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On the Biological Success of Viruses

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Abstract

Are viruses more biologically successful than cellular life? Here we examine many ways of gauging biological success, including numerical abundance, environmental tolerance, type biodiversity, reproductive potential, and widespread impact on other organisms. We especially focus on successful ability to evolutionarily adapt in the face of environmental change. Viruses are often challenged by dynamic environments, such as host immune function and evolved resistance as well as abiotic fluctuations in temperature, moisture, and other stressors that reduce virion stability. Despite these challenges, our experimental evolution studies show that viruses can often readily adapt, and novel virus emergence in humans and other hosts is increasingly problematic. We additionally consider whether viruses are advantaged in evolvability—the capacity to evolve—and in avoidance of extinction. On the basis of these different ways of gauging biological success, we conclude that viruses are the most successful inhabitants of the biosphere.

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INTRODUCTION

Ain't you hungry for success, success, success, success? Does it matter?

—The Rolling Stones, *Shattered* (1978)

Are viruses the most successful inhabitants of the biosphere? Our contention is the affirmative, and the goal of this article is to similarly convince the reader. The evidence we present is manifold. Viruses are ubiquitous, outnumbering cellular organisms and seemingly occupying every possible environmental niche that can support life. Although primarily submicroscopic, this viral multitude presents extreme biodiversity, with differing morphologies, varying strategies of host infection, and all known types of nucleic acids for genetic inheritance across generations. Perhaps most impressively, viruses appear very capable of adapting in the face of environmental changes; they can often evolve to overcome host resistance and immunity and may also prevail despite environmental perturbations experienced outside of their hosts. The repercussions of this adaptability extend beyond the viral world. Owing to the prevalence of virus-host interactions, viruses can strongly impact which host types dominate populations and communities, in turn affecting all levels of biological organization, from host-genome composition to ecosystem function. On the basis of many different ways of gauging biological success, we provide overwhelming evidence that viruses are extremely successful at what they do: continually evolving in order to harness cellular life to replicate and produce more viruses.

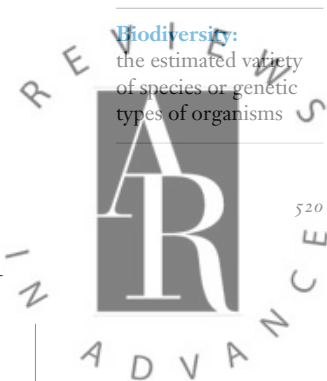
PART I: WHAT CONSTITUTES BIOLOGICAL SUCCESS?

Can it be argued, however, that viruses are the most successful inhabitants of our planet? Biological success is an old concept, predating the discovery of viruses around the turn of the twentieth century. In the mid-1800s and before, Charles Darwin (20) and earlier evolution thinkers tried to make sense of how and why some biological types persisted in nature, whereas others did not. What constitutes biological success? Most broadly, biological success has been discussed historically in terms of comparisons between groups of organisms, at taxonomic or nontaxonomic levels above that of species. For example, flowering plants—the angiosperms—are often touted as more successful than their nonflowering counterparts—the gymnosperms—because flowering plants show greater species diversity. But relative biodiversity is merely one metric for gauging the biological

Biodiversity:
the estimated variety
of species or genetic
types of organisms

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success of groups, and other bases for comparison exist; if ability to thrive in cold temperatures and nutrient-poor soils are the measures, gymnosperms instead are the more impressive land plants. Thus, in examining whether viruses are more biologically successful than Earth's other inhabitants, we take care to consider many different means for comparison, rather than a single one.

Here we examine the biological success of viruses, using metrics that include numerical abundance, environmental tolerance, biodiversity, reproductive potential and efficiency, and modes of genetic inheritance. However, our greatest focus is on viral adaptability in the face of various biotic and abiotic environmental changes; we draw heavily from our own empirical studies with RNA viruses. In addition, we consider metrics that are admittedly very difficult to assess, such as relative evolvability and persistence in the face of extinction. Judging by the evidence amassed from these different means for comparison, our contention is that on average viruses are indeed the most successful group of biological entities on the planet.

What is the utility of determining whether viruses are more successful than other organismal groups, which essentially means all of cellular life? If biological success is an ill-defined term, for which no consensus exists for making comparisons, is it even useful to spend time and energy examining this idea? Viruses are not composed of cells and therefore are not typically considered alongside other biological entities, for example, when building the universal tree of life (18). This omission is due to a dichotomy between fundamental body plans; a virus is generally composed of nucleic acid surrounded by a protein shell (capsid), whereas cellular life consists of ribosome-encoding organisms (64). However, viruses and cellular organisms both play by the same evolutionary rules: the processes of spontaneous mutation, natural selection, genetic drift, etc., that underlie the evolutionary patterns described by phylogenetic inference. This commonality indicates that a straight-up comparison is legitimate and potentially highly useful. The relative simplicity of viral genomes and life cycles provides tractable opportunities to understand how evolution proceeds in response to selective challenges. Therefore, by understanding how viruses achieve biological success—especially through adaptive evolution—we can infer by extension what promotes versus limits biological success in all evolving systems.

Viral Abundance

If sheer abundance were the only criterion for defining biological success, the argument would be over; viruses are estimated to easily outnumber all forms of cellular life combined. This comparison begins by considering the eubacteria, which are extremely ancient, having appeared roughly 3 to 4 billion years ago. Bacteria are not only ancient but also highly numerous, often achieving large population sizes in the wide variety of niches than can support metabolizing organisms; overall, bacteria number perhaps 10^{30} individuals at any one time across the planet (91). However, the vast majority of studies that relate bacterial numbers to those of bacteria-specific viruses (the bacteriophages) show that phages are numerically superior (7). In particular, surveys from aquatic environments (especially the oceans) demonstrate that viruses tend to exceed their unicellular hosts by at least an order of magnitude (6, 39, 74). Thus, just counting the phages in the virosphere, perhaps 10^{31} – 10^{32} individual viral particles exist on the planet at any one moment (39).

We are unaware of any similar attempts to compare viral numbers with those of their archaeal and eukaryotic hosts. However, one recent study employed pyrosequencing and bioinformatics methods to examine the DNA of viruses found in raw-sewage samples from around the world (14). Although this approach did not allow for numerical estimates of viral types, the study clearly showed that phages are more abundant than viruses of eukaryotes. The study also found that a huge variety of viruses remain unclassified, with no genetic match to characterized viruses described in genetic databases. These results exemplify the fact that the vast majority of viruses remain to

Evolvability: the capacity of a population to evolve via natural selection

Extinction: the disappearance of a population or species, causing reduced biodiversity

Natural selection: the process of genetic change in a population, whereby variants advantaged in survival and/or reproduction increase in frequency



be discovered, and they reinforce the notion that our understanding of the virosphere is woefully incomplete. Only greater sampling and surveillance of natural ecosystems can ultimately remedy this shortcoming. Nevertheless, these studies indicate the awesome numerical abundance of viruses on Earth and support the conclusion that they are numerically dominant in the biosphere.

Viral Biodiversity

One alternative means to gauge the biological success of viruses is to consider their known biodiversity. Earth contains a wide variety of potential habitats (available niches) that life can occupy. On the one hand, evolution of phenotypic plasticity can allow a single species to inhabit multiple varied niches. On the other hand, organisms can evolve to be highly niche specific, as shown by the process of adaptive radiation, in which a group of related species has evolved to occupy different niches. Viruses lean more toward the latter example. As a group, viruses appear to be composed of myriad species that occupy different ecological niche spaces, largely defined by the specific host(s) that provides a suitable environment for viral replication.

Viruses infect host species throughout all major branches of the cellular tree of life (61) (**Figure 1**). This means viruses share those terrestrial and aquatic environmental niches that support cellular life around the globe. This biological success extends to virus-host interactions in environments with extreme temperature and pH and with low oxygen and available light. Because so much of viral diversity remains unknown, the fundamental natural history of viruses is a very active area within virology. A prime example is the discovery and description of viruses that specifically infect archaeal hosts (archaeal phage); the often unique morphologies of these viruses have warranted naming of new viral families (**Figure 1**), which may constitute fertile territory for taxonomic discovery in viruses in the foreseeable future (60). These recent archaeal phage discoveries include the first described RNA viruses of archaea and a virus that intriguingly contains a “hybrid” genome of both RNA and DNA viral origins, not observed in any other virus to date (10, 27). Furthermore, the recent discovery of large viruses has overturned the old assumption that all viruses are submicroscopic. Rather, mimiviruses rival in size the smallest known cellular life, such as *Mycoplasma* bacteria (63). Undoubtedly, future discoveries will continue to identify novel viral families, greatly expanding their already impressive biodiversity.

Across known viral families, viruses present exceedingly diverse morphologies, ranging from icosahedrons, to rod-like filamentous particles, to sack-like structures (61) (**Figure 1**). Aside from this structural diversity, viruses feature a diversity of genetic options for inheritance across generations that is simply not present in cellular life. All cellular organisms rely foremost on double-stranded DNA as the nucleic acid that dictates phenotypic traits and cross-generational inheritance. In contrast, viruses show all possible genetic options, with genetic inheritance determined by double- and single-stranded DNA and RNA, depending on the viral family. These different inheritance strategies are used to define viruses under the Baltimore classification system, which distinguishes viruses by the replicative strategy that ultimately creates the viral mRNA translated by the host cell (4). Interestingly, these genetic options generally align with the morphological

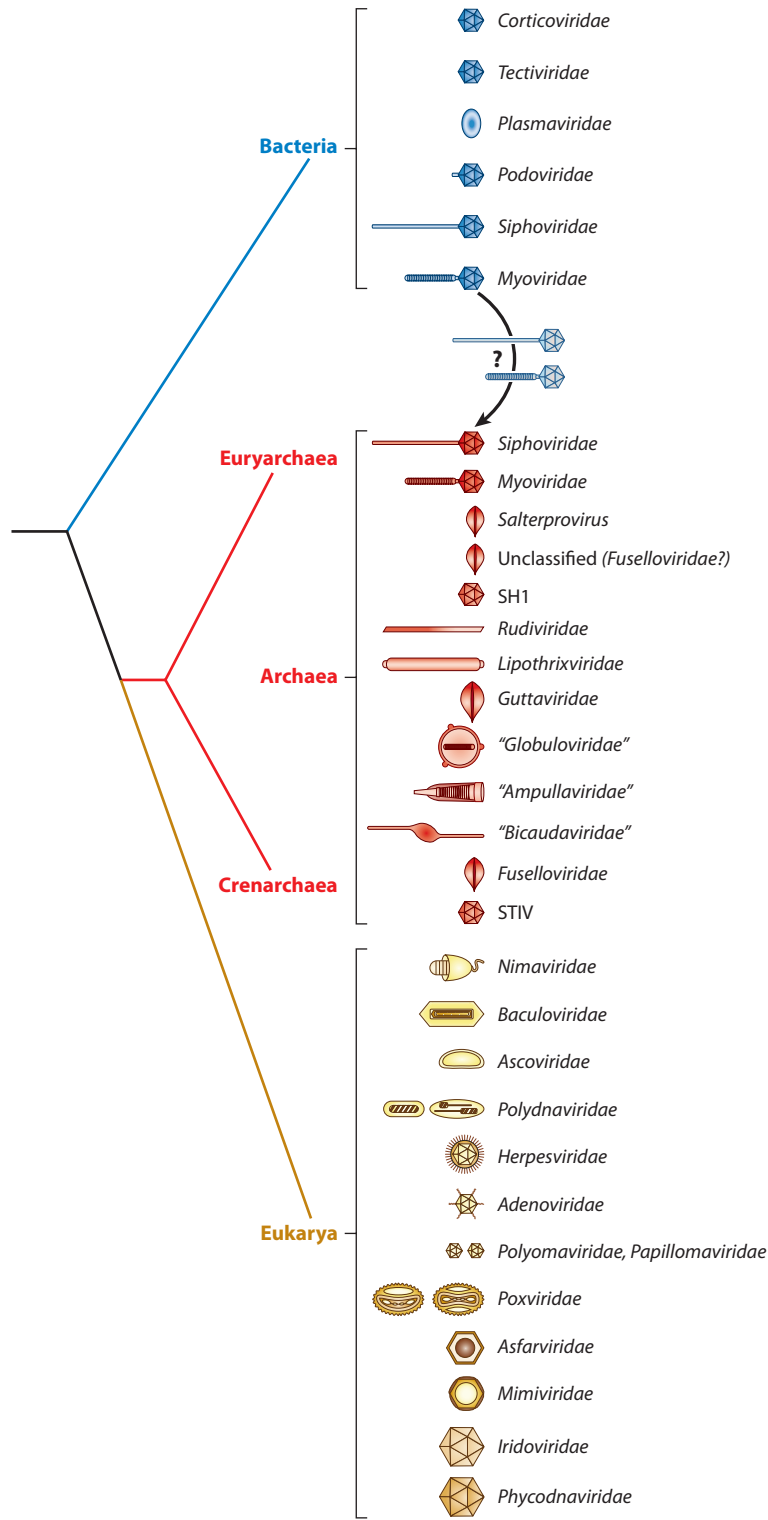
Figure 1

The families of double-stranded DNA viruses associated with the three domains of life. The viral families listed are approved by the International Committee on Taxonomy of Viruses, and the schematic representations of virions (not drawn to scale) are presented as in Reference 33. Proposed families are shown in quotation marks. Abbreviations: SH1, *Haloarcula hispanica* virus 1; STIV, *Sulfolobus* turreted icosahedral virus. Modified with permission from Reference 61.

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Adaptation: the process by which a population evolves via natural selection to gain new or modified traits that improve fitness

delimitation of viruses—the classic method used to name viral families, overseen by the International Committee on Virus Taxonomy. Clearly, viruses are more successful than cellular life when it comes to evolved genetic options for cross-generational inheritance, and the morphological diversity of viruses is vast despite their submicroscopic size.

PART II: WHY ARE VIRUSES SO SUCCESSFUL?

Viruses numerically dominate the biosphere, and even though the full extent of viral biodiversity has yet to be discovered, it is clear that they are exceedingly biodiverse. If these are reasonable metrics for gauging biological success, we can then ask: Why are viruses so successful? Compared with other biological systems, viruses show an amazing capacity to produce large numbers of progeny in a short period of time, a capacity that contributes to observations of their global densities. This efficiency can be considered from a conservation of energy standpoint, because viral reproduction exclusively utilizes the metabolic energy generated by cellular life. In addition, the efficiency is appreciable from an economy of scale standpoint, because the genetic architectures of viral genomes tend to be highly streamlined in terms of gene number, overlapping reading frames, and modularity. Moreover, the biological success of viruses must relate to adaptation via natural selection, as this proximately determines whether a population thrives in the event of inevitable environmental change, and ultimately dictates extent of biodiversity. In this section and the next, we consider the many mechanistic reasons why viruses are biologically successful, particularly their ability to adapt in the face of environmental challenges.

Many cellular organisms can produce very large numbers of offspring in a single generation, especially if their mode of reproduction is asexual. In viruses, reproduction is strictly asexual, yet these microbes are arguably the reproductive champions among biological populations. A single viral particle can infect a cell and harness the host's metabolism to drive replication and create hundreds or thousands of viral progeny in under an hour (3). In general, the mode of genome replication inside the host cell facilitates these very large numbers. Often, viral replication cycles occur via efficient genetic mechanisms, such as rolling circle replication and stamping machine replication (one template virus used to replicate all progeny genomes in the cell), that can create very large numbers of genomes off of a single template. From a population size standpoint, consider the ability of HIV to achieve extremely high titers (viremia) in the blood of a newly infected human: Roughly 5×10^3 infectious virions or 1×10^7 viral RNA units are present in every milliliter of plasma during the acute phase of HIV infection (37) (**Figure 2**). The high viral loads experienced by infected multicellular hosts are often central to the debilitating effects of viral disease. This can occur either because the rampant viral reproduction directly destroys vital tissues and organs or, in the case of HIV, because the high viral load destroys precious immune system cells, eventually leaving the host vulnerable to opportunistic infection by other pathogens.

All organisms occasionally experience some sort of resource limitation, whether of a consumable resource or, for example, of space to grow or access to mates. Evolutionary biology concerns competition between individuals for limiting resources, with the expectation that selection will favor the variants that most efficiently use available resources. For this reason, selection should tend to push organisms to harness the energy provided by others: Why waste your own energy on reproduction if you can evolve a means to have some other organism provide that energy for you?

This constant challenge to obtain limited resources has been used to explain why parasitism seems to be the dominant evolved lifestyle on our planet (62). Viruses represent the pinnacle of selfish efficiency, because they are biological entities that dispense entirely with harnessing their own resources to make energy for reproduction. Rather, they harness the metabolism of host cells, diverting their hosts' energy away from normal cellular functions and toward production of



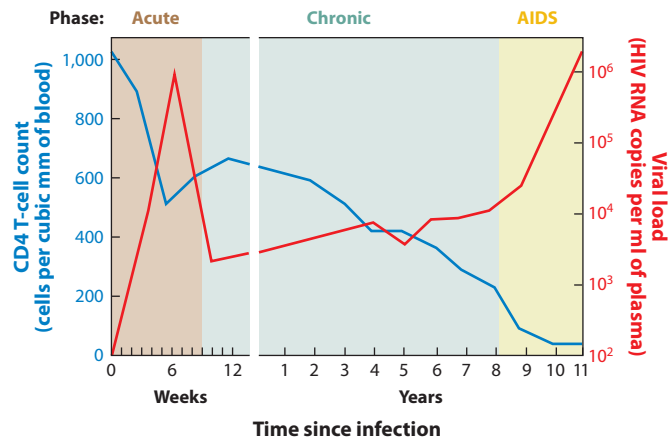


Figure 2

The general pattern of progression in an untreated HIV infection. An untreated HIV infection typically has three phases. During the acute phase, the viral load (*red*) spikes and the host may show general symptoms of a viral infection. During the largely asymptomatic chronic phase, the host mobilizes an immune response that fails to halt viral replication. During the AIDS phase, the viral load climbs again as cells of the immune system, especially CD4 T cells (*blue*), are destroyed. Modified with permission from Reference 37. Copyright © 2004 Pearson Prentice Hall, Inc.

viral particles instead. Because viruses are much less complex than cellular life, this energy can be efficiently used to make viral genomes and proteins, compared with the larger reserves needed for cellular DNA replication, protein synthesis, lipid biosynthesis, energy (ATP) synthesis, etc. Viruses can thus mechanistically outcompete a host cell for resources within the cell itself. Although the energy used to make viral progeny should be much less than that used to maintain cellular homeostasis, viruses have evolved diverse mechanisms that promote their own replication and protein synthesis while suppressing normal host function (89). These include cap-independent mechanisms (e.g., IRES) as well as specific modulation/sequestration of host protein synthesis cofactors (5). Although the thermodynamics of viral replication may give viruses a slight edge over the house, selection is not above giving them the means to further rig the game in their favor.

The efficiency of viruses is also reflected by the structure of their genomes. Like most genetic systems, viruses can experience recombination in which genes or cassettes of genes are swapped between individuals. In addition, viruses seem capable of evolving highly mosaic genomes, in which some portions of the genome derive from distantly related or completely unrelated viruses. One example is the identical RNA polymerase shared by the families *Cystoviridae* (RNA phages) and *Reoviridae* (viruses that infect eukaryotic hosts) (6). These observations show that viruses that are selected under very different circumstances may sometimes recombine, and selection will then presumably favor hybrid variants in which a large portion of the genome is summarily replaced. It is unclear how often these events occur in nature, but they certainly blur the distinctions between viruses and challenge our ability to accurately resolve deep nodes in viral phylogenetics. This modularity of viral genomes creates the possibility for efficient origin of new genome combinations, and further work is needed to determine how much it may contribute to the generation of viral biodiversity.

Viruses have evolved diverse solutions to maximizing efficiency in their genetic carrying capacity. Whereas more complex cellular organisms have been afforded some relaxation in genome size, viruses remain specifically constrained by selection to reduce nonessential genomic elements. The result is a broad array of genomic architectures in the larger known virome. One example is the

employment of overlapping reading frames (41). Increasing the carrying capacity of the genome allows viruses to increase their success through an economy of size.

The efficiency of viruses is also reflected by the multifunctionality sometimes observed for virally encoded proteins, particularly in the case of RNA viruses that are constrained in genome size. A single viral gene product can encode proteins with a diverse set of functions during the viral replication cycle. One example is the matrix protein of vesicular stomatitis virus (VSV), an arthropod-borne virus (arbovirus) in the negative-strand RNA virus family *Rhabdoviridae*. The VSV matrix protein functions both in structural organization and as a modulator of host macromolecular function (1, 8). In terms of efficient genomic architecture, many viruses encode their gene products in single open reading frames, resulting in a large polyprotein that is posttranslationally cleaved into specific subunits. In the *Togaviridae*, a family of positive-strand RNA arboviruses, the nonstructural polyprotein is uniquely functional at different stages of cleavage (**Figure 3**). The release of the polymerase subunit (nsP4) allows for complement-genome synthesis. However, further processing of the polyprotein P123 into its respective subunits is necessary for complete genome replication and subgenomic transcription (22, 46, 69, 76). Here, evolution has shaped the genomic architecture and the temporality of gene product function to regulate the larger process of viral gene expression. These various mechanisms hint at the many ways in which viruses have successfully evolved to overcome the limitations of using a small genome to achieve myriad and complex functions.

PART III: ADAPTATION AND EVOLVABILITY

Adaptation is the process by which a population is naturally selected to evolve new or modified traits, which allow individuals in the population to better meet challenges posed by their current environment. A different but related phenomenon is evolvability, which is the capacity to evolve via natural selection; a population may be relatively advantaged over other populations in its ability to adapt in the face of environmental change.

Both adaptation and evolvability are fundamentally important processes, because the vast majority of organisms seem to thrive in environments that are dynamic, rather than static, through time. These fluctuating conditions make it likely that individuals will encounter some degree of environmental change during their lifetimes and that most biological populations will be routinely challenged to adapt. Many organisms are additionally challenged because they are incapable of choosing the environment where they reside, causing them to experience different environments across space. For example, microbes may be passively moved via air, water, etc., causing them to frequently encounter environments where they may be poorly suited to survive and/or reproduce.

In general, organisms' inability to distinguish suitable habitats from unsuitable ones (or their susceptibility to passive movement between such environments) may cause them to enter an ecological trap, defined as an environment that is poor in quality for survival/reproduction and that prevents population sustainability (i.e., births that exceed deaths). The incapability of microbes to distinguish good-quality habitats from ecological traps will contribute to local extinctions. One example is that viruses must initiate infection by attaching to a cell surface receptor but

Figure 3

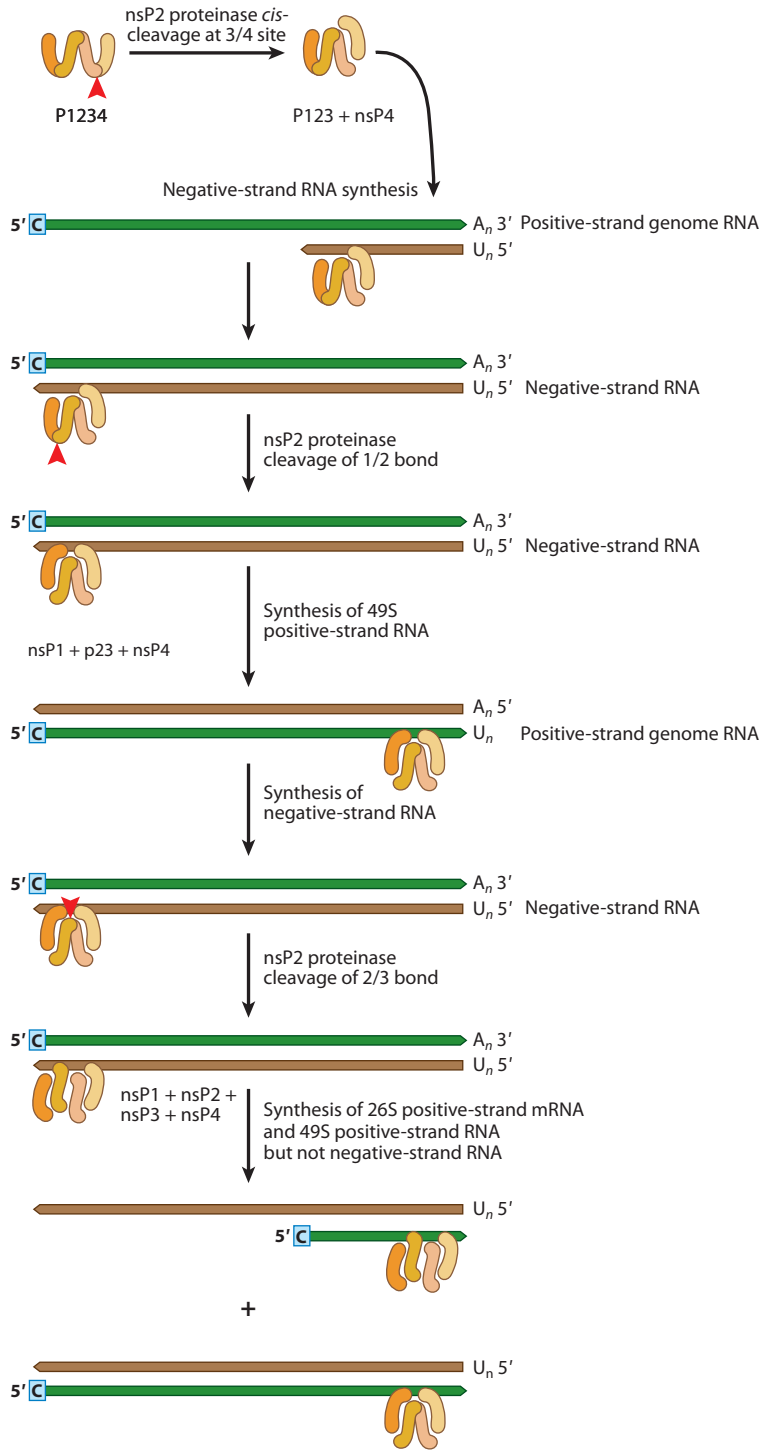
Pleiotropic gene function at the level of polyprotein cleavage. A single viral gene product (P1234) encodes three distinct RNA polymerases in alphavirus-infected cells. Proteolytic cleavage of the polyprotein modulates the template specificity and function of the RNA-dependent RNA polymerase. Adapted from Reference 73, with permission from the authors of Reference 36.

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Translation of genome RNA



are incapable of avoiding a poor host if it has the correct binding site. If the cell or tissue type is nonsupportive (nonpermissive) for productive viral replication, the infection would ultimately constitute a dead end for the virus. A viral population that contains a high frequency of individuals that enter this ecological trap should be fated for extinction (25), in the absence of any genotypic change, such as evolution of an altered attachment protein, that remedies the ecological-trap problem.

The remainder of this article addresses the ability of viruses to adapt to overcome problems such as ecological traps, as well as the question of whether some viruses may be considered more evolvable relative to other viruses or to cellular life in general. However, before addressing these topics, it is crucial to first consider whether viruses tend to exist in static versus dynamic environments across time and space, because adaptation is only necessary in the face of environmental turnover. How often do viruses experience environmental change? When encountering new environments that limit their growth or survival, what options do viruses have to cope? Do these options differ from those possessed by cellular organisms?

Biotic and Abiotic Challenges Faced by Viruses

We contend that viruses are equally or more likely than cellular organisms are to encounter environmental change, regardless of the target host genotypes or species (83). This opinion is based on the many types of environmental novelties that viruses are expected to encounter, across all levels of biological organization considered.

At the base level of molecules, the most proximate challenge for a virus is to successfully initiate cell attachment and entry. These early infection events are driven by functional viral molecules, such as proteins that permit cell attachment and enzymes important for transition into the cell. But the functional utility of these molecules may be partially or fully compromised depending on changes in the host. For example, a coevolutionary arms race between viruses and their hosts will exert selection for host modifications that prevent viral infection. Phages are incapable of infecting host bacteria that have evolved resistance due to a variety of mechanisms, ranging from cell-surface modifications that prevent binding/entry to postinfection barriers such as restriction enzymes and CRISPRs that prevent intracellular viral replication (11). Similarly, the human adaptive immune system creates changing environments for pathogens such as influenza A viruses, explaining why evolutionarily successful flu virus lineages are those with altered antigenic (hemagglutinin protein) sites that provide an advantage in the face of immune pressure (35).

Higher levels of biological organization can also present viruses with changing environmental conditions. At the levels of cells and tissues, viruses of multicellular hosts may be challenged by intrahost replication that involves cells of varying permissiveness. For example, infected cells can release chemicals that signal neighboring cells to upregulate innate immune function, which will compromise the subsequent replication and spread of viruses in host tissues. At the level of host populations, viruses may encounter variability among hosts in their susceptibility to infection. Such variability may result from differences among host genotypes or host physiologies, such as the effects of host age on strength of innate immunity (45) and the effects of adaptive immune function. These many challenges can adversely affect intrahost viral reproduction and compromise infectious viral spread among individuals in the host population. At the level of ecological communities, some viruses must undergo obligate or opportunistic replication in very different host species, as is the case with arboviruses that thrive by reproducing in both arthropod vectors and vertebrate hosts. Viruses passively encounter novel host species as they are moved through coarse-grained biotic environments, and it is increasingly recognized that, compared with other types of pathogens, such as bacteria or protozoa, viruses are more prone to successfully emerge on new hosts (16, 75). Habitat



fragmentation and other anthropogenic changes to the environment may increase the probability that viruses encounter new host species, including humans, thus augmenting emergence risk (54). These many examples of environmental change constitute only a subset of the myriad biotic challenges viruses face across different levels of biological organization, with adverse consequences for viral infection and epidemic spread.

Historically, the novel abiotic challenges viruses face have received less attention than the aforementioned biotic challenges. Nevertheless, many types of abiotic perturbations can cause viral mortality, i.e., the inability of a viral particle to successfully complete within-host replication after experiencing an environmental challenge (56). A wide variety of environmental stressors are known to somehow compromise a virus's subsequent ability to productively infect a host; examples include changes in ambient temperature, ionic strength (saltiness), UV radiation, acidity, atmospheric pressure, and moisture (2, 49, 56, 58). Although capsids function to protect viral nucleic acid from degradation by these and other environmental stressors, the protection is not absolute, and some viruses may experience very short half-lives outside of the host under these harsh conditions. Some viruses seem extremely tolerant of environmental exposure; for example, some rotaviruses can remain stable on exposed surfaces for long periods, promoting successful outbreaks in humans living in a closed habitat such as an ocean-bound cruise ship (32). However, mechanisms to explain long versus short viral half-lives remain to be discovered. For example, it is often unknown whether differences in viral mortality in the face of environmental change are better explained by resistance to degradation of nucleic acid (23) or by resistance to destruction of capsid proteins and viral enzymes used in cell entry (26).

Ecosystem-level changes have the potential to threaten the survival of all biological populations, but their possible impacts on the tiniest entities are seldom studied and remain poorly understood. Threats of global warming, rising sea levels, and ocean acidification receive plenty of attention from the popular press and are well known to the lay public. These radical widespread changes would be experienced by viruses, just as they would by other biological populations. However, only a few studies have examined the effects on viral mortality of abiotic challenges associated with proposed ecosystem changes, such as increased acidity of marine water (48) or stochastic temperature fluctuations predicted by some climate change models (2). More research is needed to identify how the survival of viruses may be compromised under radical ecosystem changes. Virus-induced mortality could regulate the size of a host population, as is proposed for cyanophage regulation of cyanobacteria populations in the ocean; arguably, this regulation would mean that the viruses indirectly dictate how much the bacteria contribute to global photosynthetic productivity (74). Thus, if increased ocean acidification were more detrimental to cyanophages than to their host cyanobacteria, this greater vulnerability could create a regulation imbalance leading to altered mean photosynthetic productivity in the ecosystem. At the least, this scenario could cause more-variable photosynthetic productivity, because blooms and crashes could occur when cyanobacteria populations are released from cyanophage regulation. This idea is speculative, but it is certainly worth researching whether ecosystem function can be upset by the effects of widespread environmental change on the most microscopic of populations. Our main message is that viruses are also vulnerable to adverse effects of ecosystem change, and the effects of such change on virus-host ecological interactions constitute vital areas for future study.

Can Viruses Adapt to Cope with Environmental Change?

Apparently, the answer to this question is a resounding “yes,” at least for an environmental challenge of extreme importance in biomedicine, agriculture, and conservation biology. Of the aforementioned biotic challenges faced by viruses, infection of new host genotypes or species has

Sex: any genetic exchange between individuals that results in new combinations of alleles

Specialist: a variant with a relatively narrow niche, allowing reproduction and/or survival under few ecological conditions

Generalist: a variant with a relatively broad niche, allowing reproduction and/or survival under many ecological conditions

received the greatest attention, due to concerns over the effects of emerging viruses on human disease morbidity/mortality, on economic losses incurred in agricultural systems, and on continued preservation of rare species threatened with extinction. Obviously, viruses must occasionally be successful in coping with this type of environmental change; otherwise, emergence (especially of viral pathogens that zoonotically transmit from wild and domesticated animals to humans) would not be recognized as a widespread problem (42). However, it bears reminding that hosts such as humans are exposed to a wide diversity of potential pathogens through the air we breathe, the food and water we ingest, etc.; only a tiny subset of these viruses can climb the “pathogen pyramid” (93) to become worrisome pathogens that successfully infect, transmit, and spread epidemically in humans. A virus does not necessarily have to change genetically to emerge on a novel host; rather, it may fortuitously infect a previously unencountered host (31, 85). Still, evidence suggests that one or more point mutations are needed for a virus to overcome a new environmental challenge, such as emergence on a new host or better survival in the face of an abiotic stressor. Therefore, drawing heavily upon our own empirical observations in RNA viruses, we confine our discussion below to examples in which viruses adapt via beneficial genetic modification, allowing them to overcome biotic and abiotic challenges. In particular, we focus on the role of genetic variation created through spontaneous mutation and sex (recombination/reassortment) in contributing to RNA virus adaptation under environmental change. We use these examples to illustrate that viral adaptability is impressive but also to emphasize that adaptation to one set of conditions may concomitantly affect viral performance under other circumstances (i.e., evolved trade-offs across environments).

Spontaneous mutations are the primary currency for evolution by natural selection. These mutations create genetic variability among individuals, which can lead to phenotypic differences that dictate which individuals are selectively favored to dominate the population in its current environment. From the perspective of inevitable environmental turnover, it is intriguing to study whether the advantageous effects of beneficial mutations are confined to the environment where they are positively selected or whether they are broadly beneficial across environments, including those previously unencountered. These circumstantial effects relate to the broad ecological concepts of environmental specialization versus generalization (47, 82). By definition, specialists are capable of thriving under relatively confined ecological conditions (narrow niche), whereas generalists can occupy a broader niche space.

We studied the specialized versus generalized role of mutations in host emergence using phage $\phi 6$, an RNA virus that infects pseudomonads, especially certain pathovars (plant-pathogenic varieties) of *Pseudomonas syringae* bacteria (31). Phage $\phi 6$ is usually grown on *P. syringae* pv. phaseolicola in the laboratory, but point mutations in the P3 attachment protein determine whether the virus can shift onto other species of host bacteria; the spectrum of mutations in P3 allowing such host shifts contains at least 10 different nonsynonymous substitutions (31, 83). Interestingly, most of these mutations allow host-niche expansion of the virus, through which it is capable of infecting a novel host but maintains the ability to infect the original *P. syringae* pv. phaseolicola host (29, 31). This property allowed us to test whether or not these emergence mutations are on average cost free. It is widely presumed that the intimacy of virus-host interactions should cause a virus to evolve strong performance on its current host, and that mutations allowing emergence on a new host should reduce performance on the old host. But this presumption assumes that emergence mutations tend to be antagonistically pleiotropic: Mutations governing infection in the novel host are assumed to compromise performance in the original host. Because P3 mutations in phage $\phi 6$ allow range expansion (incorporation of new species in the host repertoire), we could gauge whether ability to emerge on a new host tended to reduce ability to grow on the original host. Consistent with the untested assumption, most (7 of 10) mutations allowing emergence led to



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significantly poorer growth on the original host species; however, these data also indicated that antagonistic pleiotropy was not universal, because 3 of the emergence mutations were cost free on the original host (31). Clearly, there are emergence mutations that strongly reduce (31, 83) or even eliminate (29) growth on the original host. Nonetheless, these results are intriguing because they challenge the paradigm that viruses must forego strong performance across host types as they emerge; rather, they may experience mutations that expand host range at no cost. This finding should motivate studies of fitness consequences of emergence alleles in viruses of eukaryotes, such as those that infect humans and other mammals.

Genetic variation in a population can also be generated through sex: the process of exchanging genetic material between individuals, which creates new combinations of alleles. In viruses, sex can occur through classic recombination (breaking and joining of homologous nucleic acid) or through reassortment (swapping of chromosome segments between segmented RNA viruses to create new combinations) (77). Similar to influenza A virus and other segmented viruses, phage $\phi 6$ contains multiple segments per particle (86). This property allows phage $\phi 6$ genotypes that coinfect the same host cell to undergo reassortment, a process that produces hybrid genotypes containing mixtures of the three segments that were present in each coinfecting parent virus (38, 79). Phage $\phi 6$ is in the viral family *Cystoviridae*, and phylogeography studies involving natural populations of these viruses show that genetic variation generated through reassortment rivals or exceeds that generated through mutation alone (59, 71). Thus, reassortment is a key component of *Cystoviridae* biology, and the genetic variation generated through this process may play an important role in the adaptation of these viruses.

We used lab experiments to examine whether reassortment creates useful genetic variation in populations of phage $\phi 6$, allowing these populations to adapt faster than viral populations evolving through mutation alone (80). Classic ideas on the evolutionary advantage of sex assert that sex is common in natural populations because the additional genetic variation allows sexual populations to adapt faster than their asexual counterparts (34, 55, 84). We controlled the presence and absence of sex (reassortment) in phage $\phi 6$ populations by manipulating whether treatment populations experienced high versus low multiplicity of infection (MOI; ratio of viruses to cells); high MOI increases the probability that multiple viruses coinfect the same host cell and undergo reassortment, whereas low MOI allows mostly clonal (single-virus) infection of each host cell (79). When viruses were challenged to further adapt to their typical *P. syringae* pv. *phaseolicola* hosts, high-MOI lineages unexpectedly evolved more slowly than low-MOI lineages did (80), contrary to the classic prediction. This outcome was explained by viral specialization to MOI conditions, whereby high-MOI evolved lineages adapted to perform better under coinfection, which traded off with their general ability to grow well under clonal conditions (78–81). In particular, the evolved high-MOI viruses became cheaters that benefited from using intracellular products created by other viruses during coinfection, but this selfish strategy entailed less efficient production of resources when the cheaters infected cells on their own (79). These results are compelling because they indicate that the consequences of evolved specialization to one portion of the niche (i.e., coinfection) may override the possible generalized benefits of variation generated through viral sex. It is important to note that although sexual processes such as reassortment may occasionally produce beneficial variation for an evolving population, segmentation might have originally evolved for a purpose other than promoting adaptation. In particular, segmentation might have evolved to promote efficient packaging of the RNA virus genome (52), perhaps making it unlikely to observe an advantage of sex consistent with classic evolution-of-sex hypotheses (84).

One goal of the study of evolutionary biology is to make it a more predictive science. A prime example is the virus emergence problem, for which our efforts to date are largely reactive rather than proactive. If we could better predict which viral genotypes or species will successfully emerge

Fitness: the ability of an individual variant to survive and/or reproduce, relative to other variants in the population

Cheater: an individual in the population that interacts with others to gain a relative fitness advantage at the others' expense

in a host population such as humans, we could proactively prepare for inevitable pandemics similar to the current AIDS crisis caused by HIV emergence. We are a long way from accurately predicting emergence events in viral evolution (57), but recent experiments have made inroads.

To test emergence predictions, we used experiments with VSV, which is naturally transmitted between mammalian hosts via arthropod vectors. VSV is easily grown on a wide variety of cell types in laboratory tissue culture. We first used VSV to select for viral lineages that were specialized in using a single host type (versus generalized in using multiple host types) (66, 82). We then harnessed this collection of viral lineages to test a popular idea in predictions of disease emergence. This idea asserts that pathogens that are already capable of infecting multiple hosts should pose a greater threat to emerge on new hosts such as humans (16, 75), consistent with the classic notion that resource generalization is advantageous under resource uncertainty (72). We thus predicted that the lineages selected for host-use generalization would be advantaged when challenged to grow on a battery of new hosts, compared with their more specialized counterparts. Results confirmed that direct selection for host breadth fostered successful emergence on novel hosts, as assessed by measures of average growth (titer) on new hosts and estimates of variance in growth across host types (85). These results speak to predictions in evolutionary biology, suggesting that thorough knowledge of existing pathogen niche breadth (admittedly difficult information to obtain) would help identify which pathogen types are best poised to undergo host shifts.

Are Viruses More Evolvable than Cellular Life?

Despite their interesting caveats, evolution experiments with viruses clearly show the capacity of viruses to adapt successfully to environmental change. But are viruses more adaptable than cellular organisms? From the standpoint of the variation that drives natural selection, some viruses have greater access to potentially beneficial mutations simply because they mutate more often. Comparative estimates of mutation rates show that RNA and single-stranded DNA viruses mutate more readily than other evolving systems, owing to their error-prone replication and lack of proofreading to correct spontaneous mistakes in copying genetic material (28, 30). This indicates an inherent advantage for RNA and single-stranded DNA viruses when selection sorts through individual variants to find a possibly better match to current environmental challenges. Of course, this variation is not guaranteed to produce the needed adaptation, for several reasons. First, a particular variation may be useless in the face of particular challenges, indicating a constraint for natural selection to proceed. Second, natural selection can only proceed efficiently at sufficiently large population sizes (12); otherwise, genetic drift will be a more important force in evolution, and high mutation rate can create a mutational load that causes a viral population to decline in fitness rather than improve (15). Third, viruses tend to have smaller genome sizes and less genetic redundancy than cellular organisms, and therefore they possess less material that could be genetically modified through selection. Finally, a high mutation rate could be a liability if an error threshold exists, whereby the population thrives at the brink of error catastrophe (19), experiencing too many mutations per unit time (with the expectation that most mutations are deleterious as opposed to beneficial or neutral). Perhaps this brink of existence is most important if a population has reached equilibrium in a constant environment, making any nonneutral mutation a liability, but we argued above that viruses probably rarely if ever experience truly static environments. Still, many nonviral evolving populations face some of these same obstacles to selection, and available evidence strongly suggests that the higher mutation rate of some viruses creates an adaptive advantage. Anecdotally, this may explain why RNA viruses seem to emerge in new host species more often than other microbes do, despite being rarer in the biosphere. However, we must be careful in drawing this conclusion, because our view is skewed toward disease emergence



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in humans. For unknown reasons, we humans may be more susceptible to emergence by RNA viruses, whereas other hosts may not; only time-consuming and expensive surveillance of a wide variety of hosts could evaluate this possibility.

Evolvability is an intriguing process that extends beyond mere adaptation, because it involves the evolution of evolution itself; in theory, organisms might be molded by natural selection to be advantaged in their capacity to selectively adapt to future environmental changes. Although this process seems plausible, it is difficult to conceive how organisms can be naturally selected to better perform in environments they have never before encountered, especially because the prevailing notion is that evolution is blind—that is, it does not proceed toward some future goal (20, 72).

There is abundant mathematical theory on evolvability (88), but only recently have experiments rigorously addressed this process, and the majority of studies used experimental evolution of microbes (13, 49). Indeed, some of these experiments demonstrate that lineages can differ in their evolvability, gaining a relative advantage over other populations when challenged to adapt to environmental change. However, proof of the underlying mechanism(s) that drive evolvability remains mostly elusive. Here we focus on the prediction that evolved robustness is a mechanism that can drive evolvability.

Genetic robustness is defined as phenotypic constancy despite underlying genetic change (24). If a mutation changes a phenotype, we consider the gene or, more broadly, the genome, to be relatively nonrobust, or brittle, against mutational input. However, if a genome changes but phenotype remains unaffected, the genome is considered genetically robust.

If robustness affects evolvability, it should impact the ability of organisms to access evolutionary innovations. Robustness allows for easier accumulation of mutations that are neutral in the current environment; if the habitat changes, this robust genetic architecture may then promote access to a greater number of mutations that are beneficial for adaptation (87). For example, a robust population may be envisioned as residing in a region of a fitness landscape that is relatively flat, owing to the high proportion of resident genotypes in the population that are equal (neutral) in fitness (92). This creates a large neutral network of genotypes that can efficiently traverse the landscape through random drift, due to their high degree of network connectivity. If environmental change alters the fitness landscape, a robust population may experience an evolvability advantage because newly arising mutations occur in a wider diversity of genetic backgrounds, creating more-varied epistatic combinations that may prove beneficial for adaptation (40).

We studied the influence of robustness on evolvability using an empirical system in which relatively robust and brittle genotypes had been identified (53) and viruses were strongly selected to adapt to harsh environmental conditions (49). First, we identified a fundamental difference between populations of phage $\phi 6$ that had evolved at high versus low MOI (80). At high MOI, the viruses experienced complementation, a general mechanism by which viral genotypes of lower fitness can phenotypically profit from intracellular proteins made by coinfecting viruses of higher fitness (38). This complementation during coinfection should automatically buffer viral phenotypes against mutations. Thus, complementation offered a built-in mutational buffering mechanism in coinfecting viral lineages, which may have weakened selection for the viruses to maintain their individual-level robustness. This de-evolution of robustness should cause the high-MOI lineages to be more susceptible to phenotypic effects of mutational change than their low-MOI evolved counterparts are. That is, the high-MOI viruses should be brittle to mutational input, whereas the low-MOI populations should be robust. We proved that the lineages had evolved differing robustness by isolating clones at random from each treatment group and using them to found new lineages evolved under mutation accumulation. This process consisted of passage through extreme daily bottlenecks (plaque-to-plaque transfers) in which drift overwhelms selection because the small population size causes spontaneous mutations (most of them deleterious) to fix at random

Genetic robustness:
phenotypic constancy
despite underlying
genetic change



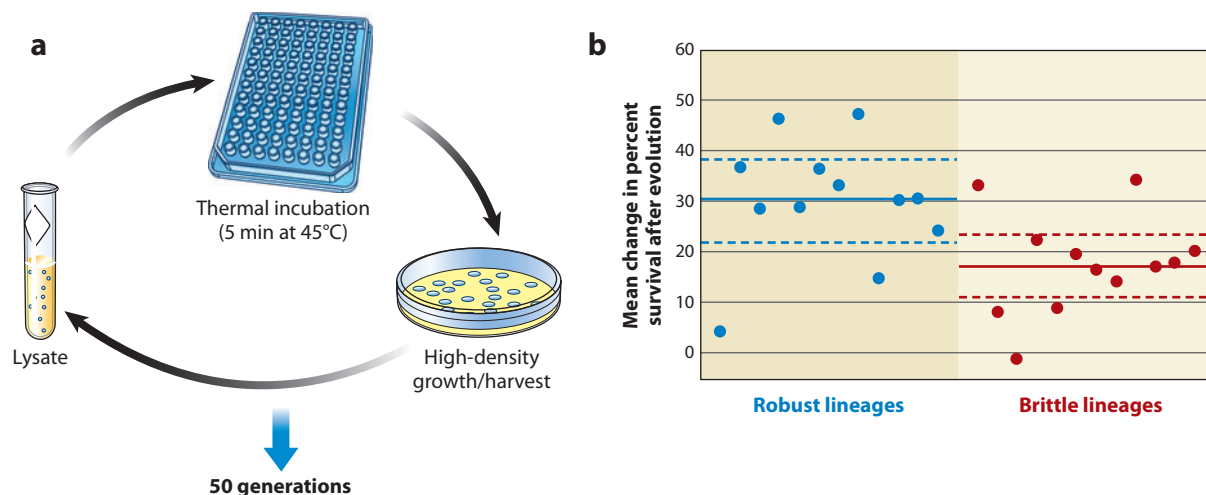


Figure 4

(a) Design for an evolution experiment in which phage lineages were selected to withstand damaging effects of heat shock. A phage $\phi 6$ lysate was exposed to 45°C incubation for 5 min, and a dilution of the surviving progeny was plated on a lawn of *Pseudomonas syringae* pv. phaseolicola bacteria. Overnight plaque formation at 25°C corresponded to 5 generations of phage evolution. The plaques were then harvested, and the process was repeated for 10 days (equivalent to 50 phage generations). (b) Robust viruses are advantaged in evolvability of thermotolerance. Mean change in percent survival after selection with heat shock (45°C) is greater for viral lineages founded by 12 robust strains (blue circles) than for lineages initiated by 12 brittle strains (red circles). All populations were subjected to 10 days (50 generations) of periodic heat shocks. The solid line is the grand mean for the group, and dashed lines are 95% confidence intervals. Both panels adapted with permission from Reference 50.

(15). The fitness effects of randomly accumulated mutations were greater and more variable in lineages founded by previously high-MOI evolved viruses, indicating that these lineages were less able to withstand the deleterious effects of mutations and confirming the prediction that they had evolved lowered robustness (53).

To determine whether robustness promoted evolvability, we studied adaptation of robust and brittle phage $\phi 6$ strains in an environment where viruses would be strongly selected to overcome harsh environmental conditions. The virus is normally cultured at 25°C, but when exposed to heat shock, such as 45°C for 5 min, roughly 80% of viral particles lose the capacity to productively infect bacteria (57, 58), owing to the poor stability of the phage lytic enzyme at high temperature (26). We allowed lineages founded by robust and brittle viruses to evolve on *P. syringae* pv. phaseolicola at 25°C, with periodic (every fifth generation) exposure to 45°C heat shock (49). Then we measured mean percent survival at 45°C for each founding clone and its derived endpoint population, to estimate the change in percent survival after 50 generations of selection to resist damaging heat shock. Results showed that the lineages founded by robust genotypes were more evolvable: They experienced greater adaptation (increase in percent survival) by the end of the study (Figure 4). Apparently, some difference in genetic architecture between the robust and brittle viral strains allows the robust viruses to more easily innovate in the face of heat shock. The exact mechanism for the robustness–evolvability link in phage $\phi 6$ has not been fully elucidated but most likely concerns the P5 lytic enzyme, because mutations in this protein are beneficial for thermotolerance (26) and presumably also function to buffer mutational effects elsewhere in the genome.

More work is needed to study generalized links between robustness and evolvability in viruses and in other biological systems, as few of these kinds of studies have been attempted to date (9, 17, 70). Equally compelling is exploration of evolvability differences among groups of organisms, such

as testing whether viruses as a group are more evolvable than cellular life. Although intriguing, it is unclear how these experiments could be adequately designed and tested. The properties of even closely related organisms are different enough that it is difficult to compare their evolvability in the face of the same challenge; ideally, they would have identical mutation rates, genetic architectures, physiologies, etc. Clearly, this would not be the case when comparing viruses with any other type of organism. Rather, we could seek indirect evidence for evolvability differences among major or minor groups of organisms. One possibility would be to determine whether species in the respective groups tend to survive for different periods of geologic time, as tested when comparing average rates of extinction between groups.

Can Viruses Better Avoid Extinction?

Extinction seems to be the ultimate fate of most species that have appeared on Earth; on the basis of the fossil record, it is estimated that well over 99.99% of all macroscopic species have gone extinct and that the average duration of their existence is roughly 10 million years (65). These are distinctly sobering statistics for humans to contemplate as a species! How long have viruses been present on Earth? The evolutionary origin of viruses is a hotly debated subject, but some ideas posit that viruses predate cellular life (61). For example, the widespread observation that viruses sometimes contain genes nonhomologous to known cellular life suggests that these are remnants of an ancient viral world, arguing against the notion that viruses originate by reductively splitting off from cellular genomes (43). Confirmation of this “virus first” claim would firmly conclude that viruses better avoid extinction than cellular organisms; perhaps they were here first and remain to this day.

But we cannot easily rely on the fossil record to test extinction hypotheses comparing viruses with nonviral life, because viruses and other microscopic, soft-bodied organisms are less prone to fossilization and therefore cannot be found in the fossil record. However, we might usefully begin by examining properties of viruses alone and asking whether viruses possess certain traits that would aid their extinction avoidance. Above, we described how viruses might be especially vulnerable to ecological traps (25). One possibility is that viral binding affinities could be traits resulting from selection to avoid improper environments; even if binding properties did not originally evolve for this purpose, they may indirectly function in this way. For example, viral coreceptors might function in this capacity. Selection for viral attachment to a primary cellular receptor would foster interaction with presumably suitable (permissive) host cells, whereas selection for a secondary coreceptor would increase the probability that the exact correct match is made. The evolution of phages that have short and long tail fibers with separate binding affinities may exemplify this phenomenon. In certain T-even phages, long tail fibers probe the cell surface to function in initial, reversible binding, whereas short tail fibers attach to additional coreceptors that function in irreversible binding (67). It would be intriguing to examine primary versus secondary binding affinities of viruses from a wide variety of viral families, to test the general hypothesis that viruses show less-specific primary binding and more-specific secondary binding.

Finally, it is compelling to consider whether viruses better avoid extinction by losing their individuality altogether. The Human Genome Project and other sequencing efforts reveal that virus-derived genes are exceedingly common in the genomes of single-celled and multicellular life. This genome incorporation is largely considered to be accidental, as is the case with transduction, in which phage genes are recombined into bacterial genomes, seemingly as mistakes, during the infection process. This loss of individuality seems like a clearly bad outcome from the standpoint of individual autonomy. But the perspective may differ if we consider selection acting at levels below the individual; from a selfish gene standpoint (21), perhaps we can view the widespread occurrence

of virus-gene incorporation as being positively driven by selection, at least some of the time, rather than purely accidental in all cases. Arguably, viruses are not very autonomous anyway. As stated above, viruses seem to be the evolutionary champions of harnessing other organisms to do their work, relying entirely on their hosts' ATP generation. If a viral gene is the target of selection, rather than the virus itself, it could conceivably be pushed by selection to become a gene or a suite of genes contained within host genomes. Of course, this is a tenuous outcome, because selection acting on the host may result in these genes being co-opted entirely for host functions, causing the virus-derived gene to lose all virus-like properties. But this selfish gene-like process may explain the widespread success of polydnviruses, which are arguably actually virus-like particles (rather than true viruses) that deliver expressed parasitoid-wasp genes into insect larvae to allow the wasp progeny to complete their life cycles by warding off antiparasitoid immunity (90). We are only beginning to appreciate the widespread occurrence of virus-derived genes in host genomes, and it is intriguing to surmise that these events may occur due to positive selection on viral genes to lose their viral autonomy.

CONCLUSION

The evidence we put forth in this article emphasizes that viruses are biologically successful, and arguably more successful than cellular life, according to the many and varied metrics we examine. The expected consequence of this apparent success is that viruses should have far-ranging effects on other organisms in the biosphere. Organisms do not live in an ecological vacuum, meaning that abundant and biodiverse entities should tend to widely ecologically interact with other inhabitants of the biosphere, having pervasive effects on the biological world. Indeed, viruses are ecologically interactive by necessity because they must rely on metabolizing hosts to complete their life cycles. Moreover, the impact of viruses on other organisms is obvious, at levels ranging from the genome to the ecosystem. Genome sequencing efforts show that virus-derived genes are commonly found in the genomes of other viruses and of cellular organisms. As sequencing efforts continue to expand the catalog of fully sequenced host-species genomes and to characterize newly identified viral genes/genomes in sequence databases, our understanding of this genome-level impact will only increase. The importance of virus-derived genes in macroevolution can be profound, evidenced by the evolution of placental mammals (of which humans are a member), which contain an assimilated retroviral gene, syncytin, that is essential for placental morphogenesis (51). As a consequence of often antagonistic virus-host interactions, molecular mechanisms have evolved that function in repelling viral attacks, including CRISPR/Cas systems, small-interfering RNAs, and cellular apoptosis, as well as cytokines, interferons, and Toll-like receptors, which function in antiviral immunity. Although evolved virus-host mutualism is possible (68), the infamous role of viruses in disease and pandemics shows their widespread adverse effects on hosts across the biosphere; human examples include the current HIV/AIDS crisis, the 1918 flu pandemic, and the decimation of Native American populations by smallpox introduced by European colonists.

We can confidently state that viruses have long interacted with cellular life, and they might have even originated before evolution of the cell. This dynamic between viruses and host species is certain to continue as natural selection pushes viruses to evolve varied and novel means to exploit cellular life across the biosphere. Viruses incidentally meet other organisms in their environments, and anthropogenic changes to habitats will increase these chance encounters, sometimes creating opportunistic emergence of viruses into humans and other hosts. Even if most of these events do not result in viral spread, their frequency and the latent adaptive potential of viruses ensure that the evolution of new virus-host interactions is inevitable (see Reference 44 for a discussion of the multiple independent evolutionary origins of HIV from simian immunodeficiency virus in



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humans). But what would be the fate of viruses in a postcellular world? Mass extinctions of cellular life due to ecosystem-wide environmental catastrophes are not without precedent (65). Would the inherent evolvability of viruses rescue them in the absence of cellular organisms, marking a successful return to the purported virus-first world that may have predated cells? We leave you with this last question to ponder, indicating an obvious weak link in the chain of otherwise overwhelming biological success of viruses.

SUMMARY POINTS

1. Viruses are arguably the most successful of all evolving entities on the planet.
2. Viral success is evident from greater numerical abundance and biodiversity that rivals that of cellular life.
3. Viruses grow more efficiently than cellular life does, because they exclusively harness host energy for viral replication rather than generating the energy themselves.
4. Viruses show a strong capacity to adapt despite often facing biotic and abiotic changes in their environments, at all levels of biological organization.
5. Natural selection allows viruses to readily adapt to new conditions, but this adaptation may trade off with performance in prior or alternative environments.
6. Elevated mutation rates and genetic robustness may provide evolvability advantages in viruses that are not experienced by cellular life.
7. The biological success of viruses is consequential for cellular life, profoundly impacting host genomes and populations as well as entire ecosystems.
8. Viruses may be more ancient than cellular life, and they seem poised for further success as evolution proceeds into the future on our planet.

FUTURE ISSUES

1. Increased natural sampling of viruses is necessary to better understand the complete virosphere (viral diversity on Earth) and to truly gauge the extent of viral success.
2. A greater understanding is needed of the mechanisms of viral adaptation, particularly in response to large-scale ecosystem changes such as global warming.
3. Functional mechanistic drivers of evolvability can be rigorously studied in viruses but remain poorly understood to date.
4. Resolving the evolutionary origins of viruses and relatedness among viral families would aid understanding of the role they play in the larger evolutionary history of the biosphere.

DISCLOSURE STATEMENT

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