Extinction rates in tumour public goods games

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Cancer evolution and progression are shaped by cellular interactions and Darwinian selection. Evolutionary game theory incorporates both of these principles, and has been proposed as a framework to understand tumour cell population dynamics. A cornerstone of evolutionary dynamics is the replicator equation, which describes changes in the relative abundance of different cell types, and is able to predict evolutionary equilibria. Typically, the replicator equation focuses on differences in relative fitness. We here show that this framework might not be sufficient under all circumstances, as it neglects important aspects of population growth. Standard replicator dynamics might miss critical differences in the time it takes to reach an equilibrium, as this time also depends on cellular turnover in growing but bounded populations. As the system reaches a stable manifold, the time to reach equilibrium depends on cellular death and birth rates. These rates shape the time scales, in particular, in coevolutionary dynamics of growth factor producers and free-riders. Replicator dynamics might be an appropriate framework only when birth and death rates are of similar magnitude. Otherwise, population growth effects cannot be neglected when predicting the time to reach an equilibrium, and cell-type-specific rates have to be accounted for explicitly.

1. Introduction

The theory of games was devised by von Neumann & Morgenstern [1], and according to Aumann [2], game theory is an ‘interactive decision theory’, where an agent’s best strategy depends on her expectations on the actions chosen by other agents, and vice versa. As a result, ‘the outcomes in question might have been intended by none of the agents’ [3]. To rank and order strategies, and to optimize individual payoffs, different systems to systematically identify equilibria have been defined. Most famously, the Nash equilibrium is a set of strategies such that no single agent can improve by switching to another strategy [4]. This concept includes mixed equilibria, which describe probability distributions over strategies. Such equilibrium concepts in game theory cover various kinds of patterns of play, i.e. simultaneous, non-simultaneous and asymmetric strategies [5]. This rich and complex framework allows for a wide application of game theory beyond economics, famously in ecology and evolution [6]. In biological context, and especially in evolutionary game theory, the focus has been on simultaneous and symmetric strategic interactions in evolving populations [7].

Evolutionary game theory replaces the idea of choice and rationality by concepts of reproduction and selection in a population of evolving individuals [8] and was conceived to study animal conflict [9]. Behavioural phenotypes are hardwired to heritable genotypes. Without the possibility of spontaneous mutation events, offspring carry the parent strategy. Evolutionary games have also been used extensively to study learning and pairwise comparison-based...
changes in strategy abundance in populations of potentially erroneous players [10–12].

Selection in evolutionary games is based on the assumption that payoff translates into Darwinian fitness, which is a measure for an individual’s contribution to the pool of offspring in the future. Complex deterministic dynamical systems arise when one considers very large populations of reproducing individuals. The most prominent example for such a system is the replicator equation [13], which focuses on the relative abundance of each strategy. The replicator equation does not model population growth specifically, but rather describes changes in relative abundances. Existence and stability of fixed points in these dynamical systems depend on the payoffs [14], and on the choice of fitness function [15]. In the study of animal behaviour, the precise measurements of payoffs, as observed from individuals’ behaviours, is difficult. Milinski et al. [16] determined all but one payoff parameter precisely, in order to observe tit-for-tat strategies in repeated Prisoner’s Dilemma games in fish. Kerr et al. showed that *Escherichia coli* bacteria can be observed to evolve according to rock–paper–scissors type of interactions, if cellular dispersal is minimal. A recent expansion of interesting theoretical considerations that apply evolutionary games to biology [17] occurred because of the ability to assess many problems in ecological and evolutionary population dynamics at least in qualitative terms, i.e. by predicting and ranking evolutionary equilibria, how population-wide coexistence can emerge from apparent individual conflict, or how fast transitions between equilibria occur.

Tumour cell populations, including cells of the tumour microenvironment, are part of a complex ecosystem [18], which can have consequences for therapeutic outcomes [19]. At the same time, it has been more widely recognized that Darwinian selection plays a key role in cancer [20]. Given the appreciated amount of both genetic and phenotypic heterogeneity in tumour cell populations [21], evolutionary games have become more widely used as a means to theoretically model tumour evolution, especially after tumour initiation [22]. Prominent examples of recent applications of replicator equations in cancer are concerned with the avoidance of the tragedy of the commons, where a sub-population of tumour cells produces a ‘tumour public good’ in form of an insulin-like growth factor [23], in form of glycolytic acid and vascular endothelial growth factor [24], or modelling the dynamic equilibrium between lactate respiration and glycolysis in tumour cells [25]. Such non-autonomous effects between tumour cells had been proposed some time ago [26], and non-cell-autonomous growth rates were recently measured empirically [27]. Similar findings and future challenges in this field have been summarized by Tabassum & Polyak [18].

We here focus on the time it takes to reach an equilibrium in different approaches to model deterministic evolutionary game dynamics. In particular, we focus on differences between logistic growth and the replicator dynamics. We show that the time to get arbitrarily close to an equilibrium, which we here call the $\varepsilon$-fixation time, might critically depend on the underlying cellular birth and death rates. We focus on two coevolving tumour cell populations, and present a discussion of the dynamics between growth factor producers $C_1$ and free-riding non-producers $C_2$. In the simplest setting, we can assume that these closely related cell types experience population doubling rate $\beta_C$, but bears a production cost $\kappa$. The respective game can be recast in the payoff matrix

\[
\begin{pmatrix}
C_1 & C_2 \\
C_1 (\alpha + \beta_C - \kappa & \alpha - \kappa) \\
C_2 (\alpha + \beta_C & \alpha)
\end{pmatrix}.
\]

We assume that the linear benefit of the public good arises through growth factor diffusion that occurs on a time scale much faster than the average times between cell divisions. In a well-mixed population with fraction $u$ of $C_1$ cells, the fitness functions of this simple game are then be given by

\[
\begin{aligned}
f_1(u) &= \alpha(1 + \beta_C u) - \kappa \\
f_2(u) &= \alpha(1 + \beta_C u).
\end{aligned}
\]

Our analysis in this paper is based on cell-type-specific doubling rates, and in the case of logistic growth, also on the apoptotic rates. We are interested in the question of when replicator dynamics, that typically only models changes in relative abundance as a result of fitness differences $f_1(u) - f_2(u)$, predicts similar $\varepsilon$-fixation times as a logistic growth dynamics, and when this is not the case. The main idea is that the replicator dynamics neglects apoptotic rates, but that these rates in turn influence the time to reach an equilibrium in a co-growing and coevolving heterogeneous cell population.

2. Methods

In this section, we introduce our model of bounded frequency-dependent growth. We define our basic deterministic framework of two co-growing cancer cell populations, derive dynamic equations for the fraction of one clone and the total size of the population, and then derive an expression for the stable manifold of the system.

2.1. Logistic growth model

The population is assumed to consist of two types, and we denote their absolute numbers by $x_1$ and $x_2$. The carrying capacity is denoted by $K$, which we consider to be a constant. It is possible to model it as a function of the strategies present in the population [28,29]. The growth rate of each type is assumed to depend on the fraction of type 1 cells $u = x_1/(x_1 + x_2)$ according to growth functions $f_1(u)$ for type 1 and $f_2(u)$ for type 2. Lastly, cells of both types die at a constant rate $\mu$. Taken together this implies that we get the following system of coupled logistic equations that describe co-growth and coevolution of the two cell types:

\[
\begin{aligned}
\frac{dx_1(t)}{dt} &= f_1(u)x_1 \left(1 - \frac{x_1 + x_2}{K}\right) - \mu x_1 \\
\frac{dx_2(t)}{dt} &= f_2(u)x_2 \left(1 - \frac{x_1 + x_2}{K}\right) - \mu x_2
\end{aligned}
\]

for $x_1, x_2 \in \mathbb{R}^+$. In the following analysis, we first assume $\mu_2 = \mu$ and $f_2(u) > 0$ for $u \in [0,1]$, i.e. the net growth rate of both cells types will always be positive. In the second part of the discussion, we will relax the assumption of equal rates and turn to the more general case of $\alpha_1 \neq \alpha_2$, $\mu_1 \neq \mu_2$, as we analyse the system implementing previously measured cellular rates of proliferation and apoptosis. Note that the logistic growth model emerges from a spatial setting that includes cellular movement if cell migration occurs on a much faster time scale compared to cell division. It has been shown that in this case spatial correlations are negligible and the population dynamics can be described using a logistic growth equation [30]. In this parameter regime, it is also justified to assume that interactions that influence the rate of cell
division become independent of specific local configurations, and depend solely on the frequency of different cell types.

2.2. Analysis
To simplify the analysis of the system (2.1), we apply the following change of variables:

\[
\begin{align*}
   u &= \frac{x_1}{x_1 + x_2} \\
   s &= \frac{x_1 + x_2}{x_1 + x_2} \tag{2.2}
\end{align*}
\]

where \(u\) is the fraction of type 1 cells and \(s\) is the total population size. By differentiating \(u\) and \(s\) with respect to time we obtain the following system of ODEs:

\[
\begin{align*}
   \frac{du}{dt} &= (f_1(u) - f_2(u))u(1 - \frac{s}{K}) \\
   \frac{ds}{dt} &= (f_1(u) - f_2(u))su(1 - \frac{s}{K}) - \mu s,
\end{align*}
\]

defined on \(u \in [0, 1]\) and \(s \in \mathbb{R}^+\). We note that in the case when \(s\) is small compared with the carrying capacity \(K\), such that \(s/K \approx 0\) the system reduces to

\[
\begin{align*}
   \frac{du}{dt} &= (f_1(u) - f_2(u))u(1 - u) \\
   \frac{ds}{dt} &= f_1(u)su + f_2(u)(1 - u) - \mu s,
\end{align*}
\]

and we see that the equation for \(u\) is independent of the population size \(s\) and \(u\) changes according to the standard replicator equation \([13,14]\). We will now proceed to a more general analysis of our model.

2.2.1. Fixed points
By solving the equations

\[
\begin{align*}
   (f_1(u) - f_2(u))u(1 - u) - \frac{s}{K} &= 0 \tag{2.3} \\
   (f_1(u) - f_2(u))su + f_2(u)(1 - u) - \mu s &= 0,
\end{align*}
\]

we see that for all growth functions \(f_1\) and \(f_2\) the system has the following set of fixed points on the boundary (see appendix A for details):

1. \((u_1, s_1) = (0, 0)\) with corresponding eigenvalues \(\lambda_1 = f_1(0) - f_2(0)\) and \(\lambda_2 = f_2(0) - \mu > 0\), which is unconditionally unstable,
2. \((u_2, s_2) = (1, 0)\) with corresponding eigenvalues \(\lambda_1 = f_1(1) - f_2(1)\) and \(\lambda_2 = f_2(1) - \mu > 0\), which is unconditionally unstable,
3. \((u_3, s_3) = (0, K(1 - \mu/f_2(0))\) with corresponding eigenvalues \(\lambda_1 = (\mu/f_2(0))(f_1(0) - f_2(0))\) and \(\lambda_2 = \mu - f_2(0) < 0\), which is stable iff \(f_2(0) < f_2(0)\), and
4. \((u_4, s_4) = (1, K(1 - \mu/f_1(1))\) with corresponding eigenvalues \(\lambda_1 = (\mu/f_1(1))(f_2(1) - f_1(1))\) and \(\lambda_2 = \mu - f_1(1) < 0\), which is stable iff \(f_2(1) < f_2(1)\).

Here, fixed points 1 and 2 are trivial in the sense that they correspond to a system void of cells. Fixed points 3 and 4 correspond to monoclonal populations and are stable if the resident type has a larger growth rate compared with the invading type.

If there are points \(u^* \in (0, 1)\) such that \(f_1(u^*) = f_2(u^*)\), then these give rise to fixed points \((u^*, K(1 - \mu/f_1(u^*)u^* + f_2(u^*)(1 - u^*))\)), which are stable if \(f_1(u^*) - f_2(u^*) < 0\) (see appendix A for proof).

We note that the stability criteria for the non-trivial fixed points at \(u = 0\) and 1, including potential internal fixed points, are identical to those of the two-type replicator equation with payoff functions \(f_1\) and \(f_2\).

2.2.2. Invariant manifold
We now focus our attention to the dynamics when the system is close to saturation \((s \approx K)\) with the aim of obtaining a simpler description of how the frequency \(u(t)\) changes in time. This can be achieved since the phase space contains a stable invariant manifold that connects all the non-trivial steady states. The invariant manifold is simply a curve \(s = h(u)\), which attracts the dynamics and once the system enters the manifold it will not leave it. This implies that the dynamics along the manifold is effectively one-dimensional, and can be captured with a single ODE for \(u(t)\).

If we write the invariant manifold as a function \(s = h(u)\), then, since it is invariant it must be tangent to the vector field \((du/dt, ds/dt)\) at every point. This implies the condition

\[
\frac{ds}{dt} = h'(u) \frac{du}{dt} \tag{2.6}
\]

which is known as the manifold equation \([14,31]\). By substituting \(ds/dt\) and \(ds/dt\) from (2.3) and letting \(s = h(u)\), we obtain the following equation for \(h(u)\):

\[
f_1(u) - f_2(u)h(u)u(1 - \frac{s}{K}) + f_2(u)h(u)(1 - \frac{h(u)}{K}) - \mu h(u) = h'(u) \left( (f_1(u) - f_2(u))u(1 - u) \left( 1 - \frac{h(u)}{K} \right) \right).
\]

This equation is a nonlinear ordinary differential equation and in order to solve it we express \(h(u)\) as a series expansion in the death rate \(\mu\), which typically is a small parameter

\[
h(u) = \sum_{i=0} a_i(u)\mu^i, \tag{2.7}
\]

where \(a_i(u)\) are coefficients that depend on \(u\). We insert this ansatz into equation (2.6) and equate powers of \(\mu\) to solve for the \(a_i's\). We do this for \(i = 0, 1, 2,\) introduce \(f(u) = u_1^1(u) + (1 - u_2^1(u), and get

\[
\begin{align*}
   a_0(u) &= K, \\
   a_1(u) &= -\frac{K}{f(u)}(1 - u), \quad a_2(u) = \frac{K}{f(u)}(f_1(u) - f_2(u))(f_1(u))
\end{align*}
\]

Numerical comparison shows that the invariant manifold is closely approximated by the first two terms, and we therefore drop all higher order terms and approximate the invariant manifold with

\[
h(u) = K \left( 1 - \frac{\mu}{f(u)} \right). \tag{2.8}
\]

Note here that the complete solution would be more complicated, as can be seen from the fact that this first order expression does not solve the original manifold equation.

The dynamics along the invariant manifold are given by replacing \(s\) with \(h(u)\) in (2.3), and we get the following expression (to first order in \(\mu\)):

\[
\frac{du}{dt} = (f_1(u) - f_2(u))u(1 - \frac{s}{K}) = \frac{\mu}{f(u)} (f_1(u) - f_2(u))u(1 - u). \tag{2.9}
\]

With the unusual prefactor that is inversely proportional to the total fitness of the population, \(f(u)\), this equation for the
frequency of type 1 cells is similar to the version of the replicator equation introduced by Maynard-Smith [32], and the one derived by Traulsen et al. [33] (if we disregard the demographic noise term). The difference compared to previous derivations is the factor $\mu$, which implies that the rate of change of $u$ along the invariant manifold is proportional to the death rate.

3. Results and discussion

It is often argued that prefactors to the replicator equation are irrelevant since the dynamic flow and fixed points remain unchanged. However, the time scale of selection leading to an equilibrium might be altered. In this section, we explore the difference between the standard replicator equation and the logistic model considered here. We examine this relationship in the context of a tumour public goods game, in which some cells produce a public good at a cost, rendering a benefit to all cells in the population.

3.1. Diffusing public goods game

Autocrine production of growth factors is a common feature of cancer cells, and has previously been modelled using evolutionary game theory [23,34]. Let us now consider two cell types that only differ in one aspect. Type 1 cells produce growth factor at a cost $\kappa$. Type 2 cells do not produce the growth factor and are termed free-riders. Otherwise, both cell types have the same growth rates, which are a linear function of growth factor availability. We assume that the growth factor production rate is given by $\rho$ and that the growth factor is bound and internalized by both cell types at rate $\delta$.

Two largely simplifying assumptions are that, first, we are describing a well-mixed system and that, second, the growth factor concentration $G$ is assumed to be uniform in space. We rely on the first assumption for mathematical convenience, as otherwise we would have to resort to non-analytical, agent-based or hybrid modelling [35]. Secondly, additional growth factor provision was shown to be rapid and leading to high levels of tumour public good, provided that the respective genetic promoter was strong [27]. In a similar study by Cleary et al. [36], who studied Wnt1-based cooperative tumour evolutionary dynamics, aberrant expression of the cooperative signalling molecule was observed on a tumour wide scale. Thus, under these simplifying but productive assumptions, the growth factor dynamics obeys the equation

$$\frac{dG(t)}{dt} = \rho x_1 - \delta G(x_1 + x_2).$$

Further, we assume that the growth factor dynamics occur on a fast time scale compared to changes in $x_1$ and $x_2$. This implies that

$$\frac{dG(t)}{dt} \approx \rho x_1 - \delta G(x_1 + x_2) \approx 0$$

and we can solve for $G$ to give

$$G = \beta \frac{x_1}{x_1 + x_2} = \beta u,$$

where $\beta = \rho/\delta$. For simplicity, we first consider a linear effect of the growth factor on the rate of cell division, as well as equal proliferation and death rates, which results in the growth functions given by equations (2.1). In order for the growth rate to be larger than the death rate for all $u$ we assume the inequality $\alpha - \kappa > \mu$. This choice of growth functions gives the following system of ODEs for the frequency of producers $u$ and the total population size $s$:

$$\frac{du}{dt} = -\kappa u(1 - u) \left(1 -\frac{s}{K}\right)$$

and

$$\frac{ds}{dt} = -\kappa u \left(1 -\frac{s}{K}\right) + \alpha (1 + \beta u) s \left(1 -\frac{s}{K}\right) - \mu s.$$

This system results from equations (2.3) and has two non-trivial steady states given by a monomorphic population of free-riders $(0, 1 - \mu/\alpha)$, and a population consisting only of producers $(1, \mu/(\alpha(1 + \beta) - \kappa))$; see analysis following equations (2.5). The eigenvalues are

$$\lambda_1 = \mu - \alpha < 0$$

and

$$\lambda_2 = -\frac{\kappa \mu}{\alpha} < 0$$

and hence the free-rider steady state is stable. For the other fixed point (producers dominate), we have

$$\lambda_1 = \frac{\kappa \mu}{\alpha (1 + \beta) - \kappa} > 0$$

and

$$\lambda_2 = \kappa + \mu - \alpha (1 + \beta) < 0,$$

making it unstable. Figure 1a shows the phase space of the system, where the open circles indicate unstable steady states and the filled circle shows the location of the single stable steady state. We note that for almost all initial conditions the dynamics rapidly converge to the invariant manifold (2.8) which is approximately given by

$$\tilde{u}(u) = K \left(1 - \frac{\mu}{f(u)}\right) = K \left(1 - \frac{\mu}{\alpha (1 + \beta u) - \kappa u}\right).$$

Once the system enters the invariant manifold the dynamics can be approximated by (2.9) which for the diffusing public goods game considered here are given by

$$\frac{du}{dt} \approx -\frac{\mu K}{\alpha (1 + \beta u) - \kappa u}(1 - u).$$

Thus, in order to assess the impact of cell death and turnover on selection, we compare our description of the public goods game (3.1) with the standard replicator equation

$$\frac{du}{dt} = (f_1(u) - f_2(u))u(1 - u) = -\kappa u(1 - u).$$

Figure 1b shows a comparison between the solution of the logistic system (3.1) and the replicator equation (3.8) for the same initial condition $u_0 = 0.75$ ($s_0 = 0.01K$) and with a death rate of $\mu = 0.1 h^{-1}$. Whereas the two solutions agree for small times (when $s \ll K$), they start to diverge as soon as the solution to the logistic system enters the invariant manifold. The solution of the replicator equation quickly converges to the steady state $u = 0$, while the fraction of producers in the logistic case decreases approximately linearly with time.

To quantify the effect of the death rate $\mu$ on the rate of selection we measured the time it takes for the logistic system to approach a steady state. For a fixed initial condition $(u_0, s_0) = (0.75, 0.01)$, we measured the time it took for the system to reach a small $\varepsilon$ neighbourhood of the fixed point, i.e. $|u(t) - u^*| \leq \varepsilon$, with $u^* = 0$ and $\varepsilon = 0.01$. We call this the $\varepsilon$-fixation time. All other parameters were fixed at...
This implies that for small \( a \approx \frac{1}{T} \) are actually follows the stable manifold. (3.6), and the light blue curve (with arrow pointing forward in time) shows the only stable steady state where the population is dominated by non-producing type 2 cells. The red line shows the invariant manifold open circles show the three unstable stationary states and the filled circle (the line is just a guide to the eye). (obtained from the logistic system and the standard replicator equation)

\[ u_{\text{ultimately}} = \frac{u_{i}}{K} \]

\[ \alpha = 1.0, \beta = 1.0, \kappa = 0.1, \quad K = 1 \quad \text{and} \quad \mu = 0.1 \quad (a,b), \]

where we chose to observe time in units of hours (h). The initial conditions are \( (u_{0}, s_{0}) = (0.75, 0.01K) \).

\[ \alpha = 1 \quad \text{and} \quad \mu = 0.1 \]

This implies that for small \( \mu \) the time it takes the system to reach the steady state can be exceedingly long. It is worth noting that the \( e \)-fixation time for the replicator equation can be obtained in the limit of \( \mu \rightarrow 0 \), performed on the logistic system, implying a never-growing population, in which the death rate equals the average birth rate.

### 3.2. Time scales of in vivo and in vitro cellular expansions

Previous studies of ecological interactions in growing tumour cell populations have observed various forms of frequency-dependent effects. These effects have then been linked to the persistence of distinct cancer cell lines that provide growth enhancing public goods to the tumour, most notably in experimental work by Marusyk et al. [27]. There, it could be shown that a mixture of certain clones could not explain tumour outgrowth in vivo by simply using superposition of individual clonal birth and death rates. Rather, synergistic tumour-driving effects can emerge, pointing to more intricate, potentially frequency-dependent growth effects, based on direct or indirect clonal interactions [18]. For the purpose of illustration, we extracted individual clonal proliferation (\( \alpha \)) and death rates (\( \mu \)) from Marusyk et al. [27], in order to predict how these rates shape the dynamics. Out of 16 clonal cell lines, each distinctively expressing a different gene, we chose four clones to calculate baseline cellular birth and death rates. The four clones, derived from the breast cancer cell line MDA-MB-468, were LoxL3 (lissyl oxidase type 3 [37], linked to breast cancer invasion and metastasis), IL11 (interleukin 11, a member of the IL6 family that plays a multifaceted role in leukemia and breast cancer [38]), and CCL5 (C-C motif ligand 5, a chemokine with emerging roles in immunotherapy [39]). The baseline cellular birth and death rates of these clones were calculated in the following way, based on in vivo growth experiments, originally performed in a mouse xenograft model (tumours formed by orthotopic trans-plantation into the mammary fat pads of immunodeficient Foxn1nu (nu) mice [27]). For all four clones, it was established that tumours grew exponentially; from longitudinal measurements and associated cellularity calculations, the net cellular doubling rates were calculated (see Ext. Data fig. 3 and SI in [27], where exponential growth rates are established that tumours grew exponentially; from longitudinal measurements and associated cellularity calculations, the net cellular doubling rates were calculated (see Ext. Data fig. 3 and SI in [27], where exponential growth rates are shown). These BrdU staining experiments measure the fraction of cells in S-phase of the whole cell cycle, as a direct estimate for the per cent of S-phase in relation to the whole cell cycle \( T \), and thus the doubling rate, which we set to \( \alpha = 1/T \). Using the relation

\[ \chi = \frac{T_{S}}{T}, \]  

we calculated the monoclonal birth rates using

\[ \alpha = \frac{\chi}{T_{S}}, \]

Thus, given the net doubling rate \( r = \alpha - \mu \), it is possible to estimate the death rate

\[ \mu = \frac{\chi}{T_{S}} - r, \]

with \( T_{S} \) fixed to 8 h. Data for \( r \) and \( \chi \) are given in appendix B. As for both \( r \) and \( \chi \), several independent measurements were performed, and we calculated distributions of \( \alpha \) and \( \mu \) for the three

\[ + \frac{1}{T_{S}} \]

\[ \frac{1}{T_{S}} \]

\[ \frac{1}{T_{S}} \]
Figure 2. (a) Birth and death rate distributions, calculated from previous experiments, where engineered breast cancer cell lines, characterized overexpressing certain cytokines, were observed to grow in vivo xenograft mouse model tumours [27]. Although net tumour growth was high, death and birth rates were similar in all clones considered. In comparison, we also show in vitro cell line rates, estimated by Juarez et al. [41]. We further used the fact that the IL11 cells are growth factor producers. (b) Using median birth and death rates from the distributions in (a), we measured the \(e\)-fixation time numerically determined using equations (3.13) (defined as the time to reach an \(e\)-neighbourhood equilibrium value of \(u\), with \(e = 0.001\), \(u_0 = 0.5\) and compared it to the \(e\)-fixation time of the standard replicator equation (3.8). Note that we used equations (3.13) for this numerical procedure. For IL11, we used \(\alpha_1 = 0.684\) d\(^{-1}\) and \(\mu_1 = 0.596\) d\(^{-1}\). For Lox3, we used \(\alpha_1 = 0.617\) d\(^{-1}\) and \(\mu_1 = 0.515\) d\(^{-1}\). For CCL5, we used \(\alpha_1 = 1.214\) d\(^{-1}\) and \(\mu_1 = 1.031\) d\(^{-1}\). \(\beta = 1\), with \(u_0 = 0.5\) and \(s_0 = 0.01/K\). Note here that the peak in \(e\)-fixation time marks the shift from \(u \to 1\) to \(u \to 0\) as the cost increases; this transition can only occur when producers and non-producers have similar birth and death rates. (c) Comparison of \(e\)-fixation times determined numerically using (3.13) to the analytical approximation (3.19), parameters the same as in (b). Time was measured in units of days (d).

cell lines described above. We contrasted these distributions to in vitro distributions of cellular birth and death rates, adapted from [41] (fig. 3 therein), which are, notably, very similar to other in vitro values, e.g. reported for the PC-9 non-small cell lung cancer cell line [42] (figure 2a). In the in vivo tumour growth experiments, exponential growth was observed within the time frame of 50–80 days, at growth rates up to two population doublings per day (net growth rate) [27]. However, in most tumours the net growth rate was more moderate, and the actual cellular birth and death rates were at least of similar order in magnitude \((\alpha/\mu \approx 1)\). This stands in contrast with the birth–death rate ratios observed in cell cultures, where birth rates often exceed death rates by an order of magnitude \((\alpha/\mu \approx 10)\) [41–44].

As a notable difference to the previous section, here we assume both \(\alpha_1 \neq \alpha_2\) and \(\mu_1 \neq \mu_2\). Thus, instead of (2.3), we now deal with the more general payoff structure

\[
C_1 = \begin{pmatrix} \alpha_1(1 + \beta) - \kappa & \alpha_1 - \kappa \\ \alpha_2(1 + \beta) - \kappa & \alpha_2 \end{pmatrix}
\] (3.12)

and obtain the following ODEs for frequency of producer cells and total size of the system:

\[
\frac{du}{dt} = \left( (\alpha_1 - \alpha_2)(1 + \beta u) - \kappa u(1 - u) \left( 1 - \frac{u}{K} \right) \right)
\]

and

\[
\frac{ds}{dt} = \left( (\alpha_1 - \alpha_2)(1 + \beta u) - \kappa s u \left( 1 - \frac{s}{K} \right) \right)
\]

\[
- s(u\mu_1 + (1 - u)\mu_2)
\] (3.13)

and we seek to estimate the time it takes to reach a small \(e\) neighbourhood of the equilibrium \(|u(t) - u^*| \leq e\), shown in figure 2b. The combination of IL11 with one other cell line was chosen because it has been established that IL11 is a growth factor producer clone, which, at least in a first approximation, renders a linear fitness benefit [27]. We here make the additional assumption that IL11 cells carry a cost associated with growth factor production, and explore the extinction process of IL11 cells as they compete with either CCL5 or Lox3 cells (figure 2).

We can calculate an estimate of the ‘time to fixation’ in the following way. Suppose the fraction of growth factor producing cells is \(z\), with \(z = 1\) and \(z^* = 1\).

\[
s_0^* = s'(u^* = 0) = K \left( 1 - \frac{\mu_s}{\alpha_2} \right)
\] (3.14)

and

\[
s_1^* = s'(u^* = 1) = K \left( 1 - \frac{\mu_s}{\alpha_1(1 + \beta) - \kappa} \right)
\] (3.15)

We now assume that the total population size remains at the stationary value, although it in fact changes (slightly) with \(u\). This assumption can be thought of as a zeroth-order approximation in \(1 - s/K\), and it implies that near the stable manifold, the frequency \(u\) obeys the ODE

\[
\frac{du}{dt} = \left( (\alpha_1 - \alpha_2)(1 + \beta u) - \kappa u(1 - u) \left( 1 - \frac{s_0^*}{K} \right) \right)
\] (3.16)

which we can solve by inserting the approximations (3.14) and (3.15) into the ODE (3.16) and get the two solutions (for two different possible endpoints)

\[
v_0(t) = \frac{1}{1 + (1/u_0 - 1)e^{-\mu_s(\alpha_1 - \alpha_2 - \kappa)/\alpha_2}}
\] (3.17)

and

\[
v_1(t) = \frac{1}{1 + (1/u_0 - 1)e^{-\mu_s(\alpha_1 - \alpha_2 - \kappa)/\alpha_1(1 + \beta) - \kappa}}
\] (3.18)
We now seek solutions of \( |v_{1,2}(\tau) - w_{0,1}^*| \leq \varepsilon \) for \( \tau \) (with the equilibrium points \( w_{0,1}^* = 0, u^2 = 1 \)), and find the following relations that approximate the \( \varepsilon \)-fixation times:

\[
\begin{align*}
\tau_{\varepsilon=0} &\approx \frac{\alpha_2}{\mu_2(\alpha_1 - \alpha_2 - \kappa)} \log \left( \frac{u_0(1-\varepsilon)}{\varepsilon(1-u_0)} \right) \\
\tau_{\varepsilon=1} &\approx \frac{\alpha_1(1+\beta - \kappa)}{\mu_1(\alpha_1 - \alpha_2 - \kappa)} \log \left( \frac{(1-\varepsilon)u_0(1-\varepsilon)}{\varepsilon u_0} \right),
\end{align*}
\]

where \( u_0 \) is the initial frequency. Note that here, we deviate from the notion of (average) fixation times in the strict stochastic sense [45], and replace the term by a threshold-based analytical approximation. Especially in a population that has reached the stable manifold, even a small fraction of remaining producer cells could still mean that there are as many cells as needed to warrant a mean field rather than a fully stochastic description.

For the \( u \to 0, s \to k(1 - \mu_2/\alpha_2) \) case, we can now compare our analytical approximations with the \( \varepsilon \)-fixation times of the full numerical solution in figure 2c, as a function of \( \kappa \). Depending on the differences in clonal birth and death rates, the approximation exhibits qualitative differences. Equation (3.19) consistently overestimates the \( \varepsilon \)-fixation time if the death rate of the producer cells is lower than that of non-producers (IL11 with CCL5, \( \alpha_1 - \mu_1 < \alpha_2 - \mu_2 \)), but it underestimates the \( \varepsilon \)-fixation time if the net growth rate of the producer cells is higher than that of non-producers as long as the cost of growth factor production does not exceed a certain threshold (IL11 with LoxL3, \( \alpha_1 - \mu_1 \approx \alpha_2 - \mu_2 \)). Hence, not only the cost of growth factor production factor influences the time to extinction of producer cells, but also the monoclonal net growth rate influences the time to extinction of producers and the impact of an assumed cost associated with growth factor production. The approximations (3.19) are of ‘zero-order’ in changes in \( s \). Yet, they are still able to reflect the basic fact that \( \varepsilon \)-fixation time can be heavily influenced by the cellular death rate of the abundant cell type. According to our rough approximation, the extinction time of producer cells (3.19) is both proportional to the ratio of birth to death rate of the non-producers, as well as inversely proportional to the death rate difference. Surprisingly, in this approximation \( \tau_{\varepsilon=0} \) does not depend on the absorption or production rate of the growth factor, captured by \( \beta \). Large differences in baseline birth rates extend growth factor producer extinction times. For larger values of \( \alpha_2/\mu_2 \), the extinction time is less sensitive to changes in the cost of growth factor production.

The two cellular death rates \( \mu_1 \) and \( \mu_2 \) have different effects on \( \varepsilon \)-fixation times. We used numerical solutions of the full system (3.13), in comparison to the replicator equation (3.8), to analyse variability of \( \varepsilon \)-fixation times (extinction of growth factor producer cells) under variable individual death rates. Thereby, we recover that higher total death rate speeds up the \( \varepsilon \)-fixation time across different initial conditions (figure 3a), and that the death rate of the ‘winner-clone’ plays a more important role (figure 3b): \( \mu_2 \) has a more pronounced impact on the \( \varepsilon \)-fixation time of non-producers. This might be connected to the fact that apoptosis-driven cell turnover of the nearly dominant cell type (i.e. the non-producer cells) governs the \( \varepsilon \)-fixation time. In accordance with this observation, the stable manifold is itself governed by the apoptotic rate of the dominant clone; compare to equation (2.8).

4. Summary and conclusion

We here have presented calculations that were concerned with the stability and time to reach a neighbourhood of equilibrium points in evolutionary game dynamics between two types of tumour cells. We focused on the dynamics of a tumour public good (tumour growth factor), in which we assumed linear fitness functions of growth factor producers and non-producers. The fitness function linearly depends on the relative abundance of growth factor producers, and production comes at a cost. We did not assume that the evolving population was at carrying capacity, as reflected in the logistic growth model.
Thus, in general, population expansion and cellular birth as well as death rates are of importance for the time the system takes to equilibrate. The standard replicator equation typically rules out explicit death effects, and thus may not accommodate the impact of these death rates on the time to reach a population equilibrium.

The use of replicator equations and birth–death processes assume constant population size [7] or a population which is growing uniformly, for example, at an exponential rate [13]. These assumptions have led to a plethora of fruitful results in evolutionary game theory [46], e.g. to the ability to understand fixation and extinction times in evolutionary 2 × 2-games [47–50], multiplayer games [51], structured populations [52] or bi-stable allelic competition [53,54]. Evolutionary games have also been used to establish rules for equilibrium selection even in complex group-coordination games [55,56], in chemical evolutionary game theory [46], e.g. to the ability to understand fixation and extinction times based on previously measured 1-fixation times [62] in the context of co-growing and coevolving bacterial species. Instead, the near-equilibrium population size and the time to reach equilibria are influenced directly by birth and death rates in the population.

We show that, for small differences between the birth and death rates, the eco-evolutionary dynamics of the mixture of two clones may be approximated by standard replicator dynamics. Analysis of previously established growth factor-dependent tumour dynamics of in vitro tumour growth showed that this parameter regime might indeed be biologically relevant (figure 2), even when the tumour population has not reached its carrying capacity. However, prominent examples of in vitro cell line expansions demonstrate that large differences between cellular death and birth rates might impact the dynamics in a different way [42–44], and in this case the replicator equation is a poor approximation of the eco-evolutionary dynamics. We used a logistic growth model that includes cell death. This system describes both co-growth, as well as coevolution of two tumour cell types. The choice of logistic growth is by no means unique, but a simple, first-order form of non-uniform growth.

We report two major findings. First, a first-order approxima- tion in death rates allows estimation of the stable manifold, and reveals linear dependence on the apoptotic rate of the more abundant cell type. Second, this knowledge can be used to inform a zero-order approximation (in constant system size) of the time to get arbitrarily close to equilibrium (ε-fixation time), which reveals that indeed the cellular turnover of the dominant cell type near equilibrium governs the ε-fixation time as the system slowly moves along the stable manifold. This framework allowed us to examine the degree of the resulting variability in ε-fixation times based on previously measured in vivo tumour cell proliferation and death rates in the context of competition between producers and non-producers of a growth factor public good.

Various aspects of cancer cell population structure, such as cellular differentiation, localization or spatial heterogeneity, point to dynamic nonlinear size changes over time, especially during treatment [63–66], and treatment can shift the evolutionary game [67]. Furthermore, selection mechanisms that go beyond relative fitness differences play a role in our understanding of other biological and clinically relevant systems, such as the hematopoietic system [68,69]. Hence, future modelling efforts that seek to apply evolutionary game theory to explain complex cancer growth patterns need to precisely disentangle complex interaction patterns between cells from the overall growth kinetics of a tumour. Detailed understanding of tumour growth kinetics is especially important in co-growing populations, as we here show that the convergence towards an equilibrium—which sets the time scale for potential treatment and relapse effects—sensitively depends on the microscopic cellular growth rates. The often performed, and mathematically convenient rescaling of time that leads to replicator equations might eliminate effects that are crucial for understanding transitions between equilibria and describing relevant time scales of tumour evolution.

Data accessibility. All data used in this paper are presented in the appendix and can be found online with the cited references.

Authors’ contributions. All authors conceived the study, analysed the data, performed mathematical and statistical modelling, and wrote the paper.

Competing interests. The authors declare no conflict of interest.

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Appendix A. Fixed points and stability

To investigate the stability of the fixed points of (2.3), we denote the right-hand sides by

\[ F(u, s) = (f_1(u) - f_2(u))u(1 - w) \left(1 - \frac{s}{K}\right) \]

and \[ G(u, s) = (f_1(u) - f_2(u))su(1 - \frac{s}{K}) + f_2(s)u \left(1 - \frac{s}{K}\right) - \mu s \]

and calculate the Jacobian at the fixed point \((u^*, s^*)\)

\[ J(u^*, s^*) = \begin{pmatrix} F_u(u^*, s^*) & F_s(u^*, s^*) \\ G_u(u^*, s^*) & G_s(u^*, s^*) \end{pmatrix} \] (A 2)

where subscript denotes partial derivative with respect to \(u\) and \(s\).

A.1. Boundary fixed points

For the boundary fixed points we find the following:

At \((u^*, s^*) = (0, 0)\), we find that

\[ J(0, 0) = \begin{pmatrix} f_1(0) - \mu & 0 \\ 0 & f_1(0) - f_2(0) \end{pmatrix} \] (A 3)

with eigenvalues \(\lambda_1 = f_1(0) - f_2(0)\) and \(\lambda_2 = f_2(0) - \mu > 0\). The last inequality holds because we assumed a positive net growth rate for both cell types for all \(u \in [0, 1]\). This fixed point is therefore unconditionally unstable.

At \((u^*, s^*) = (0, 1)\), we find that

\[ J(0, 1) = \begin{pmatrix} f_1(1) - \mu & 0 \\ 0 & f_2(1) - f_1(1) \end{pmatrix} \] (A 4)

with eigenvalues \(\lambda_1 = f_1(1) - f_2(1)\) and \(\lambda_2 = f_1(1) - \mu > 0\). Again, the inequality holds because we assumed a positive.
net growth rate for both cell types for all \( u \in [0, 1] \). This fixed point is therefore unconditionally unstable.

At \((u^*, s^*) = (1, K(1 - \mu/f_1(1)))\), we find that

\[
J\left(1, K\left(1 - \frac{\mu}{f_1(1)}\right)\right) = \left(\mu - f_1(1) \frac{K_0(\mu - f_1(1))(1 - f_1(1))}{f_1(1) - f_1(1)} - \frac{\mu f_1'(1)}{f_1'(1)}\right)
\]

with eigenvalues \( \lambda_1 = (\mu/f_1(1))(f_1(1) - f_1(1)) \) and \( \lambda_2 = \mu - f_1(1) < 0 \). This implies that the fixed point is stable iff \( f_1(1) < f_1(1) \).

At \((u^*, s^*) = (0, K(1 - \mu/f_2(0)))\), we find that

\[
J\left(0, K\left(1 - \frac{\mu}{f_2(0)}\right)\right) = \left(\mu - f_2(0) \frac{K_0(\mu - f_2(0))(1 - f_2(0))}{f_2(0) - f_2(0)} - \frac{\mu f_2'(0)}{f_2'(0)}\right)
\]

with eigenvalues \( \lambda_1 = (\mu/f_2(0))(f_1(0) - f_2(0)) \) and \( \lambda_2 = \mu - f_2(0) < 0 \). This implies that the fixed point is stable iff \( f_1(0) < f_2(0) \).

### A.2. Internal fixed points

Internal fixed points exist at points where \( f_1(u^*) = f_2(u^*) \) for \( 0 < u^* < 1 \). The corresponding \( s \)-coordinate is given by solving \( ds/dt = 0 \) in terms of \( u \) to get \( s^* = K(1 - \mu/f_1(u^*)) \).

The Jacobian at such a point is given by

\[
f(u^*, s^*) = \left(\frac{s^* u^* (f_1'(u^*)) - f_1'(u^*))}{f_1'(u^*)} - \frac{2f_1'(u^*)}{K} - \mu\right).
\]

(A5)

To say something about the stability of such a point we need to investigate the signs of the eigenvalues of \( J \). We do this by looking at the sign of each matrix entry. For now, we assume nothing about the sign of \( f_1'(u^*) - f_2'(u^*) \) and instead focus on the other factors in each matrix entry.

First, we see that

\[
s^* u^* \left(1 - \frac{s^*}{K}\right) = s^* u^* \left(1 - \frac{K}{K} \frac{1 - \mu}{f_1(u^*)}\right)
\]

\[
= s^* u^* \frac{\mu}{f_1(u^*)} > 0.
\]

(A6)

Further, we have

\[
u^* \left(\frac{s^* u^*}{K} - \frac{s^*}{K} - u^*\right)
\]

\[
= s^* u^* \frac{2}{K} - s^* u^* - u^2
\]

\[
= \left(1 - \frac{\mu}{f_1(u^*)}\right) u^2 - \left(1 - \frac{\mu}{f_1(u^*)}\right) u^2.
\]

(A8)

Here, \( 0 \leq (1 - \mu/f_1(u^*)) < 1 \) since \( f_1(u) > \mu \geq 0 \). This implies that

\[
\left(1 - \frac{\mu}{f_1(u^*)}\right) u^2 - \left(1 - \frac{\mu}{f_1(u^*)}\right) u^2 < 0,
\]

(A9)

since both terms are negative. Lastly, we see that

\[
f_1(u^*) + \frac{2f_1'(u^*)}{K} - \mu = f_1(u^*) \left(1 - \frac{2K(1 - \mu/f_1(u^*))}{K}\right) - \mu
\]

\[
= -f_1(u^*) \left(1 + \frac{2\mu}{f_1(u^*)}\right) - \mu < 0,
\]

(A11)

since \( 1 + 2\mu/f_1(u^*) > 0 \).

This implies that we can write the Jacobian as

\[
f(u^*, s^*) = \left(\frac{A\Delta f}{K}\right),
\]

(A12)

where \( A > 0 \), \( B > 0 \), \( C < 0 \) and \( \Delta f = f_1(u^*) - f_2(u^*) \). The eigenvalues of the Jacobian are given by

\[
\lambda_{1,2} = \frac{1}{2}(\Delta f \pm \sqrt{4BC\Delta f + A^2\Delta f^2}).
\]

(A12)

Now if \( \Delta f > 0 \) then \( A\Delta f > 0 \), and \( \sqrt{4BC\Delta f + A^2\Delta f^2} > A\Delta f \). This implies that \( \lambda_1 > 0 \) and \( \lambda_2 < 0 \), and hence the fixed point \((u^*, s^*) \) is unstable.

If on the other hand \( \Delta f < 0 \) then there are three possibilities, either (i) \( 4BC\Delta f + A^2\Delta f^2 > 0 \) or (ii) \( 4BC\Delta f + A^2\Delta f^2 < 0 \) or (iii) \( 4BC\Delta f + A^2\Delta f^2 = 0 \). If (i) holds then \( \sqrt{4BC\Delta f + A^2\Delta f^2} \) is complex and \( R(\lambda_{1,2}) < 0 \). Lastly, if (iii) is the case then \( \lambda_{1,2} = A\Delta f/2 < 0 \).

This shows that the stability of the stationary point at \((u^*, s^*) \) is fully determined by the sign of \( \Delta f = f_1(u^*) - f_2(u^*) \). If \( \Delta f > 0 \) the point is unstable and if \( \Delta f < 0 \) then the point is stable.

### Appendix B. Clonal population doubling rates

Here, all rates are given per day; \textit{in vivo} data taken from Marusyk \textit{et al. }[27].

For LoxL3, we used the following population doubling rates (net growth rates):

\[
\begin{align*}
0.09 & 0.083 \\
0.058 & 0.095 \\
0.103 & 0.092 \\
0.12 & 0.122 \\
0.116 & 0.113 \\
0.112 & 0.119 \\
0.13 & 0.113 \\
0.103 & 0.103 \\
\end{align*}
\]

(B1)

and the following percentage of S-phase during cell cycle \( \chi \):

\[
\begin{align*}
0.512 & 0.424 \\
0.385 & 0.349 \\
0.21 & 0.202 \\
0.195 & 0.198 \\
0.191 & 0.137 \\
\end{align*}
\]

(B2)
For IL11, we used the following population doubling rates (net growth rates):

\[
\begin{pmatrix}
0.14 \\
0.099 \\
0.055 \\
0.108 \\
0.12 \\
0.103 \\
0.084 \\
0.121 \\
0.154 \\
0.108 \\
0.123 \\
0.132 \\
0.14 \\
0.174 \\
0.029 \\
0.079 \\
0.126 \\
0.072 \\
0.075 \\
0.107 \\
0.121
\end{pmatrix}
\]

and the following percentage of S-phase during cell cycle \(\chi\):

\[
\begin{pmatrix}
0.192 \\
0.21 \\
0.207 \\
0.224 \\
0.228 \\
0.259 \\
0.309 \\
0.354 \\
0.385
\end{pmatrix}
\]

For CCL5, we used the following population doubling rates (net growth rates):

\[
\begin{pmatrix}
0.233 \\
0.216 \\
0.178 \\
0.133 \\
0.144
\end{pmatrix}
\]

and the following percentage of S-phase during cell cycle \(\chi\):

\[
\begin{pmatrix}
0.421 \\
0.482 \\
0.444 \\
0.388 \\
0.364 \\
0.282
\end{pmatrix}
\]

The distributions shown in figure 2 resulted from all possible pairs of these numbers to calculate \(a\) and \(\mu\), equations (3.10) and (3.11).

For generation of the in vitro distributions we used normally distributed rates (truncated by 0), with a mean death rate of 0.12 d\(^{-1}\) (s.d. 0.0672) and a mean birth rate of 1.32 d\(^{-1}\) (s.d. 0.048), adapted from Juarez et al. [41].

References

1. von Neumann J, Morgenstem O. 1944 Theory of games and economic behavior. Princeton, NJ: Princeton University Press.
2. Aumann RJ. 1987 Game theory. In The new palgrave: a dictionary of economics (eds EM Milgate, P Newman), vol. 2, p. 460. London, UK: Macmillan.
3. Ross D. 2010 Game theory. In The Stanford encyclopedia of philosophy (Fall 2010 Edition) (ed. EN Zalta). Stanford, CA: Stanford University. See http://plato.stanford.edu/archives/fall2010/entries/game-theory.
4. Nash JF. 1950 Equilibrium points in n-person games. Proc. Natl Acad. Sci. USA 36, 45–49. (doi:10.1073/pnas.36.1.48)
5. Myerson RB. 1997 Game theory. Analysis of conflict. Cambridge, MA: Harvard University Press.
6. Vincent TL, Brown JS. 2005 Evolutionary game theory, natural selection, and Darwinian dynamics. Cambridge, UK: Cambridge University Press.
7. Nowak MA. 2006 Evolutionary dynamics. Cambridge, MA: Harvard University Press.
8. Gintis H. 2000 Game theory evolving. Princeton, NJ: Princeton University Press.
9. Maynard Smith J, Price GR. 1973 The logic of animal conflict. Nature 246, 15 – 18. (doi:10.1038/246015a0)
10. Traulsen A, Pacheco JM, Nowak MA. 2007 Pairwise comparison and selection temperature in evolutionary game dynamics. J. Theor. Biol. 246, 522 – 529. (doi:10.1016/j.jtbi.2007.01.002)
11. Sandholm WH. 2010 Pairwise comparison dynamics and evolutionary foundations for Nash equilibrium. Games 1, 3 – 17. (doi:10.3390/g100003)
12. Wu B, Bauer B, Galla T, Traulsen A. 2015 Fitness-based models and pairwise comparison models of evolutionary games are typically different—even in unstructured populations. New J. Phys. 17, 023043. (doi:10.1088/1367-2630/17/2/023043)
13. Taylor PD, Jonker L. 1978 Evolutionarily stable strategies and game dynamics. Math. Biosci. 40, 145 – 156. (doi:10.1016/0025-5564(78)90077-9)
14. Hofbauer J, Sigmund K. 1998 Evolutionary games and population dynamics. Cambridge, UK: Cambridge University Press.
15. Sandholm WH. 2010 Population games and evolutionary dynamics. Cambridge, MA: MIT Press.
16. Milinski M. 1987 Tit For Tat in sticklebacks and the evolution of cooperation. Nature 325, 433 – 435. (doi:10.1038/325433a0)
17. Szorovkszy A. 2015 Evolutionary game theory: a case of too much theory? Blog: collective behaviour. See http://www.collective-behavior.com/evolutionary-game-theory-a-case-of-too-much-theory (cited 2017).
18. Tabasum DP, Polyak K. 2015 Tumorigenesis: It takes a village. Nat. Rev. Cancer 15, 473 – 483. (doi:10.1038/nrc3971)
19. Junttila MR, de Sauvage FJ. 2011 Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 501, 346 – 354. (doi:10.1038/nature12626)
20. Scott J, Marusyk A. 2017 Somatic clonal evolution: a selection-centric perspective. Biochim. Biophys. Acta 1867, 139 – 150. (doi:10.1016/j.jbba.2017.01.006)
21. Junttila MR, de Sauvage FJ. 2013 Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 501, 346 – 354. (doi:10.1038/nature12626)
22. Attrock PM, Liu LL, Michor F. 2015 The mathematics of cancer: integrating quantitative models. Nat. Rev. Cancer 15, 730 – 745. (doi:10.1038/nrc3621)
23. Archetti M, Ferraro DA, Christofori G. 2015 Heterogeneity for IGF-II production maintained by public goods dynamics in neuroendocrine pancreatic cancer. Proc. Natl Acad. Sci. USA 112, 1833 – 1838. (doi:10.1073/pnas.1414653112)
24. Kaznatcheva A, Scott JG, Basanta D. 2017 Cancer treatment scheduling and dynamic heterogeneity in...
social dilemmas of tumour acidity and vasculature. Br. J Cancer 116, 785–792. (doi:10.1038/bjc.2017.5)

25. Kianersy A, Vetli R, Pienta K. 2014 Critical transitions in a game theoretic model of tumour metabolism. Interface Focus 4, 20140014. (doi:10.1098/rsfs.2014.0014)

26. Tomlinson I, Bodmer W. 1997 Modelling the consequences of interactions between tumour cells. Br. J Cancer 75, 157 – 160. (doi:10.1038/bjc.1997.26)

27. Marusyk A, Tabassum DP, Altmann M, Michor F, Polyak K. 2014 Non-cell-autonomous dynamics of tumour growth supports sub-clonal heterogeneity. Nature 514, 54 – 58. (doi:10.1038/nature13555)

28. Novak S, Chatterjee K, Nowak MA. 2013 Density games. J. Theor. Biol. 334, 26 – 34. (doi:10.1016/j.jtbi.2013.05.029)

29. Gerlee P, Anderson AR. 2015 The evolution of carrying capacity in constrained and expanding tumour cell populations. Phys. Biol. 12, 056001. (doi:10.1088/1478-3975/12/5/056001)

30. Baker RE, Simpson MJ. 2010 Correcting mean-field approximations for birth-death-movement processes. Phys. Rev. E 82, 041905. (doi:10.1103/PhysRevE.82.041905)

31. Wiggins S. 2003 Introduction to applied nonlinear dynamical systems and chaos. New York, NY: Springer Science & Business Media.

32. Maynard Smith J. 1982 Evolution and the theory of games. Cambridge, UK: Cambridge University Press.

33. Traulsen A, Claussen JC, Hauert C. 2006 Coevolutionary dynamics in large, but finite populations. Phys. Rev. E 74, 011901. (doi:10.1103/PhysRevE.74.011901)

34. Gerlee P, Altmann MK. 2015 Complexity and stability in growing cancer cell populations. Proc. Natl Acad. Sci. USA 112, E2742 – E2743. (doi:10.1073/pnas.1505151112)

35. Rejniak KA, Anderson ARA. 2011 Hybrid models of tumor growth. Rev. Syst. Biol. Med. 3, 115 – 125. (doi:10.1002/wsmb.102)

36. Cleary AS, Leonard TL, Gestl SA, Gunther EJ. 2014 Tumor cell heterogeneity maintained by cooperating subclones in Wnt-driven mammary cancers. Cancer Res. 77, 3908 – 3921. (doi:10.1158/0008-5472.CAN-16-2871)

37. Maynard Smith J. 1982 Evolution and the theory of games. Cambridge, UK: Cambridge University Press.

38. Traulsen A, Hauert C. 2009 Stochastic evolutionary game dynamics. In Reviews of nonlinear dynamics and complexity, vol. II (ed. HG Schuster), pp. 25 – 61. Weinheim, Germany: Wiley-VCH.

39. Altrock PM, Gokhalne CS, Traulsen A. 2010 Stochastic slowdown in evolutionary processes. Phys. Rev. E 82, 011925. (doi:10.1103/PhysRevE.82.011925)

40. Altrock PM, Traulsen A, Gokhalne CS. 2012 The mechanics of stochastic slowdown in evolutionary games. J. Theor. Biol. 311, 94 – 106. (doi:10.1016/j.jtbi.2012.07.003)

41. Ashcroft P, Altrock PM, Gokhalne CS. 2014 Fixation in finite populations evolving in fluctuating environments. J. R. Soc. Interface 11, 20140663. (doi:10.1098/rsif.2014.0663)

42. Ashcroft P, Traulsen A, Gokhalne CS. 2015 When the mean is not enough: calculating fixation time distributions in birth-death processes. Phys. Rev. E 92, 042154. (doi:10.1103/PhysRevE.92.042154)

43. Wu B, Traulsen A, Gokhalne CS. 2013 Dynamic properties of evolutionary multi-player games in finite populations. Games 4, 182 – 199. (doi:10.3390/g4020182)

44. Altrock PM, Traulsen A, Nowak MA. 2017 Evolutionary games on cycles with strong selection. Phys. Rev. E 95, 022407. (doi:10.1103/PhysRevE.95.022407)

45. Altrock PM, Traulsen A, Reed FA. 2011 Stability properties of underdominance in finite subdivided populations. PLoS Comput. Biol. 7, e1002260. (doi:10.1371/journal.pcbi.1002260)

46. Mafessoni F, Lachmann M. 2015 Selective stolons: fixation and extinction in diploids are slower for weakly selected mutations than for neutral ones. Genetics 201, 1581 – 1589. (doi:10.1534/genetics.114.178160)

47. Hille C, Abou Chakra M, Altrock PM, Traulsen A. 2013 The evolution of strategic timing in collective-risk dilemmas. PLoS ONE 8, e66490. (doi:10.1371/journal.pone.0066490)

48. Abou Chakra M, Traulsen A. 2012 Evolutionary dynamics of strategic behavior in a collective-risk dilemma. PLoS Comput. Biol. 8, e1002652. (doi:10.1371/journal.pcbi.1002652)

49. Yeates JA, Hilde C, Zwick M, Nowak MA, Lehman N. 2016 Dynamics of prebiotic RNA reproduction illuminated by chemical game theory. Proc. Natl Acad. Sci. USA 113, 5030 – 5035. (doi:10.1073/pnas.1525273113)

50. Anderson AR et al. 2009 Microenvironmental independence associated with tumor progression. Cancer Res. 69, 8797 – 8806. (doi:10.1158/0008-5472.CAN-09-0437)

51. Basanta D, Scott JD, Rockne R, Swanson KR, Anderson AR. 2011 The role of IDH1 mutated tumour cells in secondary glioblastomas: an evolutionary game theoretical view. Phys. Biol. 8, 015016. (doi:10.1088/1478-3975/8/1/015016)

52. Archetti M, Scheuring I, Hoffmann M, Fredericke MD, Pierse NE, Yu DW. 2011 Economic game theory for mutualism and cooperation. Ecol. Lett. 14, 1300 – 1312. (doi:10.1111/j.1461-0248.2011.01697.x)

53. Kaztnacheev A, Scott JD, Basanta D. 2015 Edge effects in game-theoretic dynamics of spatially structured tumours. J. R. Soc. Interface 12, 20150154. (doi:10.1098/rsif.2015.0154)

54. Li XY, Pietichele F, Fraune S, Altrock PM, Bosch TC, Traulsen A. 2015 Which evolutionary games do growing bacterial populations play? J. R. Soc. Interface 12, 20150121. (doi:10.1098/rsif.2015.0121)

55. Bozic I et al. 2013 Evolutionary dynamics of cancer in response to targeted combination therapy. eLife 2, e00747. (doi:10.7554/eLife.00747)

56. Werner B, Scott JD, Sottoriva A, Anderson ARA, Traulsen A, Altrock PM. 2016 The cancer stem cell fraction in hierarchically organized tumors can be estimated using mathematical modeling and patient-specific treatment trajectories. Cancer Res. 76, 1705 – 1713. (doi:10.1158/0008-5472.CAN-15-2069)

57. Tang M et al. 2016 Myeloma cell dynamics in response to treatment supports a model of hierarchical differentiation and clonal evolution. Clin. Cancer Res. 22, 4206 – 4214. (doi:10.1158/1078-0145.CCR-15-2793)

58. Ibrahim-Hassim A et al. 2017 Defining cancer subpopulations by adaptive strategies rather than molecular properties provides novel insights into intratumoral evolution. Cancer Res. 77, 2242 – 2254. (doi:10.1158/0008-5472.CAN-16-2844)

59. Kaztnacheev A, Peacock J, Basanta D, Marusyk A, Scott JD. 2017 Cancer associated fibroblasts and alectinib switch the evolutionary games that non-small cell lung cancers play. (http://www.bionxiv.org/content/early/2017/08/21/179259).

60. Werner B, Beier F, Hummel S, Balabanov S, Lassy L, Orlikowsky T, Dingli D, Brümmendorf TH, Traulsen A. 2015 Reconstructing the in vivo dynamics of hematopoietic stem cells from telomere length distributions. eLife 4, e08687v2. (doi:10.7554/ eLife.08687)

61. Altrock PM, Brendel C, Renella R, Orkin SH, Williams DA, Michor F. 2016 Mathematical modeling of erythrocyte chimerism informs genetic intervention strategies for sickle cell disease. Am. J. Hematol. 91, 931 – 937. (doi:10.1002/ajh.24449)