

## Duration of detectible synchrony in a binary branching process

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### SUMMARY

An index for synchrony is defined, based on the age distribution of a population, which can be evaluated at any time, not only once per cell cycle, and which measures the characteristic of the population most relevant for the biological experimenter. The index is calculated for initially synchronized populations of cells having typical generation time distributions. From this we construct a table showing the useful duration, in number of cell cycles, of an experiment using such a population, as a function of the accuracy of the experimental measurement and the coefficient of variation of the generation time distribution.

### 1. INTRODUCTION

Cell populations where each cell splits in two before age  $\theta$  with probability  $F(\theta)$ , independent of other cells, tend to take on a stationary age distribution with density

$$a(\theta) = 2\rho e^{-\rho\theta}\{1 - F(\theta)\} \quad (\theta \geq 0), \quad (1)$$

where  $\rho$  depends only on  $F(\cdot)$ ; see Harris (1959; 1963, Chapter VI). Microbiologists have developed a number of techniques (Zeuthen, 1964) for synchronizing such populations. These techniques are designed to produce a large population where, for a short time at least, all the cells have approximately the same age; i.e. letting  $a(t, \cdot)$  be the density of the expected age distribution at time  $t$ ,

$$a(t, \theta) \sim \delta(t - [t], \theta), \quad (2)$$

where the time unit is chosen so that the mean life length  $\int \theta dF = 1$  and the number of cycles completed up to time  $t$  is  $[t]$ , the largest integer in  $t$ . By studying the biochemical, radiobiological or other behaviour of a synchronized population at time  $t$ , one is, in effect, studying the expected behaviour, many times amplified, of a single cell of age  $\theta = t - [t]$ . One of the difficulties in such studies is that if a population is initially synchronized, i.e. satisfies (2) near time  $t = 0$ , then the distribution of generation times  $F(\cdot)$  ensures a continual deterioration or decay in synchrony until finally the limiting situation represented by (1) is approached.

### 2. INDICES OF SYNCHRONY

Since the dividing of a cell, the birth of its daughter cells, is generally the only visible indication of the age of a cell, being zero for the daughter cells, biologists have proposed a number of synchrony indices, all related to the birth rate, for measuring the degree of synchrony; see Burnett-Hall & Waugh (1967). These indices, while distinguishing between synchronous growth, characterized by (2) and steady state or exponential growth, characterized by (1), and measuring more or less adequately the relative degree of synchrony in

populations, all have a common drawback. They offer no absolute criteria to the experimental scientist for deciding when a population is sufficiently synchronous to warrant the type of biochemical, radiobiological, etc., experiment mentioned previously, and when it is not.

To motivate the introduction of a new index which does offer such a criterion, we note that what matters to the scientist is the relative proportion of the population he can expect near age  $\theta = t - [t]$  at time  $t$  in excess of the proportion specified in (1). The expected value of his measurement, which is designed to detect something peculiar to cells of this age, will in general depend linearly on  $a(t, t - [t])$ ; and the measured difference between the population and an exponentially growing population will be proportional to

$$a(t, t - [t]) - a(t - [t]),$$

or to the standardized quantity

$$S(t) = \frac{a(t, t - [t]) - a(t - [t])}{a(t - [t])}. \quad (3)$$

Accordingly, if  $S(t)$  is very small, a very accurate measurement, with small variance, will be needed to distinguish between the two populations with any degree of confidence. If and only if  $S(t)$  is large, cruder techniques will suffice. We shall consider  $S(t)$  as the index of synchrony relevant to this type of experiment.

In §3 we derive  $S(t)$  for initially synchronized populations having Pearson Type III generation time distributions. In §4 we tabulate  $n_{k, \alpha}$ , the number of cycles during which  $S(t)$  remains entirely above  $\alpha$  for initially synchronized populations where the coefficient of variation of  $F(\cdot)$  equals  $k^{-\frac{1}{2}}$ . This is the duration of detectible synchrony relative to  $\alpha$ , and we may write

$$n_{k, \alpha} = \max \{ [t] \mid S(\tau) \geq \alpha \quad (0 \leq \tau < t) \}. \quad (4)$$

This tabulation indicates when an experiment on an initially synchronized population should be terminated.

### 3. PEARSON TYPE III GENERATION TIMES

Brown (1940), Kendall (1948), Harris (1959) and Burnett-Hall & Waugh (1967) have studied the kinetics of population growth where the generation time distributions are of the form

$$F(\theta) = \begin{cases} \frac{k^k}{(k-1)!} \int_0^\theta t^{k-1} e^{-kt} dt & (\theta \geq 0), \\ 0 & (\theta < 0), \end{cases} \quad (5)$$

for  $k$  a positive integer. Burnett-Hall & Waugh (1967) have suggested that the fact a generation time distribution represents a moderate departure from the form in (5) has little effect on certain other variables in the population growth process, notably the age distributions. Since our calculations involve only age distributions, we may expect that our results will at least constitute guide-lines for considering populations in a class larger than (5).

As is customary in the application of renewal theory to large populations (see, for example, Burnett-Hall & Waugh, 1967), we assume that the difference between the random functions population size, age distribution and birth rate, and their respective expected values, is

relatively insignificant. This assumption has been implicit in our definitions involving densities of age distributions, for example, (2) and (3). The assumption is at least partially justified by the fact that the population sizes concerned are usually very large even initially. Thus the random variables mentioned above are in effect the sums of large numbers of identical independent random variables, and will have very small coefficients of variation. Furthermore, the coefficients of variation of such variables are not liable to diverge; for example, the coefficient of variation of population size approaches a constant as time increases; see Harris (1959).

Now, with Harris (1963, p. 152), we observe that an object is of age  $x$  at time  $t$  ( $0 \leq x \leq t$ ) if and only if it was born at time  $t - x$  and has not itself divided by time  $t$ . Then, letting  $N(t)$  be the expected population size at time  $t$ , and  $B(t)$  the expected birth rate  $= 2(dN/dt)$ , we find that the proportion of the population near age  $\theta = t - [t]$  is the number born near time  $[t]$  less those that have split again since then, all divided by  $N(t)$ , or

$$a(t, t - [t]) = \frac{B([t]) \{1 - F(t - [t])\}}{N(t)}.$$

To write this formula it is necessary to interchange the operations of forming a quotient and taking expectations. This is only valid, of course, where our assumption about the relevant random variables and their expectations is justified. Then, from (3),

$$S(t) + 1 = \frac{B([t]) \{1 - F(t - [t])\}}{N(t) a(t - [t])}. \tag{6}$$

As may be readily verified (see references at the beginning of this section) for this type of initially synchronized population,

$$\begin{aligned} B(t) &= N(0) ck e^{-kt} \sum_{n=1}^{\infty} \frac{(ckt)^{nk-1}}{(nk-1)!} - N(0) \delta(0) \\ &= N(0) c e^{-kt} \sum_{j=0}^{k-1} \omega^j e^{c\omega^j kt} + N(0) \delta(0), \end{aligned} \tag{7}$$

where  $\omega$  is the primitive  $k$ th root of unity and  $c = 2^{1/k}$ ,

$$\begin{aligned} N(t) &= N(0) \sum_{j=0}^{k-1} c^{-j} e^{-kt} \sum_{n=0}^{\infty} \frac{(ckt)^{nk+j}}{(nk+j)!} \\ &= N(0) \frac{c e^{-kt}}{2k} \sum_{j=0}^{k-1} \frac{\omega^j}{c\omega^j - 1} e^{c\omega^j kt}, \end{aligned} \tag{8}$$

$$a(\theta) = 2k(c-1) e^{-k(c-1)\theta} \{1 - F(\theta)\}. \tag{9}$$

Substituting (7), (8) and (9) in (6), we obtain, for  $t \geq 1$ ,

$$S(t) + 1 = \frac{\sum_{j=0}^{k-1} \frac{\omega^j}{c-1} e^{c(\omega^j-1)kt}}{\sum_{j=0}^{k-1} \frac{\omega^j}{c\omega^j-1} e^{c(\omega^j-1)kt}}. \tag{10}$$

The function  $S(t)$  converges to zero as the age distribution of the population converges to the limiting distribution, but it is neither continuous nor a monotonically decreasing function.

## 4. ACCURACY OF MEASUREMENT AND DURATION OF SYNCHRONY

By knowing the accuracy of his measurement, the variance for example, the experimentalist knows the minimum value of  $\alpha$  allowable for  $S(t)$  such that he can still expect significant differences, sufficiently often, between his population and one satisfying (1). In Table 1 we show the results of computing  $S(t)$  according to (10), and using (4) to obtain  $n_{k,\alpha}$ . This is the number of cycles the scientist may reasonably extend his experiment. The columns of the table correspond to different values of the parameter  $k$  in (5). If the table is being used as a guide-line for a population not having this type of generation time distribution, the appropriate column is the one such that  $k$  is closest to {coefficient of variation of  $F(\cdot)$ }<sup>-2</sup>.

Table 1. Duration of synchrony  $n_{k,\alpha}$  in number of cycles

$\alpha \backslash k$	4	9	16	25	36	49	64	81	100
1	1	1	1	1	2	2	2	3	4
0.5	1	1	1	2	3	3	4	5	6
0.2	1	1	2	3	4	5	7	9	11
0.1	1	2	3	4	5	7	9	11	14
0.05	1	2	3	5	6	8	11	14	17
0.02	1	2	4	6	8	10	13	17	21
0.01	2	3	4	6	9	12	15	19	23
0.001	2	4	6	9	12	15	20	25	31

$\alpha$  = minimum value of  $S(t)$  tolerable.

$k^{-1}$  = coefficient of variation of generation time distribution.

In some cases, because of the nonmonotonicity of  $S(\cdot)$ , in the  $(n_{k,\alpha} + 1)$ th cycle there may be some values of  $t$  for which  $S(t) \geq \alpha$ . However, we have not tabulated these, nor have we considered values of  $k$  outside the range of biological interest. In any case, formula (10) is not difficult to compute.

## 5. REMARK

The quantity

$$R(t) = \frac{1}{N} \frac{dN}{dt}$$

is related to several of the indices of synchrony cited by Burnett-Hall & Waugh (1967). The function  $R(\cdot)$ , evaluated at successive integer values of  $t$ , gives an intermittent indication of the approach of a population to the exponential growth pattern. From (1) and (6), we may write

$$S(t) = \frac{R([t])}{\rho} \frac{N([t]) e^{-\rho[t]}}{N(t) e^{-\rho t}} - 1,$$

and hence the index  $S(\cdot)$  gives the same information as  $R(\cdot)$  at integer values of  $t$ . The function  $S(\cdot)$ , however, measures the degree of synchrony at any point in the cell cycle whereas  $R(\cdot)$  is relevant only to the division event.

REFERENCES

- BROWN, A. W. (1940). A note on the use of a Pearson Type III function in renewal theory. *Ann. Math. Statist.* **11**, 448–53.
- BURNETT-HALL, D. G. & WAUGH, W. A. O'N. (1967). Indices of synchrony in cellular cultures. *Biometrics* **23**, 693–716.
- HARRIS, T. E. (1959). A mathematical model for multiplication by binary fission. In *The Kinetics of Cellular Proliferation*, ed. F. Stohlman, Jr., New York: Springer, 368–81.
- HARRIS, T. E. (1963). *The Theory of Branching Processes*. Berlin.
- KENDALL, D. G. (1948). On the role of a variable generation time in the development of a stochastic birth process. *Biometrika* **35**, 316–30.
- ZEUTHEN, E. (Ed.) (1964). *Synchrony in Cell Division and Growth*. New York: Interscience.

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