). If the selection is for a fixed age (in fact for a narrow age class), then $[-|F|]$ will be appropriate. (See, for example, Terasima and Tolmach [8].) This will also be true for blocking techniques by which a culture is brought effectively into a single age class and subsequently released. Labelling experiments lead to a labelled subpopulation which has been effectively synchronized by selection. MacDonald [5] examines the fraction labelled mitoses curve by means of $B|S|D|\Gamma$, h and Bartlett [1] obtains large-time approximations to the moments of the total population and, hence, also approximations for grain counts using $G|G|F|\Gamma$, h (the starting condition being one cell of age zero). Radiation and chemotherapy also produce synchronous populations by selection of the cells which survive treatment.

EXAMPLE 5.4. Shifts and shocks are generally applied to cultures which are in a steady state. Their effect is to take the steady state distribution for the growth mechanism before shock as initial state for a different age dependent growth mechanism which leads to models of the forms G (or B , and so forth) $[-|G, A|]$.

One or two shifts can be treated by taking the state induced, after a period, by one shift, as the initial state for the next.

6. Models with branching growth mechanism: clock approximations

For the remainder of this paper, we shall consider models $B|S|D$ or $E|\Gamma$, h and clock models $C|S|D$ or $E|\Gamma$, h . Note that clock models can still involve stochastic effects in their initial states. Suppose the coefficient of variation of the initial state distribution is large relative to that of the life lengths in the growth mechanism. Then it is clear that stochastic variations at least in the first few generations will predominantly come from the variability of the initial state, and a clock model may be a good approximation to a stochastic branching model. The question of the "truth" of a clock model is sometimes discussed in the biological literature, but it seems desirable to replace such discussion by considerations of the goodness of the approximation.

7. Synchronization by selection: a model $B|S|D|\Gamma$, h

As indicated by the first symbol, we are considering a model in which the reproductive mechanism is a continuous time age dependent branching process. Specifically, we shall suppose this process develops by binary fission, and the life length distribution is $G(\cdot)$. For the starting conditions, let us suppose that the population was growing, before $t = 0$, according to the same branching process for long enough to establish the corresponding stable age distribution $A(\cdot)$. Selection means that there is a probability $q(u)$ that an individual of age u is retained in the new starting population. Thus, we can consider the population after time zero as commencing with a single individual whose age X has a distribution $A_0(\cdot)$ given by

$$(7.1) \quad P(X \leq x) = A_0(x) \\ = \frac{\int_0^x q(u)A\{du\}}{\int_0^\infty q(u)A\{du\}}.$$

The residual life length R of the initial individual will depend on its age X . We shall consider all conditions as being unchanged by the selection so that the conditional distribution $H(\cdot|\cdot)$ of R will be

$$(7.2) \quad P(R \leq r|X = x) = H(r|x) \\ = \frac{G(r+x) - G(x)}{1 - G(x)}.$$

If $G(x) = 1$, we shall put $P(R = 0|X = x) = 1$. Thus specified, the growth mechanism is a branching process with random start such as has been treated by Weiner [9]. The process is considered in terms of the family tree of a single individual at time $t = 0$, whence it is categorized \mathcal{S} . Equation (7.1) provides the "deletion" D and we have already referred to the continuous time and fixed conditions of growth, giving homogeneity.

8. Synchronization by selection: a clock model $C|S|D|\Gamma, h$

The reproductive mechanism will again be a continuous time binary fission branching process, but we suppose that the distribution $G(\cdot)$ is degenerate, giving probability one to the fixed life length ℓ . This can never lead to a stable age distribution, but considering the limit of a branching process with mean life length ℓ and variance of life length tending to zero, we obtain the density

$$(8.1) \quad \frac{d}{dt}A(t) = \begin{cases} (2\ell^{-1} \log 2)2^{-t/\ell} & \text{for } 0 \leq t \leq \ell, \\ 0 & \text{otherwise,} \end{cases}$$

which is usually adopted in this situation (see, for example, Campbell [3]). The age distribution $A_0(\cdot)$ after selection will be obtained from $A(\cdot)$ just as in (7.2). Modelling unchanged conditions as in Section 7, the residual life length will simply be

$$(8.2) \quad R = \begin{cases} \ell - x & \text{for } 0 \leq x \leq \ell, \\ 0 & \text{otherwise.} \end{cases}$$

The rest of the categorization of this model is the same as that in Section 7. This model can be regarded as an approximation to the former (which might be considered more realistic) as mentioned in Section 6.

9. First and subsequent fissions: synchrony in the first wave of reproduction

Let us refer to the model of Section 7 as $B/-$ and that of section 8 as $C/-$ for brevity. In both models the first fission will occur at time R . Suppose the (unconditional) distribution of R is $P(R \leq r) = F(r)$. In a parasynchronous

population starting with a number of individuals selected as in (7.1), there will be a tendency for the first wave of reproduction to occur about the time $t = E(R)$. This wave will be more concentrated (show a higher degree of synchrony) for smaller values of the standard deviation $SD(R)$. If, in the model $B/-$, the coefficient of variation of the life length distribution $G(\cdot)$ is small, we can expect a negligible contribution to the first wave of reproduction from fissions in the second and later generations. In the model $C/-$, the first wave of reproduction must occur before time $t = \ell$ and so this contribution will be zero. In $B/-$, later waves of reproduction will become blurred through the effect of generation overlap (decay of synchrony), which will not occur in $C/-$. Since in the presence of decay of synchrony, the first wave is the most coherent, its timing and effect on the transient age distribution, and so forth, are of interest if the purpose is to work with a culture in the closest available state of synchrony.

Explicit solutions for the transient or short term behavior of $B/-$ are not readily available for general life length distributions $G(\cdot)$. In this section, we shall consider the waves of reproduction in $C/-$, and in the following sections, we will examine the corresponding time dependent fluctuations in the age distribution.

Let the population size be $Z(t)$, with $Z(0) = 1$. Write $m(t) = E\{Z(t)\}$. Since the j th doubling occurs at $R + (j - 1)\ell$ for $j = 0, 1, \dots$, we get

$$(9.1) \quad P\{Z(t) = 2^j\} = F(t - j\ell + \ell) - F(t - j\ell).$$

Hence,

$$(9.2) \quad m(t) = 1 + \sum_{j=0}^{\infty} 2^j F(t - j\ell),$$

where, for fixed t , only finitely many terms are nonzero. Since $R = \ell - X$, we have $F(r) = 1 - A_0(\ell - r)$ so long as the life length distributions are continuous, whence

$$(9.3) \quad m(t) = 1 + \sum_{j=0}^{\infty} 2^j [1 - A_0\{(j + 1)\ell - t\}].$$

The first wave of fission occurs before $t = \ell$, and thus for mean growth $m^*(t)$ over the first wave, we get

$$(9.4) \quad m^*(t) = 2 - A_0(\ell - t),$$

where $0 \leq t \leq \ell$.

10. First fission: time varying age distribution during the first wave of reproduction

Still considering the model $C/-$, let the age of a randomly chosen member of the population, at time t , be X_t , with distribution function $A_t(\cdot)$. We can determine $A_t(\cdot)$ by considering the population commencing with a single individual. For the age of this individual at $t = 0$, we have $X_0 = X$ as in preceding sections, and its distribution function is $A_0(\cdot)$. There are two cases:

- (i) the first fission occurs after time t , that is, $R > t$; in such case $X_t = X + t$;

(ii) the first fission occurs before time t , and the second occurs after t , that is, $R \leq t$ and $R + \ell > t$; in such case $X = t - R$.

Thus, at least for $0 \leq t \leq \ell$, we have

$$(10.1) \quad A_t(x) = P\{R > t, X + t \leq x\} + P\{R \leq t, t - R \leq x\} \\ = P\{X < \min(\ell - t, x - t)\} + P\{\ell - t \leq X \leq \ell - t + x\}.$$

In (10.1) if $x < t$, we have $P\{X < \min(\ell - t, x - t)\} = 0$ so that

$$(10.2) \quad A_t(x) = A_0(\ell - t + x) - A_0(\ell - t).$$

If $x > t$, we have certainly $X \leq \ell < \ell + x - t$ so

$$(10.3) \quad A_t(x) = P\{X < x - t\} + P\{\ell - t \leq X\}.$$

Supposing that all distributions are continuous, this gives

$$(10.4) \quad A_t(x) = A_0(x - t) + 1 - A_0(\ell - t).$$

11. Synchronization by deletion of an early age group

A common feature of experiments involving partial destruction of a cellular population is that a brief incident such as irradiation kills some of the cells, frequently the younger ones. We shall consider in model $C/-$ synchrony by complete deletion of the age group $(0, k)$. In (7.1), we can take

$$(11.1) \quad q(u) = \begin{cases} 0 & \text{for } 0 \leq u < k, \\ 1 & \text{for } k \leq u < \ell. \end{cases}$$

Then

$$(11.2) \quad A_0(x) = \begin{cases} 0 & \text{for } 0 \leq x < k, \\ \frac{A(x) - A(k)}{1 - A(k)} & \text{for } k \leq x < \ell. \end{cases}$$

Referring to (8.1), this gives

$$(11.3) \quad A_0(x) = \begin{cases} 0 & \text{for } 0 \leq x < k, \\ \frac{2^{-k/\ell} - 2^{-x/\ell}}{2^{-k/\ell} - 1} & \text{otherwise,} \end{cases}$$

a negative exponential truncated on the left at k and on the right at ℓ . Hence, from (9.4), mean growth over the first wave of reproduction is given by

$$(11.4) \quad m^*(t) = \begin{cases} \frac{2 - 2^{-k/\ell} - 2^{-(t-t)/\ell}}{1 - 2^{-k/\ell}} & \text{for } 0 \leq t \leq \ell - k, \\ 2 & \text{for } t > \ell - k. \end{cases}$$

The time dependent behavior of the age density is given by

$$(11.5) \quad A_t(x) = \begin{cases} \frac{2^{-(t-t)/\ell} \log 2}{\ell(1 - 2^{-k/\ell})} 2^{-x/\ell} & \text{for } 0 \leq x \leq t, \\ \frac{2^{-1} \log 2}{\ell(1 - 2^{-k/\ell})} 2^{-x/\ell} & \text{for } t \leq x \leq \ell. \end{cases}$$

Finally, we will apply these results to determine an optimum policy if the destruction of cells in the young age group is the desired purpose, as in cancer chemotherapy. We can suppose that k is small relative to ℓ . Consideration of (10.1), (10.2), and (10.4) shows that the development of $A_i(x)$ with time is simply an interchange of age groups. For $x < t$ and time zero, consider the individuals in the age group $(\ell - t, \ell - t + x)$. At time t they have moved into (or, equivalently, been replaced by their offspring in) the age group $(0, x)$. The same applies to $x > t$. Thus, (10.2) and (10.4) can be treated as periodic in t of period ℓ , though the approximation is of course satisfactory only for fairly small t . With the truncated distribution (11.2), we see that at time $\ell - k$ the age distribution is just the original $A_0(x)$ translated by $\ell - k$. Furthermore, since the density $A_0(x)$ is monotone decreasing where it is nonzero, the magnitude of the age group $(0, k)$ reaches a local maximum at time $\ell - k$, and this is the optimum epoch to repeat the destructive treatment for maximum effect. The results of two successive "attacks" of this kind appear in Table II.

TABLE II

DELETION OF AN EARLY AGE GROUP
 p_i = proportion killed at i th step for $i = 1, 2$.
 q_i = proportion of total population killed up to and including i th step.

k	Step no.	p_i	q_i
	i		
0.05	1	0.07	0.07
	2	0.07	0.13
0.10	1	0.13	0.13
	2	0.14	0.26
0.15	1	0.20	0.20
	2	0.22	0.38

The increased effectiveness if the age group destroyed is broader is clear but such deletion would, in any case, require numerous repetitions to eliminate the population, especially as the blurring of the age groups corresponding to the decay of synchrony would act to make elimination less effective.

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