Cell Cycle Kinetics with Supramitotic Control, Two Cell Types, and Unequal Division: A Model of Transformed Embryonic Cells

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ABSTRACT

We develop a mathematical model of cell cycle kinetics of transformed embryonic cells. The model includes supramitotic regulation, in which decisions regarding growth control are made at a point inside the cell division cycle and their impact extends to the next decision point, located in the next division cycle. Another feature is the presence of two varieties of cells, which switch from one to the other with given transition probabilities. The third factor considered is unequal division of cells, also defined in probabilistic terms.

We provide a rigorous description of the model and derivation of its equations and analyze its asymptotic properties by defining and investigating an abstract semigroup of positive linear operators in appropriate state space. The spectral properties of the semigroup yield the balanced exponential growth law for the model.

To compare the model to experimental data, we derive basic pedigree statistics, β curves, and generation time correlations. We present numerical calculations based on measurements available for the embryonic cells. We conclude that to yield the experimentally obtained pedigree statistics, switches from one cell variety to the other must be quite infrequent.

1. INTRODUCTION

Cell cycle kinetics is of continuing interest to biologists and mathematicians. It is one of the domains of the biological sciences in which mathematical modeling is necessary to organize in a logical way new experimental findings. New measurement techniques such as flow cytometry, as well as molecular biology methods, enable deeper and more precise insight into the factors regulating cell cycle in various circumstances, in cells of

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different organisms (cf. Baserga [6], Tyson [30]). These developments have been paralleled by the evolution of mathematical tools (cf., e.g., Metz and Diekmann [23], Webb [32], Arino and Kimmel [2]).

From the mathematical viewpoint, cell cycle kinetics is a part of population dynamics, defined by specific rules of cell proliferation. Modeling of cell populations has contributed to various mathematical disciplines, including differential equations, theory of stochastic processes, and statistical inference. For the past several years, the area of mathematics that has most benefited from population studies has been the theory of operator semigroups (cf. Nagel [24]).

In this paper, we develop a mathematical model of cell cycle kinetics with supramitotic regulation, that is, a model in which decisions controlling growth of the cell are made not at the beginning of the cell division cycle but at a previous point and their impact is extended to the next decision point, which is located in the next division cycle (cf. Sennerstam [26]). The period from one decision point to the next is called the growth control cycle. In our model, the new growth control cycle is entered when the cell attains threshold size. The threshold is in general a random variable, so the model allows for imprecise control.

Another feature of the model is the presence of two varieties or types of cells. At the beginning of each growth control cycle, cells may switch from one type to the other with given transition probabilities. The third factor considered is unequal division of cells, also defined in probabilistic terms (cf. Darzynkiewicz [9, 10] and Kimmel et al. [21]).

The work on the present model has been stimulated by experiments with cultured transformed embryonic cells [26–28], the results of which imply the cell cycle mechanism described above.

We provide a derivation of model equations based on biological information (Section 2). Then we analyze the model's asymptotic properties (Section 3 and Appendix). This is accomplished by defining and investigating an abstract semigroup of positive linear operators in appropriate state space. In brief, we deduce that the semigroup is eventually compact, that its spectrum has a dominating eigenvalue determined by solution of a characteristic equation, and that the asymptotic behavior of the semigroup is determined by the dominating eigenvalue. This provides us with the asynchronous exponential growth law for our model.

To relate the model to cell kinetic data, we derive basic pedigree statistics, β curves, and generation time correlations (Section 4). We present detailed numerical calculations based on measurements available for the embryonic cells. This part of the paper is of independent interest and is written to be understandable with elementary probabilistic and statistical expertise.

The main biological conclusion of our modeling, consistent with the published pedigree statistics of cultured embryonic cells, is that switching from one cell type to the other is a necessary but relatively infrequent event.

The present analysis is a continuation of our previous related works on cell population dynamics [1–5, 19, 20].

We acknowledge the impact of ideas of Cooper and his coauthors (cf. e.g. Cooper [7]), who in a series of papers introduced and developed concepts on which our definition of supramitotic control has been based.

2. MATHEMATICAL MODEL OF CULTURED TRANSFORMED EMBRYONIC CELLS

2.1. *BIOLOGICAL BACKGROUND*

In a series of papers, Sennerstam [26] and Sennerstam and Strömberg [27, 28] provided a comprehensive picture of cell cycle dynamics of transformed mouse embryo cell lines. The findings we summarize have been obtained for the PCC3 embryonal carcinoma cells.

In the experiments reported in [26], the principal issue was the inequality of division and its contribution to the variability of cell mass and of cell generation time in the population. Mitotic cells were obtained by mitotic detachment, seeded on glass slides and stained, and investigated at intervals of 0.5–3 h after division. At each time point, 50–100 cell pairs were screened. It was found that unequal division causes the coefficient of variation of cell mass to increase by about 4% (from 13.02% to 13.55%). As an example, 0.5 h after division, the mean value of cell mass is 7.05 relative units, while the mean absolute value of the difference between sister cells is 0.42 unit. There is no visible correlation between difference of masses of sister cells and their joint mass (i.e., the mass of the mother cell).

Another striking feature of the cell kinetics is the apparent bimodality in the distributions of cell size and of cell generation time, suggesting the presence of two different types of cells.

To explore these findings, Sennerstam and Strömberg [28] analyzed the pedigree statistics of the PCC3 cells obtained by the time-lapse investigation of growing cell populations. The following observations were made:

(1) The intermitotic time distribution (the α curve) of the whole population is bimodal. The mean generation times of the two subvariants are approximately 10 and 14 h.

(2) The distribution of differences of generation times between sister cells (the β_1 curve) is unimodal.

(3) The distribution of differences of generation times between first cousin cells (the β_2 curve) is bimodal.

(4) The distribution of differences of generation times between second cousin cells (the β_3 curve) is unimodal again.

(5) The correlation coefficient between generation times of sister cells is 95%; between first cousins, 77%; between mother and daughter cells, 41%. Other correlations are close to 0.

Sennerstam and Strömberg [28] formulated a theory of cell cycle regulation for the PCC3 cells. The main ideas are that cells may switch from the "fast" to the "slow" cell cycle variant and back and that the regulation has a supramitotic character—that is, the decision that the cell belongs to given variant is made in the preceding cell division cycle. Such a hypothesis explains the observed character of the α, β_1, β_2, and β_3 curves and correlation coefficients (see Section 4).

To realize that this hypothesis is also necessary, let us consider the case of two disjoint subpopulations with generation times of 10 and 14 h. In such a situation, the "faster" cells would outgrow the other variety quite soon; besides, all the β curves would be bimodal. The purpose of our modeling is to investigate the structure of the cell cycle regulation of the cultured embryonic cells.

2.2. *DERIVATION*

The model is based on the notion of the supramitotic cell cycle regulation—a program extending from a point inside one cell division cycle to an analogous point inside the next cycle. We consider cells of type 1 (smaller) and of type 2 (larger), which may switch from type i to type j with probability p_{ij}, at a size control point between cell divisions, for example, on the G_1/S phase boundary (cf. Prescott [25] for definitions of cell cycle phases). Then they proceed to division, producing progeny of identical type.

All feasible transitions are depicted in Figure 1. The convention used there is to depict these transitions as if they occurred at the time of division. Therefore, it is necessary to consider four variants of cell division cycle, (1,1), (1,2), (2,1), and (2,2), where (i,j) denotes cells born as type i that switched to type j. Figure 1a depicts the transitions in relation to the phases of cell cycle; Figure 1b is a condensed graph. The growth and division model inside the cell cycle is depicted in Figure 2.

It is assumed that the growth rate r is constant throughout the cell cycle and identical for both cell types. Daughter cells entering G_1 at size y grow to a threshold size w_i, which is a random variable with distribution density h_i depending on cell type i. The support of h_i is the closed interval $[w_{1i}, w_{2i}]$, the ends of which are the minimum and maximum threshold value, respectively. Then the cells begin DNA synthesis; that is, they enter

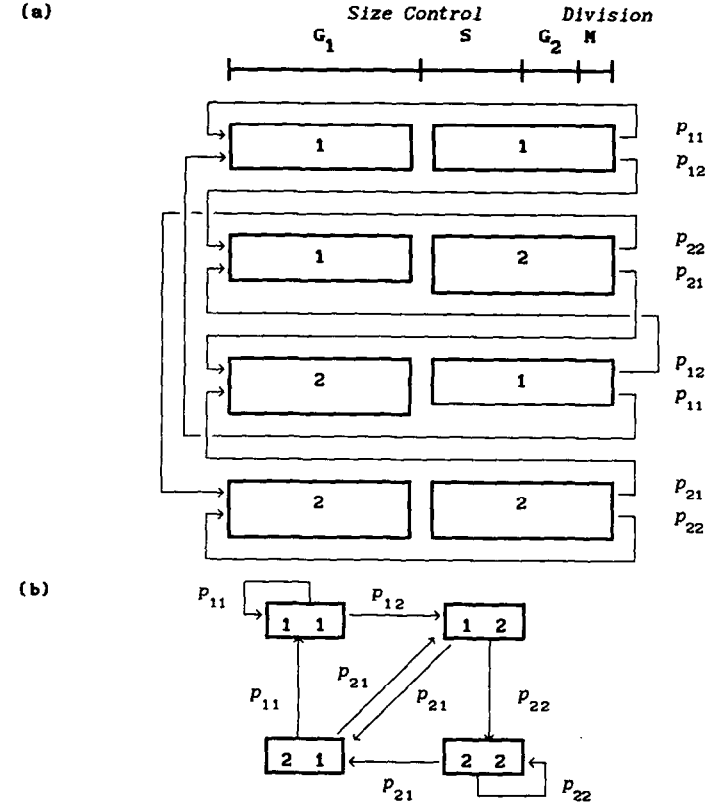

FIG. 1. The diagram depicting supramitotic cell cycle regulation and shift between two cell cycle varieties. (a) Cells switch from one cell cycle variety to the other between cell divisions, on the G_1/S boundary. Therefore, to fully describe one cell division cycle, two indices are needed, referring to the cell type before and after the G_1/S boundary is reached. Permitted transitions are from division cycle (i,j) to division cycle (j,k), with probabilities p_{jk}. (b) A simplified graph.

the S phase. Parameters of the model will be chosen in a way that excludes the possibility of a daughter cell being equal to or larger than the minimum threshold for DNA synthesis. In other words, it is guaranteed that the G_1 phase is longer than 0.

After leaving G_1, cells progress through phases S, G_2, and M toward division. Total duration of these phases is assumed to be equal to τ. During this time cells are still growing at rate r. The division is generally unequal. The size of a daughter cell derived from a mother cell of size x is a random variable y with distribution density $f(\cdot, x)$ conditional on x. The follow-

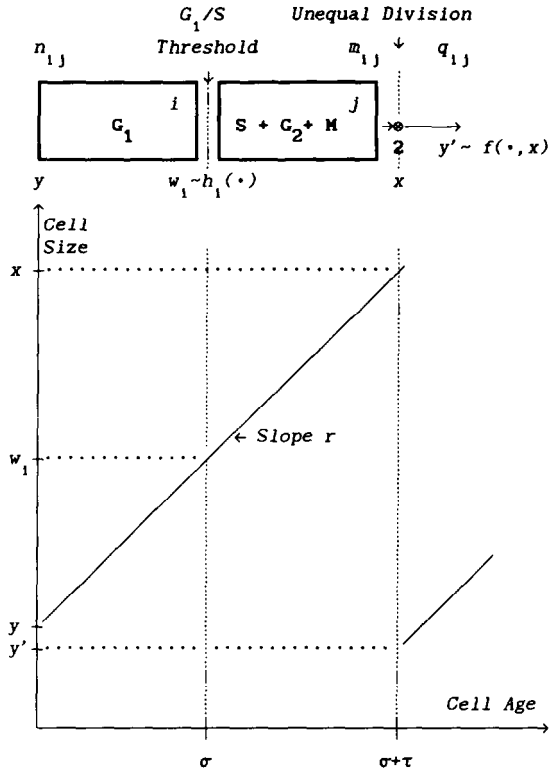

FIG. 2. The division cell cycle. Cell born as one of variety i at size y stays in G_1 for time σ, growing at rate r until it reaches the threshold size described by a random variable w_i with distribution density h_i. At this point it switches to variety j, with probability p_{ij}. Then the cell starts synthesizing DNA and passes phases S, G_2, and M in fixed time τ, growing in size at the same rate r. It divides at age $\sigma + \tau$; its size is then $x = y + r(\sigma + \tau) = y + w_i + r\tau$. The size at birth of daughter cell 1 is a random variable y' distributed with density $f(\cdot, x)$, conditional on x. The size of daughter 2 is $x - y'$. The densities of flux of the newborn and dividing (i, j) cells are denoted by n_{ij} and m_{ij}, respectively.

ing properties are satisfied: $\int_0^\infty f(y,x)\,dy = 1$; $f(y,x) = 0$, $y > x$; and $f(x-y,x) = f(y,x)$.

The dynamics of the model is described in terms of distribution densities of cell flow rates through various points of the cell cycle. First, $n_{ij}(t,y)$ is the density of flow rate of the age 0 daughter cells into G_1 phase, for the type i cells that will switch to type j at the G_1/S boundary (see Figure 2). The interpretation is that $n_{ij}(t,y)\,dt\,dy$ is equal to the number of these

cells with sizes in interval $(y, y+dy)$ that entered G_1 in time interval $(t, t+dt)$.

Analogously, $m_{ij}(t,x)$ is the density of flow rate of mother cells through division. These are cells that started as type i daughters and now are type j. Finally, $q_{ij}(t,x)$ is the flow rate density of daughter cells descending from mothers described above, before they are assigned to the G_1 phase of any of the four cell division cycle types.

The relationship between n_{ij} and q_{ij} is described by the system of equations

$$n_{ij}(t,y) = p_{ij}\left[q_{1i}(t,y) + q_{2i}(t,y)\right], \qquad i = 1,2, \tag{2.1}$$

according to the schemes in Figure 1.

The principle of unequal division implies that the relationship between q_{ij} and m_{ij} is

$$q_{ij}(t,y) = 2\int_0^\infty f(y,x)\, m_{ij}(t,x)\, dx, \qquad i = 1,2. \tag{2.2}$$

The distribution m_{ij} of the flow rate of mother cells can be found from the balance equation

$$\int_x^{x+\Delta x}\int_t^{t+\Delta t} m_{ij}(\rho,\xi)\, d\rho\, d\xi = \int_{x-\tau r}^{x+\Delta x-\tau r} h_i(w)\int_0^{w_{1i}}\int_{t-\tau-(w-\xi)/r}^{t+\Delta t-\tau-(w-\xi)/r} n_{ij}(\rho,\xi)\, d\rho\, d\xi\, dw, \tag{2.3}$$

which expresses the fact that a portion of mother cells with sizes from $(x, x+\Delta x)$ dividing in $(t, t+\Delta t)$ passed the G_1/S threshold size (w) exactly τ time units earlier and that this portion was composed of cells that were born at various sizes ξ and consequently $(w-\xi)/r$ time units before they reached the threshold size. The maximum size of the type i daughter cell is assumed to be smaller than the lowest threshold w_{1i} [hypothesis (H_*); see Section 2.3].

Dividing (2.3) by $\Delta t\,\Delta x$ and letting $\Delta t, \Delta x \to 0$ yields

$$m_{ij}(t,x) = h_i(x-\tau r)\int_0^{w_{1i}} n_{ij}\left(t - \frac{x-\xi}{r}, \xi\right) d\xi. \tag{2.4}$$

Replacing ξ by another variable, $\sigma = (x-\xi)/r - \tau$, equal to the duration

of G_1 (see Figure 2) yields

$$m_{ij}(t,x) = rh_i(x-\tau r)\int_0^{w_{1i}} n_{ij}[t-(\tau+\sigma), x-r(\tau+\sigma)]\,d\sigma. \quad (2.5)$$

For simplicity we will use the matrix-vector notation

$$n(t,y) = \begin{pmatrix} n_{11}(t,y) \\ n_{12}(t,y) \\ n_{21}(t,y) \\ n_{22}(t,y) \end{pmatrix},$$

$$H(w) = \begin{pmatrix} p_{11}h_1(w) & 0 & p_{11}h_2(w) & 0 \\ p_{12}h_1(w) & 0 & p_{12}h_2(w) & 0 \\ 0 & p_{21}h_1(w) & 0 & p_{21}h_2(w) \\ 0 & p_{22}h_1(w) & 0 & p_{22}h_2(w) \end{pmatrix}. \quad (2.6)$$

Combining equations (2.1), (2.2), and (2.5), we obtain, employing the above,

$$n(t,y) = 2r\int_0^{\infty} f(y,x)H(x-r\tau)\int n[t-(\tau+\sigma), x-r(\tau+\sigma)]\,d\sigma\,dx, \quad (2.7)$$

which is equivalent to a system of four equations (integration bounds left out for brevity).

In view of the fact that biologically there exist only two cell types, it seems intuitively true that system (2.7) should be reducible to two equations. A reduced system can be obtained if cell flows through the size control point on the G_1/S boundary are considered.

The reduction is impossible when n is used to describe model dynamics. To show this, it is sufficient to try to combine flow rates, for example, $n_1 = n_{11} + n_{21}$, $n_2 = n_{12} + n_{22}$, or $n_1 = n_{11} + n_{12}$, $n_2 = n_{21} + n_{22}$. We will use the four-dimensional model since it describes the population structure in full detail.

The expression for the total number $N_{ij}(t)$ of the (i,j)-type cells present at time t is derived in the following way. The density of cell flux into G_1, including cells born with size ξ at time ρ that will enter S after reaching threshold size w, is equal to $h_i(w)n_{ij}(\rho,\xi)$. Population at time t includes cells born before t but not earlier than one cell cycle duration before t,

that is, after $t-[\tau-(w-\xi)/r]$. Eventually,

$$N_{ij}(t)=\int_0^{w_{1i}}\int_{w_{1i}}^{\infty}\int_{t-\tau(w-\xi)/r}^{t} h_i(w)n_{ij}(\rho,\xi)\,d\rho\,dw\,d\xi, \tag{2.8}$$

where the upper bound w_{1i} reflects the fact that only cells less than minimum threshold size are allowed into G_1 [hypothesis (H_*), Section 2.3].

2.3. *ASSUMPTIONS AND SUPPORT PROPERTY*

We proceed to specify the basic hypotheses on functions f and h_i, which formalize the requirements of cell cycle dynamics.

First, we require that the inequality of division be subject to constraints. Specifically, the daughter cells are not allowed to differ too much in size.

(H_f) $f\in L^1_{\mathrm{loc}}(\mathbb{R}^2_+)$; $f\geqslant 0$; $\int_0^\infty f(y,x)\,dy=1$; $f(x-y,x)=f(y,x)$; *$f(y,x)$ is nonnegative and there exists $d_1\in(0,\frac{1}{2})$ such that $f(y,x)$ is positive if and only if $y\in(d_1x,d_2x)$, where $d_2=1-d_1$.*

Second, it is assumed that the threshold cell size required to cross the G_1/S boundary is constrained to an interval.

(H_h) *For $i=1$ and $i=2$, $h_i\in L^1_{\mathrm{loc}}(\mathbb{R}^2_+)$, $\int_0^\infty h_i(w)\,dw=1$; $h_i(w)$ is nonnegative and there exist two positive numbers w_{1i} and w_{2i}, $w_{1i}<w_{2i}$, such that $h_i(w)$ is positive if and only if $w\in(w_{1i},w_{2i})$.*

In addition, the following technical hypothesis is imposed.

$$(H_*)\qquad w_{k1}<w_{k2},\qquad k=1,2, \tag{2.9}$$

$$w_{21}>w_{12}, \tag{2.10}$$

$$w_{11}>d_2(w_{22}+r\tau). \tag{2.11}$$

The meaning of the two first conditions in hypothesis (H_*) is that cells of type 2 grow generally larger in G_1 than cells of type 1, and there is an overlap of threshold intervals of the two cell types. Condition (2.11) ensures that the model is self-consistent (see below).

As demonstrated in Appendix 1, to continue the solution n past time t, the necessary and sufficient condition is the restriction of n to the parallelogram $\mathscr{B}(t)$ defined in Equation (A1.3) in Appendix 1. We will adopt the

standard notation [16],

$$n_t(s,y) = n(t+s,y), \qquad t \geqslant 0;\ (s,y) \in \mathscr{E}(t), \tag{2.12}$$

where

$$\mathscr{E}(t) = \left\{(\rho,\xi):\ \rho \in \left(\frac{\xi}{r} + \left(t - \frac{a_2}{d_1 r}\right), 0\right);\ \xi \in [a_1, a_2]\right\} \tag{2.13}$$

is a trapezoid including parallelogram $\mathscr{B}(t)$.

From derivations in Appendix 1, it follows that the lower integration bound with respect to σ in Equation (2.7) is equal to $[x - d_2(w_{22} + r\tau)]/r - \tau$. It is nonnegative since $x \geqslant w_{11} + r\tau$ and, by assumption (2.11), $w_{11} \geqslant d_2(w_{22} + r\tau)$.

Because of support properties of n, integration over σ in Equation (2.7) can now, without complications, be carried out formally over $\mathbb{R}_+$. This simplifies many expressions.

3. ASYNCHRONOUS EXPONENTIAL GROWTH

Material of this section is of predominantly mathematical interest. Its importance for the data analysis in Section 4 stems from the fact that it is necessary to establish, in a mathematically rigorous way, the existence of asynchronous exponential growth and other fundamental relationships in our model.

3.1. *EXISTENCE OF SOLUTION AND DEFINITION AND COMPACTNESS OF SEMIGROUP*

The solution $n(t,y)$ of Equation (2.7) can be uniquely extended by steps of length θ_1 starting from initial data on $\mathscr{E}(0)$. The support of solutions does not leave the strip $\mathbb{R}_+ \times I$. The solution exists in the L^1 sense.

Let θ_1 and θ_2 ($\theta_2 > \theta_1 > 0$) be numbers such that $(-\theta_2, -\theta_1) \times I$ is the smallest rectangle containing $\mathscr{B}(0)$. This means that θ_1 and θ_2 are, respectively, the smallest and largest delay of (2.7).

The following lemma states that the solution of Equation (2.7) exists in the L^1 sense (for proof, see Appendix 2).

LEMMA 3.1

Suppose that hypotheses (H_f), (H_h), *and* (H_*) *are satisfied. If* n_0: $\mathscr{E}(0) \to \mathbb{R}_+^4$ *belongs to* $X = L^1(\mathscr{E}(0); \mathbb{R}^4)$, *then there exists a function* n: $\Omega \to \mathbb{R}_+^4$, *where* $\Omega = \mathscr{E}(0) \cup (\mathbb{R}_+ \times I)$, $n \in L^1_{\text{loc}}(\Omega)$; *which verifies* (2.7) *almost everywhere in* Ω; *and* $\lim_{t \to 0} n_t = n_0$ *in* X. *The solution is unique in the sense of an equivalence class in* $L^1_{\text{loc}}(\Omega)$.

This result also can be restated in a form more convenient for further considerations.

COROLLARY 3.1

The family of mappings $\{G(t),\ t \geqslant 0\}$,

$$G(t)\colon X \ni n_0 \to n_t \in X,$$

is a strongly continuous semigroup of positive bounded linear operators on X.

The next result requires additional technical hypotheses regarding distributions f and h_i.

$$(\mathrm{H}_\mathrm{f}') \quad f \in L^\infty_{\mathrm{loc}}(\mathbb{R}^2_+).$$

$$(\mathrm{H}_\mathrm{h}') \quad h_i \in L^\infty_{\mathrm{loc}}(\mathbb{R}_+), \qquad i = 1,2.$$

LEMMA 3.2

Under the hypotheses of Lemma 3.1 *supplemented by* (H_f') *and* (H_h'), $G(t)$ *is compact from* X *into* X *for any* $t > 3\theta_2$.

Proof of Lemma 3.2 is included in Appendix 3.

3.2. *CHARACTERISTIC EQUATION AND THE DOMINATING EIGENVALUE*

In this section, we derive and analyze the characteristic equation, which enables computation of the spectral values of the semigroup of operators $G(t)$. We also prove the existence of a dominating real eigenvalue.

The operators $G(t)$ are compact for $t > 3\theta_2$. This implies [12, theorem 11.4.1] that the spectrum of $G(t)$ is a pure point spectrum (except possibly the element $\{0\}$) composed of elements of finite multiplicity. The spectral mapping theorem for eventually compact semigroups [24, theorem A.III.6.6] implies also that all the nonzero eigenvalues are of the form $e^{\lambda t}$, where λ are the eigenvalues of the infinitesimal generator A of the semigroup (A exists because $\{G(t),\ t \geqslant 0\}$ is a strongly continuous semigroup). Since $\{G(t),\ t \geqslant 0\}$ is also a translation semigroup [15], to each eigenvalue there corresponds an eigenvector $v_\lambda \in X$ of the form $v_\lambda(\rho, \xi) = e^{\lambda\rho}\mu(\xi)$, $(\rho, \xi) \in \mathscr{E}(0)$.

The eigenvalue problem for $G(t)$ may be represented in the form

$$e^{\lambda t} v_\lambda - G(t) v_\lambda = 0, \tag{3.1}$$

which means that $e^{\lambda t} v_\lambda(0, y) = e^{\lambda t}\mu(y)$ is a solution of the evolution equa-

tion (2.7) defining the semigroup; that is,

$$e^{\lambda t}\mu(y) = 2r\int_0^\infty f(y,x)H(x-r\tau)\int_0^\infty e^{\lambda[t-(\sigma+\tau)]}\mu[x-r(\sigma+\tau)]\,d\sigma\,dx. \tag{3.2}$$

We multiply (3.2) by $e^{-\lambda t}$ and replace variable σ by $u = x - r(\sigma+\tau)$. The integration bounds for u are from $-\infty$ to $x - r\tau$. However, the support of μ is contained in $[a_1, a_2]$. Therefore, the lower bound can be set at 0. Also, if $x - r\tau \le w_{11}$, then $H(x-r\tau) = 0$; if $x - r\tau > w_{11}$, then for $u > x - r\tau$, $\mu(u) = 0$ since by (2.11) $w_{11} > a_2$. Therefore, the upper bound can be extended to ∞. Eventually,

$$\mu(y) = 2\int_0^\infty f(y,x)H(x-r\tau)e^{-\lambda(x/r)}\,dx\int_0^\infty e^{\lambda(u/r)}\mu(u)\,du. \tag{3.3}$$

Multiplying (3.3) by $e^{\lambda(y/r)}$ and integrating with respect to y, we obtain

$$\begin{aligned}(Id - B)M &\equiv \left[Id - 2\int_0^\infty\int_0^\infty f(y,x)H(x-r\tau)e^{(\lambda/r)(y-x)}\,dx\,dy\right]M \\ &= 0, \\ M &= \int_0^\infty \mu(u)\exp\left(\lambda\frac{u}{r}\right)du,\end{aligned} \tag{3.4}$$

where M is a 4-vector. This linear algebraic system has a nontrivial solution if and only if the determinant of the matrix $(Id - B)$ in (3.4) is equal to 0,

$$\det(Id - B) = 0. \tag{3.5}$$

Let us remark that $M \neq 0$, since otherwise (3.3) would imply $\mu \equiv 0$.

Condition (3.5) is equivalent to

$$I_1 I_2(1 - p_{11} - p_{22}) + p_{11}I_1 + p_{22}I_2 = 1, \tag{3.6}$$

where

$$I_i = I_i(\lambda) = 2\int_0^\infty\int_0^\infty f(y,x)h_i(x-r\tau)e^{(\lambda/r)(y-x)}\,dx\,dy, \qquad i = 1,2. \tag{3.7}$$

Equation (3.6) is the characteristic equation of our model. Its roots λ, particularly the dominating simple positive real root, provide leading terms

in the asymptotic behavior of the model. The rigorous statement is contained in the lemma below (proof in Appendix 4).

LEMMA 3.3

Under hypotheses of Lemma 3.2:

(i) *The spectrum of* $G(t)$, *for* $t > 0$, *is a pure point spectrum, consisting of isolated eigenvalues of finite multiplicity.*

(ii) *The eigenvalue* $\exp(\lambda^* t)$ *with largest absolute value is real for all* t (*i.e.*, λ^* *is real*), *and there exists no other spectral value with absolute value* $\exp(\lambda^* t)$.

(iii) *The number* λ^* *is the larger of the at most two real roots of characteristic equation* (3.6).

(iv) *The corresponding eigenvector* $v^*(\rho, \xi) = \mu^*(\xi)\exp(\lambda^* \rho)$, $v^* \in X$, *is a* 4*-vector with all the components positive.*

(v) *The function* μ^* *is obtained from*

$$\mu^*(y) = \left[2\int_0^\infty f(y,x)H(x - r\tau)e^{-\lambda(x/r)}dx\right]M,$$

where M *is a positive* 4*-vector that satisfies* $(Id - B)M = 0$.

3.3. *ASYMPTOTIC BEHAVIOR*

Throughout this section, we drop the asterisk from λ^*; it is understood that λ is the eigenvalue of the generator A with strictly dominating real part, and $\exp(\lambda t)$ is the eigenvalue of the semigroup with strictly dominating absolute value.

To state the asymptotic result about the exponential growth of the semigroup, it is sufficient to decompose the state space into the direct sum of two subspaces, the first of which is associated with the dominating eigenvalue. Trajectories on this subspace are pure exponentials $C\exp(\lambda t)$. On the complementary subspace, all the trajectories grow more slowly than $\exp(\lambda t)$. Therefore, each trajectory contains the component $C\exp(\lambda t)$, eventually "outgrowing" the remaining part of solution.

The decomposition is usually carried out by selecting as the first space the generalized eigenspace of the dominating eigenvalue. This construction is feasible if the generalized eigenspace is one-dimensional. The details are presented below.

The generalized eigenspace is defined by

$$N_\lambda = \bigcup_{k \geq 1} \operatorname{Ker}\left[(A - \lambda Id)^k\right]. \tag{3.8}$$

We know from the preceding section that $\dim \mathrm{Ker}(A - \lambda Id) = 1$. If there exists $k \geqslant 1$ such that

$$\mathrm{Ker}\left[(A - \lambda Id)^{k+1}\right] = \mathrm{Ker}\left[(A - \lambda Id)^{k}\right],$$

then $N_\lambda = \mathrm{Ker}[(A - \lambda Id)^k]$. In our case, where λ is the dominating eigenvalue of A, characterized in Lemma 3.3, this occurs with $k = 1$.

LEMMA 3.4

Under hypotheses of Lemma 3.2, $N_\lambda = \mathrm{Ker}(A - \lambda Id)$.

Proof of Lemma 3.4 is included in Appendix 5. The immediate consequence of the lemma is that N_λ is one-dimensional.

We proceed toward characterizing the asymptotic behavior of $G(t)$. Since $G(t)$ is compact (for $t > 3\theta_2$), its spectrum is pure point spectrum, composed of poles of finite order, and space X is decomposed into the direct sum of the generalized eigenspace and the generalized range of $[e^{\lambda t} Id - G(t)]$,

$$X = N_\lambda \oplus R_\lambda, \tag{3.9}$$

both of which are invariant with respect to $G(t)$,

$$G(t) R_\lambda \subset R_\lambda, \qquad G(t) N_\lambda \subset N_\lambda. \tag{3.10}$$

Therefore,

$$n_t = G(t) n_0 = G_R(t) \circ \Pi_R n_0 + G_N(t) \circ \Pi_N n_0, \tag{3.11}$$

where G_R and G_N are restrictions of G to R_λ and N_λ, respectively, and Π_R and Π_N are projections on these components of the direct sum.

For $v_\lambda \in N_\lambda$, the restricted semigroup satisfies $G_N(t) v_\lambda = e^{\lambda t} v_\lambda$. Also, $\Pi_N n_0 = \langle v'_\lambda, n_0 \rangle v_\lambda$, where v'_λ is the eigenvector of the adjoint operator G', corresponding to $\exp(\lambda t)$, chosen so that $\langle v'_\lambda, v_\lambda \rangle = 1$. The eigenvector $v'_\lambda \in L^\infty(\mathscr{C}(0))$ is nonnegative a.e. on $\mathscr{C}(0)$. Therefore,

$$\langle v'_\lambda, n_0 \rangle = \iint_{\mathscr{C}(0)} v'_\lambda n_0$$

is positive whenever $n_0 > 0$.

The spectral radius of the restriction $G_R(t)$ is $r[G_R(t)] = \exp(\omega_R t)$, where ω_R is the growth bound of the restricted semigroup (Nagel

[24, proposition A.III.1.1]),

$$\omega_R = \lim_{t \to \infty} \frac{1}{t} \ln \| G(t) \|.$$

This yields $\omega_R < \lambda$, and consequently,

$$G_R(t) \circ \Pi_R n_0 = o(e^{\lambda t}).$$

We summarize the above in a theorem.

THEOREM 3.1

Suppose that hypotheses (H_f), (H_h), (H_*), (H_f'), *and* (H_h') *are satisfied. Then, for any initial data* $n_0 > 0$, *the semigroup exhibits asymptotic exponential growth; that is,*

$$G(t)n_0 = C_{n_0} v_\lambda e^{\lambda t} + o(e^{\lambda t}). \tag{3.12}$$

λ *is the largest real root of the characteristic equation* (3.6). *The positive eigenvector* v_λ *is equal to* $v_\lambda(\rho, \xi) = e^{\lambda \rho} \mu(\xi)$, *where* $\mu(\xi)$ *is computed as in Lemma* 3.3, *and the constant* C_{n_0} *is positive if* $n_0 > 0$.

4. MODEL VERSUS DATA

4.1. *DERIVATION OF THE PEDIGREE STATISTICS*

We derive some of the basic statistical characteristics of cell population evolving according to the rules of our mathematical model. They will be compared to observations of Sennerstam [26] and Sennerstam and Strömberg [28].

The sample pedigree is presented in Figure 3. It corresponds to the experimental situation in which a cell (called the root cell of the clone or of the pedigree) is sampled at random, close to the time of its division, from an exponentially growing population and then its progeny and possibly the progeny of its progeny are recorded. Cells in the pedigree are indexed by multiindices $\sigma = (0), (0,0), (0,1), (0,0,0), (0,0,1), (0,1,0), (0,1,1), \ldots$. The number of elements in σ is equal to the generation number, and the cell number in the ith generation (from 0 to $2^{i-1} - 1$) is coded in the binary system by σ (as in Figure 3). The generation time, birthsize, G_1/S threshold size, and size at division of the cell σ are denoted, respectively, by T_σ, x_σ, w_σ, and y_σ. The type of the root cell of the pedigree is denoted (j, i_0), while for all the other cells it is either $(i_\sigma, i_{(\sigma,0)})$ or $(i_\sigma, i_{(\sigma,1)})$, depending on whether the cell's index is $(\sigma, 0)$ or $(\sigma, 1)$. The indices j and i_σ are random variables. Whenever it does not cause confusion, the parentheses will be dropped in subscripts; for example, $w_{(010)}$ will be

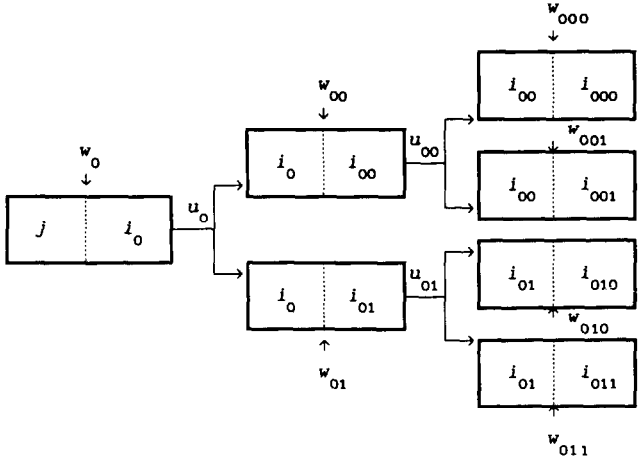

FIG. 3. The sample pedigree. The root cell of the pedigree is selected at random from the exponentially growing population. Cells in the pedigree are indexed by multiindices $\sigma = (0),(0,0),(0,1),(0,0,0),(0,0,1),(0,1,0),(0,1,1)$. The generation time, birth size, G_1/S threshold size, size at division, and the variety of the growth cycle of the cell σ are denoted, respectively, by T_σ, x_σ, w_σ, y_σ, and i_σ. Unequal division of cell σ is represented by multiplication by a random variable u_σ.

written w_{010}. The distributions of $w_{(\sigma,0)}$ and $w_{(\sigma,1)}$ are equal to h_{i_σ}. The generation time $T_{(0)}$ of the root cell cannot be measured, but its size at division can. For all the progeny cells, generation times and sizes at division can be measured.

The following assumptions are accepted to facilitate the analysis of the model.

(1) Unequal division of cell σ is represented by multiplication by a random variable u_σ. The distribution of u_σ is denoted by F_0. It has support on $[0,1]$ and is symmetric; that is,

$$F_0(0) = 0, \qquad F_0(1) = 1, \qquad F_0(u) = 1 - F_0(1-u).$$

[This yields $F(y,x) = F_0(y/x)$.] Formally,

$$y_{(\sigma,0)} = u_\sigma x_\sigma, \qquad y_{(\sigma,1)} = (1-u_\sigma)x_\sigma,$$

where u_σ is independent of x_σ.

(2) The random threshold size for a cell of type i is equal to $w_i = w_{1i} + \omega$, where the distribution of random variable ω is independent of the birth

size of the cell and of the cell type. The support of ω is $[0, w_{2i} - w_{1i}]$. It is assumed that $w_{21} - w_{11} = w_{22} - w_{12}$.

Intuitively, assumption 1 means that the inequality of cell division is proportional to its size. Assumption 2 means that the spread of the G_1/S threshold is always the same, whereas its mean depends on the cell type.

The α and β Curves. If the distribution densities h_1 and h_2 are both unimodal with sufficiently distant modes, then by Equation (2.5) the distribution of cell sizes at mitosis is bimodal. As for the β curves, let us consider the differences between lifetimes of sister and first cousin cells in the pedigree of Figure 3,

$$T_{00} - T_{01} = \frac{1}{r}\left[(w_{00} - w_{01}) - (\tilde{w} + r\tau)(2u_0 - 1)\right], \tag{4.1}$$

$$T_{000} - T_{010} = \frac{1}{r}\left[(w_{000} - w_{010}) - r\tau(u_{00} - u_{01}) - (w_{00}u_{00} - w_{01}u_{01})\right]. \tag{4.2}$$

The reasons the β_1 curve should be unimodal and the β_2 curve bimodal become apparent when expressions (4.1) and (4.2) are compared assuming equal division, that is, $u_0, u_{00}, u_{01} \equiv \frac{1}{2}$. Then,

$$T_{00} - T_{01} = \frac{1}{r}\left[(w_{00} - w_{01})\right].$$

The difference $w_{00} - w_{01}$ is unimodal because the two sister cells always have the same minimum thresholds w_{1i_0}. Consequently, the distribution of $T_{00} - T_{01}$ and the β_1 curve are unimodal also. On the other hand,

$$T_{000} - T_{010} = \frac{1}{r}\left[(w_{000} - w_{010}) - \frac{1}{2}(w_{00} - w_{01})\right]$$

includes the term $w_{000} - w_{010}$. This latter has a bimodal distribution because the minimum thresholds of two first cousins are generally different. Consequently, the distribution of $T_{000} - T_{010}$ and the β_2 curve are bimodal also. The β_3 curve, which is based on the distribution of $T_{0000} - T_{0110}$, contains contributions from one unimodal and two bimodal terms, and so it is not likely to have a distinctive bimodality. If unequal division is assumed, the analysis is complicated, but conclusions are similar.

Sister–Sister, Cousin–Cousin, and Mother–Daughter Correlations. Based on the pedigree of Figure 3 and the simplifying assumptions 1 and 2

above, the following expressions are obtained:

$$\mathrm{Cov}(T_{00},T_{01}) = \frac{1}{r^2}\Big\{\alpha_1\alpha_2(w_{11}-w_{12})^2 - \tilde{p}_1\tilde{p}_2[E(\tilde{w}_1)-E(\tilde{w}_2)](b_1-b_2) - D^2(u)E(\tilde{w}+r\tau)^2 + \Big[\frac{1}{2}-D^2(u)\Big]D^2(\tilde{w})\Big\}, \tag{4.3}$$

$$\mathrm{Cov}(T_{000},T_{010}) = \frac{\alpha_1\alpha_2}{r^2}\Big[(b_1-b_2)-\frac{1}{2}(w_{11}-w_{12})\Big]^2, \tag{4.4}$$

$$\mathrm{Cov}(T_{00},T_{000}) = \frac{1}{r^2}\Big\{{}_1\alpha_2\Big[(w_{11}-w_{12})(b_1-b_2)-\frac{1}{2}(w_{11}-w_{12})^2\Big] - \tilde{p}_1\tilde{p}_2\Big\{\frac{1}{2}[E(\tilde{w}_1)-E(\tilde{w}_2)](a_1-a_2) - \frac{1}{4}[E(\tilde{w}_1)-E(\tilde{w}_2)](b_1-b_2)\Big\} - \frac{1}{2}D^2(\omega)\Big\}, \tag{4.5}$$

$$D^2(T_{00}) = \frac{1}{r^2}\Big\{\alpha_1\alpha_2(w_{11}-w_{12})^2 - \tilde{p}_1\tilde{p}_2[E(\tilde{w}_1)-E(\tilde{w}_2)](b_1-b_2) + D^2(u)E(\tilde{w}+r\tau)^2 + \Big[2D^2(u)+\frac{1}{4}\Big]D^2(\tilde{w}) + D^2(\omega)\Big\} \tag{4.6}$$

$$D^2(T_{000}) = \frac{1}{r^2}\Big\{\gamma_1\gamma_2(w_{11}-w_{12})^2 - \alpha_1\alpha_2(w_{11}-w_{12})(b_1-b_2) + D^2(\omega) + \Big[2D^2(u)+\frac{1}{4}\Big]\Big[D^2(\omega)+\alpha_1\alpha_2(w_{11}-w_{12})^2\Big] + D^2(u)(\alpha_1w_{11}+\alpha_2w_{12}+r\tau)^2\Big\}, \tag{4.7}$$

where $D^2(u)$ denotes the identical variances of unequal division multipliers u_σ, and

$$\alpha_i = \tilde{p}_1p_{1i} + \tilde{p}_2p_{2i}, \qquad \gamma_i = \alpha_1p_{1i} + \alpha_2p_{2i},$$
$$b_i = p_{i1}w_{11} + p_{i2}w_{12}, \qquad a_i = p_{i1}b_1 + p_{i2}b_2,$$

where $i = 1, 2$. The coefficients of correlation of the sister–sister, cousin–cousin, and mother–daughter generation times can be now com-

TABLE 1

Values of Parameters of the Model of the PCC3 Cells[a]

Parameter	Symbol	Value (units)
ESS[b] *parameters*		
Probability root cell is type 1	$\tilde{p}_1$	0.518
Probability root cell is type 2	$\tilde{p}_2$	0.482
ESS G_1 /S threshold size	$E(\tilde{w})$	9.87 (rsu[c])
ESS Variance of the G_1 /S threshold	$D^2(\tilde{w})$	1.8 (rsu^2)
ESS Cell size at division	$E(\tilde{w} + r\tau)$	14.08 (rsu)
Time parameters		
Minimum time in G_1	$t_{G_1 \min}$	3 (h)
Time in $S + G_2 + M$	τ	6.9 (h)
Mean cell cycle time, type 1	$E(t_1)$	10.91 (h)
Mean cell cycle time, type 2	$E(t_2)$	14.31 (h)
Mean cell cycle time, all cells	$E(t)$	12.55 (h)
Growth and unequal division parameters		
Growth rate	r	0.61 (rsu/h)
Lower G_1 /S threshold size, type 1	w_{11}	8.87 (rsu)
Lower G_1 /S threshold size, type 2	w_{12}	10.94 (rsu)
Variance of unequal division	$D^2(u)$	0.00036

[a]Details of parameter estimation as in Section 4.3.
[b]ESS = exponential steady state.
[c]rsu = relative size unit.

puted from

$$\rho(T_{00}, T_{01}) = \mathrm{Cov}(T_{00}, T_{01})/D^2(T_{00}), \tag{4.8}$$

$$\rho(T_{000}, T_{010}) = \mathrm{Cov}(T_{000}, T_{010})/D^2(T_{000}), \tag{4.9}$$

$$\rho(T_{00}, T_{000}) = \mathrm{Cov}(T_{00}, T_{000})/\left[D^2(T_{00})D^2(T_{000})\right]^{1/2}. \tag{4.10}$$

4.2. *DATA ANALYSIS*

We have carried out numerical studies of the correlation coefficients (4.8)–(4.10) based on data in Sennerstam [26] and Sennerstam and Strömberg [28]. Table 1 is a summary of values of parameters of the model employed in the computations.

Details of Parameter Estimation. Figure 4 in [28] shows distributions of recorded 144 generation times of type 1 cells and 134 generation times of type 2 cells. From these histograms, we compute the mean cell cycle time of type 1, $E(t_1) = 10.91$ (h), and of type 2, $E(t_2) = 14.31$ (h). Then we accept the exponential steady-state (ESS) values of the probability that the root

cell is type 1, $\tilde{p}_1 = 144/(144+134) = 0.518$, and type 2, $\tilde{p}_2 = 0.482$. From this we calculate the mean ESS cell cycle time, $E(t) = \tilde{p}_1 E(t_1) + \tilde{p}_2 E(t_2) = 12.55$ (h). Based on computation in Figure 3b of [26], we accept the growth rate $r = 0.61$ relative size units (rsu) per hour. We assume that the minimum duration of the G_1 phase for the type 1 cells is $t_{G_1 \min} = 3$ (h). This last parameter is canceled out in the eventual calculations of the correlation coefficients and is introduced only for completeness.

The mean cell size at division is taken from Table 1 of [26], $E(\tilde{w} + r\tau) = 14.08$ rsu. Then we compute the minimum threshold sizes in type 1 and 2 cells,

$$w_{11} = E(\tilde{w} + r\tau)/2 + rt_{G_1 \min} = 8.87 \text{ rsu},$$

$$w_{12} = w_{11} + r[E(t_2) - E(t_1)] = 10.94 \text{ h}.$$

We assume that the mean ESS threshold sizes are not very different, and we obtain the ESS mean G_1/S threshold size,

$$E(\tilde{w}) = \tilde{p}_1 E(\tilde{w}_1) + \tilde{p}_2 E(\tilde{w}_2) = 9.87 \text{ rsu}.$$

To obtain an estimate of the variance of $\tilde{w}$, we extrapolate from the value of size variance in early G_1 provided in Table 1 of [26], 0.956^2. This yields

$$D^2(\tilde{w}) \cong 0.956^2 \left[\frac{E(\tilde{w})}{E(\tilde{w} + r\tau)/2} \right]^2 = 1.8 \text{ rsu}^2.$$

The time in phases S, G_2, and M is computed from the expression

$$\tau = [E(\tilde{w} + r\tau) - E(\tilde{w})]/r = 6.9 \text{ (h)}.$$

Finally, the variance of the unequal division multipliers u_σ is computed from the equation

$$D^2(u) = E(u^2) - \tfrac{1}{4},$$

where

$$E(u^2) = \frac{E(x^2)}{E(y^2)} = \frac{D^2(x) + E(y)^2/4}{D^2(y) + E(y)^2} = \frac{0.956^2 + 14.08^2/4}{1.833^2 + 14.08^2} = 0.00036.$$

The principal issue is now to relate the ESS values of the probabilities that root cell is type 1 or 2, $\tilde{p}_1$ and $\tilde{p}_2$, to the transition probabilities p_{ij}. Since no details about distributions h_i and of the population growth rate are known, we can obtain only very crude estimates. Therefore, we carry

out simplified computations, by assuming first that $h_1 = h_2$. This implies that the only real solution λ of the characteristic equation (3.8) satisfies $I(\lambda) = 1$. Assuming equal division and deterministic G_1/S threshold yields by (3.9),

$$I(\lambda) = 2\exp(-\lambda c),$$

where c is a positive constant.

Let us assume, as before, that $h_1 = h_2$ and $f(y,x)$ "$=$" $\delta(y - x/2)$ (Dirac's delta "function"). If we additionally assume that λ is small enough that $M \cong J\mu$, then Equation (3.4) implies

$$\begin{pmatrix} M_{11} \\ M_{12} \\ M_{21} \\ M_{22} \end{pmatrix} = \begin{pmatrix} p_{11} & 0 & p_{11} & 0 \\ p_{12} & 0 & p_{12} & 0 \\ 0 & p_{21} & 0 & p_{21} \\ 0 & p_{22} & 0 & p_{22} \end{pmatrix} \begin{pmatrix} M_{11} \\ M_{12} \\ M_{21} \\ M_{22} \end{pmatrix},$$

which yields

$$M_{12} = M_{21}, \qquad M_{11} + M_{12} = \frac{M_{12}}{p_{12}}, \qquad M_{22} + M_{21} = \frac{M_{12}}{p_{21}}.$$

If it is assumed, as we did here, that the generation times of cells of both types are similar, then the proportions of type 1 and type 2 among mothers and daughters are also similar. Therefore,

$$\tilde{p}_1 \cong \frac{M_{11} + M_{12}}{\sum_{i,j} M_{ij}} = \frac{p_{21}}{p_{21} + p_{12}}, \qquad \tilde{p} = 1 - \tilde{p}_1 \cong \frac{p_{12}}{p_{21} + p_{12}}.$$

Further computations are organized as follows. A value of p_{11} is selected from the interval $[0,1]$. Then $p_{12} = 1 - p_{11}$ and, from (4.11), $p_{21} = p_{12}\tilde{p}_1/\tilde{p}_2$. Then $p_{22} = 1 - p_{21}$. Together with the parameter estimates discussed above, this is sufficient to compute correlation coefficients from expressions (4.3)–(4.10).

Results of Computations. Numerical values of the correlation coefficients are collected in Table 2. They have been computed for values of parameter p_{11} ranging from 0.05 to 0.99. This parameter, the probability that the type 1 cell does not switch to type 2, is a measure of the "memory" present in the system.

The values of the sister–sister correlation coefficient stay positive and high for the entire range. This reflects the fact that in the model the sister cells have the variable part of their cell cycle belonging to the same type.

TABLE 2

Results of the Computations of the Correlation Coefficients of Cell Generation Times

p_{11}	Correlation coefficients of generation times		
	Sister–sister	Cousin–cousin	Mother–daughter
0.1	0.94	0.86	−0.86
0.2	0.94	0.68	−0.75
0.3	0.93	0.51	−0.63
0.4	0.92	0.36	−0.51
0.5	0.91	0.21	−0.38
0.6	0.90	0.09	−0.25
0.7	0.88	0.02	−0.10
0.8	0.85	0.01	0.07
0.9	0.81	0.16	0.29
0.925	0.79	0.25	0.36
0.95	0.77	0.35	0.44
0.975	0.75	0.54	0.53
0.99	0.73	0.68	0.55

The slow drift of this coefficient is caused by the fact that change in p_{11} is accompanied by a change in p_{22}, forced by accepting fixed values of $\tilde{p}_1$ and $\tilde{p}_2$ computed from the data.

Mother–daughter correlations increase from negative values close to -1 when p_{11} is low to positive values exceeding 0.5 when p_{11} is high. This trend is as expected, since high p_{11} implies that more frequently mother and daughter are of the same type. Cousin–cousin correlations start from high positive values when p_{11} is low, descend to a minimum for $p_{11} \cong 0.75$, and then climb to a high positive value when p_{11} is high. This behavior is caused by the fact that p_{11} low implies high probability of cell type being the same each second generation, whereas if p_{11} is high each generation is likely to be of the same type; for cousin cells high correlation is expected in both cases.

Let us note that the values of correlations computed by Sennerstam [26] from the PCC3 data are in approximate quantitative agreement with correlations in the lowest rows of Table 2, for high values of p_{11}. We return to this matter in the next section.

5. DISCUSSION

Size control has been considered repeatedly in different variants in the biological literature (cf. Fantes [14], Hola [17], Hola and Riley [18], Shields et al. [29], Tyson [30], and references therein) for cells ranging from bacteria to yeasts to mammalian cells in culture. A comprehensive paper by

Webb [32] provides mathematical references. Two principal variants have been considered, one including regulation of time in G_1, and the other regulation of the growth rate in G_1 [17, 18].

The concept of two or more alternating or successive cell cycles has been considered in the literature on unicellular organisms. An interesting example is the two-cell-type model of the population dynamics of the conical mutant of *Tetrahymena thermophila* in [22]. It is characteristic for the conical mutant that daughter cells differ significantly in size. At the temperature of 24°C, each dividing cell gives rise to the larger anterior *proter* (P) cell and to the smaller posterior *opiste* (O) cell. The generation times of the P and O cells are equal to $T_P = 3.03$ h and $T_O = 3.88$ h. The proportions of P and O cells in the proliferating population remain constant. One explanation for this is that after division the P and O cells grow to an almost identical size and then each divides into another pair of P and O cells. We omit further details, noting only the same general type of interplay between size control (not supramitotic in this case) and cell type shift as in the present model.

The unequal division models also have a long history, but their popularity seemed to be rather low, probably because of the early finding (see the review by Tyson [30]) that in bacteria the role of unequal division is negligible. More recently, precise experiments by Darzynkiewicz et al. [9, 10], followed by construction of a mathematical model [2, 21], demonstrated that unequal division is a major source of heterogeneity in Chinese hamster ovary cells in culture. Also, a major contribution of unequal division to the heterogeneity of cell counts in small colonies of cultured mouse fibroblasts (NIH 3T3 cells) has been recently discovered [20].

With respect to the PCC3 embryonal carcinoma cells considered in the present paper, the concepts of supramitotic size regulation, random shift between two cell types, and unequal division are due to Sennerstam [26] and Sennerstam and Strömberg [27, 28]. Differences in interpretation of the experimental data exist between these original communications and our present contribution. First, the assumptions of our model imply that the cell variety with higher size threshold has a longer cell cycle, opposite to what was originally assumed on the basis of indirect evidence. Second, our model does not require different growth rates for the two varieties of cells, which was originally postulated. The reason is that, in our opinion, only the existence of two different size thresholds can explain the marked bimodality in size distribution.

The interesting feature of the PCC3 cells are the bimodalities observed in the α and β_2 curves. Here, our explanation (Section 4.1) is in principle the same as that provided by Sennerstam and Strömberg [28].

The major point concerns the pedigree statistics. They do not seem to have ever been calculated before in a model of this complexity (cf. deriva-

tions in Cowan and Staudte [8]). The experimental data imply the sister–sister correlation of generation times equal to 0.95, the cousin–cousin correlation equal to 0.77, and the mother–daughter correlation equal to 0.41. Our numerical studies (Section 4.3) indicate that the present model can predict similar values only if the shift between cell types is a relatively infrequent event, that is, if the value of probability p_{11} is high.

The model discussed in this paper is of independent mathematical interest. The theory of semigroups of positive operators is as important to population models with internal structure [31] as ordinary differential equations were for the earlier models. With proper definition of the state space, various types of structures present in the population can be accommodated [23]. Since the key to basic properties of the semigroups is their strong continuity [13], the semigroup description and analysis apply most naturally to semigroups based on partial or retarded differential equations. However, it can also be successfully applied to certain Volterra equations and to other equations intermediate between integral and difference equations. Examples of these latter, stemming from cell population models, can be found in our previous works [2–4, 10].

In the present paper we carry out the analysis of a new variant of a cell cycle dynamics model based on the assumptions of supramitotic size control, random shift between two types of cells, and unequal division of cells in mitosis. The state space for the semigroup describing dynamics of this model is $L^1(\mathscr{E}(0);\mathbb{R}^4)$, where $\mathscr{E}(0)$ is a trapezoidal region in $\mathbb{R}^2$. The semigroup is strongly continuous, positive, and eventually compact. It also has support properties that are similar to irreducibility (cf. [24]) and yield analogous results: the existence of a dominating eigenvalue, which yields exponential growth in the limit. The fortunate feature of our semigroup is that the eigenvalues are obtained from a characteristic equation. Therefore, it is possible to find a relatively simple result for the mathematical object with a rich internal structure.

APPENDIX 1. THE SUPPORT PROPERTY

Support hypotheses imposed on h_i and f imply that each solution n of Equation (2.7) has the support property

$$\operatorname{supp} n(t,\cdot) \subset [d_1(w_{11}+r\tau), d_2(w_{22}+r\tau)] = [a_1,a_2] \equiv I. \quad \text{(A1.1)}$$

Indeed, on the basis of (2.4) and hypothesis (H_h),

$$\operatorname{supp} m_{ij}(t,\cdot) \subset [w_{1i}+r\tau, w_{2i}+r\tau].$$

The support of $q_{ij}(t,\cdot)$ is then $[d_1(w_{1i}+r\tau), d_2(w_{2i}+r\tau)]$ because by Equation (2.2) it is the set of all y such that $f(y,x)\neq 0$ for $x \in \operatorname{supp} m_{ij}(t,\cdot)$.

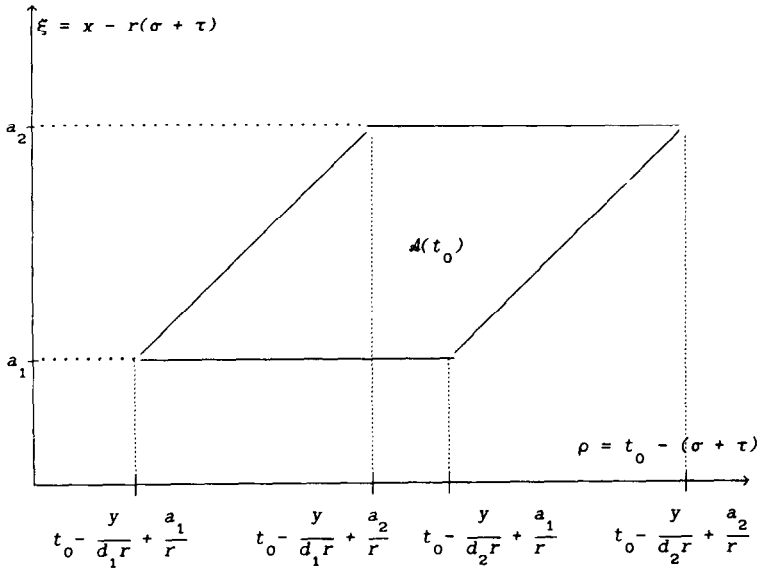

FIG. 4. Explanation of the state space of the semigroup of solutions of the model.

By hypothesis (H_f) it is necessary that $d_1 x \le y \le d_2 x$, and the last assertion follows. Finally, from Equation (2.1), we see that

$$\operatorname{supp} n_{ij}(t, \cdot) = \bigcup_{k=1}^{2} \operatorname{supp} q_{kj}(t, \cdot),$$

which yields (A1.1).

Inspection of (2.7) proves that to compute $n(t_0, y)$ it is necessary to know the restriction of n to a subset of $(-\infty, t_0) \times \mathbb{R}_+$. We will characterize this subset. Let us select $y \in I$. In Equation (2.7), integration with respect to x is carried out over interval $x \in [y/d_2, y/d_1]$ [see (H_f)]. Integration with respect to σ has to lead to $x - r(\sigma + \tau) \in I$; hence σ varies over

$$\left[\frac{x - d_2(w_{22} + r\tau)}{r} - \tau, \frac{x - d_1(w_{11} + r\tau)}{r} - \tau \right].$$

Therefore, the pair $(t_0 - (\sigma + \tau), x - r(\sigma + \tau))$ sweeps the following parallelogram $\mathscr{A}(t_0)$ (see Figure 4),

$$\mathscr{A}(t_0) = \left\{ (\rho, \xi) : \rho \in \left[\frac{\xi}{r} + \left(t_0 - \frac{y}{d_1 r} \right), \frac{\xi}{r} + \left(t_0 - \frac{y}{d_2 r} \right) \right]; \xi \in [a_1, a_2] \right\}, \tag{A1.2}$$

and when $y \in I$, it sweeps a larger parallelogram,

$$\mathscr{B}(t_0) = \left\{(\rho,\xi)\colon \rho \in \left[\frac{\xi}{r} + \left(t_0 - \frac{a_2}{d_1 r}\right), \frac{\xi}{r} + \left(t_0 - \frac{a_1}{d_2 r}\right)\right]; \xi \in [a_1, a_2]\right\}. \tag{A1.3}$$

To formally construct a solution of Equation (2.7) after time t_0, it is necessary to know it on the set $\mathscr{B}(t_0)$.

APPENDIX 2. EXISTENCE OF SOLUTIONS (PROOF OF LEMMA 3.1)

Let $|\cdot|$ denote the max norm in $\mathbb{R}^n$ or, depending on the context, the matching matrix norm in $\mathbb{R}^{n^2}$. Integrating (2.7), we obtain

$$\begin{aligned}
&\int_0^{\theta_1}\int_I |n(t,y)|\,dy\,dt \\
&\quad \le 2r\int_0^\infty |H(x-\tau r)| \int_0^{\theta_1}\int_0^\infty |n[t-(\sigma+\tau), x-r(\sigma+\tau)]|\,d\sigma\,dt\,dx \\
&\quad \le 2\int_0^\infty |H(x-r\tau)| \left\{ \iint_{\substack{\cup \mathscr{B}(t) \\ t\in(0,\theta_1)}} |n_0(\rho,\xi)|\,d\rho\,d\xi \right\} dx \\
&\quad \le 2\int_0^\infty |H(x-r\tau)| \left\{ \iint_{\mathscr{C}(0)} |n_0(\rho,\xi)|\,d\rho\,d\xi \right\} dx \\
&\quad \le 4 \iint_{\mathscr{C}(0)} |n_0(\rho,\xi)|\,d\rho\,d\xi,
\end{aligned}$$

where step 1 follows by $\int_0^\infty f(y,x)\,dy = 1$, step 2 by change of variables, $\rho = t - (\tau+\sigma)$, $\xi = x - r(\tau+\sigma)$, and by (A1.3), and step 3 by the definition of $\mathscr{C}(0)$. Step 4 is based on the estimate

$$\int |H| = \max(p_{11}, p_{12}, p_{21}, p_{22}) \int (h_1 + h_2) \le 2.$$

The result can be restated as follows:

$$\|n\|_{L^1((0,\theta_1)\times I;\mathbb{R}^4)} \le 4\|n_0\|_{L^1(\mathscr{C}(0);\mathbb{R}^4)}. \tag{A2.1}$$

We will accept $L^1(\mathscr{C}(0);\mathbb{R}^4)$ as the basic space and call it X. Equation (A2.1) yields

$$\|n_t\|_X \le 5\|n_0\|_X, \qquad t \in (0,\theta_1). \tag{A2.2}$$

Existence of solutions is obtained by iteration of the a priori estimate (A2.2). Uniqueness and nonnegativity are obvious. Continuity of n_t at $t = 0^+$ is implied by continuity of translations in L^1.

APPENDIX 3. COMPACTNESS OF THE SEMIGROUP (PROOF OF LEMMA 3.2)

Under (H_f') and (H_h'),

$$n_{|(0,\infty)\times I} \in L^\infty_{\text{loc}}\big((0,\infty)\times I;\mathbb{R}^4\big).$$

Let us consider $(t,y)\in(2\theta_2, 2\theta_2+\theta_1)\times I$ and iterate Equation (2.7) to obtain

$$\begin{aligned} n(t,y) &= 4r^2\int\int\int\int f(y,x)H(x-r\tau)f\big[x-r(\tau+\sigma),x_1\big]H(x_1-r\tau) \\ &\quad\times n\big[t-(2\tau+\sigma+\sigma_1),x_1-r(\tau+\sigma_1)\big]\,d\sigma_1\,dx_1\,d\sigma\,dx \\ &= \int\int g(t,y;\rho,\xi)n(\rho,\xi)\,d\rho\,d\xi \equiv (Kn)(t,y), \end{aligned}$$

where step 2 follows by the change of variables $(\tau_1, x_1)\to(\rho,\xi)$, $\rho = t-(2\tau+\sigma+\sigma_1)$, $\xi = x_1 - r(\tau+\sigma_1)$; and g is an L^∞ matrix function. Considering the supports of f, H, and n, it is obtained that K is a mapping from $L^\infty((0,2\theta_2)\times I,\mathbb{R}^4)$ into $L^1((2\theta_2,2\theta_2+\theta_1)\times I,\mathbb{R}^4)$.

K is compact as being defined by the integrable kernel g. Indeed, g can be approximated in the norm of L^1 by continuous functions with compact support. The corresponding operators approximate K in the operator norm from L^∞ into L^1. By the Ascoli theorem, they are compact as operators from L^∞ into C and consequently as operators from L^∞ into L^1. Therefore, K is compact as a norm limit of compact operators.

APPENDIX 4. SPECTRUM OF THE SEMIGROUP (PROOF OF LEMMA 3.3)

Let us consider real roots of the characteristic equation (3.6). First, since $\operatorname{supp} f(\cdot,x)\subset[d_1x, d_2x]$, the integration in (3.3) extends only over a region where $y-x<0$, and consequently I_1 and I_2 are strictly decreasing in λ, for λ real. Also,

$$\lim_{\lambda\to\infty} I_i(\lambda) = 0, \qquad \lim_{\lambda\to-\infty} I_i(\lambda) = \infty.$$

If $1-p_{11}-p_{22}>0$, then the left-hand side of (3.6) is strictly increasing in λ, for λ real. Therefore, there exists a single real root λ^*.

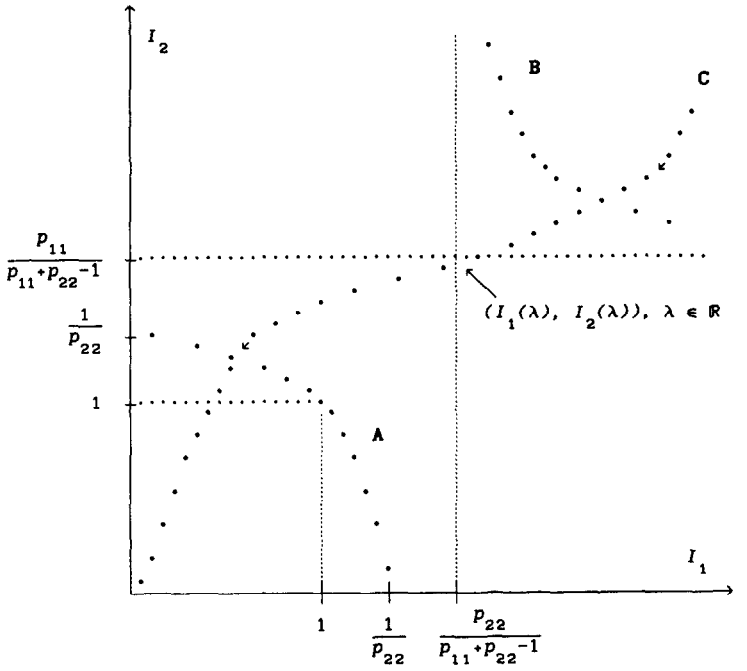

FIG. 5. Graphical interpretation of the real solutions of the characteristic equation.

The case $1 - p_{11} - p_{22} < 0$ is more complicated, since the left-hand side of (3.6) is no longer monotonous. Let us consider the points (I_1, I_2) in the positive quadrant of the plane, which satisfy (3.6) understood as an algebraic equation in I_1 and I_2. As depicted in Figure 5, these points are situated on two hyperbolic branches A and B. The curve C, the collection of points $(I_1(\lambda), I_2(\lambda))$, $\lambda \in \mathbb{R}$, intersects each of A and B in exactly one point. So, there exist exactly two real roots of (3.6) in this case, and we call λ^* the greater of them, corresponding to the intersection of curves A and C.

Existence of λ^* implies, among others, that the spectral radius of the semigroup operator $G(t)$ is positive. Since $G(t)$ is a positive and compact (for $t > 3\theta_2$) operator, the Krein–Rutman theorem (Deimling [11, theorem 6.19.2]) asserts that there exists a real eigenvalue equal to the spectral radius of $G(t)$. This eigenvalue has to be equal to $\exp(\lambda^* t)$. By the same theorem, it has a positive eigenvector $\exp(\lambda^* s)\mu^*(u)$ [which is also evident from (3.3)].

Since $\exp(\lambda^* t)$ is also the spectral radius of $G(t)$, no eigenvalue $\exp(\lambda t)$ such that $|\exp(\lambda t)| > \exp(\lambda^* t)$ may exist. We will also demonstrate that eigenvalues of $G(t)$ of the form $\exp[(\lambda^* + i\beta)t]$, $\beta \neq 0$, do not exist.

We will consider Equation (3.4). Let us denote

$$B = B(\lambda) = \left[B_{jk}\right]_{j,k=1,2,3,4},$$
$$M = M(\lambda) = \operatorname{col}\left(M_j\right)_{j=1,2,3,4},$$
$$B^* = B(\lambda^*), \qquad M^* = M(\lambda^*);$$

also,

$$|B| = \left[|B_{jk}|\right] \qquad \text{and} \qquad |M| = \operatorname{col}\left(|M_j|\right).$$

For vectors and matrices, the relation $\geqslant$ is understood componentwise, $>$ means that the inequality is strict for at least one component, and $\gg$ that it is strict for all components.

We have

$$B^*M^* = M^*, \tag{A4.1}$$

where $M^* \gg 0$. Let us suppose that $\exp[(\lambda^* + i\beta)t]$, $\beta \neq 0$, is an eigenvalue. This implies

$$B(\lambda^* + i\beta)M(\lambda^* + i\beta) = M(\lambda^* + i\beta) \tag{A4.2}$$

and

$$|B(\lambda^* + i\beta)|\,|M(\lambda^* + i\beta)| \geqslant |M(\lambda^* + i\beta)|. \tag{A4.3}$$

The definition of $B(\lambda)$ [see (3.4)] yields

$$|B(\lambda^* + i\beta)| < B^*. \tag{A4.4}$$

From (A4.3) and (A4.4), it follows that

$$B^*|M(\lambda^* + i\beta)| \geqslant |M(\lambda^* + i\beta)|. \tag{A4.5}$$

Let us suppose that this inequality is strict,

$$B^*|M(\lambda^* + i\beta)| > |M(\lambda^* + i\beta)|. \tag{A4.6}$$

The matrix B^* has a left eigenvector $N^* \gg 0$ corresponding to its eigenvalue 1, such that $N^*B^* = N^*$. Multiplying (A4.6) by N^*, we obtain

$$N^*B^*|M(\lambda^* + i\beta)| > N^*|M(\lambda^* + i\beta)|. \tag{A4.7}$$

This implies $N^*|M(\lambda^* + i\beta)| > N^*|M(\lambda^* + i\beta)|$, a contradiction. Therefore,

$$B^*|M(\lambda^* + i\beta)| = |M(\lambda^* + i\beta)|,$$

that is, $M(\lambda^* + i\beta)$ can be chosen so that

$$|M(\lambda^* + i\beta)| = M^*. \tag{A4.8}$$

This and (A4.3) imply

$$|B(\lambda^* + i\beta)|M^* \geqslant M^*. \tag{A4.9}$$

By (A4.4),

$$|B(\lambda^* + i\beta)|M^* < B^*M^* = M^*. \tag{A4.10}$$

Comparing (A4.9) and (A4.10), we obtain a contradiction, disproving the existence of eigenvalue $\exp[(\lambda^* + i\beta)t]$.

The other assertions follow.

APPENDIX 5. DIMENSION OF THE GENERALIZED EIGENSPACE (PROOF OF LEMMA 3.4)

As indicated in the beginning of Section 3.3, we drop the asterisk from λ^*. It is understood that λ is the eigenvalue of the generator A with strictly dominating real part and $\exp(\lambda t)$ is the eigenvalue of the semigroup with strictly dominating absolute value.

It is sufficient to demonstrate that the equation $(A - \lambda Id)^2 z = 0$ has the same solution as the equation $(A - \lambda Id)z = 0$. First, let us note that because the semigroup is a translation semigroup, then $z \in \mathrm{Ker}(A - \lambda Id)^2$ yields the following expression for z:

$$z(\theta, x) = e^{\lambda\theta}\big[u_0(x) + \theta u_1(x)\big], \qquad (\theta, x) \in \mathscr{O}(0),$$

and since $z \in D(A^2)$, we have $z(0, x) = \Phi(z)(x)$ and

$$[(A - \lambda Id)z](0, x) = \Phi[(A - \lambda Id)z](x),$$

where Φ is the right-hand-side operator in Equation (2.7) defining the translation semigroup at $t = 0$, that is,

$$\Phi(z)(x) = r\int_0^\infty K(x, \xi)\int_0^\infty z[-(\tau + \sigma), \xi - r(\tau + \sigma)]\, d\sigma\, d\xi, \tag{A5.1}$$

where $K(x, \xi) = 2f(x, \xi)H(\xi - r\tau)$. Consequently,

$$u_0(x) = \Phi(z), \qquad u_1(x) = \Phi\big(u_1 \otimes e^{\lambda\theta}\big). \tag{A5.2}$$

Therefore, the existence of $z \in \mathrm{Ker}[(A - \lambda Id)^2]$ is equivalent to the exis-

tence of u_0 and u_1 in $L^1(a_1, a_2)$ such that

$$u_1(x) = \Phi\big(u_1 \otimes e^{\lambda\theta}\big), \qquad u_0(x) = \Phi\big(u_0 \otimes e^{\lambda\theta}\big) + \Phi\big(u_1 \otimes \theta e^{\lambda\theta}\big). \quad (*)$$

For brevity, we denote $\Gamma_\lambda u = \Phi(u \otimes e^{\lambda\theta})$ and observe that

$$\Phi(u \otimes \theta e^{\lambda\theta}) = \frac{\partial}{\partial\lambda}\Gamma_\lambda u.$$

We observe that

$$(\Gamma_\lambda u)(x) = \int_0^\infty K(x,\xi)\exp\Big(-\frac{\lambda}{r}\xi\Big)\,d\xi\,\mathscr{L}(u), \tag{A5.3}$$

where the operator $\mathscr{L}$: $L^1((a_1, a_2), \mathbb{R}^4) \to \mathbb{R}^4$ is defined as

$$\mathscr{L}u = \int_{a_1}^{a_2} \exp\Big(\frac{\lambda}{r}\omega\Big)u(\omega)\,d\omega.$$

The 4×4 matrix B defined in (3.4) may be rewritten as

$$B = \int_{a_1}^{a_2}\int_0^\infty K(x,\xi)\exp\Big[\frac{\lambda}{r}(x-\xi)\Big]\,d\xi\,dx.$$

We have the expression

$$\mathscr{L}\circ\Gamma_\lambda = B\mathscr{L}, \tag{A5.4}$$

which will allow us to reduce Equations $(*)$ above to an equation in $\mathbb{R}^4$. Applying operator $\mathscr{L}$ to both sides of Equations $(*)$, we obtain

$$\mathscr{L}u_1 = B\mathscr{L}u_1, \qquad \mathscr{L}u_0 = B\mathscr{L}u_0 + \mathscr{L}\Big(\frac{\partial}{\partial\lambda}\Gamma_\lambda u_1\Big). \qquad (**)$$

From the first equation of $(**)$ we conclude that 1 is an eigenvalue of B associated with a positive right eigenvector (since $\mathscr{L}u_1 > 0$). Therefore, there also exists a positive left eigenvector e^* of B corresponding to eigenvalue 1, such that $e^*\mathscr{L}u_1 > 0$. Multiplication of the second equation of $(**)$ on the left by e^* yields

$$e^*\mathscr{L}\Big(\frac{\partial}{\partial\lambda}\Gamma_\lambda u_1\Big) = 0. \tag{A5.5}$$

We demonstrate that this is a contradiction. Indeed, let us note that since $H(\xi - r\tau) = 0$ if $\xi - r\tau \leq w_{11}$,

$$(\Gamma_\lambda u)(x) = \int_{w_{11}+r\tau}^{\infty} K(x,\xi)\exp\left(-\frac{\lambda}{r}\xi\right) d\xi \, \mathcal{L}(u). \tag{A5.6}$$

Therefore,

$$\frac{\partial}{\partial\lambda}(\Gamma_\lambda u)(x) = \int_{w_{11}+r\tau}^{\infty}\int_{a_1}^{a_2} K(x,\xi)(\omega-\xi)\exp\left[\frac{\lambda}{r}(\omega-\xi)\right]u(\omega)\, d\omega\, d\xi$$
$$\leq -r\tau\Gamma_\lambda u \tag{A5.7}$$

if $u \geqslant 0$. Consequently, we obtain

$$e^*\mathcal{L}\left(\frac{\partial}{\partial\lambda}\Gamma_\lambda u_1\right) \leq -r\tau e^*\mathcal{L}\left[\Gamma_\lambda(u_1)\right] = -r\tau e^*\mathcal{L}u_1 < 0,$$

which is a contradiction. The lemma is proved.

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