OPINION

Turning ecology and evolution against cancer

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Abstract | The fight against cancer has drawn researchers from a wide variety of disciplines, ranging from molecular biology to physics, but the perspective of an ecological theorist has been mostly overlooked. By thinking about the cells that make up a tumour as an endangered species, cancer vulnerabilities become more apparent. Studies in conservation biology and microbial experiments indicate that extinction is a complex phenomenon, which is often driven by the interaction of ecological and evolutionary processes. Recent advances in cancer research have shown that tumours, like species striving for survival, harbour intricate population dynamics, which suggests the possibility to exploit the ecology of tumours for treatment.

In a stable ecosystem, the lives of species seem to be perfectly coordinated, as if they were part of a larger super-organism. However, if one of the species escapes its ecological constraints and proliferates rapidly, the balance is destroyed, triggering extinctions of other species and possibly ecosystem collapse. In medicine, we call this ecological process cancer. Cancer is responsible for about one-quarter of deaths in the United States, a number that has barely changed in the past 40 years[1]. Four major factors explain why curing cancer is difficult. First, our knowledge of cellular and tissue-level processes is still limited. Second, cancer and normal cells are similar, which makes the detection and selective killing of cancer cells challenging. Third, cancer cells evolve rapidly, thereby allowing them to overcome natural tumour-suppressing mechanisms and develop resistance to anticancer drugs. Fourth, tumours can be highly diverse and include therapy-resistant clones in their heterogeneous makeup. The design of many therapeutic interventions is mostly guided by the first two factors, thereby often allowing for cancer recurrence after remission. A truly successful treatment must then be designed in a way that avoids or even exploits the evolutionary response of the tumour and its clonal diversity, while still being selective and focusing on specific molecular targets.

Molecular and epidemiological studies laid the foundation for the idea that cancer progression is driven by somatic evolution[2–7]. During a human lifetime, somatic cells divide and die to maintain functioning tissues. Inevitably, cells accumulate point mutations, copy number variations or other heritable changes, which are collectively called alterations. Some of these changes, called driver mutations or, simply, drivers, confer a selective advantage to a clonal lineage. Natural selection facilitates the progression towards cancer because it favours clones with high proliferation rates and increased survival. The selective advantage may be achieved by various phenotypes, such as suppressing apoptosis or independence from growth signals, and may eventually lead to cancer[8,9]. The rapid increase of cancer incidence with age could be a manifestation of the sequential accumulation of driver mutations[2–5], although the mathematical support for a multistage model of cancer is still debated[10]. The evolutionary nature of oncogenesis is also consistent with molecular[7] and sequencing studies of human cancers[11,12]. Indeed, evolutionary ideas have an important role in current cancer research and are used to identify driver mutations[13,14] and the order of their arrival[15]; to explain pervasive genetic instability[16–19]; and to understand the evolution of resistance to chemotherapy[20,21].

Although the evolutionary nature of cancer makes it difficult to treat, this difficulty is not unique to cancer and plagues efforts to combat some viral infections, such as HIV, and the spread of resistance to antibiotics. However, evolving populations are not invincible. A drug cocktail that suppresses the evolution of resistance can now control HIV[22,23], and an HIV vaccine is also being developed with evolutionary considerations in mind[24]. Even stronger evidence that evolving populations can be destroyed comes from a long list of endangered and extinct species that were not able to adapt to the changing environment. So, in this Opinion article, we turn to conservation biology, ecology and evolution to identify the common mechanisms that cause extinction or that inhibit adaptation.

Despite the broad appreciation of cancer as an evolutionary process, much less work has gone into characterizing the ecological aspects of cancer[2,3,6,25–27] (but see REFS 28–30). Hence, we focus on how the coupling between evolution and ecology may affect tumour dynamics. We also heavily draw on the recent experimental work with microorganisms that shows the range of possible dynamics in populations, which, similar to tumours, evolve through competition among clonal lineages.

Growth thresholds

Evolutionary modelling has provided important insights into cancer progression, but many assumptions of the models remain untested. In particular, our knowledge of ecological dynamics within tumours is limited, especially in small tumours prior to detection or after treatment. In the absence of experimental data, most models assume that tumours (or precancerous lesions) either remain constant in size or grow exponentially. These dynamics can be described by a logistic growth curve in which a population exponentially grows initially but then stabilizes at the carrying capacity. Logistic growth, however, is only one of the possible population dynamics that are observed in natural and laboratory populations. One frequent departure from logistic growth is known as an Allee effect or inverse density dependence[31–33] (BOX 1). A strong Allee effect describes a population that can grow at intermediate population density but declines when the number of organisms is either too small or too large. Such populations are then likely to collapse and become extinct if their population size falls below a certain threshold. In the context of conservation biology, such Allee effects represent a substantial challenge, because it can be exceedingly difficult to intervene and save a population that has fallen below this threshold population size. In cancer, however, the Allee effect implies the existence of a growth threshold that may be explored in therapeutics.

Allee effects are caused by a large number of mechanisms, including cooperative feeding and defence[31–33], which can potentially be relevant to cancer. Cooperation is typically inefficient when the number of organisms is small, thereby leading to a growth threshold in population dynamics, because a sufficiently high growth rate is required to

overcome inevitable deaths due to environmental factors. Cooperative feeding strategies are used by a wide variety of organisms, from African wild dogs, which hunt and feed their young in large groups[31], to baker's yeast, which digests sucrose in a collective pool outside the cell[34,35]. In cancer, cooperation among cells might be required to produce a sufficient density of diffusible growth factors needed for tumour proliferation or pro-angiogenic growth factors such as vascular endothelial growth factor A (VEGFA), which recruits blood vessels to irrigate the tumour[36]. The Allee effect is commonly seen in cell cultures. It is usually difficult to make a single cancer cell grow in isolation unless the media is conditioned by the secretions of other cells[37]. Growth thresholds are also present in micrometastases, many of which can stay dormant for long periods of time[37].

Many species also cooperate to defend against predators or harsh environmental conditions. Examples include predator avoidance by schools of fish, collective warming in colonies of the Emperor penguin and the formation of protective biofilms, which are densely packed, surface-attached communities of bacteria[31,38,39]. Similar to the bacteria in biofilms, which are considerably more difficult to treat than free-living bacteria, larger tumours are more difficult to target by drugs or radiotherapy, and this results in a potential Allee effect[39,40].

Like other aspects of population dynamics in cancer, the presence and extent of Allee effects has scarcely been investigated. Nevertheless, some of the existing studies are consistent with growth thresholds in the population dynamics of cancers. For example, xenograft transplantations of cancer cells succeed at a higher rate when a larger number of cells are injected into a mouse with a fully functional immune system. This may indicate that cooperation among cells facilitates successful initiation in these models[41]. Allee effects could also explain the low rates of cancer initiation, invasion and metastasis. It is possible that many tiny tumours are formed but that they almost always become 'extinct' before they are clinically relevant, and even before detection is possible[2]. In the presence of Allee effects, successful tumours occur owing to rare large fluctuations in the population size that take the tumours over the Allee threshold. Another possibility is that Allee effects occur after the tumour is established, owing to the emergence of new cancer subclones that are more fit at higher densities but suffer from an Allee effect at lower densities.

Some cancers recur after treatment, even if only a very low number of malignant cells remain. This phenomenon, which is known as minimal residual disease (MRD), implies that for those cancers, Allee effects are either negligible or can be removed by further evolution of cancer cells during remission[42]. Similarly, cancer can potentially be initiated from a single progenitor cell in some transgenic mouse models that were created for robust cancer initiation and could therefore be different from human cancers that typically take many years to develop.

The occurrence of Allee effects would open several new possibilities in cancer treatment (BOX 2). First, the mechanisms responsible for cooperative growth or defence could become drug targets that could supplement existing therapies. Second, the existence of a growth threshold suggests that one does not have to kill all cancer cells to cure the disease; instead, reducing the density of cancer cells below some critical value would be sufficient to ensure their eventual extinction. It would be interesting to know whether current treatments, such as chemotherapy and radiotherapy, are more successful against tumours with larger growth thresholds and whether there could be a benefit from adjusting treatment dosage based on the magnitude of a growth threshold in a tumour. Finally, a radically new approach to therapy would be to focus on the size of the threshold, rather than on the population size.

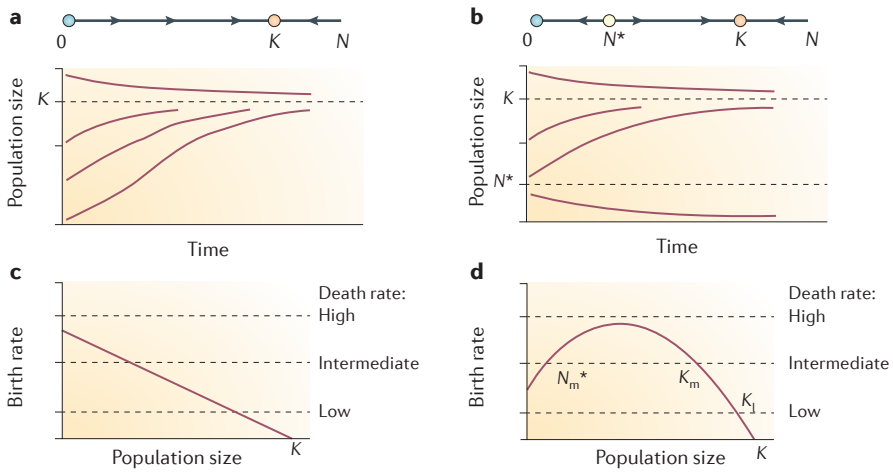
A therapy can, in principle, increase the magnitude of the Allee effect either directly or by stimulating tumour evolution towards this outcome. An immediate effect of such a treatment would be a marked reduction in the probability of metastasis, which is

Box 1 | **The Allee effect and growth thresholds**

One of the primary goals of ecology is to understand population growth or decline. Beginning with the works of Malthus to describe human population growth and the works of Lotka and Volterra to describe predator–prey interactions, ecologists have accumulated a rich set of models of population dynamics for different organisms[143]. However, most evolutionary studies, including those of cancer, have almost exclusively focused on a single type of population growth — the logistic model. The logistic model describes how population size changes with time, $N(t)$, via the differential equation $dN/dt = rN(1 - N/K)$. Here, r is the exponential growth rate and K is the carrying capacity. For small initial N ($N << K$), the population grows exponentially as $\exp(rt)$, until the population size approaches K, which is a stable equilibrium of the equation above (also known as a 'stable fixed point'; see the figure, part **a**).

However, the logistic model does not incorporate Allee effects, which are observed in many populations. An alternative model, with a strong Allee effect, is given by the differential equation $dN/dt = rN(1 - N/K)(N/N^* - 1)$, in which N^* is the Allee threshold (that is, the minimal population size required for growth). Populations with sizes greater than N^* grow to the carrying capacity, whereas populations below the growth thresholds are destined for extinction; see the figure, part **b**.

In the absence of the Allee effect, the birth rate of the population is maximal for small population sizes and decreases as the population approaches the carrying capacity. The population survives as long as the death rate is smaller than the birth rate at small population sizes (see the figure, part **c**, and part **d** for comparison). In the presence of the Allee effect, there is a peak in the per-capita birth rates at intermediate population sizes. At low death rates, the population experiences a net growth as long as $N < K_l$ (the corresponding carrying capacity). At moderate death rates, the population experiences a net decline when $N < N^*$ (the growth threshold) but grows to the carrying capacity K_m otherwise. At high death rates, the population declines for all population sizes.



often the primary cause of death. Indeed, successful metastasis requires that one or a small number of cancer cells proliferate and reach a critical population size at a new location. This process is similar to the crossing of an activation barrier in a chemical reaction, because the growth rate is negative below the threshold. As the rate of barrier crossing depends exponentially on the height of the barrier, even a modest increase in the size of the growth threshold can yield a marked reduction in the probability of successful metastasis being formed (BOX 2). Importantly, this type of approach should not select for resistance in the primary tumour, because the primary tumour is already well above the threshold, so the fitness of the primary cancer cells is not necessarily affected. An increase in the Allee threshold could also be followed by a traditional treatment that would push the primary tumour below the critical population size and lead to a rapid population 'meltdown'. Here, again, high growth thresholds could be beneficial, because they make it harder for new mutations to rescue the population[43].

Species interactions

In the wild, species rarely exist in isolation; for example, a gram of soil could contain more than 10,000 microbial species[44], and the smallest isolated ecosystem was created from three species[45]. Similarly, stromal cells and immune cells make up a substantial proportion of a tumour. This coexistence of several cell types is indicative of mutualism, predation or other types of ecological interactions. For example, tumour-derived fibroblasts can promote tumorigenesis[46,47] and macrophages can be reprogrammed by cancer cells to promote inflammation and facilitate a permissive tumour microenvironment[48,49]. In such tumours, macrophages are transformed from a predator into a commensal partner of cancer cells, thereby profoundly changing the tumour ecology.

Even more complex interactions may be at play in prostate cancer and multiple myeloma, where evolutionary game theory models suggested that three different cell types promote and inhibit the growth of each other[50,51]. In prostate cancer, stromal cells could mediate the competition between testosterone-dependent and testosterone-independent cancer subclones[50]; whereas in multiple myeloma, cancer cells may shift the physiological equilibrium that occurs between osteoclasts and osteoblasts[51]. This theoretical study predicts that competition will result in bone loss because cancer cells forge a mutualistic relationship with osteoclasts via an exchange of growth factors and inhibit osteoblasts.

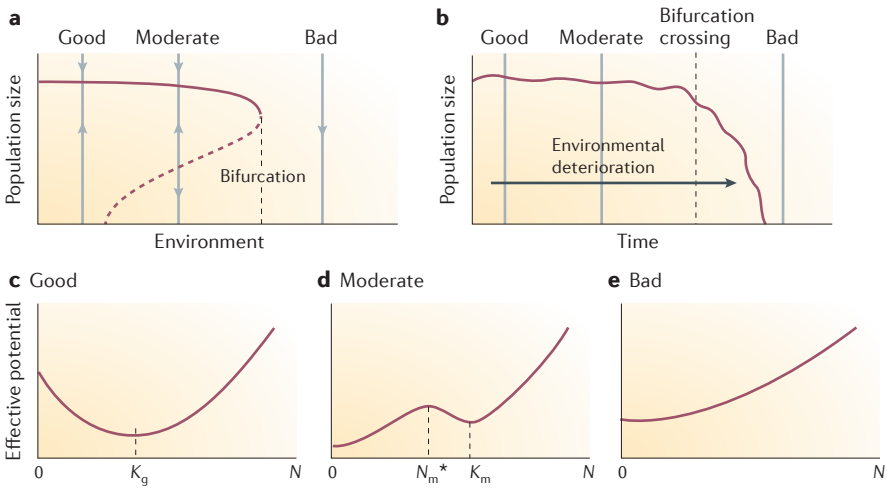
Despite the important role that stromal and immune cells have in tumours, these cells are rarely targeted by anticancer drugs that are currently used in the clinic. One exception is the success of stroma-directed therapy that inhibits the occurrence of bone metastasis in patients with breast cancer[52,53]. In addition to regular therapy, patients in these studies received daily doses of clodronate, which inhibits cancer-supporting osteoclasts and tumour cell adhesion to the bone[54,55]. Similarly, recent studies of mouse models showed that inhibition of tumour-associated macrophages leads to tumour regression because macrophages provide a permissive environment for cancer growth[48,49,56,57].

Box 2 | **Population dynamics with changing growth thresholds**

Cancer treatment would be expected to alter both the carrying capacity (*K*) and Allee threshold (*N**) of a tumour. Similar to increasing death rates, such an environmental deterioration drives the population from a monostable behaviour to bistability and eventually to extinction; see the figure, parts **a**,**b**. In the language of dynamical systems theory, the above transitions correspond to the appearance of an unstable fixed point (for $N > 0$) and then its annihilation with the stable fixed point — a phenomenon known as a bifurcation. As a bifurcation is approached, the separation between the stable and unstable fixed points diminishes. This change, however, may be mostly attributed to the increase of the unstable fixed point; for example, see the figure, part **b**, in which the population size remains nearly constant until the population starts to collapse after the bifurcation is crossed.

The change in the unstable fixed point profoundly affects the probability of metastasis, even though the change in the unstable fixed point may have a very small effect on the size of the tumour. See the figure, parts **c**–**e** for an illustration of how the effective potential changes with environmental deterioration. In good environments, the potential has a single minimum, which corresponds to a stable population at carrying capacity (K_g). Extinction under these conditions is unlikely because the population would have to climb a high potential barrier. At moderate environments, the potential has two minima: one corresponding to a population at carrying capacity (K_m) and one corresponding to an extinct population. Population extinction is more likely under these conditions because it has to cross a smaller potential barrier. More importantly, metastases that start as small populations are unlikely to succeed, because they have to cross a potential barrier to become stable. Because the probability of this barrier crossing decays as the exponent of the barrier height, even a moderate increase of the unstable fixed point could provide a marked benefit to the patient. In bad environments, extinction is the only stable state.

The changes of population stability that are described above in the context of Allee effects may also occur for evolutionary thresholds if there is bistability in the ability of the cancer to adapt. The probability of metastasis is likely to be further reduced by cooperative interactions between cancer and stromal or immune cells, as well as cooperative interactions between cancer subclones. Indeed, both cooperating partners should arrive at the same site and in sufficient quantities to establish a growing population. Increasing growth thresholds and strengthening cooperation reduces the growth of cancer cells in metastatic sites and could be an important treatment strategy. Metastases also grow and potentially overcome growth thresholds via reseeding: that is, migration of cancer cells from the primary tumour to the metastases. Inhibition of cell migration may therefore be important in preventing the growth of small metastases in addition to the better-known effect of preventing the formation of metastases.



The success of clodronate and macrophage treatments could be an example of a general strategy that seeks to decrease the fitness of stromal cells that support tumorigenesis and to increase the fitness of stromal cells that compete with cancer cells. This approach has recently been explored in the context of multiple myeloma using evolutionary game theory models[51]. Instead of exploiting the interactions to kill cancer cells, one could also strengthen the dependence of cancer cells on other cell types, thereby preventing the evolution of more aggressive cancer cells that are able to metastasize. As mutations tend to vary across patients, whereas stromal cells do not, therapies that target the stroma may have wider applicability than therapies that target somatic mutations[11,12,14,20,25,26,58]. Moreover, since stromal cells are genetically stable, they are less likely to change during treatment[58].

Genetic diversity

Tumours harbour many genetically distinct subpopulations of cancer cells. Genetic heterogeneity is a hallmark of evolving populations and typically results from the segregation of many mutations in the population at the same time. A large proportion of these mutations are likely to be neutral, because neutral mutations are common and they can persist in the population for a long time. By contrast, strongly beneficial or deleterious mutations usually have shorter lifetimes, because they either quickly spread or get eliminated by natural selection. Nevertheless, large tumours with high mutation rates could have several driver mutations segregating at the same time. This phenomenon, called clonal interference, has been repeatedly observed in evolving microbial populations[59–62]. One important consequence of clonal interference is a reduced rate of adaptation, because competing clones with similar fitness impede the fixation of each other[62,63] (FIG. 1).

Genetic heterogeneity is a determining factor in the evolutionary potential of the tumour. The level of genetic heterogeneity could be indicative of some important aspects of the internal tumour dynamics, such as mutation rates, effective population size, generation time and spatial structure, which affect the rate of tumour evolution with and without treatment. For example, tumours with more genetic variants are more likely to contain or generate a mutation encoding resistance to treatment. Recent studies of human cancers have not only found remarkably high levels of genetic diversity within tumours[12] but have also shown that the level of genetic diversity is a predictor of clinical outcome[64]. These findings have parallels with the field of ecology, in which biodiversity is known to promote the stability and resilience of ecosystems[65,66].

Although transient segregation of many mutations is a major driver of tumour heterogeneity, genetic diversity can also be maintained by long-term coexistence of different genotypes because of frequency-dependent selection. Models of cancer evolution often neglect frequency-dependent selection and assume that evolution unfolds over a fitness landscape such that every genotype has a specific fitness value that is independent of its relative abundance in the population[2,3,15,21]. Nature abounds with situations that do not fit this paradigm, in particular, situations in which different genotypes coexist because they have a higher fitness when they are rare. For instance, predators tend to attack prey of the most common appearance; therefore, prey that do not look like the others have a survival advantage[67]. The selection against the most common appearance then leads to the coexistence of a few phenotypes that are not overly abundant. Similarly, some proteins in the immune system (such as major histocompatibility complex (MHC)) tend to be diverse because pathogens evolve to avoid those that are most prevalent in the immune system[68]. Note that although frequency-dependent selection and an Allee effect are different phenomena, they are related because absolute or relative growth rates depend on the abundance of the organisms in question.

Naively, one might think that complex interactions that lead to frequency-dependent selection are quite rare and that competitive exclusion and frequency-independent selection are the norm; however, recent evolutionary experiments challenge this point of view[35,61,69–71]. In particular, one study has looked into the dynamics of beneficial mutations and surveyed almost 600 replica populations of yeast for 1,000 generations. Many beneficial mutations initially spread but did not take over the population; instead, they persisted for a long time at intermediate frequencies, which is indicative

Glossary

Allee effect
A commonly observed deviation from logistic growth, with the per-capita growth rate reaching a maximum at an intermediate population size. One often distinguishes between a strong Allee effect, when the growth rate is negative at small population sizes, and a weak Allee effect, when the growth rate at small population sizes is small but positive.

Auto-correlation time
The time that it takes deviations of a variable from its stable state to become statistically uncorrelated. This time is closely related to the recovery rate from perturbations.

Coefficient of variation
The ratio of the standard deviation to the mean. The coefficient of variation measures the relative strength of fluctuations.

Ecological dynamics
Describes interactions among species and the changes in their absolute abundances.

Evolutionary dynamics
The emergence of new genotypes and the changes in relative abundances of the existing genotypes, including possible extinctions.

Evolutionary game theory
Describes evolutionary dynamics in a polymorphic population consisting of organisms that use different strategies to succeed at a particular task and in which success depends on the strategies of other individuals, often conceptualized as a game. A typical example is a 'hawk–dove' game that describes a contest over mates. The success of an aggressive (hawk) strategy and a passive (dove) strategy depends on their relative abundance in the population and on how they fare in competition against other organisms with their own strategy and organisms with the opposite strategy.

Frequency-dependent selection
Selection that occurs when the fitnesses of species or genotypes depend on their relative abundances in the population. This type of selection can lead to stable coexistence between two species when species A is more fit than species B; when species A is rare and species B is more fit than species A; or when species B is rare.

Frequency-independent selection
Selection that occurs when the fitness of genotypes or species is independent of their relative abundance. In such situations, the genotype or species with the highest fitness takes over the population.

Genetic drift
The random changes in relative frequencies of different genotypes in a population. The primary cause of genetic drift is the stochastic variation in the number of offspring among organisms with the same fitness. Genetic drifts makes natural selection less efficient: it enables fixation of deleterious mutations, as well as the loss of beneficial mutations.

Logistic growth
A frequently used model of population growth, in which the net growth rate at population size N is $rN(1 - N/K)$. At small population sizes, such populations grow exponentially at the per-capita growth rate r, whereas, at higher population sizes, the per-capita growth is diminished until it reaches zero at $N = K$. Here, K is the stationary population size, often termed the carrying capacity. Note that the per-capita growth rate is maximal at the smallest population sizes ($N = 0$).

Population dynamics
An umbrella term that describes both ecological and evolutionary dynamics.

of frequency-dependent selection. Even after this relatively short period of evolution, stabilizing frequency-dependent selection was observed in as many as 1–10% of the populations[61]. In cancer, one sign of possible interactions among subclones is the polyclonal origin of intestinal tumours in humans and mice[72,73] and direct demonstration in WNT-driven mammary mouse cancers[144].

Niche partitioning is a common ecological mechanism for frequency-dependent selection; in this scenario, different species or subpopulations focus on different resources in the ecosystem. Niche partitioning was the probable cause for the repeated evolution of coexistence in 12 replicate *Escherichia coli* populations — the longest running laboratory-based evolution experiment[69,70]. In one population, a subpopulation of *E. coli* evolved that could digest citrate. However, instead of displacing the parent population, the new and the old clones coexisted, presumably by focusing on different sources of energy[70].

Division of labour is another possible source of frequency-dependent selection in cancer. Cancer is now widely accepted to be a disease of clonal evolution[6] (FIG. 1) in which cell lineages acquire a series of traits — the so-called 'hallmarks of cancer' (REFS 8,9). Although some of these hallmarks, such as lower rates of apoptosis, benefit individual cells, other hallmarks, such as the ability to recruit blood vessels, would benefit the tumour as a whole. These collective traits introduce the possibility that a cancer phenotype is created by several coexisting lineages that have acquired complementary adaptations[36,74]. Interdependencies among tumour subpopulations might enable one to attack the weakest cancer subclone that supports the growth of the whole tumour. Alternatively, the interdependencies could be strengthened, thereby making metastasis less likely.

Frequency-dependent selection can also arise without direct complementation between the genotypes. When cells cooperate to create 'public goods', such as diffusible growth factors, the products of the cooperation are often available to cells that might not have directly participated in their production. This favours the evolution of non-producers that benefit from the public good without incurring the cost of production. Although non-producers are likely to occur and spread, they may not entirely outcompete the producers if these producers have preferential access to the public goods that they produce. Indeed, producers and non-producers were found to coexist both in nature and in the laboratory[35,75–77].

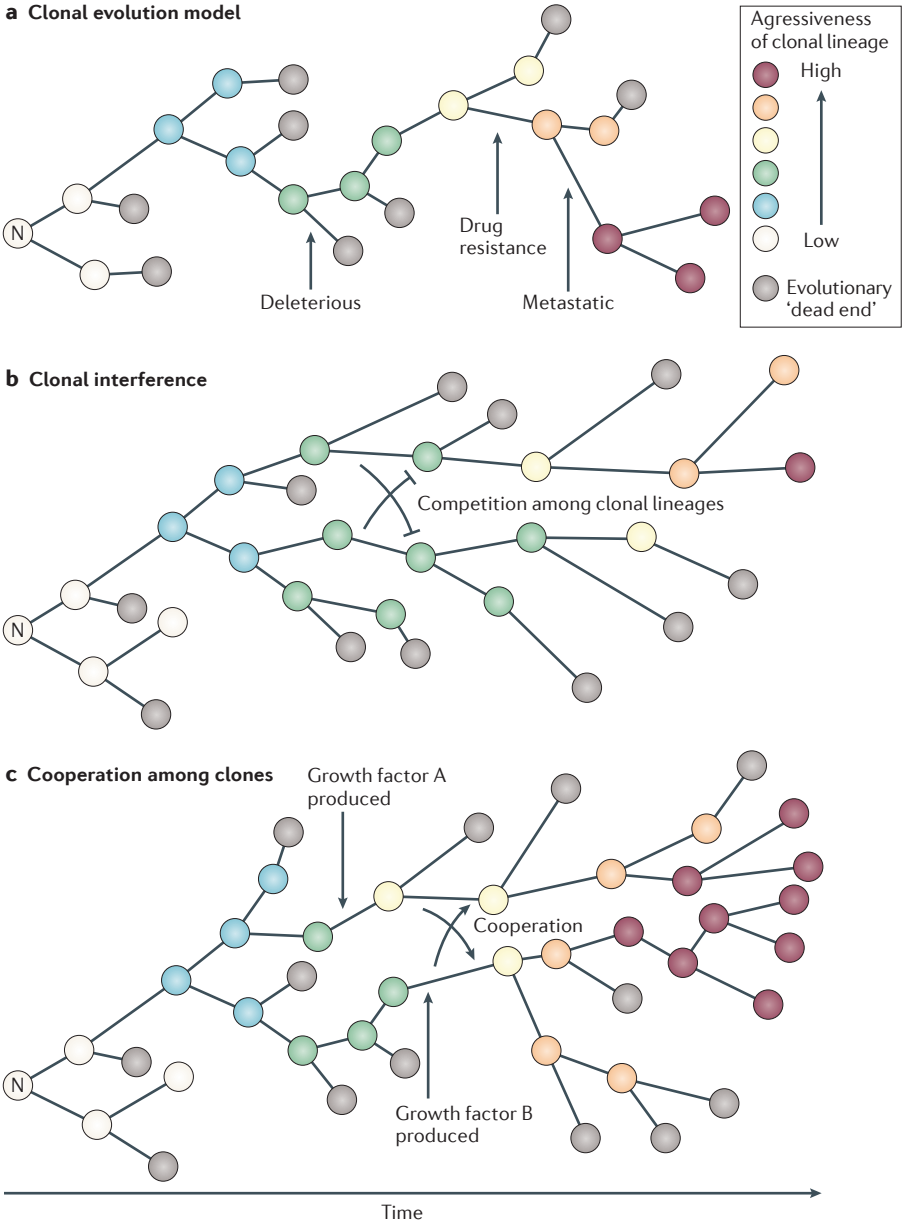


Figure 1 | **Competition and cooperation in cancer progression.** **a** | In the model of cancer as a disease of clonal evolution proposed by Nowell[6], an initially benign neoplasm (N) accumulates mutations that confer cancer phenotypes with increasing aggressiveness. Less fit lineages temporarily segregate but are typically lost through competition. **b** | Clonal lineages with similar fitness may coexist and compete within a tumour — a phenomenon known as clonal interference[62,63]. **c** | Two clones evolve complementary traits. Even though each clone is not self-sufficient, their cooperation results in malignancy[37].

The emergence of non-producers has also recently been suggested to have an important role in the context of angiogenesis[78].

Complex interactions that lead to frequency-dependent selection and coexistence can also have marked effects on population ecology, growth thresholds and resilience to perturbations. This was recently demonstrated in an experimental yeast population growing on sucrose that is hydrolysed outside the cell wall by the enzyme invertase[79]. The study compared the population growth in a pure culture of cooperators that produce invertase and a mixed culture of producers and non-producers. Although non-producers had only a minor effect on the population size, they markedly increased the critical population size required for growth and made the population more susceptible to extinction — a result that is generically expected in public goods games[80,81]. Therefore, treatments that promote the evolution of non-producers

in tumours could be advantageous because they will make tumour cells less resilient against perturbations and slow the growth of producers by reducing the rate of production of exogenous growth factors.

Compared to the relatively simple dynamics of producers by themselves (BOX 1), the response of heterogeneous tumours to treatment could be more complicated, because both the size of the tumour and its composition could change. For example, in the aforementioned yeast study, environmental stress resulted in an increase in the proportion of producers in the population. This allowed the population to survive larger perturbations than one would expect if the population composition did not change. One important consequence of these dynamics is that the time course of population disturbance was as important as the size of the disturbance. A gradual increase in environmental stress allowed the population composition to adjust to a more favourable state and rescue the population, whereas abrupt disturbances caused population extinction before their composition could change[79,82]. In the context of cancer therapy, these results suggest that a cooperatively growing tumour should be 'hit hard'.

Evolutionary thresholds

In addition to ecological thresholds caused by Allee effects and species interactions, tumours might have to overcome evolutionary thresholds due to the accumulation of deleterious mutations[83] (although the importance of deleterious mutations in cancer is still debated[16,84]). Traditionally, models focused on mutations driving cancer progression as they are the primary causes of malignancy. From the evolutionary point of view, these driver mutations are considered to be beneficial because they provide a selective advantage to the cancer cells. Because somatic cells are adapted to the developmental programme of a multicellular organism and not optimized to individual cell fitness (as for unicellular organisms), cancer cells have 'room for improvement'. Nonetheless, in the vast space of all possible mutations, beneficial mutations should be a small number compared with neutral and deleterious mutations. The low supply of beneficial mutations may be one of the main reasons why evolutionary adaptation (including cancer) requires thousands of generations. It is then not surprising that recent sequencing studies found thousands of genetic alterations in cancer genomes[11–13,83], despite the fact that only one to ten drivers are thought to be necessary for tumorigenesis[2,3,7,83]. Although these non-driver mutations are often termed passengers to indicate their irrelevance to tumorigenesis, recent evolutionary modelling and genomics analysis indicates that deleterious passengers might have an important role in limiting the progression of cancer[83].

Given that mutations are much more likely to be deleterious than beneficial, populations are always in danger of losing viability owing to a high number of deleterious mutations. 'Mutational meltdown' has been observed in empirical studies of viruses[85], mitochondria[86] and bacteria[87], and it has attracted considerable attention in theoretical evolution and conservation biology[88–90]. Recently, McFarland *et al.*[83] found that mutational meltdown is a major barrier to cancer progression.

> "Progression to cancer is then akin to overcoming the activation energy of a chemical reaction"

In very small tumours with less than around 1,000 cancer stem cells, genetic drift enables stochastic fixation of damaging passengers at a much higher rate than in large tumours, in which natural selection is more efficient at weeding out deleterious mutations. Larger tumours are also more likely to acquire a driver mutation because they have more cells that can undergo mutation. These dynamics result in a runaway process: small tumours accumulate more damaging mutations, lose fitness and get smaller, whereas large tumours accumulate more drivers, gain fitness and get bigger. Progression to cancer is then akin to overcoming the activation energy of a chemical reaction, because tumours have to rely on the stochasticity of mutation to overcome the critical population size that is necessary for deterministic expansion.

Similar to Allee thresholds, evolutionary thresholds can be exploited in treatment. The critical population size, which controls the ability of the tumour to adapt, depends on evolutionary parameters of the cancer — in particular, the mutation rate and fitness cost of deleterious passengers[83]. Treatment can, in principle, modify these parameters. Mutation rates can be increased by adding a mutagen or by inhibiting DNA repair machinery. In support of this argument, recent clinical data on patients with breast cancer suggests that a high mutation rate is associated with a better survival outcome, whereas tumours with intermediate mutation rates are the most aggressive[91]. The fitness costs of passengers can also be increased by inhibiting the cellular machinery that buffers against perturbations. Indeed, proteasome inhibitors have been approved for the treatment of multiple myeloma[92], and chaperone inhibitors are in clinical trials[93]. In both cases, the primary mechanism of action could be different from the one we suggest here; for example, chaperones might be required for proper folding of mutated KRAS, as these interventions have pleiotropic effects[94,95].

Detecting thresholds

If there are thresholds in population growth, how can they be detected? This question has attracted a lot of attention in ecology, and various detection methods have been developed for the different types of available measurements[66,79,96–99].

Thresholds are easiest to detect when they are large, so that stochastic effects (ecological or genetic drift) can be neglected and populations of different sizes can be studied in nearly identical replica experiments. Indeed, experiments started with populations below the threshold would result in extinction, and experiments started with populations above the threshold would result in growth[97,98]. Replica experiments, however, are not possible in human patients, and, even in animal models, differences in the tumour microenvironment or divergent evolution may complicate the comparison between different tumours. An alternative approach that may be applied, at least in animal models, is to perturb a tumour (for example, with a pulsed application of a drug) and observe its response. Small perturbations that decrease the tumour size but are not sufficient to push the tumour below the threshold will be followed by rapid tumour regrowth to its pre-perturbation size. By contrast, a large perturbation that decreases a tumour below its Allee threshold will lead to remission. Thus, such a bimodal response is indicative of a potential Allee threshold.

The two strategies outlined above are only appropriate when stochastic effects are negligible; otherwise, thresholds can be crossed in either direction owing to a large fluctuation. Since growth thresholds could be quite small in early tumours or metastases, stochastic effects may have a major role in the detection of growth thresholds in cancer. When replica experiments are possible, a direct way to ascertain a growth threshold is to measure how the probability of remission depends on the initial size of the tumour. This probability will decrease with the initial number of cells,

regardless of whether growth thresholds are present; however, the shape of this decrease allows one to distinguish between populations with thresholds and those without[96]. For populations without thresholds, the remission probability will decrease approximately exponentially as the initial population size grows, whereas for populations with thresholds, this decrease is more modest and can have a sigmoidal shape.

Knowledge that a tumour is close to a threshold could be quite valuable if drug dosing were altered to take this information into account. In the clinic, the proximity of thresholds can in principle be detected by measuring the time for a tumour to recover from a perturbation. When a tumour is close to a threshold, the rate of recovery from perturbations declines — a phenomenon termed 'critical slowing down' (REFS 66,97,98). It is therefore expected to take longer for a tumour to recover from a perturbation if a threshold is near.

Even in the absence of defined perturbations, critical slowing down can signal that a population is close to a threshold, because the decrease in recovery rate also alters the spatial and temporal fluctuations in population size[66,97,98,100]. This manifests as an increased auto-correlation time and length in the dynamics of the population size, as well as a higher coefficient of variation. These indicators of critical slowing down have been observed in several laboratories and even in natural populations before population collapse due to environmental deterioration[66]. In addition, a recent modelling study suggested that this approach can successfully predict transitions between distinct phenotypic states at a single cell level[101]. This observation is particularly interesting in the context of quiescent or dormant cells that can survive chemotherapy or passage through the blood stream and lead to cancer resurgence or metastasis. Indicators that a cell is about to transition to a malignant phenotype could facilitate early therapeutic intervention.

Given the complexity of cancer, it would be remarkable if such simple techniques can succeed at identifying thresholds in cancer dynamics. Nevertheless, their success in natural populations suggests that some potentially cancer-specific indicators of thresholds can be identified and deployed in laboratory-based research and in the clinic.

Spatial organization

Tumours are often classified by their shape[102], and spatial structure is likely to have a key role in tumour evolution[103]. Nevertheless, most experiments and evolutionary models of cancer neglect spatial organization. In contrast, ecologists have long appreciated the importance of space, because many ecological phenomena have spatial manifestations. Transitions to a more arid environment are accompanied by a change in spatial patterns of vegetation[104], invasive species spread as a 'population wave' across the ecosystem[105] and environmental gradients determine the boundaries of species ranges[106]. As a result, ecological studies have elucidated many effects of spatial organization on population dynamics. Most of these effects have recently been observed in microbial populations, which have remarkably similar ecology and evolution to that of tumours[107,108].

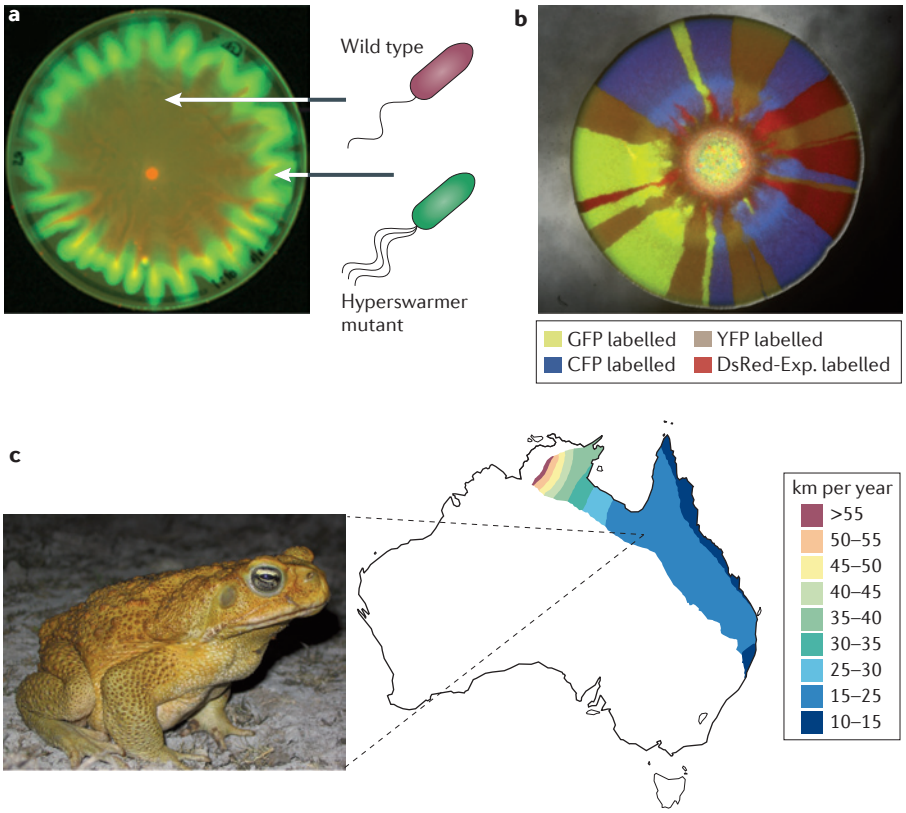


Figure 2 | **Spatial organization in populations.** **a** | In colonies of swarming bacteria, 'hyperswarmer' mutants with several flagella have a fitness advantage by being able to travel faster to the edge of the colony, where nutrient concentrations are higher. **b** | Genetic demixing in a colony of bacteria. A strain of *Pseudomonas aeruginosa* was labelled using four neutral fluorescent markers (green fluorescent protein (GFP), cyan fluorescent protein (CFP), yellow fluorescent protein (YFP) and DsRed-Express (DSRed-Exp.)), and the four labelled strains were mixed at equal proportions in the founding populations. Genetic demixing occurred as the population expanded over the agar surface on a Petri dish. The figure shows a previously unpublished experiment carried out following the protocol in REF 116. **c** | Cane toads are an invasive species in Australia, where they were introduced on the south-east coast in the 1930s. Since then, they have spread through a large part of the northern coast of Australia, and their invasion speed has markedly increased. This increase is attributed to adaptations (such as longer legs) at the leading edge. Image courtesy of B. Phillips, Department of Zoology, University of Melbourne, Australia. Figure part **a** is reprinted from *Cell Reports*, 4, Van Ditmarsch, D. *et al.*, Convergent evolution of hyperswarming leads to impaired biofilm formation in pathogenic bacteria, 697–708 © (2003), with permission from Elsevier[111].

Cancer, in fact, is similar to a geographic expansion of an invasive species. During a geographic (or range) expansion, natural selection favours not only faster growth rates but also faster dispersal (FIG. 2). A striking example is the rapid evolution of cane toads — an invasive species in Australia[109]. The rate of invasion has increased dramatically since the species was introduced[105], partly owing to the evolution of longer legs and higher stamina (FIG. 2c). However, these adaptations came with the trade-off of a weaker immune system that made the toads more susceptible to bacterial infections[110]. Similar phenomena were observed in colonies of swarming bacteria: mutants with many flagella occurred and took over the entire population owing

to their higher motility[111] (FIG. 2a), but these multi-flagellated bacteria made weaker biofilms, which are essential for their survival in the wild. Trade-offs are inevitable because organisms have to allocate limited resources among several functions that affect fitness; therefore, aggressive cancer clones are likely to have a weakness that can be exploited in treatment[112]. One such possibility is a trade-off between growth rate and drug resistance[113,114].

Another consequence of range expansions is a marked increase in demographic fluctuations (genetic drift) and a build-up of spatial heterogeneity. Experiments with neutral markers in systems as diverse as bacteria, yeast and social amoeba show that genotypes segregate in space as a colony expands[115–117] (FIG. 2b). The segregation (or genetic demixing) is caused by local stochastic extinctions of the genotypes owing to large demographic fluctuations at the expansion front. Genetic demixing results in spatial clustering of coexisting genotypes and gives a natural explanation for the spatial heterogeneity that is observed in tumours[118]. Recent modelling and experimental efforts showed how the patterns of spatial diversity can be used to quantify genetic drift and natural selection in microbial populations, as well as how they could be of value in modelling tumours[116,119,120]. More importantly, it was also shown that genetic drift and the resulting spatial structures can be controlled by nutrient limitation[119], thereby potentially allowing one to alter the parameters of cancer evolution that control adaptation thresholds. High genetic drift makes natural selection inefficient, thereby slowing the fixation of driver mutations and increasing the rate of fixation of deleterious passengers. As a result, high genetic drift should make crossing adaptation thresholds less likely in both primary tumours and metastases.

Population expansions can also have a role in the maintenance of cellular cooperative traits[119,121]. Two recent experiments with yeast show that population range expansion can be a key factor in stabilizing the cooperative digestion of sucrose. The first of these studies simulated population range expansion in a mixed population of producers and non-producers using a set of wells coupled by migration, with large population sizes and negligible genetic drift[122]. The results showed that range expansion favoured cooperation, because the producers did better at the front of the populations and could outrun a wave of non-producers as the population expanded. The second study investigated a nutrient-limited expanding colony[123]. As explained above, genetic drift during the expansion led to spatial separation of producers and non-producers. Non-producers were then outcompeted because they had only limited access to the public goods.

Another important consequence of spatial structure is the occurrence of gradients in the environmental factors. For example, transport of nutrients and waste products into and out of the tumour is often slow, which can generate gradients of growth conditions in the tumour[119,124]. Recent theoretical and experimental works suggest that evolution proceeds more rapidly in the presence of an environmental gradient because the gradient facilitates gradual adaptation from permissive to harsh environments[125–127]. In the interior of avascular tumours, cell proliferation is limited by the rate at which resources, such as oxygen or glucose, can diffuse into the tumour, and many important tumour adaptations are in response to limitations such as hypoxia[128]. Tumour heterogeneity can not only accelerate evolution but also directly select for more aggressive cancer. For example, the accumulation of lactic acid, a metabolic waste product, can lead to acidosis inside the tumour and create a selective pressure for acid-resistant clones[129,130], which could potentially be more resistant to other stresses. Theoretical models also predict that the harsh microenvironments created by the tumour itself can create a selective pressure for more aggressive cancer lineages[131]. This study also found that increasingly stressful microenvironments lead to a morphological transformation of the tumour from smooth and non-invasive margins to margins with finger-like protrusions that are associated with an aggressive and invasive phenotype.

Outlook: measuring population dynamics

Cancer was recognized as an evolutionary process more than 60 years ago[5] and, since then, evolutionary ideas have had an important role in cancer research[2,3,25,26]. However, evolutionary and ecological thinking is rarely used during drug development and in the clinic. This situation is unfortunate, as most treatments fail because of tumour evolution, and the full range of strategies to control tumour growth is not being explored. It is likely that ecological processes discussed in this Opinion article are happening simultaneously in the same tumour. The integration of ecological processes must therefore be considered. For example, the size dependency of cancer growth that is captured by the Allee effect may be linked to diversity. High clonal diversity is correlated with cancer aggressiveness[64] and, in many ecosystems, diversity correlates with population size, which together determine the adaptive potential of the population[132].

Given the potential of the ideas discussed here, we believe that it is crucial to pursue them further and make quantitative, testable and eventually useful statements about the evolutionary and ecological dynamics of cancer. Unfortunately, this effort is hampered by our limited knowledge of population dynamics inside the tumour. There is still no agreement on mutation rates in cancer, and the estimates of fitness effects of passenger and driver mutations vary by orders of magnitude[15,83,133–135]. Similarly, the growth dynamics during tumour initiation and spatial organization of larger tumours remain mostly unexplored.

The fields of ecology and evolution have also struggled to obtain reliable estimates of the parameters that control population dynamics. The rates and fitness effects of mutations were measured up to several decades after they were investigated theoretically[88]. This situation is now rapidly changing because of the progress in sequencing technologies and experiments with microbial populations[61,62,70,120]. We anticipate that similar progress will occur in cancer research.

The recent application of lineage tracing techniques to cancer is one possible avenue to uncovering population dynamics within the tumour[136–141]. Lineage tracing relies on transgenic mice with a reporter gene that can be stochastically switched on in a subpopulation of cells[136]. The changes in the average size distribution of labelled clones can then be tracked over time, thereby providing clues to the *in vivo* tissue dynamics[140]. This technology has been used to identify cancer stem cells[137–139], but it holds more promise for uncovering different aspects of evolutionary dynamics[135]. Indeed, lineage tracing is analogous to fluorescent labelling that is commonly used in microbial experiments, which has been used to measure migration rates, the strength of genetic drift and selective advantages of beneficial mutations, as well as to uncover non-trivial population dynamics[116,120,123,142]. A similar principle can be applied to investigate clonal evolution directly from human cancer samples, using methylation patterns of non-expressed genes[141]. It remains to be seen if lineage tracing[136], spatially resolved sequencing[12] or other experimental techniques can fully characterize evolutionary and ecological dynamics within the tumour, thereby opening up possibilities for accurate modelling of tumour evolution and new treatment strategies.

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doi:10.1038/nrc3712
Published online 17 April 2014

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Acknowledgements
J.B.X. is supported by National Cancer Institute Grant CA148967 through the Integrative Cancer Biology Program and by the Office of the Director, US National Institutes of Health, under Award DP2OD008440.

Competing interests statement
The authors declare no competing interests.