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Modelling viral evolution and adaptation: challenges and rewards

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Viral populations are extremely plastic. They maintain and steadily generate high levels of genotypic and phenotypic diversity that may result in different adaptive strategies. A major unknown factor in constructing realistic models of viral evolution is how mutations affect fitness, which amounts to unveiling the nature of viral fitness landscapes. Our understanding of viral complexity is improving thanks to new techniques as deep sequencing or massive computation, and to systematic laboratory assays. In this way, we are clearing up the role played by neutral networks of genotypes, by defective and cooperative interactions among viral mutants, or by co-evolution with immune systems. Models of viral evolution are thus improving their accuracy and becoming more competent from a conceptual and a predictive viewpoint.

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Introduction

The development of quantitative theories of evolution faces the challenge of synthesizing general principles from a huge number of partial observations. The biological complexity we observe is the result of a long and entangled history of collective processes, from molecules to ecosystems, unfolding at many different time and space scales [1]. Already the adaptive dynamics of a single population involves dissimilar actors and many different levels of description and selection [2,3••], among them a myriad of mechanisms causing genomic variation, various effects of mutations in phenotype [4,5], interactions between individuals and the selection of competing groups [6], the consequences of an adaptive strategy under endogenous or exogenous modifications, or the constraints imposed by environments as a function of the time scales at which they change [7]. At present, models aiming at establishing general principles bear little predictive power; models focused on a single or few observations can yield specific predictions, but suffer from restricted applicability. In all cases, unavoidable assumptions based on current experimental knowledge set the limits of the obtained results.

Viruses occupy a prominent role in the selected constellation of experimental systems employed to dive into the intricacies of evolution. They form huge, heterogeneous populations that are in perennial change and readily adapt or perish [8]. The adaptive strategies of viruses make up a large, innovative, and ingenious ensemble of mechanisms [9] that defeat our most creative expectations. A shallow overview of some of their remarkable features confronts us with many open questions in the way their populations change and adapt, as well as with the processes and mechanisms one should in principle consider in realistic models of viral evolution. Viruses, especially those with an RNA genome, perform broad explorations of genome space due to their relatively short genomes, large population sizes, and elevated mutation rates. Their dynamics are conditioned by the existence of neutral networks of genotypes (producing the same phenotype) that span genome space in a likely variable, and as yet mostly unknown, extent [10–12]. In establishing the relation between genotype and phenotype, it is critical to conceive fitness landscapes that capture the essentials of how genomic changes affect function. Viruses generate diversity through point mutations, but also through major modifications as segment deletion, non-homologous recombination or segment shuffling. In those conditions, many viruses produce a large amount of defective genomes that may thrive in appropriate environments thanks to the complementation offered by complete genomes [13]. These characteristics and several others are behind the response of pathogenic viruses to therapies, and should be taken into account when designing protocols to control the spread of viral infections.

A complete description of the complexity of a viral quasispecies is a formidable task. Though it is arguable whether this knowledge is needed before the development of general theories of viral evolution, it is undeniable that empirical knowledge guides the way. Novel techniques as ultra-deep sequencing will ease this endeavour, facilitating, for instance, a detailed characterization of viral genomic diversity [14,15]. The use of microarrays, where thousands of samples can be simultaneously analysed, could lead to the quantification of other heterogeneous features of a quasispecies, as recently demonstrated in a study of the fitness landscape and neutral space of poliovirus [16]. The steady increase in computational power available should be also of help in

establishing the much sought effect of mutations in function, systematically analysing, for instance, how mutations modify the thermodynamic stability of proteins [17•].

From viral complexity to simple models of quasispecies

Simple models might turn into powerful metaphors. This has been the case of Eigen's quasispecies model [18] which, since its application to viruses a few decades ago [19], has directed (and conditioned) the way many of us think of viral populations. Eigen's model inspired the idea of increased mutagenesis as a plausible mechanism to induce the extinction of viral infectivity, and that has proven to be an efficient strategy *in vitro* [20]. However, viral extinction can occur through mechanisms different from crossing an error threshold [21]. Alternative pathways are stochastic extinction due to the deleterious action of defective genomes [22,23], the simultaneous fading of all genomes in the population [24,25], or even the extinction due to intraspecific competition in a situation of limited resources [26]. Increases in the mutation rate, on its side, do not always impair the survival of a virus: more mutations in difficult situations might mean more lethal mutations (increasing purifying selection) and also more beneficial mutations, eventually fostering adaptation [27]. Admittedly, not every detail can be considered if a model has to be useful; however, it is essential to keep in mind those ingredients that one has left aside in order to gain understanding, and recover them whenever models and experiments disagree (see Figure 1).

The value of mutations

Despite their preeminent role in the persistence of viral populations, beneficial mutations have been often discarded in evolutionary models. Though one may believe that this approximation is sensible in highly optimized populations, experimental observations do not support it [4,28]. This is but an example (and serves as a warning) of how intuition fails in the face of evolutionary processes. In fact, beneficial and compensatory mutations may become significantly abundant in viral quasispecies displaced from mutation-selection equilibrium, notably under environmental changes such as infecting a new host [29•]. The sign and effect of a mutation depends on the genomic context where it occurs and on the current environment and, as such, cannot be assigned an absolute value.

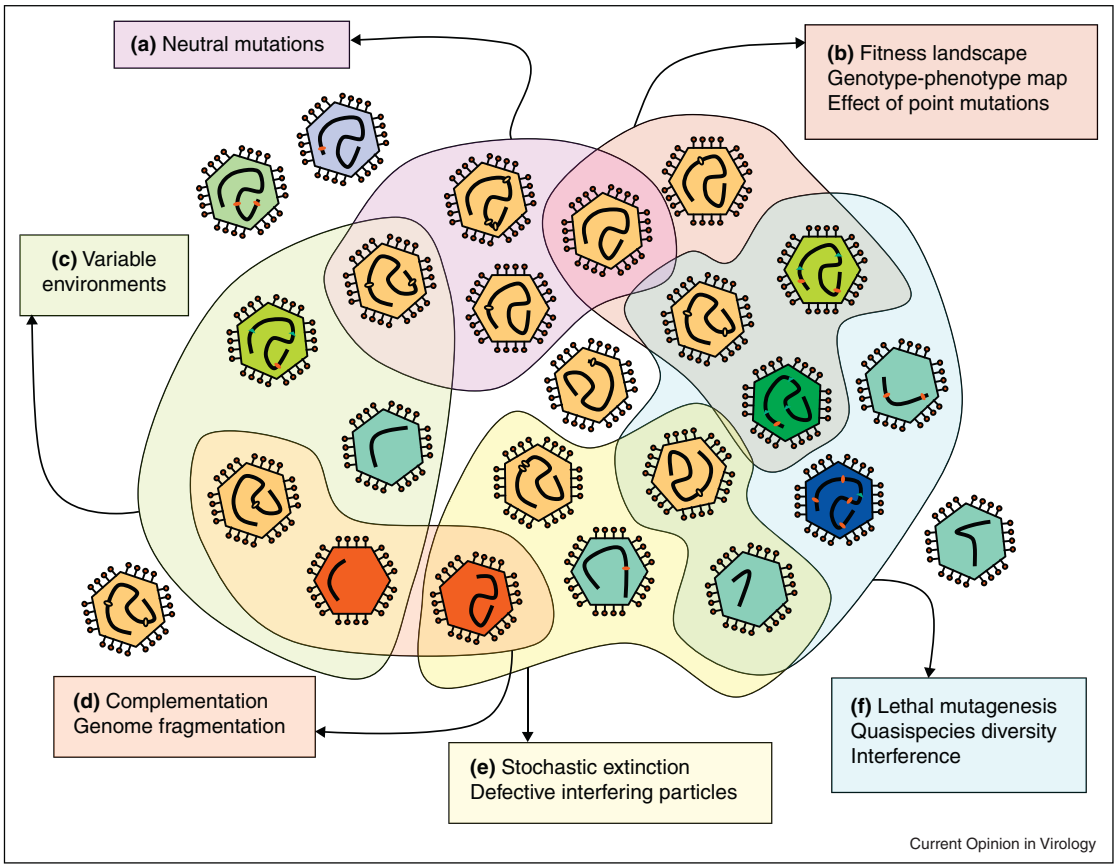
When mutations are frequent, the dynamics of asexual populations are affected by interference among mutations of different sign, and the response of the population would critically depend, among others, on the underlying distribution of fitness effects (DFE) [30]. Though the predominance of mutations with small effects has been mostly accepted [5,31] since Fisher introduced his geometrical model of adaptation [32], more abundant and more accurate measurements point to a significant abundance of mutations of average and large effect in viruses [29]. The functional form of the DFE is receiving considerable attention for obvious reasons [4], but no agreement on its mathematical form has been reached so far. Data to determine whether the DFE has a universal functional form are still insufficient, though mounting empirical evidence indicates that realistic fitness landscapes present local correlations and approach random landscapes as mutations accumulate [33], the transition between the two regimes taking place at a distance (in number of mutations) that may take appreciably large values [34••]. That scenario is qualitatively analogous (we do not know yet whether it is quantitatively equivalent as well) to the non-trivial fitness landscape of the sequence-to-RNA secondary structure map [35,36]. A reliable knowledge of the mathematical form of the DFE should help us assess the suitability of different fitness landscapes to capture the essentials of viral dynamics and molecular quasispecies [37,38,39•].

Beyond the cloud of mutants

The relative commonness of mutations of large effect in viral fitness agrees with the raising view that (quasi-) neutrality is an essential ingredient in adaptation and innovation [3••]. Large networks formed by mutually accessible genotypes with almost equal fitness should permit the costless drift of viral populations through the space of genotypes (see Figure 2(a)). Neutral networks are intimately intertwined in that high-dimensional space, implying that most phenotypes are close to one another in terms of the number of mutations that separate them. Extending Wright's metaphor, we should imagine the corresponding fitness landscape as an ensemble of layers (the networks of genotypes of equal fitness, or phenotypes) at a distance of one or few mutations in selected positions. The adaptive dynamics of viral populations on such landscapes would consist of periods of stasis — while populations drift through a neutral network —, punctuated by sudden switches between neutral networks at those times when a fitter phenotype is encountered [11,40]. This behaviour has been identified [12] and modelled [41,42•] in influenza A and in measles, where the immune system of the host forces the continued appearance of antigenic novelty if the virus is to avoid extinction.

The structure of viral populations in a genotype space fragmented into vast and interconnected neutral networks is very different from a cloud of variants structured around an optimal sequence. Emphasis should be put instead in high-fitness phenotypes represented by a large set of genomes that may be mutually distant from a genealogical point of view (Figure 2(b)). The spread of the population on the current network determines its potential to find and fix new phenotypes when selective pressures change. The co-evolution between virus and

Figure 1



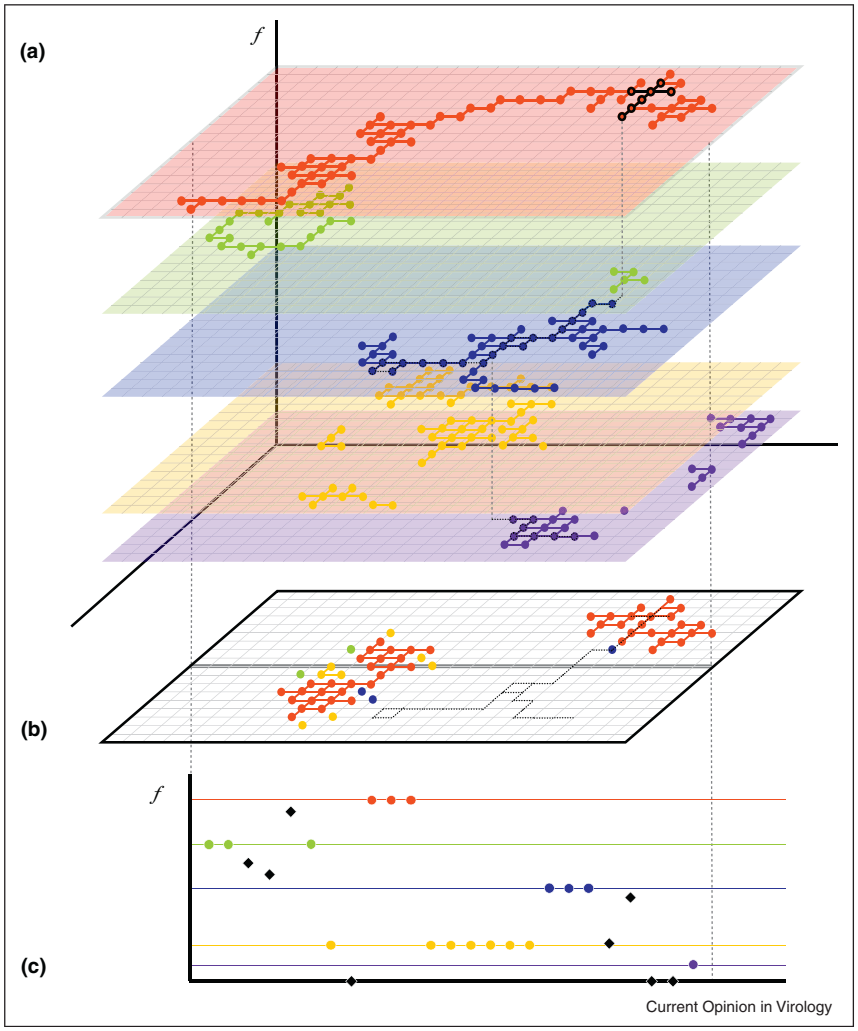
Models of viral evolution: partial insights to a complex problem. A viral population, especially if affected by large mutation rates — as it happens with RNA viruses —, is a large, heterogeneous, and complex ensemble of interrelated genomes. Tractable models of viral evolution can only describe part of the current heterogeneity. Studies on the neutral network of the most common phenotype **(a)** often discard the ensemble of neighbouring phenotypes with different fitness. Very often **(b)**, analyses of the effect of point mutations do not take into account the presence of defective particles or incomplete genomes. In characterizing the response of viral populations to variable environments **(c)**, it is the substitution of one phenotype by another in co-evolution with an immune system, the relative success of an alternative phenotype in front of the wild type under an increase in temperature, or the fixation of suboptimal phenotypes under the effect of population bottlenecks, for instance, that are analysed. How these new phenotypes emerge depends on the localization of the original population in the space of genotypes, a property that is difficult to take into account in those studies. Research on fragmented genomes and how they compete with a wild type **(d)** tends to implement only those two strategies. Defective interfering particles **(e)** are typically explored by quantifying their effect on the performance of the wild type, while heterogeneity within the subpopulation of defectors, for instance, is commonly dismissed. The consequences of increased mutagenesis **(f)** are very often investigated in models that assume a systematic negative effect of a larger mutation rate, and thus discard beneficial and compensatory mutations. By focusing on one or a few viral features, we gain understanding but loose the big picture.

immune system, as above, or the response to any other changing selective pressure could be depicted as a continuous change in the relative height of different layers, that is to say, in the fitness of possible phenotypes. The incorporation of neutral networks to models of viral evolution, together with a realistic representation of the environment, is a challenge to future evolutionary models [43].

Adaptation to therapies

Viruses have an astonishing ability to escape medical treatments aimed at causing their extinction. The question is not whether a virus will develop resistance to an antiviral drug, but when will it occur. The simultaneous administration of two or more drugs has been a successful strategy to delay the appearance of resistant mutants [44]. In the search for efficient therapeutic protocols, three fundamental issues that are aided by modelling and computational techniques are first, the characterization of the response of viral populations to antiviral drugs [45] through a more realistic implementation of their evolutionary strategies [46,47], second, how to optimize the tempo and mode of drug administration in order to minimize viral load and to maximize the time of emergence of resistant forms [48••],

Figure 2



Viral fitness landscapes. **(a)** Schematic representation of neutral networks. Each layer stands for a different phenotype, its vertical position represents its fitness *f*. Each circle corresponds to a genotype, genotypes with the same colour yielding the same phenotype. If two genotypes can be attained through mutation, a bond links them. Some phenotypes may be obtained from a large number of genotypes and the corresponding networks are connected, meaning that a viral population could attain distant regions of genotype space without paying a fitness cost (as in the red example). It is likely that rare phenotypes can be only obtained from isolated and small groups of genotypes, being thus difficult to find and fix. The black curve illustrates a possible dynamical trajectory of a population 'climbing' from the low-fitness violet phenotype to the red phenotype. At each step, fitness either remains constant or increases. Phenotypes of intermediate fitness are not necessarily visited. These trajectories substitute the image of a walker moving uphill in smooth landscapes. Evidence of such drift-and-sweep behaviour has been found in [12]. **(b)** Cartoon of the structure of a viral population. Viral populations are finite and, as such, not all genotypes compatible with a given phenotype will be present. If the phenotype extends over large regions connected by few genotypes, the viral population may be apparently formed by two genealogically distinct groups, as observed in [42]. High mutation rates imply the frequent generation of mutants of lower fitness, due to the genomic proximity of occasionally very different phenotypes (yellow, blue, and green circles). The dotted line is a projection of the adaptive trajectory shown in (a). **(c)** One-dimensional fitness landscape. Black diamonds stand for other possible phenotypes not depicted in (a). Current measures of actual landscapes suggest the presence of local correlations (there is a higher likelihood that neighbouring genotypes correspond to similar phenotypes than distant ones) and a remarkable degree of roughness [35]. In all panels of this figure, each genome has only four neighbours, while a genome of length *L* whose units come from a four-letter alphabet has 3*L* neighbours. In those spaces of high dimensionality, neutral networks have topological properties impossible to visualize in two-dimensional projections.

and third, which are the relevant interactions between the immune system and a viral population [49]. Combination therapies need to be complemented with studies to determine how dissimilar drugs interact [50]. For example, in the case of a mutagen and an inhibitor of viral replication, it may occur that the sequential administration of the two drugs is more efficient than their simultaneous administration [51]. The non-linear interaction between the two

Box 1 Viral strategies and viral games

The conspicuous heterogeneity of viral populations may lead to the emergence of subpopulations with remarkably different phenotypes and different strategies for survival. Usually, the advantage of a strategy depends on the fraction of individuals using it, permitting a formulation in terms of evolutionary games [52]. An example is the competition arising between complete and fragmented viral forms [53,54]. Under low multiplicity of infection (MOI), a viral particle needs to encapsulate a complete genome to be viable. This requirement is relaxed under high MOI, where different incomplete genomes may complement each other and eventually displace the wild-type [55]. The disadvantage of complementation may be compensated, among others, by a higher stability of the viral particle [56•]. Another strategic clash involves competitor and colonizer subpopulations [57]. The former are better at replicating within the cell, while the latter are faster at infecting new cells. In the evolutionary games of viruses there is no absolute winning strategy, since the pay-off of a game depends on the relative abundance of the competing strategy or on the current environmental characteristics. The chances of survival of a viral population should increase whenever it is able to deploy different strategies.

pharmaceuticals depends in a non-trivial way on the administered doses and on the reproductive strategy of the virus [48] (Box 1).

Conclusions

The decision to include or to dismiss a certain viral feature or process in a formal model may change in a qualitative way the nature of the dynamics and eventual fate of the (virtual) viral population. Simple models aid to develop conceptual scenarios and permit to establish a correspondence between basic mechanisms and evolutionary dynamics. They may suggest new experiments that, in case of comparing favourably with the predictions of the model, validate the latter. Nonetheless, we should be cautious when extending evolutionary models to other systems, since the original assumptions may not hold and, as a consequence, neither the expectations derived from the model.

Thanks to an always increasing body of experimental results and to the advances in genomic and computational techniques, viral populations are being characterized to an unprecedented degree of detail. These efforts should lead to a more realistic quantification of the effect of mutations in fitness. Though we have just begun to fight that challenge, it is clear that realistic fitness landscapes are neither smooth nor random, and that the conclusions obtained in too simplistic scenarios may be misleading. A subsequent step would be to construct a statistical theory able to derive models on the evolution of phenotypes from the properties of the space of genotypes.

Major rewards should come from the application of well-motivated, specific models to control the progress of viral infections. Knowing the strengths of viruses, as the quality of their diversity or the cooperative strategies they use within their populations is a first step towards designing effective protocols to limit their spread. From the synergy among a deep characterization of viral genomic diversity, knowledge of viral evolutionary strategies, drug interaction and viral co-evolution with the immune system should emerge a powerful guide to design successful therapies able to decrease the viral load and to delay the appearance of resistant forms.

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