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This is a preprint of: *Surviving evolutionary escape on complex
genotype-phenotype networks*
Journal Information: *Journal of Mathematical Biology*,
Author(s): E. Ibanez-Marcelo, T. Alarcon.
Volume, pages: 72(3) 1-25, DOI:[10.1007/s00285-015-0896-x]



Surviving evolutionary escape on complex genotype–phenotype networks

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Received: 6 October 2014 / Revised: 18 February 2015 / Published online: 23 May 2015
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Abstract We study the problem of evolutionary escape and survival of cell populations with a genotype–phenotype structure. We refer to evolutionary escape as the process where a cell of a given ill-adapted population to reach a well-adapted phenotype. Similarly, survival refers to the dynamics of the population once the escape phenotype has been reached. The aim of this paper is to analyse the influence of topological properties associated to robustness and evolvability on the probability of escape and on the probability of survival. In order to explore these issues, we formulate a population dynamics model, consisting of a multi-type time-continuous branching process, where types are associated to genotypes and their birth and death probabilities depend on the associated phenotype (non-escape or escape). We exploit the separation of time scales introduced by the the difference in reproductive ratios between the ill-adapted phenotypes and the escape phenotype. Two dynamical regimes emerge: a fast-decaying regime associated to the escape process itself, and a slow regime which corresponds to the survival dynamics of the population once the escape phenotype

Electronic supplementary material The online version of this article (doi:[10.1007/s00285-015-0896-x](https://doi.org/10.1007/s00285-015-0896-x)) contains supplementary material, which is available to authorized users.

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has been reached. We exploit this separation of time scales to analyse the topological factors which determine escape and survival probabilities. We show that, while the escape probability depends on the degree of escape phenotype, the probability of survival is essentially determined by its robustness, measured in terms of a weighted clustering coefficient.

Keywords Evolutionary escape · Survival probability · Local weighted clustering · Genotype–phenotype map

Mathematics Subject Classification 60J80 · 92D15 · 34E13

1 Introduction

Evolutionary escape is the process whereby a population under sudden changes in the selective pressures acting upon it try to evade extinction by evolving from previously well-adapted phenotypes to those that are favoured by the new selective pressure. This evolutionary process is driven by gene mutations. Examples of biological situations where this process is relevant include viruses evading anti-microbial therapy, emergence of drug resistance in cancer ([Komarova and Wodarz 2005](#)), parasites trying to infect a new host, or species attempting to invade a new ecological niche ([Iwasa et al. 2003](#)).

Earlier models of evolutionary escape have been formulated by [Iwasa et al. \(2003, 2004\)](#), their approach based on the assumption that n point mutations in some crucial parts of the genome are necessary for escape. They further assume that the genotype of the different mutants can be described by binary strings (with entries of $+1$ or -1) of length n , of which there are $2^n - 1$. It is assumed that, under the new selective pressure, most genotypes exhibit reduced proliferation ratios of sensitive mutants, $R < 1$; whereas some genotypes, the so-called escape genotypes, are such that $R > 1$. The corresponding evolutionary dynamics is modelled in terms of Galton–Watson multi-type branching process (GWMBP) ([Kimmel and Axelrod 2002](#)), where at each generation each individual of each type has a given (in general, mutant-dependent) probability of mutating, thus producing offspring belonging to a different type. The problem is to calculate the probability that an escape genotype is reached. The model proposed by Iwasa and co-workers has been analysed in more detail by [Serra \(2006\)](#), [Serra and Haccou \(2007\)](#) and [Sagitov and Serra \(2009\)](#). These authors have thus considered the process of evolutionary escape as a random search on a genotype space modelled by a hypercube: Individuals would concentrate in a given genotype and they must reach a well-adapted genotype (the escape genotype) before the population undergoes extinction. An alternative escape mechanism have been proposed in [Alarcon and Jensen \(2010\)](#) whereby escape is achieved by means of a growth-restricted (quiescent) phenotype that is insensitive to the selective pressure (e.g. a drug). This escape mechanism is relevant in cancer treatment of hypoxic tumours ([Alarcon et al. 2005](#); [Brikci et al. 2008](#); [Bristow and Hill 2008](#)) and drug resistance in bacterial populations which exhibit persistence ([Balaban et al. 2004](#); [Lewis 2007](#)).

In Ibanez-Marcelo and Alarcon (2014a), we have considered a variation of the original framework for studying escape problems. Since selective pressures act on phenotypes rather than genotypes, evolutionary escape would be best described in terms of a population dynamic that accounts for the genotype–phenotype map. This variation alters the approach proposed by Iwasa and co-workers in two significant ways. Due to evolved robustness in populations with genotype–phenotype map (Wagner 1996; Ciliberti et al. 2007a, b; Wagner 2007, 2008a, b; Jaeger and Monk 2014), not every gene mutation necessarily generates a new phenotype, i.e. many gene mutations are neutral as they do not change fitness. Furthermore, it has been shown that the topology of genotype–phenotype networks would not be well described by that associated to a regular hypercube lattice assumed by Iwasa et al. (Aguirre et al. 2011; Ibanez-Marcelo and Alarcon 2014b). In fact, we have recently shown that the corresponding phenotype network exhibits the small-world phenomenon and that, as a consequence, accelerated evolvability (relative to that of a system with no genotype–phenotype map) may emerge. We have shown that by incorporating the genotype–phenotype network structure into the study of evolutionary process a large degree of heterogeneity arises both in the probability of eventual escape and the dynamics of escape, which is absent in genotype–phenotype graphs associated to regular hypercube genotype networks (Ibanez-Marcelo and Alarcon 2014a). As a consequence, we have shown that the model of escape on genotype–phenotype graphs associated to hypercube genotype networks may lead to underscoring the escape probabilities. In this paper, we use the proposed genotype–phenotype model and its genotype–phenotype network representation [as described in detail in Ibanez-Marcelo and Alarcon (2014b), which was devoted to the topological characterisation of the genotype–phenotype network associated to a GRN model] to go one step further than in Ibanez-Marcelo and Alarcon (2014a), where we have undertaken a comparative analysis between the escape properties on a regular hypercube [i.e. the original situation proposed in Iwasa et al. (2003, 2004)] and on a complex genotype–phenotype network. Here, we analyse the escape and survival probabilities as a function of the topological properties of the escape phenotype. Our analysis is much simplified by the intrinsic time-scale separation imposed by the setting of the escape problem.

The properties and structure of genotype–phenotype maps have been established by studying several model systems (Wagner 2012): RNA, circuits of gene regulation and metabolic networks. In the RNA model of the genotype–phenotype map (Fontana and Schuster 1998), the *genotype* of each RNA molecule consists of a sequence of nucleotides. There are four such nucleotides, so for sequences of length L , the size of the genotype space is 4^L . The folded structure of an RNA sequence, which is a proxy for its *phenotype* (although it still lies far from defining its function), is determined by the sequence (genotype) in a many-to-one way, i.e. many different genotypes give rise to the same phenotype. Such non-uniqueness has led to the concept of the neutral network, first introduced in Lipman and Wilbur (1991) and Schuster et al. (1994), i.e. a network whose nodes correspond to genotypes, all with the same phenotypes, with edges between those nodes which differ by only one nucleotide (Wagner 2007). This system has been used extensively in the study of the genotype–phenotype map, in particular, those issues regarding its evolutionary properties, such as the role of

phenotypic robustness in evolvability and adaptation (Wagner 2011, 2012). Recently, the topology of the RNA genotype–phenotype space, composed by an intermingled set of neutral networks, has been analysed (Aguirre et al. 2011).

Gene regulatory networks (GRNs) have been extensively used to analyse properties of the genotype–phenotype map, in particular several variants of the model of phenotype plasticity (Wagner 1996) originally introduced by Wagner. These models are dynamical systems for the expression levels of the corresponding genes and are characterised by two elements: a matrix whose entries specify the character of the interaction between two genes (usually, activation or inhibition) and, possibly, the intensity of such interaction, and a series of rules for the time evolution of the expression levels of the genes involved. The entries of the corresponding matrix are defined as the genotype of the GRN. The associated phenotype is the steady-state gene expression yielded by the dynamics. There are many such genotypes that produce the same phenotype, which allows to extend the concept of neutral network to GRN, where nodes correspond to different matrices (producing the same steady-state) and links exist between nodes if the corresponding matrices differ only in one regulatory interaction. Models of GRNs have been used to study phenotype plasticity (Wagner 1996), robustness and innovation in circuits of gene regulation (Ciliberti et al. 2007a, b), and canalisation (Siegal and Bergman 2002; Bergman and Siegal 2003), among other issues.

Metabolic networks are the third class of systems that have been used to assess properties regarding robustness and innovation (Ndifon et al. 2009; Rodrigues and Wagner 2009; Samal et al. 2010; Rodrigues and Wagner 2011). They are formed by thousands of enzyme-catalysed chemical reactions. These networks are responsible for supplying cells with energy (i.e. ATP) and the molecule building blocks cells need to grow. The *genotype* space for this system consists of the space of all the possible metabolic networks, whereas the *phenotype* corresponds to the secondary metabolites the metabolic network is able to synthesise, the molecules they can use as energy sources, the ability to detoxify certain waste products, etc. (Wagner 2012). Innovations in these aspects not always appear as the result of gene mutations that give rise to new enzymes. They can also arise through novel combinations and utilisation of existing elements.

Genotype–phenotype networks have been extensively used in RNA and GRN models. These are networks whose set of nodes corresponds to the set of (viable) genotypes (e.g. each nucleotide sequence that yields a properly folded molecule). A link between a pair of genotypes exist if they are separated by one single mutation (e.g. an RNA molecule is linked to all its (viable) single-point mutants). The ensemble of genotypes associated to the same phenotype form its neutral network. In Ibáñez-Marcelo and Alarcón (2014b), we added to this picture the so-called phenotype nodes which correspond to the set of (viable) phenotypes. Links between genotype and phenotype nodes exist if the genotype node belongs to the neutral network of that particular phenotype.

The inclusion of the complex structure of genotype–phenotype network introduces yet another issue that needs to be considered when dealing with the escape problem. Previous studies of this problem (Iwasa et al. 2003, 2004; Serra 2006; Serra and Haccou 2007; Sagitov and Serra 2009; Ibáñez-Marcelo and Alarcón 2014a) assume that once the escape genotype is reached, escape takes place with probability 1, i.e. the proliferation ratio of the escape genotype is $R_E = \infty$. However, one could go one

step further and analyse the probability of surviving escape if $1 < R_E < \infty$ [see Weissman et al. (2009) for an example of such analysis]. This question is particularly meaningful when genotype–phenotype structure is accounted for. On the one hand, due to the multiplicity of genotypes associated to each phenotype, there exists a non-trivial population dynamics even when the population is confined within the escape phenotype. Secondly, cell populations with genotype–phenotype map exhibit evolved properties such as robustness and evolvability which affect escape (Ibanez-Marcelo and Alarcon 2014a) and survival alike: robustness, being related to resilience of phenotypes against gene mutations, is bound to affect survival, since any mutation driving cells away from the escape phenotype can be considered deleterious. Similarly, the *degree* of the escape phenotype, k_E , which is defined as the number of viable genotypes bearing the escape phenotype (Ibanez-Marcelo and Alarcon 2014b), is a measure of accessibility to the escape phenotype (roughly speaking, the higher k_E , the more access routes for the mutation-driven random search process which drives escape to reach the target phenotype). The aim of this paper is to analyse the influence of this and other topological properties associated to robustness and evolvability (Ciliberti et al. 2007a, b; Ibanez-Marcelo and Alarcon 2014b) on the probability of survival after escape.

This manuscript is organised as follows. Section 2 is devoted to a detailed description of our model and a summary of the mathematical background involved in its analysis. In Sect. 3, we report the results of our analysis and our main findings. Finally, in Sect. 4, we present our conclusions and discuss our results as well as future directions for further research.

2 Mathematical model

The classical escape model (Iwasa et al. 2003, 2004; Serra 2006; Serra and Haccou 2007; Sagitov and Serra 2009) can be summarised as follows. Each of the 2^n nodes of an n -dimensional hypercube is assumed to represent a genotype. Fitness values, represented here by the reproductive ratio, R , defined as the average per individual number of offspring, are assigned directly to genotypes. The population is assumed to be concentrated in one genotype which, prior to the change in selective pressure (as a consequence of e.g. the administration of a drug), was well adapted. After treatment commences, this initial genotype becomes ill-adapted to the new selective pressure, i.e. its reproductive ratio becomes $R < 1$. To avoid extinction the population needs to start a random, mutation driven search of the genotype hypercube, until it finds an *escape genotype*, i.e. a genotype such that its reproductive number satisfies $R > 1$. The reproductive number of all genotypes other than the escape genotype are such that $R < 1$. This implies that only the escape genotype has a positive probability of long-term survival (Kimmel and Axelrod 2002).

2.1 Genotype–phenotype network

We extend the basic framework for analysing evolutionary escape by introducing two modifications. The first one has to do with the topology of the space on which

the evolutionary dynamical process occurs. We assume that space where the escape process is performing its random search is not a regular hypercube (Iwasa et al. 2003, 2004; Serra 2006; Serra and Haccou 2007; Sagitov and Serra 2009), but a complex genotype–phenotype network (Ciliberti et al. 2007a,b; Aguirre et al. 2011; Ibanez-Marcelo and Alarcon 2014b).

In reference (Ibanez-Marcelo and Alarcon 2014b), we have formulated an evolutionary model of the genotype–phenotype network associated to gene regulatory networks (GRNs). A summary of this model is presented here with a more detailed description in the Supplementary Materials. The definition of the genotype–phenotype map is based on the idea, proposed by Stuart Kauffman, that GRNs are dynamical systems and phenotypes or differentiated states correspond to the stable attractors of these dynamical systems (Kauffman 1993). Within this framework, we represent GRNs as graphs (Wagner 1996; Siegal and Bergman 2002; Bergman and Siegal 2003; Ciliberti et al. 2007a,b) and we consider the genotype as the pair $\mathcal{G} = (G, g(0))$. G is the weighted adjacency matrix accounting for the interaction between genes, i.e. how a gene product affects the (in)activation of all other genes: $g_{ij} = \pm 1$ if there exists a link between nodes (genes) i and j , and $g_{ij} = 0$, otherwise. Positive (negative) interactions correspond to activating (inhibitory) interactions between genes. The vector $g(0)$ corresponds to the initial pattern of gene expression, which can be interpreted as heritable genetic information: the components of $g(0) = (g_i(0))$ are the states (active or inactive) of each gene when new cells are born. The phenotype is defined as the steady-state of the dynamical system defined by the following set of rules:

1. At $t = 0$, that is, at the time of birth of each cell, the initial condition $g(t = 0) = g_0$ is set.
2. At each time step, and for each gene i , the value of the quantity $I_i(t)$:

$$I_i = \sum_j g_{ji} g_j, \text{ where} \quad (1)$$

is determined

3. We determine the state of each gene at step $t + 1$ according to:

$$\begin{cases} g_i(t + 1) = 1, & \text{if } I_i(t) \geq 0 \\ g_i(t + 1) = -1, & \text{if } I_i(t) < 0 \end{cases}$$

4. Steps 2 and 3 are repeated until $t = T \gg 1$. The phenotype corresponding to the genotype $\mathcal{G} = (G, g_0)$, $\phi(\mathcal{G})$, is defined by $\phi(\mathcal{G}) = g(T)$

We further set forth conditions to discard genotypes which give rise to *unviable* phenotypes. Our viability conditions are related by those imposed by Siegal and Bergman (2002) and Bergman and Siegal (2003) [see Ibanez-Marcelo and Alarcon (2014b) for more details].

Based on this definition of the genotype–phenotype map and the viability conditions, which act as a selecting pressure against genotypes leading to unviable phenotypes, we have proposed a model which generates the corresponding genotype–phenotype network (Ibanez-Marcelo and Alarcon 2014b). Within this network, which

is an extension of the concept of *neutral network* (Wagner 2007), (viable) genotypes and phenotypes are represented by nodes of different types. Edges between nodes are also of two types. Edges between two genotype nodes correspond to *viable* gene mutations, i.e. mutations that generate a genotype associated to a viable phenotype. An edge between a genotype node, \mathcal{G}_i and a phenotype node, ϕ_j , exist if and only if $\phi(\mathcal{G}_i) = \phi_j$. In this formulation the neutral network associated to a phenotype, ϕ_j , is the set of its neighbours, i.e. all those genotypes such that $\phi(\mathcal{G}) = \phi_j$. Neutral networks corresponding to different phenotypes may be connected through genotype–genotype edges, i.e. via viable mutations producing two different (viable) phenotypes.

This pseudo-bipartite description of the genotype–phenotype network allows us to define several properties of the genotype–phenotype map in topological terms, in particular, robustness and evolvability (Ibanez-Marcelo and Alarcon 2014b). We have characterised phenotypic robustness by means of the clustering coefficient of the phenotype nodes. Our working definition of phenotypic robustness corresponds to the likelihood of a viable individual to retain its phenotype when gene mutations occur (Wagner 2007). According to this definition, the phenotype $\phi(\mathcal{G})$ of an individual with genotype \mathcal{G} is deemed to be robust if a random gene mutation, transforming the genotype $\mathcal{G} \rightarrow \mathcal{G}'$ where $\text{dist}(\mathcal{G}, \mathcal{G}') = 1$, distance between genotypes is defined as the Hamming distance between their associate matrices, G and G' , that is,

$$\text{dist}(G, G') = \sum_{i,j} \frac{|g_{ij} - g'_{ij}|}{2}, \quad (2)$$

does not affect the phenotype, i.e. if $\phi(\mathcal{G}) = \phi(\mathcal{G}')$. This definition has a topological equivalence in terms the properties of the pseudo-bipartite network defined in Ibanez-Marcelo and Alarcon (2014b) (see Supplementary Materials). Since $\text{dist}(\mathcal{G}, \mathcal{G}') = 1$ these two genotypes correspond to nodes linked by a genotype–genotype edge, $e_{\mathcal{G}, \mathcal{G}'}$. Moreover, if $\phi(\mathcal{G}) = \phi(\mathcal{G}')$ this means that both nodes \mathcal{G} and \mathcal{G}' are linked to the same phenotype node ϕ by genotype–phenotype edges. In other words, the genotype nodes \mathcal{G} and \mathcal{G}' and the phenotype node ϕ form a triangle within the pseudo-bipartite graph, that is equivalent to say that there is a connection between two nodes belonging to the neighbourhood of ϕ . Thus the number of such connections within the neutral network (also called neighbourhood) of a given phenotype (i.e. the basin of attraction of ϕ) is a direct measure of phenotypic robustness. Phenotypic robustness can thus be quantified by means of the local clustering coefficient of phenotype node ϕ , c_ϕ :

$$c_\phi = \frac{2|\{e_{\mathcal{G}, \mathcal{G}'} \mid \mathcal{G}, \mathcal{G}' \in \text{Neigh}_\phi\}|}{k_\phi(k_\phi - 1)} \quad (3)$$

where Neigh_ϕ is the neighbourhood for a node ϕ and k_ϕ is the degree of ϕ , i.e. the number of genotype nodes to which ϕ is connected.

Further topological characterisation is achieved in terms of the phenotype degree distribution, k_ϕ , i.e. the probability distribution associated to the number of genotypes connected to (i.e. bearing) a particular phenotype. We have shown that the phenotype degree distribution exhibit the fat tails characteristic of scale-free distributions

[see [Ibanez-Marcelo and Alarcon \(2014b\)](#), Figs. 4 and 5]. We have also check that c_ϕ and k_ϕ are strongly correlated: $c_\phi \approx (k_\phi - 1)^{-\alpha}$ with $\alpha \approx 1$ [as it has been done [Ibanez-Marcelo and Alarcon \(2014b\)](#)], i.e. robustness (clustering coefficient) and k_ϕ are inversely correlated: heavily connected phenotypes are less robust than poorly connected ones. Our aim is to analyse the effects of robustness and phenotypic connectedness on survival after evolutionary escape.

2.2 Population dynamics

Evolutionary escape has been studied in the context of multi-type Galton–Watson processes ([Iwasa et al. 2004](#); [Serra and Haccou 2007](#); [Ibanez-Marcelo and Alarcon 2014b](#)). This type of process is characterised by a lack of characteristic time scales, as its dynamic is generated by mere iteration of and individual progeny-generation process. Here, we aim to analyse the emergence of separation of time scales intrinsic to the evolutionary escape process and its associated consequences regarding the behaviour of the system. In order to account for the characteristic time scales, we resort to a description in terms of a continuous-time branching process with exponential life time distributions, which is closely related to the Galton–Watson process ([Kimmel and Axelrod 2002](#)).

We first define a multi-type birth-and-death process where each type is associated to a genotype, \mathcal{G}_i , so that $N_i(t)$ is the number of cells with genotype $i = 1, \dots, N_G$, where N_G is the number of viable genotypes. Our model of the genotype–phenotype map ([Ibanez-Marcelo and Alarcon 2014b](#)) assigns a phenotype to each genotype, i.e. $\phi_i = \phi(\mathcal{G}_i)$ where ϕ_i is the phenotype associated to genotype \mathcal{G}_i . Following to the model of evolutionary escape formulated in [Ibanez-Marcelo and Alarcon \(2014a\)](#), we assume that birth and death rates of each cell type depend on the phenotype rather than on the genotype. We thus consider the birth-and-death process defined by:

$$\begin{aligned} \text{Prob}(N_i(t + \Delta t) = N_i(t) + 1) &= \lambda(\phi_i)(1 - \mu)^2 \Delta t \\ \text{Prob}(N_i(t + \Delta t) = N_i(t) - 1) &= \sigma(\phi_i) \Delta t \\ \text{Prob}(N_i(t + \Delta t) = N_i(t), N_j(t + \Delta t) = N_j(t) + 1) &= 2\lambda(\phi_i)\mu(1 - \mu)\frac{a_{ij}}{d_i} \Delta t, \\ \text{Prob}(N_i(t + \Delta t) = N_i(t) - 1, N_j(t + \Delta t) = N_j(t) + 2) &= \lambda(\phi_i)\mu^2\frac{a_{ij}a_{ik}}{d_i^2} \Delta t, \end{aligned} \quad (4)$$

for all $j, k = 1, \dots, N_G$, where a_{ij} are the entries of the adjacency matrix of the genotype network, d_i is the degree of \mathcal{G}_i within the genotype network, $\lambda(\phi_i)$ and $\sigma(\phi_i)$ are the phenotype-dependent birth and death rates associated to \mathcal{G}_i , and μ is the mutation probability per division. For simplicity, we consider that $\lambda(\phi_i) = \lambda$ and $\sigma(\phi_i) = \sigma$ for all $\phi_i \neq \phi_E$ with $\lambda < \sigma$, and $\lambda(\phi_i) = \lambda_E$ and $\sigma(\phi_i) = \sigma_E$ for all $\phi_i = \phi_E$ with $\lambda_E > \sigma_E$. We can assume that $\lambda = \lambda_E$ and $\sigma \gg \sigma_E$.

We now define an associated embedded, continuous-time branching process ([Grimmett and Stirzaker 1992](#)). This branching process is characterised by the set of type-specific generating function of the probability density of the number of progeny produced by each cell: if $\phi_i \neq \phi_E$,

$$F_i(\mathbf{s}) = \frac{\sigma}{\lambda + \sigma} + \frac{\lambda(1 - \mu)^2}{\lambda + \sigma} s_i^2 + \sum_j 2 \frac{\lambda \mu (1 - \mu)}{\lambda + \sigma} \frac{a_{ij}}{d_i} s_i s_j + \sum_{j,k} \frac{\lambda \mu^2}{\lambda + \sigma} \frac{a_{ij} a_{ik}}{d_i^2} s_j s_k, \quad (5)$$

If $\phi_i = \phi_E$,

$$F_i(\mathbf{s}) = \frac{\sigma_E}{\lambda_E + \sigma_E} + \frac{\lambda_E(1 - \mu)^2}{\lambda_E + \sigma_E} s_i^2 + \sum_j 2 \frac{\lambda_E \mu (1 - \mu)}{\lambda_E + \sigma_E} \frac{a_{ij}}{d_i} s_i s_j + \sum_{j,k} \frac{\lambda_E \mu^2}{\lambda_E + \sigma_E} \frac{a_{ij} a_{ik}}{d_i^2} s_j s_k \quad (6)$$

where $F_i(\mathbf{s})$ is the probability generating function of the number of progeny generated by a cell with genotype \mathcal{G}_i and $\mathbf{s} = (s_1, \dots, s_G)$. Furthermore each genotype has an associated a survival time which is exponentially distributed with parameter $\omega = \lambda + \sigma$ if $\phi_i \neq \phi_E$ and $\omega_E = \lambda_E + \sigma_E$ if $\phi_i = \phi_E$. Note that $\omega \gg \omega_E$.

Similarly to the Galton–Watson process, the dynamics of the continuous-time branching process is generated by iterating the progeny-generation process, which mathematically translates into the following recursive equation for the generating function, $f_i(\mathbf{s}, t)$ (Kimmel and Axelrod 2002):

$$f_i(\mathbf{s}, t + \Delta t) = f_i(f_i(\mathbf{s}, t), \Delta t), \quad (7)$$

where $f_i(\mathbf{s}, t)$ is the generating function associated to the probability distribution of the population $(N_1(t), \dots, N_G(t))$ at time t generated from a single initial individual of (geno)type i . If Δt is small, then with probability close to one, the process consists of either the mother cell, provided that it survives, or its first-generation progeny, conditioned to the mother cell exhausts its life-span, i.e.

$$\begin{aligned} f_i(\mathbf{s}, \Delta t) &= s_i e^{-\omega \Delta t} + F_i(\mathbf{s})(1 - e^{-\omega \Delta t}) + o(\Delta t^2) \text{ if } \phi_i \neq \phi_E, \\ f_i(\mathbf{s}, \Delta t) &= s_i e^{-\omega_E \Delta t} + F_i(\mathbf{s})(1 - e^{-\omega_E \Delta t}) + o(\Delta t^2) \text{ if } \phi_i = \phi_E. \end{aligned} \quad (8)$$

2.2.1 Separation of time scales: quasi-steady state regime

The setting in which evolutionary escape occurs involves separation of time scales. Within this setting, all genotypes, \mathcal{G}_i , associated to phenotypes $\phi_i \neq \phi_E$ are sub-critical ($\sigma > \lambda$), whereas those genotypes associated to the escape phenotype ($\phi_i = \phi_E$) are super-critical, i.e. $\sigma_E < \lambda_E$. If we can assume that, for example, $\lambda = \lambda_E$ and $\sigma \gg \sigma_E$, which implies $\omega \gg \omega_E$, separation of time scales ensues. Consider Eq. (8) and the following re-scaled time variable $\tau = \omega_E t$. Carrying out this change of variables, Eq. (8) read:

$$\begin{aligned} f_i(\mathbf{s}, \Delta \tau) &= s_i e^{-\frac{1}{\epsilon} \Delta \tau} + F_i(\mathbf{s})(1 - e^{-\frac{1}{\epsilon} \Delta \tau}) + o(\Delta \tau^2) \text{ if } \phi_i \neq \phi_E, \\ f_i(\mathbf{s}, \Delta \tau) &= s_i e^{-\Delta \tau} + F_i(\mathbf{s})(1 - e^{-\Delta \tau}) + o(\Delta \tau^2) \text{ if } \phi_i = \phi_E. \end{aligned} \quad (9)$$

where $\epsilon = \frac{\omega_E}{\omega} \ll 1$. This implies that cell types associated to non-escape genotypes evolve much faster than those associated to escape genotypes. The former will therefore settle onto their equilibrium state, i.e. extinction, whilst the latter continue to evolve at a much slower rate. This becomes clearer if we write down the system of ODEs which govern the evolution of $f_i(\mathbf{s}, \tau)$ [see [Kimmel and Axelrod \(2002\)](#) for details of their derivation]:

$$\begin{aligned}\epsilon \frac{df_i(\mathbf{s}, \tau)}{d\tau} &= -f_i(\mathbf{s}, \tau) + F_i(f_1(\mathbf{s}, \tau), \dots, f_G(\mathbf{s}, \tau)) \text{ if } \phi_i \neq \phi_E \\ \frac{df_i(\mathbf{s}, \tau)}{d\tau} &= -f_i(\mathbf{s}, \tau) + F_i(f_1(\mathbf{s}, \tau), \dots, f_G(\mathbf{s}, \tau)) \text{ if } \phi_i = \phi_E,\end{aligned}\quad (10)$$

which explicitly shows that, under time re-scaling ($\tau = \omega_E t$), the population of non-escape genotypes evolves according to a fast dynamic and can be considered to be in (quasi-)steady state. The populations associated to escape genotypes follow a slow dynamic which evolves on a much slower time scale.

2.2.2 Separation of time scales: inner regime

Besides the long time dynamics associated to the quasi-steady state regime analysed in Sect. 2.2.1, we can study the initial, inner regime by re-scaling time: $T = \epsilon^{-1}\tau$. Under this re-scaling, Eq. (8) read:

$$\begin{aligned}f_i(\mathbf{s}, \Delta T) &= s_i e^{-\Delta T} + F_i(\mathbf{s})(1 - e^{-\Delta T}) + o(\Delta T^2) \text{ if } \phi_i \neq \phi_E, \\ f_i(\mathbf{s}, \Delta T) &= s_i e^{-\epsilon \Delta T} + F_i(\mathbf{s})(1 - e^{-\epsilon \Delta T}) + o(\Delta T^2) \text{ if } \phi_i = \phi_E.\end{aligned}\quad (11)$$

Equation (11) imply that, since $\epsilon \ll 1$, the rate of evolution of the populations associated to escape genotypes ($\phi_i = \phi_E$) is very slow, so that the most likely event is that cells with escape genotypes do not generate progeny, i.e., during this initial regime, cells associated to escape genotypes tend to stay latent (that is, they survive without producing offspring or dying). By contrast, the populations associated to non-escape genotypes ($\phi_i \neq \phi_E$) evolve at an $O(1)$ rate. The corresponding set of ODEs for the probability generating functions is:

$$\begin{aligned}\frac{df_i(\mathbf{s}, T)}{dT} &= -f_i(\mathbf{s}, T) + F_i(f_1(\mathbf{s}, T), \dots, f_G(\mathbf{s}, T)) \text{ if } \phi_i \neq \phi_E \\ \frac{df_i(\mathbf{s}, T)}{dT} &= -\epsilon (f_i(\mathbf{s}, T) - F_i(f_1(\mathbf{s}, T), \dots, f_G(\mathbf{s}, T))) \text{ if } \phi_i = \phi_E,\end{aligned}\quad (12)$$

which further confirm that, during this initial regime, the populations associated to escape genotypes stays frozen, whereas the non-escape populations evolves at a rate $O(1)$.

These properties regarding time scale separation have the consequence that, during this initial regime, population accumulates within the escape genotypes. The source of this population is mutations occurring in cells associated with non-escape genotypes but which are first neighbours of escape genotypes. According to our analysis,

these cells remain latent during the initial regime. Therefore the total number of cells accumulated within the escape phenotype during the initial regime ($T = 1$), N_0 , can be estimated by [Daniel \(2001\)](#) and [Ball et al. \(2006\)](#):

$$N_0 = Y \left(\frac{2\lambda}{\omega} \sum_{i \in \partial\phi_E} \int_0^1 \left(\mu(1-\mu) \sum_{j \in \langle \phi_E \rangle} \frac{a_{ij}}{d_i} + \mu^2 \sum_{j, k \in \langle \phi_E \rangle} \frac{a_{ij}a_{ik}}{d_i^2} \right) N_i(T) dT \right) \quad (13)$$

$Y(s)$ is a Poisson-distributed random number with parameter s and $\partial\phi_E$ is the sub-set of non-escape genotypes of the genotype networks which are first-neighbours with an escape genotype: $\mathcal{G}_i \in \partial\phi_E$ if $\phi_i \neq \phi_e$ and there exists at least one \mathcal{G}_j such that $\phi_j = \phi_E$ and $a_{ij} = 1$. Note that the cardinal of $\partial\phi_E$ is proportional to the degree of the escape phenotype, k_E , and therefore, from Eq. (13), we expect N_0 be an increasing function of k_E , too: the bigger k_E , the bigger the boundary between the escape phenotype and the remaining of the network, and the more access ways for population to enter the escape phenotype.

2.2.3 Coarse-grained population dynamics of the escape phenotype

In view of the picture arising from the discussion of Sects. 2.2.1 and 2.2.2 regarding separation of time scales, we propose a coarse-grained population dynamics of the escape phenotype, associated to the quasi-steady state regime in which the non-escape genotypes have already become extinct, and where we look at the total population of the escape phenotype, rather than looking at the populations associated to the escape genotypes. In order to formulate this coarse-grained dynamics, we invoke some of the topological properties analysed in [Ibanez-Marcelo and Alarcon \(2014b\)](#).

We have shown that, within the genotype–phenotype network defined in [Ibanez-Marcelo and Alarcon \(2014b\)](#), we can associate a clustering coefficient to each phenotype, where the clustering is associated to the proportion of genotypes which are first neighbours [i.e., according to the definition of the genotype network in [Ibanez-Marcelo and Alarcon \(2014b\)](#), genotypes that are separated by a one-hit mutation] and which share the same phenotype. If we assume that the clustering coefficient of the escape phenotype, c_E , models the probability that a gene mutation which changes \mathcal{G}_i into \mathcal{G}_j without changing phenotype, i.e. $\phi_i = \phi_j = \phi_E$: the probability of a gene mutation to produce a change of phenotype is equal to $\mu(1 - c_E)$. Similarly, the probability of a phenotype-preserving mutation is μc_E . Furthermore, we have shown that $c_E(k_E) \approx (k_E - 1)^{-\alpha}$ with $\alpha \approx 1$ ([Ibanez-Marcelo and Alarcon 2014b](#)).

By taking into account this interpretation of the clustering coefficient, we can define the following coarse-grained continuous-time branching process:

$$F_E(s) = \frac{1}{\omega_E} \left(\begin{array}{ll} \sigma_E + \lambda_E \mu^2 (1 - c_E)^2 & \text{Death} \\ + \lambda_E (2\mu(1 - c_E)(1 - \mu) + 2\mu^2 c(1 - c))s & \text{Survival} \\ + \lambda_E ((1 - \mu)^2 + 2\mu(1 - \mu)c_E + \mu^2 c_E^2)s^2 & \text{Proliferation} \end{array} \right) \quad (14)$$

with a life-time which is exponentially distributed with parameter ω_E . We have further assume that cells which mutate an change phenotype die, as they fall back into a non-escape phenotype which is sub-critical.

2.2.4 Alternative measure of robustness

Results (see Sect. 3) show that, although c_E is a measure of robustness which subtly correlates with P_S (see Fig. 6), a measure of robustness better suited for the coarse-grained, long-time dynamics of survival upon escape, must be defined. To do so, we proceed with a more detailed analysis of Eq. (10). Before proceeding we introduce the following notation: $\mathbf{1} = (1, \dots, 1)$, $\mathbf{1}_E$ is the vector with ones in the escape components and zeros otherwise, and $\mathbf{1}_N = \mathbf{1} - \mathbf{1}_E$. We define $\mathbf{g}(\tau) = (\mathbf{g}_N, \mathbf{g}_E)$ as the split vector, first components are associated to non escape genotypes, \mathbf{g}_N , and \mathbf{g}_E corresponds to indexes associated to escape genotypes.

Making some algebra we can rewrite $\mathbf{F}(\mathbf{s})$ as,

$$\mathbf{F}(\mathbf{s}) = \mathbf{1} + D(-\mathbf{1} + B \cdot \mathbf{s} \odot B \cdot \mathbf{s}) \quad (15)$$

where $B = \mu D^{-1}A + (1 - \mu)\text{Id}$, $D = \text{diag}(\gamma_i)$ ($\gamma_i = \frac{\lambda}{\lambda + \sigma}$ if $\phi_i \neq \phi_E$, $\gamma_i = \frac{\lambda_E}{\lambda_E + \sigma_E}$ if $\phi = \phi_E$) and \odot denotes the component-to-component product.

As we have shown in a previous work (Ibanez-Marcelo and Alarcon 2014a), if we define $g_i(\tau) = P(N_E(\tau) > 0 \mid \text{IC}_i)$, then $\mathbf{f}(\mathbf{1}_N, \tau) = \mathbf{1} - \mathbf{g}(\tau)$ is the complementary of the escape probability. Therefore, $g_i(\tau) \ll g_j(\tau)$ if $i \notin \phi_E$, $j \in \phi_E$, where N_E is the number of cells in the escape phenotype. Then, Eq. (10) read,

$$\epsilon \frac{d\mathbf{g}_N(\tau)}{d\tau} = -\mathbf{g}_N(\tau) + \frac{\lambda}{\lambda + \sigma} (2B \cdot \mathbf{g}_N(\tau) - (B \cdot \mathbf{g}_N(\tau) \odot B \cdot \mathbf{g}_N(\tau))) \quad (16)$$

$$\frac{d\mathbf{g}_E(\tau)}{d\tau} = -\mathbf{g}_E(\tau) + \frac{\lambda_E}{\lambda_E + \sigma_E} (2B \cdot \mathbf{g}_E(\tau) - (B \cdot \mathbf{g}_E(\tau) \odot B \cdot \mathbf{g}_E(\tau))) \quad (17)$$

We split B into four submatrices:

$$B = \begin{pmatrix} B_{NN} & B_{NE} \\ B_{EN} & B_{EE} \end{pmatrix}$$

where B_{NN} corresponds to submatrix of non escape indexes, B_{EE} is the submatrix of escape indexes and similarly for B_{EN} and B_{NE} . In terms of these submatrices, Eq. (17) can be rewritten as,

$$\begin{aligned} \frac{d\mathbf{g}_E}{d\tau} = -\mathbf{g}_E + \frac{\lambda_E}{\lambda_E + \sigma_E} [2(B_{EE} \cdot \mathbf{g}_E + B_{EN} \cdot \mathbf{g}_N) \\ - (B_{EE} \cdot \mathbf{g}_E + B_{EN} \cdot \mathbf{g}_N) \odot (B_{EE} \cdot \mathbf{g}_E + B_{EN} \cdot \mathbf{g}_N)] \end{aligned} \quad (18)$$

As $(\mathbf{g}_N)_i \ll (\mathbf{g}_E)_j$, we can neglect \mathbf{g}_N . Equation (18) then reads,

$$\frac{d\mathbf{g}_E}{d\tau} = -\mathbf{g}_E + \frac{\lambda_E}{\lambda_E + \sigma_E} [2B_{EE} \cdot \mathbf{g}_E - (B_{EE} \cdot \mathbf{g}_E \odot B_{EE} \cdot \mathbf{g}_E)] \quad (19)$$

In order to study the behaviour of \mathbf{g}_E , we first consider the simplest possible case. We assume $|\{\phi(\mathcal{G}_i) = \phi_E\}| = 1$, i.e. there is only one genotype \mathcal{G}_i in ϕ_E . This allows us to write Eq. (19) as,

$$\frac{dg_E}{d\tau} = -g_E + \frac{\lambda_E}{\lambda_E + \sigma_E} \left[2(1 - \mu)g_E - (1 - \mu)^2 g_E^2 \right] \quad (20)$$

At steady state Eq. (20) is,

$$\begin{aligned} 0 &= -g_E + \frac{\lambda_E}{\lambda_E + \sigma_E} \left[2(1 - \mu)g_E - (1 - \mu)^2 g_E^2 \right] \\ &= g_E \left(1 - \frac{\lambda_E}{\lambda_E + \sigma_E} (2 - 2\mu) + \frac{\lambda_E}{\lambda_E + \sigma_E} (1 - \mu)^2 g_E \right) \end{aligned} \quad (21)$$

with non-trivial solution

$$g_E = \frac{2}{1 - \mu} - \frac{\lambda_E + \sigma_E}{\lambda_E(1 - \mu)^2}.$$

Now, consider the case of $|\{\phi(\mathcal{G}_i) = \phi_E\}| = 2$. This implies that $B = B_{EE}$,

$$B_{EE} = \begin{pmatrix} 1 - \mu & a_{12}/d_1 \\ a_{21}/d_2 & 1 - \mu \end{pmatrix}$$

Then,

$$\text{if} = \begin{cases} a_{12} = 0 (\implies a_{21} = 0) \implies \text{we are in case } |\{\phi(\mathcal{G}_i) = \phi_E\}| = 1, \\ \quad \text{as both nodes are disconnected,} \\ a_{12} = 1 (\implies a_{21} = 1) \implies \mathbf{g}_E \text{ will depend on } d_1 \text{ and } d_2. \end{cases}$$

where, d_i is the degree of genotype \mathcal{G}_i in the genotype network. This simple case shed some light on what kind of parameters affect the value of \mathbf{g}_E . This simple case illustrates that the clustering coefficient does not characterise the evolution of \mathbf{g}_E , as we need to take the degree of the first neighbours into account.

Therefore, we define the following measure of robustness, M_E , of an escape phenotype ϕ_E :

1. Generate genotype subgraph, G_{ϕ_E} , of nodes corresponding to genotypes \mathcal{G}_i , such that, $\phi(\mathcal{G}_i) = \phi_E$.
2. Associate weights, w_{ij} for each (i, j) edge in G_{ϕ_E} .
3. Define $w_{ij} = \frac{1}{d_i} + \frac{1}{d_j}$.
4. Then,

$$M_E = \frac{\sum_{(ij)} w_{ij}}{|\{\phi(\mathcal{G}_i) = \phi_E\}|}, \quad M_E \in [0, 1].$$

Fig. 1 Two different graphs with extreme values of M_E . Graph **a** has $M_E = 0$, whereas graph **b** has $M_E = 1$. More details in Table 1

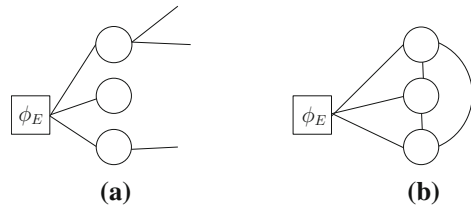
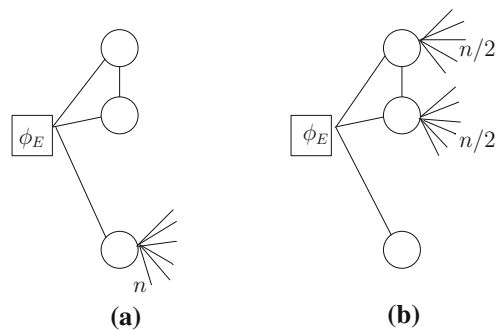


Table 1 Comparison of graphs of Fig. 1

Parameters	(a) Graph	(b) Graph
k_E	3	3
c_E	0	1
G_{ϕ_E}	$\begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$	$\begin{pmatrix} 0 & 1 & 1 \\ 1 & 0 & 1 \\ 1 & 1 & 0 \end{pmatrix}$
$G_{w\phi_E}$	$\begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$	$\begin{pmatrix} 0 & 1/2 + 1/2 & 1/2 + 1/2 \\ 1/2 + 1/2 & 0 & 1/2 + 1/2 \\ 1/2 + 1/2 & 1/2 + 1/2 & 0 \end{pmatrix}$
M_E	0	1

Fig. 2 Two different graphs with very different M_E . Graph **a** has $M_E = 2/3$, whereas graph **b** has $M_E = 4/3(n+2)$. More details in Table 2



Note that $M_E = 0$ is associated to escape phenotypes, ϕ_E , for which their escape associated genotypes are not interconnected, i.e. $M_E = 0 \implies c_E = 0$. On the other hand, if $c_E = 0$ implies that it does not exist any connection between escape genotypes. Then $M_E = 0$, in other words, $M_E = 0 \Leftrightarrow c_E = 0$. At the other extreme, $M_E = 1$ corresponds to an isolated escape phenotype, but it is not required that all possible connections between escape genotypes exist. If it is the case, then $c_E = 1$ (see Fig. 1; Table 1). In general $c_E = 1$ does not imply $M_E = 1$ or vice versa.

An intuitive handle on the meaning of M_E can be obtained by comparing the examples shown in Fig. 2. Both these graphs have $c_E = 1/3$. However, M_E takes different values and, therefore, it allows to distinguish between both cases. Note that survival is less likely in graph (b) than in graph (a). From these examples, we observe that M_E provides more information than c_E because M_E accounts for inhomogeneities between nodes. In other words, M_E carries more local information regarding the genotypes than c_E .

Table 2 Comparison parameters graphs of Fig. 2

Parameters	(a) Graph	(b) Graph
k_E	3	3
c_E	1/3	1/3
k_{nn} (second neighbours)	n	n
$G\phi_E$	$\begin{pmatrix} 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$	$\begin{pmatrix} 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$
$Gw\phi_E$	$\begin{pmatrix} 0 & 1+1 & 0 \\ 1+1 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$	$\begin{pmatrix} 0 & \frac{1}{n/2+1} + \frac{1}{n/2+1} & 0 \\ \frac{1}{n/2+1} + \frac{1}{n/2+1} & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$
M_E	$\frac{2}{3}$	$\frac{4}{3(n+2)}$

A definition for the global weighted clustering coefficient M_E , can be thought as a local clustering coefficient for a weighted network. Given an undirected, unweighted network, clustering coefficient of a node i , C_i is equal to the fraction of number of edges between neighbourhood of i . Then, given a undirected weighted network, we define C_i as the fraction between the sum of weights in edges between i -neighbourhood and the maximum value that can weights achieve, that is, k_i . On the other hand, these weights w_{ij} represent the sum of the probability of going from i to j and the one of going from j to i . This is equivalent to M_E which is normalised by k_E .

2.2.5 Redefinition of the escape phenotype coarse-grained dynamics

Using M_E as a measure of robustness, the coarse-grained dynamics of an escape phenotype is defined by the pgf:

$$F_E(s) = \frac{1}{\omega_E} \left(\sigma_E + \lambda_E \mu^2 (1 - M_E)^2 \right. \quad \text{Death} \\
+ \lambda_E (2\mu(1 - M_E)(1 - \mu) + 2\mu^2 M_E(1 - M_E))s \quad \text{Survival} \quad (22) \\
\left. + \lambda_E ((1 - \mu)^2 + 2\mu(1 - \mu)c_E + \mu^2 M_E^2)s^2 \right) \quad \text{Proliferation}$$

with a life-time which is exponentially distributed with parameter ω_E (see Sect. 2.2.3).

3 Results

Having established the separation of time scales in Sect. 2, where an (inner) fast regime associated to the escape process is followed by an outer, slower dynamics related to the within-escape phenotype dynamics, we proceed to analyse in detail the eventual survival of the population upon escape (i.e. upon the population having reached the escape phenotype). We will first study the escape process (as determined by the dynamics within the inner regime), in particular, we focus on how the escape probability and the number of cells reaching the escape phenotype depend on the degree of the escape phenotype, k_E . We then proceed to study the eventual survival

conditioned to escape, which corresponds to the outer regime and it is analysed using a coarse-grained model (see Sects. 2.2.3 and 2.2.5).

We run our population dynamical models, in particular those corresponding to fast dynamics associated to escape Eq. (11), on three different realisations of the genotype–phenotype network generated according to our multi-scale model proposed in [Ibanez-Marcelo and Alarcon \(2014b\)](#). A full account of this model is given in the Supplementary Materials.

3.1 Fast dynamics: escape properties as a function of k_E and c_E

In this Section we study escape properties during the inner regime. We show how escape probabilities can be computed and how to compute the average expected number of cells accumulated during the fast dynamics. All of these quantities are related with the degree and the clustering coefficient of the escape phenotype, we introduce in what way these correlations appears.

3.1.1 Escape probability

Given an escape phenotype ϕ_E we define the initial condition IC_i as $N_i(0) = 1$ and $N_j(0) = 0$ for all $j \neq i$ and we assume $\phi(\mathcal{G}_i) \neq \phi_E$. The probability of escape $P_E(T)$ is the probability to achieve the escape phenotype before extinction starting from the configuration IC_i and averaged for all possible initial conditions. This process happens in the inner regime. This probability can be computed in a similar way than it was done for the discrete model in [Ibanez-Marcelo and Alarcon \(2014a\)](#). Briefly, it can be checked that $P_E(T)$ coincides with the evaluation of the pgf at the parameter $\mathbf{s} = \mathbf{1}_N$. As the pgf evolves according to an ODE [Eq. (12)], we can integrate numerically it to obtain the desired values $P_E(T)$. Moreover, as we are interested in the inner regime we only compute $P_E(T)$ up to time $T = 1$.

Figure 3 shows results regarding how $P_E(T)$ changes as we vary the degree k_E (Fig. 3a, c) and the clustering coefficient ϕ_E (Fig. 3b). We observe that $P_E(T)$ is positively correlated with k_E , i.e. the larger the number of genotypes which belong the escape phenotype, the bigger (on average) is the probability of achieve the escape phenotype during the inner regime. Otherwise, the negative correlation between $P_E(T)$ and c_E is a direct consequence of $c_E(k_E) \approx (k_E - 1)^{-\alpha}$ ([Ibanez-Marcelo and Alarcon 2014b](#)).

3.1.2 Average number of cells accumulated within the escape phenotype during the initial regime

In order to calculate the average number of cells that reach the escape phenotype during the fast dynamics regime, we proceed as follows. Consider all the genotypes \mathcal{G}_k such that $\phi_E = \phi(\mathcal{G}_k)$. We are interested in computing the expectation $\mathbb{E}(N_k(T) \mid IC_i)$, which is given by [Kimmel and Axelrod \(2002\)](#):

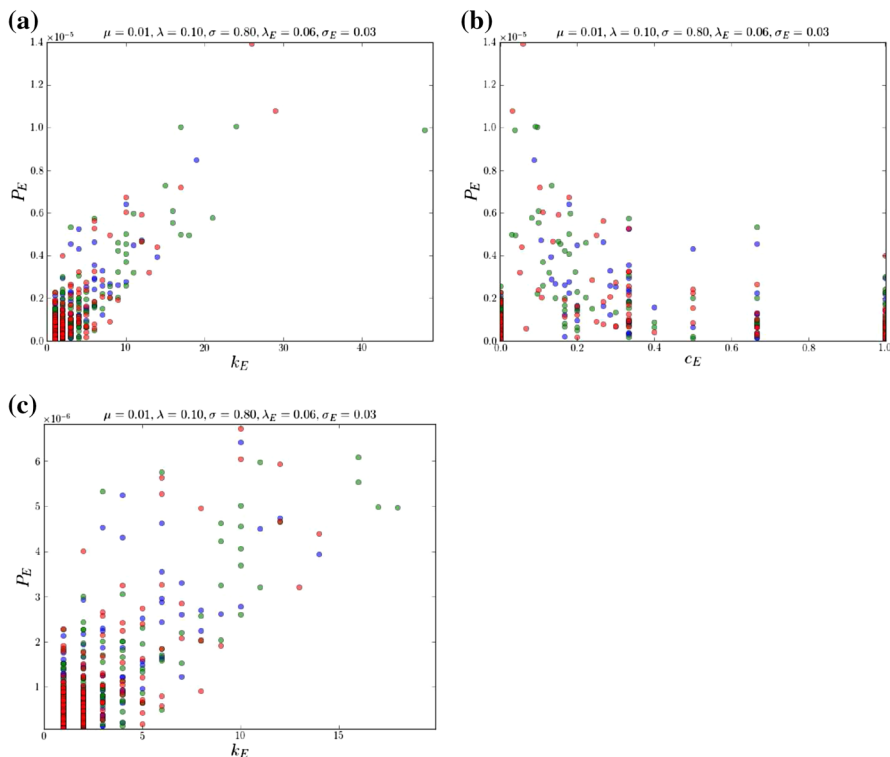


Fig. 3 Probability of escape in the time-continuous model. *Different colours* represent results for different graphs. As we expected, P_E is positive correlated with k_E (plot **a**) and negatively correlated with c_E (plot **b**), in agreement with the relation that exists between clustering coefficient and degree. Plot **c** represents a zoom of **a** (colour figure online)

$$\mathbb{E}(N_k(T) \mid \text{IC}_i) = \frac{\partial f_i}{\partial s_k}(\mathbf{1}, T), \quad (23)$$

therefore the ODE governing the evolution of this quantity can be obtained from Eq. (12). By applying ∂_{s_k} to that system,,

$$\frac{d}{dT} \frac{\partial f_i}{\partial s_k}(\mathbf{s}, T) = -\frac{\partial f_i(\mathbf{s}, T)}{\partial s_k} + \sum_l \frac{\partial F_i}{\partial s_l} \Big|_{\mathbf{f}(\mathbf{s}, T)} \cdot \frac{\partial f_l}{\partial s_k}(\mathbf{s}, T). \quad (24)$$

Evaluating at $\mathbf{s} = \mathbf{1}$, we obtain

$$\frac{d}{dT} \frac{\partial f_i}{\partial s_k}(\mathbf{1}, T) = -\frac{\partial f_i(\mathbf{1}, T)}{\partial s_k} + \sum_l \frac{\partial F_i}{\partial s_l} \Big|_{\mathbf{1}} \cdot \frac{\partial f_l}{\partial s_k}(\mathbf{1}, T) \quad (25)$$

In order to proceed further, we define the vector $\beta_k(T)$ whose components are $\beta_k(T) = (\beta_{k,i}(T), i = 1, \dots, N_G) = \left(\frac{\partial f_i}{\partial s_k}(\mathbf{1}, T), i = 1, \dots, N_G \right)$, i.e. the i th com-

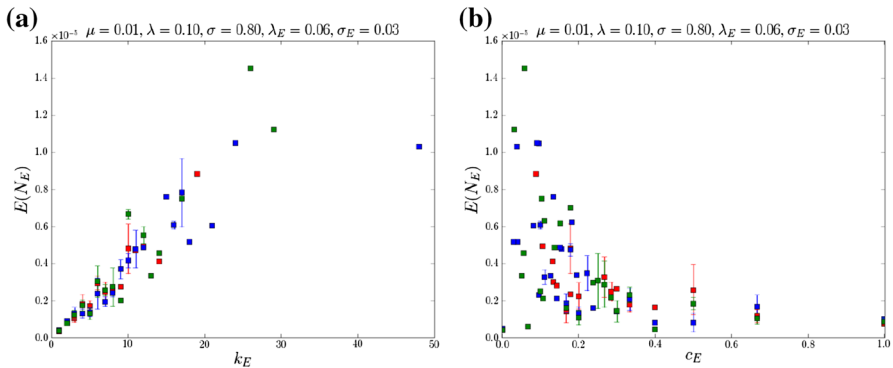


Fig. 4 Plots showing the dependence of the unconditioned average number of cells accumulated within the escape phenotype during the initial regime, $\mathbb{E}(N_E)$. Plot **a** shows how N_E varies as the degree of the escape phenotype, k_E , changes. Plot **b** shows results regarding the behaviour of $\mathbb{E}(N_E)$ as the clustering coefficient, c_E , of the escape phenotype varies. We observe that $\mathbb{E}(N_E)$ is positively correlated with k_E and negatively correlated with c_E (colour figure online)

ponent of $\beta_k(T)$ is the average population of cell of genotype \mathcal{G}_k at time T with initial conditions given by IC_i . From Eq. (25) we derive a linear ODE for the time evolution of $\beta_k(T)$:

$$\frac{d}{dT} \beta_k(T) = -\beta_k(T) + DF(\mathbf{1}) \cdot \beta_k(T) = (-\text{Id} + DF(\mathbf{1})) \cdot \beta_k(T), \quad (26)$$

with $(DF(\mathbf{1}))_{ij} = \frac{\partial F_i}{\partial s_j}(\mathbf{1})$. The solution of Eq. (26) is given by:

$$\beta_k(T) = \exp((- \text{Id} + DF(\mathbf{1})) \cdot T) \cdot \beta_k(0) \quad (27)$$

where $\beta_k(0) = (\delta_{1k}, \delta_{2k}, \dots, \delta_{N_G k})$. Finally, we define $\mathbb{E}(N_E(T=1))$ as the expected number of cells that reach the escape phenotype during the initial regime process (see Sect. 2.2.2) averaged over all possible initial conditions:

$$\mathbb{E}(N_E(T=1)) = \frac{1}{N_G - k_E} \sum_{k \in \langle \phi_E \rangle} \mathbf{1}_N \cdot \beta_k(s) \quad (28)$$

note, $\mathbf{1} \cdot \mathbf{1}_N = N_G - k_E$.

Figure 4 shows results regarding how $\mathbb{E}(N_E)$ ($N_E = N_E(T=1)$) changes as we vary the degree (Fig. 4a) and the clustering coefficient (Fig. 4b) of the escape phenotype. We observe that $\mathbb{E}(N_E)$ is positively correlated with k_E , i.e. the larger the number of genotypes which bear the escape phenotype, the bigger (on average) is the number of cells which accumulate within the escape phenotype during the initial, fast dynamics regime. This behaviour is a consequence of the fact that, in general, the larger k_E , the more likely is that cells trying to escape the ill-adapted genotypes find a route into the escape phenotype. The negative correlation between $\mathbb{E}(N_E)$ and c_E

is then a direct consequence of $c_E(k_E) \approx (k_E - 1)^{-\alpha}$ (Ibanez-Marcelo and Alarcon 2014b).

In addition to $\mathbb{E}(N_E)$, since we are interested in studying survival to escape, we need to analyse the average number of cells that reach the escape conditioned to eventual escape, $\mathbb{E}(N_E | N_0 > 0)$. To compute this quantity, we first consider $\mathbb{E}(N_k(T) | IC_i, N_0 > 0)$, i.e. the expected value of the population of the genotype \mathcal{G}_k such that $\phi(\mathcal{G}_k) = \phi_E$ with initial condition IC_i , which is given by:

$$\begin{aligned} \mathbb{E}(N_k(T) | IC_i, N_0 > 0) &= \sum_k n_k P(N_k(T) = n_k | IC_i, N_0 > 0) \\ &= \frac{\sum_k n_k P(N_k(T) = n_k; N_0 > 0 | IC_i)}{P(N_0 > 0)} \\ &= \frac{\sum_k n_k P(N_k(T) = n_k | IC_i)}{P(N_0 > 0)} \\ &= \frac{\mathbb{E}(N_k(t) | IC_i)}{P(N_0 > 0 | IC_i)} \end{aligned} \quad (29)$$

Equation (29) implies that to compute $\mathbb{E}(N_E | N_0 > 0)$ we must renormalise Eq. (28) by a factor which is equal to the escape probability, $P(N_0 > 0 | IC_i)$, computed as P_E in Sect. 3.1.1, (where we only consider those initial conditions, IC_i , for which $P(N_0 > 0 | IC_i) > 0$):

$$\mathbb{E}(N_E | N_0 > 0) = \frac{1}{N_G - k_E} \sum_{k \in (\phi_E)} \sum_{i=1}^{N_G} \frac{\beta_{k,i}(s)}{P(N_0 > 0 | IC_i)} ds \quad (30)$$

The behaviour of $\mathbb{E}(N_E | N_0 > 0)$ as k_E and c_E vary is shown in Fig. 5a, b, respectively. We find that $\mathbb{E}(N_E | N_0 > 0)$ and k_E are positively correlated (see Fig. 5a), although the observed correlation appears to be weaker for the conditioned average than for the unconditioned one (compare to Fig. 4a). Similarly, $\mathbb{E}(N_E | N_0 > 0)$ and c_E are negatively correlated (see Fig. 5b).

3.2 Slow dynamics: survival probability as a function of the clustering coefficient and the degree of the escape phenotype

We now proceed to examine the post-escape dynamics, i.e. once the fast, transient regime in which escape occurs and described by Eq. (12), a dynamical regime characterised by much longer time scales ensues in which, conditioned to the occurrence of escape, the population may still get extinct due to the within-escape phenotype population dynamics. We define $P_S(t)$ as the survival probability at time t , that is $P_S(t) = P(N_E(t) > 0 | IC_i)$, where now IC_i corresponds to an i with belongs to the escape phenotype. This probability is computed for a large time ($t \rightarrow \infty$). We model this slow, long-time regime using two different coarse-grained models. The first model, depending on clustering coefficient, is described in Sect. 2.2.3. The second model, depending on weighted clustering, M_E , is introduced in Sect. 2.2.5. In both

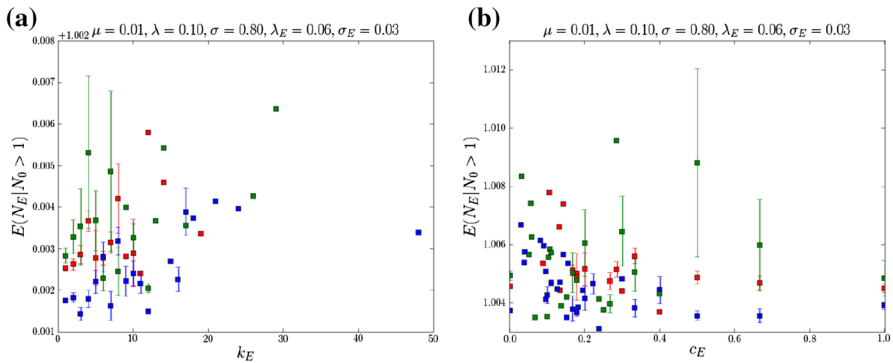


Fig. 5 Plots showing the dependence of the average number of cells accumulated within the escape phenotype conditioned to eventual escape, $\mathbb{E}(N_E | N_0 > 0)$, as we vary the degree of the escape phenotype, k_E . *Plot a* shows how $\mathbb{E}(N_E | N_0 > 0)$ varies as the degree of the escape phenotype, k_E , changes. *Plot b* shows results regarding the behaviour of $\mathbb{E}(N_E | N_0 > 0)$ as the clustering coefficient, c_E , of the escape phenotype varies. We observe that $\mathbb{E}(N_E | N_0 > 0)$ is positively correlated with k_E and negatively correlated with c_E , although the such correlation is weaker than for the unconditioned average $\mathbb{E}(N_E)$ (colour figure online)

models, the theoretical survival probability, P_S , is the complementary of the clearance probability, P_C (see Sect. 2.2.5) $P_S = 1 - P_C$. The clearance probability is given by smaller root of see [Kimmel and Axelrod \(2002\)](#):

$$F_E(P_C) - P_C = 0, \quad (31)$$

This is a second order equation which can be solved exactly. Its two roots, x_1 and x_2 , are given by

$$x_1 = 1, \quad x_2 = \frac{\sigma + \lambda\mu^2(1 - \alpha)^2}{\lambda(1 - \mu(1 - \alpha))^2},$$

where $\alpha = c_E$ in the first model, and $\alpha = M_E$ in the second model. $P_C = x_2$, therefore $P_S = 1 - x_2$. Note that P_S is the survival probability conditioned to escape, as the relation $P_S = 1 - P_C$ assumes that at least one cell has reached the escape phenotype.

In the first model, the behaviour of P_S as we vary c_E and k_E using $F_E(c_E)$ is shown in Fig. 6a, b, respectively. We find in this case that $P_S(t)$ is not well approached by theoretical P_S and its relation with c_E and k_E is not clear.

Nevertheless, in the second model, the survival probability $P_S(t)$, approached theoretically by,

$$P_S = 1 - \frac{\sigma_E + \lambda_E\mu^2(1 - M_E)^2}{\lambda_E(1 - \mu(1 - M_E))^2} = \frac{-\sigma_E - \lambda_E + 2\lambda_E(1 - \mu + \mu M_E)}{\lambda_E(1 - \mu + \mu M_E)^2} \quad (32)$$

models perfectly $P_S(t)$ as a function of M_E (see Fig. 7). Clearly, there are a strong positive correlation between $P_S(t)$ and M_E .

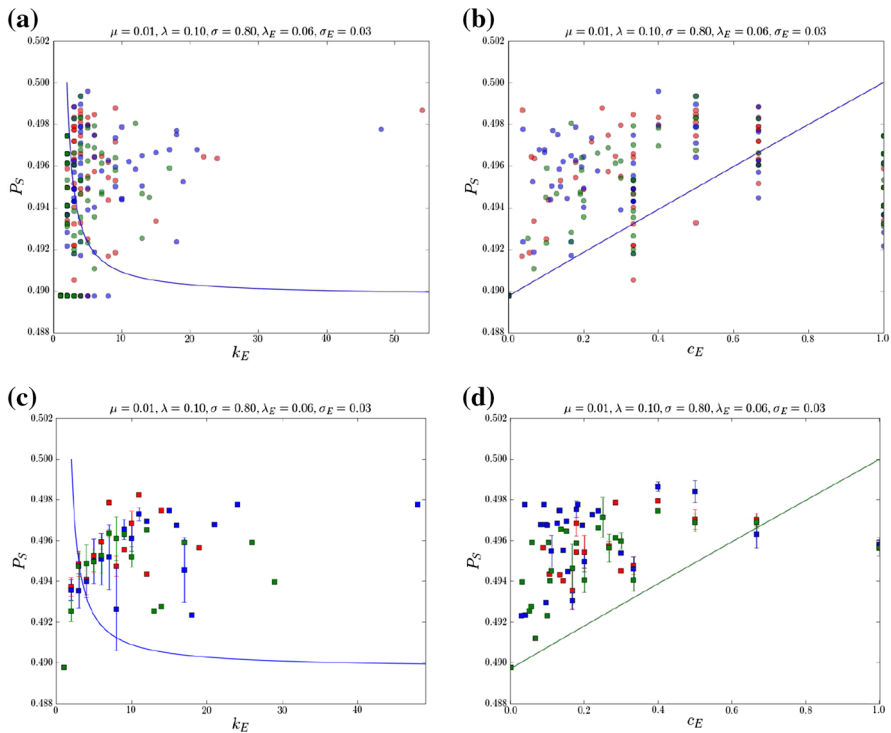
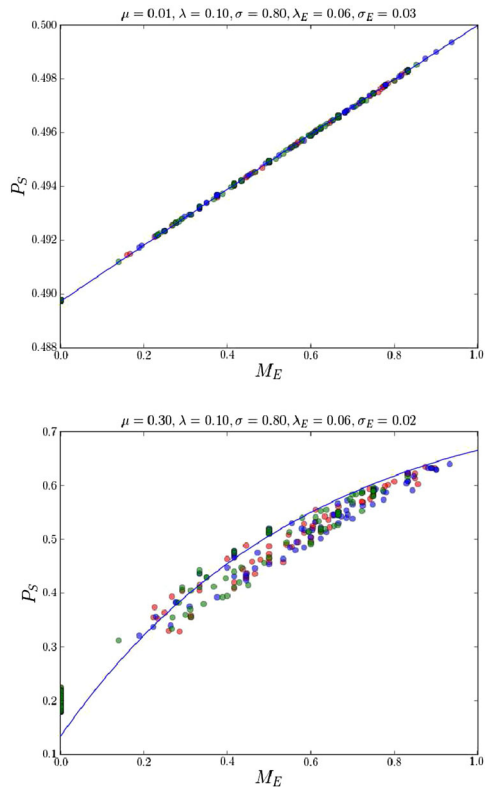


Fig. 6 Probability of survival conditioned to escape as a function of k_E (a, c) and c_E (b, d). We consider an initial condition where $N_i(\tau = 0) = 1$ for a randomly chosen \mathcal{G}_i so that $\phi(\mathcal{G}_i) = \phi_E$. Solid line represents the theoretical P_S from $(F_E(c_E))$, if we suppose that one individual go out from the escape phenotype, then is unable to reach again the escape. Top scatter plot. Bottom joining by degree (c), clustering (d). Different colours represent results for different graphs (colour figure online)

4 Conclusions

We have analysed the problem of evolutionary escape and survival for cell populations with genotype–phenotype map. Prior approaches to this problem, which do not consider populations with genotype–phenotype structure and associate fitness values directly to genotypes (Iwasa et al. 2003, 2004; Serra 2006; Serra and Haccou 2007; Sagitov and Serra 2009), have focused on the problem of estimating the probability of reaching a well-adapted (so-called escape) genotype for an initial population entirely composed of cells with ill-adapted genotypes. This point of view implicitly assumes that, once the escape genotype has been reached, the populations survives with probability one. When the genotype–phenotype map is added to the picture, the situation becomes more complex (Ibanez-Marcelo and Alarcon 2014a): genotype–phenotype structure endows complex structure to the escape phenotype which, in particular, provides robustness to the escape phenotype. Under genotype–phenotype structure, each phenotype has an associated neutral network which possess a rather reach topology (Wagner 2007; Aguirre et al. 2011; Ibanez-Marcelo and Alarcon 2014b), so that the dynamics of the system post-escape is not trivial.

Fig. 7 Plot showing the correlation between survival probability $P_S(t)$ and the new measure M_E for two different sets of parameters. *Solid line* corresponds to the theoretical P_S [approach in Eq. (32)]. *Different colours of dots* represent results for different graphs (colour figure online)



In order to explore this issue, we have formulated a population dynamics model, consisting of a multi-type time-continuous branching process, where types are associated to genotypes and their birth and death probabilities depend on the associated phenotype (non-escape or escape) via the genotype–phenotype map defined in [Ibanez-Marcelo and Alarcon \(2014b\)](#). We have shown that, within the setting associated to the escape problem, where the types associated to non-escape phenotypes are sub-critical and types associated to the escape phenotype are super-critical, separation of time scales naturally arises and two dynamical regimes emerge: a fast-decaying regime, accounting for the early stages in the evolution of the system, and associated to the escape process itself, and a slow regime which corresponds to the (survival) dynamics of the population once the escape phenotype has been reached (i.e. conditioned to escape). This separation of time scales has been exploited to analyse the topological factors which determine escape and survival. In particular, we have analysed the influence of topological properties associated to robustness and evolvability on the probability of escape and on the probability of survival upon escape.

We have shown that, while the escape probability depends on size of the neutral network of the escape phenotype (i.e. its degree), the probability of survival is essentially determined by its robustness (i.e. the resilience of the escape phenotype against genetic mutations). We have shown that the simple topological of measure pheno-

typic robustness defined in Ibanez-Marcelo and Alarcon (2014b), i.e. the clustering coefficient, is not well-adapted to describe robustness in the context of the coarse-grained dynamics of survival. Rather, a new measure in terms of a weighted clustering coefficient is necessary to accurately account for robustness under said dynamics.

Our analysis of the fast-decaying, initial regime reveals (see Fig. 3) that the escape probability, P_E , is positively correlated with the size of the neutral network associated to the escape phenotype, i.e. its degree, k_E , and negatively correlated with its clustering coefficient, c_E . This is a direct consequence of the inverse relation between both quantities $c_E \approx (k_E - 1)^{-\alpha}$ with $\alpha \approx 1$ (Ibanez-Marcelo and Alarcon 2014b). Thus, the size of borderline of escape genotypes takes an important role during the fast, initial regime, where escape actually occur. We have also calculated the average number of cells accumulated within the escape phenotype during the initial regime, both conditioned and unconditioned to eventual escape (Sect. 3.1.2, see Figs. 4, 5 respectively). Results show that $\mathbb{E}(N_E)$ and $\mathbb{E}(N_E \mid N_0 > 0)$ are identically correlated with k_E and c_E as the escape probability.

Our analysis of the slow dynamic regime, where escape has already occur and we study the dynamics of the population of the escape phenotype, reveals that the probability of survival conditioned to escape is determined by the robustness of the escape phenotype. However, we have shown that the topological measure of robustness proposed in Ibanez-Marcelo and Alarcon (2014b), i.e. the clustering coefficient, c_E , does not accurately describe the coarse-grained, quasi-steady state dynamics of the escaped population. Instead, a new measure of robustness M_E , closely related to a weighted clustering coefficient (Opsahl and Panzarasa 2009), has been defined which is better suited to defining robustness in the context of our coarse-grained dynamics in the slow regime. Results show a strong positive correlation between the survival probability, P_S , and, M_E . Moreover, this measure provides much better accuracy for the survival probability as a function of M_E (Fig. 7). The reason for the failure of the clustering coefficient to accurately describe robustness for the survival dynamics appears to be related to the fact that the clustering coefficient does not take into account the heterogeneity of the genotype nodes belonging to the neutral network of the escape phenotype (see Fig. 2). Our measure, M_E , takes into account the heterogeneity within the escape neutral network, thus providing a more detailed, better suited description of robustness.

In order to ascertain the relevance of our results it is important evaluate whether the quantities that we propose as key to determine the evolutionary dynamics on genotype–phenotype networks, i.e. the size of the neutral network, k_E , the clustering coefficient, c_E , and the weighted clustering coefficient, M_E , exhibit variations in relevant biological situations.

Regarding the size of the neutral network, k_E , there is ample evidence of its variability within genotype–phenotype networks associated to several systems, such as RNA (Aguirre et al. 2011) and gene regulatory networks (Ciliberti et al. 2007a,b). Furthermore, in a study of the genotype–phenotype network associated to a Boolean model of gene regulatory networks, Darabos et al. (2013) have defined a quantity they have dubbed *accessibility* of a phenotype. This quantity, which measures if a phenotype is relatively easy to reach, is closely related to the escape probability, P_E , which is positively correlated to k_E . Darabos et al. (2013) show that phenotype accessibility exhibits variability.

The clustering coefficient has been studied in RNA neutral networks as a quantity associated to phenotypic robustness (Aguirre et al. 2011). It has been shown to exhibit variability and a negative correlation with the size of the neutral network, although much weaker than the one we have found in our GRN genotype–phenotype network. Robustness in genotype networks associated to gene regulatory networks (Ciliberti et al. 2007a, b) has been correlated to community structure within the associated neutral networks. Such community structure is directly related with our definition of the phenotype clustering coefficient, c_E . Darabos et al. (2013) have determined that phenotypic robustness is negatively correlated with accessibility. This result is reproduced by our finding that phenotype clustering coefficient (i.e. robustness) is negatively correlated with the size of the neutral network, k_E (which is positively correlated with escape probability, i.e. accessibility).

Furthermore in reference (Ibanez-Marcelo and Alarcon 2014b), we have shown that the phenotype network is robustly connected, i.e. it is insensitive to random attacks. We associate this property to robustness of evolvability. This result is similar to the one found by Danac and Erzan (2014) where they also found that the phenotype network associated to a Boolean model of gene regulatory networks remains globally connected under random attack.

To summarise, our results show that, while the escape probability, P_E , is essentially determined by k_E , the survival probability long-time survival probability P_S depends on the robustness of the escape phenotype, as measured by our weighted clustering coefficient M_E . This weighted clustering explains much better the population dynamics of survival within the escape neutral network than the usual local clustering coefficient c_E (Fig. 6).

Acknowledgments The authors gratefully acknowledge the Spanish Ministry for Science and Innovation (MICINN) for funding under Grant MTM2011-29342 and Generalitat de Catalunya for funding under Grant 2014SGR1307.

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