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Stochastic fluctuations drive non-genetic evolution of proliferation in clonal cancer cell populations

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Evolutionary dynamics allows to understand many changes happening in a broad variety of biological systems, ranging from individuals to complete ecosystems. It is also behind a number of remarkable organizational changes that happen during the natural history of cancers. These reflect tumour heterogeneity, which is present at all cellular levels, including the genome, proteome and phenome, shaping its development and interrelation with its environment. An intriguing observation in different cohorts of oncological patients is that tumours exhibit an increased proliferation as the disease progresses, while the timescales involved are apparently too short for the fixation of sufficient driver mutations to promote an explosive growth. In this paper we discuss how phenotypic plasticity, emerging from a single genotype, may play a key role and provide a ground for a continuous acceleration of the proliferation rate of clonal populations with time. Here we address this question by means of stochastic and deterministic mathematical models that capture proliferation trait heterogeneity in clonal populations and elucidate the contribution of phenotypic transitions on tumour growth dynamics.

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1 Introduction

Evolution is one of the central unifying concepts of biology and a driving force behind life, being a cornerstone of complex systems organization [1]. It is ubiquitous through the natural world from molecules to cells, organisms and populations and in fields as diverse as zoology, botany, microbiology and oncology. Evolutionary changes in the context of asexual reproduction are mainly driven by heritable somatic mutations and epigenetic changes, genetic drift and natural selection. Evolution theory has been classically grounded in genetics and Darwinian selection processes. In the light of evolution, tumour progression has often been explained by looking at the somatic changes of cancer cells [2,3]. However, from this viewpoint, one might incur in some reductionist assumptions that are sometimes in conflict with what is observed during the real course of the disease, including treatment failure and relapse [4]. There is a growing interest in studying the evolutionary rules of cancer, and a number of important questions remain open. Increased attention has recently been given to intratumour heterogeneity as its potential role in therapeutic outcome and emergence of drug resistance [5–8]. Intratumour heterogeneity occurs at various levels, including the genome, transcriptome, proteome and phenome [9]. Research has mainly focused on mapping cancer genome instability and driver event mutations conferring a selective advantage to the affected cell clone. Non-genetic instabilities are also relevant since it is known that a single stable genotype may lead to a broad landscape of stable phenotypes [10,11]. Initial states of cancer development imply colonization of novel environments and subsequent stressful conditions [12], which may actually increase traits heritability (understanding heritability as the relation between genetic variance for the trait and the phenotypic variance for the same trait). Phenotypic variance is the result of a compendium of variances: the variance attributed to differences among genotypes, the variance associated exclusively with changes in the environment and the 'interaction variance' which represents that some genotypes might respond to the environment in a different way than others [13]. Distinct phenotypic states frequently involve differences in functional cell properties and the proportion of these phenotypes has been related to cancer grade [14,15]. This resembles what occurs in other biological contexts, where a broader population composition, which comprises a higher phenotypic diversity, increases the odds for an adaptive response to external perturbations [16].

Recent observations in both *in vivo* murine models and cohorts of cancer patients of different hystologies have found a superlinear scaling law relating proliferation and tumour size [17]. Also a longitudinal dynamics was observed implying a continuous acceleration of proliferation rates during tumours natural history, which is a dynamical counterpart of the scaling law. This fact was attributed initially to the tumour's genetic evolutionary dynamics and supported with different mathematical modelling frameworks. However, a closer look at this interpretation raises a number of questions. The results presented in [17] included two studies in animal models that displayed accelerated tumour growth dynamics in the course of one month. Longitudinal volumetric data obtained from images of cancer patients with untreated brain metastasis also showed similar growth patterns. Genetic changes seem to be necessary [18] but not sufficient to generate such an accelerated tumour growth since they require either an extremely high mutation rate, which would be restricted by cell viability, or a long time scale [19] that was not the case in the former results. For these reasons, neither the human data spanning typically few months, nor the animal models data, could be exclusively associated with tumour genetic changes because of the short time scales involved. This raises the question that we wish to explore in this paper: Could there be additional non-genetic evolutionary forces playing a role in accelerating tumour growth as observed in Ref. [17]?

The recognition of the role of phenotypic aspects in cancer evolutionary dynamics has elicited a progressive change in perspective from seeing cancer as a 'genetic disease' to a broader, 'developmental' perspective. Some analogies with embryonic development have been made, rapidly dividing tissues may have evolved increased cancer suppresion [41] and phenotypic plasticity in cancer has been biologically addressed in the literature of cancer stem cells [20]. Also

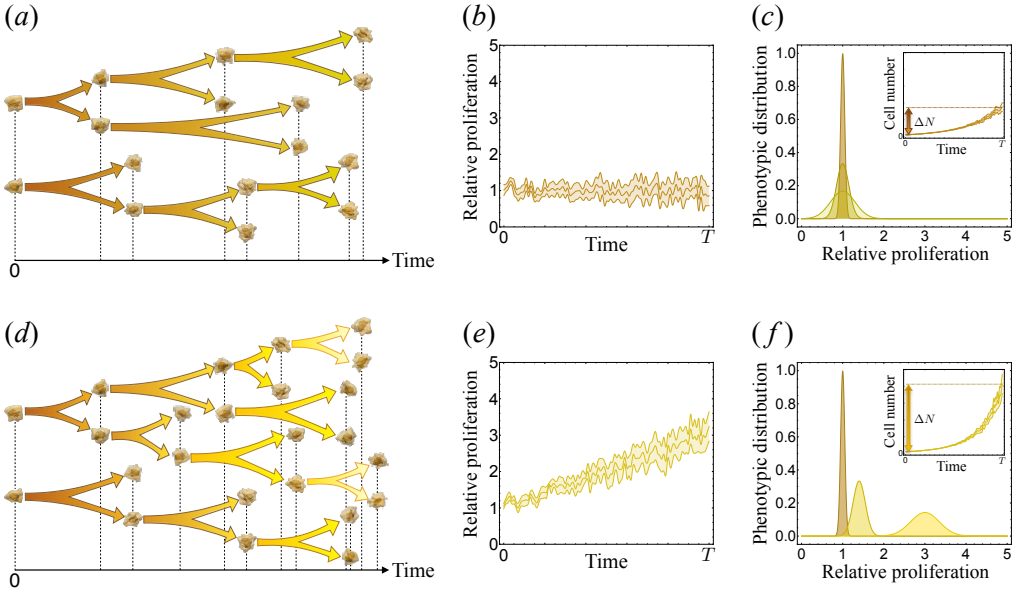

Figure 1. Stochastic fluctuations of cancer cell proliferation in two distinct scenarios: quasi-constant mean proliferation rate (*a*)-(*c*) and increasing mean proliferation rate (*d*)-(*f*). Tumour cells divide at random rates (*a*) and (*d*). Relative proliferation (*b*) and (*e*), showing the time-varying proliferation divided by the initial proliferation. Phenotypic distributions (*c*) and (*f*) at subsequent times; insets depict the net growth ΔN in the cell number during a temporal window $[0, T]$.

diapause-like states have been described as a survival mechanism against chemotherapy [21,22]. In this context, an initial clonal population will be subject to evolutionary pressures in a stochastic or environmental-induced way that finally shape the population structure.

Fluctuations in proliferation rates of clonal cancer cell populations have been observed in cultures [23–25]. The repetition of the same cell-culture experiment leads to large variations in the outcome that cannot be due only to differences in the number of cells seeded initially. This well-known fact is typically attributed to uncontrollable changes in experimental conditions. Some authors have previously accounted for these baseline variations in cell cycle duration among heterogeneous cancer cell populations [26,27] that could be indeed tightly linked to tumour response to therapy [28]. By nature, phenotypic plasticity could affect any cell trait [29], but here we will focus on proliferation as a key phenotypic characteristic in cancers. Specifically, we will study *in silico* the possibility that stochastic changes in the growth rate of clonal populations could lead to an evolutionary dynamic of that trait. Our main hypothesis would be that small phenotypic changes resulting in either faster or slower proliferation could emerge as a result of noise-induced nongenetic variability. This may lead to some variability in clonal populations that may provide the appropriate ground for selection and evolutionary dynamics. Here we will address, from a mathematical perspective, what is the outcome of those dynamics. Figure 1 illustrates the underlying rationale of how fundamentally different fluctuations in proliferation eventually impact on the net growth in cell number. The upper row (a)-(c) in Fig. 1 represents the scenario in which the cellular division time randomly varies around some basal value, as we could expect from any cellular trait. In this case, cell number shows a typical exponential growth profile (1(c)) without constraints. But, what happens if we introduce stronger fluctuations in proliferation with respect to basal value accounting for phenotypic changes? One possible, and fundamentally different, scenario is depicted in the second row (d)-(f) in Fig. 1. A significantly larger cell number change ensues with respect to the preceding scenario. We elaborate below on these qualitative settings by putting forward, both discrete and continuous, mathematical models

with the final goal of trying to answer, or at least shed light, to the question raised earlier of what is the main driving force behind the accelerated tumour growth observed in human cancers [17].

2 Materials and methods

To study the impact of phenotypic changes in proliferation on the growth dynamics of a clonal tumour cell population, we resorted to two mathematical models. The first one was based on a discrete simulator incorporating stochastic jumps between different proliferative states. The second one, consisting of a continuous reaction-diffusion parabolic equation, recapitulated the key aspects of the discrete model and allowed us to find explicit analytical formulas for the temporal dynamics of the total tumour cell number, together with the mean and the standard deviation in proliferation.

2.1 Discrete stochastic model

Let us first put forward a discrete stochastic model describing the growth dynamics of a clonal population of tumour cells having different proliferation rates, i.e. one in which not all cells divide at the same pace. To simplify the analysis, we consider a large but finite number M of allowed proliferation rates ρ_i in the interval $[\rho_{\min}, \rho_{\max}]$. Each cell belongs to a proliferative state i (with $i = 1, 2, \ldots, M$) defined by its rate $\rho_i = (i-1)\Delta\rho + \rho_{\min}$, where $\Delta\rho = (\rho_{\max} - \rho_{\min})/(M-1)$. Let $N_i(t)$ denote the number of tumour cells having phenotype i, thus corresponding to a rate ρ_i, at time t. The total number of cells at time t is $N(t) = \sum_{i=1}^{M} N_i(t)$. At time $t = 0$ the population, with N_0 being the initial cell number, is distributed in the phenotypic landscape around a characteristic proliferation rate ρ_* having a standard deviation σ_*. To simulate the population dynamics at later times, for every interval $[t, t+\Delta t]$ in steps Δt, we test whether each cell has undergone a phenotypic switch, with transition rate $\Gamma_{i\to j}$, from proliferation state i to an adjacent state $j = i \pm 1$ characterized by a proliferation rate ρ_j. No phenotypic jumps are allowed from $i = 1$ to $j = 0$ and from $i = M$ to $j = M+1$. All these switches thus give rise to a net decrease in the number $N_i(t)$ of cells having the same ρ_i at time t. Similarly, phenotypic jumps with transition rates $\Gamma_{j\to i}$ from adjacent proliferation states $j = i \pm 1$ into i result in a net increase in the cell number $N_i(t)$. Additionally, during time interval $[t, t+\Delta t]$, mitotic and apoptotic events could also take place, each either increasing or decreasing the cell number $N_i(t)$ by one unit. Combining all these stochastic processes leads to a balance equation for the number of cells that, at time $t + \Delta t$, have a proliferation rate ρ_i

$$\begin{aligned} N_i(t+\Delta t) &= N_i(t) - \Delta t\,(\Gamma_{i\to i+1} + \Gamma_{i\to i-1})N_i(t) + \Delta t\,(\Gamma_{i+1\to i}N_{i+1}(t) + \Gamma_{i-1\to i}N_{i-1}(t)) \\ &+ \Delta t\rho_i N_i(t) - \Delta t\mu N_i(t), \end{aligned} \tag{2.1}$$

with μ being the death rate (taken equal for all cells). In our numerical simulations using (2.1) we assumed for simplicity that $\Gamma_{i\to i+1} = \Gamma_{i\to i-1} = \Gamma_{i+1\to i} = \Gamma_{i-1\to i} \equiv \Gamma$, hence giving rise to symmetric transition jumps, except at the end points $i = 1$ and $i = M$.

The interplay of processes described above will result in a scenario where all phenotypic changes are *inheritable*, i.e. when a cell is committed to mitosis, its progeny will be placed in the same proliferative state. This gradually yields a progressive irreversibility from the starting characteristic proliferation rate ρ_* towards a different phenotypic landscape. In an alternative scenario, to explore the possibility of a *partial loss of inheritance* (and thus of partial reversibility), we also considered the effect of adding a decay probability to the starting characteristic proliferation rate ρ_*, equivalent to the time required to complete m_{cd} cell divisions. Computationally, this was implemented via a transition rate $\Gamma_{i\to *}$ into a localized distribution (e.g. Gaussian) centred around ρ_* for each phenotype i. In both scenarios (i.e., under *inheritance* or *partial loss of inheritance*), to monitor the dynamics of the population in the phenotype space, we evaluated the different population frequencies $f_i(t) = N_i(t)/N(t)$, with $i = 1, 2, \ldots, M$, and the mean proliferation rate $\langle\rho\rangle(t) = \sum_{i=1}^{M} \rho_i f_i(t)$.

2.2 Continuous reaction-diffusion-advection model

To derive a partial differential equation-based model that would help to better elucidate the time dynamics of the previous discrete stochastic framework, we considered the same processes albeit we extended the phenotypic switches assuming nonnegative transition rates $\Gamma_{i\to j}$ from a proliferation state i to another state j, where $i,j=1,2,\ldots,M$, and phenotypic switches with transition rates $\Gamma_{j\to i}$ from proliferation states j into i. Notice that $\sum_{j=1}^{M}\Gamma_{i\to j}=1$ and $\sum_{j=1}^{M}\Gamma_{j\to i}=1$, although the transition rates $\Gamma_{i\to j}$ and $\Gamma_{j\to i}$ are not necessarily equal in general. The balance equation (2.1) reads now as

$$N_i(t+\Delta t) \;=\; N_i(t)-\Delta t\sum_{j=1}^{M}\Gamma_{i\to j}N_i(t)+\Delta t\sum_{j=1}^{M}\Gamma_{j\to i}N_j(t)+\Delta t\rho_i N_i(t)-\Delta t\mu N_i(t). \quad (2.2)$$

Balance equation (2.2) is quite general and encompasses the inclusion over time of new subpopulations labelled by their proliferation phenotype as their sizes become nonzero as well as the extinction of others when their cell numbers vanish. Moreover, the terms $\Gamma_{i\to j}N_i(t)$ and $\Gamma_{j\to i}N_j(t)$ can be understood as outward and inward cell currents for phenotype i, respectively.

We next perform a continuous limit approximation of (2.2). This amounts to let $\Delta t\to 0$ and $\Delta\rho\to 0$ while the transition rates $\Gamma_{i\to j}\to\infty$ and $\Gamma_{j\to i}\to\infty$. In those limits, we assume that the two quantities $D=\Delta\rho^2/2\sum_{j=1}^{M}\Gamma_{i\to j}(j-i)^2$ and $v=\Delta\rho\sum_{j=1}^{M}\Gamma_{i\to j}(j-i)$ remain finite. Hence, we arrive at the following reaction-diffusion-advection equation

$$\frac{\partial n}{\partial t}=D\frac{\partial^2 n}{\partial\rho^2}-v\frac{\partial n}{\partial\rho}+\rho n-\mu n, \quad (2.3)$$

where $n=n(\rho,t)$ denotes the cell density function, such that $n(\rho,t)\,d\rho$ represents the number of tumour cells that, at time t, have a proliferation rate between ρ and $\rho+d\rho$. The first term on the right-hand side of (2.3) accounts for the fluctuations in the proliferation phenotype occurring with a diffusion constant D which is nonnegative. The second term describes the phenotypic drift in proliferation with a velocity v. Notice that this velocity may be positive or negative depending on the sign of $\sum_{j=1}^{M}\Gamma_{j\to i}(j-i)$ and is zero for fully symmetric or unbiased transitions. The third and fourth terms in (2.3) comprise the mitotic and apoptotic events. Additional mechanisms could be easily incorporated into (2.3), such as growth-limiting mechanisms preventing an unbounded increase in the total cell number. However, our main focus is to look at time scales for which the tumour has not yet achieved a large size.

The reaction-diffusion-advection equation (2.3) is further supplemented with initial and boundary conditions: $u(\rho,0)=u_0(\rho)$ and $\frac{\partial n}{\partial\rho}=0$, both at $\rho=\rho_{\min}$ and $\rho=\rho_{\max}$. These two zero-flux boundary conditions ensure that no cell will have a proliferation rate outside the interval $[\rho_{\min},\rho_{\max}]$. Rather than solving (2.3), which can be carried out by means of a Green's function formalism, it is enough for our purposes to focus on time-evolving average quantities. Specifically, the total number of cells, given by $N(t)=\int_{\rho_{\min}}^{\rho_{\max}}n(\rho,t)d\rho$, the mean proliferation rate $\langle\rho\rangle(t)=\frac{1}{N(t)}\int_{\rho_{\min}}^{\rho_{\max}}\rho\,n(\rho,t)d\rho$, and the variance $\langle\sigma\rangle^2(t)=\frac{1}{N(t)}\int_{\rho_{\min}}^{\rho_{\max}}(\rho-\langle\rho\rangle(t))^2\,n(\rho,t)d\rho$.

3 Results

3.1 Quantifying the inheritable scenario

First, we simulated the fully inheritable case in a time frame of $T=30$ days by means of the discrete stochastic model presented in Subsection 2.1. This time frame was sufficiently short to discard relevant mutational events. An example of a typical outcome is shown in Fig. 2(a). Even when the phenotypic transitions were fully symmetric, the system spontaneously drifted towards higher proliferation values, with the mean proliferation rate $\langle\rho\rangle(t)$ reaching levels more than two times larger than the initial one ρ_* [see inset Fig. 2(a)]. Also, a broadening in the phenotype landscape was apparent as time passed. In addition, the reaction-advection-diffusion

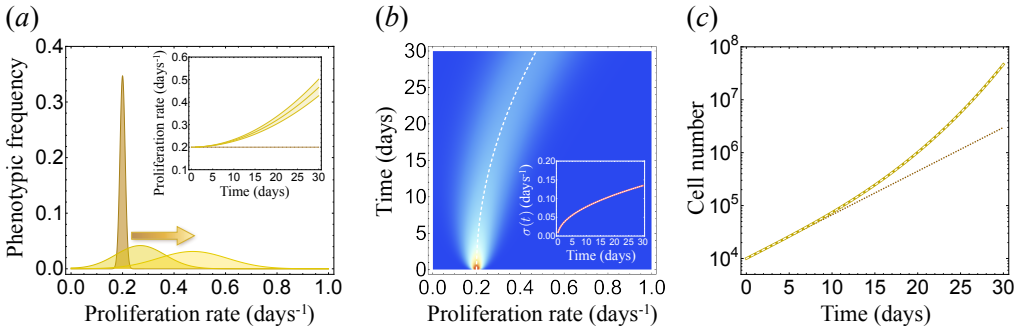

Figure 2. Computational results of the evolutionary dynamics in the landscape of the clonal tumour cell population studied in the scenario with full phenotype inheritance. (a) Phenotypic frequency at times $t = 0, 15$, and 30 days (from left to right) of a typical run using the discrete stochastic model. The inset shows a time-dependence of the mean proliferation rate as an increasing broadening band, whereas the dotted line corresponds to a constant proliferation rate $\rho_* = 0.2$ days^{-1}. (b) Pseudocolor plot of the population cell density for $\rho \in [0, 1]$ days^{-1} and $t \in [0, 30]$ days computed from the continuous reaction-advection-diffusion model (2.3). The dashed white line indicates the mean proliferation rate $\langle\rho\rangle(t)$ of the distribution as predicted by Eq. (3.5). The inset shows the standard deviation $\langle\sigma\rangle(t)$ from the solution of (2.3) (thick reddish curve) and the analytical formula (3.6) (overlapping dashed white curve). (c) Dynamics of the total cell population $N(t)$ from the solution of (2.3) (thick golden curve) and the analytical formula (3.4) (overlapping dashed white curve). The dotted line corresponds to the case of a purely exponential growth with a constant proliferation rate ρ_*. Numerical values used: For the discrete stochastic model $\Gamma = 6.0$ days^{-1}, $M = 101$ nodes, $\Delta t = 1$ h, whereas the number of simulation runs was equal to 50. For the continuous reaction-advection-diffusion model, $D = 3.0 \times 10^{-4}$ days^{-3} and $v = 0$ days^{-2}. For both the discrete and the continuous models, we used an initial Gaussian distribution centred around $\rho_* = 0.2$ days^{-1} and initial standard deviation $\sigma_* = 0.01$ days^{-1}. Also, $\mu = 0.01$ days^{-1}, $N_0 = 10^4$ tumour cells, $\rho_{\min} = 0$ day^{-1} and $\rho_{\max} = 1$ day^{-1}.

model allowed us to reproduce these features in the same time frame, as depicted in Fig. 2(b), together with the standard deviation rate [inset Fig. 2(b)]. Moreover, both models predicted that the total cell population, when plotted in a logarithmic scale, increased much faster than a simple exponential growth, as shown in Fig. 2(c).

To determine the underlying explicit time dependences of all these distinctive characteristics, and hence gain further insight, we employed the reaction-advection-diffusion model to compute the time derivatives of $N(t)$, $\langle\rho\rangle(t)$ and $\langle\sigma\rangle^2(t)$ using (2.3). These led to the following ordinary differential equations

$$\frac{dN}{dt} = -\mu N(t) + \langle\rho\rangle(t) N(t), \tag{3.1}$$

for the total cell number and

$$\frac{d\langle\rho\rangle}{dt} = v + \langle\sigma\rangle^2(t), \tag{3.2}$$

for the mean proliferation rate. In deriving (3.1) and (3.2) we neglected the boundary values $n(\rho_{\max}, t)$ and $n(\rho_{\min}, t)$, a valid assumption when the cell population does not reach significant levels at the end points of the proliferation interval. Moreover, when $n(\rho, t)$ was assumed to have a Gaussian distribution with initial mean proliferation rate ρ_* and standard deviation σ_*, we arrived at a third ordinary differential equation

$$\frac{d\langle\sigma\rangle^2}{dt} = 2D, \tag{3.3}$$

for the variance.

The three differential equations (3.1)-(3.3) constitute an exactly solvable model. The first one yields

$$N(t) = N_0 e^{-\mu t + \rho_* t + \frac{v t^2}{2} + \frac{\sigma_*^2 t^2}{2} + \frac{D t^3}{3}}, \tag{3.4}$$

with N_0 being the initial population. Thus, (3.4) shows that the total cell population evolves in a *fundamentally different* fashion than a simple exponential growth $N(t) = N_0 e^{\rho_* t}$, the latter occurring if no phenotypic changes take place in the initial proliferation rate.

For the mean proliferation rate we find

$$\langle \rho \rangle (t) = \rho_* + \left(v + \sigma_*^2 \right) t + D t^2. \tag{3.5}$$

Equation (3.5) accounts for the drift in the mean proliferation seen in all of our simulations, which is quadratic with time. Notice that even in the absence of the drift velocity ($v = 0$), $\langle \rho \rangle (t)$ still increases with time, the dominant contribution being due to the stochastic fluctuations embodied in the diffusion coefficient D and, to a lesser extent, to the initial variability σ_*.

The time dependence of the standard deviation is

$$\langle \sigma \rangle (t) = \sqrt{\sigma_*^2 + 2Dt}\,, \tag{3.6}$$

which provides another explicit and simple expression for the broadening in the phenotype landscape observed in our numerical simulations. This form for the standard deviation is characteristic of other standard diffusive processes [30].

Figures 2(b) and 2(c) also compare the numerical solutions of the mean proliferation rate, the standard deviation and the cell number obtained from (2.3) and formulas (3.4)-(3.6) giving additional confirmation of the internal consistency of our findings. Hence, in the scenario where full phenotype inheritance occurs, three distinctive features arise: a broadening in the phenotype landscape, a drift in the mean proliferation and a total cell population growing faster than a classic exponential law.

3.2 Quantifying the partially inheritable scenario

We then considered the scenario of a partial loss of inheritance in the phenotypic traits by assuming that all phenotypes have a probability $\Gamma_{i \to *}$ to revert to the basal phenotype with proliferation rate ρ_*. As in the inheritable scenario, a drift of the mean proliferation rate $\langle \rho \rangle (t)$ towards higher values with time was observed [see Fig. 3(e)], although the magnitude of this drift was smaller as the relative importance of $\Gamma_{i \to *}$ increased. Another visible difference from Fig. 2 with the full phenotype inheritance scenario is that the distribution displays a bimodal profile for a certain range of $\Gamma_{i \to *}$, evidencing the coexistence of a peak corresponding to the clonal population distributed around the initial proliferation rate ρ_* and a second broader peak comprising the more *evolved* phenotypes [see Fig. 3(a-d)]. The standard deviation of the $\langle \sigma \rangle (t)$ phenotypic distribution increases in time for all values for the *reverse transition rate*. Reasonably, this increase is less impressive as the reverse transition rate grows, but the increase in phenotypic variability is robust across all tested conditions (Fig. 3(f)]. Also, faster than a simple exponential growth occurred in the population [Fig. 3(g)] the magnitude of which was modulated by the partial phenotypic inheritance condition embodied in $\Gamma_{i \to *}$.

4 Discussion

In this study we put forward a mathematical model based on stochastic phenotypic transitions. For simplicity, and since we were mainly interested in characterising how and why tumour growth accelerates in time, we focused on proliferation. It is already known that tumour cells have a higher growth rate than proliferative non-tumoral tissues. Clonal cell lines exhibit also a higher frequency of random monoallelic expression that could increase phenotypic plasticity and spread the probability of success in a changing environment without altering the population identity.

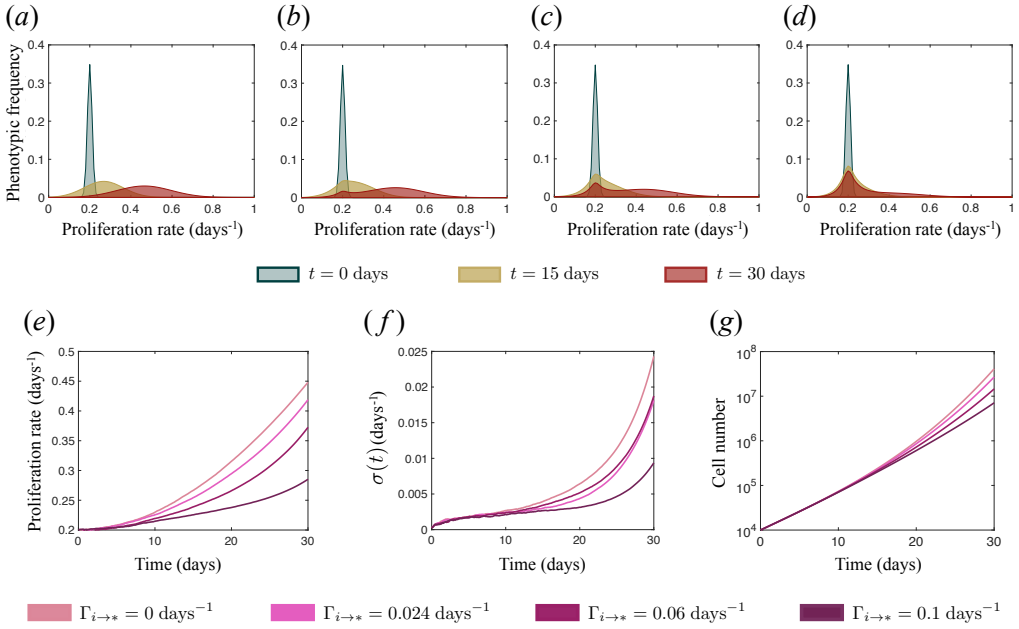

Figure 3. Computational results of the evolutionary dynamics in the landscape of the clonal tumour cell population studied in the scenario of partial loss of inheritance. (a)-(d) Phenotypic frequency at times $t = 0, 15$, and 30 days for different values of the transition rate $\Gamma_{i\to *}$: (a) $\Gamma_{i\to *} = 0$ days^{-1}, (b) $\Gamma_{i\to *} = 0.024$ days^{-1}, (c) $\Gamma_{i\to *} = 0.06$ days^{-1}, (d) $\Gamma_{i\to *} = 0.12$ days^{-1}. (e) Time-dependence of the mean proliferation corresponding to the cases (a)-(d). (f) Time-dependence of the standard deviation corresponding to the cases (a)-(d). (g) Dynamics of the total cell population corresponding to the cases (a)-(d).

In this study our initial setting consisted of a genetically homogeneous clonal population, with all cells having a growth rate concentrated around a certain value. These cells were allowed to slightly increase or decrease their growth rate in time with equal probability and thus explore a landscape of proliferation states while keeping a hypothetical common genotype. One key prediction of the mathematical models developed in this work was that the sole action of stochastic phenotypic transitions, leads to a growth of the total clonal tumour cell population that is fundamentally much faster than classical exponential growth. Besides the recent findings of explosive tumour growth in several types of cancers [17], it is also intriguing to look at other natural contexts where dramatic increases in other population species may also take place, such as cyanobacteria and algae blooms occurring in eutrophic waters [31], revealing the need for further explorations within the interplay between oncology and ecology via evolutionary theory.

Phenotypic plasticity together with noisy gene and protein levels expression has been pointed out by means of next-generation sequencing techniques such as single cell RNA-sequencing or tissue-specific differentially methylated regions (tDMRs), giving an increasing importance to the distribution of gene expression levels or epigenetics marks beyond the classical genocentric point of view [32]. This stochastic phenomenon has also been reported at many other levels in nature, including differing levels of resistance to antibiotics in genetically identical bacteria or even the stochastic mechanism underlying the development of trichromatic vision of human individual cone cells [33].

Many authors have previously analysed evolutionary cancer dynamics in phenotype-structured populations [34]. However they usually include selection pressures as a key issue to observe the consequences of this phenotypic variability. A very interesting approach to these questions from a statistical mechanics perspective suggests that the number of available states shapes tumour growth [35]. However, to the best of our knowledge, the effect of the existence of a

proliferation phenotypic landscape and its potential role as an underlying force having a *steering* effect on the natural history of tumours has not been addressed in detail.

While genomic instability and driver gene mutations play an essential role in the evolutionary dynamics of human cancers, the sustained increase in proliferation observed in [17], which our mathematical models also predict, has some resemblance with classic Darwinian selection ideas which are tightly linked to the selection of the fittest genotype (in our case phenotype). On the other hand, the process found is not of a Lamarckian-type since we did not consider phenotypic switches to be environmentally-driven in our models [7,36].

One of the novel aspects of this work is the spontaneous increase in average growth rate with time. A reduction in cell cycle duration over time had previously been described under therapy-induced cell death [28]. There it was assumed that the tumour population consisted of cells with different albeit intrinsic and fixed proliferation rates. These were inherited and microenvironmental-independent without undergoing any changes in the simulations. In our models phenotypic diversity emerges from a clonal population that continuously experiences stochastic transitions which may be small but eventually drive the tumour cells towards more proliferative phenotypes. This diffusion in phenotype landscape reminds us of spread dynamics of invasive species, in which the rate of new site colonization is not constant over time as has been proved in a variety of biological kingdoms, from virus to vertebrates [37].

Adaptive plasticity could be considered as a trait itself and consequently be subject to evolutionary processes. However, we could expect that it does not play such an important role in a 'constant', non-tumour, environment. Cancer cells are exposed to changing environmental conditions across tumour life history and this may lead to a short-term evolutionary response where genetic variation would not be the main driving force. Phenotypic diversity is a convenient strategy for the success of population expansions in a broad range of contexts. Although it is challenging to test this kind of hypothesis at the laboratory due to the required long time-scale (as a consequence of long individual lifespan), some attempts in unicellular organisms have been reported in the literature. Those evidences reflect that at short time scales, phenotypic variations are key as a strategy to succeed in fluctuating environments as shown in *Chlamydomonas* [38] and *Lactobacillus sp.* [39], also allowing for specialization in the long-term, as shown by *Escherichia coli* culture over 2000 generations under an alternating temperature regimen [40]. The behaviour of subclonal populations interacting within the constraints of the tumour microenvironment could resemble the dynamics of the interaction of functional groups of species with variation in resource exploitation ability and environmental requirements. Although phenotypic diversity implies an additional productivity cost for the functional group, a higher phenotypic variance seems to increase the long run performance [16].

Another interesting point concerns the role of inheritance of phenotypic modifications. It is already known that phenotypic modifications in somatic cells can be passed on from one generation to another by mitosis as stated previously in the fully inheritable scenario [42,43] and they do not necessarily reverse after the inducing agent ceases [44]. In fact, increasing evidence suggest that adaptation can be graded. Short-term stress would evoke tiny modifications in gene expression through signalling-mediated regulation of gene expression. On the other hand, a sustained stress situation could lead to a more radical switch in cell state, through epigenetic regulation or positive feedback loops, and hence drive to a permanent phenotypic modification [45–47]. We considered important to reflect those different inheritance patterns in our work through the partially inheritable scenario. Our results indicate that the shift towards a more aggressive average profile in the tumour phenotypic distribution is qualitatively robust across both scenarios, even when modifying the reverse transition rate. The relationship between this shift in proliferation phenotypic distribution and spatial heterogeneity remains to be explored. Microenvironmental spatial heterogeneity due, for example, to the gradients of nutrients and metabolic waste generated by tumour cells [48] might also affect that reverse transition rate. The availability of physical space and new niches for dispersal and colonization might also accelerate the shift in the tumour average proliferation rate [49–51]. Indeed, the

theoretical location of evolution at the tumour boundary has been previously reported [52] and the highest proliferation activity seems to be located also at the tumour edge, specially in poor prognosis cases, as it was recently underlined in two cohorts of breast and lung cancer patients [53].

The broadening in the phenotype landscape predicted by our models may play a key role not only in the heterogeneity of the clonal tumour cell population, but also in the emergence of resistance mechanisms under the administration of cytotoxic therapies [7]. Although these may successfully target the most proliferative cells, eventually those in the lower spectrum of the phenotype landscape would be able to repopulate the fastest proliferation cell states. Phenotypic diversity is a convenient strategy for the success of population expansions in a broad range of contexts. A better understanding of the fundamental biological processes underlying phenotypical plasticity as a source of intratumoral heterogeneity might be useful for tumour containment or implementing adaptive therapies [13,54], and, ultimately, for better design of therapeutic strategies.

In conclusion, in the context of mathematical models displaying phenotype plasticity, we have observed three distinctive features: a broadening in the phenotype landscape, a drift in the mean proliferation and a total cell population growing faster than classic exponential laws. Our models were conceptually simple and can be extended along many directions, including not only spatial effects (e.g. saturation), other traits besides proliferation, as well as more cell subpopulations (e.g. immune cells). The main predictions seem to be robust enough for experimental validation. It is remarkable that these effects emerge spontaneously in the absence of selection pressures and are independent of initial seeding and phenotypic switching probability. When phenotypic traits were allowed to be lost partially, resembling the dilution of epigenetic marks as cell division progresses, the effects were still preserved. This evolution towards a more aggressive phenotype would undoubtedly be accentuated by the presence of selection pressures in the tumour microenvironment. Furthermore, we cannot ignore that this stochastic variation is probably affecting almost any cell trait and consequently tumour cell interactions with other tumour cells and also with the surrounding tissue. However, our results indicate that the existence of this stochastic non-genetic variability in the proliferation rate seems enough to spontaneously drive to a more aggressive tumour average phenotype.

Data Accessibility. Simulations were conducted in MATLAB (version R2020a). Code files for the discrete model simulations are publicly accessible at: `https://github.com/molabEvoDynamics/rep_StochasticFluctuationsDriveNonGeneticEvolution`. [55]

Authors' Contributions. G.F.C. and V.M.P.-G. formulated the hypothesis and designed the research. C.O.-S., G.F.C. and V.M.P.-G. performed the research. C.O.-S., G.F.C. and V.M.P.-G. wrote the paper. All authors gave final approval for publication.

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