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A Trait-Based Approach to Predicting Viral Host-Range Evolvability

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Abstract

Predicting the evolution of virus host range has proven to be extremely difficult, in part because of the sheer diversity of viruses, each with unique biology and ecological interactions. We have not solved this problem, but to make the problem more tractable, we narrowed our focus to three traits intrinsic to all viruses that may play a role in host-range evolvability: mutation rate, recombination rate, and phenotypic heterogeneity. Although each trait should increase evolvability, they cannot do so unbounded because fitness trade-offs limit the ability of all three traits to maximize evolvability. By examining these constraints, we can begin to identify groups of viruses with suites of traits that make them especially concerning, as well as ecological and environmental conditions that might push evolution toward accelerating host-range expansion.

INTRODUCTION

Living through the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, researchers and the public have become interested in understanding how viruses shift host species and the factors that contribute to disease emergence. The goal is to leverage this knowledge to design pre-pandemic intervention strategies. The specifics of how emergence works vary with each virus and novel host; however, there are universal hurdles all viruses must overcome to shift hosts. The virus must gain the ability to recognize and inject genetic material into new species' cells. Once the genetic information is within the cell, it must be able to take control of cellular processes and replicate new copies of the viral genome, synthesize proteins and other molecules, and then assemble the parts into infectious particles. The virions must then escape the cell and find a new host. All of this must be completed while also avoiding host defenses. The full process involves hundreds of host-viral interactions working out in the virus's favor. For example, SARS-CoV-2 is known to interact with 332 human proteins to complete its infection cycle (1, 2). Given how many opportunities there are for misalignments between the emerging virus and its new host, it would seem nearly impossible that host shifts ever occur. Yet they do. This is in part because host species share common ancestry and maintain similar molecular pathways, which viruses adapted to other hosts can plug in to. Additionally, viruses have shown extraordinary ability to generate genetic variation that allows them to ameliorate host incompatibilities and switch host species (**Figure 1**).

For this review, we focused on understanding the evolutionary aspects of the host-range expansion and viral emergence. Multiple exceptional reviews have already been published on the evolution of host-range expansion that provide a thorough background on the subject (3–6). Given this, we narrowed our focus to the topic of viral evolvability. Evolvability is the capacity of lifeforms to adapt, and because viral adaptation plays an important role in host-range expansion, it is reasonable to expect that more evolvable viruses are more likely to emerge in new hosts. Viral species are known to vary greatly in their evolvability (e.g., low mutation rates of DNA-based versus RNA-based viruses), but even viruses separated by just a single mutation can vary in their evolutionary potential (7). Given the variation in evolvability and its likely importance in predicting host shifts, identifying drivers of evolvability could play an important role in identifying the factors that contribute to viral host shifts.

To tackle the subject of drivers of host-range evolvability, we started by searching for viral traits that could enhance evolvability. We then focused on the subset that have a well-developed theory and/or direct experimental evidence to support their role and settled on mutation rate, recombination rate, and protein stability. For each trait, we explored the theory behind how the trait affects evolvability, the relationship between trait values and evolvability, and empirical evidence to support the trait's influence on evolvability. Next, we explored constraints on the traits' evolution by determining possible trade-offs and the types of environments that could tip the scales toward increased or decreased evolvability.

MUTATION RATES

Often the barrier for viruses to shift hosts is a small number of mutations that help the virus ameliorate incompatibilities with the new host (**Figure 2**). The following are a few examples to help visualize the role mutation plays in host-range expansion. Many bacterial cells are resistant to the bacteriophage λ because the cells do not express the outer membrane protein LamB, which λ uses as its receptor. Four mutations in the host-recognition protein can allow λ to gain access to these hosts by interacting with a completely new surface protein, OmpF (8, 9). Similar mutations

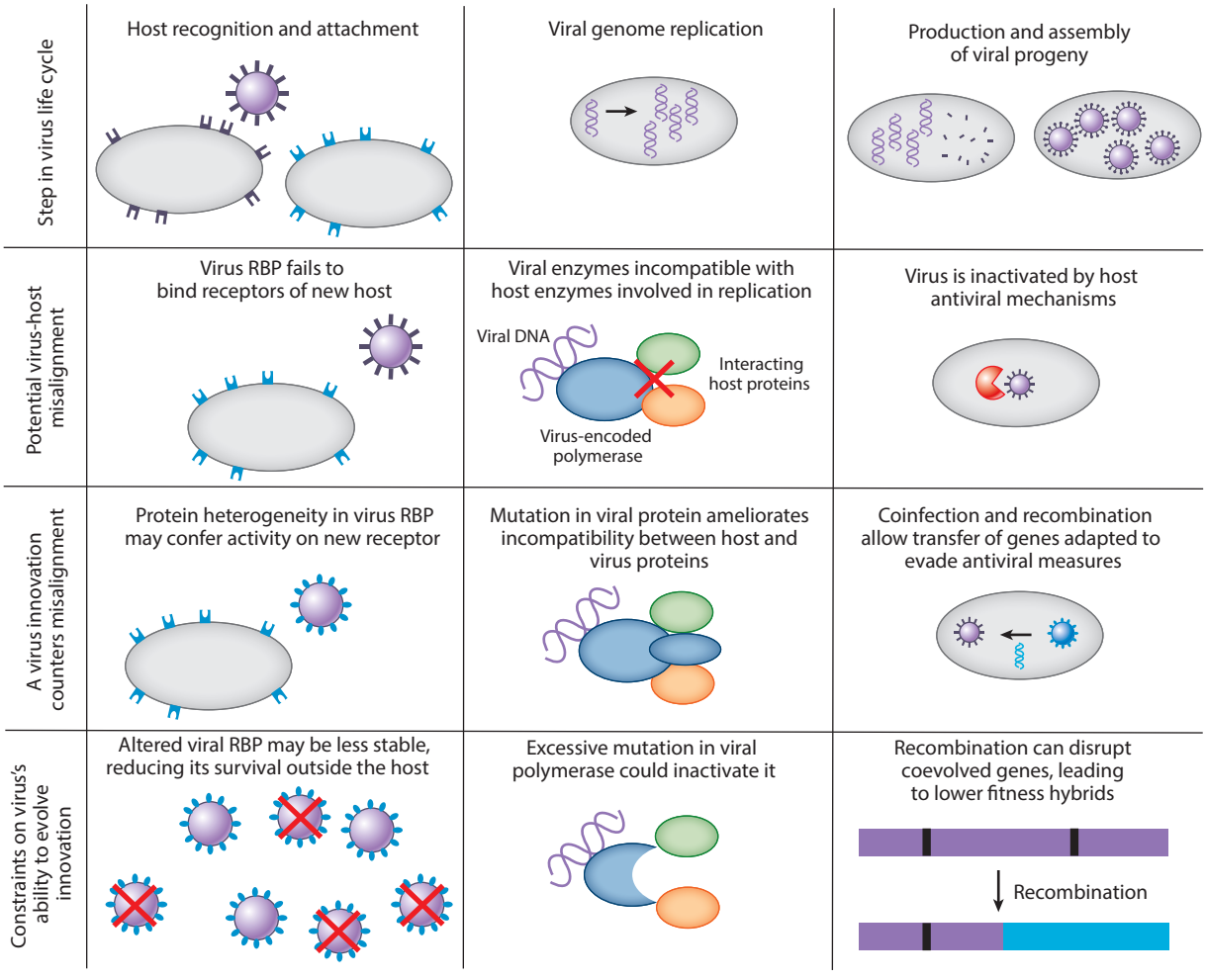

Figure 1

An approach to predicting virus host-range expansion evolvability by examining intrinsic traits and constraints. In this review, we examine the many possible misalignments between a virus and a novel host species or cell type at various steps in the virus life cycle. We examine several intrinsic traits that can enhance the ability of viruses to evolve functional innovations that correct misalignments and permit infection of a new host. Traits that promote innovation can come with associated costs, causing evolvability to be constrained. These constraints enable speculation about the conditions that might promote host-range expansion. Several hypothetical examples are illustrated here to demonstrate how each trait could enhance evolvability by ameliorating misalignments and how constraints could limit that ability. Abbreviation: RBP, receptor binding protein.

in the tail fiber of other bacteriophage have been linked to host-range expansions to new strains (10–12) or entirely new species (13–15). This type of evolution also underlies mammalian virus shifts to humans (16–20). Incompatibilities may also arise internal to the cell (21, 22), but these can also be repaired by relatively few mutations. For example, λ is unable to infect bacteria that lack DnaJ that is involved in λ-DNA replication and ManXYZ involved in transporting λ DNA into the cytoplasm (23). However, λ can overcome these missing elements and successfully infect the hosts by gaining mutations that allow the virus to no longer rely on these host proteins during replication (24).

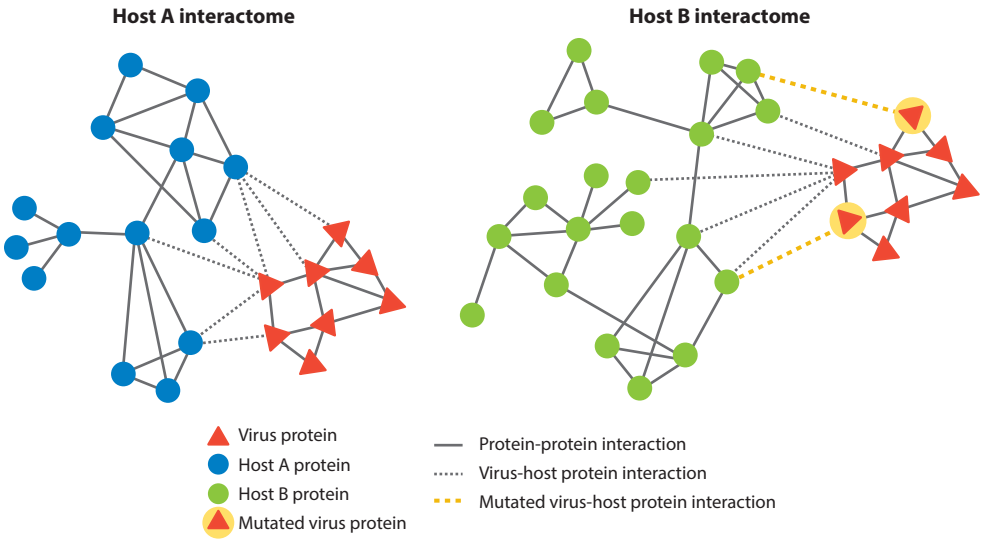

Figure 2

Mutations can alter the shape of virus-host protein interactomes. The interactions between the virus and proteins from host A determine the virus's ability to mount an infection. If the interactome of another potential host is similar, then the virus may be able to gain infectivity on that host with few mutations. Here, we show a schematic representation of how mutated virus proteins might facilitate a host-range shift from its natural host, A, to a novel host, B. The mutated proteins gained the ability to interact with host B's proteins, allowing infection. Figure adapted from images created with BioRender.com.

Given that so many host-virus cellular incompatibilities can be solved with relatively few mutations, it is reasonable to expect that viruses with higher mutation rates would be more likely to expand their host range. This effect should be amplified when a virus has multiple incompatibilities because the chance of gaining multiple mutations simultaneously is the product of the mutation rate (e.g., 10-fold increase in mutation rates will increase the probability of uncovering two mutations by 100-fold, 1,000-fold for three, and so on). Viruses also have enormous variation in mutation rates, with RNA viruses mutating on average 1 in 10^3 bases per replication, and DNA viruses at 1 in every 10^8 (25). Furthermore, viruses can evolve increased mutation rates through mutations that alter the activity of the enzymes that synthesize genetic material or that carry out proofreading (26). Given this variation and their capacity to evolve increased mutation rates, mutation rate is expected to be an important driver of host-range evolvability.

Increased mutation rates may increase host-range evolvability through less intuitive mechanisms as well. For example, a computational model of viral host-range evolution showed that high viral mutation rates may help viruses maintain broad host ranges (27). Under low host diversity conditions, in which a virus serially infects the same species, natural selection is expected to eliminate energetically costly traits that allow the virus to infect unavailable hosts. Viruses with high mutation rates can maintain a broad host range in the face of this selection pressure because host-range expansion mutations are generated faster than natural selection can purge them. This provides the viral population with genetic variation that can be readily employed if a new host is encountered.

If mutation is key to host-range evolvability, does that mean that viruses with the highest mutation rates are most likely to shift hosts? Not necessarily. A large fraction of mutations are deleterious and can slow adaptive evolution. This cost is especially pronounced in viruses. It is estimated

that 20–41% of mutations are lethal in five very different viruses (DNA and RNA viruses that infect bacterial, plant, and animal hosts) (28). These surprisingly high values were confirmed by a second study in which three DNA and three RNA bacteriophages were studied side by side. The average percentage of deleterious mutations in DNA viruses was 29% and 20% for RNA viruses (29). Additional methods were used to measure the average nonlethal mutation fitness effect, which was deleterious for DNA (−0.027) and RNA (−0.047) viruses. These findings are also in line with a human virus, influenza A, where researchers found 31.6% of mutations are lethal, but most of the nonlethal mutations are deleterious (30). Taken together, viruses experience high numbers of deleterious mutations, and increasing mutation rates too high will impart a significant fitness cost on viruses and limit their evolvability.

Moreover, certain mutations can reshape viral fitness landscapes through epistasis and restrict the availability of host-range expansion mutations. This creates a second-order effect of the accumulation of mutations on reducing evolvability. This idea was explored through experiments conducted on three genotypes of bacteriophage φ6 that typically infects *Pseudomonas syringae pv. phaseolicola* but is known to evolve to infect multiple new *Pseudomonas* species (31). Two of the φ6 genotypes studied had previously evolved the ability to infect the novel host *Pseudomonas syringae pv. tomato*, and the third was their ancestor. Through sequencing populations descended from these three genotypes, researchers found that the evolved genotypes had access to fewer mutations for expansion to a third host, *Pseudomonas syringae pv. atrofaciens*. This showed that the accumulation of mutations, even beneficial mutations, can alter viral genomes in ways that increase epistasis and can restrict their host-range evolvability.

Natural patterns of viral variation lead to the hypothesis that increases in mutation rates increase evolvability, but only to a certain point. In 2012, an analysis was published by Rafael Sanjuán (25) that studied 84 different viruses that span the full spectrum of mutation rates. Sanjuán found a strong positive correlation between mutation rate and evolutionary rate, wherein an increase in mutation showed a proportional increase in evolutionary rate, up to a point after which it plateaued. Using a mathematical theory based on expected fitness effects of mutations, Sanjuán showed that evolutionary rate should begin to decline with increases in mutation rates. In line with this prediction, results from laboratory experiments that increase mutation rates beyond natural levels show that viral fitness rapidly declines beyond the predicted threshold and at high enough levels that the viruses can even go extinct (32–34). Indeed, lethal mutagenesis is the basis of viral therapies, including molnupiravir for SARS-CoV-2 treatment (35). Altogether this shows that viruses with higher natural mutation rates are more likely to evolve expanded host ranges, but this is expected to be true only to a certain point.

Most viruses exist below the threshold where increases in mutation rate will cause a decline in fitness. Thus, there is presumably opportunity for many viruses to increase their mutation rates to become more evolvable. How might the environment cause viruses to increase their mutation rates, either directly through exposure to mutagens or indirectly by selecting for viruses with elevated mutation rates? This question is especially relevant in the context of anthropogenic global change because the changing environment could cause higher mutation rates and ultimately increase the risk of emerging diseases. This topic was explored for the A/H1N1 strain influenza virus, where researchers assessed the effects of temperature, population density, precipitation, and social development on genomic substitution rate (36). Researchers examined 11,721 cases of H1N1 from locations across the globe. They assessed the nucleotide substitution rate by comparing the genetic sequences of the focal viruses to the sequence of the earliest reported isolate from each location. Minimum annual temperature had a nonlinear association with mutation, with mutation peaking at 15°C. Population density was found to have a positive association with substitution rate. In contrast, no correlations were found between precipitation and social development.

Taken together, the environment has a role in shaping viral evolvability and global change can alter viral evolvability. This is particularly problematic because global change, such as deforestation, is predicted to also cause humans to encounter more zoonotic disease, which will further increase the chance of disease emergence. If those diseases have heightened mutation rates or are generally more evolvable, then this could help tip the scales toward increased frequency of host shifts. More work along these lines is warranted, especially studies that more directly assess mutation rates and consider additional environmental variables and their interactions.

RECOMBINATION RATES

Point mutations play an important role in driving host-range evolution. However, they require time and, if multiple mutations are required, relatively smooth paths of incremental gains in the fitness landscape to evolve (37, 38). Recombination provides an opportunity to transfer large amounts of genetic material between genomes, driving more rapid genetic divergence. In the context of virus host-range evolution, recombination could facilitate increased evolvability of host-range expansions if genomes exchange genetic elements that confer infectivity on new hosts.

In viruses, there are many mechanisms for recombination that have been reviewed extensively elsewhere (39), so we will briefly highlight just a few. In viruses with DNA genomes, recombination typically occurs via pathways related to DNA replication and repair (40–42). Some viruses rely on host-encoded recombination machinery, while others encode their own recombination proteins (43). Two of the most thoroughly understood virus-encoded recombination systems are the λ Red system of bacteriophage λ (44) and the T4 recombination system (45). Viruses with RNA genomes primarily use a copy choice mechanism, in which the RNA-dependent RNA polymerase or reverse transcriptase jumps from one piece of RNA to another (46, 47). For both DNA and RNA viruses, the extent to which homology plays a role in determining the sites of recombination is highly variable; indeed, recombination between viral genomes can occur even with substantial sequence divergence (43, 48–50).

How might recombination rates themselves evolve? Elevated recombination rates may result from the use of virus-encoded recombination systems. In a study of lambdoid phages, those that had evolved their own recombination machinery tended to have more genomic mosaicism than related viruses that relied on host-encoded recombination systems (43). For viruses that use host-recombination machinery, modulating the use of host enzymes that ensure fidelity (51) could also be used to increase or decrease recombination. In RNA viruses, recombination rate could be modulated indirectly via RNA secondary structure evolution (52). For example, in human immunodeficiency virus, RNA secondary structure influences the rate of recombination (53). If an increased recombination rate enables an ancestral virus to generate more diverse progeny, descendants with adaptive variation would also carry the trait of high recombination, causing it to be indirectly selected for (54).

Elevated recombination rates might enhance evolvability of host-range expansion (55). One group of animal viruses thought to be particularly prone to host-range evolution via recombination is the coronaviruses (56–59). There have been three separate emergences of coronaviruses in humans this century, and there is evidence that in all three instances, the strains responsible arose via a combination of point mutations and recombination in the spike proteins. Recombination is most clearly implicated in the evolution of the strain that caused the 2003 severe acute respiratory syndrome coronavirus pandemic (60, 61). Recombination also clearly occurred in the recent evolutionary history of the strain of Middle East respiratory syndrome coronavirus that caused the 2012 outbreak (62), and subsequent recombination among strains circulating in humans likely increased its transmissibility (63). Investigations into the origins of the strain that caused the SARS-CoV-2 pandemic are ongoing. One hypothesis is that recombination enabled emergence in humans by

replacing the receptor binding motif in a bat coronavirus with that of a pangolin coronavirus capable of binding the human angiotensin converting enzyme-2 (ACE2) receptors (64). An alternative hypothesis is that SARS-CoV-2 is descended with little change from a bat coronavirus that already possessed the key ACE2-binding residues, and the immediate progenitors to SARS-CoV-2 have simply not been sampled (65).

There is evidence for recombination's role in host shifts in other animal viruses and by mechanisms other than altered host recognition. A well-known example occurred when an eastern equine encephalitis virus and a sindbis-like virus recombined and produced a strain with new antigen specificity (66). In baculoviruses, host-range expansion has been observed to occur via recombination in natural infections (67), and host range can be intentionally engineered via recombination of the helicase genes from two different baculovirus (68). Recombination has also been widespread in the evolution of plant geminiviruses and has likely contributed to host switching in agriculturally important hosts (69, 70). Another intriguing possibility is that recombination might provide a mechanism for rapidly excising genomic elements that trigger host antiviral response (71). Positive-stranded RNA viruses, which more readily undergo recombination compared to negative-stranded RNA viruses, were more evolvable in escaping host antiviral defenses (71).

Extensive work on T-even bacteriophages has shown that genes encoding the tail fiber proteins that determine host range readily recombine, even between relatively divergent sequences (10, 72). Transferring entire tail fiber genes, or, more rarely, specific regions within genes, between phages with distinct host ranges conferred the host range of the donor upon the recipient phage (10, 72, 73). Recombinants were generated under laboratory conditions, but it is possible that similar reshuffling of host-specificity regions could allow naturally evolving viruses to expand host range.

Understanding the conditions that favor host-range evolvability in bacteriophages also has applications to phage therapy, in which it would be useful to intentionally broaden the host range of phages. One approach to generating broad host-range phages uses conditions that favor recombination to accelerate host range evolution (74). Iterative rounds of evolution with a cocktail of different phages yielded a phage with a host range that is even broader than the sum of the ranges of the initial cocktail (74). Remarkably, the evolved phage with the broadest host range underwent at least 48 recombination events between two of the initial cocktail strains (74). A different study exploring the use of training to pre-evolve λ phage for use in phage therapy applications identified a highly suppressive variant that contained both point mutations and a recombination in the host-recognition protein (75). Intriguingly, the recombination occurred not with a coinfecting phage but with a relic prophage encoded in the host genome (75), a phenomenon that has been observed elsewhere (76).

Recombination may generate genetic variation favorable for evolution, but high rates of recombination likely come with costs, such as the production of defective progeny. One way this can occur is if recombination occurs within the coding sequence of a protein, resulting in a nonfunctional protein due to frameshifts or a chimera. The viability of chimeric proteins depends on the similarity of the peptide sequences and the location of the break point, but many chimeric proteins are nonviable due to disruptions in protein folding (77, 78). Recombination can also cause genome truncation, rendering some progeny incapable of carrying out a complete infection cycle (79). In some viruses, the disadvantage is compounded because the nonviable particles can interfere with the production of viable particles (80, 81). Another potential cost of recombination is the production of incompatible hybrids. Even in highly related virus genotypes, recombination can create incompatibilities between genes (82) or even within the same gene (83). This was observed between two closely related λ genotypes that specialize for different *Escherichia coli* receptors (83). When host-recognition protein mutations from different genotypes were engineered into a single hybrid protein, the resulting phage was inviable (83).

Virus genomes may have evolved properties that minimize the costs of recombination. Computational and experimental studies have shown that intragenic recombination, occurring within protein coding sequences, appears to be less disruptive than might be expected (77, 78, 84). It is not clear whether this is because nonviable chimeric proteins are purged by selection or because viruses have evolved recombination hotspots at domain boundaries where break points will be less disruptive (78, 84). It has also been observed that genome-wide recombination break points are more likely to occur at gene boundaries than would be expected by chance (85). This could be partially explained by selection purging nonviable recombinants (86), but it is also possible that viral genomes may have also undergone evolution to favor genomic architecture that minimizes the disruption of coevolved genes with interacting functions. In maize streak virus, recombination was more favorable when the genome fragment being exchanged did not have extensive interactions with the rest of the genome (87). Many virus genomes are organized such that genes with related functions are positioned together (88). For example, in the genome of bacteriophage λ, the genes coding for the tail shaft proteins, tail tip proteins, and side tail fiber proteins are clustered together (89), and the same is true for T4-related phages (90). It is thought that virus evolutionary history has been shaped by exchanging functional modules (91). Viruses with genomes characterized by spatial modularity might more easily transfer the elements necessary to exploit novel hosts, potentially making them more evolvable with respect to host-range expansion (**Figure 3**).

There are several host-related, ecological, and environmental factors that might shape the relationship between recombination and evolvability. High multiplicity of infection (MOI) could enhance evolvability via recombination by increasing the potential for increasing sequence

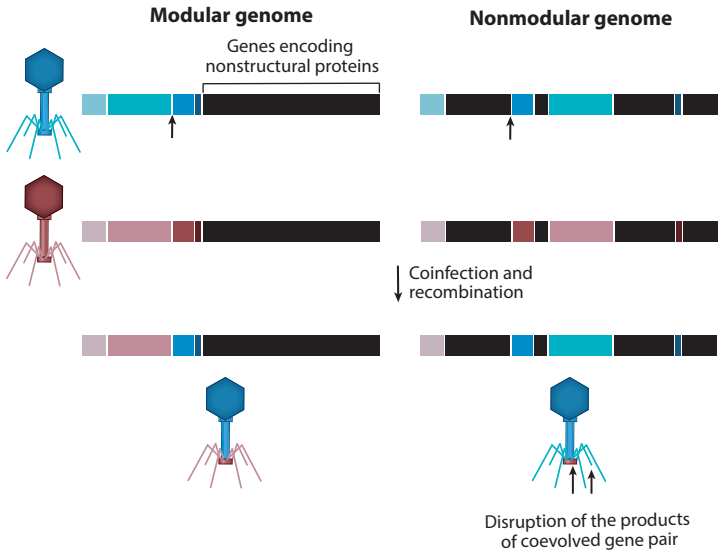

Figure 3

Genome modularity may prevent the costs of recombination, facilitating host-range expansion. Many viruses have genomes that are remarkably modular, with functionally similar proteins encoded by genes spatially clustered in the genome. In this hypothetical schematic, we show how this organization might facilitate the transfer of functional modules between virus genomes. Viruses with modular genomes might more readily exchange the genes that confer infectivity on novel host species or cell types, such as an ensemble of tail proteins in a bacteriophage. Modular organization would help avoid the cost of breaking up gene pairs that have coevolved to interact with each other, such as the tail tip protein and the tail fiber proteins of a bacteriophage.

diversity (92). However, high MOI does not always result in increased viral evolvability. For example, in a study in which phage Φ2 was grown with its host bacteria, *Pseudomonas fluorescens*, under varying MOI, infectivity did not evolve faster at high MOI (93). However, this study examined adaptation to the current host rather than host-range expansion. Because recombination can occur between related viruses when they coinfect the same host, industrial agriculture and the wildlife trade likely create conditions that facilitate the evolution of novel viruses through recombination (94).

PHENOTYPIC ROBUSTNESS AND HETEROGENEITY

Mutation and recombination provide the genetic variation that can allow viruses to infect new hosts. However, virus host shifts cannot be predicted solely based on the supply of genetic variation, in part because of higher-order mechanisms, such as genetic robustness and nongenetic phenotypic heterogeneity, which tune the extent to which genetic changes affect the virus phenotype. Genetic robustness suppresses the effects of mutation on the phenotype (95), which in the immediate term might be expected to hinder the production of novel phenotypes, potentially slowing adaptation. However, because a high fraction of mutations are deleterious (96), robustness can also favor the accumulation of cryptic genetic diversity that may lead to adaptation in the longer term (97, 98). In essence, genetic robustness allows viruses to traverse otherwise insurmountable fitness valleys, enabling them to eventually ascend fitness peaks. The second mechanism, phenotypic heterogeneity, allows organisms to generate a range of phenotypes from a single genotype without underlying genetic variation and therefore can be conceptualized as the opposite of robustness. A genotype with the ability to express multiple phenotypes can bypass the delay associated with acquiring adaptive mutations and therefore might experience greater adaptability (99, 100). Although robustness and heterogeneity tune phenotypic response in opposite directions, there is evidence that both can enhance evolvability. Most studies on this topic have been performed on enzymes (101–103), but because the structural components of viruses are mostly proteins, insights gained from these studies can inform drivers of viral evolvability.

A consensus has emerged that genetic robustness enhances enzyme evolvability (104). Because robustness can be difficult to characterize in living systems, many studies approached the question by manipulating thermostability, a trait that is intrinsically linked to robustness. Thermostability is a measure of resistance to heat, and it is thought that thermostable proteins tend to also be robust to mutation (105). Enzymes with high thermostability are buffered against the destabilizing effects of mutations, allowing the proteins to evolve more mutations and increasing the likelihood that an adaptive mutation is uncovered (101–103).

There is some evidence that robustness might also promote evolvability in viruses, but this evidence is not unequivocal. Robustness increased the evolvability of thermotolerance in bacteriophage ϕ6 (106), although robustness and thermotolerance are traits that tend to be correlated (107), and it is possible that this pattern would not generalize to the evolvability of other traits. In one study in line with the stability-evolvability link, a vesicular stomatitis virus that had been selected for thermotolerance exhibited enhanced antigenic diversification and antibody escape (108). However, in a different study comparing the host-range evolvability of two vesicular stomatitis viruses, the less robust strain host range evolved faster (109).

In contrast to robustness, there is a less extensive literature on the role of phenotypic heterogeneity in protein evolvability. However, recent advances in structural methods capable of detecting heterogeneity have renewed interest in the implications of heterogeneity for evolvability. Conceptually, it seems plausible that proteins with more structural heterogeneity may have immediate access to phenotypes that carry out new functions (110, 111). There is evidence to support

this assertion in laboratory studies. For example, decreasing structural rigidity by removing amino acid contacts resulted in a more evolvable scaffold for designing a novel metallo-β-lactamase via directed evolution (112). Similarly, a mutation that stabilizes a non-native, alternative conformation in PSD95, a synaptic scaffolding protein, allows the protein to recognize multiple classes of ligands at once, thereby acting as an evolutionary bridge between an ancestral protein that recognizes only one class and a double mutant protein that recognizes only a second class (113). Another study revealed that the ability of antibody proteins to achieve specific recognition of a diverse set of targets depends on precursors that are conformationally heterogeneous. Subsequent mutations introduce contacts that increase the structural rigidity of a single conformation, generating specificity to a single target in the mature antibody (114). It is intriguing to speculate that this pattern may be mirrored in the evolution of other protein types, with metamorphic intermediates capable of folding into multiple conformations playing a key role in functional and structural transitions (115).

One way in which phenotypic heterogeneity of proteins could impact virus evolvability is if receptor binding proteins, which are responsible for host recognition, can evolve the capacity to produce multiple conformations with differing binding specificity, like the precursor antibodies (114). There is some evidence that an analogous process occurs in bacteriophage λ during its evolution from a single-receptor specialist to a dual-receptor generalist, followed by subsequent specialization on either the old or new receptor (8, 83, 116). As predicted, the evolution from specialist to generalist was accompanied by a loss of stability, and the new specialist genotypes that evolved from the generalist regained stability (116). The generalist also displayed properties consistent with the production of phenotypic heterogeneity because multiple phenotypic subpopulations of phage particles were detected in an isogenic culture (116). In addition, λ selected for enhanced thermostability were less evolvable and required additional destabilizing mutations to gain the use of the new receptor (7).

If genetic robustness and phenotypic heterogeneity act in opposite directions, how can both promote evolvability? There have been several attempts to reconcile the effects of robustness and phenotypic heterogeneity through theoretical and computational models. One approach focused on the timescale on which new functions evolve, concluding that robustness is favorable in the long term but not necessarily in the short term (117). For this, robustness of an individual sequence is defined by the number of one-mutation-away sequences that encode the same phenotype. Use of the theoretical framework of neutral networks demonstrated that robustness does in fact correlate negatively with the evolvability of that sequence. However, robust phenotypes, defined as those that can be encoded by many different sequences, facilitate the proliferation of diverse sequences. This, in turn, increases the likelihood that one of the many sequences encoding the phenotype will have one-mutation-away sequences that encode a novel phenotype (118). Under this framework, phenotypic robustness, but not genotype robustness, should promote evolvability. Phenotypic robustness could be encoded by disordered protein regions, in which structural changes may actually be tolerated more easily, allowing for more sequence diversity and rapid evolution. For example, Nodamura viral polymerase can tolerate high levels of sequence disruption in its structurally disordered C-terminal region without losing function (119). Finally, it may be that the effect of robustness depends on the extent of change in the distribution of fitness effects between an old and new environment (120). Through these efforts, it is understood that the relationship between stability and evolvability is likely far more complex than a simple linear correlation.

We consider whether fitness trade-offs provide another perspective on this problem. In our hypothetical model, the relationship between stability and evolvability is unimodal, with an optimal stability at which evolvability is highest (**Figure 4*a***). From the optimum, evolvability decreases as a protein moves toward both extremes of stability, low and high (**Figure 4*a***). This framework would

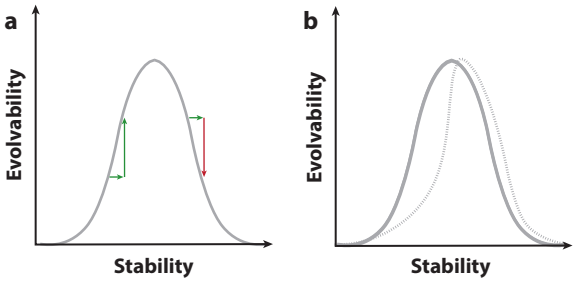

Figure 4

Virus evolvability is in many ways shaped by the ability of viral proteins to evolve new functions. Stability is a trait intrinsic to proteins that is thought to affect evolvability, but the relationship is complex. (*a*) We propose that protein evolvability, and by extension virus evolvability, is constrained at both extremes. At low stability, evolvability is constrained by protein misfolding, whereas at high stability, it is constrained by excessive rigidity that prevents structural dynamism. Depending on where a protein is on the continuum of stability, enhancing stability could increase or decrease evolvability. (*b*) Proteins evolved under different environments might have differently shaped stability-evolvability curves. A protein evolved under thermal stress might have a right-shifted curve because low stability would be more costly.

reconcile conflicting empirical results from different studies, as the effect of changing stability (i.e., increasing or decreasing) would have a different effect on evolvability (i.e., either positive or negative) depending on where the protein is on the curve (**Figure 4*a***). The exact shape of the curve might be different depending on the evolutionary history of the protein (**Figure 4*b***) but is fundamentally driven by trade-offs. Thus, at low stability, evolvability is constrained by unfolding and aggregation, whereas at high stability, evolvability is constrained by excessive rigidity preventing the exploration of novel folds. Applied to virus evolvability, these constraints on protein evolvability may manifest at various levels of the viral life cycle, particularly during particle production and transmission (**Figure 1**), which we address next.

The main constraints restricting evolvability at low stability are the production of viable progeny particles and survival outside the host cell during transmission between hosts (**Figure 1**). During virus production inside the cell, particles that contain unstable proteins might not assemble properly, might be subject to degradation by host quality control machinery, or might be more reliant on host chaperones to fold properly (121). Once the progeny viruses are produced and assembled, they are released from the intracellular environment into the external environment and must survive until encountering the next susceptible cell. Unstable viruses might be more likely to become deactivated by environmental forces outside the host, potentially hindering transmission between host individuals. There are several documented cases of stabilizing mutations resulting in increased viral transmission rates (122, 123). In animal viruses, increased sensitivity to temperature might restrict a virus from infecting hosts with higher body temperatures (124), inhibiting initial spillover to a novel host. The trade-off between phenotypic heterogeneity and transmission raises the question of how enhanced evolvability could possibly outweigh the seemingly high cost of reduced transmissibility. One possibility is that chance destabilizing mutations introduce conformational heterogeneity into viral proteins, generating an incipient function, such as binding to a new host receptor. Additional mutations might then rapidly tune performance of the new function and restabilize the protein, minimizing the number of transmission events that would be required with the less stable particle. Evidence for this model comes from bacteriophage λ, in which destabilizing mutations that arose in a stable, single receptor specialist allowed activity

on a novel receptor, and subsequent mutations restored stability and simultaneously increased specialization to the new receptor (116).

A constraint on evolvability at high stability could be reduction in the capacity to produce the phenotypic flexibility necessary for novel activity. At the protein level, high stability has been associated with structural rigidity and reduced conformational flexibility (125, 126). Consistent with this, stabilizing mutations sometimes reduce activity in enzymes (127, 128), and mutations that confer new abilities are often destabilizing, although perhaps not more destabilizing than the average mutation (129). The hypothesis that stability can constrain activity is also consistent with results from the non-enzymatic bacteriophage λ host-recognition protein. When stabilizing mutations were inserted into the receptor binding proteins of generalists able to use two different receptors, they lost function on one receptor (7). Together, these results suggest that high stability can constrain new activity.

Given these constraints, we can begin to speculate about the ecological and environmental conditions that might favor or impede virus evolvability via protein heterogeneity. Host density has been shown to be an important determinant of viral transmission (130). At high host density, a viral particle may not need to persist for long periods of time outside of the cell before finding a new host. Thus, selection for stability would be relaxed and favor evolvability via phenotypic heterogeneity. Similarly, the diversity of hosts in the environment, specifically the ratio of susceptible to resistant hosts, could shape the response. Host diversity has been shown to influence the evolution of host range in bacteriophage T7 (131) and novel phage øJB01 (132), although potential links to robustness and phenotypic heterogeneity were not explored.

An abiotic condition that could influence evolvability by phenotypic heterogeneity is temperature. Under conditions of high temperature, it seems reasonable to predict that phenotypic heterogeneity would be more constrained due to selection for thermostability. This might manifest as a shift in the optimal stability for evolvability toward higher values (**Figure 4*b***). Rapidly rising global temperatures will undoubtedly alter the selection pressures faced by viruses, and it is interesting to speculate about whether virus evolution might shift toward increasing thermotolerance (133). However, because viruses are intracellular parasites, the extent to which they experience selection from the external environment might be modulated by host processes. Unstable viruses could potentially be shielded from misfolding by host protein-folding chaperones (121). For example, an amino acid change in the influenza nucleoprotein known to destabilize the protein and enhance immune evasion resulted in severe fitness costs at febrile temperatures, but only when a host heat shock factor was inhibited (134). In viruses that use multiple host species to complete their life cycles, such as arboviruses, viral proteins face the additional challenge of folding and functioning at vastly different temperatures (135).

CONCLUSION

A unimodal relationship between trait values and evolvability was uncovered for all three traits examined. Such relationships make it difficult to predict viral evolvability because it is often unclear whether the viral variants being scrutinized reside on the increasing or decreasing slopes of the relationship. Unimodal relationships suggest that there are limits to virus evolvability and that environmental pressures that push those limits could have a significant impact on reducing viral evolvability. These pressures could occur naturally and might indicate areas of less concern to focus surveillance efforts on locations where conditions favor evolvable viruses. Or the pressures could stem from human interventions designed to mitigate the risk of disease emergence. Certainly, more research is necessary to understand the drivers of viral host-range evolvability.

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