Invasion in multi-type populations: The role of phenotypic robustness and fluctuations

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Abstract

We study the invasion dynamics of a mutant population characterised by an increased phenotypic variability with respect to the incumbent population, with an emphasis on the effects of increased phenotypic robustness and fluctuations induced by small system size. This model is based in recent experimental and theoretical results which hint at the possibility that inactivation of certain genes, either by the effect of mutations or by pharmacological intervention, leads to the liberation of cryptic genetic variation which, in turn, produces a host of new phenotypic variants. We present a theoretical framework based on the so-called evolutionary formalism where the population dynamics can be analysed in terms of a suitably defined entropy function. This analytical framework allows us to ascertain the effect that increased phenotypic robustness of the

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new phenotypes has on the invasion probability and show that increased phenotypic robustness improves the fixation probability of the mutant population. The stochastic effects due to small population size are evaluated by means of numerical simulations. Our results are discussed in the light of recent results concerning the effect of diversity on the progression from a premalignant condition, such as the Barret’s esophagus, to fully a developed tumour.

1 Introduction

Cancer is an evolutionary process where the homeostatic mechanisms regulating normal tissue function break down, where successive genetic and epigenetic alterations and adaptations to the environmental conditions thereby lead to the emergence of cellular phenotypes capable to outgrow their normal counterparts [22, 3].

The paradigm of the evolutionary dynamics of cancer is the Fearon-Vogelstein diagram [11], Fig. 1. This genetic model of colon cancer consists of a linear pathway of genetic mutations which are then associated to phenotypic modifications leading from normal colon epithelium to invasive cancer.

This model has been the key stone in many arguments about the evolutionary dynamics, but the first model of the evolution of cancer that develops a mathematical framework which allows the evolutionary dynamics to be put into context is due to Gatenby and co-workers [13, 14, 26]. They consider a model of cellular dynamics that defines the interactions between cellular and sub-cellular events and tissue-level changes, in which subsequent cancer development is possible due to relaxation in growth constraints and adaptations to changes in micro-environmental conditions (e.g. in epithelial tissues, as a consequence of the separation of the tumour population from its blood supply by an intact basal membrane). Their results are consistent with an experimental model in which the carcinogenesis process is considered to happen in three stages: initiation, which is due to the exposure to mutagens and where tissue or cell morphology changes very little but a permanent susceptibility to cancer formation is acquired, promotion, which follows non-mutagenic tissue disruption such as wounding or inflammation, and progression, where the lesion evolves into a fully
Figure 1: Diagrammatic representation of the Fearon-Vogelstein evolutionary dynamics of cancer.
malignant tissue [26]. According to their model, the earliest mutations in tumour development would affect tumour-suppressor genes which confer minimal changes to the cellular phenotype with minimal proliferation enhancement. Subsequent progression towards a malignant tissue occurs in two phases: one of them dominated by mutations that lower barriers for proliferation (oncogenes, tumour-suppressor genes, senescence genes) and the second one governed by micro-environmental factors such as blood flow and local concentrations of oxygen, glucose and H⁺.

In addition, other issues such as the role of the micro-environment [13, 26], the complexity of phenotype dynamics [16], and the role of fluctuations and spatial degrees of freedom [17] are basic and fundamental for a proper understanding of the evolutionary dynamics of cancer.

It has recently been suggested that diversity may be a risk factor for neoplastic, premalignant lesions to evolve into fully malignant tumours is diversity. In particular, Maley et al. [21] have studied the factors involved in predicting the evolution of the Barrett’s oesophagus into an adenocarcinoma. They have found, using measures of diversity such as the Shannon entropy, that the best predictor for the progression to malignancy is clonal diversity, outperforming all the usual genetic markers.

Issues related to the complex interaction between the tumour and the micro-environment has been analysed using models from population biology and game theory in [13, 14]. In particular, they discuss how normal cell populations can evolve to sustain different coexisting cell types and how the introduction of a small population of (non-evolving) cancer cells can dramatically change the fitness landscape of the normal population, thus rendering it susceptible of being invaded when cells are allowed to evolve.

So far, a thorough exploration of the factors involved in these observations has not been carried out. The analysis carried out in this paper constitutes a first attempt to explain some of the mechanisms involved in the role of diversity in progression to cancer.

To this end, we analyse the stochastic process of invasion of a resident population which can be of any of \( d_I \) different types (e.g. the different types of cells composing a tissue) by a mutant population that, by inactivation of an evolutionary capacitor, has acquire a num-
ber $d_M > d_I$ of different phenotypes. We evaluate the ability of the mutant population to invade the resident one in terms of three key parameters, namely, $d_M$, the size of the populations, and the phenotypic robustness of the new phenotypes. In the context of this paper the term \textit{robustness}, refers to the stability of the phenotype with respect to genetic mutations [18, 6, 27]. As explained in more detail in Section 2, phenotypic robustness is characterised by a parameter, $\delta$, which quantifies the probability, that upon division and as a consequence of a genetic mutation, an individual bearing a particular phenotype produces offspring with a different phenotype. We show that, generally speaking, increased phenotypic robustness leads to more invasive populations. In particular, both phenotypic robustness and small system size can compensate for slightly deleterious mutations, as the corresponding invasion probability under highly robust phenotypes and/or small system size is very similar to that of a neutral mutation.

This paper is organised as follows. In Section 2, we present our population dynamics model, which corresponds to a multi-type branching process with resource limitation. In Section 3 we introduce the evolutionary formalism developed by Demetrius and co-workers [2] and explain how it can be used to obtain the demographic parameters (growth rate and the corresponding variance) of a given population. These parameters are then used to calculate the fixation probability of the mutant population using the diffusion approximation. In Section 4 we apply these results to the particular case in which both the incumbent and the mutant population evolve according to the aforementioned multi-type branching process. The effects of small population size are analysed by means of numerical simulations. Finally, in Section 5 we summarise our results and discuss their relevance for the study of the evolutionary dynamics of cancer.

2 Multi-type branching process with resource limitation

In order to advance further our discussion, we focus on a particular type of population dynamics, namely, a multi-type branching process with resource limitation. In the context of this paper, the different types in which the population divides correspond to the (robust) pheno-
notypes. We further assume that all the (pheno)types yield offspring with the same probability: that of an individual in our original population model. For the sake of example, we will consider two different populations. The incumbent population is defined by an strategy whereby the population splits in $d_I$ types. The average number of offspring to be produce by a given individual is given by:

$$A = 2e^{-\mu N} \begin{pmatrix} 1 - \nu & \frac{\nu}{d_I-1} & \cdots & \frac{\nu}{d_I-1} \\ \frac{\nu}{d_I-1} & 1 - \nu & \cdots & \frac{\nu}{d_I-1} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\nu}{d_I-1} & \frac{\nu}{d_I-1} & \cdots & 1 - \nu \end{pmatrix}.$$  \hspace{1cm} (1)

The prefactor $e^{-\mu N}$ describes the resource limitation, $N$ is the total number of individuals. In general $\nu_{ij}$ is the mutation probability per generation from type $i$ to type $j$. For simplicity we will assume $\nu_{ij} = \nu \forall i, j$.

The mutant population adopts a strategy with a larger variety of available types ($d_M$ different types) but which may have an increased phenotypic robustness (smaller type mutation probability). Otherwise, the proliferation probability is the same as for the incumbent population. Thus:

$$B = 2e^{-\mu N} \begin{pmatrix} 2e^{\phi}(1 - \nu + \delta) & 2e^{\phi}\frac{\nu - \delta}{d_M-1} & \cdots & 2e^{\phi}\frac{\nu - \delta}{d_M-1} \\ \frac{\nu - \delta}{d_M-1} & 1 - \nu + \delta & \cdots & \frac{\nu - \delta}{d_M-1} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\nu - \delta}{d_M-1} & \frac{\nu - \delta}{d_M-1} & \cdots & 1 - \nu + \delta \end{pmatrix}.$$  \hspace{1cm} (2)

In Eq. (2), $\delta$ is a measure of the increase in phenotypic robustness of the corresponding phenotype. The parameter $\phi$ corresponds to an increment in the growth rate of one of the phenotypes, which can be either positive or negative. The remaining phenotypes produce offspring at the same rate as the phenotypes of the incumbent population.

The matrices Eqs. (1) and (2) determine the deterministic average dynamics of the incumbent and mutant populations, $u_I(t+1) = Au_I(t)$ and $u_M(t+1) = Bu_M(t)$, respectively.
3 Evolutionary formalism and invasion probability

We now address the question whether a small population of mutants with can take over an incumbent population. Demetrius and co-workers [7, 8] have developed a formalism based on the application of ideas from ergodic theory to evolutionary problems [2] and the diffusion approximation [12]. This formalism allows us to estimate the fixation probability of a mutant population in the presence of an incumbent species. Here we generalise this formalism in order to apply it to the problem of whether new phenotypes generated by a genetic inactivation can invade the incumbent population.

The central quantity of the evolutionary formalism is the so-called **evolutionary entropy**, $H$. This quantity characterises the complexity of the structure of the population and it can be understood as a dynamical generalisation of the Shannon entropy. Whilst the latter quantity only deals with the variability within the population in terms of the occupancy of the different types that compose the population, the evolutionary entropy also characterises the flow of population between the different types, thus offering a much richer description of the system.

The relation between evolutionary and Shannon entropy is well-established for certain population dynamical models such as the Leslie model, where it has been shown that both quantities a simply proportional to each other [8]. Since in the present paper, we are only interested in presenting the qualitative mechanisms involved in the role of phenotypic diversity and robustness on the dynamics and stability of populations, we leave the discussion of the actual analytical relation between evolutionary and Shannon entropy for a multi-type branching process for future work.

The starting point of this formalism is the following fundamental equation:

$$ r = H + F, $$

where $r = \log(\Lambda_0)$ is the growth rate (or Malthusian parameter), with $\Lambda_0$ is the dominant eigenvalue of $A$, whereas $H$ and $F$ are the entropy and the reproduction potential, defined as:
\[ H = - \sum_{i, \ell} \pi_i p_{i\ell} \ln p_{i\ell} \]

\[ F = \sum_{i, \ell} \pi_i p_{i\ell} \ln a_{i\ell} \]  \hspace{1cm} (4)

where \( p_{i\ell} \) is defined as:

\[ p_{i\ell} = \frac{a_{i\ell} V_{i\ell}}{\Lambda_0 V_i} \]  \hspace{1cm} (5)

with \( V A = \Lambda_0 V \), and \( \pi \) is the stationary distribution associated to \( \mathcal{P} = (p_{ij}) \): \( \pi_i = \mathcal{V}_i \mathcal{U}_i \), with \( \mathcal{U} \) given by \( \mathcal{A} \mathcal{U} = \Lambda_0 \mathcal{U} \). Let \( S = \{1, 2, \ldots, n\} \) be the finite set of states of our system. A simple infinite system is defined by a sequence consisting of assigning \( \phi_i \in \{1, \ldots, n\}: \ldots \phi_{-2} \phi_{-1} \phi_0 \phi_1 \phi_2 \ldots \), so that a configuration of the system is defined by a sequence such that \( \phi = \{\phi_i\} \in \prod_{n=-\infty}^{\infty} S \equiv \Phi \). The phase space is then defined by as \( \Sigma = \{\phi \in \Phi : a_{\phi_i, \phi_{i+1}} > 0\} \). Eqs. (3)-(5) are derived from a variational principle [2], namely

\[ r = \sup_{\nu \in M} \left( H_\nu(\tau) + \int \gamma d\nu \right) \]  \hspace{1cm} (6)

where \( M \) is the set of invariant measures under under the transformation \( \tau : \Sigma \to \Sigma \), defined by \( (\tau \phi)_i = \phi_{i+1} \), \( \phi \in \Sigma \) and \( i \) being a natural number, \( H(\alpha) = -\sum_{j=1}^{n} \nu(A_j) \log \nu(A_j) \) where \( \alpha = (A_j, j = 1, \ldots, n) \) is a partition of the phase space of the system. Given two partitions \( \alpha \) and \( \beta \) we can define a new partition \( \alpha \lor \beta \) whose sets are given by \( (A_i \cap B_j, A_i \in \alpha, B_j \in \beta) \) provided that a set is omitted if it is empty or of zero measure. Consider the shift \( \tau \) and let \( \alpha^* \) denote a new partition defined as \( \alpha^* = \alpha \lor \tau^{-1} \alpha \lor \tau^{-2} \alpha \lor \cdots \lor \tau^{-m} \alpha \). We now define the average information per step:

\[ H(\alpha, \tau) = \lim_{m \to \infty} \frac{1}{m} H(\alpha^*) \]

The entropy \( H_\nu(\tau) \) of the corresponding dynamical system \((\Sigma, \nu, \tau)\) is defined as:

}\]
\[ H_\nu(\tau) = \sup_{\alpha} H(\alpha, \tau) \]

Furthermore \( \gamma = \log a_{\phi_0\phi_1} \) where \( a_{x_0x_1} \) is the transition rates between two states of the system, \( \phi_0 \) and \( \phi_1 \). The phase space \( \Sigma \) is defined in the following way. The solution to this variational problem produces Eqs. (3)-(5) [2, 4].

3.1 **Demographic parameters of the incumbent and mutant populations**

This section is devoted to calculate the demographic parameters (essentially, the growth rate and the variance) for our two populations and their relation to the evolutionary entropy. We follow the work by Demetrius and co-workers [2, 8] with some significant modifications so that their formalism can be adapted to our case. Since the growth rate is given by Eq.3, in what remains of this section we calculate the variance.

Our analysis starts from the definition of the reproduction potential, \( F \):

\[ F(\gamma) = \int \gamma d\mu \]  

(7)

where \( \gamma = \log(a_{\phi_0\phi_1}) \) and \( \mu \) is a probability measure defined on the spaces of genealogies (see [2, 8] for full details). We define the space of all possible (phenotypic) genealogies \( \Sigma_n \) of length (number of generations) \( n \). An element of this space \( \sigma_n = (\phi_0, \phi_1, \ldots, \phi_n) \) with \( \phi_i = 1, \ldots, m + 1 \) describes a trajectory of successive phenotypes. Let \( \tau : \Sigma_{n-1} \rightarrow \Sigma_n \) the shift operator. Now consider the quantities [8]:

\[ S_n \gamma(\sigma_n) = \sum_{i=0}^{n-1} \gamma(\tau^i \sigma_n) = \sum_{i=0}^{n-1} \log(a_{\phi_i\phi_{i+1}}) \]

\[ Z_n \gamma = \sum_{\sigma_n} \exp(S_n \gamma(\sigma_n)) = \sum_{\sigma_n} a_{\phi_0\phi_1} a_{\phi_1\phi_2} \cdots a_{\phi_{n-1}\phi_n} \]

\[ r = \lim_{n \to \infty} \frac{1}{n} \log Z_n \gamma \]  

(8)
where the last of Eqs. (8) follows from the identity $Z_n \gamma = ||A^n||$. One can now define a probability measure $\mu(\sigma_n)$ defined on $\Sigma_n$:

$$
\mu(\sigma_n) = \frac{1}{Z_n \gamma} \exp(S_n \gamma(\sigma_n))
$$

In the thermodynamic limit, and with the population dynamics prescribed by the matrix $A$, the measure Eq. (9) is the corresponding Markov measure [2]:

$$
\mu(\sigma_n) = \mu(\phi_0, \phi_1, \ldots, \phi_{n-1}) = \pi_{\phi_0}p_{\phi_0\phi_1}p_{\phi_1\phi_2} \cdots p_{\phi_{n-1}\phi_{n-1}}
$$

With this definition we can explicitly calculate the quantity $F$ (see Eq. (7)):

$$
F_I = \langle S \gamma_I \rangle = \lim_{n \to \infty} \frac{1}{n} \sum_{\sigma_n} \mu_I(\sigma_n) S_I \gamma_I(\sigma_n)
$$

$$
= \lim_{n \to \infty} \frac{1}{n} \sum_{i=0}^{n-1} \sum_{\sigma_n} \mu_I(\sigma_n) \log(a_{\phi_i\phi_{i+1}}).
$$

Using Eq. (11), we obtain:

$$
F_I = \lim_{n \to \infty} \frac{1}{n} \sum_{i=0}^{n-1} \sum_{\sigma_n} \pi_{\phi_i}p_{\phi_i\phi_{i+1}} \cdots p_{\phi_{n-1}\phi_{n-1}} \log(a_{\phi_i\phi_{i+1}})
$$

$$
= \sum_{\phi_i\phi_j} \pi_{\phi_i}p_{\phi_i\phi_j} \log(a_{\phi_j\phi_i}).
$$

In order to study invasion of the incumbent population we need to calculate the variance associated with the reproduction potential for each species:

$$
\sigma^2 = \langle (S \gamma)^2 \rangle - \langle S \gamma \rangle^2.
$$

Where $\langle S \gamma^2 \rangle$ is given by:

$$
\langle S \gamma^2 \rangle = \lim_{n \to \infty} \frac{1}{n^2} \sum_{\sigma_n} \mu(\sigma_n)(S \gamma(\sigma_n))^2
$$
To calculate $\sigma^2$, i.e. the variance of the reproduction potential, we resort to one of the standard tricks in the Statistical Mechanics toolkit [5], namely, we introduce two new conjugate quantities, a field and the corresponding response variable. In the thermodynamics limit, the size of the fluctuations as measured by the aforementioned variance is then given by the second derivative of the corresponding thermodynamic potential, i.e. $n^{-1} \log(Z_n \gamma)$, which in the thermodynamic limit gives the growth rate $r$ (see Eq. 8), after the field tending is taken to zero.

In the present case, since we are interested in calculating the variance of the reproduction potential, $S_n \gamma$, we perform the perturbation: $\Gamma(h) = \gamma + h \varphi$, which corresponds to perturbing the matrix $A$ in the following manner: $A(h) = (a_{ij}(h)) = (a_{ij}b_{ij}^{(1)})$, where $h$ is a small perturbation.

From the last of Eqs. (8), we have:

$$r(h) = \lim_{n \to \infty} \frac{1}{n} \log(Z_n \Gamma(h)) \quad (15)$$

where $Z_n \Gamma(h)$ is defined by Eq. (8) with $\gamma = \Gamma(h)$. From Eqs. (8), (10) and (15), it follows that [8]:

$$r'(0) = \frac{d}{dh} \left( \lim_{n \to \infty} E_n S_n \varphi \right) = \int \varphi d\mu$$

$$r''(0) = \frac{d^2}{dh^2} \left( \lim_{n \to \infty} (E_n (S_n \varphi)^2 - (E_n S_n \varphi)^2) \right) = \sigma^2(\varphi) \quad (16)$$

If we now take $b_{ij} = a_{ij}$, we have that $A(h) = (a_{ij}^{1+h})$ and, consequently, $\varphi = \gamma$. Thus, $F(\gamma) = r'(0)$ and $\sigma^2(\gamma) = r''(0)$.

On the other hand, from the ergodic theorem proved in [2], we know that $r(h) = H(h) + \int \Gamma(h) d\mu(h) = H(h) + \int \gamma d\mu(h) + h \int \gamma d\mu(h)$. Therefore:

$$r'(0) = \left. \frac{dH(h)}{dh} \right|_{h=0} + \left. \frac{d}{dh} \int \gamma d\mu(h) \right|_{h=0} + \int \gamma d\mu \quad (17)$$

where $\mu \equiv \mu(h = 0)$. Comparing Eqs. (16) and (17), we obtain:

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1 In our case, the notion of the thermodynamic limit is equivalent to taking the limit $n \to \infty$. 

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11
\[ \frac{d^2}{dh^2} \int \gamma d\mu(h) \bigg|_{h=0} = - \left( \frac{dH(h)}{dh} \right) \bigg|_{h=0} \]  

(18)

Taking a second derivative with respect to \( h \) we obtain:

\[ r''(0) = \frac{d^2 H(h)}{dh^2} \bigg|_{h=0} + \frac{d^2}{dh^2} \int \gamma d\mu(h) \bigg|_{h=0} + \frac{d}{dh} \int \gamma d\mu(h) \bigg|_{h=0} \]  

(19)

Now, if \( h << 1 \), we can consider that all the quantities involved in the above equation are linear in \( h \). Under this approximation, the first two terms in Eq. (19) can be neglected. Finally, by using Eqs. (16) and (18), we obtain:

\[ \sigma^2 = - \left( \frac{dH(h)}{dh} \right) \bigg|_{h=0} \]  

(20)

Hence, in the linear approximation with \( H(h) \) given by \( H(h) = H + hH_h \), we have that \( \sigma^2(\gamma) = -H_h \), which is given by:

\[ H_h = - \sum_{i,j} \pi_i^{(1)} p_{ij}(1 + \log p_{ij}) + \pi_i p_{ij}^{(1)} \log p_{ij} \]  

(21)

where \( \pi_i(h) = \pi_i + h\pi_i^{(1)} \) and \( p_{ij}(h) = p_{ij} + h p_{ij}^{(1)} \). The details of how these quantities are calculated is given in Appendix A.

### 3.2 Diffusion approximation: Invasion probability

According to Demetrius et al. [8], the fixation the fixation or invasion probability as a function of the initial concentration of mutants, \( p \), can be obtained by means of a diffusion approximation where stochastic differential equations are obtained for the quantities \( N(t) = N_I(t) + N_M(t) \) and \( p(t) = N_M(t)/N(t) \), where \( N_I \) and \( N_M \) are the populations corresponding to the resident and mutant populations, respectively. These equations are given in terms of the growth rate and variance for the incumbent population, \( r_I \) and \( \sigma^2_I \), which correspond to number of phenotypes equal to \( d_I \) and \( \delta = 0 \), and the growth rate and variance for the mutant population, \( r_M \) and \( \sigma^2_M \), which correspond to number of phenotypes equal to \( d_M \) and \( \delta > 0 \). The parameters. Assuming that for \( t \to \infty \), \( N(t) \) settles down to an approximately constant value (which
Figure 2: Comparison between the fixation probability, $P_I(p)$, as predicted by the diffusion approximation with simulation results. Black circles and squares correspond to $\mu = 0.0001$ and $\delta = 0$ and $\delta = 0.498$, respectively. Blue diamonds and triangles correspond to $\mu = 0.001$ and $\delta = 0$ and $\delta = 0.498$. The red and green lines correspond to the theoretical prediction with $\delta = 0$ and $\delta = 0.498$, respectively.
is satisfied in our case), namely \( \langle N \rangle \), we can analyse the dynamics of \( p(t) \) in terms of the backward Kolmogorov equation, whose solution corresponds to the extinction probability of the resident population as a function of \( p(t = 0) = p \), i.e.:

\[
P_I(p) = \frac{1 - \left(1 - \frac{\Delta \sigma^2}{\sigma^2_M} p \right)^{\frac{2\langle N \rangle}{\Delta \sigma^2} + 1}}{1 - \left(1 - \frac{\Delta \sigma^2}{\sigma^2_M} \right)^{\frac{2\langle N \rangle}{\Delta \sigma^2} + 1}} \tag{22}
\]

where \( s \) is defined by:

\[
s = \Delta r - \frac{\Delta \sigma^2}{\langle N \rangle} \tag{23}
\]

where, \( \Delta r = r_M - r_I, \ \delta \sigma^2 = \sigma^2_M - \sigma^2_I, \ \langle N \rangle \) is the average stationary population. In the case of the branching process we consider in this paper, \( \langle N \rangle \) is given by \( 2e^{-\mu} = 1 \), i.e. \( \mu \langle N \rangle = \log 2 \).

We further define the invasibility, \( I \), as the area below the curve defined by \( y = P_I(x) \):

\[
I = \int_0^1 P_I(x) dx \tag{24}
\]

This quantity is such that \( 0 \leq I \leq 1 \) and allows us to formulate the following rule of thumb: If \( 0 \lesssim I < 0.5 \), invasion is very unlikely to happen. If, on the other hand, \( 0.5 < I \lesssim 1 \) then invasion almost certainly occurs. If \( I \approx 0.5 \), then we are in a nearly-neutral situation where invasion is a purely random event and the invasion probability is equal to the initial concentration of mutants. Note that the quantity \( I \) correlates with \( s \): If \( s > 0 \) then \( I > 0.5 \), if \( s < 0 \) then \( I < 0.5 \).

The accuracy of the diffusion approximation as a function of system size is evaluated in Fig. 2, by comparing Eq. (22) with computer simulations. As we can see from these results, the diffusion approximation performs poorly when the size is small (\( \mu = 0.001 \)) but as system size increases the approximation becomes more accurate (compare with the results for \( \mu = 0.0001 \)).
4 Results

Small system size increases fixation probability of slightly deleterious phenotypes. Since the diffusion approximation is only valid for very large systems, it is interesting to address the issue of how small systems behave. In this case, we use numerical simulations of the corresponding stochastic process of invasion. The corresponding results are shown in Figs. 2, 3 and 4, where results are given from simulations with $\mu = 0.001$, i.e. a total population of around 700 individuals.

From Fig. 2, we see that the simulation results in the slightly deleterious case is always bigger than the predicted by the diffusion approximation, i.e. finite size makes fixation of slightly deleterious mutants more likely. We can also observe that the smaller the system, the more likely is fixation to occur. Furthermore, when phenotypic robustness is increased, the fixation probability increases quite dramatically. For $\mu = 0.001$, our simulations predict that increasing phenotypic robustness makes fixation almost as likely as in the neutral case (see Figs. 3 and 4). Thus, the co-operation between small system size and increased phenotypic robustness can compensate for a small decrease in proliferation probability. This effect is less dramatic for slightly advantageous mutations (see Figs. 3 and 4).

These results regarding fixation of mutants with slightly deleterious phenotypes due to small-system size-induced fluctuations are consistent with a number of other results previously reported in the literature (see, for example, [23, 19, 20, 24]).

Robustness increases the invasibility of mutant population and prevents an error catastrophe. Fig. 5 shows results obtained within the diffusion approximation for the invasibility variable $I$, Eq. (24). In this case we have considered a resident population with $d_I = 10$ and have analysed the behaviour of the system as function of $d_M$ and $\delta$. Fig. 5(a) shows results corresponding to $\delta = 0$, i.e. no increase in phenotypic robustness with respect to the resident population. In the slightly deleterious scenario, this always leads to worse-than-neutral invasibility. However, as a function of $d_M$, we observe a non-monotonic behaviour: invasibility initially increases until a maximum is reached and then a decrease in invasibility ensues. This is
Figure 3: Numerical results for invasion simulations. Panel (a) shows the value of the dominant eigenvalue for the mutant population as a function of $\delta$ (i.e. phenotypic robustness). Red circles and black squares correspond to the slightly deleterious situation and the slightly fitter case, respectively. Panel (b) shows the relative increase the fixation probability: $R_I \equiv \frac{P_I(\delta=0.5) - P_I(\delta=0)}{P_I(\delta=0)}$. Red circles and black squares correspond to the slightly deleterious situation and the slightly fitter case, respectively. Panel (c) shows the fixation probability $P_I$ as a function of the initial concentration of mutants, $p$. Blue circles correspond to the slightly fitter scenario with $\delta = 0$, orange squares, to the neutral case, red diamonds, to the slightly deleterious case for $\delta = 0.5$, and, finally, the green triangles, to the slightly deleterious situation with $\delta = 0$. Other parameter values: $d_I = d_M = 3 \nu = 0.5$, $\mu = 0.001$, $\phi = 0.01$.\
Figure 4: Numerical results for invasion simulations. Panel (a) shows the value of the dominant eigenvalue for the mutant population as a function of $\delta$ (i.e. phenotypic robustness). Blue circles correspond to the slightly fitter scenario ($\phi = 0.01$ with 4 types), orange squares to the neutral case ($\phi = 0$ with 3 types), and green triangles to the slightly deleterious case ($\phi = -0.01$ with 4 types). The orange triangles and green squares correspond to the slightly fitter scenario and the slightly deleterious case, respectively, with $d_I = 3$ and $d_M = 4$. Panel (b) shows the fixation probability $P_I$ as a function of the initial concentration of mutants, $p$, with $d_I = 3$ and $d_M = 4$. Violet stars correspond to slightly fitter scenario with $\delta = 0.5$, blue circles to the slightly fitter scenario with $\delta = 0$, red diamonds, to the slightly deleterious case with $\delta = 0.5$ and green triangles to the slightly deleterious case with $\delta = 0$. Orange squares correspond to the neutral case. Other parameter values for both panels: $d_I = 3$, $d_M = 4$, $\nu = 0.5$, $\mu = 0.001$, $\delta = 0.5$. 
due to the fact that, for small $d_M$ the probability of some of the population belonging to slightly unfit phenotype is larger. As $d_M$ grows, the amount of the population with this phenotype decreases and, therefore, the chance for invasion improves. On the contrary, for the slightly fitter scenario, the invasibility decreases monotonically as $d_M$ increases. In particular, at some point, $I$ becomes smaller than $1/2$ and keeps on decreasing, which means that at this point the slight advantage in terms of proliferation is not enough to compensate for the increase in variance and the mutant looses its ability to invade the resident population. We call this transition an error catastrophe, as it resembles the one described by Eigen & Schuster within the context of the quasi-species model [9]. The term error catastrophe, although lacking a precise definition, is usually taken to mean that certain tolerances has been exceeded triggering a transition in the system [10]. In the original quasi-species model, there is a limiting value of error or mutation rate that must not be surpassed if the wild type is to be kept stable. In the present case, the limiting value of error refers to phenotypic variability: By increasing such quantity the mutant population looses its ability to take over the resident population. This is resemblant to the antiviral therapeutic approach whereby a mutagenic substance is administered that pushes the virus over the error threshold and stops the infection to take over [10].

A rather different scenario is revealed when phenotypic robustness is let to increase in the mutant population, as shown in Fig. 5.(b). In the slightly deleterious case we can observe that $I$ increases until it reaches quasi-neutrality, that is $I \simeq 0.5$. This is due to the fact that, since phenotypic robustness has increased, the population initially in the slightly unfit phenotype is unlikely to mutate into a different one and therefore will eventually become extinct. This, in turn, leads to an increase in population in the remaining phenotypes which have the same proliferation rate as in the resident population, leading to this quasi-neutral scenario. Regarding the slightly fitter case ($\phi > 0$), phenotypic robustness protects the mutant from the error catastrophe: $I > 0.5$ for all $d_M$. The mechanism in this case is that the initial population in the fitter phenotype remains in it and, due to its increased proliferation probability, eventually takes over the whole mutant population (Recall that the total population is resource limited so if the population in the phenotype with larger proliferation rate increases,
the population in all the other phenotypes must decrease).

By comparing Figs. 5.(a) and (b), we observe that, in general, phenotypic robustness increases the ability of the mutant to invade the resident population.

**ΔH is a predictor of the behaviour of the system.** We discuss here how the evolutionary formalism predicts the behaviour of the system. Looking at the results shown in Fig. 6 it is clear that $\Delta \sigma^2$ and $\Delta H$ are positively correlated, i.e. $\Delta \sigma^2 \Delta H > 0$. We also observe that $d(\Delta \sigma^2)/dd_M$ and $d(\Delta H)/dd_M$ are positively correlated (at least for large $d_M$) too: $\frac{d(\Delta \sigma^2)}{dd_M} \frac{d(\Delta H)}{dd_M} > 0$.

By comparing Figs. 5 and 6 we can deduce a number of properties that relate $\Delta H$ with the outcome of the competition between the resident population and the mutant population. The first of this property is that if $\Delta H(d_M)$ changes sign, i.e. if there exists a value of $d_M = d_0$ such that $\Delta H(d_0) = 0$, then $s$ will change sign too and therefore $I$ will cross through its neutral value $I = 0.5$. To see this compare the insets in Fig. 5 (a) and (b), which plots $s$ as a function of $d_M$ with the corresponding plots in Fig. 6. It also follows from that $\Delta \sigma \Delta H > 0$ together with the definition of $s$ Eq. (23): if $\Delta \sigma$ changes sign (and therefore so does $\Delta H$) $s$ must change sign. This implies that $\Delta H$ can be used to predict the existence of the error catastrophe as characterised earlier in this Section and shown in Fig. 5.(a) for the slightly fitter case.

The sign of $\frac{d(\Delta H)}{dd_M}$ determines whether a particular phenotype within the mutant population will take over. Fig. 6.(b), which corresponds to the slightly fitter, increased phenotypic robustness case reveals that $\frac{d(\Delta H)}{dd_M} < 0$. As we have discussed earlier, in this case the population accumulates in the phenotype with larger proliferation rate, this phenotype will therefore eventually takes over the population (see Fig. 5.(b), red line). All other cases considered in Figs. 5 are such that $\frac{d(\Delta H)}{dd_M} > 0$, which implies that the population tends to spread over the large numbers of phenotypes. The effect of this on the ability of the mutant to invade the resident population depends on the particular case considered: in the slightly fitter, non-increased phenotypic robustness case (Fig. 5.(a), red line) it leads to an error catastrophe. On the contrary, in the slightly deleterious and increased phenotypic robustness case, the ability of the mutant to is increased (Fig. 5.(b), red line).
Figure 5: Plot (a) shows the invasibility, $I$, as a function of $d_M$ corresponding to $\phi = 0.01$ (red dashed lines) and $\phi = -0.01$ (black solid line) with $\delta = 0$. Plot (b): The same as for panel (a) except now $\delta = 0.49$. Plot (c) shows the difference in the growth rate between the mutant and the incumbent population $\Delta r = r_M - r_I$ as a function of $d_M$ for $\delta = 0$. Plot (d): The same parameters except $\delta = 0.49$. The insets in plots (a) and (b) depict the corresponding dependency of the quantity $s$ on $d_M$. 

20
Figure 6: The above panels correspond to $\Delta \sigma^2$ (black line) and $\Delta H$ (red line) as a function of $d_M$ for $dI = 10$. Parameter values: $\mu = 0.0001$ and $\nu = 0.5$. Other parameter values ($\delta$ and $\phi$) as indicated on top of the corresponding pannels.
Note that all these phenomenology cannot be understood in terms of $\Delta r$, i.e. the differential the growth rate (Figs. 5.(c) and (d)), alone.

5 Discussion

In this paper we have presented both analytical and numerical results on the outcome of the competition between two populations as a function of the number of phenotypes and phenotypic robustness of the mutant population. This investigation is motivated by recent results regarding the role of diversity in progression to malignancy [21].

Our results are in good agreement with results reported by Maley and coworkers [21] on the Barret’s esophagus, in particular on predictors for the progression from the neoplasm state to a fully developed adenocarcinoma. The Barret’s esophagus is a premalignant condition that has been recognised as neoplasm, as it is generally clonal and hyperproliferative and carries a 30-fold increase risk of progression to cancer. Genetic instability occurs at high frequency and yet less than 5% of the cases progress to malignant cancer, with the Barret’s esophagus staying in the premalignant state for the whole of the patient’s life span in all the other cases. Maley et al. have found, by applying measures of diversity borrowed from evolutionary ecology, such as the Shannon entropy, that clonal diversity is a better predictor than the usual genetic markers (e.g. tumour-suppressor genes, oncogenes, genetic instability). Our results can be interpreted in terms of the results of Maley et al.: our results confirm that diversity improves the chances of the mutant population to invade the incumbent one, as long as phenotypic robustness is also increased.

We have shown that, in the particular case in which the population dynamics is represented by a multi-type branching process, fluctuations and a relative increase in phenotypic robustness both increase the likelihood of fixation of the mutant population. Our analysis is based on an application of the evolutionary formalism developed by Demetrius and co-workers [2, 7, 8] to the problem at hand. The use of this formalism allows us to characterise the outcome of the competition between the incumbent and mutant populations in terms of the entropy difference between the two populations, $\Delta H$.

We have shown that increased phenotypic robustness in a slightly
fitter mutant population protects it from a type of error catastrophe when the number of mutant phenotypes increases beyond some threshold (see Figs. 5.(a) and (b)). In the case of a slightly deleterious mutation, phenotypic robustness can compensate for smaller growth rate so that the likelihood of invasion of the mutant may become as large as for a neutral mutation (see Figs. 5.(a) and (b)).

The biological mechanisms giving rise to more diverse premalignant lesions remain largely unknown and, thus, this issue is beyond the scope of the present study. Our analysis on the role of diversity is based on the observation by Maley et al. [16] according to which phenotypic diversity appears to be increased in a number of Barrett’s esophagus cases which happen to be the more likely to evolve into malignant tumours.

The fact that fluctuations (Fig. 2, where we show that the fixation probability increases as $\mu$ increases) and phenotypic robustness (see Figs. 5 and 6 where it is shown that larger phenotypic robustness leads to bigger $I$) increases the ability of a mutant population to invade the resident population may have some importance in the evolutionary dynamics of cancer. The scenario put forward by the Fearon-Vogelstein diagram has been challenged by a number of authors (see [3] and references therein). Recently, results reported in [28] have revealed a scenario much more complex than the one proposed by Fearon & Vogelstein [11]. Wood et al. [28] have performed a genetic screening of numerous samples of breast and colorectal cancer to determine which mutations are present in those cancers and with which frequency. The structure of this landscape is much more complex than expected from the Fearon-Vogelstein model with genes as APC, KRAS and P53 (which are part of the Fearon-Vogelstein diagram) being mutated in many of the samples examined, but also numerous genes that are mutated with smaller frequency, thus suggesting a much more heterogeneous picture. In fact, comparison of two different samples reveals that the overlap between the set of genes mutated in both cases is very small [28]. Whereas historically cancer research has been focused on the most frequently mutated genes, it now appears that cancer evolution could actually be driven by the less frequently mutated genes each associated with a small change in fitness.

The observed sample-to-sample heterogeneity [28] suggests an important role for randomness and therefore for nearly neutral mutations.
In this context, our results on the effect of fluctuations and increased phenotypic robustness on the behaviour of slightly deleterious mutants may be particularly relevant as they provide a mechanism for some of the genetic variability observed in tumour samples, as they could help to fix mutant varieties that, in different circumstances, would be lost.

These results fit well within the framework put forward by Gatenby and co-workers [14, 26] where initial genetic modifications which do not necessarily entail an increase in growth rate are, at a later stage, exposed to an environment that favours the growth of these new variants. Furthermore, the wider variety of phenotypes produced by gene inactivation leads to a greater variability for this environmental changes to operate on.

To summarise, our results stress the importance of diversity, phenotypic robustness and fluctuations for the survival and invasion of a mutant population. Further exploration of issues such as how phenotypic robustness evolves as genetic and epigenetic modifications accumulate. To address this point, however, requires different methods of analysis where the dynamics of both the corresponding gene regulatory networks and the population dynamics are explicitly modelled [1, 15, 16]. We postponed this issue for future investigation.

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**A Linear theory**

In this appendix we provide, by means of non-degenerate perturbation theory of eigenvalue problems [25], explicit expressions for the parameters needed to calculate the variance of the proliferating potential as per Eq. (21).

The perturbed matrix $\mathbf{A}(h) = (a_{ij}^{(1+h)})$ is, up to first order in $\delta$, given by $\mathbf{A}(h) = (a_{ij}) + h(a_{ij} \log a_{ij}) = \mathbf{A} + h\mathbf{A}_h$. The quantities $\pi_i^{(1)}$
and \( p_{ij}^{(1)} \) are given in terms of the dominant eigenvalue of \( \mathcal{A}(h) \) and the corresponding right and left eigenvalues. We thus treat \( \mathcal{A}_h \) as a small (first order) perturbation to \( \mathcal{A} \) and solve the corresponding eigenvalue problem perturbatively up to first order.

Consider the eigenvalue problem:

\[
\sum_j a_{ij}(h)u_j(h) = \Lambda_0(h)u_i \\
\sum_i a_{ij}(h)v_i(h) = \Lambda_0(h)v_j
\]  \hspace{1cm} (25)

The first order correction to \( h = 0 \) corresponding to the right eigenvector is:

\[
\sum_j \left( a_{ij}u_j^{(1)} + a_{ij}^{(1)}u_i \right) = \Lambda_0 u_i^{(1)} + \Lambda_0^{(1)}u_j
\]  \hspace{1cm} (26)

where \( u_i(h) = u_i + hu_i^{(1)} \), \( \Lambda_0(h) = \Lambda_0 + h\Lambda_0^{(1)} \), and \( a_{ij}^{(1)} \equiv a_{ij} \log a_{ij} \), where the superscript denotes the first order correction in \( h \). \( \Lambda_0 \) and \( \mathbf{U} = (u_i)^T \) are the dominant eigenvalue and corresponding right eigenvector of \( \mathcal{A} \). Multiplying Eq. (26) by \( \mathbf{V} = (v_i) \), i.e. the left eigenvector of the unperturbed matrix, we obtain:

\[
\Lambda_0 \mathbf{V} \cdot \mathbf{U}^{(1)} + \sum_{ij} v_i a_{ij}^{(1)} u_j = \Lambda_0 \mathbf{V} \cdot \mathbf{U}^{(1)} + \Lambda_0^{(1)} \mathbf{V} \cdot \mathbf{U}
\]  \hspace{1cm} (27)

Since the first two terms on the right and left hand sides of Eq. (27) cancel and \( \mathbf{V} \cdot \mathbf{U} = \sum_i v_i u_i = \sum_i \pi_i = 1 \), we finally obtain the following expression for the linear correction to the dominant eigenvalue, \( \Lambda_0^{(1)} \):

\[
\Lambda_0^{(1)} = \sum_{ij} v_i a_{ij}^{(1)} u_j
\]  \hspace{1cm} (28)

The starting point to obtain first order correction to the right eigenvector is the eigenvalue equation Eq. (25), and the identity [25]:

\[
\sum_n \mathbf{V}(\Lambda_n) \mathbf{U}(\Lambda_n) = \mathbf{I}
\]  \hspace{1cm} (29)
where the summation runs over all the eigenvalues of the unperturbed matrix $\mathcal{A}$, and $\mathbf{I}$ is the identity matrix, which means that we can write the following identity:

$$
\sum_j a^{(1)}_{ij} u_j = \sum_{j,k} a^{(1)}_{kj} u_j \sum_n v_k(\Lambda_n) u_i(\Lambda_n) = \\
\sum_{n \neq 0} \sum_{j,k} a^{(1)}_{kj} u_j v_k(\Lambda_n) u_i(\Lambda_n) + \left( \sum_{j,k} a^{(1)}_{kj} u_j \right) u_i(30)
$$

According to Eq. (28), the term in parenthesis is equal to $\Lambda^{(1)}_0$, which means that Eq. (30) reads:

$$
\sum_j a^{(1)}_{ij} u_j - \Lambda^{(1)}_0 u_i = \sum_{n \neq 0} u_i(\Lambda_n) \sum_{j,k} a^{(1)}_{kj} u_j v_k(\Lambda_n) u_i(\Lambda_n) \tag{31}
$$

Using Eq. (26) the left hand side of Eq. (31) can be rewritten, leading to:

$$
\Lambda_0 u^{(1)}_i - \sum_j a_{ij} u^{(1)}_j = \sum_{n \neq 0} u_i(\Lambda_n) \sum_{j,k} a^{(1)}_{kj} u_j v_k(\Lambda_n) \tag{32}
$$

By multiplying Eq. (32) by $\mathcal{V}(\Lambda_m)$, and using that $\mathcal{V}(\Lambda_m) \cdot \mathcal{U}(\Lambda_n) = \delta_{m,n}$, we get:

$$
\mathcal{V}(\Lambda_m) \cdot \mathcal{U}^{(1)} = \frac{\sum_{j,k} a^{(1)}_{kj} u_j v_k(\Lambda_n)}{\Lambda_0 - \Lambda_m} \tag{33}
$$

and, therefore, finally we obtain that:

$$
\mathcal{U}^{(1)} = \sum_{n \neq 0} \frac{\sum_{j,k} a^{(1)}_{kj} u_j v_k(\Lambda_n)}{\Lambda_0 - \Lambda_n} \mathcal{V}(\Lambda_n) \tag{34}
$$

Similarly, for the first order correction to the left eigenvalue, $\mathcal{V}^{(1)}$, we obtain:

$$
Ohta\mathcal{V}^{(1)} = \sum_{n \neq 0} \frac{\sum_{j,k} a^{(1)}_{kj} v_k u_j(\Lambda_n)}{\Lambda_0 - \Lambda_n} \mathcal{U}(\Lambda_n) \tag{35}
$$
Eqs. (28), (34) and (35) allows us to calculate $\pi_i^{(1)}$ and $p_{ij}^{(1)}$, which can be obtained from their respective definitions (see Eq. (5) and below):

$$\pi_i^{(1)} = v_i u_i^{(1)} + v_i^{(1)} u_i$$

$$p_{ij}^{(1)} = \frac{1}{\Lambda_0 v_i} \left( a_{ji}^{(1)} v_i + a_{ji} v_i^{(1)} - \frac{\Lambda_0^{(1)} v_i + \Lambda_0 v_i^{(1)}}{\Lambda_0 v_i} a_{ji} v_j \right).$$ 

(36)

In the general case, and for our population dynamics model of choice (a multi-type branching process), these quantities cannot be obtained in closed, analytical form\(^2\). We have written a script in Matlab that allows us to calculate all the quantities of interest.

References


\(^2\)With the exception of the neutral case (i.e. all types proliferate at the same rate) where we can compute everything analytically


