Bulletin of Mathematical Biology (2006) 68: 1573–1599
DOI 10.1007/s11538-005-9046-8

ORIGINAL ARTICLE

Spatial Stochastic Models for Cancer Initiation and Progression

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Received: 24 March 2005 / Accepted: 15 June 2005 / Published online: 11 July 2006

Abstract The multistage carcinogenesis hypothesis has been formulated by a number of authors as a stochastic process. However, most previous models assumed "perfect mixing" in the population of cells, and included no information about spatial locations. In this work, we studied the role of spatial dynamics in carcinogenesis. We formulated a 1D spatial generalization of a constant population (Moran) birth–death process, and described the dynamics analytically. We found that in the spatial model, the probability of fixation of advantageous and disadvantageous mutants is lower, and the rate of generation of double-hit mutants (the so-called tunneling rate) is higher, compared to those for the space-free model. This means that the results previously obtained for space-free models give an underestimation for rates of cancer initiation in the case where the first event is the generation of a double-hit mutant, e.g. the inactivation of a tumor-suppressor gene.

Keywords Moran process · Mathematical model · Tumor suppressor gene · Multistage carcinogenesis

Abbreviations TSG: Tumor suppressor gene · APC: Adenomatous polyposis coli · ODE: Ordinary differential equation

1. Introduction

The multistage carcinogenesis hypothesis states that cancer proceeds as a sequence of mutations and waves of clonal expansion. This theory was first proposed by Nordling (1983) and by Armitage and Doll (1954), and further developed and refined by Day and Brown (1980), Brown and Chu (1983) and by Moolgavkar (1978). Further mathematical treatments of somatic mutation in multistage carcinogenesis have emphasized a Darwinian evolutionary perspective to the process

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(Greaves, 2002; Nowak et al. , 2002; Gatenby and Vincent, 2003; Michor et al., 2004a; Komarova and Wodarz, 2004; Frank, 2005). From the evolutionary point of view, there are different types of mutational events that have been described as milestones on the pathway to cancer (Vogelstein and Kinzler, 2004, 1997). Some mutations directly lead to the generation of *advantageous mutants*. This is characteristic of the "gain-of-function" mutations (see e.g. Strachan and Read, 1999) which activate oncogenes. The corresponding cells have an advantageous phenotype and have a tendency to expand. This scenario can be described by the following simple diagram:

$$A_{(1)} \xrightarrow{u} B_{(r)}. \tag{1}$$

Here, type "A" is the wild type, "B" is the one-hit mutant, and u is the rate of mutation. The fitness of type "B" is denoted by r and, in the case of oncogenes, it is larger than the fitness of the wild type (which is normalized to 1). Other types of mutations may not lead to a direct selection advantage but are nonetheless necessary milestones on the way to the next clonal expansion. An example of such a mutation can be an inactivating ("loss-of-function," Strachan and Read, 1999) mutation in a tumor suppressor gene (TSG) (Vogelstein and Kinzler, 2004). Upon the first hit, when one of the copies of the TSG is inactivated, the fitness of the cell is similar to that of wild-type cells, because there is still one functional copy of the TSG remaining. However, a second inactivating hit in such a cell will lead to a drastic increase in the fitness as the TSG is now completely turned off. The mutation diagram describing these molecular events is the following:

$$A_{(1)} \xrightarrow{u} B_{(r)} \xrightarrow{u_1} C_{(R)}. \tag{2}$$

Here, type "B" represents the phenotype with one copy of the TSG inactivated (and the fitness r equal to that of type "A"), and type "C" has no functioning copies of the TSG, and an increased fitness, $R > 1$. Processes described by diagram (2) can also be discussed in the context of the phenomenon of genetic instability (Lengauer et al., 1998). If the first mutation, "A"→"B," is an event by which a cell acquires an unstable, *mutator* phenotype (Loeb, 1991, 2001), then the fitness of type "B" (the unstable cells) could be equal to that of the wild type. Alternatively, it could be lower (Komarova et al., 2003; Komarova and Wodarz, 2004), because instability results in a higher chance to create non-viable offspring, and thus confers a disadvantage to the cell. However, the next event could happen at an accelerated rate, u_1, and leads to a fast generation of type "C" cells, which may have a selective advantage.

Inspired by Knudson's two-hit hypothesis (Knudson, 1971), Moolgavkar (1978) has created a mathematical two-hit model which describes the probability of accumulation of two mutations in a population of cells. Initially the theory was limited to an exponentially growing (on average) population, and later it was modified to include other laws of overall population growth (Heidenreich et al., 1997), and extended to multi-hit scenarios (Luebeck and Moolgavkar, 2002). For two hits (of which the second one is highly advantageous) some interesting analytical results

on the hazard function have been obtained (Dewanji et al., 1991). In application to colon cancer, the following question has been asked: what is the rate of inactivation of the tumor suppressor gene, APC (Komarova et al., 2003)? It was found that if the mutation rate is small compared with the inverse of the population size, the description of a two-hit model can be simplified significantly by looking only at "long-lived," homogeneous states. Other stochastic models have been proposed, including Little and Wright (2003), Nowak et al. (2004), Tan (1991), and Tan and Chen (1998). Models which distinguish between stem cells and differentiated daughter cells have also been analyzed (Yatabe et al., 2001; Michor et al., 2004b; Komarova and Wang, 2004).

All the models described above assume "perfect mixing" in the population of cells. There is no information about spatial locations, and no spatial dynamics. This may not be a shortcoming if we talk about liquid tumors (like leukemia). However in a discussion of solid tumors spatial considerations must play a role. On the other hand, many spatial mechanistic models of cancer spread have been proposed (e.g. see the reviews Araujo and McElwain, 2004; Bellomo and Preziosi, 2000; Chaplain, 1996), but typically these models do not take account of the evolutionary nature of cancer growth. Evolutionary dynamics of a spatial tumor is what we study in this paper.

As an attempt to include spatial considerations in the stochastic dynamics of selection and mutations, we have designed a one-dimensional spatial generalization of the space-free Moran birth–death process. In this spatial model, the total number of cells in the organ is kept at a constant level, and a continuous-time discrete state-space birth–death process describes proliferation, death and mutations of cells. In the present study we ask two specific questions: (i) For a system where cells of type "A" can mutate into type "B," diagram (1), what is the rate of fixation of type "B" depending on the relative fitness of this type and the total number of cells in the constant population? (ii) What is the rate of generation of double-hit mutants, "C," depending on the fitness of type "B," mutation rates and the total number of cells (diagram (2))? For each question, we consider a space-free model as well as a one-dimensional stochastic spatial model, and compare the results. We obtain the following insights.

(i) The fixation of a one-hit mutant (type "B," diagram (1)) will happen faster in a space-free model, if the mutant is either disadvantageous or advantageous. In the case of a neutral mutant, the fixation rate is the same with and without spatial effects (except for a thin "boundary layer," where a mutant has a lower chance to reach fixation).
(ii) The result for a two-hit model, diagram (2), is different. It turns out that for certain important regimes, the generation of a double-hit mutants happens *faster* in the spatial model compared to the space-free model.

The second result implies that the results previously obtained for space-free models give an underestimation for rates of cancer initiation in the case where the first event is the generation of a double-hit mutant, e.g. the inactivation of a tumor-suppressor gene.

This paper is organized as follows. In Section 2 we review our results for a space-free model and come up with a new method of calculations which can be extended to the spatial model. In Section 3 we formulate the spatial model and investigate its dynamics. We calculate the rate of fixation for one-hit (diagram (1)) and two-hit (diagram (2)) mutants. For processes of type (2) we consider the cases where the intermediate type (type "B") is neutral or disadvantageous. We derive exact applicability conditions for our method, as well as a rigorous definition of neutrality. Section 4 is reserved for conclusions and discussion.

2. Stochastic non-spatial models

We assume the following process. Each of the N cells in a well-mixed population belongs to one of the three types, "A," "B" or "C." The cells can divide (possibly with mutations) and die. The rate of cellular division is governed by their relative fitness. We take the fitness of type "A" to be 1 (this sets the time-scale of the process). Relative to this, the fitness of type "B" is r and the fitness of type "C" is R. We assume that $R \gg 1$, and r can be smaller, equal to, or greater than 1. The mutation network is given by diagram (2). Note that we do not allow back mutations. Each time a cell dies, it is immediately replaced by means of cell division, so that the population is kept constant (this is a Moran process, see Komarova et al., 2003). Cells are chosen for death at random. Reproduction happens proportional to the cell's fitness. The Moran process is illustrated in Fig. 1.

We say that the system is in the state j, $0 \leq j \leq N$ if (1) the number of cells of type "B" is equal to j and (2) there are no cells of type "C" present. In order to account for the production of double-mutants (cells of type "C"), we add state $j = E$ to the system. $j = E$ means that there is at least one cell of type "C" in the population. We assume that this state is absorbing, that is, once one mutant of type "C" is produced, it will take over the population (this is equivalent to the assumption $R \gg 1$).

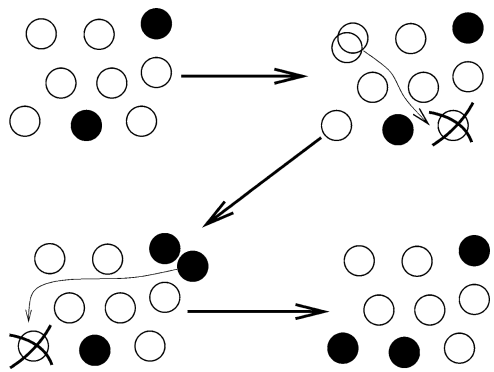

Fig. 1 The Moran process: a cell is chosen for death at random, and is immediately replaced by division of another cell. Cells for division are selected proportional to their fitness. There is no notion of space, or distance between cells. In the diagram, white and black circles represent two different cellular types, say types "A" and "B."

During a short time interval, Δt, the following processes can happen:

- Transition $j \to j+1$ with probability $\Delta t\, P_{j\to j+1}$, where

$$P_{j\to j+1} = \frac{N-j}{N}\frac{rj(1-u_1)}{N-j+rj} + \frac{N-j}{N}\frac{(N-j)u}{N-j+rj}, \quad 0 \le j \le N-1. \tag{3}$$

  Here, the first term is the probability for a cell of type "A" to be chosen for death times the probability for a cell of type "B" to be chosen for reproduction and reproduce faithfully. The second term is the probability of death of a type "A" cell times the probability for a cell of type "A" to reproduce with a mutation.
- Transition $j \to j-1$ with probability $\Delta t\, P_{j\to j-1}$, where

$$P_{j\to j-1} = \frac{j}{N}\frac{(N-j)(1-u)}{N-j+rj}, \quad 1 \le j \le N. \tag{4}$$

  Here, we multiply the probability of a cell of type "B" to die by the probability of a cell of type "A" to reproduce without a mutation.
- Transition $j \to E$ with probability $\Delta t\, P_{j\to E}$, where

$$P_{j\to E} = \frac{rju_1}{N-j+rj}, \quad 0 \le j \le N, \tag{5}$$

  which is just the probability for a cell of type "B" to be chosen for reproduction and to reproduce with a mutation. It does not matter which cell type is chosen for death in this case. We also have
- $E \to E$ with probability 1 and $j \to j$ for $j \neq E$ with probability $1 - \Delta t(P_{j\to j+1} + P_{j\to j-1} + P_{j\to E})$.

All other transitions have zero probability.

2.1. Invasion probability in a two-species model

We start our analysis with a review of simple but important results on the behavior of a two-species model.

Let us first consider a simple system where all mutations are suppressed ($u = u_1 = 0$). We start from one cell of type "B" ($j = 1$). The states reachable from $j = 1$ are $j \in \{0, 1, \ldots, N\}$, and there are two absorbing states: $j = 0$ (extinction of type "B") and $j = N$ (fixation of type "B"). The first question we address is the following: what is the probability that starting from one mutant of type "B," the system will get absorbed in state $j = N$? This is an important question as it gives the probability of mutant *invasion* starting from low numbers. It can be calculated by standard methods, see Komarova et al. (2003). Let us suppose that π_i is the probability to get absorbed in state N starting from state i. We have

$$\pi_i = P_{i\to i+1}\pi_{i+1} + P_{i\to i-1}\pi_{i-1} + (1 - P_{i\to i+1} - P_{i\to i-1})\pi_i, \quad 0 \le i \le N,$$

which simplifies to

$$r\pi_{i+1} + \pi_{i-1} - \pi_i(r+1) = 0,$$

with boundary conditions

$$\pi_0 = 0, \quad \pi_N = 1.$$

The solution is given by

$$\pi_i = \frac{1 - 1/r^i}{1 - 1/r^N}, \tag{6}$$

and in particular,

$$\pi_1 \equiv \rho(r) = \frac{1 - 1/r}{1 - 1/r^N}. \tag{7}$$

We have $\rho(r) = 1/N$ for $r = 1$, which can also be shown from simple symmetry considerations.

Next, let us include the possibility of mutations from "A" to "B," that is, $u > 0$; the second type of mutations is still suppressed ($u_1 = 0$, see diagram (1)). There is only one absorbing state in this system, $j = N$. The probability to be absorbed by time t can be approximated by

$$1 - \mathrm{e}^{-Nu\rho t}, \tag{8}$$

where we scaled the time with N, that is, effectively we measure time in terms of *generations* of type "A" cells (this is equivalent to introducing a new time variable $\tilde{t} = Nt$; in what follows the tildes are omitted). The average number of new mutations arising in a unit time is given by Nu. Out of these, the fraction ρ, Eq. (7), will proceed to fixation. Formula (8) holds if the mutation rate is sufficiently small. The precise condition for this was derived in Komarova et al. (2003). For neutral mutants, such that $|1 - r| \ll 1/N$, approximation (8) holds if

$$uN \ll 1, \tag{9}$$

and for disadvantageous mutants, such that $r < 1$ and $|1 - r| \gg 1/N$, the condition is weaker,

$$uN \ll r^{-(N-1)}. \tag{10}$$

(Condition (9) is a sufficient condition for both cases; condition (9) follows from condition (10) for r close to one). These conditions mean that if mutations are rare, then the production of new mutants can be considered independent of the dynamics of the existing mutants. The characteristic time for a mutant to go extinct

is a lot smaller than a characteristic time of mutant production. Therefore, the rate of fixation is a composite of the production of mutations, Nu, and the probability for each mutant to reach fixation, ρ.

2.2. A three-species model

Let us now consider a positive mutation rate $u_1 > 0$, and investigate the process of fixation in the state E, which is the only absorbing state of system (2). It is possible to show that if condition (10) holds, then the dynamics can be characterized by only a small number of "long-lived," homogeneous states. Let us denote the probability to find the system in state $j = 0$ by x_0, the probability to find it in state $j = N$ by x_1 and the probability to find it in state $j = E$ by x_2. Here, x_0, x_1 and x_2 correspond to the all "A", all "B" and all "C" states respectively, and the subscripts refer to the number of mutations (0, 1 or 2) that each type of cells has. Under assumption (10) we have $x_0 + x_1 + x_2 = 1 - O(1/N)$. The dynamics can be characterized by hopping between these states, as in the following system:

$$\dot{x}_0 = -R_{0\to 1}x_0 - R_{0\to 2}x_0, \tag{11}$$

$$\dot{x}_1 = R_{0\to 1}x_0 - R_{1\to 2}x_1, \tag{12}$$

$$\dot{x}_2 = R_{1\to 2}x_1 + R_{0\to 2}x_0, \tag{13}$$

with the initial condition

$$x_0(0) = 1, \quad x_1(0) = x_2(0) = 0.$$

Note that here and in what follows we use the *generation* time-scale; that is, we scale time with N, the total number of cells, so that we have on average N cell-divisions per time-unit. In system (11)–(13) we have introduced the three rate constants, $R_{0\to 1}$, $R_{1\to 2}$ and $R_{0\to 2}$. The first two rates are found very easily. Indeed, the rate $R_{0\to 1}$ is given by

$$R_{0\to 1} = Nu\rho, \tag{14}$$

formula (8). This rate reflects the production of mutants followed by their successful fixation. The rate $R_{1\to 2}$ is obtained similarly, except the probability of fixation of mutants of type "C" is considered to be equal to 1, yielding

$$R_{1\to 2} = Nu_1. \tag{15}$$

The processes reflected in constants $R_{0\to 1}$ and $R_{1\to 2}$ are shown in Fig. 2a. First the system gets into the all "B" state, after which another mutation brings it to the all "C" state. We will call this scenario a *two-step process*.

The third constant appearing in system (11)–(13), $R_{0\to 2}$, has the meaning of *tunneling*: the system reaches the all "C" state without first pausing at the all "B" state, see Fig. 2b.

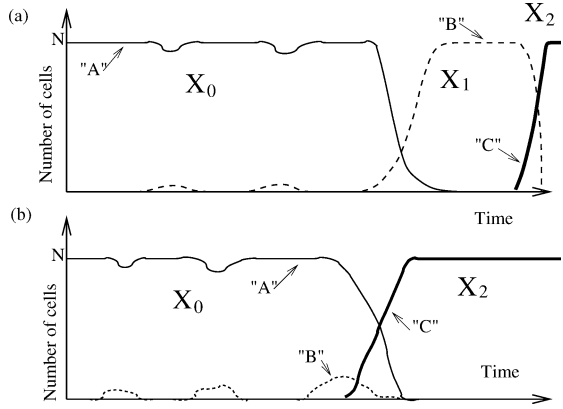

Fig. 2 Typical dynamics of cells. The thin solid line corresponds to the number of cells of type "A," the dashed line is type "B" and the thick solid line is type "C." (a) A two-step process, where fixation of type "B" is followed by fixation of type "C." (b) Tunneling, where fixation of type "B" does not occur.

2.3. The approximation of a doubly-stochastic process

In order to calculate the rate at which tunneling occurs, we will use the approximation of a doubly-stochastic process. Starting with all cells in state "A," let us denote the probability to have a double mutant at time t as $P_2(t)$. We will view the stochastic process as a sequence of subprocesses, each of which is a mutation generating one cell of type "B," and its subsequent evolution. Each such subprocess describes the lineage starting with a single mutant of type "B." We will assume that these lineages are independent, that is, the total number of mutants of type "B" is small compared to N. This assumption holds most of the time unless a mutant of type "B" reaches fixation, in which case we have a two-step process with rates $R_{0\to 1}$ and $R_{1\to 2}$. The exact condition for tunneling to be important is given in Section 2.5. If $P_1(t)$ is the probability to obtain a double mutant by time t within a lineage starting from 1 cell of type "B," then we have, under the assumption of independence of different lineages,

$$P_2(t) = 1 - \exp\left[-uN\int_0^t P_1(t')\mathrm{d}t'\right] \tag{16}$$

(see e.g. Parzen, 1999 for a derivation). The probability $P_1(t)$ was calculated by Komarova et al. (2003) and then by Iwasa et al. (2004). The function $P_1(t)$ is a monotonically increasing function of time, which starts off as linear and then reaches a saturation level, P_1^∞. It turns out that it is the saturation level that gives the main contribution to the integral in (16), see Section 2.5. Here we develop a concise and instructive method for calculation of P_1^∞, which we will later use for the spatial model.

For the number of cells of type "B" we have a one-dimensional Markov process. The state $j = 0$ corresponds to extinction, which is an absorbing state. An

additional value $j = E$ is included in the state space to indicate that a cell of type "C" has been produced. We will assume that this is also an absorbing state. The transition probabilities are given by formulas (3), (4) and (5). The main feature of tunneling is that fixation of the type "B" does not occur. In fact, for most realizations, the number of cells of type "B" is small compared to N. Using this fact, the expressions for transition rates can be simplified to yield,

$$P_{j\to j+1} = \lambda j, \quad P_{j\to j-1} = \mu j, \quad P_{j\to E} = \beta j. \tag{17}$$

These transition coefficients are obtained by taking the highest-order term in the expansion of coefficients (3), (4) and (5) in terms of the small parameter j/N:

$$\lambda = r(1-u_1), \quad \mu = 1, \quad \beta = u_1 r.$$

Note that an extra term N contained in the denominator in formulas (3), (4) and (5) is cancelled by using the generation time-scale. For generality purposes we will carry out all the calculations for general constant values of λ, μ and β.

Let us denote by $\xi_i(t)\Delta t$ the probability to jump to state E during the time interval $(t, t+\Delta t)$, given that at time 0 we are in state i. This function satisfies the following equation:

$$\xi_i(t+\Delta t) = \lambda i\,\Delta t\xi_{i+1}(t) + \mu i\,\Delta t\xi_{i-1}(t) + \beta i\,\Delta t\xi_E(t) + (1 - i\,\Delta t(\lambda+\mu+\beta))\xi_i(t),$$

which is equivalent to the ODE,

$$\dot{\xi}_i = \lambda i\xi_{i+1} + \mu i\xi_{i-1} + \beta i\xi_E - i(\lambda+\mu+\beta)\xi_i, \quad 2 \le i.$$

The boundary condition is given by

$$\dot{\xi}_1 = \lambda\xi_2 + \mu\xi_0 + \beta\xi_E - (\lambda+\mu+\beta)\xi_1,$$

where obviously $\xi_0(t) = 0$ and $\xi_E(t) = 0$. Let us take the Laplace transform of the ODE, where

$$\mathcal{L}\xi_i(t) = f_i(s).$$

Using $\mathcal{L}\delta(t) = 1$ and $\mathcal{L}\dot{\xi}_i = sf_i - \xi_i(0)$, we obtain the following system of equations,

$$i\,[\lambda f_{i+1} + \mu f_{i-1} - (\lambda+\mu+\beta)\,f_i] - f_i s = -i\beta, \quad i > 1, \tag{18}$$

$$[\lambda f_2 - (\lambda+\mu+\beta)\,f_1] - f_1 s = -\beta. \tag{19}$$

2.4. Mutation probability in each lineage

In order to obtain $P_1(t)$, the mutation probability in each lineage, we would need to find $f_1(s)$ (we start from one cell of type "B"), and evaluate $\mathcal{L}^{-1}[f_1(s)/s]$. Indeed, the cumulative probability $P_1(t)$ is related to the function $\xi_1(t)$ as $\xi_1 = \frac{\mathrm{d}P_1}{\mathrm{d}t}$. Therefore their Laplace transforms differ by a factor s. However, it is the value

$P_1^\infty = \lim_{t\to\infty} P_1(t)$ that gives the main contribution to probability (16)). This value is given simply by $P_1^\infty = f_1(0)$. We can solve system (18)–(19) analytically in the special case where $s = 0$. We have,

$$\lambda f_{i+1} + \mu f_{i-1} - (\lambda + \mu + \beta) f_i = -\beta, \quad i > 1, \tag{20}$$

$$[\lambda f_2 - (\lambda + \mu + \beta) f_1] = -\beta. \tag{21}$$

The general solution is given by

$$f_i = 1 + A\alpha_-^i + B\alpha_+^i,$$

where the numbers $\alpha_\pm$ are the roots of the quadratic equation, $\lambda\alpha^2 - (\lambda + \mu + \beta)\alpha + \mu = 0$. We can see that $\alpha_+ > 1$ and $0 < \alpha_- < 1$. Using the boundary condition for f_1 and boundedness of f_i, we obtain $A = -1$ and $B = 0$, such that $f_i = 1 - \alpha_-^i$, and

$$f_1(0) = 1 - \frac{1}{2\lambda}(\lambda + \mu + \beta - \sqrt{(\lambda + \mu + \beta)^2 - 4\lambda\mu}).$$

We have three cases:

1. The mutant "B" is negatively selected, that is, $\mu > \lambda$ and $\beta(\lambda + \mu) \ll (\lambda - \mu)^2$. In this case, $\alpha_- = 1 - \frac{\beta}{\mu - \lambda}$ and $f_1(0) =$

$$P_1^\infty = \frac{\beta}{\mu - \lambda} = \frac{r u_1}{1 - r}.$$

2. The mutant "B" is neutral, that is, $\beta(\lambda + \mu) \gg (\lambda - \mu)^2$. In this case we obtain $\alpha_- = \sqrt{\beta/\lambda}$, and the tunneling rate,

$$P_1^\infty = \sqrt{\beta/\lambda} = \sqrt{u_1}.$$

3. The mutant "B" is positively selected, that is, $\mu < \lambda$ and $\beta(\lambda + \mu) \ll (\lambda - \mu)^2$. In this case,

$$P_1^\infty = 1 - \frac{\mu}{\lambda} + \frac{\mu\beta}{\lambda(\lambda - \mu)} = 1 - \frac{1}{r} + \frac{u_1}{r - 1}.$$

It is noteworthy that one can obtain the same result for the tunneling rate (in cases 1 and 2 above) by solving system (20)–(21) in a continuous limit. Indeed, let us set $\mu = \lambda - \epsilon$. Equations (20)–(21) can be rewritten as

$$\lambda(-2 f_i + f_{i-1} + f_{i+1}) + \epsilon(f_i - f_{i-1}) - \beta f_i = -\beta, \tag{22}$$

$$\lambda(f_2 - f_1) + (\beta + \epsilon - \lambda) f_1 = -\beta, \tag{23}$$

which in continuous limit reads

$$\lambda f'' + \epsilon f' - \beta f = -\beta, \tag{24}$$

$$\lambda f'(0) + (\beta + \epsilon - \lambda) f(0) = -\beta, \tag{25}$$

where $f(x)$ is a continuous equivalent of f_i. This system can be solved exactly, by setting

$$f(x) = 1 + A\mathrm{e}^{-\gamma x},$$

where $\gamma = 1/2[(\lambda - \mu) + \sqrt{(\lambda - \mu)^2 + 4\lambda\beta}]$. From the initial condition we have

$$A = \frac{2(2\beta - \mu)}{\lambda + \mu - 2\beta + \sqrt{(\lambda - \mu)^2 + 4\beta\lambda}}.$$

The value $f(0)$ gives the estimate for P_1^∞. In the limit where $\lambda < \mu$ and $\beta(\lambda + \mu) \ll (\lambda - \mu)^2$ we have

$$P_1^\infty = \frac{\beta}{\mu - \lambda}, \tag{26}$$

if $\beta(\lambda + \mu) \gg (\lambda - \mu)^2$ we have

$$P_1^\infty = \sqrt{\beta/\lambda}, \tag{27}$$

and finally for $\lambda > \mu$ and $\beta(\lambda + \mu) \ll (\lambda - \mu)^2$ we have

$$P_1^\infty = 1 - \frac{\mu}{\lambda} + \frac{\lambda^2 + (\lambda - \mu)^2}{\lambda^2(\lambda - \mu)}\beta. \tag{28}$$

2.5. The tunneling rate in the space-free model

In order to estimate expression (16), we need to know the time-scale of change of the function $P_1(t)$. It approaches its saturation level around the time $t_c \sim \sqrt{\beta\lambda}$ in the case of neutral mutants and $t_c \sim \mu - \lambda$ in the case of disadvantageous mutants (see the derivation in Appendix A).

Let us focus our attention on time-scales comparable to the value $t_{1/2}$ such that $P_2(t_{1/2}) = 1/2$. We have $P_2(t) \sim 1/2$ and therefore

$$uN \int_0^t P_1(t')\mathrm{d}t' \sim \log 2.$$

In order for our approximation of the independence of lineages to hold, condition (9) or (10) must be satisfied, which means that for the time-scale of interest,

$\int_0^t P_1(t')\mathrm{d}t' \sim (uN)^{-1}$. Since $P_1(t) \leq P_1^\infty$ for all values of t, we have $\int_0^t P_1^\infty \,\mathrm{d}t' > 1$, or

$$t > \frac{1}{uNP_1^\infty}.$$

For neutral mutants this yields $t > \sqrt{\lambda/(uN\beta)}$, and for disadvantageous mutants we have $t > (\mu - \lambda)/(uN\beta)$. In both cases we can see that because $uN < 1$, we have for the time-scale of interest,

$$t \gg t_c.$$

Therefore the function $P_1(t)$ under the integral in Eq. (16) can be replaced by its saturated value:

$$P_2(t) = 1 - \mathrm{e}^{-uNP_1^\infty t}.$$

This means that the rate of tunneling, $R_{0\to 2}$ in Eqs. (11)–(13) is given by the following expressions:

- Disadvantageous mutants "B," that is, $\mu < \lambda$ and $\beta(\lambda + \mu) \ll (\lambda - \mu)^2$. In this case, we have

$$R_{0\to 2} = uN\sqrt{\beta}\mu - \lambda = \frac{uu_1 rN}{1-r}. \tag{29}$$

- Neutral mutants "B," that is, $\beta(\lambda + \mu) \gg (\lambda - \mu)^2$. We have

$$R_{0\to 2} = uN\sqrt{\beta/\lambda} = u\sqrt{u_1}N. \tag{30}$$

Tunneling is the dominant process if the inequality $R_{0\to 1} < R_{0\to 2}$ holds, Wodarz and Komarova (2005), which is equivalent to condition $\rho < P_1^\infty$. A simple calculation shows that this can be rewritten in the following way:

$$N > N_{\mathrm{tun}}, \tag{31}$$

where in the case of disadvantageous mutants,

$$N_{\mathrm{tun}} = \frac{\log u_1 + 2\log[r/(1-r)]}{\log r}, \tag{32}$$

and in the case of neutral mutants,

$$N_{\mathrm{tun}} = \frac{1}{\sqrt{u_1}}. \tag{33}$$

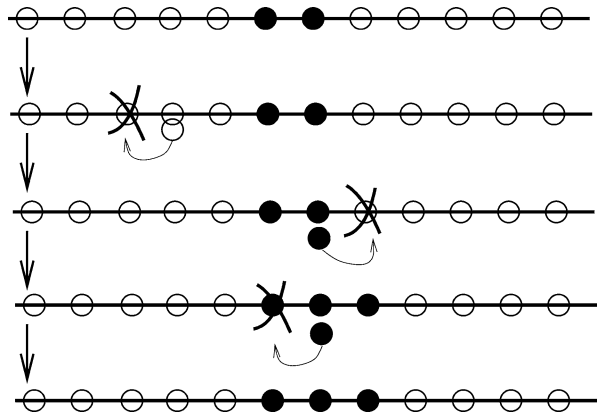

Fig. 3 The Moran process generalized to the one-dimensional space: a cell is chosen for death at random, and is immediately replaced by a division of one of the two neighboring cells (chosen proportional to their fitness).

3. The basic spatial model of cell division

Let us build a 1D model where cells are aligned along a regular grid, at locations $1, 2, \ldots, N$, see Fig. 3. We assume that the total number of cells does not change. Cells are randomly chosen for death. Each cell death is followed by a cell division of one of its two neighboring cells, which places its daughter cell at the empty slot. Cell death occurs randomly and division is proportional to the relative fitness of the cells.

3.1. A two-species model

Let us assume that there is a mutant cell with the relative fitness r at position j. The mutant cell produces mutant cells upon reproduction. Wild-type cells produce other wild type cells (the mutation rate is set to zero). If any cell at position $1, \ldots, j-2$ or $j+2, \ldots, N$ dies, then there can be no change in configuration. A change can occur only in two cases:

- Death occurs at position j, in which case the mutant disappears.
- Death occurs at position $j+1$ or $j-1$. Then the number of mutants can increase by one if the mutant cell is chosen for division.

Similarly, if we have several mutant cells at sequential positions from i through j, then a change of the number of mutants can only happen if death occurs at positions $i-1$, i, j or $j+1$ (see Fig. 3). In this model, a mutant colony which originated as one cell can only occupy adjacent slots (a joint set). A change in the position of this set can only be caused by cell death at its boundary.

We can characterize the states of the system by the positions of the leftmost and the rightmost mutant, i and j, such that

$$1 \le i \le j \le N. \tag{34}$$

The transition matrix is given by the following. If the left boundary of the mutant domain is at 1 (or the right boundary is at N), then these boundaries cannot move anymore. Otherwise, if the number of mutants is larger than one, then the probabilities to expand the mutant domain to left and right are given by $P_{i,j\to i-1,j} = P_{i,j\to i,j+1} = \frac{1}{N}\frac{r}{1+r}$, and the probabilities to reduce the domain on left and right are given by $P_{i,j\to i+1,j} = P_{i,j\to i,j-1} = \frac{1}{N}\frac{1}{1+r}$. Finally, if there is only one mutant (i.e., $i = j$), then we have $P_{i,i\to i-1,i} = P_{i,i\to i,i+1} = \frac{1}{N}\frac{r}{r+1}$, and the probability to lose the mutant is $\frac{1}{N}$. All the rest of the elements of the matrix are equal to zero.

We can envisage the dynamics as a two-dimensional Markov random walk inside domain (34), with an additional absorbing state which can be reached from the diagonal $i = j$; this state corresponds to the extinction of the mutant and is denoted by $\tilde{E}$. The other absorbing state is the fixation of the mutant, $(0, N)$. The random walk is governed by the matrix above. We can set i to be the horizontal and j the vertical coordinate of the position of the walker, and then the above probabilities can be referred as $P_{ij}^{\rightarrow}$, $P_{ij}^{\leftarrow}$, $P_{ij}^{\uparrow}$ and $P_{ij}^{\downarrow}$.

3.2. *Probability of mutant invasion*

We can calculate the probability of absorption in $(0, N)$ starting from a state (i, j), which we call u_{ij}. We have the following system of equations,

$$\begin{aligned} u_{ij} &= u_{i-1,j}P^{\leftarrow} + u_{i+1,j}P^{\rightarrow} + u_{i,j-1}P^{\downarrow} + u_{i,j+1}P^{\uparrow} \\ &\quad + u_{ij}[1 - (P^{\leftarrow} + P^{\rightarrow} + P^{\downarrow} + P^{\uparrow})], \qquad (35) \\ &\quad 1 < i < j < N, \qquad (36) \\ u_{1j} &= u_{1,j+1}P^{\uparrow} + u_{1,j-1}P^{\downarrow} + u_{1j}[1 - (P^{\uparrow} + P^{\downarrow})], \quad 1 < j < N \qquad (37) \\ u_{iN} &= u_{i-1,N}P^{\leftarrow} + u_{i+1,N}P^{\rightarrow} + u_{iN}[1 - (P^{\leftarrow} + P^{\rightarrow})], \quad 1 < i < N, \qquad (38) \\ u_{jj} &= u_{j-1,j}P^{\leftarrow} + u_{j,j+1}P^{\uparrow} + u_{jj}[1 - (P^{\leftarrow} + P^{\uparrow} + P^{\tilde{E}})], \quad 1 < j < N, \qquad (39) \\ u_{11} &= u_{12}P^{\uparrow} + u_{11}[1 - (P^{\uparrow} + P^{\tilde{E}})], \qquad (40) \\ u_{NN} &= u_{N-1,N}P^{\leftarrow} + u_{NN}[1 - (P^{\leftarrow} + P^{\tilde{E}})], \qquad (41) \\ u_{1N} &= 1. \qquad (42) \end{aligned}$$

The quantities u_{ii} are probabilities of invasion starting from one mutant at position i.

The results for this model must be compared with the probabilities of invasion in a space-free model, Eqs. (6) and (7). Numerical solutions for the probabilities of absorption show that quantities u_{ii} are symmetric one-hump (flat) functions which are smallest for $i = 1$ and $i = N$, where they are equal to $1/2\rho$, see Fig. 4. The inner values of this function are approximately constant, and they approach $1/N$ for neutral mutants. What is interesting is that if the mutant is either advantageous

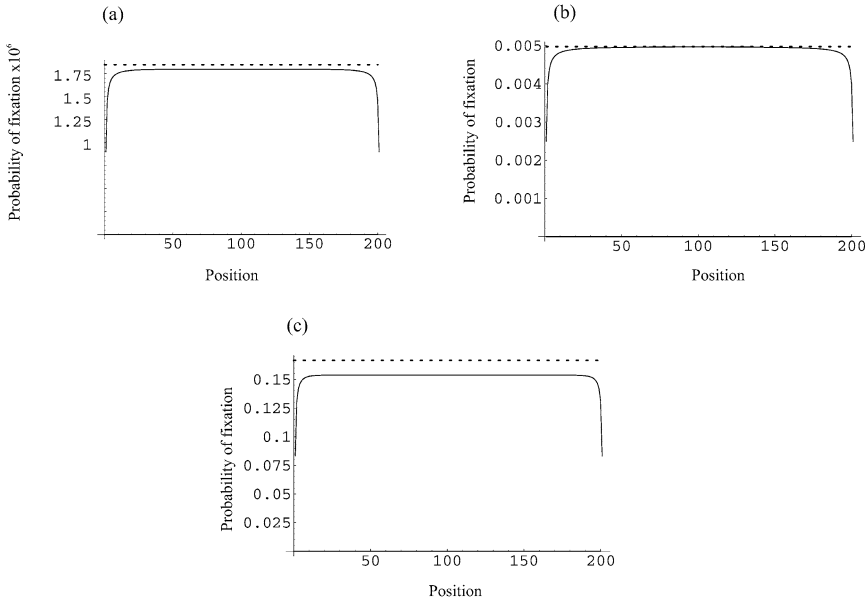

Fig. 4 Numerical solutions of system (35)–(42), where the probability of absorption u_{ii} is plotted as a function of the position of the mutant, i, in a system of $N = 200$ cells. (a) Disadvantageous mutants, $r = 0.95$; (b) neutral mutants, $r = 1$; (c) advantageous mutants, $r = 1.2$. The dotted horizontal line corresponds to the value $\rho(r)$, the probability of fixation for the space-free system.

or disadvantageous, then the inner values of u_{ii} are smaller than the space-free prediction, ρ.

It turns out that a slight change in Eqs. (39) and (41) can simplify the description and allow for an analytical solution. The idea is that if point (i, j) is sufficiently far away from the boundary, then the boundary effects are not felt and u_{ij} only depends on $|j - i|$ rather than on the initial position of the mutant interval. In order to solve the problem away from the boundary, we can use the following trick. We will replace Eqs. (39) and (41) with the following:

$$u_{11} = u_{12}P^{\uparrow} + u_{11}[1 - (P^{\uparrow} + P^{\tilde{E}}/2)], \tag{43}$$

$$u_{NN} = u_{N-1,N}P^{\leftarrow} + u_{NN}[1 - (P^{\leftarrow} + P^{\tilde{E}}/2)]. \tag{44}$$

We observe that with the new boundary conditions, (43) and (44), quantities u_{ij} do not depend on the position of the mutant interval, but only on its length. This is the consequence of the fact that the transition probability obey certain symmetries, such as

$$P^{\uparrow} = P^{\leftarrow}, \quad P^{\downarrow} = P^{\rightarrow}.$$

Let us denote by g_j the probability that the mutant will invade starting from a mutant interval of length $j + 1$. We have a self-consistent system of equations for

the probabilities g_i,

$$g_i(P^{\uparrow} + P^{\downarrow}) = P^{\uparrow} g_{i+1} + P^{\downarrow} g_{i-1}, \quad 0 < i < N-1, \tag{45}$$

$$g_0(P^{\uparrow} + P^{\bar{E}}/2) = P^{\uparrow} g_1, \tag{46}$$

$$g_{N-1} = 1. \tag{47}$$

This system can be solved by setting $g_i \propto \alpha^i$, and finding $\alpha = 1$ and $\alpha = P^{\downarrow}/P^{\uparrow} = 1/r$. Therefore, we have

$$g_i = A + B/r^i,$$

and the constants A and B are found from the boundary conditions,

$$A = \left(1 - \frac{r+1}{r^{N-1}(3r-1)}\right)^{-1}, \quad B = \frac{r^{N-1}(r+1)}{1+r+r^{N-1}-3r^N}.$$

The probability to invade starting from only one mutant cell is given by $g_0 = A + B$. We have,

$$g_0 = \frac{2r^{N-1}(1-r)}{1+r+r^{N-1}-3r^N}.$$

We can compare this quantity with the invasion probability, ρ, without spatial dynamics,

$$g_0 = \rho \, \frac{2r(1-r^{N-1})}{1+r+r^{N-1}-3r^N}.$$

In particular, for neutral mutants such that $|r-1| \ll 1/N$, we have $g_0 = \rho = 1/N$. For large values of N, we obtain, in the case of advantageous mutants ($r > 1$, $|r-1| \gg 1/N$),

$$g_0 = \frac{2r}{3r-1}\rho < \rho, \tag{48}$$

and in the case of disadvantageous mutants ($r < 1$, $|1-r| \gg 1/N$) we have

$$g_0 = \frac{2r}{1+r}\rho < \rho. \tag{49}$$

3.3. Three-species model

Next, let us formulate the dynamics for a three-species model, diagram (2), in a one-dimensional space. Again, we will use a homogeneous state approximation,

and describe the behavior of the system by means of Eqs. (11)–(13). The applicability conditions for this approximation are now somewhat more restrictive and they are derived in Section 3.5. The rate constants $R_{0\to1}$ and $R_{1\to2}$ can be calculated in the same way as for the non-spatial model. We have, instead of formula (14),

$$R_{0\to1} = Nug_0 = Nu\frac{2r^N(1-r)}{1+r+r^N-3r^{N+1}},$$

where g_0 is the probability of successful fixation of a mutant starting from one cell of type "B." Approximations for neutral and disadvantageous mutants are given by formulas (48) and (49). Similarly, we calculate the second rate in the two-step process, which is the same as in the space-free model, Eq. (15),

$$R_{1\to2} = Nu_1.$$

Finally, we need to find the tunneling rate, $R_{0\to2}$.

3.4. Doubly-stochastic approximation

In order to find the rate of tunneling, we use the doubly-stochastic approximation once more, formula (16). Each (independent) lineage of type "B" spreads as a one-dimensional spot. The size of the spot is given by the random variable i. The state $i = 0$ is equivalent to the state $\tilde{E}$ of Section 3.1, i.e. extinction of the mutant. The state $i = E$ corresponds to the creation of a mutant of type "C." The probability $P_1(t)$ in formula (16) is the probability to acquire a second mutation among the lineage of a single cell of type "B." For the dynamics within a lineage, the transition probabilities are given by

$$\begin{aligned}
P_{i\to i+1} &= \frac{r(1-u_1)}{(r+1)}, \quad 0 \le i \le N-1,\\
P_{i\to i-1} &= \frac{1}{(r+1)}, \quad 1 \le i \le N,\\
P_{1\to0} &= \frac{1}{2},\\
P_{N\to N} &= N(1-u_1),\\
P_{i\to E} &= \frac{4ru_1}{(r+1)} + (i-2)u_1, \quad 3 \le i \le N,\\
P_{2\to E} &= \frac{4ru_1}{(r+1)},\\
P_{1\to E} &= \frac{2ru_1}{(r+1)},
\end{aligned}$$

where time is measured in terms of generations. In what follows we will simplify the problem so that the transition probabilities are

$$P_{i\to i+1} = \lambda, \quad P_{i\to i-1} = \mu, \quad P_{i\to E} = \beta i. \tag{50}$$

These are spatial analogues of formulas (17). Note that in formulas (17), the coefficients λ, μ and β were obtained by taking the lowest order term in the Taylor series in terms of the small i/N. In formula (50), the probabilities represent a "model" of the real situation rather than an approximation. Indeed, in Eqs. (50), with

$$\lambda = r(1-u_1)/(r+1), \quad \mu = 1/(r+1), \quad \beta = 3ru_1/(r+1),$$

we neglect several subtleties that we discovered for spatial propagation of mutants. For instance, we ignore the fact that $P_{1\to 0} \neq P_{i\to i-1}$ for $i > 1$. We also ignore the fact that the probability for exiting into state E from state i is not exactly proportional to i: for $i < 3$ it does not depend on i, and for larger i it has a constant (in i) term.

We will use the same method as we developed for the space-free system, Sections 2.3 and 2.4. Let us denote by $\xi_i(t)\Delta t$ the probability to be absorbed in E during the interval $(t, t+\Delta t)$ starting from i at $t = 0$. We have the following equations for ξ_i:

$$\dot{\xi}_i = \lambda\xi_{i+1} + \mu\xi_{i-1} + \beta i\xi_E - (\lambda+\mu+\beta i)\xi_i, \quad 2 \le i,$$

with the boundary condition

$$\dot{\xi}_1 = \lambda\xi_2 + \mu\xi_0 + \beta\xi_E - (\lambda+\mu+\beta)\xi_1.$$

The Laplace transform yields the system,

$$[\lambda f_{i+1} + \mu f_{i-1} - (\lambda+\mu+s)f_i] - f_i\beta i = -i\beta, \quad i > 1, \tag{51}$$

$$[\lambda f_2 - (\lambda+\mu+s)f_1] - f_1\beta = -\beta. \tag{52}$$

This is similar to an inhomogeneous discrete Airy equation. Let us denote

$$h_i = f_i - 1,$$

and set $\mu = \lambda - \epsilon$. We have for the function h_i,

$$\lambda(h_{i+1} - 2h_i + h_{i-1}) + \epsilon(h_i - h_{i-1}) - sh_i = \beta i h_i, \tag{53}$$

$$\lambda(h_2 - h_1) - h_1(\lambda - \epsilon + \beta + s) = \lambda - \epsilon. \tag{54}$$

Using the continuous limit, we obtain the system

$$\lambda h'' + \epsilon h' - sh = \beta x h, \tag{55}$$

$$\lambda h'(0) - h(0)(\lambda - \epsilon + \beta + s) = \lambda - \epsilon; \tag{56}$$

for the second boundary condition we use the boundedness of the solution for large x. This system can be solved exactly in terms of the Airy function Ai and its derivative. Here we present the stationary solution corresponding to $s = 0$. We have

$$h(x) = \frac{2e^{-\epsilon x/(2\lambda)}(\epsilon - \lambda)Ai[K(x)]}{(2\beta - \epsilon + 2\lambda)Ai[K(0)] - 2\beta(\beta/\lambda)^{-2/3}Ai'[K(0)]},$$
$$K(x) = \frac{\epsilon^2 + 4\beta\lambda x}{4(\beta/\lambda)^{2/3}\lambda^2}.$$

Setting $x = 0$, we obtain

$$h(0) = \frac{2(\epsilon - \lambda)}{2\beta - \epsilon + 2\lambda - 2\beta(\beta/\lambda)^{-2/3}R\left(\left[\frac{\epsilon}{2\lambda(\beta/\lambda)^{1/3}}\right]^2\right)}, \quad R(z) = \frac{Ai'(z)}{Ai(z)}.$$

Depending on the fitness of type "B," this expression has a different limiting behavior. We have three cases:

- Type "B" is disadvantageous: $\epsilon < 0$ and $\epsilon \gg (\beta/\lambda)^{1/3}$. In this case, the argument of the function $R(z)$ tends to infinity and we can use the standard asymptotic expansion of the Airy function and its derivative for a large argument. We obtain $h(0) = -1 + \frac{(\lambda-\mu)^2+\lambda^2}{(\lambda-\mu)^2\mu}\beta$, or $f(0) = 1 + h(0) =$

$$P_1^\infty = \frac{(\lambda - \mu)^2 + \lambda^2}{(\lambda - \mu)^2\mu}\beta = 3ru_1\frac{(r-1)^2 + r^2}{(r-1)^2}. \tag{57}$$

  This rate has the same order of magnitude as the corresponding rate in the nonspatial calculation, Eq. (26).
- Type "B" is neutral, that is, $\epsilon \ll (\beta/\lambda)^{1/3}$. In this case, the argument of the function $R(z)$ tends to zero. We obtain

$$P_1\infty = \left(\frac{3\beta}{\lambda}\right)^{1/3}\frac{\Gamma(2/3)}{\Gamma(1/3)} = (9u_1)^{1/3}\frac{\Gamma(2/3)}{\Gamma(1/3)}. \tag{58}$$

  This rate is larger than the one found for the neutral mutant in the space-free model, see expression (27).
- Type "B" is advantageous: $\epsilon > 0$ and $\epsilon \gg (\beta/\lambda)^{1/3}$. We have

$$P_1^\infty = 1 - \frac{\mu}{\lambda} + \frac{\mu(\lambda^2 + (\lambda - \mu)^2)}{(\lambda - \mu)^2\lambda^2}\beta = 1 - \frac{1}{r} + 3ru_1\frac{r^2 + (1-r)^2}{(1-r)^2r^2},$$

  which is reminiscent of Eq. (28).

3.5. *The rate of tunneling in the spatial model*

Now we can calculate the tunneling rate for the cases where type "B" is neutral or disadvantageous. We will use the same method as in Section 2.5. Suppose t_c is the time where the function $P_1(t)$ comes close to its saturation value, P_1^∞. The values for t_c are found in Appendix B. Again, we will set the time-scale of the process such that the probability of acquiring a mutant of type "C" is about 1/2. This is equivalent to the estimate $t \sim 1/(P_1^\infty uN)$, see Eq. (16). The condition $t \gg t_c$ will guarantee that the tunneling rate is simply uNP_1^∞. For the case of disadvantageous mutants, this condition is identical to

$$uN \ll \frac{r-1}{r+1}. \tag{59}$$

In the case of neutral mutants, we obtain

$$uN \ll \left(\frac{u_1}{3}\right)^{1/3} \left(\frac{\Gamma(1/3)}{\Gamma(2/3)}\right)^2 \frac{r}{r+1}. \tag{60}$$

If these conditions of are satisfied, we get the following result for the two cases.

- Type "B" is disadvantageous: $r < 1$ and $\frac{1-r}{1+r} \gg (3u_1)^{1/3}$. If condition (59) holds, then the tunneling rate is given by

$$R_{0\to 2} = 3rNuu_1 \frac{(r-1)^2 + r^2}{(r-1)^2}. \tag{61}$$

- Type "B" is neutral, that is, $\frac{1-r}{1+r} \ll (3u_1)^{1/3}$. If condition (60) holds, then the tunneling rate is given by

$$R_{0\to 2} = uN(9u_1)^{1/3} \frac{\Gamma(2/3)}{\Gamma(1/3)}. \tag{62}$$

Note that the rate of tunneling in the case of disadvantageous mutants, (61), is always larger than that for the space-free model, Eq. (29). It has the same order of magnitude in terms of small u_1. Regarding the case of neutral mutants, it is interesting that the rate of tunneling in the spatial model, (62), has a *larger order of magnitude* than that in the space-free model, Eq. (30). In both cases, tunneling happens faster in the spatial model compared to the space-free model.

In Fig. 5 we present a numerical simulation where these effects are demonstrated. We ran a stochastic spatial and non-spatial models in the regime where tunneling played an important role (compared to a two-step process, see the conditions below). The mutant "B" was taken to be neutral ($r = 1$). The initial condition was the all "A" state, and the simulations were stopped as soon as the first mutant of type "C" was created. For each model, we performed 10, 000 runs and

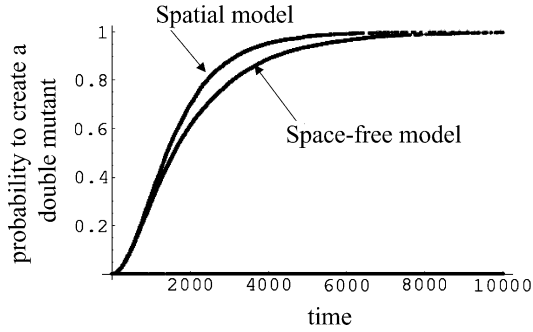

Fig. 5 Cumulative probability distribution function for the generation of a mutant of type "C," in spatial and non-spatial models (numerical results). Here, $r = 1$, $N = 100$, $u = 0.005$ and $u_1 = 0.02$. The simulation was performed for a discrete-time Markov process.

found out numerically the distribution of times of generation of a double-hit mutant. One can see that for the spatial model, the generation of a double-hit mutant occurs earlier, that is, at a higher rate, than that for the space-free model.

The next remark is on the definition on neutrality in different models. It is the same for spatial and space-free descriptions in the regime where a two-step process dominates (or if we have only two types, "A" and "B" in the system). In this case mutants with fitness satisfying $|1 - r| \ll 1/N$ can be considered neutral. Indeed, in the expansion of the fixation probability of a mutant in terms of r around the value $r = 1$, the highest-order term is given by $1/N$, and the next term is $(N - 1)(r - 1)/2N$ without spatial effects, and $(N - 1)^2(r - 1)/2N^2$ in the spatial model. The smallness of the second term compared to the first term is a criterion of neutrality.

The meaningful definition of neutrality changes in the regime where tunneling is important. There, it is not the time-scale of fixation, but rather the rate of tunneling which is the dominant factor. Now, the definition is different in the spatial model compared to that in the space-free model. In the latter case, neutral mutants were defined by the condition

$$|1 - r| \ll \sqrt{u_1}. \tag{63}$$

In the spatial case, we have

$$|1 - r| \ll (3u_1)^{1/3}. \tag{64}$$

That is, a larger region of fitnesses around $r = 1$ qualifies as neutral.

The relative importance of tunneling can be obtained by comparing the rates $R_{0\to1}$ and $R_{0\to2}$. As in the space-free model, condition (31) implies that typically, mutants of type "C" are generated before fixation of type "B" occurs. For disadvantageous mutants of type "B," N_{tun} is given by

$$N_{\text{tun}} = \log\left[\frac{3}{2}\,\frac{u_1 r(r+1)[(r-1)^2 + r^2]}{(1-r)^3}\right] / \log r. \tag{65}$$

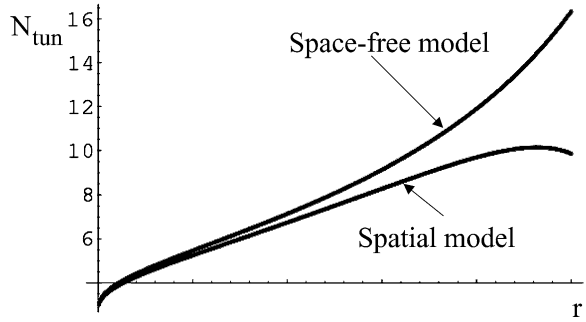

Fig. 6 The quantity N_{tun} for disadvantageous mutants, for the spatial and space-free models, calculated from formulas (65) and (32) respectively. For this graph, $u_1 = 10^{-3}$; the range of r is from zero to 0.75, so that the mutant is disadvantageous in the spatial model.

In Fig. 6 we present the comparison of N_{tun} for the space-free (see formula (32)) and spatial models, for a fixed value of $u - 1$, and different values of r. In the case of neutral intermediate mutants, we have

$$N_{\text{tun}} = \frac{\Gamma(1/3)}{\Gamma(2/3)} \frac{1}{(9u_1)^{1/3}}, \tag{66}$$

compare with formula (33) for the space-free model. We can see that N_{tun} is always smaller for the spatial model compared to that for the space-free model.

Conditions (59) and (60) define whether the homogeneous state approximation holds. They are a stronger version of the conditions (9) and (10) which were derived for the space-free model. There, in order to be able to approximate the system by three long-lived states, it was enough to require that the first mutation rate is small compared to $1/N$. Now, an additional factor comes in. The conditions for applicability of system (11)–(13), inequalities (59) and (60), can be written as one condition,

$$uN \ll \max\left\{\frac{1-r}{1+r}, (3u_1)^{1/3}\right\}. \tag{67}$$

If condition (67) is violated, then the tunneling in this case happens according to a different scenario, and system (11)–(13) does not apply. In particular, if the relevant time-scale is very small compared to the saturation time, t_c, then the function $P_1(t)$ can be approximated as a linear function of time, $P_1(t) \approx \gamma t$, and we would have

$$P_2(t) = 1 - \mathrm{e}^{-uN\gamma t^2/2},$$

see also Nowak et al. (2004).

4. Conclusions

We have studied the role of space in fixation of one-hit mutants and in generation of double-hit mutants, in a stochastic birth–death process with a constant population. A spatial (1D) generalization of the Moran process was defined and described analytically. We investigated the mutation-selection networks of types (1) and (2) and found the following results:

- Fixation probabilities of one-hit mutants are lower for disadvantageous (Eq. (49)) and advantageous (Eq. (48)) mutants. They are the same for neutral mutants.
- Generation of double-hit mutants can be described by using a homogeneous-state approximation (Eqs. (11)–(13)). In this case, the generation of type "C" occurs by means of a two-step process or by means of stochastic tunneling.
- For neutral intermediate mutants, the rate of generation of double-hit mutants is higher in the spatial setting. More precisely, the two-step process happens at the same rate, but the rate of tunneling scales as $u_1^{1/3}$ (Eq. (62)) as opposed as $u_1^{1/2}$ in the space-free model (Eq. (30)). Here u_1 is the second rate of mutation which generates type "C" from type "B."
- In the case of disadvantageous mutants of type "B," the two-step process is slower in the spatial model, but the tunneling is again faster (it has the same order of magnitude in terms of small u_1, see Eqs. (29) and (61)). In the regime where tunneling predominates, the generation of double-mutants occurs faster in the spatial model.
- We also obtained results on the applicability of a homogeneous state-approximation (Eq. (67)), the definition of neutrality (Eq. (64) compared to (63)) and the relative contribution of tunneling (Eqs. (31), (65) and (66)) for the spatial model. All these differ from those found in the space-free model.

Perhaps the most interesting result is that the generation of double-hit mutants occurs faster in the spatial model. There is an intuitive explanation for this. Let us consider the probabilities for the number of cells of type "B" to increase or decrease by 1 in the spatial and non-spatial models. For the latter, the probability to increase/decrease during the time-interval Δt is given by $P(j \to j+1) \approx rj\Delta t$; $P(j \to j-1) \approx j\Delta t$. For the spatial model we have $P(j \to j+1) \approx r\Delta t$; $P(j \to j-1) \approx \Delta t$. For these formulas we assumed $j \ll N$, which is true in the tunneling regime. We can see that if $j > 1$, then the motion happens on a faster time-scale for the space-free case, as the probabilities are multiplied by the number of mutants of type "B." In the spatial model, these probabilities are independent of j. Therefore, we can say that once a colony of type "B" reaches a size larger than one, it tends to "linger around" for a longer period of time in the spatial model. And of course, such colonies are a breeding ground for mutants of type "C." The longer they persist, the higher is the chance to create a mutant of type "C" as a result of each individual mutation "A"→"B." The rate of generation of mutants of type "B" is the same in both models. Therefore, this argument explains why the rate of generation of mutants of type "C" is higher in the spatial setting.

Compared to space-free models, the present model is an improvement in terms of how well it describes real tumors. However, in order for a model to give accurate quantitative predictions of the observed reality, two or three spatial dimensions have to be used. Analytical results in two and three dimensions are much harder to obtain and this is a focus of future research. However, some important lessons learned from the 1D model will apply in higher dimensions. In particular, the above argument which explains why the generation of double-hit mutants in a spatial model happens faster than in a space-free model will hold in 2D and 3D, so we can expect an acceleration of "tunneling" in a real tumor compared to that predicted by a complete-mixing model.

Appendix A: Characteristic time-scale of the probability $P_1(t)$ for the non-spatial process

Let us study the applicability of approximation (20) to Eq. (18). The values of s for which the corresponding term can be ignored compared to other terms in Eq. (20) will give a reciprocal of a time-scale where the solution approaches its steady state value. We will assume for this analysis that $\beta \ll 1$. Let us write Eq. (20) in the following way:

$$\lambda(f_{i+1} - 2f_i + f_{i-1}) - (\mu - \lambda)(f_i - f_{i-1}) + \beta(1 - f_i) = \frac{f_i}{i}s. \tag{A.1}$$

For the cases where the mutant of type "B" is neutral or disadvantageous, we have $\alpha_- = 1 - \delta$, with $\delta \ll 1$, where $\delta = \sqrt{\beta/\lambda}$ and $\delta = \beta/(\mu - \lambda)$ respectively. We have for the terms on the left hand side of Eq. (A.1),

$$\lambda\delta^2, \quad (\mu - \lambda)\delta, \quad \beta,$$

where for each term we took the highest contribution in terms of δ. The right hand side can be estimated as δs. For the case of neutral mutants, we obtain that the three terms on the left are

$$\beta, \quad (\mu - \lambda)\sqrt{\beta/\lambda}, \quad \beta,$$

that is, in order for the s-term to be smaller than these, we must have $s < \sqrt{\beta\lambda}$. For the case of disadvantageous mutants, we have

$$\lambda^3 \frac{\beta^2}{(\mu - \lambda)^2}, \quad \beta, \quad \beta.$$

We can see that the first of these terms is actually small compared to the rest, and we can ignore it, as our approximation only takes account of terms of the order of $\beta/(\mu - \lambda)$. To get the right balance, we need to have $s < \mu - \lambda$. Therefore, we have the following time-scales of the process: for neutral mutants,

$$t_c \sim (\sqrt{\beta\lambda})^{-1}, \text{ "B" neutral,}$$

and for disadvantageous mutants,

$$t_c \sim (\mu - \lambda)^{-1}, \text{ “B” disadvantageous.}$$

Appendix B: Characteristic time-scale of the probability $P_1(t)$ for the spatial process

We would like to obtain the estimate for the time when the solution $P_1(t)$ approaches its steady-state level. This is equivalent to finding the maximum s for which the corresponding terms can be ignored in Eq. (53). We cannot use the same method as in the space-free system because we do not have an analytical solution for the discrete problem. Instead, we will work with the continuous Eq. (55). We can write down the exact solution with $s > 0$ and compare it with the solution for $s = 0$. In the case of disadvantageous mutants, we have an additional term in expansion (57), namely,

$$P_1^{\infty} = -\frac{s}{\lambda - \mu} + \frac{(\lambda - \mu)^2 + \lambda^2}{(\lambda - \mu)^2 \mu}\beta.$$

This suggests that for $s \ll \frac{(\lambda-\mu)^2+\lambda^2}{(\lambda-\mu)\mu}\beta$, the solution is close to its saturation level. We obtain

$$t_c = \frac{(\lambda - \mu)\mu}{(\lambda - \mu)^2 + \lambda^2}\beta^{-1}, \quad \text{“B” disadvantageous.}$$

In the neutral case, we also have an additional term in expansion (58),

$$P_1^{\infty} = \left(\frac{3\beta}{\lambda}\right)^{1/3} \frac{\Gamma(2/3)}{\Gamma(1/3)} + \left(\frac{\Gamma(2/3)}{\Gamma(1/3)}\right)^2 \left(\frac{3}{\lambda}\right)^{2/3} \frac{s}{\beta^{1/3}}.$$

This suggests that the values of s must satisfy $s \ll \frac{\Gamma(1/3)}{\Gamma(2/3)}(3/\lambda)^{-1/3}\beta^{2/3}$, which means that

$$t_c = \frac{\Gamma(2/3)}{\Gamma(1/3)}(3/\lambda)^{1/3}\beta^{-2/3}, \quad \text{“B” neutral.}$$

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