

Massachusetts Institute of Technology

Sloan School of Management

Working Paper

**Estimation for Replicative Senescence via a
Population Dynamics Model of Cells in Culture**

Lawrence M. Wein

Joseph T. Wu

April 18, 2000

Working Paper Number 4111

Contact Address:

Prof. Lawrence M. Wein

Sloan School of Management

Massachusetts Institute of Technology

Cambridge, MA 02142-1343

Lwein@mit.edu

<http://web.mit.edu/lwein/www/>

Estimation of Replicative Senescence via a
Population Dynamics Model of Cells in Culture

Lawrence M. Wein, Ph.D.
Sloan School of Management, MIT, Cambridge, MA, USA

and

Joseph T. Wu, B.S.
Operations Research Center, MIT, Cambridge, MA, USA

Corresponding Author:

Lawrence M. Wein
Sloan School of Management, E53-343
MIT
Cambridge, MA 02142, USA
Phone: 617-253-6697
Fax: 617-258-7579
Email: lwein@mit.edu

Running title: Estimation of Replicative Senescence

Abstract

A simple mathematical model is developed for determining the time-varying fraction of senescent cells in culture in terms of the underlying probability distribution of the number of population doublings until senescence. This functional relationship is inverted, which allows for the estimation of the probability distribution of the number of population doublings until senescence given experimental data on the time-varying fraction of senescent cells. The relationship – in particular, the lag – between these two quantities is analyzed under the assumption that the number of population doublings until senescence follows the Weibull distribution. If the number of population doublings until senescence is geometrically distributed (i.e., the Weibull with shape parameter equal to one) then the cell culture appears immortal.

Key Words: Replicative senescence, Mathematical model, Stochastic processes

A measurement goal of many *in vitro* studies is the number of population doublings until replicative senescence [1]. Typically, this random quantity is not quantified directly, but is estimated via the time-varying fraction of cells in culture that are incapable of further proliferation. This paper develops and analyzes a simple mathematical model of the relationship between these two quantities. We construct the probability distribution for the number of population doublings until senescence from the time-varying fraction of senescent cells, and explore the implications of assuming that the number of population doublings until senescence takes on certain common probability distributions (uniform or Weibull).

Materials and Methods

The mathematical model tracks the population dynamics of cells in culture. Inter-generation times are assumed to be constant and identical for all cells, giving rise to a totally synchronized system that evolves in discrete time. Time is indexed by the generation number n , and N replicating cells are initially present in generation $n = 0$. In generation $n = 1, 2, \dots$, all replicating cells from the previous generation divide into two cells, and each daughter cell is (independently of one another) a replicating cell with probability $1-h_n$ and is a senescent cell with probability h_n , where $h_0 = 0$. Senescent cells are incapable of further proliferation. Let p_n be the probability that a cell divides in generations $0, \dots, n-1$, and fails to divide thereafter; we refer to the probability mass function $\{p_n, n = 1, \dots\}$ as the *senescence time distribution*. The probabilities p_n and the hazard rates h_n are related via

$$p_n = h_n \prod_{i=0}^{n-1} (1 - h_i). \quad (1)$$

Let R_n be the number of replicating cells and S_n be the number of senescent cells at time (i.e., generation) n . Then the stochastic process (R_n, S_n) is a two-type decomposable Galton-Watson branching process [2]. The expected values of these quantities are given by

$$E[R_n] = N2^n \prod_{i=1}^n (1 - h_i) \quad \text{and} \quad E[S_n] = N \sum_{i=1}^n 2^i h_i \prod_{j=1}^{i-1} (1 - h_j). \quad (2)$$

The primary quantity of interest is the mean fraction of cells that are senescent in generation n , $E[\frac{S_n}{R_n+S_n}]$. We approximate this quantity by the ratio of the means,

$$f_n = \frac{E[S_n]}{E[R_n] + E[S_n]} = \frac{\sum_{i=1}^n 2^i h_i \prod_{j=1}^{i-1} (1 - h_j)}{\sum_{i=1}^n 2^i h_i \prod_{j=1}^{i-1} (1 - h_j) + 2^n \prod_{i=1}^n (1 - h_i)}, \quad (3)$$

and refer to f_n as the *fraction of senescent cells*. This approximation should be quite accurate because of the large number of cells typically involved. Figure 1 depicts the quantities p_n and f_n for a hypothetical example. The goal of our analysis is to understand the relationship between the p_n 's in (1) and the f_n 's in (3).

Results

Equation (3) is a triangular system of equations, and hence can be inverted to solve for the h_n 's in terms of the f_n 's. This inversion yields

$$h_1 = f_1, \quad (4)$$

$$h_n = \frac{2f_n - f_{n-1}f_n - f_{n-1}}{2(1 - f_{n-1})} \quad \text{for } n = 2, 4, 6, \dots, \quad (5)$$

and

$$h_n = \frac{f_n(4 - 2f_{n-2} - 2f_{n-1} + f_{n-2}f_{n-1}) - f_{n-1}(2 - f_{n-2})}{2(2 - f_{n-2} - 2f_{n-1} + f_{n-2}f_{n-1})} \quad \text{for } n = 3, 5, 7, \dots \quad (6)$$

Substituting (4)-(6) into (1) gives an explicit but complex expression for the p_n 's in terms of the f_n 's.

Because it is difficult to obtain any general insights from equations (4)-(6), we now let p_n take on an assumed form, and derive the resulting fraction of senescent cells, f_n . Perhaps the simplest case is to suppose that the cell senescence time is uniformly distributed on the integers between a and b . Then $p_n = \frac{1}{b-a+1}$ for $n = a, \dots, b$, and $h_n = \frac{1}{b-n+1}$ for $n = a, \dots, b$.

It follows from equation (3) that

$$f_n = \frac{2^{n-a+1} - 1}{2^{n-a+1} - 1 + 2^{n-a}(b-n)} \approx \frac{2}{2+b-n} \quad \text{for large } n. \quad (7)$$

Note that f_n is increasing and convex in n for $n < b$, and all cells are senescent by time b . To assess the lag between p_n and f_n , we compute two quantities. The first quantity is $f_{(a+b)/2}$, which is the fraction of senescent cells at the mean senescence time. The second quantity is the generation $n_{0.5}$ such that $f_{n_{0.5}} = 0.5$; i.e., the generation when 50% of the cells are senescent. Using the approximation in (7), we get

$$f_{(a+b)/2} \approx \frac{2}{2+w}, \quad (8)$$

where $w = \frac{b-a}{2}$ is the half width of the uniform interval, and

$$n_{0.5} \approx b - 2. \quad (9)$$

As a hypothetical example, suppose that the number of population doublings for human embryonic fibroblasts is uniform between $a = 50$ and $b = 70$. Then, as shown in Figure 2, there is a significant lag in the observed fraction of senescent cells: $f_{60} \approx 0.167$ and $n_{0.5} = 68$.

A more appropriate senescence time distribution is the Weibull, which is the most popular parametric family of distributions in survival analysis [3] and failure analysis [4]. The discrete-time version of this distribution has probability mass function $p_n = \theta^{(n-1)^\alpha} - \theta^{n^\alpha}$ and hazard rate $h_n = 1 - \theta^{n^\alpha - (n-1)^\alpha}$ for $n = 1, \dots$ [5]. This distribution is characterized by the shape parameter α and the scale parameter θ , where $\alpha > 0$ and $0 < \theta < 1$. The special case $\alpha = 1$ is the geometric distribution, which is the discrete-time counterpart to the exponential distribution, with constant hazard rate $1 - \theta$. More generally, the hazard rate is monotone increasing (decreasing, respectively) in n for $\alpha > 1$ ($\alpha < 1$, respectively). The mean senescence time under the Weibull assumption is

$$\sum_{i=0}^{\infty} \theta^{i^\alpha}. \quad (10)$$

Figure 3 displays the discrete Weibull distribution for several different values of α . The Weibull's popularity stems from its flexibility in describing a variety of behaviors.

Using equation (3), we find that the fraction of senescent cells is given by

$$f_n = 1 - \frac{2^n \theta^{n^\alpha}}{1 + \sum_{i=0}^{n-1} 2^i \theta^{i^\alpha}}. \quad (11)$$

If $\alpha = 1$ then $E[n] = (1 - \theta)^{-1}$ and f_n converges to $2(1 - \theta)$ as n gets large, as long as $\theta > 0.5$ (i.e., the mean senescence time is greater than two generations). For example, if $\theta = 0.98$ (the mean senescence time is 50 generations) then $f_1 = 0.020$, $f_2 = 0.030$, $f_3 = 0.035$, $f_5 = 0.039$, and the asymptotic limit of 0.04 is approached very quickly. If $\alpha > 1$ then f_n in (11) converges to 1 as n gets large. However, the convergence can be slow if the shape parameter α is sufficiently small, as shown in Figure 4.

Discussion

This paper is concerned with estimating the probability distribution of the number of population doublings until replicative senescence (referred to here as the senescence time) from experimental data on the time-varying fraction of cells in culture that are senescent. If all cells in a culture had the same senescence time then the percentage of senescent cells would instantaneously shift from 0% to 100%. In this case, the estimation problem is trivial. However, many factors (e.g., environmental conditions, growth factors, cell-to-cell interactions, intrinsic ageing) affect the proliferative behavior of cells, resulting in some heterogeneity in the senescence time. This variation among cells complicates the relationship between the senescence time distribution and the time-varying fraction of senescent cells. More specifically, the fundamental asymmetry involved – the senescent cells do not repopulate whereas the nonsenescent cells proliferate exponentially – causes the fraction of senescent cells to lag behind the senescence time distribution. This lag has not been well quantified and may be underestimated (due to the highly nonlinear nature of the asymmetry) by experimental researchers.

From a pragmatic standpoint, our results (see equations (1) and (4)-(6)) can be used

to construct a probability mass function for the senescence time from experimental data that dynamically tracks the fraction of senescent cells. However, the nature of the lag between these two key quantities is more effectively elucidated by making probabilistic assumptions about the senescence time distribution and analyzing the resulting fraction of senescent cells. If the senescence time is uniformly distributed then the lag is easily quantified (see equations (8) and (9)) and can be significant; i.e., the percentage of senescent cells at the mean senescence time can be considerably less than 50%. Because the uniform distribution is unlikely to provide a good fit to experimental data, we also considered the discrete time version of the Weibull distribution, which is a very flexible family of distributions that is widely used to measure lifetimes in medical and industrial settings.

Our most interesting result shows that if the senescence time is geometrically distributed (i.e., a discrete Weibull with shape parameter one) then the fraction of senescent cells rapidly converges to two divided by the mean senescence time; hence, if the mean senescence time was 50 generations then the fraction of senescent cells would peak at 0.04, which may be below the level of detection; i.e., these cells would appear immortal. More generally, although the fraction of senescent cells converges to 1 for the discrete Weibull distribution with shape parameter greater than 1, convergence can be slow if the shape parameter is not large.

While some studies have suggested that cells, before differentiation into somatic and germ cells, are capable of unlimited proliferation in culture, these results have been criticized and remain controversial (see [1] for a review). Our analysis may shed some light on this controversy, by showing that immortality can be achieved by somewhat modest heterogeneity (more specifically, by the exponentially decaying right tail) among cells in their senescence times.

Our mathematical model contains two simplifying assumptions. The most restrictive

assumption is that all intergeneration times are constant. There is evidence (summarized in [1]) that the reduction in cell proliferation is due not only to an increasing fraction of cells that are incapable of dividing, but also to progressively larger intergeneration times as cells age. If the elongation of intergeneration times varied deterministically in time (it appears to be roughly linear in the generation number in Figure 2 of [7]), then one can simply translate time into generation number and use our results directly. However, if intergeneration times elongate in a variable manner – as suggested in [7] – then a more complicated continuous-time branching process model would be required to determine the relative impact of larger intergeneration times and increasing fractions of nonreplicating cells on the declining proliferative capacity of cells in culture. Our model also ignores the subcultivation that typically occurs during the early stages of cell culture experiments. If some of the senescent cells are not successfully transplanted during the replating process then an underestimation of the fraction of senescent cells and an overestimation of the lag between this quantity and the senescence time distribution will occur.

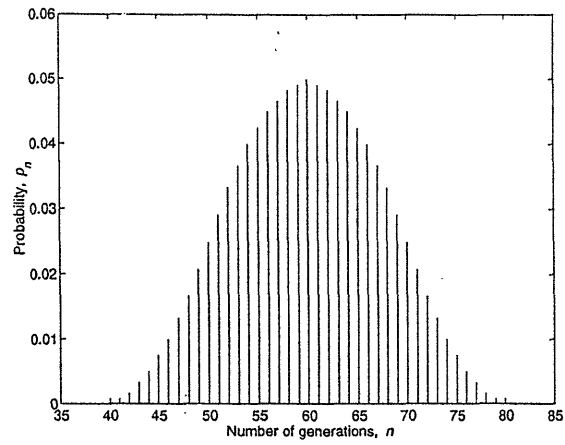
Despite these two shortcomings of the model, our analysis suggests that the lag between the fraction of senescent cells and the time to replicative senescence may be larger than previously thought, and shows how an exponential right tail in the senescence time distribution could, in theory, generate an immortal culture.

Acknowledgments

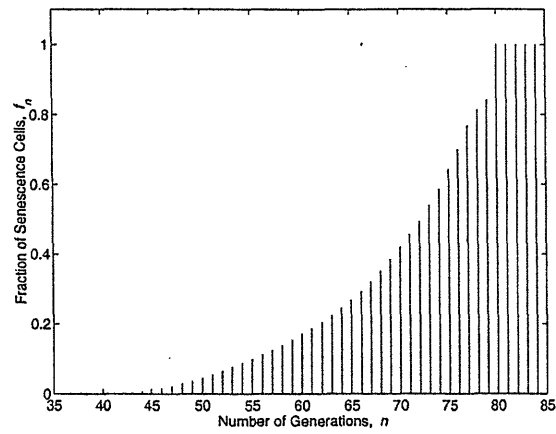
The first author thanks Bob Weinberg for posing this problem to him and for valuable discussions.

References

- [1] Cristofalo V. J., and Pinolo, R. J. (1993). Replicative senescence of human fibroblast-like cells in culture. *Physiol. Rev.* **73**, 617-638.
- [2] Harris T. (1963). "The Theory of Branching Processes," Springer-Verlag, Berlin.
- [3] Armitage, P., and Berry, G. (1987). "Statistical Methods in Medical Research," 2nd ed., Blackwell Scientific Publications, Oxford.
- [4] Barlow, R. E., and Proschan, F. (1975). "Statistical Theory of Reliability and Life Testing, Probability Models," Holt, Reinhart and Winston, Inc., New York.
- [5] Kalbfleisch, J. D., and Prentice, R. L. (1980). "The Statistical Analysis of Failure Time Data," John Wiley & Sons, New York.
- [6] Pawelec, G., Sansom, D., Rehbein, A., Adibzadeh, M., and Beckman, I. (1996). Decreased proliferative capacity and increased susceptibility to activation-induced cell death in late-passage human CD4⁺ TCR2⁺ cultured T cell clones. *Exp. Gerontol.* **31**, 655-668.
- [7] Absher, P. M., Absher, R. G., and Barnes, W. D. (1974). Genealogies of clones of diploid fibroblasts. *Exp. Cell Res.* **88**, 95-104.

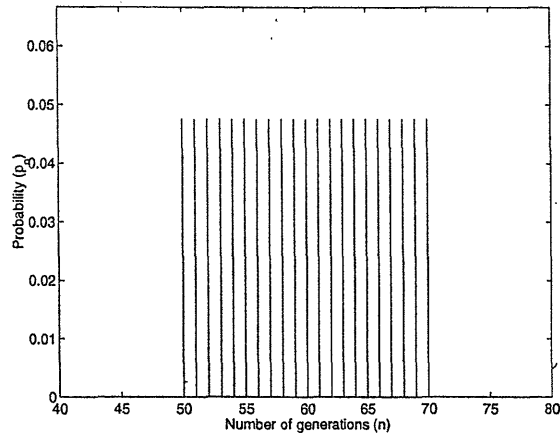


(a)

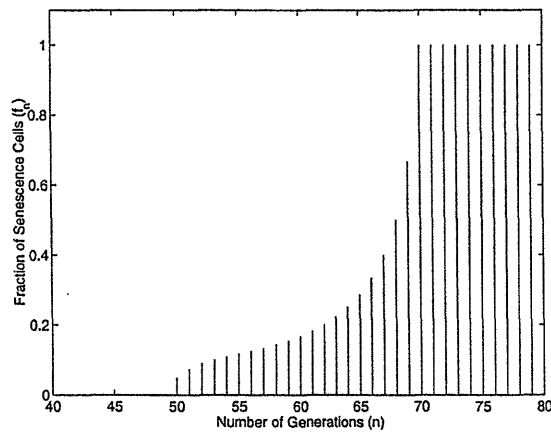


(b)

Figure 1. Figure (a) contains the probability mass function p_n for the number of generations until senescence. Given p_n , the fraction of senescent cells in culture is shown in Figure (b).



(a)



(b)

Figure 2. The lag between p_n and f_n , where p_n is uniformly distributed between 50 and 70 population doublings. Only one out of six cells are senescent at the mean senescence time (generation 60), and it is not until generation 68 that 50% of the cells become senescent.

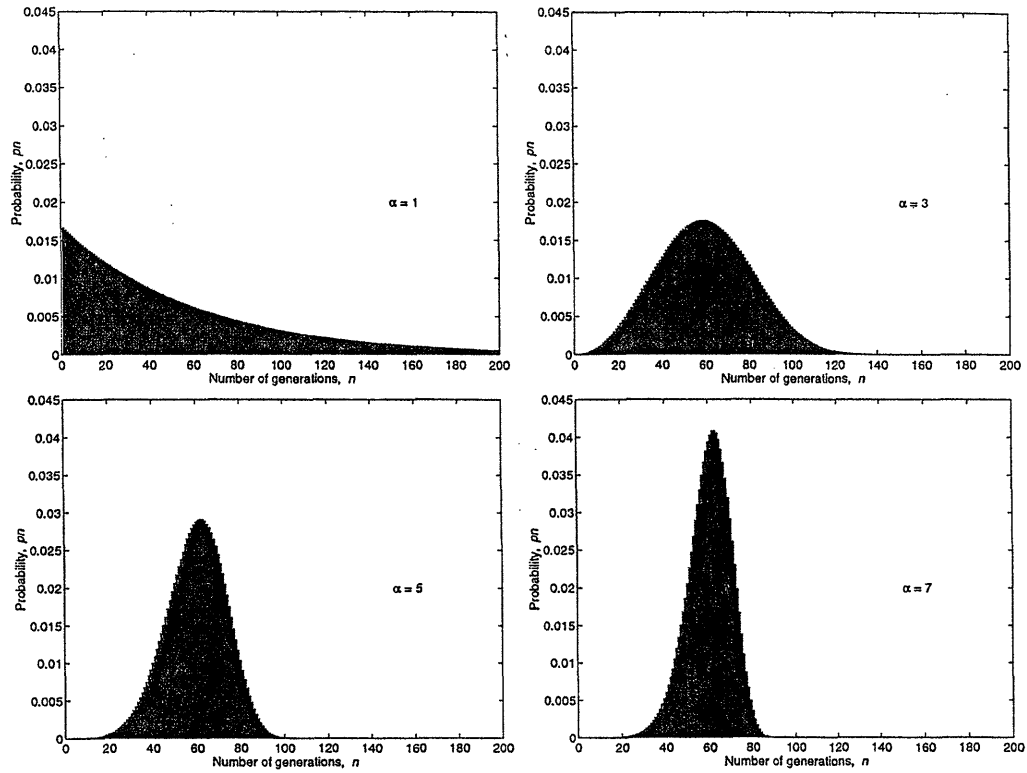


Figure 3. The probability mass function p_n of the discrete Weibull distribution with various shape parameter values α , scaled so that the mean is 60 generations.

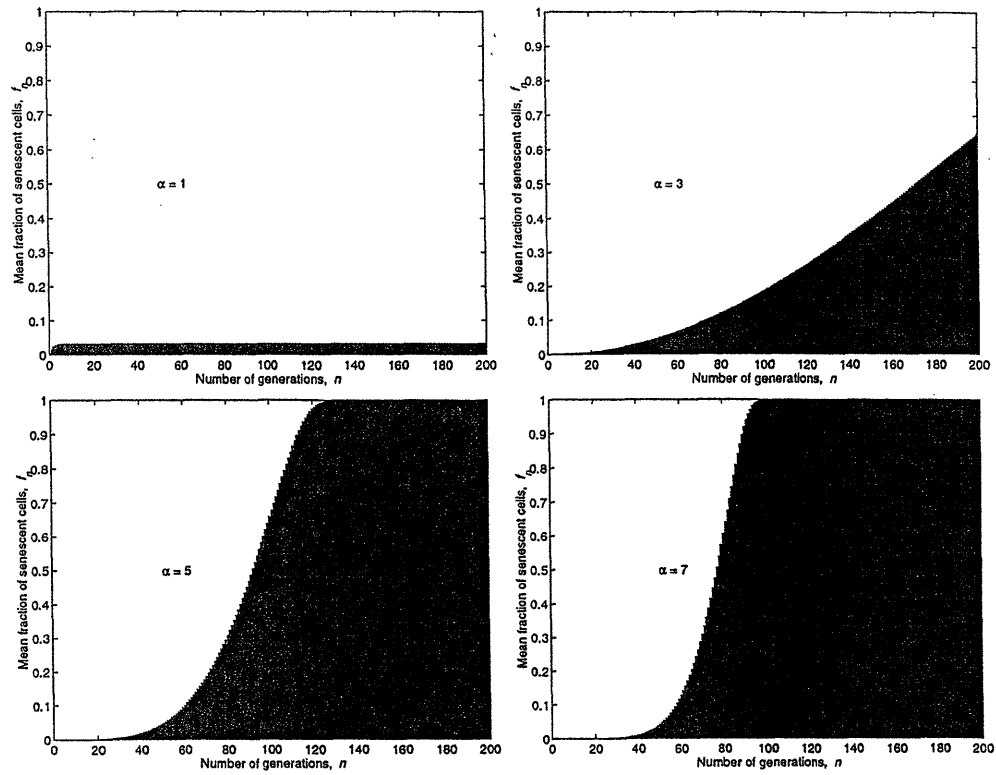


Figure 4. The fraction of senescent cells f_n corresponds to the four values of α used in Figure 3.