ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Modelling cell lifespan and proliferation: is likelihood to die or to divide independent of age?

by

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A. Schematic for Eq. (2.11)

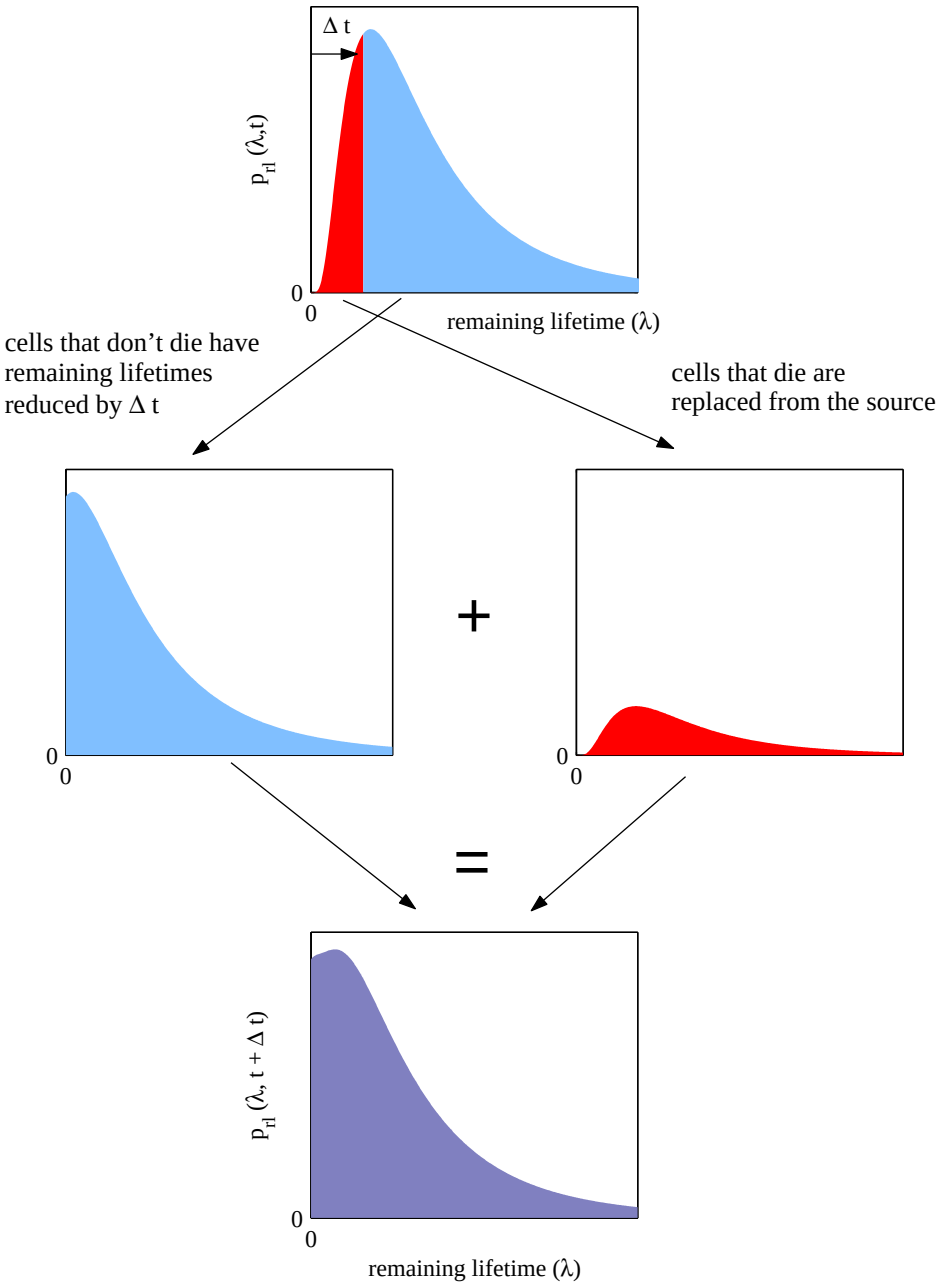

Figure 1. Diagram representing the evolution of the distribution of remaining lifetimes under Eq. (2.11). In time Δt some of the cells die (coloured red) and the same amount are replaced from the source with a fixed distribution of lifetimes. Meanwhile the cells that don't die (coloured blue) have remaining lifetimes reduced by Δt. The distribution of remaining lifetimes at a time Δt later (shaded purple) is the sum of these two contributions. Eq. (2.11) describes the continuous ($\Delta t \to 0$) limit of this process.

B. Full Models

This section is primarily the contribution of co-author Dejan Milutinović.

Dying population with external replacement

Let us denote by $P(\lambda, l, t)$ the density distribution of cells with remaining time λ and total lifetime l at the time t. The total lifetime of a cell is always greater than its remaining lifetime, i.e., $l \geq \lambda \geq 0\ \forall l, \lambda \in \Re$. The time evolution of $P(\lambda, l, t)$ distribution is defined over $\lambda \times l$ plane, and $l \geq \lambda \geq 0$ defines the region $\Omega \in \lambda \times l$ where $P(\lambda, l, t) \geq 0$. The two boundaries of the region Ω are defined by $\lambda = 0$ and $\lambda = l$, $\lambda, l \geq 0$. Outside of the region Ω, $P(\lambda, l, t) = 0$.

Let us consider a small region $S \in \Omega$ bounded by the contour C. The increase of the density distribution $P(\lambda, l, t)$ in S is equal to the flux of $P(\lambda, l, t)$ through the contour C. This may be written as

$$\begin{aligned} \int_S \frac{\partial P(\lambda, l, t)}{\partial t} \mathrm{d}S &= -\oint_C \vec{f} \cdot P(\lambda, l, t) \mathrm{d}(C\vec{n}_0) \\ &= -\int_S \nabla \cdot (\vec{f} P(\lambda, l, t)) \mathrm{d}S \quad (.1) \end{aligned}$$

where $\vec{f}$ is the vector field that produces the flux. The minus sign results from the reference direction for the vector $\vec{n}_0$ which is normal to the contour C and is chosen to point outside the region S. The last term of this equation results from Gauss' theorem (Evans 1998). Symbol ∇ is the operator $[\frac{\partial}{\partial\lambda}\vec{\lambda_0}, \frac{\partial}{\partial l}\vec{l_0}]$ with the unit vectors $\vec{\lambda_0}$ and $\vec{l_0}$ pointing in the direction of λ and l axes, respectively. The operation "$\cdot$" denotes the scalar product of the vectors. In our case the flux is the result of the remaining time λ decreasing with time, and therefore $\vec{f} = -\vec{\lambda_0}$. If we apply the limit $S \to 0$ to expression (.1) we find that the evolution of $P(\lambda, l, t)$ inside the region Ω is defined by the following partial differential equation

$$\frac{\partial P(\lambda, l, t)}{\partial t} = \frac{\partial P(\lambda, l, t)}{\partial \lambda} \quad (.2)$$

Taking into account that the total amount of cells is constant, the following boundary condition has to be satisfied

$$P(\lambda = l, l, t) = L(l) \int_0^\infty P(0, l', t) \mathrm{d}l' \quad (.3)$$

which reflects the fact that the amount of the cells leaving the region Ω at the boundary $\lambda = 0$ enters the same region at the boundary $\lambda = l$. The probability density function of the cells having lifetime l on the boundary $\lambda = l$ is given by $L(l)$. Therefore, at $t = 0$, $P(\lambda, l, t = 0) = 0$ for all $\lambda, l \in \Omega$ except for the boundary $\lambda = l$ for which $P(\lambda, l, t = 0) = L(l)\delta(\lambda - l)$, where the function $\delta(x)$ is Dirac pulse function.

In the steady-state the time derivative in (.2) is equal to zero

$$0 = \frac{\partial P^{\mathrm{ss}}(\lambda, l, t)}{\partial \lambda} \Rightarrow P^{\mathrm{ss}}(\lambda, l) = \phi(l) \quad (.4)$$

therefore, the steady-state density distribution $P^{\mathrm{ss}}(\lambda, l)$ is a function, denoted as $\phi(l)$, of one variable l. This functional dependence is also valid on the boundary $\lambda = l$, and using boundary condition (.3) we can write

$$P^{\mathrm{ss}}(\lambda = l, l) = \phi(l) = L(l) \int_0^\infty P^{\mathrm{ss}}(0, l') \mathrm{d}l' \quad (.5)$$

Now we introduce

$$P^{\mathrm{ss}}_{\mathrm{rl}}(\lambda) = \int_0^\infty P^{\mathrm{ss}}(\lambda, l') \mathrm{d}l'$$

and having in mind that $P^{\mathrm{ss}}(\lambda, l) = 0$ for $\lambda > l$, we obtain

$$P^{\mathrm{ss}}_{\mathrm{rl}}(\lambda) = \int_\lambda^\infty P^{\mathrm{ss}}(\lambda, l') \mathrm{d}l' \Rightarrow P^{\mathrm{ss}}_{\mathrm{rl}}(0) = \int_0^\infty P^{\mathrm{ss}}(0, l') \mathrm{d}l'$$

where the last integral of this expression is also the part of Eq. (.5) from which follows

$$\phi(l) = L(l) P^{\mathrm{ss}}_{\mathrm{rl}}(0) \quad (.6)$$

Let us introduce now

$$P^{\mathrm{ss}}_{\mathrm{tl}}(l) = \int_0^\infty P^{\mathrm{ss}}(\lambda, l) d\lambda$$

since we know that $P^{\mathrm{ss}}(\lambda, l) = 0$ for $\lambda > l$ and taking into account that $\phi(l)$ is not a function of λ

$$P^{\mathrm{ss}}_{\mathrm{tl}}(l) = \int_0^l \phi(l) \mathrm{d}\lambda = l\phi(l)$$

Finally, using Eq. (.6) we obtain

$$P^{\mathrm{ss}}_{\mathrm{tl}}(l) = l L(l) P_{\mathrm{rl}}(0)$$

After the normalisation of $P^{\mathrm{ss}}_{\mathrm{tl}}$, we obtain Eq. (2.13). Eq. (2.12) also follows from Eq. (.4) and Eq. (.6), which is a good check of consistancy with the derivation in Sec. 2.

Dividing population

To derive this model, we may follow the same way of reasoning as in the previous subsection. For the density distribution function we have $P(\delta, d, t) = 0$ except in the region $d \geq \delta \geq 0$ of $\delta \times d$ plane where $P(\delta, d, t) \geq 0$. Two boundaries of this region are $\delta = 0$ and $\delta = d$. Inside this region the evolution of density distribution is given by

$$\frac{\partial P(\delta, d, t)}{\partial t} = \frac{\partial P(\delta, d, t)}{\partial \delta} \quad (.7)$$

In order to take into account the cell division, the following boundary condition must be satisfied

$$P(\delta = d, d, t) = 2D(d) \int_0^\infty P(0, d', t) \mathrm{d}d' \quad (.8)$$

The probability density function of the cells having division time d on the boundary $\delta = d$ is $D(d)$. Therefore, at $t = 0$, $P(\delta, d, t = 0) = 0$ except for the boundary $\delta = d$ for which $P(\delta, d, t = 0) = D(d)\delta(\delta - d)$ where function $\delta(x)$ is Dirac pulse function.

Similarly to Sec. 3 we look for steady-state solutions of the form

$$P(\delta, d, t) = N(t)p^{\mathrm{ss}}(\delta, d).$$

By substituting this expression into the model we obtain Eq. (3.7) for the population size and the steady state probability denisty function $p^{ss}(\delta, d)$

$$p^{\mathrm{ss}}(\delta, d) = 2k\mathrm{e}^{-k(d-\delta)}D(d), \tag{.9}$$

where $k = \int_0^\infty \mathrm{d}dP^{\mathrm{ss}}(0, d)$ is given by Eq. (3.10).

From this solution we can find the steady-state marginal density $p^{\mathrm{ss}}_{\mathrm{tnd}}(\delta)$, Eq. (3.9), plotted in Fig. 5, and

$$p^{\mathrm{ss}}_{\mathrm{td}}(d) = 2(1 - \mathrm{e}^{-kd})D(d), \tag{.10}$$

plotted in the same figure.

C. Numerical Methods

In this section we describe the numerical method we used for the simulations presented in this paper. This method is based on the intuitive way in which the differential equations are derived and is thus the simplest one might think of for numerically solving those equations. The simulations in this paper are simply illustrative of the approach to equilibrium and thus the conclusions, which are based on the analytic steady state, are not dependent on the numerical method. We mainly describe the numerical simulation of the dying population with replacement of Sec. 2, and briefly mention how it is modified for the dividing population of Sec. 3.

We wish to simulate the evolution of the joint probably distribution $p(\lambda, l, t)$ of remaining lifetimes and total lifetimes within the population according to the model, Eq. (.2) and Eq. (.3). We adopt a fundamental time step Δt, chosen to be small enough that the simulation approximates a continuous process. The time step Δt is used as the time step in the forward-time evolution, and in the discretisation of the probability distributions. We represent $p(\lambda, l, t)$ as an $M \times M$ *distribution matrix* $[p_{i,j}]$, where

$$p_{i,j}(t) = \int_{(i-1)\Delta t}^{i\Delta t} \mathrm{d}\lambda \int_{(j-1)\Delta t}^{j\Delta t} \mathrm{d}lp(\lambda, l, t) \tag{.11}$$

is the proportion of cells with age between $(i-1)\Delta t$ and $i\Delta t$ and lifetime between $(j-1)\Delta t$ and $j\Delta t$. Clearly $p_{i,j} = 0$ for $i > j$ because remaining lifetime can never exceed total lifetime. The maximum lifetime that is represented in the numerical simulation is $M\Delta t$, so M must be chosen large enough such that there is only a very small fraction of the population with lifetimes greater than $M\Delta t$.

Initially all cells have their entire lifetimes remaining, so

$$p_{i,j} = p_i\delta_{i,j}, \tag{.12}$$

where $\delta_{i,j}$ is the kroneker delta ($\delta_{i,j} = 1$ if $i = j$, $\delta_{i,j} = 0$ if $i \neq j$). We choose $p_i = \int_{(i-1)\Delta t}^{i\Delta t} \mathrm{d}lL(l)$, where $L(\cdot)$ is the pre-defined source distribution (lognormal or gamma in this paper). This choice of p_i is not strictly necessary as any initial condition will evolve to the same steady-state solution, however it is a sensible choice.

As time evolves dying cells are replaced by new cells whose lifetimes are distributed according to $L(\cdot)$. In time Δt all cells represented by $p_{1,j}$ will die, as they have a remaining lifetime less than Δt. The total proportion of dead cells is

$$x = \sum_{j=1}^{M} p_{1,j}. \tag{.13}$$

All other cells decrease their remaining lifetime by Δt, while their total lifetimes do not change. Hence the distribution matrix at time $t + \Delta t$ is given by

$$p_{i,j}(t + \Delta t) = p_{i+1,j}(t) + xl_i\delta_{i,j}, \tag{.14}$$

where $l_i = \int_{(i-1)\Delta t}^{i\Delta t} \mathrm{d}lL(l)$.

After evolving for a sufficient number of time steps (typically $\sim M$) the distribution approaches a steady state. We are able to extract the distributions of remaining lifetimes and total lifetimes in the population from the numerical simulations, and compare with our analytic expectations in steady state, Eq. (2.12) and Eq. (2.13), respectively. In general we find excellent agreement by making Δt sufficiently small and M sufficiently large. Apart from checking our analytic solutions in steady state, we can also study the approach to equilibrium, for which we were not able to obtain analytic solutions.

The simulations for a dividing population, Eq. (.7) and Eq. (.8), are very similar. The number x now represents the number of cells that divide in the time step Δt, and the *population density matrix*, $[P_{i,j}]$, updates according to

$$P_{i,j}(t + \Delta t) = P_{i+1,j}(t) + 2xl_i\delta_{i,j}. \tag{.15}$$

The total size of the population is given by

$$N(t) = \sum_{i=1}^{M}\sum_{j=1}^{M} P_{i,j}(t), \tag{.16}$$

which grows in time as described in the main text.

REFERENCES

Evans, L.C. 1998 *Partial Differential Equations.* American Mathematical Society. Providence.