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Sample path properties of the average generation of a Bellman–Harris process

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Abstract

Motivated by a recently proposed design for a DNA coded randomised algorithm that enables inference of the average generation of a collection of cells descendent from a common progenitor, here we establish strong convergence properties for the average generation of a super-critical Bellman–Harris process. We further extend those results to a two-type Bellman–Harris process where one type can give rise to the other, but not vice versa. These results further affirm the estimation method's potential utility by establishing its long run accuracy on individual sample-paths, and significantly expanding its remit to encompass cellular development that gives rise to differentiated offspring with distinct population dynamics.

Keywords Bellman–Harris process · Sample-path properties · Average generation inference · Average tree depth inference · DNA coded algorithm · Two-type process

Mathematics Subject Classification 92D25 · 60J85

1 Introduction

Consider a collection of cells subject to proliferation, differentiation and death. Define the generation of each descendent to be the number of divisions that led to that cell. Generation dependent behaviour has been implicated in the risk of cancer and its evolution (Frank et al. 2003; Merlo et al. 2006; Tomasetti and Vogelstein 2015), as well as being a determiner in the complex differentiation dynamics of proliferating cell systems (Hodgkin et al. 1996; Tangye et al. 2003; Turner et al. 2008; Hills et al. 2009; Duffy et al. 2012; Zhang et al. 2013; De Boer and Perelson 2013; Marchingo

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et al. 2014). If a cell population expands asynchronously or is subject to death as well as division, then the average generation of a collection of cells cannot be inferred solely from knowledge of cell numbers, Fig. 1a, and additional information is needed to determine this quantity Fig. 1b.

A range of experimental techniques have been developed that allow evaluation or estimation of the generations of cells. Entire lineages can be recorded in vitro via time lapse microscopy so long as cells can be continuously tracked (Powell 1955; Smith and Martin 1973; Sulston et al. 1983; Hawkins et al. 2009; Gomes et al. 2011; Giurumescu et al. 2012; Richards et al. 2013). An alternate methodology is to stain initial cells with a fluorescent dye (Lyons and Parish 1994; Lyons 2000; Hawkins et al. 2007a; Quah and Parish 2012) such that with each division cells inherit approximately half of the molecules from their parent and thus fluoresce with half their intensity. A cell's generation can thus be inferred from its luminous intensity via flow cytometry. This high throughput approach is suitable for adherent cells that cannot be tracked optically, and can be used for in vivo adoptive transfer experiments where cells are transplanted from one animal into another. The transplanted cells typically exhibit a heritable marker, e.g. constitutive expression of a fluorescent protein (Hawkins et al. 2016; Duarte et al. 2018), so that their offspring are distinguishable from endogenous cells in the new animal. Experiments based on adoptive transfer have been instrumental, for example, in understanding immune responses (Buchholz et al. 2013; Marchingo et al. 2014), as well as reconstitution of the blood system after bone marrow transplant (Akinduro et al. 2018).

In most applications division tracking dyes are used to determine the distribution of a population across generations, but recent developments have created an experiment design where the offspring of individual clones can be identified via colour multiplexes of distinct division diluting dyes (Marchingo et al. 2016; Horton et al. 2018). Genetically modified mice also exist that enable an inducible equivalent of a division diluting dye in vivo without the need for adoptive transfer of ex-vivo stained cells (Tumbar et al. 2004; Foudi et al. 2009; Mascré et al. 2012). These methods enable 6–10 generations to be followed before fluorescent signal-to-noise ratio is too low for a cell's generation to be reliably determined.

Methods to estimate replicative tree depth in vivo have been proposed that involve measurement of average telomere length (Harley et al. 1990; Allsopp et al. 1992; Vaziri et al. 1994; Weinrich et al. 1997; Rufer et al. 1999; Hills et al. 2009) or the number of somatic mutations introduced during DNA duplication (Shibata et al. 1996; Tsao et al. 2000; Shibata and Tavaré 2006; Wasserstrom et al. 2008; Reizel et al. 2011; Carlson et al. 2012). Methods in this direction rely on inference rather than direct determination, but they offer the possibility of tracing more than 10 generations in vivo.

We recently proposed a new design for in vivo inference of average generation that relies on a DNA coded randomised algorithm (Weber et al. 2016). For illustration, consider a single initial cell at time $t = 0$. As in Fig. 1a, b, let $Z(t)$ be the number of offspring alive at time t and $G(t)$ be the sum of the generations of all living cells at that time. The proposal to infer $G(t)/Z(t)$ in Weber et al. (2016) is to equip the initial cell with a neutral label, i.e. one whose presence or absence has no ramifications for population dynamics, such that during each cell's lifetime, immediately prior to
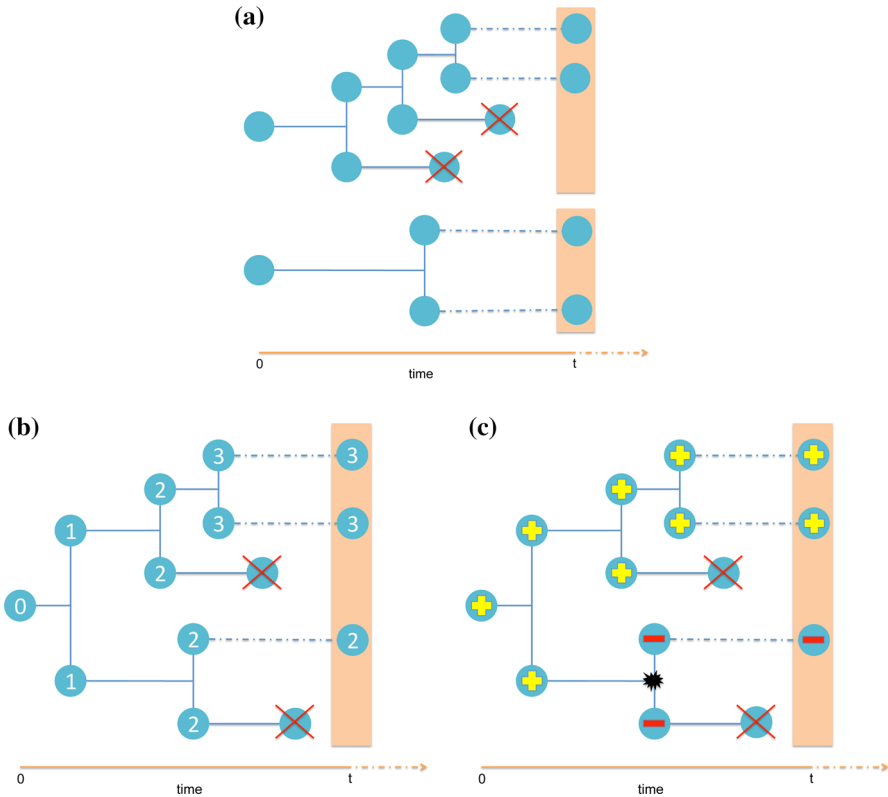
Fig. 1 Average generation. **a** If a population of cells grows asynchronous or is subject to death, knowledge of the number of cells alive at a single time (orange box, time t, $Z(t) = 2$) does not uniquely determine the average number of divisions that lead to to the living cells (i.e. the depth of the family tree). **b** With the progenitor being defined to be in generation 0, the total generation of the process at any time is the sum of the generations, the number of edges back to the root of the tree, of living cells (orange box, $G(t) = 3 + 3 + 2 = 8$) and the average generation is the total generation divided by the number of living cells, $G(t)/Z(t) = 8/3$. **c** The randomised algorithm proposed in Weber et al. (2016) for inferring $G(t)/Z(t)$ is based on having a neutral label in the initial cell that is independently lost with probability p during each cell's lifetime (indicated by the black cloud) and is not regained by further offspring once lost. If the proportion of label-positive cells can be measured and the probability of label loss, p, is small, then the following relationship holds $G(t)/Z(t) \approx -1/p \log(Z^+(t)/Z(t))$ in two approximate senses more fully explained in the main text (colour figure online)

cell division, with a small probability p the label is irrevocably and heritably lost. Thus either all the offspring of a label-positive cell have the label, which occurs with probability $1 - p$, or all do not, which occurs with probability p. With $Z^+(t)$ denoting the number of label positive cells at time t, as in Fig. 1c, the suggested estimator is

$$\frac{G(t)}{Z(t)} \approx -\frac{1}{p} \log\left(\frac{Z^+(t)}{Z(t)}\right), \text{ assuming } p \text{ is small.} \tag{1}$$

This surprising formula is desirable for a number of reasons: (1) it allows for cell death; (2) it does not require knowledge of cell cycle times; and (3) for inference it requires only a proportional measurement rather than absolute numbers. Moreover, to infer the relative developmental depth of two populations equipped with the system, one does not need to know p, the probability of label loss per cell lifetime, if it is the same for both. A DNA coded randomised algorithm, based on the existing FUCCI cell cycle reporter (Sakaue-Sawano et al. 2008), to realise the design is proposed in Weber et al. (2016).

Two distinct derivations of the approximation (1) are provided in Weber et al. (2016). One, based on properties of cumulant generating functions, establishes that for an arbitrary lineage relationship between the cells constituting $Z(t)$, the expected number of label-positive cells, $\mathbb{E}(Z^+(t))$, over all possible delabellings recovers the correct value as the probability of label loss goes to zero:

$$\frac{G(t)}{Z(t)} = \lim_{p \to 0} -\frac{1}{p} \log \left(\frac{\mathbb{E}(Z^+(t))}{Z(t)} \right).$$

For a single realisation of the delabelling process, as would occur experimentally, this provides no assurance. To establish such a result, some structure is needed on the family tree. Consequently, a complementary result is also established in Weber et al. (2016) within the context of the standard model of an asynchronously developing tree, the Bellman–Harris branching process (Harris 1963; Athreya and Ney 1972; Jagers 1975; Haccou et al. 2005). That is, a growing tree model where cells have i.i.d. lifetimes and independent i.i.d. numbers of offspring numbers at the end of their lives. With $Z(t)$ being number of cells alive at time t in a super-critical Bellman–Harris branching process, so long as the label-positive sub-population $Z^+(t)$ is super-critical, it is established in Weber et al. (2016) that

$$\lim_{t \to \infty} \frac{\mathbb{E}(G(t))}{t\,\mathbb{E}(Z(t))} = \lim_{p \to 0} \lim_{t \to \infty} -\frac{1}{pt} \log \left(\frac{Z^+(t)}{Z(t)} \right), \text{ almost surely if } \liminf_{t \to \infty} Z^+(t) > 0. \tag{2}$$

The right hand side of this equation says that as long as the label-positive sub-population continues to exist, ultimately the estimate of average generation converges on each single path of the process. To provide an intuitive explanation as to how this equality arises in the context of a branching process, we require some additional concepts. With L being the lifetime distribution and $h > 1$ being the average number of offspring, let $\alpha := \alpha(h)$ be the Malthusian parameter, i.e. the solution to

$$h\mathbb{E}(e^{-\alpha(h)L}) = 1. \tag{3}$$

Proposition 1 of Weber et al. (2016) proves that $\alpha(h)$ is a real analytic function of h and hence it coincides with its Taylor expansion. Based on well-known almost sure convergence results (Jagers 1969b; Athreya and Kaplan 1976), Theorem 1 of Weber et al. (2016) establishes that the behaviour of $Z^+(t)$ for small p is given by

$$Z^+(t) \approx \mathcal{Z}_p^+ e^{\alpha(h(1-p))t} = \mathcal{Z}_p^+ e^{[\alpha(h)-h\alpha'(h)p+O(p^2)]t},$$

where $\mathcal{Z}_p^+ := \lim_{t\to\infty} e^{-\alpha(h(1-p))t} Z^+(t)$ is a random pre-factor. Using the exponential growth rate of $E(Z(t))$ (e.g. Harris 1963, pg. 142), by identifying the growth rate of $E(G(t))$ (Weber et al. 2016, Theorem 2), it is shown that both sides of (2) converge to the constant $h\alpha'(h)$. While this branching process result further motivates the estimator, the left hand side of (2) is not entirely satisfactory. It is an average quantity over realisations of the branching process and it forms the ratio of expectations, $\mathbb{E}(G(t))/\mathbb{E}(Z(t))$, rather than the expectation of the ratio $\mathbb{E}(G(t)/Z(t)))$.

In the present paper we make two mathematical advances that further enhance the promise of the proposed method. We first rectify this shortcoming by proving a substantially stronger result: that for a Bellman–Harris branching process the sample-path average generation divided by time converges almost surely to a constant, giving

$$\lim_{t\to\infty} \frac{G(t)}{tZ(t)} = \lim_{p\to 0} \lim_{t\to\infty} -\frac{1}{pt} \log\left(\frac{Z^+(t)}{Z(t)}\right), \text{ almost surely if } \liminf_{t\to\infty} Z^+(t) > 0.$$

$$(4)$$

The convergence result on the left hand side greatly strengthens the only previous result we are aware of, that proved in Samuels (1971) where convergence in probability of average generation is established for processes in which there is no death. Given the ubiquity of Bellman–Harris processes, it is likely to be of interest for other reasons, but for our purposes it is most significant in providing extra support for merits of the proposed average generation inference methodology.

In order to establish this fact we prove a collection of surprising results for the paired processes $(Z(t), G(t))$ of a super-critical Bellman–Harris process. In particular, we show that

$$\lim_{t\to\infty} \left(e^{-\alpha t} Z(t), t^{-1} e^{-\alpha t} G(t)\right) = (c_1 \mathcal{Z}, c_2 \mathcal{Z}), \tag{5}$$

where $\mathcal{Z}$ is a random variable and c_1, c_2 are constants. Namely, even though the total generation advances at a different rate to the population size, the random element of the prefactor is the same for both, and properties of the ratio $G(t)/Z(t)$ follow.

To establish those results we use a combination of both old and novel arguments, essentially following the methodology described by Harris (1963), but relying on a peculiar renewal theorem, inspired by results of Asmussen (1998), for what are is known as defective probability measures, which are measures whose total mass is smaller than 1 (Resnick 2013, Chapter 3). The Malthusian parameter can be thought of as determining an exponential tilt that identifies a measure with density $h \exp(-\alpha t) dP(L \le t)$. That is a probability measure as it integrates to 1 thanks to Eq. (3). Defective probability measures, however, naturally arise in the study of the higher moments of branching processes as one encounters renewal equations with more extreme exponential tilts, $\exp(-k\alpha t)$ for $k > 1$, resulting in measures that integrate to less than 1. The new results allow us to obtain an integral formulation for the probability generating functions of the prefactors described above. To clinch the

result, we essentially insert the guess that the randomness in the prefactors of the two processes is the same.

The second contribution of the present paper is to provide mathematical support that significantly extends the remit of the average generation estimation scheme by considering a two-type super-critical Bellman–Harris process with one-way differentiation, where cells of the first type can differentiate into cells of the second, but not vice versa (e.g. Fig. 2). Assuming, as before, a cell of the first type is equipped with a neutral label that is heritably lost at each division with a given probability, we establish that a relationship akin to that given in (4) holds for both cell types irrespective of the relative size of their Malthus exponents. Namely, if one starts with a single cell of one type that can differentiate and generate a second type, one can ultimately drawn inferences about the average generation of each cell type. This encompasses, for example, scenarios where healthy cells may give rise to quickly growing cancer cells, or quickly expanding multipotent progenitors that give rise to slowly dividing terminally differentiated cells.

In the current article, our focus is on the relationship in a closed population between the population size, and the sum of the generations of all living cells. As a result, we employ the original Bellman–Harris branching process framework where cells only give rise to offspring at the end of their lives, and the key quantities recorded are population number and, for our study, total generation. For the applications we have in mind, this suffices.

Since their introduction, however, age dependent branching processes have been subject to extensive mathematical study, and naturally arise in diverse applications from the life-sciences to queueing theory and beyond. Those studies have resulted in substantial generalisations of the framework that include, for example, populations with exogenous immigrants, populations where individuals can give rise to offspring during their lives or can have offspring number distributions that depend upon lifetimes, multi-type populations that consist of individuals of more than one type, each with distinct proliferation and differentiation parameterizations. Other important mathematical developments include the treatment of branching random walks and generalisations that allow the study of general functionals of the population (Athreya and Ney 1972; Jagers 1975, 1989, 1992; Asmussen and Hering 1983; Haccou et al. 2005; Athreya and Jagers 2012).

So many of these developments have found application in the study of cell systems that it is not possible to provide a comprehensive list, and so we give only a few illustrative examples. The classical, experimentally-supported model of cell cycle model, the Smith–Martin Model (Smith and Martin 1973), has been studied through the lens of a multi-type branching process (Nordon et al. 2011). The widely used Cyton Model (Hawkins et al. 2007b) of lymphocyte can be naturally described as a binary branching process where offspring numbers are not independent of life-times and has been analysed as such (Subramanian et al. 2008; Duffy and Subramanian 2009). A recent high-profile study drawing inferences on the differentiation order in immune responses relied on emperical fitting of distinct multi-type branching process to data (Buchholz et al. 2013).
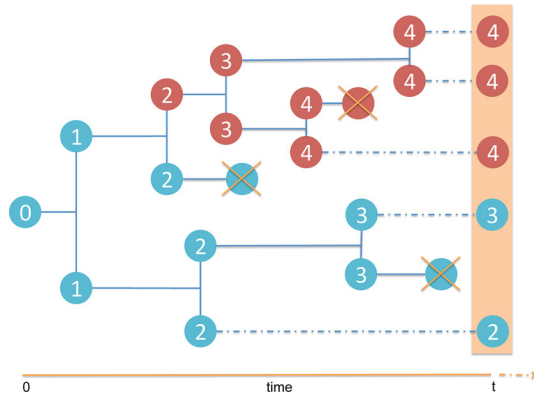
Fig. 2 Two-type process. In addition to division and death, a cell may differentiate into another type (indicated here by a change in colour) with distinct proliferation properties. For many scientific questions, one is interested in the average generation of cells of each type. The figure describes the growth of a population that starts with one cell of type-1 at time 0 and, after consecutive divisions, consists of 5 cells at time t. The average generation of cells of type-1, the blue cells, is $(3 + 2)/2 = 2.5$, while for cells of type-2, the red cells, it is 4 (colour figure online)
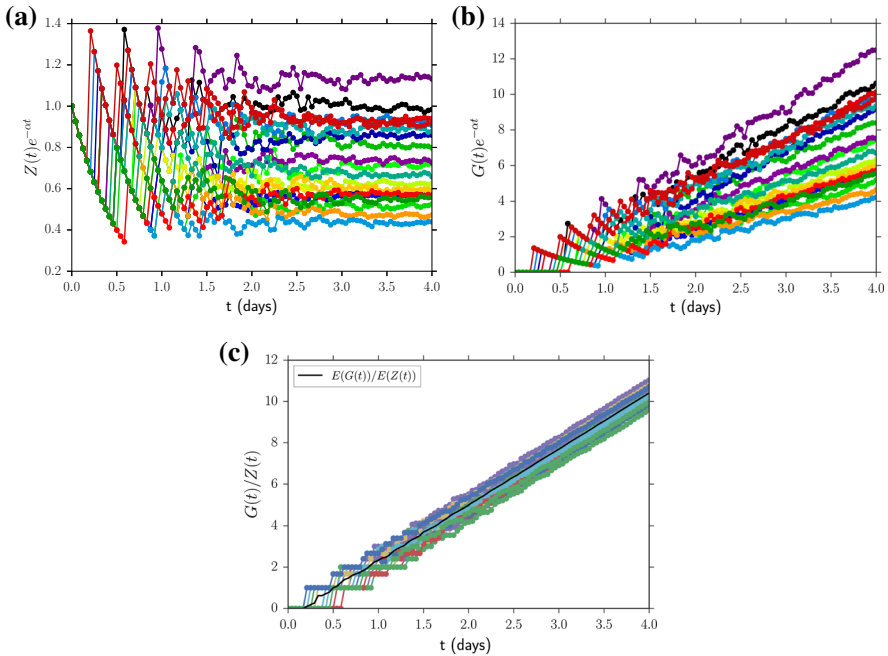
2 Motivation for the main mathematical result

A time-dependent model of a family tree is necessary to investigate the temporal dyamics of average generation. Analysis is trivial in the simplest such stochastic model, the Galton–Watson branching process (Watson and Galton 1875; Harris 1963; Kimmel and Axelrod 2002). It assumes that all cells of a given generation share a common lifetime at the end of which they produce i.i.d. numbers of offspring for the next generation. If t_n is the time of birth of the nth generation, then the total generation is simply $G(t_n) = nZ(t_n)$. Consequently, the well known result for the limit behviour of $Z(t_n)$ as n becomes large in the super-critical case (Harris 1963, Chapter 1) also describes the prefactor on front of the distribution of $G(t_n)$,

$$\lim_{n \to \infty} \frac{Z(t_n)}{h^n} = \mathcal{Z} \implies \lim_{n \to \infty} \frac{G(t_n)}{nh^n} = \mathcal{Z} \tag{6}$$

where $h > 1$ is the average number of offspring, $\mathcal{Z}$ is a non-negative random variable such that $\mathbb{E}(\mathcal{Z}) = 1$, and the equalities in (6) are meant in distribution.

On relaxing the constraint that all lifetimes are equal, however, there seems to be little *a priori* reason why the analogous quantity to $\mathcal{Z}$ in (6), which is $\mathcal{Z}$ in (5), should be shared by both $Z(t)$ and $G(t)$. Moving away from synchronicity, if the lifetimes of cells are i.i.d. positive and non-lattice random variables, the development forms a Bellman–Harris branching process (Harris 1963; Kimmel and Axelrod 2002). In that setting, cells are spread across generations and the ratio $G(t)/Z(t)$ is no longer deterministic. As $\mathbb{E}(G(t))/(t\mathbb{E}(Z(t)))$ converges to a constant (Weber et al. 2016), it is reasonable to suspect that the average generation will still grow linearly in time. That possibility is also suggested by Fig. 3, where, for independent simulations of a super-critical Bellman–Harris process with Malthusian parameter α defined in (3),

Fig. 3 Growth rates of population size, $Z(t)$, total generation, $G(t)$, and average generation, $G(t)/Z(t)$, of a super-critical Bellmann–Harris process. Each plots present 20 Monte Carlo simulations of a Bellmann–Harris branching process starting at $t = 0$ with a single cell, where paths are conditioned to have living cells at the final time-point of the simulation. Lifetimes are lognormal with mean 9.3 hours and standard deviation 2.54, which coincide with those measured for murine B cells stimulated in vitro with CpG DNA Hawkins et al. (2009). At the end of each cell's life it gives rise to no cells with probability 1/5 and two with probability 4/5. **a** With $Z(t)$ being the population size at time t and $\alpha > 0$ being the Malthusian parameter defined in Eq. (3), this figure plots the evolution of $Z(t)/e^{\alpha t}$, which is known to converge almost surely and in mean square to a random variable A (Harris 1963). **b** With $G(t)$ denoting the total generation of the process (see Fig. 1) at time t, for the same paths this plot shows $G(t)/e^{\alpha t}$, which grows linearly over time with a random slope B. Results in Sect. 3.4 establish that A and B are almost surely the same, up to a multiplicative constant, on a path-by-path basis. Thus the average generation process, $G(t)/Z(t)$, grows linearly in time, but with the same slope for every path. This can be seen empirically in panel **c** where 20 instances of this process are plotted (solid lines with markers), as well as $\mathbb{E}(G(t))/\mathbb{E}(Z(t))$ (solid black line)

$Z(t)e^{-\alpha t}$, $G(t)e^{-\alpha t}$, and $G(t)e^{-\alpha t}/Z(t)e^{-\alpha t}$ are plotted, illustrating the factor t in the ratio between the first two.

Collating observations across multiple simulations, however, Fig. 4 suggests something analogous to (6) is taking place. Figure 4a plots the empirical cumulative distribution function of the renormalised total cell numbers and total generation at a large time, suggesting equality in distribution. Figure 4b displays a scatter plot of the per-simulation prefactors of those quantities for large t. There is a strong positive correlation in these values, hinting at their relatedness. Finally Fig. 4c shows sample-paths of the difference between the renormalised total cell numbers less renormalised total generation, which appears to be converging to zero. This further suggests convergence in probability of the sample-path average generation of a Bellman–Harris

(a)

(b)

(c)
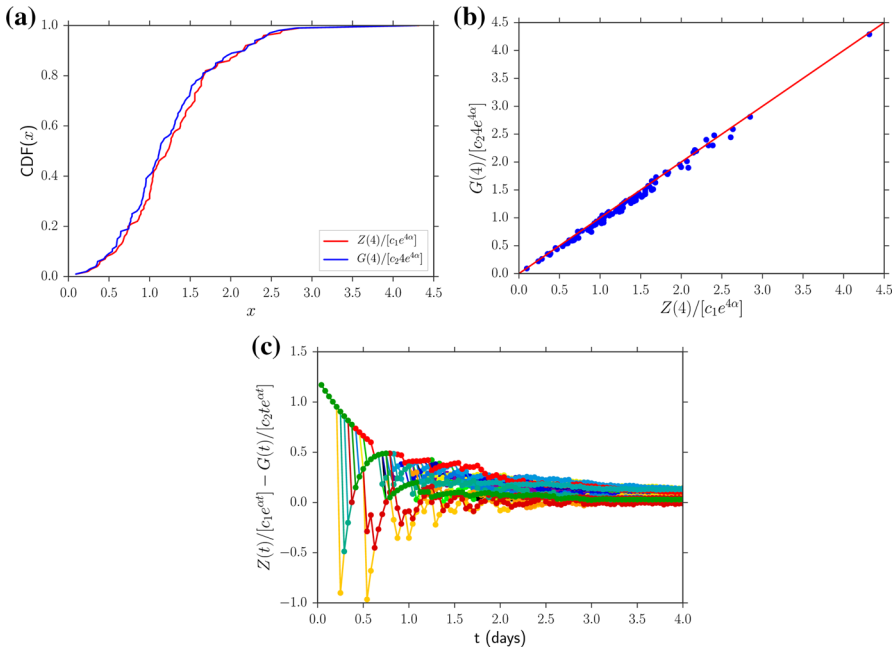
Fig. 4 Comparison between simulations of $Z(t)/e^{\alpha t}$ and $G(t)/(te^{\alpha t})$. These show results from 100 Monte Carlo simulations of a Bellman–Harris process with paramaterization as in Fig. 3. **a** At $t = 4$ days, empirical cumulative distribution function (ECDF) of $Z(t)/(c_1 e^{\alpha t})$ and $G(t)/(c_2 t e^{\alpha t})$ are shown, where c_1 and c_2 are constants that normalise the limit behaviour of means of the two processes and are computed numerically. The ECDFs of the prefactor on the population size and the slope of the total generation process are similar suggesting that they follow the same distribution. **b** Also at $t = 4$ days, the scatter plot of $Z(t)/(c_1 e^{\alpha t})$ versus $G(t)/(c_2 t e^{\alpha t})$ on a path-by-path basis suggests a stronger result, that there is equality almost surely. This impression is further informed by plot (c) where 20 paths describing the evolution over time of $Z(t)/(c_1 e^{\alpha t}) - G(t)/(c_2 t e^{\alpha t})$, which appear to converge to zero as t increases, are displayed

process, conditional on survival. Thus, even though $G(t)/Z(t)$ is no longer deterministic, the randomness in $G(t)/Z(t)$ does not reside in the linear term, but in something smaller. This is something that we formally establish in this paper. It arises as consequence of Theorem 3 and is stated formally in Corollary 3.

3 Convergence of the normalised average generation of a super-critical Bellman–Harris process

3.1 Assumptions, notation and previous results

The following notation and assumptions are in force throughout Sect. 3. We consider a Bellman–Harris branching processes with strictly positive non-lattice lifetime random variable L and non-negative offspring random variable N. We define $h := \mathbb{E}(N)$ and $v := \mathbb{E}(N(N-1))$, and assume that both are finite. We work within the super-critical

case, $h > 1$, so that the population has a positive probability of escaping extinction (Harris 1963).

We make use of the Malthusian parameter α defined in (3). As $h > 1$, $\alpha > 0$ exists and is unique. For $h > 1$, it is established in Proposition 1 of Weber et al. (2016) that the Malthusian parameter α is a real analytic function of h. For our purposes, we don't need to consider α as a function of h, but we will sometimes use the notation α' to indicate the value $d\alpha(x)/dx|_{x=h}$. To study the limit behaviour of the scaled version of the process $(Z(t), G(t))$ we use standard notions of convergence in distribution (D), in mean square (L^2), and almost surely (a.s.) (Rudin 1976; Feller 1968). Convolution between functions will be denoted by the operator $*$. Occasionally in the text we will refer to the underlying measurable space or the probability space, which we denote as $(\Omega, \mathcal{B}(\Omega))$ and $(\Omega, \mathcal{B}(\Omega), \mathbb{P})$, respectively. Example constructions of such spaces can be found in Harris (1963), Chapter VI.2.

A brief summary of known results concerning $Z(t)$ and $G(t)$ will follow. According to Harris (1963), Jagers (1969b), under the above assumptions, the limit behaviour of $Z(t)$ satisfies

$$\frac{Z(t)}{e^{\alpha t}} \xrightarrow{a.s., L^2} c\mathcal{Z}, \tag{7}$$

where $\mathcal{Z}$ is a non-negative random variable such that $\mathbb{E}(\mathcal{Z}) = 1$, and

$$c = \lim_{t \to \infty} \frac{\mathbb{E}(Z(t))}{e^{\alpha t}} = \frac{\int_0^\infty \mathbb{P}(L > t)e^{-\alpha t}dt}{h \int_0^\infty ue^{-\alpha u}d\mathbb{P}(L \le u)} = \frac{h - 1}{h^2\alpha \int_0^\infty ue^{-\alpha u}d\mathbb{P}(L \le u)}.$$

For the expected value of $G(t)$, the following is proven in Theorem 2 of Weber et al. (2016)

$$\lim_{t \to \infty} \frac{\mathbb{E}(G(t))}{te^{\alpha t}} = h\alpha'c, \text{ where } \alpha' = \frac{1}{h^2 \int_0^{+\infty} ue^{-\alpha u}d\mathbb{P}(L \le u)}. \tag{8}$$

There, we find also information concerning the asymptotic covariance of $G(t)$ and $Z(t)$ and the ratio of their expectations,

$$\lim_{t \to \infty} \frac{\mathbb{E}(G(t)Z(t))}{te^{2\alpha t}} = c^2h\alpha'k \text{ and } \lim_{t \to \infty} \frac{\mathbb{E}(G(t))}{t\mathbb{E}(Z(t))} = h\alpha',$$

$$\text{where } k = \frac{v \int_0^\infty e^{-2\alpha u}d\mathbb{P}(L \le u)}{1 - h \int_0^\infty e^{-2\alpha u}d\mathbb{P}(L \le u)}, \tag{9}$$

recalling that $v = \mathbb{E}(N(N - 1))$. The scaling of means in Eqs. (7) and (8) suggest the definition of normalised versions of the processes $Z(t)$ and $G(t)$,

$$\mathcal{Z}_t := \frac{Z(t)}{ce^{\alpha t}} \text{ and } \mathcal{G}_t := \frac{G(t)}{ch\alpha'te^{\alpha t}}, \tag{10}$$

whose use will simplify notation in the proofs.

In order to establish one of the main results of the paper, Eq. (4), stated in Corollary 3 of Sect. 3.5, we study the limit behaviour of the process $\{\mathcal{G}_t\}$. We do that in two steps: first, in Sect. 3.3 we consider $\{\mathcal{G}_t\}$ as an L^2 process and determine its mean square limit; then, in Sect. 3.5 we reinforce that result by proving that the convergence is also valid with probability 1 under a condition on the speed of the L^2 convergence. In Sect. 3.3, we make extensive use of a particular version of Key Renewal Theorem for defective measures that we establish in Sect. 3.2. Once we prove in Sect. 3.4 that $\mathcal{G}_t$ and $\mathcal{Z}_t$ share the same random prefactor on front of their dominant term for large t, we are finally able to characterise the limit behaviour of $G(t)/(tZ(t))$.

3.2 A new renewal theorem for defective measures

In order to prove (8) in Weber et al. (2016), a version of the Renewal Theorem, Theorem 6.2(b), of Asmussen (1998), is used in a fundamental way. In this section we generalise that theorem to make it applicable for defective measures, i.e. measures with total mass less than one, which appear in Renewal Theory in the context of transient renewal processes (Resnick 2013, Chapter 3). Before going to the main result of the section, Theorem 1, we first state a non-standard version of the classical Dominated Convergence Theorem (DCT), which can be applied to a collection of sequences of functions $\{(f_{t,\tau})_{t\in\mathbb{R}_{\geq 0}} : \tau \in \mathbb{R}_{\geq 0}\}$, each one converging pointwise, when $t \to \infty$, to a same function f, uniformly for $\tau \geq 0$. This can be proved essentially repeating the same steps of the classical DCT, including the use of Fatou's lemma, but this time the hypothesis of the uniformity in τ allows a stronger conclusion. This proposition is followed by a lemma that depends on it.

Proposition 1 (Non-standard DCT) *Let $(\mathbb{R}, \mathcal{B}(\mathbb{R}), \mu)$ be a measure space, and for every $\tau \geq 0$ let $(f_{t,\tau})_{t\geq 0}$ be a sequence of functions in $L^1(\mu)$ that converges pointwise to f uniformly for $\tau \in [0, \infty)$, i.e. given $\epsilon > 0$ and $u \in \mathbb{R}$ there exists a $t_{\epsilon,u} > 0$ s.t. for every $t \geq t_{\epsilon,u}$ and $\tau \geq 0$ we have $|f_{t,\tau}(u) - f(u)| < \epsilon$. Assume there is $g \in L^1(\mu)$ s.t. $|f_{t,\tau}(u)| \leq g(u)$ for every t, τ, and u. Then, $f \in L^1(\mu)$ and*

$$\lim_{t\to\infty} \int_{\mathbb{R}} f_{t,\tau}(u)d\mu(u) = \int_{\mathbb{R}} f(u)d\mu(u) \qquad \text{uniformly for } \tau \geq 0,$$

i.e. given $\epsilon > 0$ there exists a $t_\epsilon^* > 0$ s.t. for every $t \geq t_\epsilon^*$ and $\tau \geq 0$ we have $|\int_{\mathbb{R}} f_{t,\tau}(u) - f(u)d\mu(u)| < \epsilon$.

We are now going to use this version of the DCT to study the limit behaviour of convolutions between functions and probability measures. We are interested in these particular structures because we will show that the moments of $G(t)$ can be written in that form.

Lemma 1 (Convolution with a finite measure doesn't change convergence rates) *Consider $f = f(t, \tau) : \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0} \to \mathbb{R}$ locally bounded in t and s.t., for every $\tau \geq 0$, $f(t, \tau)/[t^p(t + \tau)^q] \to c_1$ when $t \to \infty$, with $c_1 < \infty$, $p, q \geq 0$, and let μ be a finite measure on $(\mathbb{R}_{\geq 0}, \mathcal{B}(\mathbb{R}_{\geq 0}))$. Then, for every $\tau \geq 0$*

$$\lim_{t \to \infty} \frac{1}{t^p (t+\tau)^q} \int_0^t f(t-u, \tau)\mu(du) = c_1 \mu([0, \infty)). \tag{11}$$

Furthermore, if $|f(t, \tau)| \le f_1(t) f_2(t+\tau)$, with $f_i(t): \mathbb{R}_{\ge 0} \to \mathbb{R}_{\ge 0}$ locally bounded functions for $i \in \{1, 2\}$, $f_1(s)/s^p \to a_1$, $f_2(s)/s^q \to a_2$, and $f(t, \tau)/[t^p(t+\tau)^q] \xrightarrow[t \to \infty]{} c_1$ uniformly for $\tau \ge 0$ with $a_1, a_2, c_1 < \infty$ and $p, q \ge 0$, then (11) is true uniformly for $\tau \in [0, \infty)$.

Proof We only prove the second part of the lemma, as the first part follows from the same rationale with the use of the classical Dominated Convergence Theorem instead of Proposition 1.

For the following, we extend the functions f, f_1, and f_2 to $\mathbb{R} \times \mathbb{R}_{\ge 0}$, $\mathbb{R}$, and $\mathbb{R}$, respectively, by defining $f(t, \tau) = f_1(t) = f_2(t) = 0$ when $t < 0$. If we can establish that $|f(t-u, \tau)|/[t^p(t+\tau)^q]\mathbf{1}_{[0,t)}(u)$ is bounded by a constant M, for every $u \in \mathbb{R}$, $\tau \ge 0$, and t sufficiently large, we can apply the DCT in Proposition 1 and conclude that Eq. (11) holds uniformly for $\tau \in [0, \infty)$.

Given $\epsilon > 0$, from the hypotheses made, we know that there exists $u_\epsilon > 0$ s.t. for every $u \ge u_\epsilon$ we have $f_1(u)/u^p \le a_1 + \epsilon$ and $f_2(u)/u^p \le a_2 + \epsilon$. Without loss of generality we can suppose $t \ge t_{\epsilon,u} := \max\{u_\epsilon, 1\}$. So, for every $u \in \mathbb{R}$, we have

$$
\begin{aligned}
0 \le g_t(u) := \frac{f_1(u)}{t^p}\mathbf{1}_{[0,t)}(u) &= \frac{f_1(u)}{t^p}\mathbf{1}_{[0,u_\epsilon)}(u) + \frac{f_1(u)}{t^p}\mathbf{1}_{[u_\epsilon,t)}(u) \\
&\le f_1(u)\mathbf{1}_{[0,u_\epsilon)}(u) + \frac{f_1(u)}{u^p}\mathbf{1}_{[u_\epsilon,\infty)}(u) \\
&\le \sup_{[0,u_\epsilon)} f_1(u) + a_1 + \epsilon = M_1 < \infty, \tag{12}
\end{aligned}
$$

where in the last equality we have used the fact that f_1 is a locally bounded function. From (12), we have that $g_t(u)$ is dominated by M_1 for every $u \in \mathbb{R}$ and $t \ge t_{\epsilon,u}$. So, the same will be true for $g_t(-u)$, and for its translation $g_t(t-u)$. A similar reasoning can be done with f_2, obtaining

$$\frac{f_1(t-u)}{t^p}\mathbf{1}_{[0,t)}(u) \le M_1, \qquad \frac{f_2(t-u)}{t^q}\mathbf{1}_{[0,t)}(u) \le M_2,$$

for every $u \in \mathbb{R}$ and $t \ge t_{\epsilon,u}$. Remembering that for hypothesis $|f(t, \tau)| \le f_1(t) f_2(t+\tau)$, for every $u \in \mathbb{R}$, $t \ge t_{\epsilon,u}$, and $\tau \ge 0$ we have

$$\frac{|f(t-u, \tau)|}{t^p(t+\tau)^q}\mathbf{1}_{[0,t)}(u) \le \frac{f_1(t-u)}{t^p}\mathbf{1}_{[0,t)}(u)\frac{f_2(t+\tau-u)}{(t+\tau)^q}\mathbf{1}_{[0,t+\tau)}(u) \le M_1 M_2 =: M$$

That concludes the proof. $\qquad \square$

Armed with that lemma, we can now prove the main result of this section.

Theorem 1 (A defective measure version of Theorem 6.2(b) Asmussen (1998)) *Consider the integral equation*

$$K(t, \tau) = f(t, \tau) + \int_0^t K(t - u, \tau)\rho(du), \qquad (13)$$

where $K, f : \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0} \rightarrow \mathbb{R}$, and ρ is a positive defective measure on $(\mathbb{R}_{\geq 0}, \mathcal{B}(\mathbb{R}_{\geq 0}))$, i.e. $\rho([0, \infty)) < 1$. If $f(t, \tau)$ is locally bounded in t and s.t., for every $\tau \geq 0$, $f(t, \tau)/[t^p(t + \tau)^q] \rightarrow c_1$ when $t \rightarrow \infty$, with $c_1 < \infty$, $p, q \geq 0$, then for every $\tau \geq 0$

$$\lim_{t \rightarrow \infty} \frac{K(t, \tau)}{t^p(t + \tau)^q} = \frac{c_1}{1 - \rho([0, \infty))}. \qquad (14)$$

Furthermore, if f is s. t. $|f(t, \tau)| \leq f_1(t)f_2(t + \tau)$, with $f_i(t) : \mathbb{R}_{\geq 0} \rightarrow \mathbb{R}_{\geq 0}$ locally bounded functions, $i \in \{1, 2\}$, s.t. $f_1(t)/t^p \rightarrow a_1$, $f_2(t)/t^q \rightarrow a_2$, and $f(t, \tau)/[t^p(t + \tau)^q] \xrightarrow[t \rightarrow \infty]{} c_1$ uniformly for $\tau \geq 0$ with $a_1, a_2, c_1 < \infty$ and $p, q \geq 0$, then (14) is true uniformly for $\tau \geq 0$.

Proof From (Resnick 2013, Theorem 3.5.1), the only solution of (13) that is bounded on every finite interval of t has the form

$$K(t, \tau) = (U * f)_\tau(t) = \int_0^t f(t - u, \tau)U(du), \qquad (15)$$

where $U([0, t)) = \sum_{n=0}^\infty \rho^{*n}([0, t))$, $\rho^{*n}([0, t)) = (\rho * \rho^{*(n-1)})([0, t))$, and $\rho^{*0}([0, t)) = \mathbb{1}_{[0,\infty)}(t)$. Using Lemma 1 and the fact that $U([0, \infty)) = 1/(1 - \rho([0, \infty)))$ (Resnick 2013, Sect. 3.11), we obtain (14). □

Thanks to the linearity of integration, we have the following mild generalisation.

Corollary 1 *If in Theorem 1 we substitute the condition $|f(t, \tau)| \leq f_1(t)f_2(t + \tau)$ with $|f(t, \tau)| \leq \sum_{i=1}^n f_{2i-1}(t)f_{2i}(t + \tau)$, where f_i are locally bounded functions s.t. $f_{2i-1}(t)/t^p \rightarrow a_{2i-1}$, $f_{2i}(t)/t^q \rightarrow a_{2i}$ and $a_{2i-1}, a_{2i} < \infty$ for every $1 \leq i \leq n$, then the conclusions of Theorem 1 hold.*

3.3 Mean square convergence

Equation (8) states that $\mathbb{E}(\mathcal{G}_t) \rightarrow 1$. A natural question that this result rises is whether there exists a non-negative random variable $\mathcal{G}$, s.t. $\mathbb{E}(\mathcal{G}) = 1$, to which $\mathcal{G}_t$ converges in mean. Studying the behaviour of the second moment of $\mathcal{G}_t$, in Theorem 2, the main result of the section, we will prove something stronger than that: the convergence is true also in L^2. To achieve that we will need a version, stated in Proposition 2, of one of the results presented in Weber et al. (2016) concerning the Probability Generating Function (PGF) of $(G(t), Z(t))$, that better fits our purpose. We use it in Lemmas 2 and 3 where a study of the covariance between $G(t)$ and $Z(t)$, and of the relation

between different terms of the total generation process is made. This will lead us to
Corollary 2, which allows us to finally prove Theorem 2.

Proposition 2 (A reformulation of Theorem 2 of Weber et al. (2016)) *For $s_1, s_2, r_1, r_2,$*
$t, \tau \in \mathbb{R}_{\geq 0}$, define $F(s_1, s_2, r_1, r_2, t, \tau) := \mathbb{E}(s_1^{G(t)} s_2^{G(t+\tau)} r_1^{Z(t)} r_2^{Z(t+\tau)})$. Then, we
have

$$F(s_1, s_2, r_1, r_2, t, \tau) = r_1 r_2 \mathbb{P}(L > t + \tau)$$
$$+ r_1 \int_t^{t+\tau} \rho_N \left(\mathbb{E} \left(s_2^{G(t+\tau-u)} (s_2 r_2)^{Z(t+\tau-u)} \right) \right) d\mathbb{P}(L \leq u)$$
$$+ \int_0^t \rho_N \left(F(s_1, s_2, s_1 r_1, s_2 r_2, t - u, \tau) \right) d\mathbb{P}(L \leq u), \quad (16)$$

where $\rho_N(s) = \mathbb{E}(s^N)$, the probability generating function of the offspring number,
N.

Using Proposition 2, we analyse the limiting behaviour of the covariance between
$Z(t)$ and $G(t)$.

Lemma 2 (Limit behaviour of the covariance of $\mathcal{G}_t$ and $\mathcal{Z}_t$) *Using the previous nota-*
tion, we have

$$\lim_{t \to \infty} \mathbb{E}(\mathcal{G}_t \mathcal{Z}_{t+\tau}) = k = \lim_{t \to \infty} \mathbb{E}(\mathcal{G}_{t+\tau} \mathcal{Z}_t) \qquad \textit{uniformly for } \tau \geq 0, \quad (17)$$

where k is defined in (9).

Proof We prove only the first of the equalities in (17) as the other one can be obtained
in a similar way.

Consider the integral Eq. (16) and take the derivative first for s_1, secondly for r_2,
and then evaluate it at $(1, 1, 1, 1, t, \tau)$. We obtain that

$$\mathbb{E}(G(t)Z(t + \tau)) = v \int_0^t [\mathbb{E}(G(t - u))\mathbb{E}(Z(t + \tau - u))$$
$$+ \mathbb{E}(Z(t - u))\mathbb{E}(Z(t + \tau - u))] \, d\mathbb{P}(L \leq u)$$
$$+ h \int_0^t \mathbb{E}(Z(t - u)Z(t + \tau - u)) d\mathbb{P}(L \leq u)$$
$$+ h \int_0^t \mathbb{E}(G(t - u)Z(t + \tau - u)) d\mathbb{P}(L \leq u),$$

where we recall that $h = \mathbb{E}(N)$ and $v = \mathbb{E}(N(N - 1))$. Multiplying both sides of this
equation by $e^{-\alpha t} e^{-\alpha(t+\tau)}$, and denoting

$$K(t, \tau) := \frac{\mathbb{E}(G(t)Z(t + \tau))}{e^{\alpha t} e^{\alpha(t+\tau)}},$$
$$d\overline{\mathbb{P}}(L \leq u) := h e^{-2\alpha u} d\mathbb{P}(L \leq u), \quad d\mathbb{P}'(L \leq u) := v e^{-2\alpha u} d\mathbb{P}(L \leq u),$$

$$f(t, \tau) := \int_0^t \left[\frac{\mathbb{E}(G(t-u))}{e^{\alpha(t-u)}} \frac{\mathbb{E}(Z(t+\tau-u))}{e^{\alpha(t+\tau-u)}} + \frac{\mathbb{E}(Z(t-u))}{e^{\alpha(t-u)}} \frac{\mathbb{E}(Z(t+\tau-u))}{e^{\alpha(t+\tau-u)}} \right]$$

$$\times \, d\mathbb{P}'(L \leq u) + \int_0^t \frac{\mathbb{E}(Z(t-u)Z(t+\tau-u))}{e^{\alpha(t-u)}e^{\alpha(t+\tau-u)}} d\overline{\mathbb{P}}(L \leq u), \qquad (18)$$

we have that

$$K(t, \tau) = f(t, \tau) + \int_0^t K(t-u, \tau)d\overline{\mathbb{P}}(L \leq u). \qquad (19)$$

Observe that $\overline{\mathbb{P}}$ is a defective measure. In fact,

$$\int_0^{+\infty} d\overline{\mathbb{P}}(L \leq u) = h \int_0^{+\infty} e^{-2\alpha u} d\mathbb{P}(L \leq u) < h \int_0^{+\infty} e^{-\alpha u} d\mathbb{P}(L \leq u) \overset{(3)}{=} 1. \qquad (20)$$

As $\mathbb{E}(\mathcal{G}_t \mathcal{Z}_{t+\tau}) = \mathbb{E}(G(t)Z(t+\tau))/[h\alpha'c^2 t e^{\alpha t} e^{\alpha(t+\tau)}]$, in order to conclude the proof, we would like to apply Theorem 1 at (19) with $p = 1$ and $q = 0$. So, we need to prove that the hypotheses on $f(t, \tau)$ are verified.

Note that $f(t, \tau)$ is the sum of three integrals, where each integrand, divided by t, converges to a constant when $t \to \infty$, uniformly for $\tau \geq 0$ (see (8),(7), and (Harris 1963, pg. 145)). Furthermore, each of these integrands is dominated by the product of two locally bounded functions (the moments of $Z(t)$ and $G(t)$ are locally bounded solutions of integral equations of the type in Eq. (19), see (Harris 1963, pg. 142) and (Weber et al. 2016, Theorem 2)), one depending on t and another one depending on $t + \tau$ (for the last integrand, use the Cauchy–Schwartz inequality to see it). As these dominant functions satisfy the hypotheses of Lemma 1 with $p = 1$ and $q = 0$ (see (8) and (7)), we can conclude that

$$\lim_{t \to \infty} \frac{f(t, \tau)}{t} = h\alpha'c^2 \int_0^\infty d\mathbb{P}'(L \leq u) = h\alpha'c^2 v \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)$$
uniformly for $\tau \geq 0$.

Moreover, if we consider the first of the integrals in (18) and apply the Cauchy–Schwartz inequality, we obtain

$$\int_0^t \frac{\mathbb{E}(G(t-u))}{e^{\alpha(t-u)}} \frac{\mathbb{E}(Z(t+\tau-u))}{e^{\alpha(t+\tau-u)}} d\mathbb{P}'(L \leq u)$$

$$\leq \left(\int_0^t \left| \frac{\mathbb{E}(G(t-u))}{e^{\alpha(t-u)}} \right|^2 d\mathbb{P}'(L \leq u) \right)^{1/2}$$

$$\times \left(\int_0^{t+\tau} \left| \frac{\mathbb{E}(Z(t+\tau-u))}{e^{\alpha(t+\tau-u)}} \right|^2 d\mathbb{P}'(L \leq u) \right)^{1/2} =: f_1(t) f_2(t+\tau),$$

with $f_1(t)$ and $f_2(t)$ satisfying the hypotheses of Theorem 1. As the same reasoning holds for the other integrals in (18) (for the last integral we use Cauchy–Schwartz inequality twice), thanks to Theorem 1, with $p = 1$ and $q = 0$, and Corollary 1 we obtain

$$\lim_{t \to \infty} \frac{K(t)}{t} = \frac{h\alpha' c^2 v \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \le u)}{1 - h \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \le u)} \quad \text{uniformly for } \tau \ge 0.$$

Recalling the definition of k, $\mathcal{G}_t$, and $\mathcal{Z}_{t+\tau}$ at (9) and (10), we have completed the proof of the first inequality in (17). $\qquad\square$

We now study the covariance between the total generation process at two distinct times, for which we will need to use Lemma 2.

Lemma 3 (Limit behaviour of the covariance of $\mathcal{G}_t$ and $\mathcal{G}_{t+\tau}$) *Using the previous notation, we have*

$$\lim_{t \to \infty} \mathbb{E}(\mathcal{G}_{t+\tau} \mathcal{G}_t) = k \qquad \text{uniformly for } \tau \ge 0,$$

where k is defined in (9).

Proof The proof is similar to that in Lemma 2, so some details are omitted.

If we take the derivative of Eq. (16) first for s_1, secondly for s_2, and then evaluate it at $(1, 1, 1, 1, t, \tau)$, we obtain

$$\begin{aligned}
\mathbb{E}(G(t+\tau)G(t)) = v &\int_0^t \mathbb{E}(G(t+\tau-u))\mathbb{E}(G(t-u))d\mathbb{P}(L \le u) \\
&+ v \int_0^t \Big[\mathbb{E}(Z(t+\tau-u))\mathbb{E}(Z(t-u)) + \mathbb{E}(G(t+\tau-u)) \\
&\times \mathbb{E}(Z(t-u)) + \mathbb{E}(Z(t+\tau-u))\mathbb{E}(G(t-u)) \Big] d\mathbb{P}(L \le u) \\
&+ h \int_0^t \Big[\mathbb{E}\big(G(t+\tau-u)Z(t-u)\big) + \mathbb{E}\big(Z(t+\tau-u)G(t-u)\big) \\
&+ \mathbb{E}\big(Z(t+\tau-u)Z(t-u)\big) \Big] d\mathbb{P}(L \le u) \\
&+ h \int_0^t \mathbb{E}(G(t+\tau-u)G(t-u))d\mathbb{P}(L \le u). \quad (21)
\end{aligned}$$

Multiplying both sides of this equation by $e^{-\alpha t} e^{-\alpha(t+\tau)}$ and denoting

$$K(t, \tau) := \frac{\mathbb{E}(G(t+\tau)G(t))}{e^{\alpha t} e^{\alpha(t+\tau)}}, \quad d\overline{\mathbb{P}}(L \le u) := he^{-2\alpha u} d\mathbb{P}(L \le u),$$

$$d\mathbb{P}'(L \le u) := ve^{-2\alpha u} d\mathbb{P}(L \le u),$$

$$f(t, \tau) := \int_0^t \frac{\mathbb{E}(G(t+\tau-u))}{e^{\alpha(t+\tau-u)}} \frac{\mathbb{E}(G(t-u))}{e^{\alpha(t-u)}} d\mathbb{P}'(L \le u)$$

$$+ \int_0^t \left[\frac{\mathbb{E}(Z(t+\tau-u))}{e^{\alpha(t+\tau-u)}} \frac{\mathbb{E}(Z(t-u))}{e^{\alpha(t-u)}} + \frac{\mathbb{E}(G(t+\tau-u))}{e^{\alpha(t+\tau-u)}} \frac{\mathbb{E}(Z(t-u))}{e^{\alpha(t-u)}} \right.$$

$$+ \frac{\mathbb{E}(Z(t+\tau-u))}{e^{\alpha(t+\tau-u)}} \frac{\mathbb{E}(G(t-u))}{e^{\alpha(t-u)}} \Big] d\mathbb{P}'(L \leq u)$$

$$+ \int_0^t \left[\frac{\mathbb{E}\big(G(t+\tau-u)Z(t-u)\big)}{e^{\alpha(t+\tau-u)}e^{\alpha(t-u)}} + \frac{\mathbb{E}\big(Z(t+\tau-u)G(t-u)\big)}{e^{\alpha(t+\tau-u)}e^{\alpha(t-u)}} \right.$$

$$+ \frac{\mathbb{E}\big(Z(t+\tau-u)Z(t-u)\big)}{e^{\alpha(t+\tau-u)}e^{\alpha(t-u)}} \Big] d\overline{\mathbb{P}}(L \leq u), \tag{22}$$

we have that

$$K(t,\tau) = f(t,\tau) + \int_0^t K(t-u,\tau) d\overline{\mathbb{P}}(L \leq u). \tag{23}$$

As already observed in (20), $\overline{\mathbb{P}}$ is a defective measure. In order to conclude the proof, we would like to apply Theorem 1 to (23), and so we need to prove that the hypotheses on $f(t,\tau)$ are verified. This will be easier by proving a weaker version of Lemma 3 which states that $\lim_{t\to\infty} \mathbb{E}(G(t)^2)/[t^2 e^{2\alpha t}] = (h\alpha'c)^2 k$. This result, that now we prove, is obtained applying the first part of Theorem 1 to (23), when $\tau = 0$.

For $\tau = 0$, we have that $K(t,0) = \mathbb{E}(G(t)^2)/e^{2\alpha t}$ and

$$f(t,0) = \int_0^t \left[\frac{\mathbb{E}(G(t-u))^2}{e^{2\alpha(t-u)}} + \frac{\mathbb{E}(Z(t-u))^2}{e^{2\alpha(t-u)}} + 2\frac{\mathbb{E}(G(t-u))}{e^{\alpha(t-u)}} \frac{\mathbb{E}(Z(t-u))}{e^{\alpha(t-u)}} \right] d\mathbb{P}'(L \leq u)$$

$$+ \int_0^t \left[2\frac{\mathbb{E}\big(G(t-u)Z(t-u)\big)}{e^{2\alpha(t-u)}} + \frac{\mathbb{E}(Z(t-u)^2)}{e^{2\alpha(t-u)}} \right] d\overline{\mathbb{P}}(L \leq u). \tag{24}$$

Notice that all five terms inside the integrals in (24) are locally bounded in t (the moments and the covariance of $Z(t)$ and $G(t)$ are locally bounded solutions of integral equations of the type (23), see (Weber et al. 2016, Theorem 2)) and, divided by t^2, they converge to constants. So, we can use Lemma 1 with $p = 2$ and $q = 0$, obtaining

$$\lim_{t\to\infty} \frac{f(t,0)}{t^2} = (h\alpha'c)^2 \int_0^\infty d\mathbb{P}'(L \leq u) = (h\alpha'c)^2 v \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u). \tag{25}$$

As $f(t,0)$ is locally bounded in t (it is finite sum of convolutions of locally bounded functions), Eq. (25) allows us to apply Theorem 1 obtaining

$$\lim_{t\to\infty} \frac{K(t,0)}{t^2} = \lim_{t\to\infty} \frac{\mathbb{E}(G(t)^2)}{t^2 e^{2\alpha t}} = \frac{(h\alpha'c)^2 v \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)}{1 - h \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)} = (h\alpha'c)^2 k. \tag{26}$$

Let's go back to the proof of Lemma 3 and see that $f(t,\tau)$ satisfies the hypotheses of Theorem 1. In (22), each of the seven integrands, when divided by $t(t+\tau)$, converges

to a constant when $t \to \infty$, uniformly for $\tau \geq 0$ (see (8),(7),(17), and Harris 1963, pg. 145). Furthermore, each of these integrands is dominated by the product of two locally bounded functions, one depending on t and another one depending on $t + \tau$ (use the Cauchy–Schwartz inequality for the last three integrands to see it). As these functions satisfy the hypotheses of Lemma 1 (see (8),(7), and (26)), we can conclude that

$$\lim_{t \to \infty} \frac{f(t, \tau)}{t(t + \tau)} = (h\alpha'c)^2 \int_0^\infty d\mathbb{P}'(L \leq u) = (h\alpha'c)^2 v \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)$$

uniformly for $\tau \geq 0$.

Moreover, using the Cauchy–Schwartz inequality (for the last three integrals we have to use it twice), each of the integrals in (22) are dominated by the product of two functions, one depending on t and the other one on $t + \tau$, which satisfy the hypotheses of Theorem 1. So, Corollary 1 implies

$$\lim_{t \to \infty} \frac{K(t)}{t(t + \tau)} = \frac{(h\alpha'c)^2 v \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)}{1 - h \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)} \quad \text{uniformly for } \tau \geq 0.$$

The definitions of k and $\mathcal{G}_t$ at (9) and (10), respectively, allow to conclude the proof.
□

An immediate consequence of this lemma is the following.

Corollary 2 ($\mathcal{G}_t$ is a Cauchy sequence in L^2) *Using the previous notation, we have*

$$\lim_{t \to \infty} \mathbb{E}((\mathcal{G}_{t+\tau} - \mathcal{G}_t)^2) \to 0 \quad \text{uniformly for } \tau \geq 0.$$

Proof From Lemma 3, uniformly for $\tau \geq 0$, we have that

$$\lim_{t \to \infty} \mathbb{E}((\mathcal{G}_{t+\tau} - \mathcal{G}_t)^2) = \lim_{t \to \infty} \left[\mathbb{E}(\mathcal{G}_{t+\tau}^2) + \mathbb{E}(\mathcal{G}_t^2) - 2\mathbb{E}(\mathcal{G}_{t+\tau}\mathcal{G}_t) \right] = k + k - 2k = 0$$

□

We have just proved that $\mathcal{G}_t$ is a Cauchy sequence in L^2, i.e. for every $\epsilon > 0$ there exists a $t_\epsilon > 0$ s.t. for every $t > t_\epsilon$ and $\tau \geq 0$ we have $\mathbb{E}((\mathcal{G}_{t+\tau} - \mathcal{G}_t)^2) < \epsilon$. Thanks to the completeness of the L^2 space, we can now easily prove Theorem 2.

Theorem 2 (Mean square convergence of $G(t)$) *There exists a non-negative random variable $\mathcal{G} \in L^2$ such that*

$$\lim_{t \to \infty} \mathbb{E}((\mathcal{G}_t - \mathcal{G})^2) = 0,$$

with $\mathbb{E}(\mathcal{G}) = 1$ and $Var(\mathcal{G}) = k - 1 = [(v + h) \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u) - 1]/[1 - h \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)] > 0$.

Proof The existence of a such $\mathcal{G}$ follows from Corollary 2, the fact that the L^2 space is complete, and that $\mathcal{G}_t$ satisfies the Cauchy criterion for convergence in L^2. Using (8) and the fact that $L^2 \subset L^1$, we know that $\mathbb{E}(\mathcal{G}) = \lim_{t \to \infty} \mathbb{E}(\mathcal{G}_t) = 1$, so it remains only to compute the variance. From the L^2 convergence we have that $\mathbb{E}(\mathcal{G}^2) = \lim_{t \to \infty} \mathbb{E}(\mathcal{G}_t^2)$. Then,

$$\mathrm{Var}(\mathcal{G}) = \mathbb{E}(\mathcal{G}^2) - \mathbb{E}(\mathcal{G})^2 = \lim_{t \to \infty} \mathbb{E}(\mathcal{G}_t^2) - 1$$

$$= k - 1 \overset{(9)}{=} \frac{(v + h) \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u) - 1}{1 - h \int_0^\infty e^{-2\alpha u} d\mathbb{P}(L \leq u)}. \tag{27}$$

The positivity of (27) follows from the same argument used by Harris in (Harris 1963, pg. 146). Indeed, there he proved that the process $\mathcal{Z}_t$ converges a.s. to a random variable $\mathcal{Z}$ with the same mean and variance as $\mathcal{G}$. □

Theorem 2 gives us the mean square convergence of $\mathcal{G}_t$, which implies also the convergence in probability and in mean. In Sect. 3.5 we will see that the convergence is also true with probability one.

3.4 Functional equation for the MGF of $(\mathcal{G}, \mathcal{Z})$

A surprising consequence of Theorem 2 and (Harris 1963, Theorem 19.1) is that the processes $\mathcal{G}$ and $\mathcal{Z}$ share the same mean and variance. In this section, using the Moment Generating Function (MGF) of the pair $(\mathcal{G}, \mathcal{Z})$, we prove that these two variables are actually almost surely equal. That is, on a path-by-path basis, the prefactor for the normalised population size and for the normalised total generation is the same with probability one.

Theorem 3 ($Z(t)$ and $G(t)$ have same randomness in their dominant terms) *Given*

$$\frac{G(t)}{ch\alpha' t e^{\alpha t}} = \mathcal{G}_t \overset{L^2}{\to} \mathcal{G} \text{ and } \frac{Z(t)}{ce^{\alpha t}} = \mathcal{Z}_t \overset{a.s.}{\to} \mathcal{Z}$$

we have that

$$\mathcal{G} = \mathcal{Z} \quad a.s.$$

Proof The proof is divided in two parts: first, we prove that $\mathcal{G}$ and $\mathcal{Z}$ are equally distributed, then that they coincide with probability one.

Theorem 2, together with (7), imply that $(\mathcal{G}_t, \mathcal{Z}_t) \overset{D}{\to} (\mathcal{G}, \mathcal{Z})$ in distribution. So, we can characterise the distribution of the pair $(\mathcal{G}, \mathcal{Z})$ studying the MGF of $(\mathcal{G}_t, \mathcal{Z}_t)$ when $t \to \infty$.

Proposition 2 gives us an equation solved by the PGF of the vector $(G(t), G(t + \tau), Z(t), Z(t + \tau))$. Evaluating this equation in $(s_1, 1, r_1, 1, t, 0)$, we obtain the following expression solved by the PGF $F(s_1, r_1, t)$ of $(G(t), Z(t))$

$$F(s_1, r_1, t) = \mathbb{E}(s_1^{G(t)} r_1^{Z(t)}) = r_1 \mathbb{P}(L > t) + \int_0^t \rho_N\Big(F(s_1, s_1 r_1, t - u)\Big) d\mathbb{P}(L \leq u).$$

(28)

Replacing s_1 with $\exp(-s/[hc\alpha' t e^{\alpha t}])$ and r_1 with $\exp(-r/[ce^{\alpha t}])$, for $s, r \geq 0$, we obtain an expression solved by the MGF $\phi(s, r, t)$ of $(\mathcal{G}_t, \mathcal{Z}_t)$:

$$\phi(s, r, t) = \mathbb{E}\Big(e^{-\frac{sG(t)}{hc\alpha' t e^{\alpha t}}} e^{-\frac{rZ(t)}{ce^{\alpha t}}}\Big)$$

$$= e^{-\frac{r}{ce^{\alpha t}}} \mathbb{P}(L > t) + \int_0^t \rho_N\Big(\mathbb{E}\Big(e^{-\frac{(t-u)se^{-\alpha u}}{t} \mathcal{G}_{t-u}} e^{-\frac{(s+hr\alpha' t)e^{-\alpha u}}{h\alpha' t} \mathcal{Z}_{t-u}}\Big)\Big) d\mathbb{P}(L \leq u)$$

$$= e^{-\frac{r}{ce^{\alpha t}}} \mathbb{P}(L > t) + \int_0^t \rho_N\Big(\phi\Big(\frac{(t-u)}{t} se^{-\alpha u}, \frac{(s+hr\alpha' t)}{h\alpha' t} e^{-\alpha u}, t - u\Big)\Big) d\mathbb{P}(L \leq u).$$

Taking the limit for $t \to \infty$ of $\phi(s, r, t)$, we obtain that $\mathbb{E}(\exp(-s\mathcal{G})\exp(-r\mathcal{Z}))$ solves the integral equation

$$\phi(s, r) = \int_0^\infty \rho_N\Big(\phi\Big(se^{-\alpha u}, re^{-\alpha u}\Big)\Big) d\mathbb{P}(L \leq u) \quad s, r \geq 0.$$

(29)

This means that if we consider $r = 0$, the function $\mathbb{E}[\exp(-s\mathcal{G})]$, that represents the MGF of $\mathcal{G}$, solves the integral equation

$$\psi(s) = \int_0^\infty \rho_N\Big(\psi\Big(se^{-\alpha u}\Big)\Big) d\mathbb{P}(L \leq u), \quad s \geq 0$$

(30)

with $\psi(0) = 0$ and $\psi'(0) = -1$. The uniqueness of the solution of this problem (Levinson 1960, Theorem 4.1) and the fact that the MGF of the variable $\mathcal{Z}$ solves (30) too (Harris 1963, pg. 146), give us that the MGFs of $\mathcal{Z}$ and $\mathcal{G}$ coincide for $s \geq 0$. Using a result proved by Mukherjea et al. (Mukherjea et al. 2006, Theorem 2), we can conclude that $\mathcal{Z}$ is equal in distribution to $\mathcal{G}$.

Now, if we consider $r = s$ in (29), we can see that the function $\mathbb{E}[\exp(-s(\mathcal{G}+\mathcal{Z}))]$, that represents the MGF of $\mathcal{G} + \mathcal{Z}$, solves (30) but with the initial conditions $\psi(0) = 0$ and $\psi'(0) = -2$. Another solution of (30) with the same initial conditions is given by $2\mathcal{Z}$. Also in this case, the uniqueness of the solution and (Mukherjea et al. 2006, Theorem 2) allows us to conclude that $2\mathcal{Z} \overset{D}{=} \mathcal{Z} + \mathcal{G}$.

These last two results give us that $\mathcal{Z} \overset{a.s.}{=} \mathcal{G}$. In fact, $\mathcal{Z} \overset{D}{=} \mathcal{G}$ implies that $\text{Var}(\mathcal{Z}) = \text{Var}(\mathcal{G})$, and

$$2\mathcal{Z} \overset{D}{=} \mathcal{Z} + \mathcal{G} \implies 4\text{Var}(\mathcal{Z}) = \text{Var}(\mathcal{Z}) + \text{Var}(\mathcal{G}) + 2\text{Cov}(\mathcal{Z}, \mathcal{G})$$

$$\implies \text{Var}(\mathcal{Z}) = \text{Cov}(\mathcal{Z}, \mathcal{G}) \implies \text{Corr}_{\mathcal{Z}, \mathcal{G}} := \frac{\text{Cov}(\mathcal{Z}, \mathcal{G})}{\sqrt{\text{Var}(\mathcal{Z})}\sqrt{\text{Var}(\mathcal{G})}} = 1,$$

where in the last inequality we have used the definition of Pearson's correlation coefficient. The correlation coefficient equal to 1 implies that $\mathcal{G} = a\mathcal{Z} + b$ a.s., for $a \geq 0$,

$b \in \mathbb{R}$ (Casella and Berger 2002, Theorem 4.5.7). From $\mathcal{Z} \overset{D}{=} \mathcal{G}$, we obtain $a = 1$ and $b = 0$, i.e. $\mathcal{Z} \overset{a.s.}{=} \mathcal{G}$. This conclude the proof. □

Thus, from Theorem 3, $\mathcal{Z}$ can be used in lieu of $\mathcal{G}$ from here on.

3.5 Almost sure convergence of $G(t)$

We have gathered the results needed to establish one of the significant results of the article: the almost sure convergence of a normalised version of the process $\{G(t)\}$. In order to prove that, we will assume something concerning the speed of convergence of $\mathcal{G}_t$ to $\mathcal{Z}$ as L^2 functions. This assumption is equivalent to the one made by Harris in (Harris 1963, Chapter VI, Theorem 21.1) concerning the size of the population, which—for the population size—was later established by Jagers (1969b) to be unnecessary.

Theorem 4 (Almost sure convergence of $G(t)$) *If $\int_0^\infty \mathbb{E}((\mathcal{G}_t - \mathcal{Z})^2)dt < \infty$, we have that*

$$\frac{G(t)}{h\alpha'cte^{\alpha t}} = \mathcal{G}_t \xrightarrow[t \to \infty]{a.s} \mathcal{Z}.$$

Proof We start with the additional hypothesis $p_0 = \mathbb{P}(N = 0) = 0$ in order to have $G(t)$ as a finite, non-decreasing step function of t. Using Fubini's theorem on $\int_0^\infty \mathbb{E}((\mathcal{G}_t - \mathcal{Z})^2)dt < \infty$, we obtain that $\mathbb{P}(\int_0^\infty (\mathcal{G}_t - \mathcal{Z})^2 dt < \infty) = 1$. Since $G(t)$ is non-decreasing in t, we have

$$\mathcal{G}_{t+\tau} = \frac{G(t+\tau)}{hc\alpha'(t+\tau)e^{\alpha(t+\tau)}} \geq \frac{t}{(t+\tau)e^{\alpha\tau}} \frac{G(t)}{hc\alpha'te^{\alpha t}} = \frac{t}{(t+\tau)e^{\alpha\tau}}\mathcal{G}_t, \quad (31)$$

where the inequalities are true for every realisation of the random variables.

Let's suppose that $\mathcal{G}_t \xrightarrow[t \to \infty]{a.s.} \mathcal{Z}$ is not true . If $(\Omega, \mathcal{B}(\Omega), \mathbb{P})$ is the probability space where $\mathcal{G}_t$ and $\mathcal{Z}_t$ are defined, then there exists a set $A \subseteq \{\omega \in \Omega \,|\, \lim_{t \to \infty} \mathcal{G}_t(\omega) \neq \mathcal{Z}(\omega)\}$ that is measurable and such that $\mathbb{P}(A) > 0$. Since $\mathcal{Z} > 0$ a.s. (Harris 1963, Remark 1, Section 20), we can also suppose that $\mathcal{Z}(\omega) > 0$ for every $\omega \in A$.

For every $\omega \in A$ we have that at least one between $\limsup_{t \to \infty} \mathcal{G}_t(\omega) > \mathcal{Z}(\omega)$ and $\liminf_{t \to \infty} \mathcal{G}_t(\omega) < \mathcal{Z}(\omega)$ is true. We will see that in both cases we will have $\int_0^\infty (\mathcal{G}_t(\omega) - \mathcal{Z}(\omega))^2 dt = +\infty$, leading to the contradiction $\mathbb{E}(\int_0^\infty (\mathcal{G}_t - \mathcal{Z})^2 dt) = +\infty$.

Let us start fixing $\omega \in A$ and assuming $\limsup_{t \to \infty} \mathcal{G}_t(\omega) > \mathcal{Z}(\omega)$. This implies that there exist a $\delta > 0$ and a sequence $(t_i)_{i \in \mathbb{N}}$, with $\lim_{i \to \infty} t_i = \infty$, such that $\mathcal{G}_{t_i}(\omega) > (1 + \delta)\mathcal{Z}(\omega)$, $i \in \mathbb{N}$. If we consider $0 < \epsilon < \delta$, without loss of generality we can choose this sequence such that

$$t_{i+1} - t_i > \frac{(\delta - \epsilon)t_i}{1 + \epsilon + \alpha t_i(1 + \delta)} := b_i.$$

Note that δ, ϵ, and t_i depend on ω and that $(b_i)_{i \in \mathbb{N}}$ and $(t_i)_{i \in \mathbb{N}}$ are monotonically increasing.

Using (31) and the relation $e^{-\alpha \tau} \geq 1 - \alpha \tau$, we obtain for every $i \in \mathbb{N}$

$$
\begin{aligned}
\mathcal{G}_{t_i + \tau}(\omega) &\overset{(31)}{\geq} \frac{t_i}{(t_i + \tau)e^{\alpha \tau}} \mathcal{G}_{t_i}(\omega) > \frac{t_i}{t_i + \tau}(1 - \alpha \tau)(1 + \delta)\mathcal{Z}(\omega), && \tau \in (0, \infty) \\
&\geq (1 + \epsilon)\mathcal{Z}(\omega) && \tau \in (0, b_i) \\
&\geq (1 + \epsilon)\mathcal{Z}(\omega) && \tau \in (0, b_1),
\end{aligned}
\tag{32}
$$

where we have used the fact that the function $t_i(t_i + \tau)^{-1}(1 - \alpha \tau)(1 + \delta)$ is decreasing in τ, that for $\tau = b_i$ it is equal to $(1 + \epsilon)$, and that $(b_i)_{i \in \mathbb{N}}$ is an increasing sequence.

Hence, using (32), we have for every i that

$$
\begin{aligned}
\int_{t_i}^{t_{i+1}} (\mathcal{G}_t(\omega) - \mathcal{Z}(\omega))^2 dt &\geq \int_{t_i}^{t_i + b_1} (\mathcal{G}_t(\omega) - \mathcal{Z}(\omega))^2 dt \\
&= \int_0^{b_1} (\mathcal{G}_{t_i + \tau}(\omega) - \mathcal{Z}(\omega))^2 d\tau \geq (\epsilon \mathcal{Z}(\omega))^2 b_1 > 0.
\end{aligned}
$$

This allows us to say that $\int_0^\infty (\mathcal{G}_t(\omega) - \mathcal{Z}(\omega))^2 dt = +\infty$.

Same conclusion can be obtained assuming $\liminf_{t \to \infty} \mathcal{G}_t(\omega) < \mathcal{Z}(\omega)$. Indeed, for the definition of lim inf we have that there exist $\delta \in (0, 1)$ and a sequence $(t_i)_{i \in \mathbb{N}}$, with $t_i > 1$ and $\lim_{i \to \infty} t_i = \infty$, such that $\mathcal{G}_{t_i} < (1 - \delta)\mathcal{Z}$. We can also pretend that $t_{i+1} - t_i > a > 0$, where a is chosen in order to satisfy the following inequalities for i big enough

$$
\begin{aligned}
0 < \mathcal{G}_{t_i - \tau} &\overset{(31)}{\leq} \frac{t_i}{t_i - \tau} e^{\alpha \tau} \mathcal{G}_{t_i} < (1 - \delta) \frac{t_i}{t_i - \tau} e^{\alpha \tau} \mathcal{Z} && \tau \in (0, t_1) \\
&\leq (1 - \epsilon)\mathcal{Z} && \tau \in (0, a),
\end{aligned}
$$

where ϵ is a constant s.t. $0 < \epsilon < \delta$. The existence of such a is consequence of the fact that $\psi(t, \tau) := (1 - \delta)e^{\alpha \tau} t/(t - \tau)$, as long as $\tau < t$, is increasing in τ and decreasing in t. Indeed, this implies that there exists $a > 0$ s.t. for $\tau \in [0, a]$ $(1 - \delta) = \psi(1, 0) \leq \psi(1, \tau) \leq (1 - \epsilon)$, from which we can conclude that for $\tau \in [0, a]$ and $t \geq 1$, we have $(1 - \delta) = \psi(t, 0) \leq \psi(t, \tau) \leq (1 - \epsilon)$.

Then, we have

$$
\begin{aligned}
\int_{t_{i-1}}^{t_i} (\mathcal{Z}(\omega) - \mathcal{G}_t(\omega))^2 dt &\geq \int_{t_i - a}^{t_i} (\mathcal{Z}(\omega) - \mathcal{G}_t(\omega))^2 dt \\
&\geq \int_0^a (\mathcal{Z}(\omega) - \mathcal{G}_{t_i - \tau}(\omega))^2 d\tau \geq (\epsilon \mathcal{Z}(\omega))^2 a.
\end{aligned}
$$

As before, this implies that $\int_0^\infty (\mathcal{G}_t(\omega) - \mathcal{Z}(\omega))^2 dt = +\infty$.

So, for every $\omega \in A$ we have $\int_0^\infty (\mathcal{G}_t(\omega) - \mathcal{Z}(\omega))^2 dt = +\infty$ and, because $\mathbb{P}(A) > 0$, we have $\mathbb{E}(\int_0^\infty (\mathcal{G}_t - \mathcal{Z})^2 dt) = +\infty$. This contradicts the hypothesis of the theorem and so we have proved that $\lim_{t\to\infty} \mathcal{G}_t = \mathcal{Z}$ with probability 1 under the condition $p_0 = 0$.

When $p_0 \neq 0$, we can observe that $G(t) = G_B(t) - G_D(t)$, where $G_B(t)$ and $G_D(t)$ are the sum of the generation of the cells born and dead before or at time t, respectively. Also for these processes we can find integral equations for the probability generating function similar to the one found for $G(t)$ and repeat all the previous steps. Thanks to the monotonicity of $G_B(t)$ and $G_D(t)$, this time we don't need the assumption $p_0 = 0$, obtaining the almost sure convergence of $G_B(t)/n_1 t e^{\alpha t}$ and $G_D(t)/n_2 t e^{\alpha t}$ to the random variables $\mathcal{Z}_B$ and $\mathcal{Z}_D$ respectively, where n_1, n_2 are positive constants. This allows us to conclude that $\mathcal{G}_t$ converges to $\mathcal{Z}_B + \mathcal{Z}_D$. □

Having established the almost sure result for the limiting behaviour of the total generation process $G(t)$, we are in a position to make the final deduction of the section that leads to Eq. (4). Thanks to Eq. (7), Theorem 4, and the Continuous Mapping Theorem, we have the following corollary.

Corollary 3 (Almost sure average generation inference) *If* $\mathbb{E}(N^2) < \infty$, *$\liminf_{t\to\infty} Z^+(t) > 0$, and $\int_0^\infty \mathbb{E}((\mathcal{G}_t - \mathcal{Z})^2) dt < \infty$, we have that*

$$\lim_{t\to\infty} \frac{G(t)}{t Z(t)} = h\alpha' = -\lim_{p\to 0} \lim_{t\to\infty} \frac{1}{pt} \log\left(\frac{Z^+(t)}{Z(t)}\right) \quad almost\ surely, \quad (33)$$

where the right hand side is established in (Weber et al. 2016, Theorem 1).

Thus the average estimation scheme firstly proposed in Weber et al. (2016) is almost surely correct on a path-by-path basis for a Bellman–Harris branching process. The principle behind this result is that for almost all paths $G(t)/Z(t) = h\alpha' t + o(t)$ for large t. That is, the randomness in the average generation is not contained in the linear factor but in something asymptotic smaller (see Fig. 3c). On the other hand, so long as the Z^+ population persists, $-1/t \log(Z^+(t)/Z(t)) \approx \alpha(h(1-p)) - \alpha(h)$ for large t. However, as the Malthus parameter is real analytic (Weber et al. 2016, Proposition1), $\alpha(h(1-p))$ coincides with its Taylor expansion around $p = 0$, $\alpha(h(1-p)) = \alpha(h) - h\alpha'(h)pt + O(p^2)$. Thus $-1/(pt) \log(Z^+(t)/Z(t)) \approx h\alpha'(h)$, the same constant as appears for the time-rescaled average generation.

4 A two-type Bellman–Harris process subject to one-way differentiation

In addition to division and death, cells often undergo changes in cell-type. For example, many tissues are formed through progressive stages of proliferation and change in cell-type, called cellular differentiation, from stem cells (Kondo et al. 1997; Akashi et al. 2000), while cancer cells arise as mutants with abherent DNA from healthy cells (Mendelsohn et al. 2015; Hong et al. 2010). Changes in cell-type are often accompanied by changes in population kinetics (Akinduro et al. 2018), and to better

understand these differentiation processes it can be desirable to obtain information on the average generation of each population as they are often reported as being division-linked (Hodgkin et al. 1996; Deenick et al. 1999; Duffy et al. 2012; Pauklin and Vallier 2013).

As a basic model of changes in cell type, in the present section we extend the previous results to a two-type Bellman–Harris branching process subject to one-way differentiation, a model first considered in Jagers (1969a) where cells of one type can give rise to another but not vice-versa. These results significantly extend the remit and utility of the inference of average generation by random delabelling. In particular, if the initial cell is equipped with a neutral label that is heritably lost with a fixed probability per division, we prove that the average generation of each cell-type can be inferred from knowledge of that probability and the proportion of label positive cells. Before stating the results, we introduce notation that is consistent with that used in Sect. 3.1 and with that employed in Jagers (1969a), where sample path results for the population size were first established in this two-type setting. More general classes of multi-type branching processes have been studied, both with directed, one-way changes in type as considered here, and also when cycles of cell types are possible. Extensive early synopses of these results are gathered in Mode (1971) and Athreya and Ney (1972) while a comprehensive recent treatment appears in Athreya and Jagers (2012).

As in Fig. 2, consider a cell population whose members are from two types, type-1 and type-2. Each cell lives a random type-dependent lifetime $L_i, i \in \{1, 2\}$, after which it dies or divides generating N_i offspring. We assume L_i and N_i are independent for each cell, and amongst all cells. Furthermore, we suppose that only type-1 cells can generate cells of both types, i.e. N_1 takes values in $\mathbb{N}^2$ and has PGF ρ_1, whereas the offspring of type-2 cells are exclusively type-2 cells, so that N_2 takes value in $\mathbb{N}$ and has PGF ρ_2. We denote by $h_i := (\partial/\partial x_i)\rho_1(1, 1)$ the average number offspring of type-i generated from a type-1 cell and, with $\mu := d/dx\,\rho_2(1)$, the average number of offspring obtained from a type-2 cell. As in the single-type case, we suppose that h_1 and μ are greater than 1 so that both populations are super-critical.

We assign a generation to each cell, the integer that records how many divisions led to that cell (Fig. 2). We define cells a time zero as being in generation zero. Furthermore, we suppose the cells in the initial population are equipped with a neutral label (i.e. one that does not influence population dynamics) that, independently for each cell, is heritably lost immediately prior to a cell's division with probability p. For $i \in \{1, 2\}$, we denote by $Z_i(t)$ the total number of type-i cells in the population at time t, by $G_i(t)$ the total generation of type-i cells at time t, and by $Z_i^+(t)$ the size of type-i label-positive at time t. To describe the growth rates of these processes, we will need the Malthusian parameters, α_1 and α_2, that are the solutions of the equations

$$h_1 \mathbb{E}\left(e^{-\alpha_1 L_1 t}\right) = 1 \quad \text{and} \quad \mu \mathbb{E}\left(e^{-\alpha_2 L_2 t}\right) = 1. \tag{34}$$

The existence and the uniqueness of the solutions of these equations are guaranteed by the hypotheses $h_1 > 1$ and $\mu > 1$. As in Sect. 3.1, we denote the derivatives of the Malthus parameters as a function of the average offspring number by

$$\alpha_1' = \frac{1}{h_1^2 \int_0^{+\infty} te^{-\alpha_1 t} d\mathbb{P}(L_1 \le u)} \quad \text{and} \quad \alpha_2' = \frac{1}{\mu^2 \int_0^{+\infty} te^{-\alpha_2 t} d\mathbb{P}(L_2 \le u)}.$$

The population dynamics of type-1 cells are unaffected by type-2 cells and, treating differentiation as death, behave as a single type process. If the starting population only has type-2 cells, the system is again in the single type setting. Thus the interesting setup is when the system is initiated with cells of type-1 and queries are of the population size and average generation of type-2 cells.

Let $\mathbb{P}_i$ and $\mathbb{E}_i$ denote the probability and the expectation conditional on the population starting with a single cell of type $i \in \{1, 2\}$. The growth of the type-2 population size given one initial type-1 cell, $Z_2(t)$ under $\mathbb{P}_1$, is studied in Jagers (1969a). Those results can be immediately applied to study $Z_2^+(t)$, given the first cell is type-1 and label-positive. Analogous results for $G_2(t)$ can be obtained by repeating the steps made in the single type case. In particular, adapting the integral Eq. (16) to the two-type problem, using Lemma 1 and Theorem 1 we can establish the growth rates of $\mathbb{E}_1(G_2(t)Z_2(t))$, $\mathbb{E}_1(G_2(t)^2)$, $\mathbb{E}_1(Z_2(t)Z_2(t + \tau))$, $\mathbb{E}_1(G_2(t)Z_2(t + \tau))$, $\mathbb{E}_1(G_2(t+\tau)Z_2(t))$, and $\mathbb{E}_1(G_2(t)G_2(t+\tau))$. These results enable us to conclude the mean square limit of $G_2(t)$ under $\mathbb{P}_1$. Stepping from the L^2 result to the almost sure one is achieved in the same way as Theorem 4. As this line of reasoning is essentially a replication of what is done in the single type case, the details are omitted. From these, starting with one label-positive type-1 cell, the in-expectation result relating the average generation to the proportion of labelled cells follows immediately:

$$\lim_{t \to \infty} \frac{\mathbb{E}_1(G_2(t))}{t\mathbb{E}_1(Z_2(t))} = -\lim_{p \to 0} \lim_{t \to \infty} \frac{1}{pt} \log \left(\frac{\mathbb{E}_1(Z_2^+(t))}{\mathbb{E}_1(Z_2(t))} \right).$$

This equation says that, on average, the average generation of the type-2 population can be determined from averages of the delabelling proportion. To obtain stronger convergence results, one notes that a combination of (Harris 1963, Theorems 19.1 and 21.1), Theorem 2, and Theorem 4 gives that

$$\lim_{t \to \infty} \frac{Z_i(t)}{c_i e^{\alpha_i t}} \stackrel{L^2, a.s.}{=} \mathcal{Z}_i \quad \text{and} \quad \lim_{t \to \infty} \frac{G_i(t)}{d_i t e^{\alpha_i t}} \stackrel{L^2, a.s.}{=} \mathcal{Z}_i \quad \text{under } \mathbb{P}_i, \qquad (35)$$

where

$$c_1 = \frac{h_1 - 1}{h_1^2 \alpha_1 \int_0^\infty te^{-\alpha_1 t} d\mathbb{P}(L_1 \le t)}, \qquad c_2 = \frac{\mu - 1}{\mu^2 \alpha_2 \int_0^\infty te^{-\alpha_2 t} d\mathbb{P}(L_2 \le t)},$$

$d_1 = c_1 h_1 \alpha_1'$, $d_2 = c_2 \mu \alpha_2'$, and assuming $\int_0^\infty \mathbb{E}[(G_i(t)/(d_i te^{\alpha_i t}) - \mathcal{Z}_i)^2] dt < \infty$ for the almost sure results concerning $\{G_i(t)\}$ in (35). Moreover, from Weber et al. (2016) we have also that, if $\lim_{t \to \infty} Z_i^+(t) > 0$

$$\lim_{p \to 0} \lim_{t \to \infty} -\frac{1}{pt} \log \left(\frac{Z_i^+(t)}{Z_i(t)} \right) \stackrel{a.s.}{=} \begin{cases} h_1 \alpha_1' & \text{if } i = 1 \\ \mu \alpha_2' & \text{if } i = 2 \end{cases} \quad \text{under } \mathbb{P}_i,$$

where we supposed that the first cell is label positive.

We present two sets of results depending on whether $\alpha_1 > \alpha_2$ or vice versa. If $\alpha_1 < \alpha_2$, which would model, for example, the creation of cancer cells, the growth rate of the type-2 cells is greater than the type-1 cells and their average generation is determined by the derivative of the latter Malthus parameter.

Proposition 3 $(\alpha_1 < \alpha_2)$ *If* $(\partial/\partial x_i x_j)\rho_1(1, 1)$, *for* $1 \le i \le j \le 2$, *and* $(\partial/\partial x^2)\rho_2(1)$ *are finite, we have that*

$$\lim_{t \to \infty} \frac{Z_2(t)}{c_{1,2}e^{\alpha_2 t}} \stackrel{L^2, a.s.}{=} \mathcal{W}, \quad \text{and} \quad \lim_{t \to \infty} \frac{G_2(t)}{d_{1,2}te^{\alpha_2 t}} \stackrel{L^2}{=} \mathcal{W} \quad \text{under } \mathbb{P}_1, \qquad (36)$$

where

$$c_{1,2} = \frac{h_2 c_2 \int_0^\infty e^{-\alpha_2 t}d\mathbb{P}(L_1 \le t)}{1 - h_1 \int_0^\infty e^{-\alpha_2 t}d\mathbb{P}(L_1 \le t)}, \qquad d_{1,2} = c_{1,2}\mu\alpha_2', \qquad (37)$$

and $\mathcal{W}$ *is a non-negative random variable such that* $\mathbb{P}_1(\mathcal{W} = 0) = \mathbb{P}_1(\lim_{t \to \infty} Z_1(t) = 0, \lim_{t \to \infty} Z_2(t) = 0)$ *and* $\mathbb{E}_1(\mathcal{W}) = 1$.

If $\int_0^\infty \mathbb{E}_1[(G_2(t)/(d_{1,2}te^{\alpha_2 t}) - \mathcal{W})^2]dt < \infty$, *the second limit in* (36) *is also true almost surely. Assuming the initial cell is of type-1, i.e.* $Z_1^+(0) = 1$ *and* $Z_2(0) = G_1(0) = G_2(0) = 0$, *we have*

$$\lim_{t \to \infty} \frac{G_2(t)}{tZ_2(t)} \stackrel{a.s.}{=} \mu\alpha_2' \stackrel{a.s.}{=} \lim_{p \to 0}\lim_{t \to \infty} -\frac{1}{pt}\log\left(\frac{Z_2^+(t)}{Z_2(t)}\right) \quad \text{if } \lim_{t \to \infty} Z_2^+(t) > 0.$$

If $\alpha_2 < \alpha_1$, as might occur with the production of terminally differentiated cells, the growth rate of the type-1 cells is greater than the type-2 cells and their average generation is determined by the derivative of the former Malthus parameter. That is, in this setting, so long as the type-1 population continues to exist, the average generation of the type-2 cells is dominated by immigrants from the type-1 population.
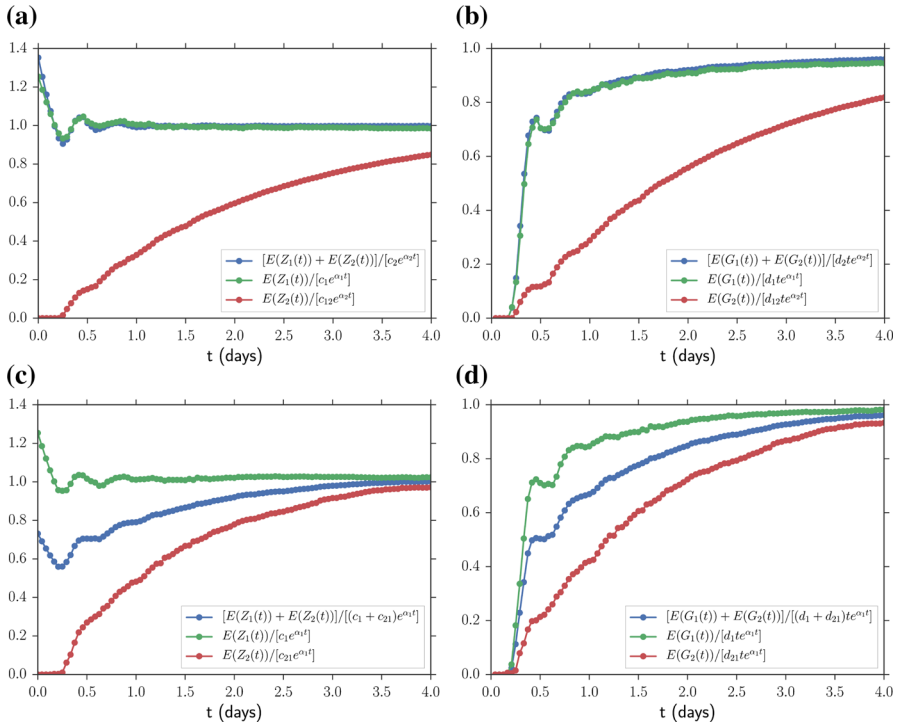
Proposition 4 $(\alpha_2 < \alpha_1)$ *If* $(\partial/\partial x_i x_j)\rho_1(1, 1)$, *for* $1 \le i \le j \le 2$, *and* $(\partial/\partial x^2)\rho_2(1)$ *are finite, we have that*

$$\lim_{t \to \infty} \frac{Z_2(t)}{c_{2,1}e^{\alpha_1 t}} \stackrel{L^2, a.s.}{=} \mathcal{Z}_2 \quad \text{and} \quad \lim_{t \to \infty} \frac{G_2(t)}{d_{2,1}te^{\alpha_1 t}} \stackrel{L^2}{=} \mathcal{Z}_2 \quad \text{under } \mathbb{P}_1, \qquad (38)$$

where

$$c_{2,1} = \frac{h_2(1 - \int_0^\infty e^{-\alpha_1 t}d\mathbb{P}(L_2 \le t))}{h_2^2\alpha_1(1 - \mu\int_0^\infty e^{-\alpha_1 t}d\mathbb{P}(L_2 \le t))}, \qquad d_{2,1} = c_{2,1}h_1\alpha_1', \qquad (39)$$

and $\mathcal{Z}_2$ *random variable defined in* (35) *with* $\mathbb{P}_1(\mathcal{Z}_2 = 0) = \mathbb{P}_1(\lim_{t \to \infty} Z_1(t) = 0)$ *and* $\mathbb{E}_1(\mathcal{Z}_2) = 1$.

(a)

(b)

(c)

(d)

Fig. 5 Average growth rates of population sizes and total generations of each type starting with a single type-1 cell and using the scalings in Propositions 3 and 4. Cells have lognormal lifetime with mean 9.3 hours and standard deviation 2.54 (Hawkins et al. 2009). Type-1 cells give rise to type-1 cells with probability 5/6 and to type-2 cells with probability 1/6. Means are computed averaging the results of 1000 Monte Carlo simulations of populations growing for four days. **a, b** These illustrations are in the case $\alpha_1 < \alpha_2$ and cells of both types always have two offspring. **c, d** These are in the setting $\alpha_2 < \alpha_1$, obtained by setting $N_1 = 2$ and $\mathbb{P}(N_2 = 0) = 2/5 = 1 - \mathbb{P}(N_2 = 2)$

If $\int_0^\infty \mathbb{E}_1[(G_2(t)/(d_{2,1}te^{\alpha_2 t}) - \mathcal{Z}_2)^2]dt < \infty$, the second limit in (38) *is also true almost surely. Assuming the initial cell is of type-1, i.e.* $Z_1^+(0) = 1$ *and* $Z_2(0) = G_1(0) = G_2(0) = 0$, *we have*

$$\lim_{t \to \infty} \frac{G_2(t)}{tZ_2(t)} \stackrel{a.s.}{=} h_1\alpha_1' \stackrel{a.s.}{=} \lim_{p \to 0} \lim_{t \to \infty} -\frac{1}{pt} \log\left(\frac{Z_2^+(t)}{Z_2(t)}\right) \quad if \lim_{t \to \infty} Z_1^+(t) > 0.$$

We conclude the paper by presenting some simulated results that illustrate the features of these two-type results, both for average generation and for its inference. Figure 5 provides average normalised paths of the processes $Z_i(t)$ and $G_i(t)$. In Fig. 5a, b, $\alpha_1 < \alpha_2$, but despite the fact the type-2 population is the fastest growing on average, it is the slowest one to converge. This occurs due to the random delay in the production of any type-2 cells. Note also that the total population of both type-1 and type-2 cells behave as a single-type branching process with $N = 2$ and lognormal lifetime distribution. Hence, the growth rates of $Z(t) = Z_1(t) + Z_2(t)$ and
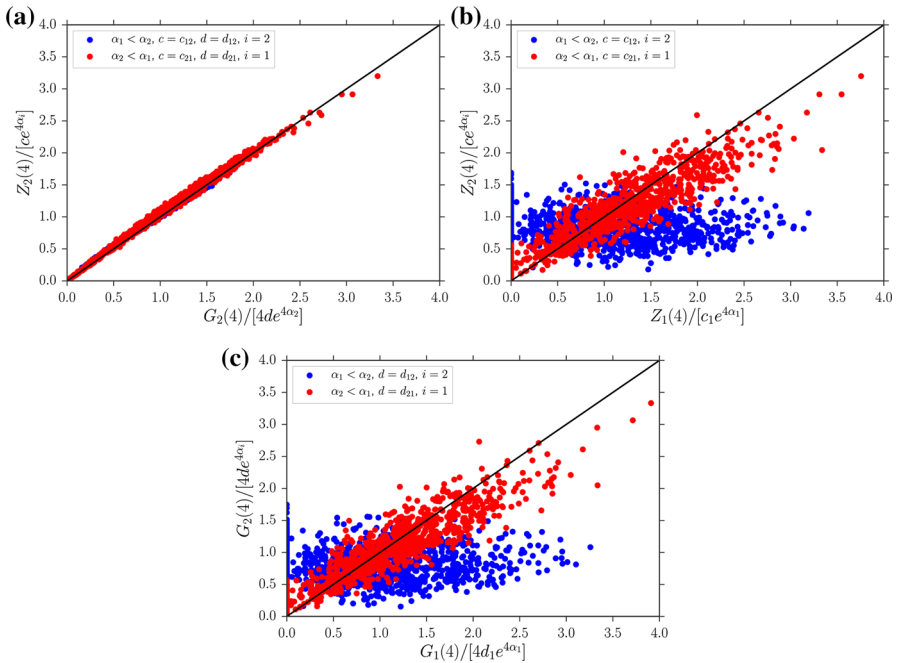
Fig. 6 Relationships in per-path randomness. Plots were created using the same 1000 Monte Carlo simulations used to generate Fig. 5. Blue points correspond to $\alpha_1 < \alpha_2$, while red ones to $\alpha_2 < \alpha_1$. **a** Scatter plot of normalised versions of $Z_2(t)$ and $G_2(t)$ is displayed at $t = 4$ days. Pearson correlation coefficient for both blue and red points is 0.99. **b** Scatter plot of normalised versions of $Z_1(t)$ and $Z_2(t)$ is displayed at $t = 4$ days. Pearson correlation coefficient for blue and red points is -0.19 and 0.94, respectively. **c** Scatter plot of normalised versions of $G_1(t)$ and $G_2(t)$ is displayed at $t = 4$ days. Pearson correlation coefficient for blue and red points is -0.09 and 0.94, respectively (colour figure online)

$G(t) = G_1(t) + G_2(t)$ are the same as if the type-2 population was started with one type-2 cell. In Fig. 5c, d, $\alpha_1 > \alpha_2$. Here, the second population is dominated by differentiation from the first cell type, with both populations have the growth rate of the type-1 population. The behaviour of $Z(t)$ and $G(t)$ for the entire population is the sum of the corresponding processes for the two types.

Turning to the relatedness in random prefactors, Fig. 6a is consistent with the deduction that there is equality almost surely between the rescaled limit of the population size and total generation of the second type. Figure 6b shows the prefactor for type-1 and type-2 population sizes. Consistent with results in (Jagers 1969a) red dots are suggestive that when $\alpha_2 < \alpha_1$ both normalised processes converge to the same random variable. For $\alpha_1 < \alpha_2$, however, this is not the case for the blue dots and the random variables appear uncorrelated. Figure 6c is analogous to Fig. 6d but for total generation, with the same deduction as for the population size holding where when $\alpha_1 > \alpha_2$, the randomness is common to both types and otherwise it is not.

Part of the significance of Propositions 3 and 4 is that they provide an instrument by which one can infer the average generation of each of the populations in a two-type Bellman–Harris branching process, generalising the results in (Weber et al. 2016)

(a)

(b)

(c)

(d)
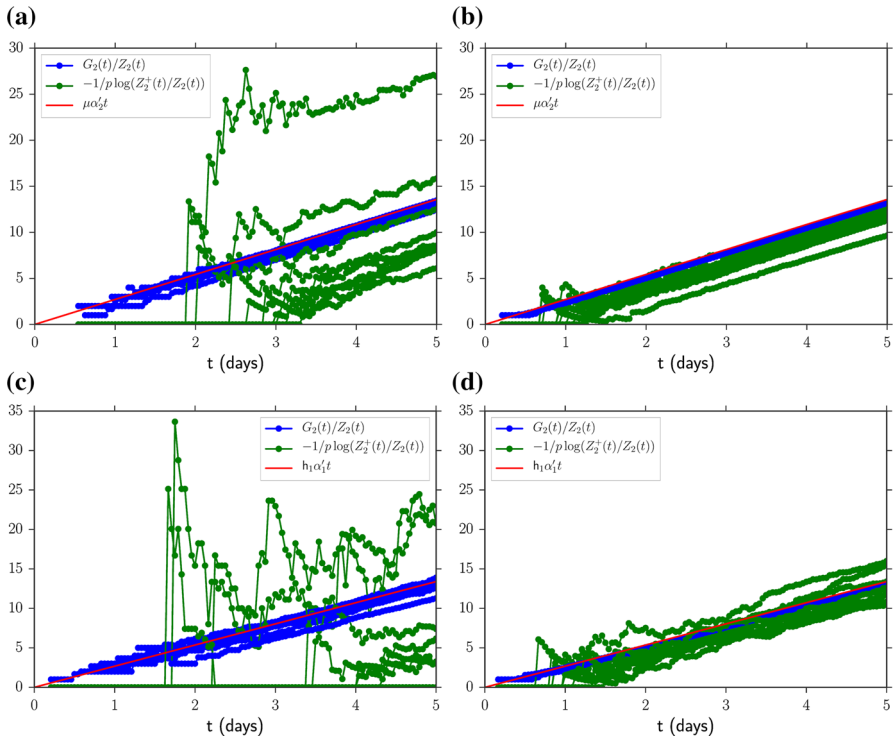
Fig. 7 Sample-path estimation of average generation. For each sub-panel, ten Monte Carlo simulations of a two-type population are presented. These employ the same parameterisation in Fig. 5, with the exception of the initial population size in the two right hand side panels. Each initial cell is equipped with a neutral label that doesn't alter population dynamics, and which is lost irrevocably to all subsequent offspring with probability $p = 10^{-2}$ per cell division. The red line indicates the theoretical prediction of the mean average generation. Blue lines indicate the development of the per-path average generation, while the green lines are the estimates from the delabelling formula (1). **a, b** Plots are in the setting $\alpha_1 < \alpha_2$ case, but start with one and 100 type-1 cells at $t = 0$, respectively. **c, d** Equivalent of (**a, b**) but with $\alpha_2 < \alpha_1$

Proposition 2. In the presence of cells equipped with a neutral label that is heritably lost with a fixed probability at each division, the average generation and a function of the proportion of label-positive cells of each type share the same dominant term. The mathematical results say that the slope of the average generation and the slope of the estimator are the same when the probabilistic regularity of a large population takes hold. Figure 7a, b illustrate this relationship for the type-2 population via the use of some Monte Carlo simulations in the presence of a single initial label positive cell of type-1. In this setting the large population regularlity only takes hold at later times. Starting with more than one initially labelled cell, illustrated with 100 in Fig. 7c, d, results in the desired asymptotic equivalence occuring at a much earlier time. For true cellular systems, the cell numbers are likely to be significantly larger again.

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