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Evolutionary dynamics of invasion and escape

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Abstract

Whenever life wants to invade a new habitat or escape from a lethal selection pressure, some mutations may be necessary to yield sustainable replication. We imagine situations like (i) a parasite infecting a new host, (ii) a species trying to invade a new ecological niche, (iii) cancer cells escaping from chemotherapy, (iv) viruses or microbes evading anti-microbial therapy, and also (v) the repeated attempts of combinatorial chemistry in the very beginning of life to produce self-replicating molecules. All such seemingly unrelated situations have a common structure in terms of Darwinian dynamics: a replicator with a basic reproductive ratio less than one attempts to find some mutations that allow indefinite survival. We develop a general theory, based on multitype branching processes, to describe the evolutionary dynamics of invasion and escape.

Keywords: Multi-type branching process; Quasispecies; Mutation; Emerging infection; Cancer therapy; Vaccination

1. Introduction

At the very beginning of life (on earth or elsewhere), organic chemistry produced some macromolecules with random sequences. According to the RNA first hypothesis (Eigen and Schuster, 1977, 1982; Cairns-Smith, 1982; Szathmary and Maynard Smith, 1997; Orgel, 1998), these polymers had a limited ability of self-replication. Only a small subset of the random sequences had a basic reproductive ratio, R, greater than one, which means that one sequence could produce on average more than one offspring per lifetime. The overwhelming majority of random sequences had $R<1$, and therefore lead to lineages that would eventually go to extinction. Hence the physical and chemical environment of this world generated many short sequences capable of replication. We want to calculate the probability that one or many of those sub-critical lineages will mutate to a sustainable sequence with $R>1$.

Imagine a virus of one host species that is transferred to another host species, such as recent epidemics like HIV or SARS. In the new host, the virus has a basic reproductive ratio less than one (Anderson and May, 1992). Some mutations may be required to generate a virus mutant that can lead to an epidemic in the new host species. There will be repeated attempts to invade the new host. We want to calculate the probability that such an attempt succeeds in producing a mutant virus that initiates a new epidemic.

Suppose a successful HIV vaccine is found. Vaccinated hosts become exposed to a viral quasispecies. If the vaccine is effective, then most virus mutants will have a basic reproductive ratio less than one in the vaccinated host (Nowak and May, 2000). There will be some mutants, however, that can break through the protective immunity of the vaccine. We want to calculate the probability that a virus quasispecies of a given size finds (or already contains) an escape mutant that establishes an infection and thereby causes vaccine failure.

Cancer therapy often involves surgery or radiation to remove the main tumor followed by chemotherapy to eliminate remaining cancer cells. In the case of effective chemotherapy the majority of those cancer cells have a basic reproductive ratio less than one. Those cells are sensitive to this particular therapy. Genetic heterogeneity in the population of cancer cells could mean that some mutants have a basic reproductive ratio in excess of one. Those cells are resistant. Furthermore, sensitive

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cells could mutate to give rise to resistant cells. For example, resistance to Gleevec is caused by point mutations in the Bcr-Abl oncogene (Gorre et al., 2001; Sawyers, 2001). Other resistance mutations involve the inactivation of p53 or other tumor suppressor genes (Ozols, 1989; Keshelava et al., 2000; Kigawa et al., 2001). We want to calculate the probability of success or failure of anti-cancer therapy.

These are the main applications we have in mind when constructing our theory. More generally we describe situations where a genetically heterogeneous population of replicators is under selection to invade a new niche or repopulate a niche under a major selection pressure (such as vaccination or chemotherapy). Our approach is based on the theory of multi-type branching processes (Athreya and Ney, 1972; Seneta, 1970). The main assumption is that lineages behave independently of each other. Thus the fitnesses of individual genotypes are constant and not frequency or density dependent. Recombination as a mutational mechanism has to be excluded because its effective rate is intrinsically frequency dependent.

In Section 2 we outline the basic theory calculating the probability of non-extinction/escape for lineages starting from single individuals. We perform the calculation for various cases of increasing complexity ranging from single types to sequential mutations, networks and quasi-species (Section 3). In Section 4 we calculate the non-extinction probability when starting with heterogeneous populations.

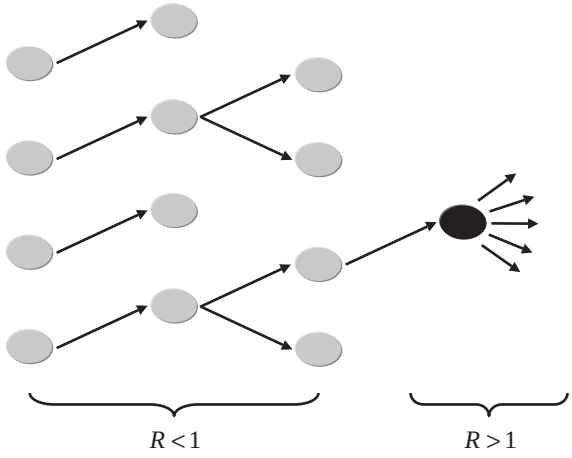

Fig. 1. Extinction or escape. Individual replicators undergo reproduction and death at random times. All birth and death events occur independently of each other: there is no frequency or density-dependent fitness. A lineage can either die out or escape from selection pressures to survive indefinitely. Persisting replicators arise by mutations in sub-critical lineages under selection pressure or preexistence prior to the onset of selection pressure.

2. Non-extinction of lineages

Consider a continuous-time branching process. Individual replicators undergo reproduction or death at random times (Fig. 1). There are different mutants with different fitnesses. In accordance with the fundamental assumption of branching processes, individuals behave independently of each other: there is no frequency- or density-dependent fitness. All birth and death events occur independently of each other.

2.1. A single type

Let us first explore the simplest case with a single type of replicator. Let $N(t)$ be the number of individuals at time t. We want to calculate the probability that the population will become extinct or survive indefinitely, i.e. escape. Consider the generating function

$$g(z,t) = E(z^{N(t)}|N(0)=1). \tag{1}$$

Here, z is a positive parameter satisfying $0<z\leqslant 1$. Within a short time interval of length Δt, an individual dies with probability Δt, produces an offspring with probability $R\Delta t$, and remains unchanged otherwise. R is the basic reproductive ratio, defined as the expected number of offspring produced from a single individual during its lifetime. We obtain

$$g(z,t+\Delta t) = \Delta t + g(z,t)[1-(1+R)\Delta t] + g(z,t)^2 R\Delta t.$$

We have used $E(z^{N(t)}|N(0)=2) = E(z^{N(t)}|N(0)=1)^2 = g(z,t)^2$ because two lineages starting from different cells at a given time behave independently. In the limit of infinitesimal Δt, we obtain the ordinary differential equation

$$\frac{dg}{dt} = (1-g)(1-Rg)$$

with initial condition, $g(z,0)=z$, because $N(0)=1$. We can derive the time-dependent solution of the generating function. At $z=0$, the generating function is equal to the probability of extinction. The rate of ultimate extinction is given by $g(0,\infty) = Pr(N(\infty)=0|N(0)=1)$. Therefore the probability of non-extinction (escape) is

$$\xi = 1-g(0,\infty) = \begin{cases} 0 & R\leqslant 1, \\ 1-1/R & R>1. \end{cases} \tag{2}$$

Escape is not possible if $R\leqslant 1$. If $R>1$ there is a certain probability of escape. Note that in this case the expected population size increases with time, but the escape probability is less than 1, because demographic stochasticity causes random extinction.

We call a replicator with $R>1$ sustainable, while a replicator with $R<1$ is not sustainable. In terms of escape from anti-cancer therapy, a cell with $R>1$ is resistant (is an escape mutant) while a cell with $R<1$ is sensitive to therapy. In certain applications of the theory it also makes sense to consider the neutral case, $R=1$.

2.2. Two types

Next we examine the situation of two types. Type 1 mutates to type 2 with a small probability u. There is no back mutation. Within a short time interval, Δt, an individual dies with probability Δt. Type 1 reproduces with probability $R_1\Delta t$. Type 2 reproduces with probability $R_2\Delta t$. Type 1 generates a type 1 offspring with probability $1-u$, and a type 2 offspring with probability u. If the mutation rate u is small compared to all other parameters, we can expand the escape probability with respect to u and consider the leading order term only. Let ξ_1 denote the probability of non-extinction (escape) when starting from a single individual of type 1. If $R_1 \leqslant 1$ but $R_2 > 1$, then type 1 individuals will eventually go extinct, but mutations can lead to type 2 individuals that can increase indefinitely. Let ξ_2 be the probability of non-extinction when starting with a single individual of type 2. We can calculate these probabilities by using a generating function for two types. From the recurrence formula for a multi-type generating function, we derive the equation for the non-extinction probabilities of different types (Appendix A). If the mutation rate u is much smaller than all other parameters, we obtain analytical expressions. The leading order terms for a small mutation rate, u, are

$$\xi_1 = 1 - 1/R_1 \quad \text{if } R_1 > 1 \text{ and } R_1 - 1 \gg \sqrt{u\xi_2}, \tag{3a}$$

$$\xi_1 = \sqrt{u\xi_2} \quad \text{if } R_1 \approx 1 \text{ and } |1 - R_1| \ll \sqrt{u\xi_2}, \tag{3b}$$

$$\xi_1 = \frac{R_1}{1-R_1}u\xi_2 \quad \text{if } R_1 < 1 \text{ and } 1 - R_1 \gg \sqrt{u\xi_2}. \tag{3c}$$

Eq. (3a) implies that if type 1 itself is an escape mutant ($R_1 > 1$), then the escape probability is of the order of 1, which is much larger than mutation rate u. In contrast, if $R_1 \leqslant 1$, then the escape probability is zero in the absence of mutation. With mutation, the dominant term of the escape probability is of the order of $\sqrt{u}$ if $R_1 \approx 1$, or of the order of u if $R_1 < 1$ (Eqs. (3b) and (3c)). The escape probability of a single type 2 individual with $R_2 > 1$ is $\xi_2 = 1 - 1/R_2$.

2.3. Sequential mutations

In the following, we calculate the probability of non-extinction/escape for a sequence of $m-1$ mutations (Fig. 2). Consider the situation where all intermediate mutants, $i = 1, \ldots, m-1$, have $R_i < 1$ and $1 - R_i \gg \sqrt{u}$. The final type, m, has $R_m > 1$ and is therefore an escape mutant. Let us introduce the notation $a_i = R_i/(1-R_i)$. Denote by u_i the mutation rate from type i to type $i+1$. Other mutations (from i to j where $j \neq i+1$) are not possible. The escape probability starting from a single type i via this chain of mutations is

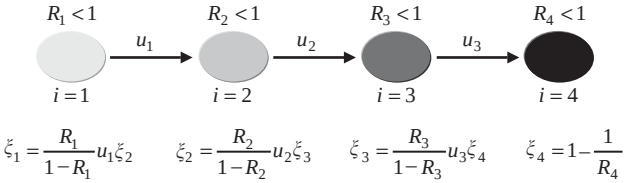

Fig. 2. Probabilities of escape. Consider a sequence of $m = 3$ mutations. An individual of type $i = 1$ mutates with probability u_1 to an individual of type $i = 2$. An individual of type $i = 2$ mutates with probability u_2 to an individual of type $i = 3$ which mutates with probability u_3 to an individual of type $i = 4$. The basic reproductive ratios, defined as the expected number of offspring produced by a single individual during its lifetime, of individuals of type $i = 1, \ldots, 4$ are given by $R_1, \ldots, R_4$. R_1, R_2, and R_3 are less than one, while R_4 is greater than one. The probabilities of escape of individuals of type $i = 1, \ldots, 4$ are given by $\xi_1, \ldots, \xi_4$ (see Eq. (3)).

$$\xi_i = \xi_m \prod_{j=1}^{m-1} a_j u_j \quad i = 1, \ldots, m,$$
$$\xi_m = 1 - 1/R_m. \tag{4}$$

If the mutation rates are the same for all steps and equal to u, the escape probability starting from a single type 1 is proportional to u^{m-1}, where $m-1$ is the number of mutational steps required to reach the escape mutant, m.

2.4. Mutation networks

Let us consider a mutation network of m types. The mutation from i to j is given by u_{ij}. Let $u_{ii} = 0$. The first n types have a basic reproductive ratio less than 1, all remaining types have a basic reproductive ratio greater than one. Thus $R_i < 1$ for $i = 1, \ldots, n$ and $R_1 > 1$ for $i = n+1, \ldots, m$. The escape probabilities are given by the linear system

$$\xi_i = \frac{R_i}{1-R_i}\left(\sum_{j=1}^{m} u_{ij}\xi_j\right) \quad i = 1, \ldots, n,$$
$$\xi_i = 1 - 1/R_i \quad i = n+1, \ldots, m. \tag{5}$$

2.5. Quasispecies

From an information theoretic perspective and without loss of generality, we can describe genomes as binary sequences of a given length. A heterogeneous ensemble of genomes is called a quasispecies (Eigen and Schuster, 1977). Here we study stochastic quasispecies dynamics (McCaskill, 1984; Nowak and Schuster, 1989) in a situation of invasion or escape.

Suppose mutations in n positions are required to reach an escape mutant. There are $m = 2^n$ mutants which we label $i = 1, \ldots, m$. The escape mutant is denoted by index m. Basic reproductive ratios are given

by $R_i<1$ for $i=1,\ldots,m-1$ and $R_m>1$. If the mutation rate per bit is denoted by u, then the probability to mutate from sequence i to sequence j is given by $u^{h_{ij}}(1-u)^{n-h_{ij}}$. The Hamming distance, h_{ij}, counts the number of point mutations between two sequences.

As shown in Appendix A, we can express the escape probability as the sum of contributions from different paths leading to the escape mutant m. Paths that include a smaller number of mutational steps are more important than those with a larger number of steps. For small mutation rates, u, we only have to consider those paths with the minimum number of steps. For example, suppose $n=3$ and the escape mutant is 111 (Fig. 3). Starting from 000, the following paths are examples of paths that have the minimum number of mutational steps: (i) 000 to 010 to 011 to 111; (ii) 000 to 101 to 111; (iii) 000 to 111. All those paths include three steps. Observe that multiple simultaneous mutations (as shown in path (ii) and (iii)) can be as important as sequential single mutations (path (i)). The following path has 4 mutational steps and can be neglected in our analysis: 000 to 110 to 011 to 111. The minimum number of steps is given by the Hamming distance between starting sequence and escape sequence.

The escape probability starting from a single type m is given by $\xi_m=1-1/R_m$. The escape probability starting from a single type i is

$$\xi_i=\xi_m\sum_{p\in P_i}v(p). \tag{6a}$$

The set P_i contains all those paths, p, that connect sequence i and m with the minimum number of steps, h_{im}. Consider a particular path

$$p: i=k_1\to k_2\to k_3\to\cdots\to k_g=m. \tag{6b}$$

The value of path p is given by

$$v(p)=u^{h_{im}}\prod_{j=1}^{g-1}a_{k_j}. \tag{6c}$$

As before, we have $a_i=R_i/(1-R_i)$. In general, simultaneous mutations at k loci occur at rate u^k per cell division. This contribution has the same order of magnitude $O(u^k)$ as a chain of k one-step mutations. All paths of successive mutations from 00..0 to 11..1 where the number of 1 digits increases at each step have the same order of magnitude with respect to u.

2.5.1. Sequence length two

As a first specific example, let us suppose mutations in two loci are relevant for escape (Fig. 4). The mutation rates in these two loci are u_1 and u_2. We consider 4 types, 00, 01, 10 and 11. The basic reproductive ratios R_{00},R_{01},R_{10} are less than 1, while $R_{11}>1$. As before we use $a_i=R_i/(1-R_i)$. The probabilities for escape starting with a single individual of type 00, 01, 10 or 11 are

$$\begin{aligned}\xi_{00}&=u_1u_2a_{00}(1+a_{01}+a_{10})\xi_{11},\\ \xi_{01}&=u_1a_{01}\xi_{11},\\ \xi_{10}&=u_2a_{10}\xi_{11},\\ \xi_{11}&=1-1/R_{ii}.\end{aligned} \tag{7}$$

In the expression for ξ_{00}, the first term in brackets indicates the contribution of direct mutation from 00 to 11, while the second and third terms indicate sequential mutations via 01 and via 10, respectively. Direct mutation from 00 to 11 is more important than

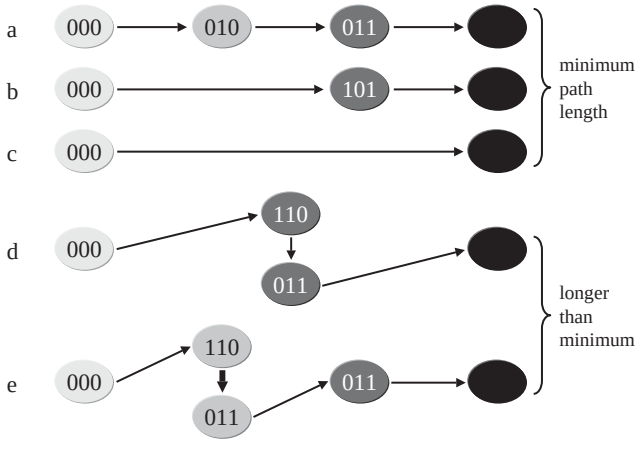

Fig. 3. Minimum number of mutational steps. The minimum number of steps is given by the Hamming distance between starting sequence, here 000, and escape sequence, here 111. The Hamming distance, h_{ij}, counts the number of point mutations between two sequences. Here we have $h_{000,111}=3$. Panels (a)–(c) are examples for mutational paths containing the minimum number of mutational steps, because they all include three steps. Panels (d) and (e) are examples for mutational paths containing more than 3 mutational steps. Such paths can be neglected in our analysis.

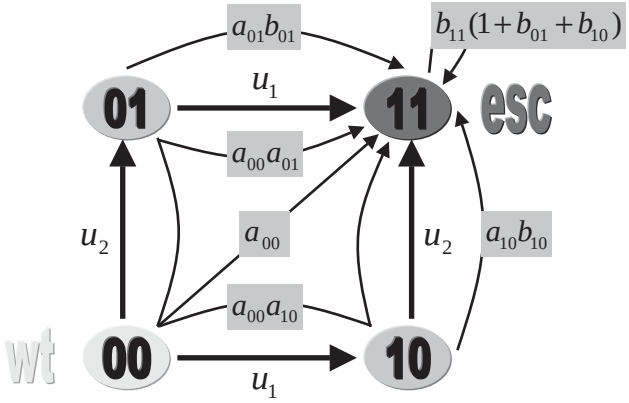

Fig. 4. Two mutations to escape. The mutation rates in the two positions are u_1 and u_2. We consider $i=4$ types, 00, 01, 10 and 11. Type 00 is the wild type, type 11 the escape mutant. The basic reproductive ratios R_{00}, R_{01}, and R_{10} are less than one, while $R_{11}>1$. Here $a_i=R_i/(1-R_i)$ and $b_i=1/(1-w_i)$ for $i=00$, 01, 10, 11. The parameter w_i denotes the fitness of type i prior to the onset of selection pressure. The probabilities of escape starting with a single individual of type 00, 01, 10 or 11 are given by Eq. (7).

sequential mutation from 00 to 01 to 11, if $1 > a_{01}$ which is equivalent to $R_{01} < 1/2$. Direct mutation from 00 to 11 is more important than sequential mutation via either 01 or 10 if $R_{01} + R_{10} - (3/2)R_{01}R_{10} < 1/2$. The more deleterious the intermediate steps are the more important is direct mutation. Interestingly the relative importance of direct versus sequential mutations does not depend on a comparison between R_{01}, R_{10} and R_{00}, but only on the absolute values of R_{01} and R_{10}.

2.5.2. Sequence length n

Let us now expand the previous calculation to binary sequences of length n, but assuming identical basic reproductive ratios, $R<1$, for all sequences $i = 1, \ldots, m-1$, and identical mutation rate, u, for all positions. The escape mutant, m, has basic reproductive ratio $R_m > 1$. We derive the following formulas in Appendix C. Let $f_i(x)$ be the polynomial of ith order, which is recursively defined as

$$f_i(x) = x\sum_{j=0}^{i-1}\binom{i}{j}f_j(x) \quad \text{and} \quad f_0(x) = 1. \tag{8a}$$

We have $f_1(x) = x$, $f_2(x) = x + x^2$, $f_3(x) = x + 6x^2 + 6x^3$, $f_4(x) = x + 14x^2 + 36x^3 + 24x^4$. The escape probability of type i is:

$$\xi_i = \xi_m u^{h_{im}} f_{h_{im}}(a). \tag{8b}$$

Here, $a = R/(1-R)$ and $\xi_m = 1 - 1/R_m$. The Hamming distance between sequences i and m is denoted by h_{im}.

3. Populations

We can also calculate the probability of non-extinction starting from a heterogeneous population of size N. Let us revisit the example of Section 2.5.1 with four genotypes denoted by 00, 01, 10 and 11. Suppose the relative abundances of the individual types in the initial population are given by x_{00}, x_{01}, x_{10} and x_{11}. The probability of non-extinction of the population is

$$P = 1 - \exp[-N(x_{00}\xi_{00} + x_{01}\xi_{01} + x_{10}\xi_{10} + x_{11}\xi_{11})].$$

The initial distribution of genotypes could be the consequence of a mutation selection balance. If we consider the situation of an infectious agent attempting to invade a new host, then the x_i values could be determined by mutation-selection forces in the original host. Suppose the mutation rates are intrinsic to the infectious agent and are, therefore, the same in the old and in the new host. Suppose w_i denotes the fitness of type i in the old host. Assume $w_{00} > 1$, but w_{01}, w_{10}, w_{11} are all less than 1. Hence 00 is the wild-type sequence with the highest fitness in the original host, but in the new host only 11 is a sustainable replicator. We want to calculate the probability that a population of size N will succeed in infecting the new host. Let us introduce the parameters $b_i = 1/(1-w_i)$. The equilibrium distribution in the old host is approximately given by

$$x_{00} = 1,$$
$$x_{01} = u_2 b_{01},$$
$$x_{10} = u_1 b_{10},$$
$$x_{11} = u_1 u_2 b_{11}(1 + b_{01} + b_{10}),$$

(see Appendix B for the derivation in general cases). These are leading order terms in the expansion of small mutation rates. With this initial distribution the probability of escape of a population of size N is

$$P = 1 - \exp(-NCu_1u_2). \tag{9a}$$

The risk factor, C, is given by

$$C = a_{00}(1 + a_{01} + a_{10}) + a_{01}b_{01} + a_{10}b_{10} + b_{11}(1 + b_{01} + b_{10}). \tag{9b}$$

3.1. Quasispecies with sequence length n

Let us now consider the situation of Section 2.5.2. In the original host the wild-type sequence is 00..0; its fitness is 1. All other sequences have fitness, $w<1$. Let $b = 1/(1-w)$. The fraction of sequence i in the initial population is

$$x_i = u^{h_{0i}} f_{h_{0i}}(b), \tag{10}$$

where $f_i(x)$ are the very same polynomials as in Eq. (8a). The escape probability can be written as

$$P = 1 - \exp[-NC_n u^n \xi_m]. \tag{11a}$$

The risk factor is given by

$$C_n = \sum_{i=0}^{n}\binom{n}{i}f_{n-i}(a)f_i(b). \tag{11b}$$

See Appendix C for derivation. Table 1 shows examples of the dependence of the population size compatible with a 99% probability of success on basic reproductive

Table 1
99% probability of success. The number of mutational steps is denoted by m. R denotes the basic reproductive ratio of types $0, \ldots, m-1$ during exertion of the selection pressure. The fitness values of types $1, \ldots, m$ before the onset of treatment is denoted by w. The table shows the dependence of the population size compatible with a 99% probability of success on R and w

	$N99$			
m	$R=0$ $w=0$	$R=0$ $w=0.9$	$R=0.9$ $w=0.01$	$R=0.9$ $w=0.9$
1	10^2	10	10	5
2	3×10^5	5×10^3	5×10^3	2×10^3
3	8×10^8	2×10^6	2×10^6	5×10^5
4	1×10^{12}	4×10^8	5×10^8	9×10^7
5	2×10^5	7×10^{10}	1×10^{11}	2×10^{10}

ratios during the selection pressure and fitness values prior to the onset of the selection pressure.

4. Extensions

4.1. (Almost) Neutral intermediate steps

We have analysed cases where all replicators could be clearly separated into escape mutants with $R_i > 1$ and non-escape mutants with $R_i < 1$. The calculations are valid if all basic reproductive ratios are either less than $1 - O(\sqrt{u})$ or greater than $1 + O(\sqrt{u})$ (see Appendix A for detail). Under this assumption, all escape mutants have escape probability $\xi_i = 1 - 1/R_i$, which is determined only by their own reproductive ratio and is independent of mutation rates. For non-escape mutants, we trace the shortest paths leading to escape mutants, calculate their values, and the sum over all such paths provides the probability of escape.

When both the mutation rate of type j and the escape probability of cell types of the destination of the mutant are small, we can derive the following formula (see Appendix A):

$$\xi_j = -\frac{1}{2a_j} + \sqrt{\frac{1}{(2a_j)^2} + \sum_{i \neq j} u_{ji}\xi_i}, \tag{12}$$

As before we have $a_j = R_j/(1 - R_j)$. Formulas $\xi_i = a_i \sum_{j \neq i} u_{ij}\xi_j$ and $\xi_i = 1 - 1/R_i$ are two limiting cases of Eq. (12) when R_i is clearly away from 1. If R_i is close to 1, we must use Eq. (12). The escape probability ξ_i does not satisfy the simple matrix formula, but it can be expressed using a nonlinear function of the sum of mutation rates and escape probability. In particular, if there is a chain of mutations that lead to the escape mutant m, and all the intermediate types are neutral ($R_0 = R_1 = R_2 = \cdots = R_{m-1} = 1$, and $R_m > 1$), we have

$$\xi_0 = u_0\sqrt{u_1\sqrt{u_2 \ldots \sqrt{u_{m-1}\xi_m}}} \tag{13}$$

and $\xi_m = 1 - 1/R_m$ instead of Eq. (4).

4.2. Connection to tunneling

In branching process models, the total number of individuals is not regulated. If the population does not go to zero, it will increase indefinitely (Athreya and Ney, 1972). In some applications, however, the total number of cells or individuals can be regulated to maintain a finite value. An example is given by the interesting connection between the results of this paper and stochastic tunneling of cancer progression (Nowak et al., 2002; Komarova et al., 2002, 2003; Michor et al., 2003; Iwasa et al., 2003a, b). Consider a population of N cells following a Moran process. Initially all cells are of type 0 and have relative fitness 1. They mutate to type 1 cells with fitness $R_1 \leqslant 1$. Type 1 cells mutate to type 2 cells with fitness $R_2 > 1$. The mutation rates from 0 to 1 and from 1 to 2 are given by u_1 and u_2, respectively. We are interested in the probability that type 2 cells have taken over the population by time t. If the population size N is not very large, type 1 cells will be generated by mutation and eventually be fixed in the population. Subsequently type 2 cells will be produced and become fixed. The population moves from all-0 to all-1 to all-2. If, on the other hand, the population size is large, then type 2 cells will emerge before type 1 cells reach fixation: type 1 mutants temporarily increase and generate a type 2 cell before becoming extinct again. The system moves from all-0 to all-2 without ever visiting all-1. This process is called stochastic tunneling. The rate of tunneling can be derived from the branching process calculations shown in this paper. The system moves from state all-0 to all-2 at rate $Nu_1\xi_1$. The rate of production of type 1 mutants is given by Nu_1. If $R_1 < 1$, then the probability of non-extinction of a lineage starting from a single type 1 mutant is given by $\xi_1 = R_1/(1 - R_1)u_2\xi_2$. If $R_1 = 1$, then this probability is given by $\xi_1 = \sqrt{u_2\xi_2}$. The probability of non-extinction of a lineage starting from a single type 2 mutant is given by $\xi_2 = (1 - 1/R_2)/(1 - 1/R_2^N)$. For large N, this becomes $\xi_2 = 1 - 1/R_2$. For the range of validity of these expressions and correction terms we refer to Iwasa et al. (2003a, b).

There is a connection between tunneling and the concept of 'hitch-hiking'. If type 1 is deleterious, then it is doomed to extinction unless it gets a lift from the advantageous type 2 mutation. In population genetics, this effect is called hitch-hiking (De Visser, 2002). It is important if two loci are strongly linked. Hitch-hiking is more important in asexual populations or in the absence of recombination (Charlesworth, 1978). One of the reasons why tunneling is important in the somatic evolution of cancer is the absence of meiotic recombination.

4.3. Synchronized generations

We have discussed continuous-time branching processes where individuals die and reproduce at random times. We can also describe populations with discrete and non-overlapping generations. Individuals reproduce independently of each other and are replaced by their offspring. Individuals produce a number of offspring that follows a Poisson distribution with mean R. The escape probability of a lineage starting from a single type is the largest solution of the transcendental equation

$$-\ln(1 - \xi) = R\xi. \tag{14}$$

The solution is positive if $R > 1$, and zero if $R \leqslant 1$. This property is the same as for the corresponding escape

probability, $1-1/R$, in the continuous time branching process. There are, however, some differences. In the continuous time branching process, the number of offspring of one individual has a variance larger than the Poisson distribution with the same mean, and hence the effect of random drift (stochasticity caused by small population size) is stronger, making selection less effective. We can also develop the multi-type branching process for discrete generations (Iwasa et al., 2003a, b). All the equations remain the same, except the probability of non-extinction of the escape mutant is given by Eq. (14) instead of $1-1/R$. In the discrete time model, exactly the same results as for the continuous time model can be obtained, if the number of offspring follows a geometric distribution rather than a Poisson distribution. Hence, the main difference between continuous and discrete time branching processes comes from the probability distribution for the number of offspring produced by a single individual in its lifetime.

5. Discussion

Branching process models have been used in a number of different contexts in biology. In ecology, for example, branching processes have been developed to calculate the success of invasion of a species into a new habitat, but without consideration of evolutionary change. Environmental fluctuations can lead to time dependent fitness (Iwasa and Mochizuki, 1988). If the environment fluctuates according to a stationary process, then the population size is described by a doubly stochastic process, where the population size follows a branching process with temporally fluctuating growth rate (Haccou and Iwasa, 1995).

Branching process models have also been used for explaining the age-dependent incidence of cancer (Knudson, 1971; Moolgavkar and Knudson, 1981; Little, 1995; Luebeck and Moolgavkar, 2002; Little and Wright, 2003). During tumorigenesis, cells receive multiple mutations and undergo clonal expansion. Usually, these models are analysed numerically. Here we provide analytical solutions based on the assumption that mutation rates are small.

In the present paper, we calculate evolutionary escape dynamics for populations struggling for survival under a strong selection pressure. This selection pressure can be exerted by an immune system combating infectious agents, by chemotherapy directed against cancer cells, by hostile or changing environments complicating the (re)population of invading or persisting organisms. Initially, the population can be heterogeneous: diverse genetic backgrounds and partial as well as full escape mutants can be present at the time of onset of the selection pressure. The initial distribution can be caused by a mutation-selection processes. The probability of escape is shaped by the following parameters: the total population size, the mutation rates of individuals within the population, the basic reproductive rates of individuals during the exertion of the selection pressure, and the fitness values of individuals prior to the onset of the selection pressure. With our theory, we can quantify how escape depends on pre-existence versus emergence of resistant mutants.

Our theory can be applied to the spread of replicating organisms both in single individuals and in host populations. It holds for arbitrarily complex mutational networks and fitness landscapes. The fitness values of individual mutants subject to selection pressure can be time dependent. The model can be extended to include spatial compartments with different extinction probabilities. There can also be latently infected cells or latent cancer cells such as cancer stem cells. However, there are some limitations to our approach. The basic assumption of multi-type branching processes is independence of the lineages that accumulate mutations. Hence, mutational mechanisms such as recombination and horizontal gene transfer as well as frequency- or density-dependent fitness have to be excluded. All of these phenomena can be important in certain situations of escape dynamics. Our study provides a general analytical theory for the evolutionary dynamics of escape. It is a point of departure for more specific and complex models that deal with particular situations arising in populations under a strong selection pressure.

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Appendix A

A.1. Escape probability in general cases

We start with the generating function for a multi-type branching process:

$$g_j(z_1, z_2, \ldots, z_n, t) = E[z_1^{N_1(t)} z_2^{N_2(t)}, \ldots, z_n^{N_n(t)} | N_i(0) = \delta_{ij}], \tag{A.1}$$

where $z_1, z_2, \ldots, z_n$ are positive constants satisfying $0 < z_i \leqslant 1$ for $i = 1, 2, 3, \ldots, n$. The average is calculated

for the trajectories starting with a single cell of type j at time $t=0$. The extinction probability is obtained by setting the parameters $z_1, z_2, \ldots, z_n$ equal to zero: $g_j(0,0,\ldots,0,\infty)=Pr[N_1(\infty)=N_2(\infty)=\cdots=N_n(\infty)=0|N_i(0)=\delta_{ij}]$. We consider the case in which there is only a single escape type m, $R_m>1$; all the others are sensitive to the exerted selection pressure, $R_i<1$ for $i\neq m$. Here the fitnesses are clearly different from 1 (see the inequality condition given in Eq. (3a)). Let u_{ij} be the mutation rate from type i to type j.

We consider events occurring in a short time interval of length Δt, and expand the generating function as

$$g_j(\mathbf{z},t+\Delta t)=\Delta t\bullet 1+R_i\Delta t g_j(\mathbf{z},t)\left\{\sum_{i=1}^{n}u_{ji}g_i(\mathbf{z},t)\right\}+(1-(1+R_i)\Delta t)g_j(\mathbf{z},t).$$

Here we used

$$E\left[z_1^{N_1(t)}\ldots z_n^{N_n(t)}\middle|\begin{array}{c}\text{one cell of type } i \text{ and}\\ \text{another of type } j \text{ at } t=0\end{array}\right]$$
$$=E\left[z_1^{N_1(t)}\ldots z_n^{N_n(t)}\middle|\begin{array}{c}\text{one cell of type } i\\ \text{at } t=0\end{array}\right]\times E\left[z_1^{N_1(t)}\ldots z_n^{N_n(t)}\middle|\begin{array}{c}\text{one cell of type } j\\ \text{at } t=0\end{array}\right]$$
$$=g_i(\mathbf{z},t)g_j(\mathbf{z},t)$$

and

$$E[z_1^{N_1(t)}\ldots z_n^{N_n(t)}|\text{two cells of type } i \text{ at } t=0]=E\left[z_1^{N_1(t)}\ldots z_n^{N_n(t)}\middle|\begin{array}{c}\text{one cell of type}i\\ \text{at } t=0\end{array}\right]^2=g_i(\mathbf{z},t)^2.$$

In the limit of very small Δt, we have

$$\frac{\mathrm{d}}{\mathrm{d}t}g_j=(1-g_j)+\left\{u_{jm}(g_m-1)+\sum_{\substack{i\neq j\\ i\neq m}}u_{ji}(g_i-1)+(g_j-1)\left(1-\sum_{i\neq j}u_{ji}\right)\right\}g_jR_j.$$

By setting $\xi_j=1-g_j$ and $t\to\infty$, we have

$$\xi_j=\left\{u_{jm}\xi_m+\sum_{\substack{i\neq j\\ i\neq m}}u_{ji}\xi_i+\left(1-\sum_{i\neq j}u_{ji}\right)\xi_j\right\}(1-\xi_j)R_j,$$

which can be rewritten as

$$\left\{\frac{1}{R_j(1-\xi_j)}-1\right\}\xi_j=u_{jm}\xi_m+\sum_{\substack{i\neq j\\ i\neq m}}u_{ji}\xi_i-\sum_{i\neq j}u_{ji}\xi_j. \tag{A.2}$$

We here assume that all the mutation rates (u_{ij}) are small, and of the order of magnitude $O(u)$. The sum $\sum_{i\neq j}u_{ji}\xi_j$ is of a higher order with respect to u than the terms on the left-hand side, and can therefore be neglected. The first and second term on the right-hand side are of $O(u)$ or smaller. Then we can derive

$$\xi_j=\frac{1}{2}\left[-\left(\frac{1}{R_j}-1\right)\pm\sqrt{\left(\frac{1}{R_j}-1\right)^2+4y}\right], \tag{A.3}$$

where $y=u_{jm}\xi_m+\sum_{i\neq j,\,i\neq m}u_{ji}\xi_i$, by neglecting terms of higher order with respect to y.

When one of the two terms in the square root of Eq. (A.3) is much larger than the other, we can simplify the expression as follows. We have three cases:

Case I: *advantageous*. If $R_j>1$ and $1-1/R_j\gg 2\sqrt{y}$, we have

$$\xi_j=1-\frac{1}{R_j}+[\textit{smaller terms}]. \tag{A.4a}$$

Here the escape probability ξ_j is of $O(1)$, and it is independent of the mutation rate.

Case II: *nearly neutral*. If R_j is close to 1, or more exactly $|1-1/R_j|\ll 2\sqrt{y}$, then we have

$$\xi_j=\sqrt{u_{jm}\xi_m+\sum_{i\neq j,\,i\neq m}u_{ji}\xi_i}+[\textit{smaller terms}], \tag{A.4b}$$

Case III: *deleterious*. *If* $R_j<1$ and $1/R_j-1\gg 2\sqrt{y}$, we have

$$\xi_j=\frac{R_j}{1-R_j}\left(u_{jm}\xi_m+\sum_{\substack{i\neq j\\ i\neq m}}u_{ji}\xi_i\right)+[\textit{smaller terms}]. \tag{A.4c}$$

These three cases correspond to the three cases of Eq. (3) in the main text when there are only two cell types.

In the following, we consider the case in which mutation rates are very small, and the fitnesses of mutants are classified to be either advantageous or deleterious. The escape probability of advantageous mutants is independent of mutation rates and of the order of 1. We consider the case in which there is a single escape mutant, denoted by m, and n deleterious mutants that are connected with each other and with the escape mutant m by rare mutation. Let $(\xi_1,\xi_2,\ldots,\xi_n)^{\mathrm{T}}$ be a column vector of the escape probabilities; the superfix T indicates transposition of a vector or a matrix. We also introduce a diagonal matrix,

$$A=diag\left[\frac{R_1}{1-R_1},\frac{R_2}{1-R_2},\ldots,\frac{R_n}{1-R_n}\right]$$

and the matrix of mutation rates, $U=\{u_{ij}\}$, where the diagonal element is taken to be 0. Eq. (A.4c) becomes

$$(\xi_1,\xi_2,\ldots,\xi_n)^{\mathrm{T}}=A\{(u_{1m},u_{2m},\ldots,u_{nm})^{\mathrm{T}}\xi_m+U(\xi_1,\xi_2,\ldots,\xi_n)^{\mathrm{T}}\}, \tag{A.5}$$

which is rewritten as Eq. (5) in the main text. After matrix calculation, we have the following results:

$$(\xi_1, \xi_2, \ldots, \xi_n)^{\mathrm{T}} = (A^{-1} - U)^{-1}(u_{1m}, u_{2m}, \ldots, u_{nm})^{\mathrm{T}}\xi_m = (A + AUA + AUAUA + AUAUAUA + \cdots) \times (u_{1m}, u_{2m}, \ldots, u_{nm})^{\mathrm{T}}\xi_m. \quad \text{(A.6)}$$

By examining the term on the right-hand side, we get the interpretation of the contribution of different paths with the waiting factor given by Eq. (6c) in the main text.

Appendix B

B.1. Distribution prior to infection

The distribution prior to infection is determined as a result of the mutation-selection balance. We consider the case in which there is a single wild type individual, $i = 0$, with fitness 1 and n mutants, $i = 1, 2, \ldots, n$. Here the mutation rates among strains are the same as those after infection, but the selection coefficients are different.

Type i mutant has fitness w_i, which is less than 1 for non-wild types ($i = 1, 2, 3, \ldots, n$). The wild type has fitness $w_0 = 1$. Mutation rates of different types are the same as those after infection, u_{ij}. Let x_i be the abundance of type i mutants. We have

$$\mathrm{d}x_i/\mathrm{d}t = -(1 - w_i)x_i + \sum_{j=1}^{m} x_j u_{ji} + u_{0i}x_0 + [\text{small terms}], \quad \text{(B.1)}$$

where the small term includes contribution from the mutation out of the focal type.

Let $\mathbf{x} = (x_1\ x_2\ \ldots\ x_m)^{\mathrm{T}}$ be the column vector for the equilibrium distribution. At equilibrium, it satisfies

$$0 = -\mathbf{x}B^{-1} + \mathbf{x}U + x_0(u_{01}\ u_{02}\ \ldots\ u_{0m}),$$

where

$$B = diag\left[\frac{1}{1 - w_1}, \frac{1}{1 - w_2}, \ldots, \frac{1}{1 - w_{m1}}\right]$$

is a diagonal matrix. After using some matrix algebra, we have

$$x = x_0(u_{01}\ u_{02}\ \ldots\ u_{0m})(I - BU)^{-1}B = x_0(u_{01}\ u_{02}\ \ldots\ u_{0m})(B + BUB + BUBUB + \cdots). \quad \text{(B.2)}$$

Considering $x_0 \sim 1$, Eq. (B.2) is rewritten as

$$x_i = \sum_{q:0\to i} \tilde{v}(q), \quad \text{(B.3)}$$

where the sum is calculated over all the paths connecting the wild type to type i, such as $q : 0 = k_1 \to k_2 \to \cdots \to k_m = i$. The value of the path is defined as $\tilde{v}(q) = u_{k_1k_2}b_{k_2}u_{k_2k_3}b_{k_3}\ldots u_{k_{m-1}k_m}b_{k_m}$ with $b_i = 1/(1 - w_i)$. If all mutational steps are of $O(u)$, x_i is of $O(u^{d(0,i)})$ where $d(0, i)$ is the Hamming distance between the wild type and the focal type i, or the number of sites differing between them.

Appendix C

C.1. Formula for n-bits string

We here consider the case in which genotypes are expressed as i-bits strings, such as 000…0, 100..0, 010,000..0, and 111..1. Among these, the all-0 string is the wild type, and the all-1 string is the escape mutant. The fitness of each non-wild type is a common constant, $w < 1$; the fitness of non-escape mutants also is a common constant, $R < 1$. In this case, we can calculate the total risk. We first derive a recursive formula based on the number of bits i. Let ξ_0^i be the escape probability for the wild type 0000..00 when the total length of bit sequence is i. The first step of the mutation may include changes at either a single locus or multiple loci. The result of such a change is a sequence of j 0's and $i - j$ 1's. There are $\binom{i}{j}$ possible sequences of this kind. The escape probability of these cell types is equal to ξ_0^j, or equal to one when the sequence consists of j bits. From Eq. (A.5), the escape probability ξ_0^i is the sum of the products of a fitness factor, a, the mutation rate, and the escape probability of the resulting cell type. Taken together, we have

$$\xi_0^i = a\sum_{j=0}^{i-1}\binom{i}{j}u^{i-j}\xi_0^j. \quad \text{(C.1)}$$

By setting $\xi_0^i = u^i f_i(a)(1 - 1/R_m)$, we get for $f_i(a)$:

$$f_i(a) = a\sum_{j=0}^{i-1}\binom{i}{j}f_j(a).$$

We also note that $\xi_0^0 = 1 - 1/R_m$, which is the escape probability for the escape mutant. This produces $f_0(a) = 1$. Hence the polynomial $f_i(x)$ is determined recursively as specified by Eq. (10b).

Consider the initial distribution with n-bit strings in which the wildtype, 000…00, has fitness 1 and all mutants have fitness $w < 1$. Following a procedure similar to the calculation above, we get

$$x_i = \sum_{j<0}^{i-1} x_j\binom{i}{j}u^{i-j}b,$$

where $b = 1/(1 - w)$. With $x_0 \sim 1$, we can derive $x_i = x_0u^i f_i(b)$. From this we can obtain Eq. (10).

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