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Evolution of fast mutating replicators—RNA viruses and the RNA world

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Abstract

In the last two decades, viruses have become the model system to witness evolution in the laboratory. Large population sizes, high mutation rates, and short generation times are the three features that permit to carry out in vitro experiments under controlled conditions. In this contribution we briefly review a number of recent experiments that open new prospectives in our understanding of molecular evolutionary mechanisms, in their dependence with population dynamics and quasispecies organization, and in the interaction between heterogeneous populations and the environment. One of the possible origins of RNA viruses is a hypothetical RNA world, previous to our present DNA world, where information coding and catalytic functions would be simultaneously performed by RNA molecules.

Keywords: RNA viruses; Evolutionary dynamics; Molecular quasispecies; RNA world

Viruses are obligated cellular parasites. They have compact and small genomes that can only be replicated inside a host cell, where they take advantage of several cellular metabolic processes. However, due to the low fidelity of the enzyme RNA polymerase, always encoded in the viral genome, RNA virus replication is a very error prone process: on the average, every copied genome differs in one nucleotide from its parental genome. As a result, RNA virus populations are highly heterogeneous, a property that is directly responsible for their adaptive ability.

Heterogeneity occurs at the genetic level, as a direct expression of high mutation rates, but also affects molecular structure and eventually the phenotype (the fitness) of the viral particle. A viral quasispecies is thus a complex ensemble of viruses characterized by a broad distribution of fitness values. The precise shape of the fitness distribution of a quasispecies depends on the mutation rate and on the environment where evolution takes place. That is to say, on the selection pressures that the population is subjected to.[1]

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[1]A simple formal description of heterogeneity is provided by quasispecies theory. If we consider an ensemble with $i = 1, \ldots, N$ different fitness types and transition rates w_{ij} between types when replication takes place, the so-called mutation-selection equilibrium (the stationary distribution of fitness classes) is given by the components of the right eigenvector corresponding to the largest eigenvalue of the matrix w_{ij}. Different selection pressures could be described by different elements of the transition matrix w_{ij} [3].

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The study of viral evolution in laboratory conditions involves as a first step the adaptation of sample populations of in vivo viruses to cellular cultures. Typically, cells grown in flasks or on Petri dishes are subsequently infected with a virus. After a variable development time for the infection, and possibly a number of sequential transfers of samples of the virus to fresh cellular cultures, several quantifications (e.g. sequencing, evaluation of viral yield, competition with a reference viral population) are carried out. In the following, we describe three in vitro experiments where evolution and adaptation of viral populations has been monitored. In the two first experiments, infections are of the lytic type, that is, cells are lysed (killed) by the virus when a critical concentration is achieved inside the cell. In the third experiment, infection is persistent, such that the cell continuously releases viral particles to the extracellular medium. The different competition strategies allowed in one or another case are very relevant to determine the fate of the quasispecies. While in lytic infections viral particles have to compete to efficiently infect new fresh cells, in persistent infections the most relevant competition occurs for resources and replication inside the infected cell. In other words, the selection pressures for fast replication or infection of new cells are at least quantitatively different in each situation. We will focus on the original motivation to carry out those three experiments and on the main results obtained. The interested reader is referred to the published papers to obtain further information (see also the review [1] and references therein).

Experiment 1. *Muller's ratchet and population bottlenecks*: Muller's ratchet theory states that mutations are unavoidably fixed in small asexual populations due to stochastic events [2]. Whenever a population is optimized in a given environment, new mutations will be mostly deleterious, such that their progressive accumulation may result in steady degradation and eventual extinction of the population.

The effect of Muller's ratchet can be enhanced by subjecting a population to repeated bottleneck events. In its extreme form, a single viral particle can give rise to a whole population, such that all the mutations carried by the seed virus will be transmitted to the progeny. The a priori expected result of such a procedure is a monotonous decrease in viral fitness until the population becomes no longer viable. A systematic experiment with foot-and-mouth disease virus infecting cellular monolayers has followed that protocol for several years. As expected, an initial decrease in fitness was identified. However, the average fitness reached a statistically stationary state after a number of population bottlenecks had been applied. The viral yield (measured as the number of plaque forming-infective-viral particles in the population) displayed large fluctuations, implying that fitness could be recovered even in a situation where mutations steadily accumulated in the genome.

The fluctuations in the viral yield followed a stretched exponential distribution. Analytically, this function can be obtained if two elements are considered: the population grows multiplicatively during development between bottlenecks, and the fitness value of the seed particle takes random values according to the diversity generated during population growth [4]. Recovery of fitness is explained by the presence of compensatory mutations that outweigh the negative effect of mutations already present [5]. The obtained results speak for a high robustness of viruses (bottlenecks are indeed a perturbation frequently encountered in Nature) and highlight the degeneracy existing between genome and fitness spaces: there might be many different sequences coding for the same phenotype.

Experiment 2. *Epidemic spreading with complete and fragmented genomes*: In contrast with population bottlenecks, massive population passages from plate to plate are the current protocol used to optimize viral fitness. The fraction of defective genomes[2] stably maintained in the viral population might increase under application of massive transfers. This implies that new interactions between subpopulations, as defection or altruism, might play relevant roles in evolution.

On theoretical grounds, it can be expected that fragmented (thus defective) genomes could be a winning evolutionary strategy when the amount of viral particles per cell is in excess. One reason might be the advantage conferred by sex to eliminate deleterious mutations. Another one could be a faster replication of shorter, complementary fragments, when compared with the full genome. Though the advantage of fragmenting the genome is not yet clear empirically, the transition from a full genome to a fragmented type

[2]Defective forms of a virus have incomplete genomes or do not code properly for all required proteins. They might use the resources provided/codified by complementary genomes in the population if the latter are simultaneously present. They behave in practice as a parasite of the cellular parasite.

(two complementary, uncomplete genomes, encapsidated in different particles) has been already observed in massive passages of foot-and-mouth disease virus [6].

The relative performance of the two systems, the first one constituted by viral particles with full genomes and the second one formed by populations of two complementary particles[3] was quantified in cell killing assays. Those assays consist in adding an amount n_0 of infective particles to a monolayer of susceptible cells. The time $T(n_0)$ required to kill all of the cells depends on n_0 and quantifies the virulence of the virus. It was observed that the functional form of the killing time depended qualitatively on the viral type: $T(n_0) \propto \ln(n_0)$ for full genome particles, while $\ln T(n_0) \propto \ln(n_0)$ for complementary ones, where coinfection was required.

A numerical and analytical investigation of the mechanisms involved in epidemics spread in those cases revealed that, with high probability, it is the transport of infective particles far from the original plaque where they are produced what determines the functional form of $T(n_0)$. Local diffusion is present in both cases, and causes the growth of individual plaques. Long-range transport is also present in a low amount, but it implies strong dilution of viral particles and thus is only effective in the case of full genomes: coinfection becomes extremely rare in very diluted environments, as is the case [7].

Experiment 3. *Mutagens and the lethal effect of defective populations*: According to quasispecies theory, the mutation rates of RNA viruses place them very close to the so-called error threshold. Beyond that threshold, replication of genomes is not accurate enough to maintain the information in the dominant genome, and a quasispecies would lose its biological identity. As a result, enhanced mutagenesis has been proposed as an alternative strategy to induce viral extinction. Two immediate results of treatments of viral infections with mutagens are indeed a reduction of viral infectivity (preceding extinction in most cases) and a broadening of the mutant spectrum. Though quite efficient, the use of high amounts of mutagens has the shortcome that also cellular activity is heavily impaired, what partly prevents their clinical use.

However, at low amounts of mutagen a new form of extinction has been described [8]. In persistent infections of cells with lymphocytic choriomeningitis virus, the addition of small quantities of the mutagen 5-fluorouracil induces the extinction of infectivity at about three days post infection. Interestingly, quantifications of the RNA present in the cells and in the medium did not yield decreases in the genomic RNA. This indicates that, though infectivity is suppressed, replication of RNA continues inside the cell. The interpretation of this observation needs to take into account the characteristics of the environment. In a persistent infection of this kind, competence between viral genomes occurs inside the cell. The presence of sequences most effective in sequestering the products necessary to replicate will be enhanced. However, phenotypic features not subject to selection, such as the ability to encapsidate or to recognize cellular receptors for infection of new cells, might disappear, since they are useless in the current context. As time elapses, the fraction of fast replicators grows and that of fully viable genomes decreases inside the cell. Extinction of infectivity supervenes due to the lethal effect of the growing defective subpopulation.

The simultaneous presence of fully viable and partly defective forms cannot be avoided in a natural quasispecies, so infectivity and replicative ability cannot be decoupled in the laboratory experiment. Numerical simulations where those two traits were independently affected by the mutagen revealed that only in a situation where the viral population is structured in different subclasses, with more than one phenotypic trait, can extinction of one of the traits (not selected for in the given environment) occur as observed. At sufficiently high amounts of mutagen, also the replicative ability is suppressed and both traits undergo extinction.

Though quasispecies theory has been repeatedly applied to viral evolution since viral quasispecies were first identified [9], it has its roots in theoretical studies of prebiotic chemistry [3]. At early evolutionary times, proof-correcting mechanisms during copying were absent, and the first replicating molecules were necessarily subjected to high mutation rates. Hence, molecular quasispecies were the kind of populations present at those times, and the evolutionary mechanisms displayed at present by RNA viruses can give us clues about evolution, adaptation and eventual complexification in a hypothetical RNA world [10]. Prebiotically speaking, that scenario partly solves one of the chicken-and-egg problems of present genomics. If DNA codes for the genetic information, but proteins (codified by DNA) are required to perform almost all catalytic functions,

[3]In the former case, a single viral particle entering a cell produces infection. In the latter case, the simultaneous coinfection of the same cell is required to cause disease.

among them replication of DNA, what was first? Nowadays, it is known that, though with an efficiency usually lower than proteins, RNA can perform a pletora of catalytic activities, on the one hand, and serves as the repository of genetic information in RNA viruses, on the other hand. This is possible because RNA, apart from encoding information in a way similar to DNA, in a nucleotidic sequence (its genotype), is a very plastic molecule that can adopt many different structural conformations than translate into different functional activities (its phenotype). Hence, genotype and phenotype are represented by a single molecule, with all the implications that this has for molecular selection and evolution.

Improvements in the amount of data that can be simultaneously dealt with by computers, and in their calculation speed, allow detailed numerical studies of the evolutionary dynamics of large populations of RNA molecules. RNA sequences can be reliably folded into their secondary structure (identified in a first approximation with the phenotype of the molecule), such that selection pressures can be applied on the phenotype, and not on the genotype. This gives rise to new evolutionary dynamics, and permits to quantify, among others, the relationship between sequence and structure spaces [11,12].

Foreseeable progress in our understanding of evolutionary mechanisms will come from three different approaches. The combination of sequencing techniques and genomic analyses sheds light on the molecular changes experienced along evolution by evolving populations. Secondly, numerical studies including explicit representations of molecular structure give further insight on the relevance of selection mechanisms acting on the phenotype, and on the paths followed by sequences to solve adaptation to new environments. And, thirdly, new experiments with RNA viruses and with RNA molecular populations will yield new data on population evolution and on the relevant mechanisms at play in molecular evolution.

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