Getting to Know Viral Evolutionary Strategies: Towards the Next Generation of Quasispecies Models

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Abstract Viral populations are formed by complex ensembles of genomes with broad phenotypic diversity. The adaptive strategies deployed by these ensembles are multiple and often cannot be predicted a priori. Our understanding of viral dynamics is mostly based on two kinds of empirical approaches: one directed towards characterizing molecular changes underlying fitness changes and another focused on population-level responses. Simultaneously, theoretical efforts are directed towards developing a formal picture of viral evolution by means of more realistic fitness landscapes and reliable population dynamics models. New technologies, chiefly the use of next-generation sequencing and related tools, are opening avenues connecting the molecular and the population levels. In the near future, we hope to be witnesses of an integration of these still decoupled approaches, leading into more accurate and realistic quasispecies models able to capture robust generalities and endowed with a satisfactory predictive power.

Contents

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1 Introduction

Our intuition on the origin and mechanisms of adaptation often fails when confronted with the products of evolution. We tend to depict the adaptation of populations, and the strategies that permit it, in the way an engineer would do. But natural selection, relying on an apparently simple trial-and-error principle, prospers through the deployment of a large number of unforeseeable solutions for the survival of organisms. Viruses, in their quality of fast evolving entities, count among the most creative adapters on Earth. And they are as well excellent examples of how our educated guesses do not succeed at predicting the outcome of evolutionary experiments or the strategies used in adaptation.

Very often, we resort to three features of viruses that underlie their enormous plasticity (large population numbers, high mutation rates—especially in RNA viruses—and short generation time) to "explain" their adaptive success. But these are just the very plain ingredients of the receipt. Viral populations are diverse not only because they contain a variety of self-sustained genotypes, but especially because several different phenotypes might coexist in the same quasispecies. In this sense, the idea of a fittest genome (often known as the "master sequence"), and of its preeminence above all other mutants, loses meaning. Perhaps concepts borrowed from ecology, where organisms with different roles are necessary to sustain the system in a dynamic equilibrium, would offer a better picture of the complex relationships established among the genomes that integrate a single viral population. In the context of a viral ecology, it would be easier to integrate cheaters, defectors or altruistic cooperators as different phenotypes that simultaneously thrive in quasispecies. Understanding the strengths of a heterogeneous ensemble of mutants is a step forward towards understanding the diversity of strategies that viruses might use to ensure their survival.

There are abundant examples of viral adaptation that challenge classical theories and a priori expectations of viral behaviour. Let us illustrate this statement with three examples. Our first example regards the origin of fitness gain in bipartite viruses. Multipartite viruses have fragmented genomes encapsidated in different particles and represent about 50 % of all viruses infecting plants (Hull 2013). To complete an infection cycle, at least one representative of each of the fragments must be present inside the cell, a condition that usually requires a very high multiplicity of infection (MOI). The success of multipartite viruses in front of non-fragmented counterparts requires that the cost of high MOI be compensated by an advantage originating from the fragmentation and separated encapsidation of the genome. Classical theories suggested that an increase either in the speed of replication or in higher copying fidelity due to shortened genomes might compensate for the disadvantage of mandatory coinfection (Nee 1987; Chao 1991). However, a series of experiments where a complete, wild-type genome of foot-and-mouth disease virus (FMDV) was displaced at high MOI by a cognate, bipartite form evolved in vitro (García-Arriaza et al. 2004) failed to detect any of those putative advantages. After several years of efforts, the advantageous mechanism could be

eventually identified. Viral particles in the bipartite form of FMDV enjoy a higher stability, likely due to a decreased pressure exerted in the capsid by genomes of smaller size (Ojosnegros et al. 2011). Higher stability translates into longer average lifetime, sufficient to displace the wild type at an MOI that permits frequent coinfection. Subsequent studies have shown, however, that the initial transition towards genome segmentation was favoured by the accumulation of mutations during the evolution of the standard genome and that those mutations conferred higher replicative fitness to the segmented genome version than to the standard genome (Moreno et al. 2014). The relative stability of the wild-type versus the bipartite form is related in a specific functional manner to the MOI, setting limits to the emergence of multipartite viruses from complete cognate wild types (Iranzo and Manrubia 2012). Several questions on the adaptive strategies of multipartite viruses remain open, since the mechanism leading to the fixation of bipartite forms described in the previous studies (segment deletion followed by competition with a non-fragmented counterpart) cannot explain the success of multipartite viruses with more than three fragments or the recently identified imbalance in the frequencies of genomic fragments of Alfalfa mosaic virus (Sánchez-Navarro et al. 2013) and Faba bean necrotic stunt virus (Sicard et al. 2013).

Our second example regards a potentially lethal role played by defective viral genomes in the population. Replication of RNA viable viral genomes steadily generates defective mutants able to replicate through the use in *trans* of essential resources provided by complementary genomes. A puzzling observation arose in an experiment with persistent infections of lymphocytic choriomeningitis virus (LCMV) under the action of mild doses of a mutagenic drug: while the intracellular levels of RNA seemed unaffected by the mutagen, the ability of the virus to infect new cells systematically declined until extinction (Grande-Pérez et al. 2005). There were some important elements in those experiments that caused the final output. First, a persistent infection permits the accumulation of defective, replicatively competent forms that may lose the ability to infect, since this latter trait is not actively selected in that scenario. Second, there is a competition within the quasi-species for replicative resources, used by all genomes but produced only by the "altruists" that survive. Third, since the number of genomes inside a cell is finite, and the replicative process is by nature affected by severe fluctuations in the numbers of defectors versus wild-type genomes, there is a nonzero probability that defectors take over and, subsequently, the population goes extinct due to the absence of basic resources (Iranzo and Manrubia 2009). In the experiment with LCMV, the collective dynamics of the population, together with the particular environment, is essential to determine the final outcome, which would be much alleviated in the case of a lytic infection or if the system would be described through a model with an infinitely large population—as assumed in many mathematical models of viral evolution. There are other formal scenarios where intraspecific competition might lead to potential threats to the survivability of viral populations, e.g. when there is a limitation in the number of susceptible cells for infection, which may cause decreases in diversity (Aguirre and Manrubia 2008) and eventually the extinction of the population if resistant cells appear (Cuesta et al. 2011). In many

cases, the consideration of a limited number of viral particles, and even of extreme population bottlenecks, becomes essential to our understanding of the dynamics and fate of viral populations in many realistic situations (Lázaro et al. 2006; Manrubia and Lázaro 2006).

Our third example has to do with how compensatory mutations act to balance the accumulation of deleterious mutations (and thus to avoid sustained degradation) in small populations. Muller's ratchet theory (Muller 1964) posits that small asexual populations must accumulate mutations in an irreversible way. Assuming that the least mutated genomes are the fittest ones, an irreversible process of fitness loss might be triggered by the systematic application of extreme bottlenecks. With this proviso, an experiment was designed to force the accumulation of mutations in FMDV with the aim of quantifying the process of extinction (Escarmís et al. 2002). Unexpectedly, after several passages, the virus stopped losing fitness and entered a regime with a well-defined average population size subjected to strong fluctuations (Lázaro et al. 2003), the sustained accumulation of mutations notwithstanding. The only explanation for this behaviour is that, contrary to expectations, not all mutations fixed have a deleterious effect in fitness, as assumed by theory. Actually, the fraction of compensatory, even beneficial, mutations increases as the average fitness of the population decreases. Eventually, a balance between the size of the bottlenecks and the degree of optimization of the population is established (Lázaro et al. 2002).

The previous examples illustrate the complex behaviour of viruses at the population level and the many factors that may contribute to the collective organization and response of those populations to different selection pressures. There is, however, a number of underlying molecular mechanisms, also with unexpected effects at the level of phenotypes, that make possible and at the same time condition population dynamics. Most empirical efforts have been devoted to the description and understanding of the effect of specific mutations in particular genomes, as reviewed in the first sections of this contribution. Only a deeper understanding of the quality and quantity of interactions between mutations, of the genotypic and phenotypic diversity of quasispecies, of the effect of different mutational mechanisms and also of the interactions between coexisting viral phenotypes can lead to a reliable theory of viral evolution and adaptation, eventually endowed with the ability to predict the responses of viral populations to changing environments. A new generation of quasispecies models should emerge from the integration of molecular information with population behaviour (Manrubia 2012).

2 The Complex Response of RNA Viruses to Increases in the Mutation Rate

Early quasispecies theory predicted the existence of a maximum error rate—the error threshold, compatible with the maintenance of genetic information (Eigen 1971; Biebricher and Eigen 2005). Above that threshold, the population is displaced towards regions of the sequence space occupied by similar, low-fitness

genomes. At that point, selection is no longer efficient and genetic information cannot be maintained (Eigen 2002). The predicted value of the error threshold depends strongly on the specific features considered, among others the length of genomes, the roughness of the fitness landscape, the abundance of lethal mutants or the molecular degradation rate (Saakian and Hu 2006; Saakian et al. 2006; Takeuchi and Hogeweg 2007). Theoretical expectations motivated empirical studies aimed at evaluating the likelihood that artificial increases in the error rate could cause the extinction of viral populations (Eigen 2002). In vitro experiments to test this so-called lethal mutagenesis hypothesis have achieved remarkable success (Agudo et al. 2009; Crotty et al. 2001; Dapp et al. 2009; Grande-Pérez et al. 2002; Holland et al. 1990; Loeb et al. 1999; Severson et al. 2003; Sierra et al. 2000), demonstrating that viral extinction can indeed be caused by sufficiently large increases in the mutation rate (reviewed in Perales et al. 2011b; Domingo et al. 2012). This success nonetheless, there is as yet no convincing proof that the observed extinctions are the consequence of crossing an informational error threshold of the kind described in Eigen's quasispecies theory (Bull et al. 2007; Manrubia et al. 2010; see also Chaps. 13 and 14).

In general, well-adapted viral populations propagated in constant environments under the action of high concentration of mutagens are extinguished or reduce their infectivity (Moreno et al. 2012; Perales et al. 2011a; Arias et al. 2013; Arribas et al. 2011). However, when the viral population is allowed to adapt to gradual increases in mutagen concentration, variants that better resist the drug can be selected, and the population may escape extinction (Arias et al. 2008; Arribas et al. 2011; Agudo et al. 2010; Pfeiffer and Kirkegaard 2003). In addition to raising concerns on the efficacy of antiviral therapies based on lethal mutagenesis (Iranzo et al. 2011; Perales et al. 2012), these mutants have shown that the high error rates of RNA viruses are a necessary condition for efficient adaptation to complex selective pressures (Pfeiffer and Kirkegaard 2005; Vignuzzi et al. 2006). There could even be circumstances in which increases in the error rate might occasionally enhance virus performance. For instance, a study carried out with an RNA bacteriophage showed that the lytic plaques developed from a low-fitness mutant in the presence of a mutagenic nucleoside analogue had higher titres than those developed from the same virus at standard error rate (Cases-González et al. 2008). Those results suggest a positive effect of the increase of the error rate in low-fitness viruses, more prone to experience beneficial mutations. A similar effect might be observed when mutagenic treatments are applied in changing environments. At the first stages of an adaptation process, there is a larger availability of beneficial mutations, again potentially implying a beneficial action of an increased error rate. Some viruses that replicate in alternating hosts experience higher mutation rates in one of them (Coffey et al. 2011; Vasilakis et al. 2009), maybe responding to the need of generating a larger genetic diversity to permit adaptation. The effects of mutagens might be confounded due to their capacity to simultaneously inhibit virus replication (Pariente et al. 2003). For example, a study carried out with VSV subjected to a mutagenic treatment showed decreased fitness and adaptability (Lee et al. 1997), two typical outcomes of inhibited viral replication.

Mutation rates vary across viruses and even within the same virus when growing in different environments and are dependent on the genetic background. These dependencies draw a complex picture where the response to additional increases in the error rate may differ largely between populations. The estimated error rate for RNA viruses ranges from 10^{-5} to 10^{-3} errors per nucleotide per cell infection (Drake and Holland 1999; Gago et al. 2009; Holland et al. 1982; Sanjuán et al. 2010). The host cell may induce variations of the viral error rate depending on factors such as nucleotide composition (Julias and Pathak 1998), presence of mutagenic reactive oxygen species derived from the metabolism of the cell (Seronello et al. 2011) or expression of genes such as APOBEC3 or ADAR, which cause hypermutability in several viruses (Holtz and Mansky 2013; Harris et al. 2003). Differences in the viral mutation rates have also been observed across hosts or cell type infected (Pita et al. 2007). Actually, lethal mutagenesis studies yield many examples of the variety of responses elicited from RNA viruses subjected to artificial increases in the error rate: fitness decreases and extinction (Perales et al. 2011a; Domingo et al. 2012), genomic shifts in sequence space (Perales et al. 2011c), alterations of the network of interactions within the mutant spectrum (Grande-Pérez et al. 2005) or selection of resistant mutants (Arias et al. 2008; Arribas et al. 2011; Agudo et al. 2010; Pfeiffer and Kirkegaard 2003; Iranzo et al. 2011). The evolutionary history of a viral population eventually determines the region of the sequence space it occupies (its genomic diversity), a fact that affects genetic robustness, viral plasticity and the effect of new mutations (Sanjuán et al. 2007; Graci et al. 2012).

Augmenting the error rate also disturbs current interactions within the mutant spectrum, as shown in the case of lethal defective particles (Grande-Pérez et al. 2005; Iranzo and Manrubia 2009). The role of defective interfering genomes was further demonstrated also in FMDV and other viruses (Perales et al. 2007). The transition from the mainly cooperative interactions that occur in optimized virus quasispecies to the defective interactions in preextinction quasispecies can be understood as a continuum driven by the error rate (Domingo et al. 2005). High multiplicities of infection may favour interactions among mutants and thus require higher error rates to achieve extinction. In this sense, population bottlenecks could be very effective to clean viable genomes from the poisoning effect of hypermutated mutant spectra (Manrubia et al. 2010; Lázaro et al. 2006). Mutagenic treatments enlarge the region of sequence space explored by a virus (Ojosnegros et al. 2008) due to an increase in the diversity of the population. Examination of the FMDV mutant spectrum in the presence of ribavirin showed an increase in the frequency of A/G and C/U transitions, causing a displacement towards regions of sequence space where the virus was more prone to experience deleterious mutations (Perales et al. 2011c). A ribavirin-resistant mutant isolated in that same virus restored the normal transition pattern without affecting the average mutation frequency or the incorporation of ribavirin by the replicase (Agudo et al. 2010). This kind of displacements could be very effective at moving populations to unexplored regions of the sequence space and could have unexpected evolutionary consequences, particularly if the increase of the error rate is transitory and the hypermutated variants have the

opportunity to recover fitness through compensatory mutations that stabilize the population in the new genomic region. These are excellent examples of the complex interplay between molecular changes and population dynamics, and a step forward towards connecting these two levels.

3 The Effect of Mutations on Fitness

The development of useful theories of viral evolution and adaptation depends on our knowledge of the effects of mutations on phenotype. The examples of the previous section illustrate a non-trivial collective response of viral populations when the mutation rate increases, including enhanced adaptability. Natural mutation rates suffice in most cases to guarantee the latter, often not only through point mutations, but also through other mutational mechanisms that cause segment deletions, genome recombination or genome fragment shuffling. In this section, we review the empirical efforts addressed at quantitatively characterizing the effect of point mutations in fitness, and their dependence on the genetic background and on the current environment.

Studies carried out with site-directed randomly chosen single mutants of vesicular stomatitis virus (VSV) (Sanjuán et al. 2004a), bacteriophage Qβ (Domingo-Calap et al. 2009) and tobacco etch virus (TEV) (Carrasco et al. 2007) show that a high fraction—from 0.20 to 0.41—of the mutations that arise in the genome is lethal. Focusing on viable mutations, deleterious mutations are much more frequent than beneficial, and mutations of mild effect are more likely than those of large effect (Sanjuán 2010). The high amount of deleterious and lethal mutations might be partly due to the small size of most RNA virus genomes, which imposes a high degree of compaction of the genetic information in overlapping reading frames and coding sequences that simultaneously hold structural functions (Holmes 2003). Despite this apparently low tolerance to mutations, RNA viruses maintain high heterogeneity and adaptability, due to the multifactorial and context-dependent effect of mutations. In particular, interactions between mutations modulate their value, such that the fitness of a viral genome is continuously redefined along evolution.

Mutations that occur in genomes differing in more than a single substitution permit the appearance of interactions among mutations, or epistasis (Phillips 2008). Epistatic interactions show up when the combined effect of different mutations in the same genome does not equal the sum of the effects of each independent mutation. Several classes of epistasis arise depending on whether the mutations that interact are beneficial or deleterious, and on whether the sum of effects is higher or lower than expected by simple addition of their individual effects (case of no epistasis). Particular cases are synergistic (antagonistic) epistasis, where the total effect of two mutations increasing fitness is larger (smaller) than the sum of the individual effects, sign epistasis (a deleterious mutation reverts its negative effect in the context of a positive mutation) and reciprocal sign epistasis (two deleterious

mutations have a positive effect when they co-occur). Epistasis has been detected in many RNA viruses such as bacteriophage Φ6 (Burch and Chao 2004), TEV (Lalić and Elena 2012), FMDV (Elena 1999), Chikungunya virus (Tsetsarkin et al. 2009), VSV (Sanjuán et al. 2004b) and Human immunodeficiency virus (HIV) (Martínez et al. 2011; Bonhoeffer et al. 2004; Kouyos et al. 2012). Compensatory mutations are a case of reciprocal sign epistasis that likely plays an essential role in evolution and adaptation (Covert et al. 2013). Fitness changes monitored in several clones of FMDV transmitted through successive population bottlenecks, where the fixation of mutations proceeded independently of their selective value, were interpreted on the basis of a higher availability of compensatory mutations in low-fitness genomes (Lázaro et al. 2002; 2003). Compensatory mutations also balance the fitness cost of drug-resistant mutants, causing the permanence of the resistant phenotype in the absence of the drug (Buckheit 2004). The dynamics of adaptation to new selective pressures, showing the largest fitness gains at the beginning of the process, has been ascribed, at least partially, to a diminished effect of beneficial mutations as fitness increases, which is also a form of epistasis (Bull et al. 2000; Rokyta et al. 2011).

The sign and the magnitude of the effect of mutations are also strongly influenced by the environment and in the particular case of viruses by the host they infect. Often, adaptation to a host has a cost that translates into worsened performance in alternative hosts (Turner and Elena 2000; Weaber et al. 1999; Lalić et al. 2011). In other cases, however, evolution in a particular host does not entail a cost in others (Núñez et al. 2007) and can even result in fitness gains (Remold et al. 2008; Coffey and Vignuzzi 2011). A cost of fitness across environments has been also observed in drug-resistant mutants, which usually pay a fitness cost when the drug is absent (Armstrong et al. 2011). In the case of escape mutants, genomic changes carried by resistant phenotypes typically target critical virus functions and are accompanied by fitness decreases (Das et al. 2011). This kind of fitness trade-offs can be due to antagonistic pleiotropy, that is to mutations beneficial in the selected environment but deleterious elsewhere, or to the accumulation of mutations neutral in one environment but disadvantageous in others. Whereas these studies clearly demonstrate that a particular combination of mutations does not perform equally in different environments, they usually do not identify the contribution of individual mutations to the overall effect. This question was addressed by Elena and co-workers in a study where they characterized a collection of twenty single-nucleotide substitution mutants of TEV across a set of eight host environments, five of which were natural hosts for the virus (Lalić et al. 2011). They found that the fraction of lethal, deleterious, neutral and beneficial mutations depended on the specific host, and this dependence varied with the phylogenetic distance among hosts. In the same way that the fitness effects of mutations change with the environment, there is no reason to expect that epistatic interactions are independent of the environment or of the overall genomic context. Another study carried out with TEV virus compared the effect of pairs of mutations in different hosts, showing that epistasis was indeed affected by the degree of genetic divergence between the primary and the alternative hosts (Lalić and Elena 2013).

4 Fitness Landscapes

A fitness landscape is a mapping from the genomic, multidimensional space, to a real value that represents fitness (Wright 1931; see also Chap. 4). The empirical observations described up to now offer glimpses of the local structure of viral fitness landscapes and of the short-term consequences of their structure. Often, only mutants differing in one or few sequence positions have been studied, and even landscapes as small as that in Fig. 1b have not been fully described. Further, the dependence of fitness on the endogenous environment and on current selective pressures extraordinarily complicates the picture. At this point, there are two different approaches one can take in order to advance in the understanding of viral evolution. First, one can target particular model systems and the value of precise mutations. These studies are relevant to understand the effect of specific mutations in the studied context and may have predictive power regarding the system under study. On the negative side, these models are difficult to generalize. Second, one can take a statistical point of view, where emphasis is put on the average effects of mutations in fitness and the typical structure of fitness landscapes in order to derive expectations on the dynamics of populations. In this second case, the ability to perform specific predictions diminishes, but the results might be applicable, as a first approximation, to a larger number of systems and evolutionary contexts. It is likely that new techniques soon yield quantitative information on much larger regions of the sequence space, working towards a convergence of those currently separated approaches.

There have been abundant formal attempts to derive a functional relationship between the mutations acquired by a genome and its fitness, which represents a first step towards characterizing the structure of fitness landscapes. Early on, simple dependencies led to assuming that evolution was occurring on a smooth, Fujiyama-like landscape (Kimura and Maruyama 1966), where epistasis was absent. Populations would steadily accumulate beneficial mutations until the unique, global fitness maximum would be reached. At the other extreme, there was the assumption of a random landscape, which ignored correlations between the phenotype yielded by a genome and that of its mutational neighbours, and predicted an exponential shape for the distribution of beneficial effects (Orr 2003). The study of the functional form of the effect of mutations in fitness is actually a complementary way to probe the structure of a fitness landscape. In highly correlated landscapes (e.g. Fujiyama-like), most mutations have a small effect on fitness and yield similar phenotypes; mutations with a large effect are exponentially rare, implying that they are not to be detected in practice. In random landscapes, the effect of a mutation cannot be predicted, in the sense that any change in fitness is possible. As it could have been guessed, a realistic fitness landscape lies somewhere in between those extremes: most mutations have a small effect in fitness (Orr 1998; Lourenço et al. 2011), but the probability to have a mutation with a medium-to-large effect is non-negligible (Lalić et al. 2011). Significant advances in this direction might arrive soon in the light of recent achievements regarding the

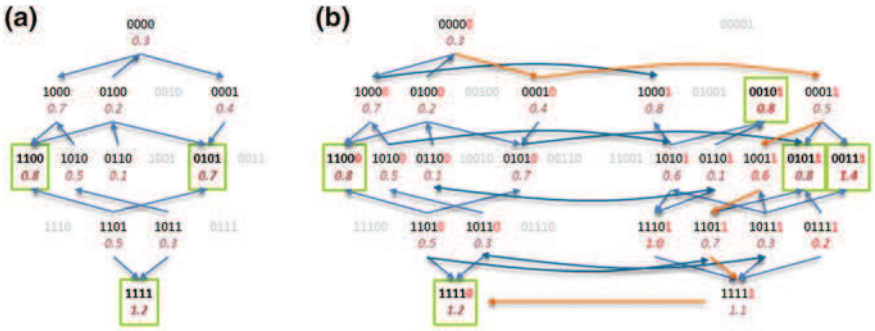

Fig. 1 Epistasis and roughness of fitness landscapes. Fitness is indicated below each of the sequences. Sequences of zero fitness are represented in *pale grey*. *Arrows* point towards increases in fitness. Local maxima appear in *bold face* within a *green square*. **a** Example of fitness landscape for a genome of length 4. This landscape has three maxima, two local and one global. The latter (1111) cannot be reached through point mutations from most other sequences (1101 and 1011 are the exceptions). There are several cases of epistasis, which make the landscape rough. An example of negative sign epistasis is the group {0000,1000,0100,1100} and one of reverse sign epistasis {1100,0100,0101,1101}. **b** When longer sequences are considered, new pathways for adaptation appear, and the position of global and local maxima might change. Now, the former global maximum 11110 can be attained through point mutations from 00000, as indicated by *orange arrows*, and from several other sequences. However, it has become a local maximum, and it is likely that the population either attains the new global maximum 00111 or gets trapped at the local maximum 01011. Sequences {00101,10101,01101,11101} form an example of reverse sign epistasis and {00010,00011,01010,01011} of no epistasis (thus not affecting landscape roughness). There are also two neutral mutations, between pairs {01100,01101} and {10110,10111}. Note that those same mutations change value when the genomic context varies

overall structure of viral fitness landscapes (Kouyos et al. 2012; Acevedo et al. 2014). A precise description of the shape of the distribution of fitness effects (Good et al. 2012), and by extension of the structure of fitness landscapes, is essential to understand the evolution and adaptation of viral populations. Quantitative information on the local and global structure of fitness landscapes will probably experience a boost in the near future thanks to the extended use of next-generation sequencing technologies (Beerenwinkel and Zagordi 2011; Radford et al. 2012).

Epistasis and roughness of fitness landscapes are two sides of the same coin. Depending on the type of epistasis dominant in natural populations, fitness landscapes can rank from smooth (no epistasis or magnitude epistasis) to rough, with multiple adaptive peaks. Meta-analyses of empirically characterized fitness landscapes point to the existence of certain general properties in landscapes described to date that make them compatible with a rough, Fujiyama-like structure (Szendro et al. 2013). In the case of RNA viruses, the abundance of sign and reverse sign epistasis speaks for rugged fitness landscapes, with many peaks and valleys a few mutational steps away (Withlock et al. 1995; Poelwijk et al. 2011). Epistatic interactions are also responsible for the appearance of ridges in the fitness landscape, that is sets of genomes of similar fitness that permit an easy exploration of

the space of genotypes. In the limit where genomes have equal fitness and are mutually accessible through mutations, it has been shown that populations tend to select the most connected regions of this neutral network, where they attain maximal robustness (de Visser et al. 2003; Huynen et al. 1996). Robustness may be a property only observable in large populations that have evolved for a sufficiently long time at high error rate (Lauring et al. 2013). Though rarely observed, this collective behaviour has been detected in micro-RNAs (Borenstein and Ruppin 2006), viroids (Codoñer et al. 2006) and RNA viruses (Sanjuán et al. 2007).

5 Discussion and Prospects

The adaptive potential of RNA viruses depends on the interplay among many factors, such as high mutation rates, epistasis (dependence of mutations on the genomic context), robustness (or tolerance to mutations) or interactions within the mutant spectrum. Adaptation depends on environmental conditions, which determine the number and strength of selective pressures applied to viruses and promote the appearance of specific solutions (of which resistance to drugs is a particular case). A complete theory with predictive power seems a formidable task at the moment, though empirical knowledge and steady improvements in quasispecies models (be they applied to specific experimental scenarios or aimed at capturing general features of viral evolution) are widening our view of the problem and, hopefully, offering a broadening and increasingly complete picture (see Chaps. 1, 3, and 4).

The properties of the fitness landscape determine the nature of the evolutionary trajectories available to populations (Poelwijk et al. 2007). Our current picture is that of a correlated (relatively smooth) landscape at the local scale, crossing over to a rougher and eventually random landscape at large genomic distances (Kouyos et al. 2012). Local correlations, that is the presence of mutations that affect little or do not affect fitness, might construct long pathways that, as proposed by Maynard Smith decades ago, should permit the almost costless navigation of the genome space (Maynard Smith 1970). Regions of high robustness to mutations support a larger diversity of the population and consequently grant access to a larger number of evolutionary innovations (novel phenotypes), eventually promoting adaptation (Wagner 2012). There is indirect evidence that the number of different genotypes yielding an almost invariable phenotype is a large set that under very general conditions might span most of the genome space (Wagner 2011). If this is so, most phenotypes might be a few mutational steps away. At present, we have a handful of examples where evolution consisting in periods of drift along quasineutral networks of genotypes, followed by the identification and fixation of a phenotype of higher fitness, has been described (Koelle et al. 2006; Woo and Reifman 2012), but the way towards characterization of this behaviour in other systems is paved.

As it occurs in most of the empirical and theoretical analysis performed to date, we have mostly reviewed the effect of point mutations in evolution and adaptation

—with the exception of the few examples on the role of large genomic deletions in population dynamics. If we assume single mutations as the mechanism that drives movement on genotype spaces, only contiguous genotypes can be accessed. However, other mechanisms as recombination modify the accessibility of different genotypes and the simple image of diffusive movement on a landscape. The final states of the population are significantly modified when recombination comes into play: mutation–selection equilibrium is no longer unique and depends on the evolutionary history of the population. Other mutational mechanisms and sources of variability, and their effects when coupled to a particular genomic, populational or environmental context, should be included in future conceptual and formal approaches to viral evolution.

Our hope for a meaningful theory of viral quasispecies relies in the existence of generic mechanisms or, at least, in a reduced number of evolutionary classes. The identification of universal patterns in the fitness landscape of viruses and other evolutionary systems (as proteins or whole genomes) should prove a tremendous advance in the development of evolutionary theories of broad applicability. The first steps in that direction are encouraging and promise very exciting challenges and advances in the near future.

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