FEMS Microbiology Reviews, 2023, 47, 1–10
DOI: 10.1093/femsre/fuad038
Advance access publication date: 8 July 2023
Review Article

Drivers and consequences of bacteriophage host range

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Editor: [Eddy J. Smid]

Abstract

Bacteriophages are obligate parasites of bacteria characterized by the breadth of hosts that they can infect. This "host range" depends on the genotypes and morphologies of the phage and the bacterial host, but also on the environment in which they are interacting. Understanding phage host range is critical to predicting the impacts of these parasites in their natural host communities and their utility as therapeutic agents, but is also key to predicting how phages evolve and in doing so drive evolutionary change in their host populations, including through movement of genes among unrelated bacterial genomes. Here, we explore the drivers of phage infection and host range from the molecular underpinnings of the phage–host interaction to the ecological context in which they occur. We further evaluate the importance of intrinsic, transient, and environmental drivers shaping phage infection and replication, and discuss how each influences host range over evolutionary time. The host range of phages has great consequences in phage-based application strategies, as well as natural community dynamics, and we therefore highlight both recent developments and key open questions in the field as phage-based therapeutics come back into focus.

Keywords: phage–host interactions, host range, bacteriophage

Upon their discovery by Twort and d'Herelle at the beginning of the 20th century, viruses infecting bacteria, the bacteriophages, were a focal point in the battle against bacterial infections. The first successful use of phages in human medicine in Western literature was reported in 1919 to treat bacterial dysentery in patients (Sulakvelidze and Alavidze 2001). This interest expanded beyond human health to agriculture. In 1924, for example, phages were used in field trials to contain black rot disease in brassicas (Mallmann and Hemstreet 1924). Even though their discovery was revolutionary in the pre-antibiotic era, allowing us to fight bacterial infections for the first time, phages were found to be unreliable, especially in comparison to broad-spectrum antibiotics discovered only a few years later. This rapid shift away from phage therapy in the Western World (Pirnay 2020) meant little to no active research in this area for many decades, but as we once again find ourselves in need of new anti-bacterial treatments due to the spread of antibiotic resistance, such efforts are once again on the rise.

Underlying the historical difficulties in applying phages as a broad antimicrobial to treat disease is the limited host range of these viruses. Like any other parasite or pathogen, a phage's host range is characterized by the subset of hosts, the number of species and strains, that it can infect. This host range is dependent on the phage, the host, and the environmental conditions under which infection occurs (Hyman and Abedon 2010). Phages have been found to vary dramatically in their apparent host range, with some specialists infecting only a narrow subset of bacterial hosts and generalists infecting a wide spectrum of hosts. However, the narrow versus broad host range concept is quite controversial and inconsistent (de Jonge et al. 2019). In the literature, the term "broad" host range can be used to describe phages infecting multiple species of bacteria, but also when a phage can reproduce on a broad range of strains within one species (Ross et al. 2016). If we define a strain as a bacterial isolate belonging to a species but characterized by a genetic diversity that lies within the species threshold (Simar et al. 2021), then we can delineate phages that infect only within versus across species. However, the myriad of techniques that are employed to determine the host range of a phage, and the necessary limits of detection of host range based on examination used, make such clear delineations a challenge (Mirzaei and Nilsson 2015). Nevertheless, the study of phage host range and how it is determined at the genetic and environmental levels is critical to understanding how phages can target specific bacterial populations while leaving other bacterial members of the community relatively unaffected. In the case of phage applications, a narrow host range means a higher likelihood that phage therapy treatment will fail due to a mismatch between the pathogen strain and the phage therapeutic, while more generalist phages pose serious risks in fermentation and can cause problems in factory settings for decades (Lavelle et al. 2018, Jolicoeur et al. 2023). Beyond specific phage applications, host range is a key determinant of how phages impact microbial diversity in natural settings, including within the microbiome (Morella et al. 2018).

In this review, we introduce the concept of intrinsic, transient, and environmental drivers of phage infection and, in extension, host range. These drivers greatly influence the ecology of microbial communities, their function, and their future evolutionary trajectory, and as such give us a framework to rationally design phage applications and engineer both microbial and viral communities drawing from basic phage biology. In this review, we build from molecular underpinnings of phage–host interactions and ex-

Received 3 May 2023; **revised** 5 July 2023; **accepted** 7 July 2023

pand to environmental drivers of host range and evolutionary responses of phages and their hosts to examine the consequences of phage host range from application to natural community dynamics. We then highlight emerging research and prominent questions in this area as phage-based applications come back into focus.

Molecular underpinnings of phage host range

As obligate cellular parasites, viruses fully rely on the host's extracellular and intracellular machinery to complete their life cycle, underlining the intimate interaction between the host and its parasite. The interaction and infection are characterized by different steps ranging from attachment of the phage particle to the cell, injection of the genetic material, host takeover, replication, production of new phage particles, and lysis of the cell (Fig. 1). The outcome of the infection, however, depends on environmental factors along with the phage genome itself. Temperate phages encode proteins facilitating the potential incorporation of the phage genome into the bacterial genome causing a latent infection, referred to as lysogeny. Lytic phages, on the other hand, complete their life cycle upon entering, and ultimately lysing, the bacterial host cell. Dependent on environmental conditions, including host abundancy, temperate phages can opt for a latent or a lytic infection (León-Félix and Villicaña 2021). There is an array of alternative infection strategies that phages employ described to date, but remarkably understudied, including pseudolysogeny, the phage carrier state, and chronic infections in the case of filamentous phages (Mäntynen et al. 2021). In this review, we focus on the drivers of host range shaping phages in the lytic cycle, where the majority of the research has been done.

During the initial step of phage infection, phages need to recognize and successfully adsorb to the bacterial cell wall. The molecular structures that act as phage receptors vary among bacterial strains. Sugar residues on the cell surface, like capsular polysaccharides, teichoic acids, and lipopolysaccharides, motility-associated structures, such as flagella and pili, and/or proteins and porins have all been reported to function as sites of attachment either as primary or secondary receptors (reviewed in Mangalea and Duerkop 2020). Most phages depend on such chromosomally encoded factors, but plasmid-dependent phages have been described that exploit plasmid-encoded conjugation proteins (Quinones-Olvera et al. 2023). While monovalent phages attach to one receptor, polyvalent phages can recognize multiple receptors on the cell surface or a conserved component among distinct hosts, significantly broadening their overall host range (Gonzalez et al. 2018, Vasquez et al. 2023). The presence, absence, and diversity of these molecular structures, along with the diversity of the phage's receptor recognition machinery and mechanisms inhibiting the phage from reaching the receptor, act as key determinants of virion attachment and initiation of infection. As such, genetic signatures of phage receptor use can be leveraged to predict phage infection, as has been shown for *Xanthomonas campestris* pv. *campestris*, where the LPS biosynthetic gene cluster was representative for predicting phage infectivity (Holtappels et al. 2022). Protein structure predictors have also been used to explore how receptor-binding proteins influence phage host range. For example, AlphaFold2 was used to explore the architecture of host-binding machineries from mycobacteriophages, and the predicted structures indicated that mycobacteriophages would preferentially use cell surface polysaccharides and lipids to recognize and attach to their hosts (Cambillau and Goulet 2023). For a detailed overview of how receptor-binding proteins influence phage host range and their implications on phage ecology and engineering, see de Jonge et al. (2019).

The evolution of phage receptors and susceptibility markers in general as an adaptation to resist phage has been reported in multiple phage–host combinations and is a main driver of phage resistance observed *in vitro*, often resulting in a fitness cost and impacting the evolutionary adaptability of the bacteria (Sumrall et al. 2019, Holtappels et al. 2020, Warring et al. 2022). Yet, there are bacterial-encoded defenses described, such as TraT in *Escherichia coli*, that shield rather than alter the phage receptor, reducing virion attachment, and causing a reduced infection efficiency and potential resistance against the phage (Riede and Eschbach 1986, Labrie et al. 2010, Egido et al. 2022). Moreover, shielding the phage receptor by an overproduction of cell surface proteins, as well as operons involved in the production of extracellular polymeric substances, has been reported to protect cells from a potential phage infection (Yuan et al. 2022). Besides the primary and secondary phage receptors, some phages require the presence of additional factors in the bacterial cell wall to successfully infect. These additional structures, as well as the receptors themselves, act as susceptibility markers and influence the ability of a phage to infect a given bacterial isolate. *Escherichia coli* phage HK97, for example, requires the interaction of an inner membrane glucose transporter (PtsG) and periplasmatic chaperone (FkpA) with the phage tape measure protein for DNA injection (Cumby et al. 2015).

Once inside the cell, the phage's genetic material is subjected to an array of different bacterial- and viral-encoded defense systems, as reviewed elsewhere (Egido et al. 2022). Recent efforts in the field have resolved phage-encoded mechanisms that counteract these bacterial defenses, such as anti-CRISPRs and others (Pawluk et al. 2018, Srikant et al. 2022). However, the lack of transcription and translation of these mechanisms, as well as proteins responsible for host takeover, and especially the synchronization thereof can result in an aborted infection. Inability to synchronize transcription and translation resulted in an unsuccessful infection, potentially due to host proteases degrading phage-encoded proteins (Howard-Varona et al. 2018, de Jonge et al. 2019). Members of the *Schitoviridae*, however, bypass the need of the host's RNA polymerase (RNAP) by coinjecting a virion-associated RNAP (vRNAP), facilitating the transcription of early phage proteins. In the case of *Agrobacterium* spp., this resulted in a broader host range of these *Schitoviridae* phages compared to the other phages isolated (Fortuna et al. 2023). Other phages have been reported to encode proteins interacting directly with the transcription machinery of the host. It has been hypothesized that these proteins either direct the host RNAP to the viral DNA [gp12 of phage 14-1 and gp23 of phage LUZ24 (Van Den Bossche et al. 2014)] or shut it down [gp2 of phage LKA1 (Wagemans and Lavigne 2012)]. On the same note, there are also proteins reported that influence the translation (Robertson et al. 1994) and RNA degradation (Van Den Bossche et al. 2016, Dendooven et al. 2017). In general, numerous examples are described of how phages shuttle the host metabolism to support their own reproduction. Processes such as replication, the cytoskeleton, DNA silencing, cell division, motility, and metabolism, among others, are targeted and directed to sustain phage reproduction (De Smet et al. 2017). The inability to take over the host metabolism will eventually result in a decreased infection efficiency and potential exclusion from the phage's host range, and thus offer additional opportunities for the evolution of host resistance.

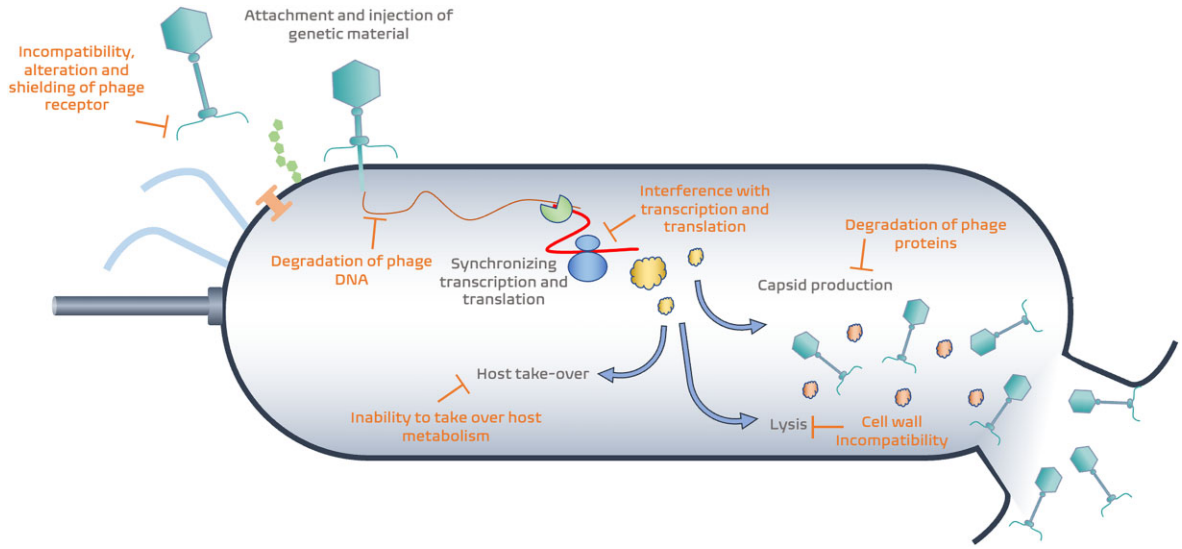

Figure 1. Molecular underpinnings of phage infection and phage host range. The first step in the phage infection is adsorption to the bacterial cell. Incompatibility between the phage receptor and the phage tail fiber will inevitably lead to an interruption of the phage infection. Next, the phage injects its genetic material, which will be transcribed and translated into viral proteins. Inability to express the phage proteins due to out-of-sync transcription and translation results in an abortion of the infection. As phages are obligate parasites, they require use of the bacterial metabolism. If there is no interaction between the phage and the host proteins, then phages are reduced in their incapable of host takeover. Near the end of the infection cycle, phages produce capsid proteins. Host proteases can interfere with this process, reducing the amount of viral progeny that is produced. Finally, the phage produces specialized enzymes to degrade the host's cell wall (lysis). If the cell wall is incompatible with the enzymes produced, then it will limit the number of phage progeny produced.

In the final step of infection, new phage particles are produced along with specialized proteins (endolysins and holins) that degrade the bacterial cell wall, releasing the newly formed phage particles into the environment. Inability to efficiently degrade the cell wall will inevitably result in a halted infection and a reduction of further transmission and infection efficiency. Endolysins are responsible for degrading the peptidoglycan in the cell wall, while holins permeabilize the cell membrane, allowing endolysins to reach the peptidoglycan layer (Cahill and Young 2019). Endolysins can be globular or modular, depending on whether they contain a single or multiple domains. In the latter case, endolysins consist of a catalytic and one or multiple cell wall-binding domain(s) (CBD; Gerstmans et al. 2018). These CBDs direct the endolysin to the peptidoglycan layer, increasing the activity compared to endolysins lacking a CBD (Walmagh et al. 2013). CBDs are known to interact with specific molecules associated with the peptidoglycan layer and thus introduce a degree of specificity to the endolysin (Loessner et al. 2002). Recently, it has been shown that endolysins and different domains can be exchanged through homologous recombination between different *Lactococcus* phages coinfecting the same cell, and that these newly introduced endolysins evolved via mutations accumulating in both the catalytic and CBDs, allowing the enzyme to adapt to the new phage and host (Oechslin et al. 2022). Hence, we can postulate that acquisition and adaptation of these enzymes influence the phage's host range.

At every step of the infection, ranging from the attachment, injection, reproduction, and lysis of the host, there are factors that determine the outcome and success of infection, and thus whether or not a bacterial isolate is considered part of the host range of a particular phage. These drivers of infection and host range can be categorized as predetermined, evolutionarily transient, or environmental drivers based on their taxonomic constraints, (co)evolutionary history, and environmental flux.

Drivers of phage infectivity and host range of phages

Intrinsic drivers of host range and nonhost resistance

When evaluating infection on large collections of bacteria and phages isolated from diverse environments, the host range often shows some degree of modularity. In a modular network, nodes can be partitioned into subsets, and most connections occur within these subsets rather than between the subsets (Beckett and Williams 2013). In bipartite phage–host interaction networks, this means that a set of phage isolates typically infect a subset of bacterial isolates, but there is little to no infection observed of bacteria outside of this specific subset. For example, a study on 286 genetically uncharacterized hosts and 215 phages isolated from the Atlantic Ocean demonstrated an evident modularity in the interaction, where the interaction network consisted of 49 modules from which half of the modules indicate single phage–host pairs (Flores et al. 2013). Similar results were obtained when the infectivity of 248 *Vibrio* phages was evaluated on a collection of 279 hosts (Kauffman et al. 2022). This modularity was also uncovered at the genus level of *Staphylococcus* and within the *Agrobacterium* species complex (Göller et al. 2021, Fortuna et al. 2023). In the latter case, a phylogenetic analysis of hosts revealed that there was a relationship between phage infectivity and bacterial relatedness. In current phage host range analyses, the host phylogeny, especially at a whole genome level, is seldom included. Notably, phages that do infect across the species levels, either natural or engineered, seem to adapt most readily to phylogenetically related bacteria. Through genetic engineering, multiple authors were able to efficiently expand the host range of phages, but generally, the host range remained within the same taxonomical order (reviewed in Dunne et al. 2021). We can argue that such taxonomic separation is likely the result of fundamental differences in cell wall structures, transcription–translation machin-

ery, and general metabolism between bacterial families and genera that contribute to an incompatibility between phage and bacterium, generally referred to as nonhost resistance (Antonovics et al. 2013). We consider these conserved and fundamental differences predetermined drivers of phage host range (Fig. 2A). Overcoming these barriers requires substantial alteration to the phage that is unlikely to result from mutations and the acquisition of external genetic material. This is further supported by the idea of a constrained viral evolution due to relatively small genome sizes (Belshaw et al. 2008), even though these ideas could be less applicable to giant phages characterized by extravagant large genomes, which opens a new research field about the host range specificity of these phages.

Transient drivers of host range

At more fine-grained taxonomic levels, phages and their hosts are entangled in a coevolutionary (arms) race. Due to the evolutionary pressure phages exert on host populations, they drive the evolution of their hosts. Arms race dynamics are generally characterized by a more nested bipartite network, as bacteria evolve ever increasing resistance against previous phage types. The nestedness of a network or matrix is a measure of whether positive interactions of each row or column are a subset of all positive interactions in the other rows or columns, where a perfectly nested network has each row or column as a strict subset of the next (Beckett and Williams 2013). In Ecology, the nestedness of a network is an indication of specialization in which generalist phages can infect a wide array of bacterial isolates and specialist phages infect only a limited or even a single isolate that is, on average, also targeted by the generalist phages. Such a high degree of nestedness has been observed during the *in vitro* coevolution of *P. aeruginosa* and two different phages, LUZ19 and 14-1 (Gurney et al. 2017), as well as *E. coli* with a lytic mutant of phage λ (Gupta et al. 2022). A more recent detailed study on the modularity and nestedness resulting from coevolution between phage λ and *E. coli* revealed that the interaction network can be nested, modular, or nested within the modules depending on the coevolutionary timepoint being observed (Borin et al. 2023). The overall nestedness resulted from evolution of the host receptor LamB, followed by phage escaping the resistance by adapting to another receptor OmpF. The modules emerged as the coevolution further shaped the interaction of the extramembrane hoops of OmpF and the central tail fiber protein J. Additional amino acid substitutions in OmpF and the phage tail fiber then contributed to intermodular nestedness (Borin et al. 2023). Numerous examples in literature show the adaptation of phage receptors, and even a historical contingency of these mutations, embedded in bacterial genomes multiple generations after the phage is removed from the system (Debray et al. 2022). A schematic is illustrated in Fig. 2B.

In addition to resistance evolved via mutational change, resistance mechanisms can be acquired through horizontal gene transfer or through acquisition of CRISPR spacers from the phage genome (Koonin et al. 2017). Individual genomes have been reported to carry an arsenal of different defense mechanisms encoded, and recent efforts in the field showed that, on average, prokaryotes encode five antiviral defense mechanisms in their genome (Tesson et al. 2022). During a screening of 2778 genomes of cheese-associated microbial communities, individual genomes encoded on average 7.5 defense mechanisms, suggesting that these communities are under high phage predation and evolutionary flux (Somerville et al. 2022). Phages have different ways of overcoming these defense mechanisms, either by preventing activation of the mechanisms, becoming resistant to the action of the system, or by acquiring or modifying a factor inhibiting the system. In T4, for example, the phage was able to surpass a toxin–anti-toxin system in *E. coli* by acquiring segmental amplifications of *tifA*, which encodes an inhibitor of the bacterial-encoded toxin. However, these amplifications promote large deletions in the phage genome, resulting in the loss of other phage accessory genes important to mitigate the effects of other phage defense mechanisms (Srikant et al. 2022). This example demonstrates the viral trade-off of overcoming one phage defense mechanism but constraining the phage's overall host range.

Beyond bacterium-encoded defense systems, there are also virus-encoded mechanisms described that interfere with the infection of other viruses, referred to as superinfection immunity and exclusion. While superinfection immunity describes the event in which a phage prevents the completion of a second (typically similar) phage's life cycle after DNA injection, superinfection exclusion is defined as the immunity related to the blocking of the initial infection of a secondary phage. The former is typical for prophages expressing repressors of the lytic cycle, and the latter is described for a myriad of phages, including lytic and filamentous phages (Abedon 2022). *Escherichia coli* phage T5, for example, produces the lipoprotein Llp, which masks FhuA, the receptor of T5 and thus causing superinfection exclusion (Decker et al. 1994, Van Den Berg et al. 2022). Phage T4 has been reported to encode multiple superinfection exclusion mechanisms that interfere with the infection of other T-even phages (Lu et al. 1993, Lu and Henning 1994, Shi et al. 2020), and in *Streptococcus thermophilus*, a protein encoded by prophages showed a similar effect and prevented the injection of genetic material by superinfecting viruses (Ali et al. 2014). Similar exclusions were described for filamentous phages infecting *P. aeruginosa* in which phage Pf influences the infectivity of multiple phages with different infection strategies (Wang et al. 2022a). These filamentous phages form crystalline structures around rod-shaped bacterial cells, which are hypothesized to protect the cell from harm like potentially phage infections (Tarafder et al. 2020). Other virus-like elements, referred to as phage satellites, are described parasitizing phages by using their genetic machinery to complete their own life cycle. These include P4-like, phage-inducible chromosomal islands (PICIs), and PICI-like elements (PLEs), the last of which completely interrupts the infection of a lytic *Vibrio cholerae* phage ICP1 (O'Hara et al. 2017) and shapes phage evolution in terms of the presence and absence of defenses countering PLEs (Angermeyer et al. 2022). These genetic islands can give an evolutionary advantage to the bacterial host in terms of virulence, biofilm formation, and toxins, or thye can provide protection to phage infections (Ibarra-Chávez et al. 2021).

All of these examples, both bacterial- and viral-encoded defenses, illustrate the myriad ways of how phage host range can be impacted by evolutionary dynamics, either through mutations and genomic alterations or the acquisition of mobile genetic elements (through transduction, horizontal gene transfer, or lysogenic conversion). We categorize these drivers as transient drivers of host range. Phages overcome these hurdles by accumulating mutations in different proteins, restriction recognition sequences (Pleška and Guet 2017), and protospacers (Künne et al. 2018), or by incorporating additional genes counter-acting defenses like anti-CRISPR systems (Marino et al. 2020). Hence, we can hypothesize that these transient drivers require fewer substantial adaptations of the phage to infect the isolate compared to the fixed drivers.

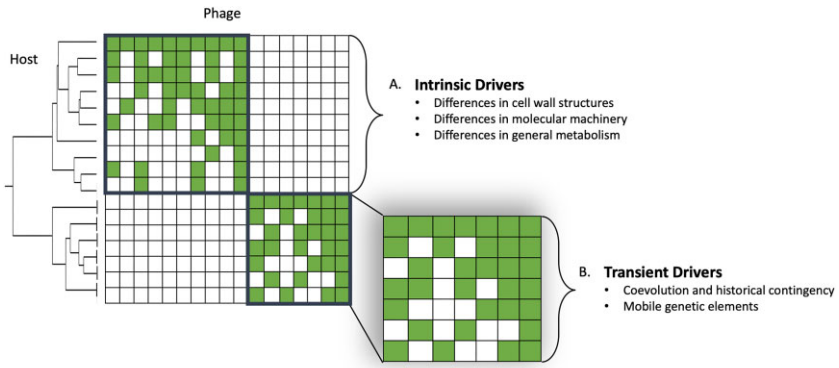

Figure 2. (A) Intrinsic drivers of host range create modularity in a PBIN (*x*-axes are different phage isolates, *y*-axes are different bacterial isolates, green indicates infection, and white shows resistance) caused by nonhost resistance as cell wall structures, the molecular machinery in the cell, and the general metabolism between distinct bacteria are incompatible with different phages. (B) Transient drivers influence phage infectivity due to coevolution dynamics and the acquisition of mobile genetic elements that shape the phage–host interaction.

Environmental drivers of host range

When considering the host range of phages, we should consider not only who *can* be infected by whom (e.g. in ideal circumstances such as an *in vitro* culture), but also who *will* be infected by whom in the natural environment. These two might differ, for example, as a result of factors influencing the physical ability of a phage to reach the bacterial host, as well as those influencing the stability of the phage particle, the molecular interaction between the tail fiber and its receptor, or the physiology of the host along with phenotypic plasticity resulting in phage tolerance. The most obvious of environmental drivers is spatial structure, which can influence the direct interaction between the phage and its host (Koskella et al. 2022). Physical separation by microenvironments, for example in biofilms, will inevitably result in the lack of a phage infection (Fig. 3A). Bacteria often coalesce in multispecies biofilms and form complex communities (Joshi et al. 2021), and these biofilms introduce a degree of spatial structure that makes individuals embedded deep in the biofilm inaccessible for phages (Simmons et al. 2020). Even though phages can carry depolymerases degrading and restructuring biofilms (Pires et al. 2016), the potential effect of spatial structure may not be underestimated in *ex vitro* systems.

Mathematical models have predicted that diffusion of the phage particle can be considered a main driver of phage infectivity in structured environments that greatly influences population dynamics (Simmons et al. 2018, Sousa and Rocha 2019). This raises questions about the performance of "bulky" phages in these structured environments. The importance of spatial structure has further been showed experimentally using one phage-resistant and one phage susceptible-strain of *P. aeruginosa* along with the phage, demonstrating that bacteria embedded in biofilms experience less evolutionary pressure exerted by phages as they escape phage predation (Fig. 3B) (Testa et al. 2019). In a multispecies biofilm composed of *E. coli* and *V. cholerae*, phage-susceptible *E. coli* was shielded from phage T7 by the formation of microenvironments in the biofilm, protecting the cells from phage predation (Winans et al. 2022). Furthermore, the biofilm matrix itself changed as *E. coli* produced fewer curli proteins. On the contrary, synergistic effects between phages have been reported where they support each other's infection as one phage degrades the biofilm matrix, making the host accessible for the second phage to attach (Born et al. 2011). These examples demonstrate the importance of spatial structure in the succession of a phage infection, as well as the presence of other members in the community to initiate and facilitate infection.

Other environmental drivers (abiotic and biotic factors) impact phage infectivity and host range, either by influencing the phage or by shaping bacterial physiology. Strobel and colleagues found, for example, that the evolutionary history of phage λ and its local adaptation to temperature influences its ability for host range expansion, where an increased thermostability of protein J from phage λ resulted in a reduced host range and adaptability (Strobel et al. 2022). Among the abiotic factors known to influence phage infections are temperature, UV light, hydrogen peroxide, salinity, nutrient availability, aeration, and pH, all of which can influence lysogenic and lytic switches in temperate phages such as phage λ (Fig. 3C) (León-Félix and Villicaña 2021). Similar observations were found for filamentous phages in salt stress conditions where the growth of *Vibrio alginolyticus* strains was more impacted by filamentous phages compared to nonstress conditions (Goehlich et al. 2019). The importance of abiotic factors in other alternative lifestyles such as pseudolysogeny and the phage carrier state remains an open question. In lytic infections, ions—especially Ca^{2+} and Mg^{2+}—were found necessary for phage T4 and MS2 to complete their life cycle (Carlson et al. 2023). Indeed, divalent cations increase the affinity of the phage tail fiber and its receptor (Rountree 1955). Furthermore, a model of the diffusion of these cations pointed out that at a distance beyond 1 μm of a lysing bacterial cell, the concentration of these ions dropped below the EC50 necessary for infection and hence reduced the efficiency (Fig. 3D) (Carlson et al. 2023). This example illustrates not only the importance of ions for phage infection but also the influence of space in an ion-depleted environment.

The biotic environment can also (re)shape phage host range, for example, by quorum sensing (QS) and quorum quenching. Prophages that integrate into the host's genome, for example, respond to *N*-acyl-homoserine lactones and other QS molecules and switch to a lytic infection cycle when the quorum is reached (Ghosh et al. 2009, León-Félix and Villicaña 2021). The opposite reaction was found for *Vibrio* phage H20-like prophage p47, where the phage preferred a temperate cycle of a lytic in high host abundancy (Tan et al. 2020). QS has also implications for lytic phages. Phage LUZ19, for example, requires an active PQS system to complete its life cycle (Hendrix et al. 2022). On the other hand, bacterial cell surfaces are under the regulation of QS as well as proteases, phage defense systems such as CRISPR-Cas, and abortive infection systems (León-Félix and Villicaña 2021, Ahator et al. 2022, Wang et al. 2022b). Hence, we can hypothesize that neighboring cells in the bacterial community protect one another against

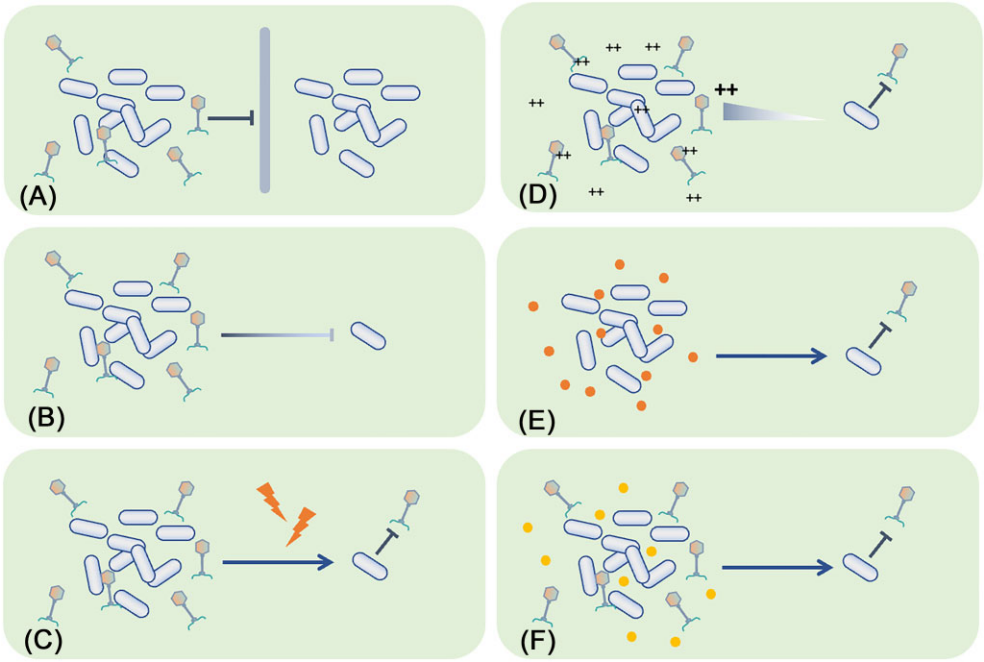

Figure 3. Environmental drivers of phage host range. (A) spatial structure impacts the ability of phages to encounter their host and initiate the infection. (B) Limited diffusion of the phage particle in complex environments such as biofilms impairs the infection. (C) Environmental stresses, such as abiotic factors like temperature, pH, and osmotic pressure, among others, influence the attachment of the phage to the cell and the host's physiology. (D) Ions are necessary for the phage to efficiently attach to the host. (E) Quorum sensing influences the host's physiology, changing cell wall structures, the expression of proteases, phage resistance mechanisms, and abortive infection mechanisms. (F) Phage communication systems like arbitrium change the phage physiology in the host cell.

a potential phage attack and build tolerance (Fig. 3E). This is further illustrated by Tzipilevich and colleagues, who showed that *Bacillus subtilis* responded to phage predated neighboring cells, resulting in the expression of the stress-response RNA polymerase sigma factor σ^X and inhibition of phage infection (Tzipilevich et al. 2022). This phage tolerance response is a part of the physiology of survivor effects, the overarching term to refer to phenotypic and physiological plasticity of bacteria in proximity of phage infections as reviewed elsewhere (Letarov and Letarova 2023).

Besides host abundance, phages are known to regulate one another's life cycles and infections, referred to as the arbitrium system as discovered in *Bacillus* phages phi3T and Wβ (Erez et al. 2017, León-Félix and Villicaña 2021). Upon infection, phi3T produces in low concentrations AimP and its interaction partner AimR. Due to the low concentration of AimP early in the phage propagation, AimR dimerizes and binds to the upstream region of *aimX*, favoring a lytic infection. After several rounds of infection, the concentration of AimP increases, binding to AimR and favoring the expression of AimX, which results in a lysogenic life cycle (Erez et al. 2017, Aframian et al. 2022). In other words, the arbitrium system helps the phage make discissions on a lytic versus a temperate lifestyle depending on phage abundance (Fig. 3F). This example opens the question whether there are similar phage–phage communication systems that shape phage infectivity.

All drivers, as we have defined them ranging from intrinsic to transient and the environment, have a clear impact on phage infection and host range. While the intrinsic and transient drivers are more genetically deterministic, environmental drivers influence bacterial physiology and hence the tolerance to phage infections. Even though the different drivers are distinct for a multitude of reasons, they shape the bacterium–host interaction, shaping the evolutionary trajectory of phages, their role in ecology, and application in biotechnology.

The impact of phage host range in ecology and biotechnology

Microbial communities form complex ecological networks. Phages are believed to act as puppet masters of these communities, either by infecting and killing members of the community or by steering bacterial evolution and physiology (Breitbart et al. 2018). Phage host range is essential in shaping these processes and how the community will respond to these dynamics. Different ecological models like kill-the-winner, piggyback-the-winner, kill-the-competitor, arms race dynamics, and fluctuating selection dynamics are described to explain the influence of phages on microbial populations and communities (Brown et al. 2023), and all of these models are influenced by the host range. Experimental phage depletion of microbial communities establishing on plant leaves was characterized by a reduced diversity (Morella et al. 2018), suggesting that in the presence of phage, a community gains in its diversity by influencing the growth of the most abundant members as predicted by kill-the-winner dynamics (Thingstad 2000). But phage impacts at the community level are generally only inferred from correlative analyses, and thus many open questions remain.

Current host range analyses mainly focus on pairwise interaction networks, losing information on how phages are steering the overall community, especially in *in situ* environments (Koskella et al. 2022). Moreover, current technologies and culture-based host range analyses only scratch the surface of what is happening in a microbial community. New techniques such as epicPCR allow the *in situ* host range analyses on environmental samples such as

estuarine water and reveal temporal phage–host interaction dynamics for actinophages (Sakowski et al. 2021). Other, unbiased *in silico*-based interaction networks are currently being developed by means of alignment-dependent approaches, nucleotide similarity, viral marker genes, and machine learning as reviewed elsewhere (Coclet and Roux 2021). Using host range data, researchers are training artificial intelligence algorithms by means of phage infectivity data, codon usage, protein domains, protein interactions, phage susceptibility markers, and defense mechanisms (Lood et al. 2022). As bigger datasets are generated, multilayer models are established and increase the power and specificity of these algorithms, allowing us to successfully predict phage–host interactions by means of digital phagograms. These developments are key to positively impact the future of phage therapy and phage biocontrol.

As we move forward, the importance of coevolution on host range in an ecological environment should not be underestimated, especially in light of trade-off effects related to phage resistance. Numerous examples in the literature demonstrate a reduced bacterial virulence or increased susceptibility to antibiotics when a host gains phage resistance (Meaden et al. 2015, Chan et al. 2016, Sumrall et al. 2019, Holtappels et al. 2020, Łusiak-Szelachowska et al. 2022, Warring et al. 2022, Marchi et al. 2023). These trade-off effects are considered a major advantage in the application of phages as biocontrol agents in different fields, such as medicine and agriculture. To date, knowledge on these trade-off effects and their impact on the competitiveness of a member in the community is lacking, as well as the actual resistance development *in situ*. In complex environments, not only do phages exert an evolutionary pressure on the bacterial host, but also the environment, the eukaryotic host, and interactions with other competing microbes. Future research should focus on identifying the importance of each evolutionary pressure in this complex dynamic framework.

These concepts become more evident still in the field of probiotics, synthetic microbial communities, and phage therapy. Phages naturally associated with the host potentially undo the beneficial properties that are attributed with inoculated microbes. Lytic phages infecting probiotic strains *L. paracasei*, for example, are described (Mercanti et al. 2016), yet we do know fully understand the impact of phages on the beneficial properties of these inocula. Developments in genetic engineering, and especially the incorporation of non-natural nucleotides, will lead to new revolutions to develop beneficial strains resistant to phages or beneficial phages resistant to bacterial defenses (Shandell et al. 2021, Nyerges et al. 2023). As we aim to manipulate microbiomes through microbial amendments, we need detailed information on how the *in situ* viral community responds to and impacts the applied communities, as well as to understand how we can potentially use viral communities to alter bacterial communities associated with dysbiosis (e.g. Lin et al. 2019). Mechanistic understanding of microbial and viral communication and the impact on bacterial physiology will further help us to design application strategies that are efficient and sustainable. As more research unravels the complex interaction between phages and their hosts, our insights into phage host range expand and will guide us to design efficient antimicrobial strategies and manipulate microbiomes to our benefit.

Conclusion

Phages—as obligate bacterial parasites—are defined by their host range. Mechanistic, applied, and ecological research has demonstrated that intrinsic, transient, and environmental drivers shape phage–host interactions and ultimately host range. While intrinsic drivers mitigate off-target effects of therapeutic phages, transient drivers pose challenges for generic phage applications because of resistance development. In a similar way, environmental drivers shape *in situ* phage–host interactions, impacting the efficacy and outcome of phage treatments. Not only do these drivers challenge phage applications, but they determine how phages are interacting with bacterial communities and their function. Drawing from basic mechanistic and ecological insights, we are beginning to understand the complex interplay between phages and their hosts, but expanding this knowledge will be essential to both engineering phage host range to our benefit and to designing sustainable biotechnological applications to face future challenges in agriculture, food, the environment, and medicine.

Author contributions

D.H.: conceptualization, preparation of original draft, and writing of the manuscript at draft. P.A.Z.: writing of the manuscript at draft. B.K.: conceptualization and writing of the manuscript at draft.

Conflict of interest. The authors declare no conflict of interest.

Funding

This research was funded by an NSF and USDA/NIFA Career Award (NSF # 1942881 and USDA/NIFA # 1024053), BK is a CZ Biohub Investigator and PAZ holds a scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES_PrInt # 88887.194842/2018-00).

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Received 3 May 2023; **revised** 5 July 2023; **accepted** 7 July 2023