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The Size and Scar Distributions of the Yeast

Saccharomyces cerevisiae

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A model for the growth of populations of *Saccharomyces cerevisiae* is formulated and analysed. The probability of bud emergence is assumed to depend on the size of the cell. Under certain conditions on birth size the model can be reduced to a single renewal equation. Using Laplace transform techniques and renewal theory we establish the existence of a stable scar and size distribution under certain conditions on the growth rate of individual cells. The steady state values for the relative frequencies of unbudded and budded cells in the various scar classes are given.

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1. INTRODUCTION

The yeast *Saccharomyces cerevisiae* is a single cell organism, which reproduces by budding. Early in the cell cycle the future daughter cell appears as a bud on the surface of the mother cell. The bud grows in size throughout the rest of the cell cycle and eventually at the conclusion of the cycle it separates from the mother cell. After each cell separation a bud scar is left on the wall of the mother cell. The cells of the yeast population are thus naturally divided into different so-called scar classes according to the number of scars on the cell wall or, equivalently, the number of divisions the cell has undergone. We say that a cell with j daughters and hence with j bud scars belongs to the j :th scar class. By counting the scars using electronmicroscopy one can determine to which class a particular cell belongs. A new bud can never be formed at the site of an old scar. As a consequence every cell can produce only a finite number of daughters, simply because when the surface of the cell is completely covered by scars no new bud can emerge, Bartholomew and Mittwer (1953) (cited from Beran (1968)) estimated the theoretical maximum number of bud scars to 100. Cells with over 40 scars have been observed (Mortimer and Johnston (1959)). For a description of the cell cycle of *S. cerevisiae* and its various phases we refer to Hartwell (1974).

Cells belonging to different scar classes are not equal. Both the unbudded and budded phase is longer for virgin cells (i.e., cells which have not yet divided) than for cells which have divided at least once. Mothers of the same size but belonging to different scar classes get daughters of different size (cf. Lord and Wheals (1981)). The size of newborn daughters of mothers belonging to the same class is widely distributed as is the cell size at bud emergence (Lord and Wheals (1981)). Regarding these facts it is clear that a reliable mathematical model for the dynamics of populations of budding yeast cannot be very simple. Although the Malthusian law of exponential growth holds during steady state, it is not adequate to describe the transient behaviour of yeast populations. Any realistic model

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must take the individual differences of the cells into account.

The simplest, structured population model for the growth of *S. cerevisiae* is a model where the different scar classes are considered as distinct but interacting subpopulations with different death and division rates. A model of this kind has been presented and investigated by Gani and Saunders (1977). That model was governed by a linear difference equation on \mathbb{R}^n . The model predicted that the relative frequencies of the scar classes (i.e., the number of cells in a particular scar class divided by the total number of cells in the population) approach constant values and that the convergence towards steady state is monotone. Gyllenberg (1985) pointed out that the continuous time analogue of the Gani and Saunders model does not show oscillations either. However, experiments show that the relative frequencies of the scar classes of populations of *S. cerevisiae* in batch cultivation with substrate excess perform damped oscillations until equilibrium is reached (Beran, Streiblova, Leiblova (1969)).

The next step towards a realistic growth model of budding yeast is to distinguish cells within the same scar class from each other by means of a new variable and in this way take the differences between cells in the same scar class into account. The time evolution of the new variable should in some sense describe the progress of an individual cell through the cell cycle.

From a mathematical point of view the easiest way to model progression through the cell cycle is to introduce age as a new variable because there exists a well developed theory of age structured population dynamics. In the case of populations of *S. cerevisiae* the age of a cell belonging to the j :th scar class is usually defined as the time elapsed since the cell entered the j :th class. Models with continuous age have been investigated by Hamada, Kanno and Kano (1982), Hamada and Nakamura (1982), Tuljapurkar (1983) and Gyllenberg (1985). They all showed that the age dependent model predicted the observed damped oscillations. Adams, Rothman and Beran (1981) divided the cell cycle into a finite number of subintervals, each of which represented a certain stage in the cycle. They showed that this discrete age model predicted damped oscillations at least for some special choices of initial age distributions.

The biological origin of the oscillatory behaviour of the relative frequencies of the scar classes lies in the fact that the newborn daughter cells are much smaller than the mother cells that produced them and that a cell must have a certain critical size before it can possibly become budded. (Johnston, Pringle and Hartwell (1977)). Cells which have divided at least once have already reached the critical size whereas virgin cells have to grow before bud emergence can take place. This implies that virgin cells have longer cycle time than cells with bud scars. There is also experimental evidence of differences between cycle times of higher scar classes. (Hartwell and Unger (1977)).

In the approach using age structure the difference in cycle time is modelled by choosing different age specific fission rates for the various scar classes. If the fission rates are taken to be the same in all classes, then the age model does not predict oscillations (Gyllenberg (1985)). Remembering that the age-independent model predicts monotone approach to equilibrium one could therefore say that the oscillations are due to the differences in cycle time between scar classes and the age dependent behaviour within the class.

Although the age dependent model gives a qualitatively rather correct description of the transient state of yeast populations it can be criticized for several reasons. Firstly, it models a *consequence* of the size dependent behaviour of the population and not the size dynamics itself. Secondly, the age of a cell is hard, if not impossible, to measure whereas size is easy to observe. Moreover, Johnston et al. (1977) found that growth, rather than progress through the DNA division cycle, is rate limiting for cell proliferation and Lord and Wheels (1981) categorically stated that any model without a size related component is incompatible with data.

The purpose of this paper is to construct and analyse a size structured model for the growth and scar class distribution of *S. cerevisiae* during batch cultivation with an excess of substrate. A size dependent growth model has previously been presented and investigated by Hjortso and Bailey (1982, 1983). They assumed that at constant environmental conditions all daughter cells are born with the same size and that all cells traverse "start" (an event occurring just before bud emergence) exactly

when they reach the critical size. These assumptions are not in accordance with the observations of Lord and Wheals (1981).

The organization of the paper is as follows. In Section 2 we derive the basic model which is formulated as a possibly infinite system of first order hyperbolic PDE:s with time delays and transformed size-arguments. In Section 3 we show that under a certain condition on the range of birth sizes this system can be reduced to a single renewal equation. Section 4 is devoted to the investigation of the location of roots of the characteristic equation and the determination of the large time behaviour of solutions. In Section 5 we study the steady state in some detail and finally in Section 6 we indicate possible generalizations of the model and mention alternative techniques which could be used when a reduction to an integral equation cannot be performed.

2. DERIVATION OF THE MODEL

Let t and x denote time and size, respectively. By size we understand any quantity, such as volume mass, DNA-content, etc., which is conserved at cell separation, that is, the sum of the sizes of the mother and daughter cell immediately after cell separation equals the size of the budded mother cell immediately before cell separation. The state of an unbudded cell is assumed to be characterized by its size and number of bud scars. We therefore introduce the size-density $n_j^0(t, x)$ of unbudded cells in the j :th scar class, that is, the integral $\int_{x_1}^{x_2} n_j^0(t, x) dx$ is the number of unbudded cells with j bud scars and size between x_1 and x_2 . We allow j to attain integral values from 0 to k , where $k \leq \infty$. The (more realistic) case $k < \infty$ corresponds to the assumption that there is a maximum number of divisions a cell can possibly undergo, whereas no such restriction is imposed if $k = \infty$.

We let $b(x)$ denote the per capita rate at which cells of size x become budded. We assume that this rate depends on size alone and not on the number of scars. As mentioned in the introduction there exists a critical size, denoted by x_0 , which must be reached by a cell before it can enter the budded phase. We therefore require $b(x)$ to equal zero for x less than x_0 . We also assume the existence of a maximal size, normalized to 1, of unbudded cells. This means that cells must become budded before reaching size 1. The mathematical condition corresponding to this assumption says that $b(x)$ must approach infinity as x tends to 1 in a way to be specified in hypothesis (H_b) below.

We let $g(x)$ denote the individual growth rate of unbudded cells. Although one might expect g to depend on j , we shall assume that the individual growth rate is the same for all scar classes. We make this assumption only for the sake of notational convenience. All calculations could be carried out in the case of scar class dependent growth rate and they would lead to results analogous to those of the present paper. But the price one would have to pay for the gain in generality would be too high: Awfully more complicated formulas and expressions with less obvious biological interpretations.

We assume that all cells with the same number of scars spend the same time in the budded phase. This is suggested by the results of Lord and Wheals (1981, p. 366) which showed a very small (≈ 0.1) coefficient of variation (standard deviation divided by mean) compared with the corresponding value for the unbudded phase (≈ 0.5). The constant duration of the budded phase in the j :th scar class is denoted by r_j . There is experimental evidence that $r_0 \geq r_j$, $j = 1, 2, \dots$ (Lord and Wheals (1918)).

The state of a budded cell is not - as opposed to unbudded cells - fully characterized by its size and number of scars. This is a consequence of the fact that cells enter the budded phase with different sizes. We therefore introduce yet another variable, a , which denotes the age of the bud - a quantity defined as the time elapsed since bud emergence. We assume that all new biomass produced during the budded phase goes to the bud (cf. Hartwell and Unger (1977)). Consequently the main part of the cell (i.e., everything except the bud) has the same size throughout the budded phase (and also immediately after bud separation) as it had at the time it became budded. We let $n_j^1(t, a, x)$ denote the density of budded cells in the j :th scar class having bud age a and mother size x . The growth of the bud is assumed to depend on the age and size of the bud and size of the mother and is governed by the initial value problem

$$\frac{d}{da} m_j(a, x) = g_j^1(a, x, m_j(a, x)) \quad (2.1)$$

$$m_j(0, x) = 0, \quad (2.2)$$

where $m_j(a, x)$ is the size of a bud of age a , whose mother has j bud scars and size x and g_j^1 is a function, which is assumed to be known. We do not impose any particular conditions on g_j^1 ; we simply assume that the problem (2.1), (2.2) is well posed so that we can define

$$y_j(x) = m_j(r_j, x) \quad (2.3)$$

and formulate our hypotheses in terms of y_j . $y_j(x)$ is the size of the bud at time of bud separation and hence the size of the newborn daughter cell whose mother had j bud scars and size x at time of bud emergence.

Since, as explained above, a cell has size between x_0 and 1 when it enters the budded phase, $y_j(x)$ is defined for $x \in [x_0, 1]$. We shall assume that y_j is strictly increasing with $y_j(x_0) > 0$ and $y_j(1) < 1$. $y_j^{-1}(x)$ is then defined on $[y_j(x_0), y_j(1)]$ and represents, of course, the size of a mother whose daughter has size x at cell separation.

We are now ready to write down the balance equations for the different scar classes and phases. We refer to the book of Metz and Diekmann (to appear) for a detailed description of how to derive balance equations of population dynamics in general. We have

$$\frac{\partial}{\partial t} n_0^0(t, x) + \frac{\partial}{\partial x} (g(x) n_0^0(t, x)) = \quad (2.4)$$

$$-b(x) n_0^0(t, x) + \sum_{j=0}^k \frac{1}{y_j'(y_j^{-1}(x))} n_j^1(t, r_j, y_j^{-1}(x)),$$

$$g(\alpha) n_0^0(t, \alpha) = 0,$$

$$\frac{\partial}{\partial t} n_j^0(t, x) + \frac{\partial}{\partial x} (g(x) n_j^0(t, x)) = -b(x) n_j^0(t, x) + n_{j-1}^1(t, r_j, x), \quad (2.5)$$

$$j = 1, 2, \dots, k,$$

$$g(x_0) n_j^0(t, x_0) = 0, \quad j = 1, 2, \dots, k,$$

$$\frac{\partial}{\partial t} n_j^1(t, a, x) + \frac{\partial}{\partial a} n_j^1(t, a, x) = 0, \quad j = 0, 1, \dots, k, \quad (2.6)$$

$$n_j^1(t, 0, x) = b(x) n_j^0(t, x), \quad j = 0, 1, \dots, k. \quad (2.7)$$

In equation (2.4) the domain of x ranges from $\alpha := \inf_{0 \leq j \leq k} y_j(x_0)$ to 1 and in (2.5) from x_0 to 1. In equation (2.6) x is just a dummy variable which has a fixed value in $[x_0, 1)$. The domain of a is $[0, r_j]$.

Consider the group of unbudded cells (in some particular scar class) of size x at time t . The left hand side of (2.5) (or (2.4)) is the rate at which the population of this group is changing in time. The first term of the right hand side is a sink term - it describes the loss due to transition to the budded phase. The source terms describe what happens at bud separation: The mother cell becomes an unbudded cell with the same size in the next scar class (eq. (2.5)) whereas the bud becomes a completely new cell with no scars (eq. (2.4)). The j :th term in the sum in eq. (2.4) should be interpreted as zero for $x \notin [y_j(x_0), y_j(1)]$. The boundary conditions supplementing (2.4) and (2.5) express, respectively, the facts that there is no flux of virgin cells through size α and no cells with at least one scar pass size x_0 . Equation (2.6) just reflects the fact that the age of the bud increases equally with time. The boundary condition (2.7) is the balance equation for the event of bud emergence.

In order to get a well posed problem we need the following initial conditions.

$$n_j^0(0, x) = v_j^0(x), \quad \begin{array}{ll} \alpha < x < 1 & \text{if } j = 0 \\ x_0 < x < 1 & \text{if } j = 1, \dots, k. \end{array} \quad (2.8)$$

$$n_j^1(0, a, x) = v_j^1(a, x), \quad 0 \leq a \leq r_j, \quad x_0 < x < 1, \quad j = 0, 1, \dots, k. \quad (2.9)$$

About the given functions we make the following hypotheses

$$(H_y) \quad y_j \in C^1[x_0, 1], \quad y_j' > 0, \quad y_j(1) \leq x_0 \\ \text{for all } j = 0, 1, \dots, k, \quad \alpha := \inf_{0 \leq j \leq k} y_j(x_0) \geq 0$$

$$(H_g) \quad g \in C[\alpha, 1], \quad g(x) > 0 \quad \text{for all } x \in [\alpha, 1]$$

$$(H_b) \quad b \in C[\alpha, 1], \quad b(x) = 0 \quad \text{for } x \in [\alpha, x_0], \\ b(x) > 0 \quad \text{for } x \in (x_0, 1),$$

$$\lim_{x \uparrow 1} \int_{x_0}^x b(s) ds = \infty$$

$$(H_r) \quad \inf_{0 \leq j \leq k} r_j > 0.$$

$$(H_v) \quad v_0^0 \in C[\alpha, 1], \quad v_j^0 \in C[x_0, 1], \quad j = 1, \dots, k,$$

$$v_j^0(x) \exp\left(\int_{x_0}^x \frac{b(s)}{g(s)} ds\right) \text{ is bounded on } [x_0, 1]$$

and

$$\sum_{j=0}^k \sup_{x \in [x_0, 1]} v_j^0(x) \exp\left(\int_{x_0}^x \frac{b(s)}{g(s)} ds\right) < \infty.$$

$$v_j^1 \in C([0, r_j] \times [x_0, 1]), \quad j = 0, 1, \dots, k$$

and

$$\sum_{j=0}^k \sup_{a \in [0, r_j]} \int_{x_0}^1 v_j^1(a, x) dx < \infty.$$

The condition (H_v) imposed on the initial distributions not only guarantee that the total number of cells at time zero,

$$\int_{\alpha}^{x_0} v_0^0(x) dx + \sum_{j=1}^k \int_{x_0}^1 v_j^0(x) dx + \sum_{j=0}^k \int_0^{r_j} \int_{x_0}^1 v_j^1(a, x) dx da,$$

is finite but also that the number of cells entering the budded phase per unit of time,

$$\sum_{j=0}^k \int_{x_0}^1 b(x) v_j^0(x) dx,$$

is indeed finite in the beginning.

3. THE INTEGRAL EQUATION

In this section we shall show that our assumption that all newborn daughter cells are smaller than the smallest mother cells allows us to reduce the problem (2.4)-(2.9) to a single integral equation of renewal type, even if $k = \infty$. The reason why this reduction is possible is that when $y_j(1) \leq x_0$ holds, then all reproducing cells were either present in the initial population or have passed size x_0 as unbudded cells in the zeroth scar class. This fact allows us to base our book-keeping on the flux

$$B(t) = g(x_0)n_0^0(t, x_0) \quad (3.1)$$

of unbudded virgin cells passing size x_0 . $B(t)$ will play a rôle very similar to that of the birth rate in the familiar Lotka-McKendrick system for age-dependent population dynamics. We refer to Diekmann (1985) for the derivation of an analogous integral equation in the context of a model for the size distribution of a population reproducing by fission into equal parts.

As is the case for the Lotka-McKendrick model, the basic technique used in deriving the integral equation is integration along characteristics. The characteristic line through (t_0, a_0) of eq. (2.6) is of course the straight line $a - a_0 = t - t_0$. To define the characteristic curves of eq. (2.4) and (2.5) let

$$G(x) = \int_x^{\infty} \frac{d\xi}{g(\xi)} \quad (3.2)$$

and define

$$X(t, x) = G^{-1}(G(x) + t), \quad 0 \leq G(x) + t \leq G(1). \quad (3.3)$$

G^{-1} is well defined since $g > 0$. Observe that $X(t, x)$ is the solution of the initial value problem

$$\frac{dX}{dt} = g(X), \quad X(0, x) = x \quad (3.4)$$

and hence $X(t, x)$ represents the size of an unbudded cell which belonged to the same scar class and had size x t time units ago. $G(x) - G(\xi)$ is the time it takes for an unbudded cell to grow from size ξ to size x provided it remains unbudded. The curve in the (t, x) -plane defined by $x = X(t - \tau, \xi)$ or equivalently by $t = \tau + G(x) - G(\xi)$ is called the characteristic curve passing through (τ, ξ) . During its residence in the unbudded phase a cell travels along a characteristic curve.

Integrating eq. (2.6) along characteristics, and taking (2.7) and (2.9) into account one obtains ($x_0 < x < 1$, $0 \leq a \leq r_j$)

$$n_j^1(t, a, x) = \begin{cases} v_j^1(a - t, x), & 0 \leq t < a, \\ b(x)n_j^0(t - a, x), & a < t. \end{cases} \quad (3.5)$$

Because of assumption (H_j) the sum in eq. (2.4) is zero for $x > x_0$. One can therefore by integration along characteristics express the solution $n_0^0(t, x)$ in the region $t > 0$, $x > x_0$ in terms of $B(t)$ and $v_0^0(x)$. Substituting this expression into (3.5) one obtains $n_0^1(t, r_j, x)$. Then one substitutes the $n_0^1(t, r_j, x)$ found in this way into (2.5), solves for $n_1^0(t, x)$ and obtain $n_1^1(t, r_{mj}, x)$ from (3.5). This procedure can be repeated and one eventually obtains $n_j^0(t, x)$ ($t > 0$, $x_0 < x < 1$) for all $j = 0, 1, \dots, k$ in terms of $B(t)$ and the initial data. The result is as follows.

$$n_j^0(t, x) = \begin{cases} \Phi_j(t, x), & 0 \leq t < r_0 + \dots + r_{j-1} + G(x) - G(x_0) \\ H_j(x, x_0) \frac{E(x)}{g(x)} B(t - [r_0 + \dots + r_{j-1} + G(x) - G(x_0)]), & t > r_0 + \dots + r_{j-1} + G(x) - G(x_0). \end{cases} \quad (3.6)$$

Empty sums should be interpreted as zero and sums of the form $r_l + \dots + r_j$ with $l = j$ as r_l . In (3.6) we have employed the following notation.

$$E(x) = \exp\left(-\int_{x_0}^x \frac{b(\xi)}{g(\xi)} d\xi\right), \quad x_0 \leq x < 1. \quad (3.7)$$

$$H_0(x, z) = 1, \quad x_0 \leq z \leq x < 1 \quad (3.8)$$

$$H_j(x, z) = \int_z^x \frac{b(\xi)}{g(\xi)} H_{j-1}(\xi, z) d\xi, \quad x_0 \leq z \leq x < 1, \quad j = 1, \dots, k. \quad (3.9)$$

$$\Phi_j(t, x) = \sum_{l=0}^j \phi_{jl}^0(t, x) + \sum_{l=0}^{j-1} \phi_{jl}^1(t, x) \quad (3.10)$$

$$\phi_{jl}^0(t, x) = \begin{cases} H_{j-l}(x, \eta_{jl}) \frac{g(\eta_{jl})}{g(x)} \frac{E(x)}{E(\eta_{jl})} v_l^0(\eta_{jl}), & t \in [r_l + \dots + r_{j-1}, r_l + \dots + r_{j-1} + G(x) - G(x_0)], \\ 0, & t \notin [r_l + \dots + r_{j-1}, r_l + \dots + r_{j-1} + G(x) - G(x_0)], \end{cases} \quad (3.11)$$

$$\phi_{jl}^1(t, x) = \begin{cases} 0 \leq l \leq j, \quad j = 0, \dots, k, \\ \int_0^{\min\{t, r_l\}} H_{j-l-1}(x, \xi_{jl}) \frac{g(\xi_{jl})}{g(x)} \frac{E(x)}{E(\xi_{jl})} v_l^1(r_l - s, \xi_{jl}) ds, & t \in [r_{l+1} + \dots + r_{j-1}, r_l + \dots + G(x) - G(x_0)], \\ 0, & t \notin [r_{l+1} + \dots + r_{j-1}, r_l + \dots + G(x) - G(x_0)], \end{cases} \quad (3.12)$$

$$0 \leq l \leq j-1, \quad j = 1, \dots, k.$$

$$\xi_{jl} = X(-t + s + r_{l+1} + \dots + r_{j-1}, x), \quad 0 \leq l \leq j-1, \quad j = 1, \dots, k. \quad (3.13)$$

$$\eta_{jl} = X(-t + r_l + \dots + r_{j-1}, x), \quad 0 \leq l \leq j, \quad j = 0, \dots, k. \quad (3.14)$$

Next we interpret the introduced functions in terms of probabilities. Consider an unbudded cell of size z in class l . The probability that it remains unbudded at least until it reaches size ξ is given by $E(\xi)/E(z)$. The chance density that bud emergence occurs when the cell has size x is $b(\xi)/g(\xi)$. Here the factor $1/g(\xi)$ accounts for the conversion of chance per unit of time to chance per unit of size. The probability that the cell after cell separation will stay in the $(l+1)$:th unbudded phase until it reaches size x is $E(x)/E(\xi)$. Summing up over all possible sizes ξ at which bud emergence can occur (i.e. $z < \xi < x$) we find that the probability that an unbudded cell of size x will be present in the following unbudded phase when it has size $x > z$ is given by

$$\int_z^x \frac{E(\xi)}{E(z)} \frac{b(\xi)}{g(\xi)} \frac{E(x)}{E(\xi)} d\xi = \frac{E(x)}{E(z)} H_1(x, z).$$

Repeating this argument we see that given that an unbudded cell has size z in class l , the probability that it is present in the $(l+j)$:th unbudded phase when it has size $x > z$ is

$$\frac{E(x)}{E(z)} H_j(x, z).$$

$\xi_{jl} [\eta_{jl}]$ is the size an unbudded cell, which at time t is in the j :th class with size x , had at time s [0], provided it at that instance was an unbudded cell in class $l+1$ [l]. Thus, $\phi_{jl}^0(t, x)$ [$\phi_{jl}^1(t, x)$] is the density of unbudded cells in the j :th scar class which were initially present as unbudded [budded] cells in class l , $l=0, \dots, j$ [$l=0, \dots, j-1$]. $\Phi_j(t, x)$ is the total contribution of the initial population to $n_j^0(t, x)$.

As a final step we must solve eq. (2.4) for $n_0^0(t, x)$ in the region $(\inf_{0 \leq j \leq k} y_j(x_0), x_0)$. Since $n_j^0(t, x)$ - and hence $n_j^1(t, r_j, x)$ by (3.5) - are now known for $x_0 < x < 1$, this is possible. Actually, it suffices to determine the flux $B(t) = g(x_0)n_0^0(t, x_0)$. The rate at which budded cells in class j give birth to new cells of size x in class 0 is given by

$$A_j(t, x) = \frac{1}{y_j'(y_j^{-1}(x))} n_j^1(t, r_j, y_j^{-1}(x)).$$

Using (3.5) and (3.6) one obtains

$$A_j(t, x) = \begin{cases} \frac{1}{y_j'(y_j^{-1}(x))} v_j^1(r_j - t, y_j^{-1}(x)), & 0 \leq t \leq r_j \\ \frac{b(y_j^{-1}(x))}{y_j'(y_j^{-1}(x))} \Phi_j(t - r_j, y_j^{-1}(x)), & r_j \leq t \leq r_0 + \dots + r_j + G(y_j^{-1}(x)) - G(x_0) \\ \frac{K_j(y_j^{-1}(x))}{y_j'(y_j^{-1}(x))} B(t - [r_0 + \dots + r_j + G(y_j^{-1}(x)) - G(x_0)]), & \end{cases} \quad (3.15)$$

$$r_0 + \dots + r_j + G(y_j^{-1}(x)) - G(x_0) < t,$$

where

$$K_j(x) = \frac{b(x)}{g(x)} H_j(x, x_0) E(x). \quad (3.16)$$

The unbudded cells in class 0 passing size x_0 at time t are exactly those who were present at time $t=0$ as unbudded virgin cells with size $X(-t, x_0)$ plus those who were born with size η $t - [G(x_0) - G(\eta)]$ time units ago. Summing up over all scar classes and all possible birth sizes one finds

$$B(t) = \sum_{j=0}^k \int_{\max\{y_j(x_0), X(-t, x_0)\}}^{y_j(1)} A_j(t - [G(x_0) - G(\eta)], \eta) d\eta + B_0(t), \quad (3.17)$$

where

$$B_0(t) = \begin{cases} g(X(-t, x_0)) \nu_0^0(X(-t, x_0)), & 0 \leq t < G(x_0) \\ 0, & G(x_0) < t. \end{cases} \quad (3.18)$$

Substituting the expression (3.15) for A_j into eq. (3.17) and performing the change $x = y^{-1}(\eta)$ of variables one obtains

$$B(t) = \sum_{j=0}^k \int_{x_0}^1 K_j(x) B(t - [r_0 + \dots + r_j + G(x) - G(y_j(x))]) dx + \Phi(t), \quad (3.19)$$

where

$$\begin{aligned} \Phi(t) = & \sum_{j=0}^k \int_{x_0}^1 \nu_j^1(r_j - t + G(x) - G(y_j(x)), x) dx \\ & + \sum_{j=0}^k \int_{x_0}^1 b(x) \Phi_j(t - [G(x_0) - G(y_j(x))] - r_j, x) dx + B_0(t), \end{aligned} \quad (3.20)$$

with the convention that $B(t) = 0$ for $t < 0$, $\nu_j^1(a, x) = 0$ for $a \notin [0, r_j]$ and $\Phi_j(t, x) = 0$ for $t \notin [0, r_0 + \dots + r_{j-1}]$

It is easily seen that the kernel K_j is an L^1 function of norm 1. This is obvious for $j=0$, since K_0 is simply $(b/g)E$ and a standard induction argument using the recursion formula (3.9) yields the general result. K_j is the probability density for the random variable defined as the size at which an originally virgin unbudded cell enters the budded phase in the j :th scar class.

We still observe the following facts. Even in the case $k = \infty$ the sum on the right-hand side of eq. (3.19) contains for each t at most a finite number of nonzero terms. Hence there is no convergence problem for this sum. That the first sum in the definition of Φ converges follows immediately from (H_r) . Using the definitions (3.10)-(3.12) and hypothesis (H_r) one finds that each integral in the latter sum is finite and that this sum, too, contains at most a finite number of terms. Thus $\Phi(t)$ is well defined. It represents the total contribution of the initial population to the cohort of unbudded virgin cells passing size x_0 . Notice that according to our hypotheses Φ is continuous with compact support if $k < \infty$ and is bounded and continuous in the case $k = \infty$.

If the solution $B(t)$ of eq. (3.19) is known, one immediately obtains the size distributions and age-size distributions of the various phases and scar classes from formulae (3.6) and (3.5). The complicated system (2.4)-(2.9) involving a possibly infinite number of partial differential equations with delays has thus been reduced to the single integral equation (3.19). In the following section we shall investigate the asymptotic behaviour of solutions of (3.19). By the preceding remarks information concerning the ultimate behaviour of $B(t)$ automatically yields corresponding results for the solutions of the original problem (2.4)-(2.9).

4. THE CHARACTERISTIC EQUATION AND THE ASYMPTOTIC BEHAVIOUR OF SOLUTIONS

The standard method to obtain qualitative information about the solution $B(t)$ of eq. (3.19) is to use Laplace transformation. One first shows that the Laplace transform \hat{B} of B is a meromorphic function with all its poles to the left of some vertical line in the complex plane. The inversion formula and an application of the residue theorem then yield the desired information of the behaviour of $B(t)$ as t tends to infinity. This approach works nicely if \hat{B} has a real pole λ_d and all other poles have real part less than $\lambda_d - \epsilon$ for some $\epsilon > 0$. If this is not the case, it seems better to apply the renewal theorem of Feller (1971). We shall therefore start by examining the location of the poles at \hat{B} and then draw the conclusions for the behaviour of the solutions of (3.19) and (2.4)-(2.9).

Taking the Laplace transform of both sides at (3.19) one obtains

$$\hat{B}(\lambda) = \sum_{j=0}^k e^{-\lambda[r_0 + \dots + r_j]} \tilde{K}_j(\lambda) \hat{B}(\lambda) + \hat{\Phi}(\lambda) \quad (4.1)$$

where $\hat{}$ denotes Laplace transform and

$$\tilde{K}_j(\lambda) = \int_{x_0}^1 e^{-\lambda[G(x) - G(y_j(x))]} K_j(x) dx. \quad (4.2)$$

Clearly $\tilde{K}_j(\lambda)$ is defined and analytic for all $\lambda \in \mathbb{C}$ and hence the formula

$$\pi(\lambda) = \sum_{j=0}^k e^{-\lambda[r_0 + \dots + r_j]} \tilde{K}_j(\lambda) \quad (4.3)$$

defines an entire function if $k < \infty$. In the case $k = \infty$ the series in (4.2) is dominated by $\sum_{j=0}^{\infty} e^{-\lambda[r_0 + \dots + r_j]}$ for $\operatorname{Re} \lambda \geq \epsilon > 0$. Since $\inf_{0 \leq j < \infty} r_j > 0$ by (H_r) , the ratio test assures convergence of the majorant, hence the original series converges uniformly for $\operatorname{Re} \lambda \geq \epsilon$ by Weierstrass's M -test. It follows that π is well defined and analytic for $\operatorname{Re} \lambda > 0$.

Provided $\pi(\lambda) \neq 1$ $\hat{B}(\lambda)$ is given by

$$\hat{B}(\lambda) = \frac{\hat{\Phi}(\lambda)}{1 - \pi(\lambda)}. \quad (4.4)$$

Obviously $\hat{B}(\lambda)$ is a meromorphic function with poles at the roots of the characteristic equation

$$\pi(\lambda) = 1. \quad (4.5)$$

It is easily seen that eq. (4.5) has a unique real root λ_d which is positive and simple and that all other roots appear as pairs of complex conjugates with real parts less than or equal to λ_d . λ_d is called the *dominant* root of the characteristic equation. We say that λ_d is *strictly dominant* if all other roots have real part less than λ_d . If there exists an $\epsilon > 0$ such that $\operatorname{Re} \lambda < \lambda_d - \epsilon$ for all nonreal roots, then λ_d is called *strongly dominant*. The following proposition gives rather precise information about the location of the other characteristic roots in relation to the dominant one. Recall that real numbers τ_j ($j = 0, 1, \dots, k \leq \infty$) are *commensurable* if there exists a $\tau > 0$ such that all τ_j are integral multiples of τ . The largest number τ with this property is called the *generator* of the commensurable numbers τ_j .

PROPOSITION 4.1.

- If there exists at least one j $0 \leq j \leq k$ such that $G(x) - G(y_j(x))$ is not constant on the entire interval $[x_0, 1]$, then the dominant root λ_d of the characteristic equation is *strongly dominant*.
- If $G(x) - G(y_j(x)) = \tau_j = \text{constant}$ on $[x_0, 1]$ for all $j = 0, 1, \dots, k$ and if the numbers $r_0 + \dots + r_j + \tau_j$ are commensurable with generator τ , then there exist infinitely many roots of the characteristic equation on the vertical line $\operatorname{Re} \lambda = \lambda_d$ located at $\lambda = \lambda_d + i n 2\pi/\tau$, and there are no other roots.

- (c) If $G(x) - G(y_j(x)) = \tau_j = \text{constant}$ on $[x_0, 1]$ for all $j = 0, 1, \dots, k$ and if the numbers $r_0 + \dots + r_j + \tau_j$ are not commensurable, then the dominant root λ_d of the characteristic equation is strictly dominant but in the case $k < \infty$ not strongly dominant.
- (d) Suppose $k < \infty$. The characteristic equation has infinitely many roots in the complex plane.

PROOF. Consider the equation

$$\pi(\lambda_d + i\omega) = 1. \quad (4.6)$$

We are looking for solutions $\omega \neq 0$ of (4.6). If (4.6) is to hold, then the real part of $\pi(\lambda_d + i\omega)$,

$$\operatorname{Re} \pi(\lambda_d + i\omega) = \sum_{j=0}^k \int_{x_0}^1 e^{-\lambda(r_0 + \dots + r_j + G(x) - G(y_j(x)))} K_j(x) \quad (4.7)$$

$$\cos \omega[r_0 + \dots + r_j + G(x) - G(y_j(x))] dx,$$

must equal 1. We observe that if there is at least one j such that $G(x) - G(y_j(x))$ is not constant on $[x_0, 1]$, then by the continuity of G the corresponding cosine term in (4.7) must be less than one on a subinterval of $(x_0, 1)$. Since $K_j(x) > 0$ for $x \in (x_0, 1)$ it follows that $\operatorname{Re} \pi(\lambda_d + i\omega) < 1$ for $\omega \neq 0$ and that $\operatorname{Re} \pi(\lambda_d + i\omega)$ is bounded away from 1 as $\omega \rightarrow \pm\infty$. Moreover we see that $\operatorname{Re} \pi(\lambda_d + i\omega) = 1$ if and only if all the cosine terms are equal to 1 on the entire interval $[x_0, 1]$ which happens if and only if $G(x) - G(y_j(x)) = \tau_j = \text{constant}$ for all j and $r_0 + \dots + r_j + \tau_j$ are commensurable with generator $\tau = 2\pi/\omega$. On the other hand, if this is the case, then $\sin \omega[r_0 + \dots + r_j + \tau_j] = 0$ for all j , hence $\operatorname{Im} \pi(\lambda_d + i\omega) = 0$ and it follows that (4.6) is satisfied with $\omega = n \frac{2\pi}{\tau}$ ($n \in \mathbb{Z}$). Similar arguments show that there are no other roots of (4.5). We have now proved (b) and showed that λ_d is strongly dominant in the cases (a) and (c).

To prove that λ_d is strongly dominant in case (a), observe that since π is an analytic function no bounded sequence of roots of eq. (4.5) can approach the line $\operatorname{Re} \lambda = \lambda_d$ and since $\operatorname{Re} \pi(\lambda_d + i\omega)$ is bounded away from 1 as $\omega \rightarrow \pm\infty$ and $\pi'(\lambda)$ is bounded on the line $\operatorname{Re} \lambda = \lambda_d$ neither can an unbounded sequence.

To complete the proof of (c) suppose that $r_0 + \dots + r_j + \tau_j$ are constants which are not commensurable. Since

$$\int_{x_0}^1 K_j(x) dx = 1$$

for all j $\pi(\lambda)$ takes the form

$$\pi(\lambda) = \sum_{j=0}^k e^{-\lambda(r_0 + \dots + r_j + \tau_j)}. \quad (4.8)$$

In the case $k < \infty$ it is known (see Bellman and Cooke (1963, p. 403)) that if λ varies in a set which is bounded away from the 1-points of π then $\pi(\lambda)$ is bounded away from 1 in that set. Being a sum of periodic functions, the mapping $\omega \rightarrow \pi(\lambda_d + i\omega)$, is an almost periodic function on \mathbb{R} . It attains the value 1 only once, at $\omega = 0$. It follows from Bohr's characterization of almost periodic functions that there exists a sequence $\omega_n \rightarrow \infty$ such that $\pi(\lambda_d + i\omega_n) \rightarrow 1$ (cf. Fink (1974, p. 8)). Thus, $\pi(\lambda)$ is not bounded away from 1 on the vertical line $\operatorname{Re} \lambda = \lambda_d$ and hence this line cannot be bounded away from the set of roots of eq. (4.5), which means exactly that λ_d is not strongly dominant.

We finally prove (d). Suppose $k < \infty$. Then π is an entire function of order $\rho = 1$ (recall that the order of an entire function f is the infimum of all positive numbers s such that $|f(\lambda)| < \exp(|\lambda|^s)$ for all large enough $|\lambda|$). We already know that eq. (4.5) has infinitely many roots if $G(x) - G(y_j(x)) = \tau_j = \text{constant}$ for all j so suppose this is not the case. If eq. (4.5) had only a finite

number of roots $\lambda_d, \lambda_1, \dots, \lambda_n$ with multiplicity m_j ($j=1, \dots, n$) then $\pi(\lambda)$ would have the form

$$\pi(\lambda) = 1 + (\lambda - \lambda_d)(\lambda - \lambda_1)^{m_1} \dots (\lambda - \lambda_n)^{m_n} \exp(Q(\lambda)), \quad (4.9)$$

where $Q(\lambda)$ is a polynomial of degree not greater than $\rho=1$ (cf. Markushevich (1965, p. 267)). The absolute value of the right hand side of (4.9) approaches either 1 or ∞ as $|\lambda|$ tends to infinity along the line $\operatorname{Re} \lambda = \lambda_d$. But as noticed above, this is not true for the left hand side. This contradiction shows that the characteristic equation has infinitely many roots if $k < \infty$. \square

We can now prove the following

THEOREM 4.2. *Suppose the condition*

$$G(x) - G(y_j(x)) = \tau_j = \text{constant on } [x_0, 1] \text{ for all } j \text{ and } r_0 + \dots + r_j + \tau_j \text{ commensurable} \quad (4.10)$$

is not satisfied. Then the solution B of equation (3.19) has the form

$$B(t) = (C + r(t))e^{\lambda_d t}, \quad (4.11)$$

where C is a constant depending only on the initial state and $r(t) \rightarrow 0$ as $t \rightarrow \infty$. If $G(x) - G(y_j(x))$ is not constant on $[x_0, 1]$ for at least one j , then $r(t)$ decays exponentially, that is

$$|r(t)| \leq M e^{-\epsilon t} \quad (4.12)$$

for some constants $M < \infty$ and $\epsilon > 0$. If (4.10) holds then for fixed $t \geq 0$

$$B(t + n \frac{2\pi}{\tau}) \rightarrow C e^{n \frac{2\pi}{\tau} \lambda_d} \sum_{p=1}^{\infty} e^{-p \frac{2\pi}{\tau} \lambda_d} \Phi(t + p \frac{2\pi}{\tau}) \quad (4.13)$$

as the integer n tends to ∞ .

PROOF. Consider first the case where $G(x) - G(y_j(x))$ is not constant for at least one j . Then λ_d is strongly dominant by proposition 4.1 (a). It is easily seen from (3.19) that B grows no faster than an exponential function. Since $\hat{B}(\lambda)$ defined by (3.23) is analytic for $\operatorname{Re} \lambda > \lambda_d$ we can apply the complex inversion formula, which together with the residue theorem implies (4.11) with r satisfying (4.12) for some $M < \infty$ and some $\epsilon > 0$ (see Hoppensteadt (1975) or Gurtin and McCamy (1979)).

Suppose now that $G(x) - G(y_j(x)) = \tau_j = \text{constant}$ for all j . After the transformation

$$Z(t) = e^{-\lambda_d t} B(t) \quad (4.14)$$

equation (3.19) reduces to the difference equation

$$Z(t) = \sum_{j=0}^k e^{-\lambda_d [r_0 + \dots + r_j + \tau_j]} Z(t - [r_0 + \dots + r_j + \tau_j]) + e^{-\lambda_d t} \Phi(t). \quad (4.15)$$

Then sum in (4.15) is the convolution of Z and the discrete probability measure with mass $e^{-\lambda_d [r_0 + \dots + r_j + \tau_j]}$ at $r_0 + \dots + r_j + \tau_j$. In the terminology of Feller (1971) this measure is arithmetic with span $2\pi/\tau$ if and only the numbers $r_0 + \dots + r_j + \tau_j$ are commensurable with generator τ . Since the probability measure has finite expectation and $e^{-\lambda_d t} \Phi(t)$ is integrable the renewal theorem of Feller (1971, p. 363) yields (after a transformation back to $B(t)$) the result (4.11) for the noncommensurable case and (4.13) in the commensurable case. \square

It is easily found by elementary separation of variables in the system (2.4)-(2.7) that this system admits a product solution (also called steady state) of the form

$$n_j^0(t, x) = C e^{\lambda_d t} \psi_j^0(x) \quad (4.16)$$

$$n_j^1(t, x) = C e^{\lambda_d t} \psi_j^1(a, x), \quad (4.17)$$

where the size distributions $\psi_j^0(x)$ and age-size distributions $\psi_j^1(a, x)$ are given by

$$\psi_j^0(x) = e^{-\lambda_d[G(x)-G(x_0)+r_0+\dots+r_{j-1}]} \frac{E(x)}{g(x)} H_j(x, x_0), \quad (4.18)$$

for $j=0, 1, \dots, k, x_0 < x < 1$,

$$\psi_0^0(x) = \frac{1}{g(x)} \sum_{j=0}^k e^{-\lambda_d[G(x)-G(x_0)+r_0+\dots+r_j]} \int_{x_0}^{\min(y_j^{-1}(x), 1)} K_j(\xi) e^{-\lambda_d[G(\xi)-G(y_j(\xi))]} d\xi \quad (4.19)$$

for $\alpha < x < x_0$, and

$$\psi_j^1(a, x) = e^{-\lambda_d[G(x)-G(x_0)+r_0+\dots+r_{j-1}+a]} K_j(x) = e^{-\lambda_d a} b(x) \psi_j^0(x) \quad (4.20)$$

for $j=0, \dots, k, x_0 < x < 1$. If λ_d is strictly dominant it is a consequence of the asymptotic behaviour of $B(t)$, expressed by (4.11) that all solutions of the system approach this product solution as t tends to infinity. This is stated more precisely in the next theorem.

THEOREM 4.3. Suppose condition (4.10) does not hold and let $n_j^0(t, x)$, $n_j^1(t, a, x)$ be the solution of system (2.4)-(2.9) for some given initial distributions $v_j^0(x)$, $v_j^1(a, x)$. Then there exists a continuous function r vanishing at infinity such that

$$|e^{-\lambda_d t} n_j^0(t, x) - C \psi_j^0(x)| dx \leq r(t) \quad (4.21)$$

$$\int_{x_0}^1 |e^{-\lambda_d t} n_j^1(t, a, x) - C \psi_j^1(a, x)| dx \leq r(t) \quad (4.22)$$

for all $j=0, \dots, k$, all $x \in (x_0, 1)$ [$x \in (\alpha, 1)$ when $j=0$ in (4.21)], all $a \in [0, r_j]$ and all $t > 0$. Moreover, to each $x \in (x_0, 1)$ corresponds an $M(x) < \infty$ such that

$$|e^{-\lambda_d t} n_j^1(t, a, x) - C \psi_j^1(a, x)| \leq M(x) r(t). \quad (4.23)$$

If $K_1(x)$ is bounded on $(x_0, 1)$, then $M(x)$ can be chosen independently of x . If there exists at least one j such that $G(x) - G(y_j(x))$ is not constant on $[x_0, 1]$, then $r(t)$ can be chosen to satisfy

$$r(t) \leq M e^{-\epsilon t} \quad (4.24)$$

for some constants $M < \infty$ and $\epsilon > 0$.

The proof of Theorem 4.3 is a straightforward application of formulae (3.5), (3.6) and (4.11) and is therefore omitted.

We have already seen how the crucial condition (4.10) entered the proofs of Proposition 4.1 and Theorem 4.2. At this point it seems appropriate to have a closer look at the biological significance of this condition. Consider an unbudded virgin cell of size x_0 at time $t=0$. Suppose it enters the j :th budded phase with size x_{j+1} . It will enter the zeroth budded phase at time $G(x_1) - G(x_0)$ and leave it at time $r_0 + G(x_1) - G(x_0)$. It will enter the second budded phase at time $r_0 + G(x_1) - G(x_0) + G(x_2) - G(x_1) = r_0 + G(x_2) - G(x_0)$ and leave it at time $r_0 + r_1 + G(x_2) - G(x_0)$. By induction we find that the $(j+1)$:th daughter will be born at time $r_0 + \dots + r_j + G(x_j) - G(x_0)$ and it will reach size x_0 at time $T_j := r_0 + \dots + r_j + G(x_j) - G(y_j(x_j))$. If T_j is a constant function of x_j , then no matter at what time and with what size the mother cell has entered the different budded phases, its $(j+1)$:th daughter will reach size x_0 exactly at time T_j . In other words, if the initial population consists entirely of virgin cells of size x_0 , then all the $(j+1)$:th daughter cells will have exactly the same size x_0 at time T_j . If all the T_j are constant and moreover commensurable, we find that the first daughters of the j :th daughters of the initial population as well as the second daughters of the $(j-1)$:th daughters of the initial population and so on all have size x_0 at time T_j . In short, the size distribution of the zeroth class is concentrated at x_0 at time T_j . If the T_j 's are not commensurable then at each time the size distribution of the zeroth class will consist of a

finite sum of Dirac distributions and as time elapses the support of this distribution will eventually become dense in $[\alpha, 1]$ (this follows from Feller (1971, Lemma 2, p. 147). Of course, initial distributions of Dirac type are not permitted by (H_r) but could be replaced by a smooth function which has a high peak at x_0 and which vanishes outside some neighbourhood of x_0 . The conclusion is that in the commensurable case no dispersion in cell size occurs and consequently the initial distribution cannot converge towards the product solution (4.16), where ψ_j^0 is a smooth function which vanishes only at $x=1$. If the T_j are not commensurable, then there is enough dispersion to allow for convergence towards a stable size distribution.

Under what circumstances can condition (4.10) actually hold? In models for populations of cells reproducing by fission studied by Diekmann, Heijmans and Thieme (1984) and Gyllenberg and Heijmans (1985) the most important exceptional case in which convergence towards a stable size distribution does not take place corresponds to the situation where individual cells grow exponentially throughout the cell cycle. The assumption of exponential individual growth is also commonly made by microbiologists. We shall therefore investigate whether or not this assumption implies (4.10).

Suppose for the sake of simplicity that $r_j=r$ for all $j=0,1,\dots,k$, that is the budded phase has the same duration in all scar classes. Exponential individual growth in the unbudded phase means that g is a linear function. After scaling the time we may take $g(x)=x$. Assuming that budded cells grow exponentially at the same rate as unbudded we take $y_j(x)=(e^r-1)x$ for all $j=0,1,\dots,k$. Then we have

$$G(x)-G(y_j(x)) = \int_{(e^r-1)x}^x \frac{d\xi}{\xi} = \ln(e^r-1)^{-1} = \tau = \text{constant} \quad (4.25)$$

for all $j=0,1,\dots,k$. The numbers $r_0+\dots+r_j+\tau_j=(j+1)r+\tau$ are commensurable if and only if there are positive integers m and n such that

$$\frac{r}{\ln(e^r-1)^{-1}} = \frac{m}{n}. \quad (4.26)$$

It is easily proved that (4.26) holds for a countable set of values of r which is dense in $(0, \ln 2)$. Notice that this is precisely the interval to which r must belong since, in the time scale we have chosen, $\ln 2$ is the size doubling time which is certainly greater than r . We conclude that in the case of exponential individual growth for almost all (with respect to Lebesgue measure) value of r there exists a stable size distribution whereas for a dense set of values of r convergence does not take place.

Finally we point out that the durations r_j of the budded phases are measured quantities which are some kind of averages. From a practical point of view statements about the commensurability of the numbers $r_0+\dots+r_j+\tau_j$ are therefore completely meaningless - they can never be checked. Moreover, as noticed above, an arbitrarily small perturbation of r will destroy the stability. In the case of exponential individual growth the system is thus essentially unstable. It is worth noting that we have based our theory on the assumption that all the budded phases have constant durations r_j . In the real world this is not so, there is always a variance, albeit small, in r_j and this variance implies a dispersion in cell size sufficient to ensure convergence towards a stable size distribution even in the case of exponential individual growth.

5. THE STEADY STATE

In the preceding section we showed that unless condition (4.10) holds the solution of system (2.4)-(2.9) will approach a steady state given by (4.16)-(4.20). During the steady state the size distributions and age-size distributions of the various scar classes and phases remain constant in shape and the total number of cells in each class and phase grows exponentially with the same exponent. This exponent is the real root λ_d of the characteristic equation and we call it the *Malthusian parameter* of the population.

Below we shall derive expressions for the relative frequencies of cells in the various phases. Questions concerning these quantities have attracted much attention in the microbiological literature, see for instance Beran (1968) and Beran et. al. (1969).

We let

$$N_j^0(t) = \int_{\alpha_j}^1 n_j^0(t, x) dx$$

($\alpha_0 = \alpha$, $\alpha_j = x_0$ for $j \geq 1$) denote the total number of unbudded cells and

$$N_j^1(t) = \int_0^{r_j} \int_{x_0}^1 n_j^1(t, a, x) dx da$$

the total number of budded cells in the j :th scar class at time t . Thus $N_j = N_j^0 + N_j^1$ is the total number of cells in the j :th scar class and $N = \sum_{j=0}^k N_j$ is the total number of cells in the population. We shall also use the symbols $N^0 = \sum_{j=0}^k N_j^0$ and $N^1 = \sum_{j=0}^k N_j^1$ for the total number of unbudded and budded cells, respectively. The relative frequencies are defined as follows:

$$f_j^l = N_j^l / N, \quad j=0, 1, \dots, k, \quad l=0, 1. \quad (5.1)$$

In (5.1) either the sub- or superscripts could also be missing. The following quantities will play an important role in what follows. Their biological interpretation will be explained at the end of this section.

$$\beta_j = e^{-\lambda_d[r_0 + \dots + r_j]} \int_{x_0}^1 K_j(x) e^{-\lambda_d[G(x) - G(x_0)]} dx, \quad (5.2)$$

$$\beta = \sum_{j=0}^k \beta_j. \quad (5.3)$$

If $k = \infty$ the series in (5.3) converges by the ratio test. A fortiori $\beta_j \rightarrow 0$ as $j \rightarrow \infty$ and the symbol $\beta_k = \beta_\infty$ is interpreted as 0.

One could of course obtain the values of f_j^l at steady state from (5.1) after integrating the steady state solutions given by (4.16)-(4.20) over all sizes and ages. However, especially the expression for $\psi_0^0(x)$ is rather complicated, so we prefer to proceed in a different way and first derive balance equations for N_j^l and N at steady state.

The gain in the total number of cells in the populations is due to births and is given per unit of time by

$$\sum_{j=0}^k \int_{x_0}^1 n_j^1(t, r_j, x) dx = \sum_{j=0}^k \int_{x_0}^1 b(x) n_j^0(t - r_j, x) dx = C e^{\lambda_d t} \beta. \quad (5.4)$$

The first term in (5.4) is the number of cells leaving the budded phase per unit of time and it is equal to the number of births per unit of time. The first equality in (5.4) is simply a consequence of the boundary condition (2.7) which states that the cells leaving the j :th budded phase are exactly those who entered it r_j time units earlier. In obtaining the latter equality we have substituted the steady state solution given by (4.16), (4.18) for n_j^0 . The loss in the total number of cells is due to deaths and equals per unit of time the flux of individuals leaving the last (k :th) budded phase, that is,

$$\int_{x_0}^1 n_k^1(t, r_k, x) dx = \int_{x_0}^1 n_k^0(t - r_k, x) dx = C e^{\lambda_d t} \beta_k. \quad (5.5)$$

If $k = \infty$ there are no deaths, which is in agreement with our convention $\beta_\infty = 0$. From (5.4) and (5.5) we obtain the following balance equation for the total numbers of cells:

$$\frac{dN}{dt} = C e^{\lambda_d t} [\beta - \beta_k]. \quad (5.6)$$

By reasoning analogous to that above we can derive the following balance equations.

$$\frac{dN_j}{dt} = Ce^{\lambda_d t} [\beta_{j-1} - \beta_j], \quad j \geq 1, \quad (5.7)$$

$$\frac{dN_0}{dt} = Ce^{\lambda_d t} [\beta - \beta_0], \quad (5.8)$$

$$\frac{dN_j^1}{dt} = Ce^{\lambda_d t} [e^{\lambda_d \tau_j} - 1] \beta_j, \quad j \geq 1. \quad (5.9)$$

By subtracting (5.9) from (5.7) and (5.8) we obtain, respectively

$$\frac{dN_j^0}{dt} = Ce^{\lambda_d t} [\beta_{j-1} - e^{\lambda_d \tau_j} \beta_j], \quad j \geq 1, \quad (5.10)$$

$$\frac{dN_0^0}{dt} = Ce^{\lambda_d t} [\beta - e^{\lambda_d \tau_0}]. \quad (5.11)$$

The relative frequencies are now immediately obtained from (5.6)-(5.11) by noting that certainly

$$f_j^i = \left(\frac{dN_j^i}{dt} \right) / \frac{dN}{dt}.$$

The result is presented in Table 1.

TABLE 1. Relative frequencies at steady state

scarclass \ phase	unbudded	budded	total
0	$\frac{\beta - e^{\lambda_d \tau_0} \beta_0}{\beta - \beta_k}$	$\frac{(e^{\lambda_d \tau_0} - 1) \beta_0}{\beta - \beta_k}$	$\frac{\beta - \beta_0}{\beta - \beta_k}$
j	$\frac{\beta_{j-1} - e^{\lambda_d \tau_j} \beta_j}{\beta - \beta_k}$	$\frac{(e^{\lambda_d \tau_j} - 1) \beta_j}{\beta - \beta_k}$	$\frac{\beta_{j-1} - \beta_j}{\beta - \beta_k}$
total	$\frac{2\beta - \beta_k - \sum_{j=0}^k e^{\lambda_d \tau_j} \beta_j}{\beta - \beta_k}$	$\frac{\sum_{j=0}^k e^{\lambda_d \tau_j} \beta_j - \beta}{\beta - \beta_k}$	1

Let τ_j be the random variable defined as the time at which an originally (at $t=0$) virgin cell of size x_0 leaves the j :th budded phase. Observe that this means that the random variable $\tau_j - \tau_{j-1}$ ($j \geq 1$) is the length of the j :th cell cycle (= the duration of the unbudded + budded phase in the j :th scar class). If the cell enters the j :th budded phase with size x (which happens with probability density $K_j(x)$), then $\tau_j = G(x) - G(x_0) + [r_0 + \dots + r_j]$. Thus β_j is the expectation of $e^{-\lambda_d \tau_j}$, in symbols

$$\beta_j = \mathbb{E}(e^{-\lambda_d \tau_j}). \quad (5.12)$$

The probability interpretation of the results in Table 1 is easily understood if one assumes that the population grows in a chemostat with dilution rate equal to the Malthusian parameter λ_d . The intrinsic growth of the population is thus exactly balanced by harvesting and the total number of cells in the various phases and classes remain constant. Moreover, we shall assume that $k = \infty$. With these

conventions we get

$$f_0^0 = \mathfrak{E}(1 - e^{-\lambda_d(\tau_0 - r_0)})/\beta, \quad (5.13)$$

$$f_j^0 = \mathfrak{E}(e^{-\lambda_d \tau_{j-1}}(1 - e^{-\lambda_d(\tau_j - \tau_{j-1} - r_j)}))/\beta, \quad j \geq 1, \quad (5.14)$$

$$f_j^l = \mathfrak{E}(e^{-\lambda_d(\tau_j - r_j)}(1 - e^{-\lambda_d r_j}))/\beta, \quad j \geq 0, \quad (5.15)$$

From the definition (5.3) of β and the definition (4.3) of π and the fact that λ_d is the real root of the characteristic equation (4.5) it follows that $\beta > 1$. Let us assume for a moment that $\beta = 1$. This corresponds to the case where all new born cells have equal size $y_j(x) = x_0$, which is certainly in conflict with our hypotheses. As an example we examine the content of (5.15) when $\beta = 1$. $e^{-\lambda_d(\tau_j - r_j)}$ is the fraction of cells surviving (not being harvested) up to time $\tau_j - r_j$. If the cell was in class 0 with size x_0 at time $t = 0$, then $\tau_j - r_j$ represents the time at which the cell enters the j :th budded phase. $(1 - e^{-\lambda_d r_j})$ is the fraction of cells, present at some instant t , which will be harvested in the time interval $[t, t + r_j]$. Thus we conclude that f_j^l is the probability that a cell which enters the j :th budded phase will be harvested during that phase. Generally we have that f_j^l is the probability that a cell which enters the l :th phase ($l = 0, 1$ stands for unbudded and budded, respectively) in the j :th scar class is harvested during that phase. f_j^j is the probability that a cell which enters the j :th scar class is harvested when it still belongs to that class. Since β in fact is greater than 1 the value of f_0^0 is actually greater than that obtained for $\beta = 1$ and the values of the other f_j^l are correspondingly smaller. The biological reason for this is of course that virgin cells have to spend some time as unbudded before they reach the critical size x_0 .

It should also be noted that in the case $r_j = r$ for all j we have $f^0 = 2 - e^{\lambda_d r}$ and $f^1 = e^{\lambda_d r} - 1$, a result also predicted by the model of Lord and Wheals (1980).

As mentioned in the introduction observations show that during the transient state the relative frequencies approach the equilibrium values f_j^l in an oscillatory manner. In was proved in Proposition 4.1 (d) that in the most realistic case of finitely many scar class ($k < \infty$) the characteristic equation has infinitely many nonreal roots. Thus the series representation of the solution $B(t)$ of eq. (3.19) (cf. Feller (1941)) certainly contains periodic terms. It follows easily as in the proof of a related proposition in Gyllenberg (1985) that the relative frequencies exhibit the same kind of damped oscillations as $B(t)$.

6. CONCLUDING REMARKS

It was the hypothesis $y_j(1) \leq x_0$, $j = 0, 1, \dots, k$, which made the reduction of the model to a renewal equation and the analysis of Section 4 possible. This hypothesis stated that all new born cells have size less than the smallest size at which bud emergence can occur. The results of Lord and Wheals (1981, p. 368) clearly show that there is a small overlap in the size distribution at birth and at bud emergence. Thus the procedure followed in the present study is not strictly supported by data. Fortunately there are stronger mathematical tools - the theory of semigroups - available for handling the general case. In a recent paper Gyllenberg and Heijmans (1985) studied an equation corresponding to the system (2.4)-(2.9) in the simplest case $k = 0$ without the restriction $y_0(1) \leq x_0$. In that paper we showed that the solution of the system defined a strongly continuous semigroups of linear operators on the Banach space of all continuous functions from $[-r_0, 0]$ into an infinite dimensional Banach space X . We showed further that under a condition corresponding to (4.10) of the present paper (since we considered a system with only one delay we did not have to bother about commensurability) the semigroup is compact after finite time and that the infinitesimal generator of the semigroup has a strictly dominant algebraically simple real eigenvalue λ_d . Using results from the theory of strongly continuous semigroups we were then able to prove convergence of solutions towards a stable size distribution. The methods used in the paper of Gyllenberg and Heijmans (1985) could be adapted to the full system (2.4)-(2.9) without the condition $y_j(1) \leq x_0$. The results of Theorem 4.3 would essentially remain valid.

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