Spatial interactions and cooperation can change the speed of evolution of complex phenotypes
Spatial interactions and cooperation can change the speed of evolution of complex phenotypes

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Complex traits arise from the interactions among multiple gene products. In the case where the complex phenotype is separated from the wild type by a fitness valley or a fitness plateau, the generation of a complex phenotype may take a very long evolutionary time. Interestingly, the rate of evolution depends on nontrivial ways on various properties of the underlying stochastic process, such as the spatial organization of the population and social interactions among cells. Here we review some of our recent work that investigates these phenomena in asexual populations. The role of spatial constraints is quite complex: there are realistic cases where spatial constrains can accelerate or delay evolution, or even influence it in a nonmonotonic fashion, where evolution works fastest for intermediate-range constraints. Social interactions among cells can be studied in the context of the division-of-labor games. Under a range of circumstances, cooperation among cells can lead to a relatively fast creation of a complex phenotype as an emerging (distributed) property. If we further assume the presence of cheaters, we observe the emergence of a fully mutated population of cells possessing the complex phenotype. Applications of these ideas to cancer initiation and biofilm formation in bacteria are discussed.

Results
Cancer, Tumor Suppressor Genes, and Evolutionary Models with Rigid Homeostatic Control. Our first example of a complex phenotype acquired by cells via a fitness valley/plateau crossing belongs to the field of oncology. A very common pattern in carcinogenic transformation is the inactivation of a tumor suppressor gene.

The concept of tumor suppressor genes was developed by Alfred Knudson who analyzed population data for retinoblastoma, and proposed his famous “two-hit hypothesis” (8). According to his theory, in the early onset version of retinoblastoma, children inherit a defective Rb gene from one parent. These children are halfway to getting the disease the moment they are born. In contrast, children who develop retinoblastoma later in childhood are born with two functional copies of the gene but subsequently acquire two hits in both copies of the gene, resulting in a later cancer onset. The tumor suppressor gene hypothesis of oncotogenesis was a revolutionary concept, suggesting that cancer was not caused by the presence of an activated, cancer-producing gene, but rather the absence of a gene. Many genes with such properties were subsequently discovered, including p53, WT1, BRCA1, BRCA2, and APC.

The mechanism by which a typical tumor suppressor gene is inactivated is sometimes referred to as a loss-of-function mutation. This process results in a gene product having less or no function. Two independent loss-of-function mutations are necessary to inactivate a gene, because after the first mutation, the second copy of the gene is still active. Thus, we can think of cells with a tumor suppressor gene inactivated as a complex phenotype. The inactivation of only one copy of the tumor suppressor gene results in a phenotype that is either neutral or negatively selected compared with the wild type. A fitness disadvantage could be the result of the deletion of the first copy by a loss-of-heterozygosity event, whereby a large chunk of genetic material is lost, giving the cell a fitness disadvantage. The (complex) phenotype with both copies inactivated has a significant fitness advantage.

The stochastic Moran process is a useful tool to study early oncotogenesis both analytically and numerically: it describes cellular turnover in constant populations, where cell divisions are subject to a rigid homeostatic control. An elementary update consists of a cell death (whereby the cell is chosen for death randomly with a uniform probability), followed immediately by a cell division. Each cell is characterized by a fitness parameter, and divisions happen with probability proportional to the cell fitness. Upon division, with a small fixed probability a cell can mutate, such that one of the daughter cells has a different phenotype (and thus may have a different fitness). This versatile

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process can be adapted to describe many biologically relevant situations. Fig. 2 shows different types of the Moran process, from the simple mass action, to a spatial process, to a hierarchical process that keeps track of stem cells and differentiated cells in tissues.

**Three Modes of Evolution in Mass-Action Systems.** The crossing of fitness valleys/plateaus in the case of only two mutational steps have been studied in refs. 5 and 9–11. As described above, this system has important applications in oncology. Several authors studied it in the context of mass-action dynamics, where spatial locations of dividing cells do not play a role. This very simple and relatively abstract system is an important case study because it allows a rigorous mathematical description of the ways of evolution in the simple case. It turns out that the results allow understanding of several more complex and more biologically relevant scenarios, including the mutation dynamics in spatial and hierarchically organized cell populations (12–15).

Complex phenotype generation can occur by three basic mechanisms (5):

i) For large mutation rates (or for large populations, the condition for $m = 2$ and neutral intermediate mutants is $u >> 1/N$), the dynamics of valley crossing is nearly deterministic, with mutants rising in a steady fashion.

ii) For intermediate mutation rates (restricted by $1/N^2 << u << 1/N$ for neutral intermediate mutations), the advantageous type can reach fixation following temporary low-level drift of intermediate mutants. This has been called “stochastic tunneling” (9) and is likely to occur in larger populations where deleterious mutants cannot reach fixation.

iii) For small mutation rates ($u << 1/N^2$), the complex trait emerges following the sequential fixation of intermediate mutants by drift.

Interestingly, the trends observed for the case of two mutations also hold for complex phenotypes that contain more steps to evolve (Fig. 3).

**Rigid Homeostatic Control: Spatial Restrictions Accelerate the Crossing of Fitness Valleys and Plateaus.** To understand the role of space in the rate of complex phenotype generation, we follow the development presented in ref. 6, where we study the spatial Moran process formulated on a 2D square grid of size $N$, with periodic boundary conditions. In this process, individuals die at random, independently of their phenotype, and are immediately replaced by the progeny of one of the nearby individuals, selected randomly from the square neighborhood of a fixed size, $M$. The probability to be selected for reproduction is proportional to the fitness of each phenotype, and mutations happen with a given probability ($u$) for each of $m$ sites upon reproduction. The intermediate mutants are either neutral or disadvantageous, and the final $m$-hit mutant has a strong advantage.

We investigate the role of spatial interactions by varying the neighborhood size $M$ where the individuals can place their offspring. If for a given individual, the neighborhood only includes the eight surrounding spots on the square grid, this corresponds to the nearest-neighbor situation. If the neighborhood is as big as the whole population, then this is the mass-action scenario.

In the case of neutral or disadvantageous intermediate mutants, the rate of $m$-hit mutant production decreases with the neighborhood size—i.e., tight spatial interactions (small values of $M$) lead to a faster $m$-hit mutant production. (Interestingly, in the case of slightly advantageous intermediate mutants, the result is the opposite, and spatial interactions tend to slow down the production of $m$-hit mutants; see ref. 6 for further details.)

An intuitive explanation for this phenomenon evokes the concept of mutant islands. Consider the case of fitness valley/plateau crossing. Under mass-action rules, all types are mixed randomly (Fig. 4A). However, if reproduction is only allowed within a small neighborhood, spatial structures tend to form, where the nearby individuals are likely to have identical genotypes (Fig. 4B). For example, in the case of $m = 2$, where one-hit mutants are neutral or disadvantageous, once a one-hit mutant is generated, its clone (if it has a chance to form) will be located in the vicinity of the

![Diagram](image-url)
original de novo mutation. It can be argued that such localized clones are, on average, longer lived than spatially dispersed clones because a dead mutant is more likely to be replaced by the progeny of another mutant than a wild-type individual if most of its neighbors are mutants. In turn, longer-lived clones of intermediate mutants are more likely to produce $m$-hit mutants, which speeds up the process of complex phenotype generation.

How tight the mutant islands are depends on the neighborhood size. For very small neighborhoods, the dynamics are the most localized, and the islands are more pronounced than in systems with larger neighborhood sizes. The degree to which space influences the rate of evolution depends on the parameters (6).

i) For large mutation rates, the difference between the spatial and mass-action systems is very small because for very high mutation rates, new mutants are produced very frequently, and are accumulated mostly by de novo production rather than by mutant reproduction (the nearly deterministic regime described above). In this case, the role of mutant islands is negligible, and the waiting time is not changed by the neighborhood size.

ii) For intermediate mutation rates, where evolution occurs largely by stochastic tunneling, the accelerating role of space in $m$-hit mutant generation becomes more pronounced, because de novo mutant generation is less frequent, and the mutant island effect becomes more important, as it drives the tunneling rate.

iii) For small mutation rates, there is another change in behavior, which is related to the system entering the regime of sequential fixation of intermediate mutants. For neutral intermediate mutants, the accelerating role of space decreases for very low mutation rates, and for disadvantageous mutants, it merely stops increasing (see ref. 6 for more details).

As discussed above, the assumptions of the Moran model correspond well to the evolutionary dynamics that occur in healthy tissues. Healthy tissue is tightly regulated by feedback factors, such that the number and density of cells remains constant over time, with all of the available tissue space filled with cells (16). Most tissue in the human body exhibits strong spatial structure; interestingly, our theory suggests that this can decrease the time until a certain number of mutations have accumulated in a cell, and this could reduce the time to cancer. At the same time, however, extensive cell migration occurs in many tissues, and migration has been shown to lead to similar properties as mass action (14). Also, it has recently been shown that a hierarchical organization of cell lineages can significantly reduce the rate of double-hit mutant generation (15). Thus, it is possible that though strong spatial structure is necessary for tissue function for other reasons, patterns of cell organization and cell migration have evolved to counter the tumor-promoting effect of spatial structure.

**Bacteria, Biofilms, and Models with Less Rigid Homeostatic Control.**

Though the Moran process is a good description of highly regulated tissue growth, where space is packed with cells, it is probably a less accurate description of many ecological systems, where not all of the space of a habitat is occupied by organisms. Instead, there is likely to be a certain spatial distribution of individuals, with the occurrence of empty space. This situation likely applies to free living organisms—e.g., microbial communities.

It is well known that genetic adaptation in bacteria is central to their survival in changing and often hostile environments. The flexibility of the bacterial genome expression allows bacterial colonies to successfully transition between life in the environment and in the human or animal host, characterized by different availability of nutrients. Defense against the host’s immune system is also an important factor for continual survival. One particularly notable example of bacterial adaptation is their ability to grow as part of a sedentary, sessile community called a biofilm.

Studying biofilms is clinically relevant because of their implications in many bacterial diseases, such as dental caries, middle ear infection, and lung infections in cystic fibrosis patients, to name just a few (17). Understanding the formation of biofilms is important, because they are extremely difficult to eradicate. For example, bacteria in an established biofilm can withstand antibacterial drugs at concentrations up to three orders of magnitude higher than those needed to kill genetically equivalent planktonic bacteria (17).

To describe evolutionary processes in bacterial colonies, it is more appropriate to use the so-called “contact process” than the Moran process (18, 19). In a square 2D grid of size $N$, nodes can be either unoccupied or occupied with cells of different types. Each time step consists of $\nu N$ elementary updates, where $\nu$ is the mean density of individuals and $\nu N$ is the total number of occupied sites. At each elementary update, an individual is picked at random. With a given probability $D$, this individual is removed, and with probability $L = 1 - D$ it attempts reproduction. Reproduction proceeds as follows. A random site in the neighborhood of size $M$ of this

**Fig. 3.** Three dynamical regimes of fitness valley/plateau crossing: (A) nearly deterministic regime, (B) stochastic tunneling, and (C) sequential fixation. Simulations are performed for a mass-action Moran process with $m = 4$ mutations, a $50 \times 50$ grid, and neutral intermediate mutants. Wild types are shown in black, intermediate one-hit, two-hit, and three-hit mutants are shown in blue, green, and pink, respectively. The advantageous four-hit mutant is shown in red. The mutation rates are (A) $u = 10^{-4}$; (B) $u = 10^{-4}$; and (C) $u = 10^{-5}$.

**Fig. 4.** After ref. (6): Spatial configuration of the Moran process for the case of (A) mass-action and (B) nearest-neighbor spatial process. In the presence of spatial structure, islands of intermediate mutants are observed. The chosen parameters were grid size $N = 50 \times 50$, $m = 2$, intermediate mutants were neutral, $u = 10^{-5}$.
individual is picked, and if it is occupied, the reproduction is aborted, and the update is complete. If the site is empty, the offspring (possibly with a mutation) of the individual is placed at the site, which completes the update. The Moran process described earlier can be viewed as the limit of the contact process where \( L \gg D \), in which case the grid is completely filled with live cells at all times, and the dynamics are driven by the death events, each of which is immediately followed by a division event.

**Less Rigid Homeostatic Control: Spatial Restrictions Can Either Accelerate or Decelerate Evolution.** In ref. 6, we investigated how spatial interactions influence the rate of complex phenotype generation. Let us start with the simpler case of slightly advantageous intermediate mutants. There, tight spatial interactions slow down the \( m \)-hit mutant generation both in the Moran and in the contact process. Interestingly, for neutral and disadvantageous intermediate mutants, the effect of spatial structure on the rate of emergence of the \( m \)-hit mutant is different and more complicated in the contact process than that in the Moran process model. In the contact process, spatial structure can either accelerate or delay the generation of the complex phenotype, depending on parameters. In some cases, there is even an optimal neighborhood size that maximizes the rate of emergence of the \( m \)-hit mutant—i.e., evolution works fastest for intermediate-range interactions.

To explain this complicated behavior, we note that there are two different mechanisms that govern the spatiotemporal dynamics of the \( m \)-hit mutant generation. On the one hand, the formation of mutant islands is facilitated by tight spatial interactions. This is exactly the same trend as observed and explained in the context of the Moran model, and it results in the increase of the tunneling rate for the nearest-neighbor model. On the other hand, in contrast to the Moran model, the population development over time in the contact process is nonuniform and is defined by the equilibrium density of individuals. For smaller values of \( M \), the mean equilibrium density of individuals is smaller, a consequence of macroscopic structures forming in the population. Thus, the total number of events is also lower. Therefore, the rate of evolution (measured, e.g., by the rate of tunneling) for the mass-action model is faster compared with the nearest-neighbor model. Combining the two opposite effects, we can show that depending on parameters, the following patterns arise (6):

- **i)** For large mutation rates, the crossing of the fitness valley/plateau happens the fastest in the mass-action model, because the mutant island effect does not play a significant role in this regime, and the time scale of the events is faster for mass action.
- **ii)** For intermediate mutation rates, the two trends trade off, and the mean time for fitness valley crossing experiences a minimum for intermediate values of \( M \).
- **iii)** For small mutation rates, spatial structure decreases the time until the \( m \)-hit mutant emerges. Evolution occurs slowest for the mass-action scenario and fastest for the nearest-neighbor scenario. As the mutation rate decreases, the magnitude of this effect rises.

We apply this theory to biofilm formation in bacteria. In ref. 17, four different factors are quoted that may be responsible for the “decision” of bacteria to enter a sessile mode of growth: (i) defense: biofilm formation can serve as a stress response; (ii) colonization: biofilm formation could be a mechanism to remain in a favorable niche; (iii) community: biofilm providing an opportunity to take better advantage of cooperation, selfless behavior, or gene transfer in bacteria; and (iv) biofilm appears to be a default mode of bacterial growth. To these four factors, our theory adds a fifth one, which is related directly to the ability of the colony to evolve quickly and to optimize its flexibility: (v) the speed of adaptation changes depending on the spatial organization, as explained below.

As mentioned, the effect of space on the rate of evolution of complex traits in a contact process is different compared with the Moran process. Space can accelerate or slow down the rate of evolution, or there can be an optimal degree of spatial restriction that maximizes the rate of evolution. How fast microbial populations can adapt by crossing fitness valleys likely is an important force that drives their evolution. Sedentary bacterial growth is characterized by strong spatial restrictions, whereas planktonic growth is characterized by a strong degree of mixing. According to our analysis, sedentary growth could be favored if mutation rates are relatively low (compared with a threshold value defined by other parameters, such as the population size, the number of mutational steps, and the fitness of intermediate mutants); this is because in this case, populations that show distinct spatial structure can adapt faster through accelerated crossing of fitness valleys, compared with populations that mix well. If, however, mutation rates are higher than a threshold, evolution is faster under mass action than under spatial restrictions. Therefore, for such bacteria, selection could favor a planktonic lifestyle.

**Fitness Valley Crossing in the Presence of Cooperation and Cheating.** In the context of asexual populations, the fitness valley crossing dynamics investigated so far requires the sequential accumulation of mutations within individuals. However, if individuals share gene products as public goods, a form of parallel evolution can occur in which the complex trait arises not within one individual, but as an emergent property among cooperating individuals through division-of-labor dynamics (20–22). Production of public goods is a well-known occurrence among microorganisms (23, 24), and has been documented to occur in tumor cell populations (25–28). Therefore, next we examine fitness valley crossing dynamics in the context of division of labor, focusing on the time it takes for the complex trait to arise. Following ref. 7, we show that the complex trait can invade significantly faster as an emerging property of a cooperating population than through sequential evolution. Interestingly, the evolution of “cheaters,” which destroy the cooperation dynamics, enables all of the mutations to accumulate within one individual on a time scale that is much faster than that for sequential evolution.

As our modeling setup, we again consider a 2D square grid of size \( N \), and assume a contact process of divisions and deaths, where parameter \( M \) defines the degree of spatial interactions, as described previously. Several kinds of individuals are considered that differ in their genetic makeup and replication kinetics. As before, assume the existence of \( m \) genes that express certain products. Wild types carry unmutated genes in all sites and have fitness \( R \); if all \( m \) genes are mutated, the individual gains a fitness advantage compared with the wild type (fitness \( R^+ > R \)). If only a subset of the genes is mutated, the individual carries a fitness cost compared with the wild type (fitness \( R^- < R \)).

Further, in a division-of-labor scenario, we assume that these partial mutant types can share gene products and cooperate with each other through public goods to increase fitness (Fig. 5A). Hence, if within a defined cooperation neighborhood (which may or may not have size \( M \)), the missing products are provided by at least one individual per site, these can be used by the cell conferring a fitness advantage compared with the wild type. In this case, the enhanced fitness is an emergent property of a cooperating population. The size of the radius determines how many agents a given cooperater can help. Because producing public goods typically confers a fitness cost (29, 30), an amount \( f \) is subtracted from the fitness of an agent for each cooperating site (Fig. 5C).

The model also takes account of cheating with respect to the individual sites. A cheater produces a gene product at reduced levels, only available to itself, and thus avoids the fitness cost \( f \) (Fig. 5B and C). We explore two scenarios in which wild types
do and do not benefit from public goods; both can be realistic, depending on the biological system.

First, assume the generation of cooperators, but the absence of cheaters for the case where wild types do not benefit from public goods. In contrast to sequential evolution scenario, the complex trait can arise not necessarily within one individual, but also as an emergent property of a cooperating population with enhanced fitness, which becomes dominant; this happens significantly faster than the emergence of the m-hit mutant in sequential evolution (Fig. 6A). The simultaneous presence of at least one cooperator for each site within the cooperation radius, and thus a sufficiently large radius and mutation rate, are required for this to be possible. Note, however, that the m-hit mutant cooperating at each site will never invade because it carries the maximal fitness cost. If we assume that wild types can also benefit from public goods, the complex phenotype cannot arise as an emerging property of cooperating individuals that become dominant (Fig. 6B) because cooperation increases the wild-type fitness, and cooperating agents pay a cost and thus have a relative disadvantage; they are maintained by the wild type through mutations. Because wild type and cooperators enjoy increased fitness from the public goods, however, they still persist at significantly higher levels compared with the partial mutants in the sequential evolution scenario.

Next, assume the generation of cheaters. In this case, the dynamics are similar regardless of whether the wild-type cells benefit from public goods. In contrast to sequential evolution scenario, the complex trait can arise as an emerging property among cooperating individuals. Nevertheless, cooperator–cheater dynamics significantly accelerate the emergence of the m-hit mutant compared with the sequential evolution scenario. Parameters were chosen as follows: grid size = 100 × 100; m = 5; R = 0.15; R+ = 0.5; R− = 0.135; Δ = 0.1; f = 1/70; u = 3.17 × 10−3; M = 100.

Fig. 5. Cooperating and cheating phenotypes. (A) A mutation diagram in the absence of cheaters, depicting cooperation between partially mutated cooperators, in the case of m = 2. Wild-type genes are denoted by lowercase letters, and cooperators by uppercase letters. (B) Adding the possibility of cheaters; the cheaters are denoted by uppercase letters with asterisks. The simplest case of m = 1 is presented. (C) For the case m = 2, all of the types are cataloged, and their fitness in the absence and in the presence of cooperation is identified.

Fig. 6. After ref. (7): Distribution of times until the complex phenotype reaches 90% of the total population, based on repeated runs of the computer simulation. (A) Scenario where wild types do not benefit from shared goods. Time until emergence of the m-hit mutant is longest for the sequential evolution scenario. Cooperator–cheater dynamics significantly speed up the emergence of the m-hit mutant. In this case, even before the m-hit mutant arises, the complex phenotype arises as an emerging property among cooperating individuals. (B) Scenario where wild types do benefit from shared goods. In this case, the complex phenotype cannot become dominant as an emerging property among cooperating individuals. Nevertheless, cooperator–cheater dynamics significantly accelerate the emergence of the m-hit mutant compared with the sequential evolution scenario.
become more dominant (Fig. 6). Cooperators are maintained by cheaters and wild types through mutation, at a level given by

\[ N_c \approx \frac{R^+}{T} u M \left(1 - \frac{D}{R^+}\right) \]  

per site per cooperating neighborhood (this approximation is valid if \( u f << 1 \)). If the level \( N_c \) is relatively low, the cooperating phenotype at any given locus goes extinct frequently within the local interaction radius through stochastic effects. The local extinction leads to a local crisis of the cooperators/cheaters, their average fitness plunging. If at this time, an \( m \)-hit mutant is present, this reduction in average fitness will allow it to be selected for and to quickly become the dominant population (Fig. 7). In this setting the \( m \)-hit mutant arises significantly faster than in the sequential evolution scenario (Fig. 6) for two reasons: (i) high-level replication of cooperators/cheaters ensures fast generation of mutants and (ii) cheaters reduce the average fitness of the cooperator/cheater population, thus conferring a selective advantage to the \( m \)-hit mutant, without which it would not surge to dominance.

The higher the number of involved loci, the more the cheating pathway speeds up the invasion of the \( m \)-hit mutant compared with sequential evolution. Further, the following mutation rate dependencies take place (7):

- \( i \) For large mutation rates, the cooperators never disappear from the system, and the \( m \)-hit mutants never come to dominate the system. In this case, the sequential dynamics are faster than those governed by cooperation and cheating.
- \( ii \) For intermediate mutation rates, cooperation and cheating significantly accelerate evolution, as described above.
- \( iii \) For small mutation rates, no cooperating population can get established and the dynamics are identical to those of sequential evolution.

For simplicity, in Fig. 6 it was assumed that the cooperation and reproduction radii are identical; this need not be the case, as explored in refs. 31 and 32, in the context of imitation dynamics of unrelated individuals. In ref. 7 it was shown that even for unequal cooperation and reproduction radii, one can still find the accelerated evolution of the complex phenotype through the cooperator/cheater pathway. There are, however, some interesting trends that are observed as we vary the two radii relative to each other. For example, if we keep the replication radius constant, and increase the cooperation radius, the cooperator population within the radius rises, and this makes cheater-induced extinction of individual cooperator types less likely. The chances of cooperator extinction, however, are increased as the mutation rate is lowered. Therefore, for larger cooperation radii, an accelerated emergence of the \( m \)-hit mutant requires lower mutation rates. For relatively small cooperation radii and small mutation rates, the cooperator/cheater pathway does not accelerate the emergence of the \( m \)-hit mutant, because not enough cooperating types are generated by mutations within the cooperation radius, such that the division of labor dynamics cannot emerge.

Cooperation and Cheating in Cancer. The idea that cooperation could be implicated in carcinogenesis was first proposed in ref. 25; there, two conceptual models of carcinogenesis are contrasted. In the first traditional model of multistage carcinogenesis, mutations are accumulated sequentially, leading to transformation of tissue from normal to premalignant, and further to increasing degrees of malignancy. The second conceptual view allows for cooperation among cells in an inhomogeneous population. In particular, it is suggested that intratumor cooperation can occur among partially transformed mutant cells that have complementary needs, such as two growth factors. Cross-feeding soluble gene products is a form of cooperation that enables each participating cell type to survive and proliferate. Another examples where cooperation among cancer cells can be very relevant is sharing VEGF factors for recruiting blood cells in angiogenesis (26). A further example pertains to the phenomenon of metastases and involves sharing factors that allow survival under loss of contact inhibition and degrading extracellular matrix. This and other examples are described by ref. 28.

![Diagram](https://www.pnas.org/cgi/doi/10.1073/pnas.1400828111)
Though several forms of cooperation among cancer cells have been identified by a number of authors, the presence of both cooperation and cheating in cancer has not been previously conceptualized. To highlight a possible example (suggested by H. Jain), we consider the development of treatment resistance in prostate cancer. Prostate cancers depend on androgens for growth and survival, and androgen ablation therapy causes them to regress (Fig. S A and B). Cancers that are not cured by surgery eventually become androgen independent, rendering antiandrogen therapy ineffective (33–35). Several types of mutants have been identified. Two of the more common types are of particular interest for the present construction. The first type of mutation facilitates local biosynthesis of androgen; it raises the local androgen level, thus enhancing the fitness of all cancer cells in the neighborhood (Fig. 8C). In terms of our model, this type of mutation can be identified as a cheater mutation. A different type of mutation reduces the activation threshold for the androgen receptor for the affected cell (Fig. 8D). In terms of our model, this is a cheater mutation, because it provides a growth advantage for the affected cell but not for its neighbors.

**Conclusion**

In this paper we attempted to summarize some evolutionary aspects of the emergence of complex phenotypes. In the case where this process requires a crossing of a fitness valley or a plateau, evolution has to overcome a serious barrier before an advantageous, multiple-hit mutant appears and spreads.

Not very surprisingly, the timing of this process depends on the parameters such as the number of fitness values of the intermediate mutants, the mutation rates, and the population size. More interestingly, the rate of evolution also depends in nontrivial ways on further properties of the underlying stochastic process, such as the spatial organization of the population and social interactions among cells.

The role of spatial constraints is complex. In a process under rigid homeostatic control (the Moran process), spatial constraints accelerate the crossing of fitness valleys and plateaus. In a less rigid system (the contact process), space can either accelerate or delay the crossing of fitness valleys and plateaus, depending on parameters. In some cases, there is even an optimal neighborhood size—i.e., evolution works fastest for intermediate-range interactions. The degree to which spatial restrictions affect the rate of evolution depends on the mutation rates (with respect to thresholds defined in terms of other system parameters). Generally, spatial constraints make the largest difference for the intermediate mutation rates also characteristic of the process of stochastic tunneling.

The social life of cells, which in this paper is understood in the narrow sense of division of labor games, also changes the speed of fitness valley/plateau crossing. It is possible to show that under a range of circumstances, cooperation among cells can lead to a relatively fast creation of a complex phenotype as an emerging (distributed) property. In the absence of cheaters, although, all of the mutations do not “meet” in the same cell. If we further assume the presence of cheaters, we observe the emergence of a fully mutated population of cells possessing the complex phenotype.

Apart from being an important concept in the Darwinian biology, the generation of complex phenotypes has relevance for several biomedical systems, such as cancerous cells and biofilm formation. Understanding the principles of evolution in these and other systems has a very concrete and practical importance.