Interactions between bacterial and phage communities in natural environments

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Abstract
We commonly acknowledge that bacterial viruses (phages) shape the composition and evolution of microbial communities in nature and therefore play important roles in ecosystem functioning. This view stems from the 1990-2000s which revealed high viral abundance, diversity and virus-induced mortality in aquatic ecosystems as well as an association between collapses in bacterial density and peaks in phage abundance. The recent surge in metagenomics analyses has provided deeper insight into the abundance, genomic diversity and spatiotemporal dynamics of phages in a wide variety of ecosystems, ranging from deep oceans to soil and the mammalian digestive tract. However, the causes and consequences of variations in phage community compositions remain poorly understood. Here we review our current knowledge on the composition and evolution of phage communities, as well as their roles in controlling the population and evolutionary dynamics of microbial communities. We discuss the need for greater ecological realism in laboratory studies to capture the complexity of microbial communities that thrive in real environments.

Introduction
Viruses that infect bacteria (phages) are the most abundant biological entities on this planet. They were estimated to reach a total number of 10^{31} particles[1], which represents a biomass of 200 million tons[2,3]. This number results from the product of two factors: the number of prokaryotic cells on Earth, which was estimated to be over $4x10^{30}$ [4], and the virus-to-microbe ratio, which was estimated from direct counts of extracellular virus-like particles (VLPs) in seawater samples. These counts revealed an average 10-fold excess of VLPs compared to microbial cells. As sampling of Earth habitats is increasing and techniques to estimate virus and cell abundances are progressing, these figures may need to be re-evaluated. For example, it is becoming clear that the virus-to-microbes ratio varies with microbial with cell density[5] and between different environments. That being said, recent re-calculations estimated that the total number of phages on Earth remains close to 10^{31} [6,7].

Phage abundance varies between ecosystems: it is relatively homogeneous in seawater, with 10^5-10^7 particles/ml, more heterogeneous in soil with 10^3-10^9 particles/g, depending on soil type[8], and constitutively high in the mammalian gut where it reaches 10^8-10^{10} particles/g in faecal material[9,10]. Phages are found virtually everywhere, even in extreme environments such as the Antarctic soil, deserts or within ancient samples (e.g. in mummies[11]), and are extremely diverse both in their virion structures and genomic content[9]. Yet, our knowledge of the composition of phage communities in different environments has remained incomplete.

The recent surge in large-scale viral metagenomic studies is providing deeper insight into the abundance, taxonomic diversity and distribution of phages across a wide range of ecosystems[12,13]. Overall, viromics studies have emphasized that we have only uncovered the tip of the iceberg, with many more viral sequences remaining to be discovered, and that a given environment hosts a large diversity of genetically-distinct phage populations (most viral sequences cannot be taxonomically classified)[13]. This observation raises the question of how this exceptional diversity is generated. The advances in comparative genomics in the 2000's revealed the remarkable mosaic architecture of phage genomes, where blocks of genes can be found across several phage genomes that otherwise share no identity[14]. This suggests a high degree of horizontal gene transfer (HGT) between phages, resulting in complex evolutionary histories[14]. Besides HGT, other factors drive the evolution and diversification of phages, namely the interactions with their bacterial hosts and the (social) interactions with other phages.

Due to their ubiquity and abundance, phages are thought to be responsible for 20-40% of bacterial lysis, although this is a relatively crude estimate that is likely to depend on the environmental conditions[15,16], and therefore are a major force in shaping the composition of microbial communities. Given that bacteria account for approximately 15% of the global biomass on Earth[7], it becomes clear that phages play an important role in the functioning of many ecosystems and, on a larger scale, have key implications for the flux of carbon and the recycling of biomass (e.g. viral shunt)[17]. Beside shaping the composition of bacterial populations, phages are important drivers of bacterial evolution, not only because their parasitic nature imposes high selective pressures on their hosts, but also through mechanisms including lysogenic conversion, transduction and host gene disruption when they integrate into the bacterial genome as prophages.

Here we review our current knowledge of the composition and dynamics of phage communities in natural environments (e.g. soil, ocean, mammalian gut) based on latest observational (metagenomic) studies. In addition, we refer to recent laboratory work that went beyond the frame of traditional phage-bacteria pairwise studies and discuss how these findings advance our understanding of phage-bacteria interactions outside the laboratory. In particular we will discuss current insights into (i) the compositions and (ii) evolution of phage communities and (iii) their multi-faceted roles in bacterial ecology and evolution. Finally, we will discuss the factors that influence phage and bacteria coevolution in nature.

I – Ecology of phage communities

Phage diversity and distribution

A changing view of the virosphere

Phages are highly diverse and vary in their virion structures (tailed, non-tailed, enveloped, filamentous)[9], types of genetic material (double or single-stranded DNA or RNA)[9] and gene content. Phages containing dsDNA are, by far, the most well-studied and among them, tailed phages (*Caudovirales*) account for >90% of all phages that have been described to date. This bias likely resulted from isolation and observation procedures and the introduction of culture-independent methods (sequencing) has recently provided a better knowledge of phage diversity by uncovering, amongst other, lineages of abundant non-tailed dsDNA and diverse subfamilies of ssDNA phages[18–21] (see below). At the same time, these metagenomic studies provide insight into the gaps in our knowledge of the diversity of the virosphere[22]. Indeed, a large majority of viral-associated sequences in metagenomic datasets do not align to any known sequence and constitute the so-called "the viral dark matter"[23]. Despite these advances,

there is still a strong bias against certain types of phages (typically RNA phages are poorly detected) owing to sample processing (i.e. extraction, concentration and purification) and sequencing techniques (reviewed in REF 24). Recent studies expanded the number of complete RNA phage sequences from 16 in 2015 (in comparison with >1000 complete DNA phage genomes at that time) to over a thousand[25,26], suggesting that RNA phages are more abundant and diverse than previously thought.

Phages can also be classified according to their lifecycles, which may be qualified as chronic, lytic or lysogenic (described in Figure 1). While these three archetypal infection cycles may well reflect how phages replicate in the laboratory, there is mounting evidence that these models may not be representative of phage-bacteria interactions in nature. Recent papers argue that phage infection strategies may be seen as a continuum (rather than categories) ranging from efficient productive infections (i.e. releasing new virions) to persistent non-productive infections[27,28], which do not produce new phage particles but still spread phage genome in the bacterial population (i.e. transmitted from mother to daughter cell).

Chronic cycle

The majority of chronic phages known to date belongs to the *Inoviridae* family which is composed of filamentous ssDNA phage species. A recent survey discovered more than ten thousand putative inoviruses in 35% of available metagenomes (>6400 metagenomes), expanding the number of known inovirus sequences by two orders of magnitude. Even though the abundance of inoviruses in a defined ecosystem may be overestimated (due to the amplification protocols used prior library preparation which introduce a positive bias towards small ssDNA), their diversity is remarkable given that they have been associated with hosts across the domains Archaea and Bacteria (including almost all bacterial phyla)[29]. In addition, they have been detected in virtually all biomes ranging from aquatic to terrestrial and host-associated[18]. Chronic phages associated with eukaryotic hosts may have significant impacts on host health, owing to their physical properties (e.g. the filamentous virions produced by Pf phage favours the formation of robust biofilms by the host *Pseudomonas aeruginosa*[30]), the toxins and virulence factors they may carry, which increase the pathogenicity of their bacterial host (e.g. Vibrio phage CTX which encode the cholera toxin) and their interaction with the mammalian immune system[31] (**Box 2**). Overall, these findings suggest that a large fraction of phages in the biosphere are produced through chronic cycles, but whether these cycles are associated with specific environments or ecological conditions remains unexplored.

Lytic and Lysogenic cycles

Lytic and lysogenic cycles are distributed unevenly across ecosystems, which is thought to be driven by host density, at least partly. Indeed, lytic replication is favoured under conditions where hosts can proliferate and reach high densities while lysogeny is favoured when host abundance is low. While this long-established concept is well-supported by theoretical and experimental studies[32,33], data from environmental studies are more ambiguous. Some reported a positive (/negative) correlation between the frequency of lytic (/lysogenic) phages and bacterial density[34,35], but others reported weak or no correlations[36]. Also contradicting this view, the nutrient-rich, bacterial-dense ecosystem of the mammalian gut is dominated by lysogenic bacteria[37,38] and copiotroph species tend to have more prophages[39]. Recent studies are therefore exploring which ecological factors, in addition to host density, influence the relative frequencies of lysis and lysogeny. Some have suggested that the fitness benefits associated with lysogenic and chronic infections (e.g. improved bacterial growth or survival) may best determine when phages should be lysogenic[27,40]. These benefits can emerge from

homoimmunity, which protects bacteria carrying a prophage against infection by similar phages, or from phage-encoded accessory genes which include genes that provide resistance to unrelated phages (**Box 1**). A proposed scenario is the following[27,40]: lytic infections suppress fast-growing bacteria, enabling the invasion of bacteria carrying prophages which provide protection against lytic phages. As the density of these lysogens increases, lysogenic phages may acquire mutations allowing them to escape homoimmunity[41] and subsequently control the lysogen population. Further development of tools allowing to detect whether prophages are active or dormant based on metagenomic data[42] will likely provide valuable information about the frequencies of lysis and lysogeny in nature and how they depend on the ecological context[42].

Pseudolysogeny

Alongside their canonical lifecycles, phage may use an alternative mode of persistence that has been described as a "phage carrier state". Upon injection of its genome, the phage engages neither in a lytic nor a lysogenic cycle and instead remains in the host cell as a non-replicative extrachromosomal element that is asymmetrically transferred to one of the daughter cells after bacterial division. Different phage types have been found to enter this carrier state: virulent or temperate dsDNA phages, dsRNA[43] or ssRNA phages[44]. This phenomenon, which has been termed pseudolysogeny, usually occurs when hosts are in a nutrient-depleted environment and a lytic, productive cycle may resume when hosts encounter more favourable conditions[45]. This carrier state might be a way for phages to be protected from environmental physico-chemical conditions (e.g. UV, temperature) that can damage phage particles.

Virome variations in time and space

The composition of environmental viromes from distinct geographic origins are generally very different, whether it be in aquatic[12] or terrestrial[8] environments, even on local scales[35,46]. For example, in the oceans, viromes vary with the depth of the water column (both in terms of species and gene content)[46,47]. The local distributions of viruses in soil are less documented, because soil viromes are challenging to study. Nonetheless, recent studies on permafrost suggested that the composition of phage communities differ along the permafrost thaw gradient, correlating with host community composition and variations in abiotic parameters (e.g. pH, soil moisture)[48,49]. Similarly, human-associated viromes differ between individuals, though smaller differences in virome richness are observed between individuals from the same geographic origin[50–52]. Nonetheless, in all these environments some "cosmopolitan" phages (i.e. found in a vast diversity of unrelated environments) have been identified[8,12]. In some instances, nearly-identical phage genomes have been found in samples originating from similar but very distant locations[12,53]. A well-documented example is that of the crAss-phage group[50], which is a widespread phage family found in the gut of virtually all individuals.

In contrast to their variability across space, virome compositions seem relatively stable in time. Abundant phage species can persist over 1-2 years, as suggested by metagenomic time-series surveys performed on seawater samples[47,54] and human feces[51]. Some phages may linger for long periods of time as demonstrated by one comparative genomics study, which repeatedly found the same cyanophages in seawater samples collected from the same location over a decade, with very little genomic variation[55]. Some phage species only peak at specific times of the year. Several studies reported seasonal successions of viral communities[56–58], with some communities switching from lysogeny to lytic replication when the season changes[34,56]. These seasonal virome patterns often (but not always) mirror the

seasonal variations in host abundances related to weather conditions[56,58,59], and in some cases, may be a consequence of pseudolysogeny. It has been proposed that when the host population is under nutrient restrictions, phages may enter a carrier state (because commitment to a lytic or lysogenic cycle is too energy-consuming) which allows their maintenance for a prolonged time and favours phage-bacteria coexistence in an ecological niche with limited resources[45]. Once favourable conditions are met, phage replication may resume (through a lytic or lysogenic cycle). The human gut virome was found to remain stable in healthy adults, although changes in richness occur with age. A dynamic succession of viral species (predominantly temperate[38]), yielding high viral diversity, occurs during infancy[60] and is followed by the establishment of a more stable gut virome, dominated by a set of abundant phage species (e.g. crAss-like and *Microviridae* families) that persist during adulthood[38,51,52,61]. While the gut virome is generally thought to be dominated by temperate phages, recent reports suggest that virulent phages may be abundant in the adult gut, mainly based on the high prevalence of crAss-like phages[61]. However, if crAss-like phages do not display hallmarks of lysogenic lifestyle, their virulent nature remains questionable. Latest findings indicated that they likely use unusual infection strategies allowing them to replicate without hampering their host[62].

Many questions about the spatio-temporal variations of virome compositions and how they are linked to the ecological context remain open. For example, whether there is a core phageome in the human gut[63] or not[51] remains unclear. This is in part due to biases in the detection of certain types of phages (as discussed above) and in part due to the difficulties to compare metavirome datasets between different studies, owing to differences in sample processing and analytical methods, the lack of a universal reference database for viruses and the large amount of viral dark matter in these datasets. Development of new bioinformatics tools, standardised protocols and viral reference databases[52,64] will help to overcome these challenges.

II – Phage evolution in communities

In natural communities, phage evolution is influenced by the density and diversity of bacterial populations but also by the presence of other phages competing for the same host resources.

Host range

Phages vary in their host range. Many are specialised on a relatively small set of strains while few generalist phages infect a broader range of hosts[65], sometimes spanning different bacterial genera[66]. Multiple *in vitro*[67–71] and recent *in vivo* evolution experiments[72,73] have shown that host range is a highly evolvable trait and that density, diversity and quality of hosts are key parameters that determine host-range, which can expand or contract[67,68]. Intuitively, expanding the host range is advantageous for phages since they can infect more hosts and this may depend on the overall diversity of the bacterial community (i.e. the more diversity, the higher selective pressure for generalist phenotypes)[71]. However, becoming a generalist may come with different types of costs. Specifically, phage with an extended host range may have a lower growth rate (*i.e.*, produces less phage progenies or replicates more slowly) in its novel host than in its original host (ecological cost)[74]. Furthermore, the ability to infect a novel host can result in a lower performance on the original host (evolutionary cost)[74].

In contrast, phages may reduce their host range, resulting in specialisation or avoidance, when either good quality or poor hosts are abundant within the microbial community, respectively[67,70]. A key step in host range evolution is the ability to bind new receptors, which

often requires several mutations in genes encoding phage tail proteins[68] and may be facilitated by the presence of "intermediate" hosts[72]. In the presence of hosts with different receptors, a phage population may evolve different genotypes that have non-overlapping host ranges[69]. A classic example is that of phage Lambda, which may use two alternative receptors (LamB and OmpF). When evolving on two *Escherichia coli* host genotypes that expressed either the LamB or OmpF receptor, the phage population split into different lineages that specialized on each receptor type[69]. Interestingly, some phages encode diversity-generating retroelements (DGRs) that generate variability in their tail genes to accelerate their adaptation to new hosts.

While the impact of bacterial diversity on phage host range evolution has been well studied, the impact of phage diversity is less understood. In nature, phages exist within diverse communities, and this can influence the host range of a focal phage. For example, the presence of phages encoding enzymes degrading bacterial capsules can help other phages to adsorb to encapsulated hosts[75]. Another example is that of mixed coinfection by coliphages T2 and T4, where a fraction of phage progeny has the genotype of one phage and the host range of the other, or a hybrid host range[76]. Phage diversity can also indirectly affect the host range of other phages by driving evolutionary changes in the host that affect their susceptibility to other phages[77].

Social interactions and genetic exchanges

Phages from different taxonomic groups (as defined by the International Committee on Taxonomy of Viruses) and with different lifestyles can infect a common host, which potentially leads to within-host phage-phage interactions. Recent large-scale environmental metagenomic and single-cell studies estimated that 35% - 50% of infected bacteria contain multiple phages[78,79]. Ultimately, the likelihood of coinfections depends on the environmental context (e.g. phage-bacteria densities and phage-to-host ratio), on phage lifestyle (e.g. temperate phages are more often involved in coinfections than virulent phages because they can remain in the host for longer periods of time) and can be influenced by phage themselves[79,80]. For example, dsRNA phage phi6 can enhance coinfection by inducing the upregulation of a phage receptor, which results in phi6 attaching faster to infected cells compared to uninfected cells[81]. Coinfections can shape the composition and the behaviour of phages communities because they impact lysis/lysogeny decisions in temperate phages (**Figure 2a**)[82–84] or lysis timing in virulent phages (e.g. lysis inhibition) and because coinfecting phages compete for intra-cellular host resources (**Figure 2b**)[85]. In some cases, mutant phages that exploit proteins produced by coinfecting phages may evolve in the population (**Figure 2c and Box 1)**[86,87]. In other cases, phages may evolve traits that benefit[88,89] or exclude[90,91] one another (**Figure 2c and Box 1**). The recent discoveries of multiple social-like interactions in phages indicate that these behaviours may be common in nature, with important ecological and evolutionary consequences[92,93].

Coinfections also have important consequences for the evolution of phage communities as they allow direct genetic exchange between phages (**Figure 2d**). The hypothesis of a high frequency of HGT in phages, which was initially based on comparative genomics analyses[14], has been confirmed by metagenomic studies[53,94]. Signatures of recombination were found to be widespread, with specific genes being more likely to undergo recombination than others, including genes involved in host recognition and anti-defence systems (e.g. anti-restriction modification)[53,94]. These observations suggest that recombination is likely an important driver of phage diversity and adaption in natural communities[53,94]. The frequency of HGT depends on phage lifestyle and is particularly high between temperate phages (although this is not true for

all temperate phages)[95]. Although less frequent, HGT has been reported between virulent phages from close taxonomic groups and notably concerned genes belonging to the early-transcribed regions (generally involved in host takeover)[96]. Finally, little is known about genetic exchanges between temperate and virulent phages. A recent study has suggested they have important consequences for the spread of genes between distant phage taxa (because virulent phages may have a larger host range than temperate phages), which can fuel functional innovations and genetic diversification in phage genomes[97].

Lifecycle parameters

Competition between phages can drive the evolution of various life-history traits including their transmission mode or lysis timing, which ultimately affect the impact they have on their bacterial host[98]. For example, temperate phages may acquire mutations that allow them to overcome homoimmunity. These so-called "ultravirulent" mutants carry a mutation which prevents the protein that represses the lytic cycle from binding its operator sequence[41]. As a result, these phages can kill hosts that already carry a prophage. However, this increased infection capacity is likely transient as compensatory mutations may later emerge to restore lysis repression[41]. Theory has suggested that such cycles of lysis derepression/restoration may emerge in a coevolutionary arms race[41]. Consistently, a recent study investigating the coevolution between *Roseburia intestinalis* and its prophages in the mouse gut showed that "ultravirulent" mutants of Shimadzu prophage systematically invaded the population, and, in some cases, these mutants later acquired compensatory mutations restoring lysogeny (and resistance to the ultravirulent mutants)[73]. The abundance of suitable hosts within the microbial community also influences evolution of phage lifecycles. When access to hosts is limited, phages were found to evolve towards more prudent strategies of exploitation (e.g. less damaging or less infective), avoiding the complete depletion of host resources. Related to this, structured environments, where phage dispersal and immigration of susceptible hosts are limited, generally favour the evolution a reduced infection efficiency (e.g. reduced adsorption or delayed lysis)[98,33].

III - Consequences of phage communities

Phages can impact the composition of microbial communities, the evolution of constituent species, and the interactions that bacteria have with one another and with other organisms (e.g. eukaryotic host) (**Box 2**). Variations in the compositions of phage communities may therefore have far-reaching consequences.

Impact on microbial ecology

Phages are thought to shape the taxonomic and functional composition of microbial communities as well as their stability[99], but different phage types have very different impacts. Virulent phages have been thought to drive density- and frequency-dependent dynamics, following the "Kill-the-winner" model, where they suppress the most common bacteria which enables minority populations to rise in frequency[100]. These effects tend to maintain diversity in bacterial communities as they offset the proliferation of a single dominant species. However, this model is overly simplified as it does not consider spatial variations or demographic stochasticity[101]. For example, virulent phage infections may not be efficient, or enter a pseudolysogenic state, leading to fluctuations in the size of the phage population[102]. These predator-prey dynamics, where peaks in bacterial densities are followed by a rise in phage abundance and a subsequent collapse in bacterial densities, have been observed in a wide range of environments, such as freshwater ecosystems[103], saline ponds[104] and in the

context of "self-limiting" cholera outbreaks, where phages likely regulate epidemics seasonality[105,106]. These cycles of negatively-correlated, fluctuating phage and bacteria abundances can last over long periods of time as illustrated by a decade of time-series data collected in the Sargasso Sea[107]. Virulent phages can also redirect bacterial metabolism through the expression of auxiliary metabolic genes (AMGs)[108]. With an estimated 10^{23} infections per second on Earth[15], it is likely that the metabolic activity of virocells alters the functioning of bacterial communities and their ecosystems. AMGs have been best described in marine phages and are involved in a variety of processes such as photosynthesis, carbon metabolism and nitrate reduction[17,109,110]. For example, genes encoding photosystem II proteins, commonly found in cyanophages, likely contribute to the maintenance of photosynthesis during infection, which otherwise impairs the expression of host-encoded photosynthetic genes[111]. Mining of metagenomic datasets has revealed hundreds of novel putative AMGs, which are unevenly distributed, some being restricted to specific biomes while other are more widespread[8,112,113]. These high diversities and abundances of AMGs suggest that phages play important roles in biogeochemical cycles[112], but how much they contribute to matter and energy production remains challenging to quantify.

Temperate phages are often thought to have a limited impact on bacterial population dynamics. Because they can transmit both horizontally and vertically (**Figure 1**), the fitness interests of temperate phages and their hosts are more aligned, leading to more mutualistic (or less damaging) behaviours compared to virulent phages. However, temperate phages can lead to significant mortality in bacterial populations, as a result of prophage induction[34] or due to the evolution of ultravirulent mutants[73]. Also, they can impact the dynamics of microbial communities by sustaining inter-bacterial competition. When temperate phages stochastically lyse a fraction of the lysogenic host population (prophage induction), they can promote the liberation of host-produced toxins (bacteriocins) which inhibits other bacteria in the community[114] or the virions released can themselves kill sensitive bacterial competitors[115] (hence providing a fitness advantage to the lysogenic strain) until these competitors become in turn lysogenized and, therefore, resistant to phage attack (e.g. due to homoimmunity)[116]. However, in at least some cases where competitors become lysogenised, temperate phages may deliver toxins that would inhibit competitors' growth, providing an advantage to the original lysogenic host population[117].

Impact on microbial evolution

Selection of resistance mutations

Phages can drive rapid genetic and phenotypic changes in bacteria. In particular, experimental *in vitro* studies extensively showed that bacteria quickly evolve resistance against phages[68], for example through the modification of surface structures that serve as phage receptors, or through the insertion of phage-derived sequences into bacterial CRISPR-Cas loci (called spacers), which enables the recognition and cleavage of phages carrying the cognate sequence upon reinfection[118]. These two anti-phage defence mechanisms are often found to evolve rapidly in the laboratory (receptor modification being the most frequently observed), but many more exist in nature[119,120]. Importantly, the resistance mechanisms that evolve in test tubes are not necessarily the ones that evolve in more complex natural environments (**Box 3**). The selection of one defence system over another potentially has downstream impacts on bacterial communities. For example, mutations of phage receptor often have pleiotropic effects because these receptors have important physiological roles including motility, metabolites transport, or surface adhesion[121]. While the associated fitness costs may be small in laboratory culture, they are often amplified in natural environments, where the value of these costs further

depends on abiotic (e.g. temperature, nutrient availability)[122,123] and biotic contexts (**Box 3**). For example, receptor mutations become more costly in the presence of other bacterial species competing for the same ecological niche[124] as well as in the presence of a diversity of phages[125,126], depending on the degree of cross-resistance that these mutations provide against these different phages (and hence the number of receptor mutations needed for full-resistance).

Modulation of bacterial mutation rate

Coevolution between a phage and its host can increase the rate of molecular evolution of the host[127]. Bacterial clones with high mutation rates may be positively selected in the presence of phages, because they adapt more easily to phage predation[127]. By increasing the level of ecological complexity in their experimental design, recent studies showed that the presence of multiple phage species may further accelerate bacterial molecular evolution[128] and accentuates the selection for hypermutators[129] (**Figure 3a**). Host mutational background may in turn have important impacts on phage-bacteria interactions as it may also influence the type of resistance that evolves[130]. For example, hypermutator populations of *Pseudomonas aeruginosa* were shown to contain high proportion of pre-existing mutants with a modified phage receptor, which, at least in the short term, limits the evolution of CRISPR-mediated immunity[130]. High mutation rates can favour bacterial adaptation in changing stressful environments and therefore, may be positively selected in nature. For example, hypermutator strains are often isolated in the context of chronic infections[131]. However, it remains unclear whether phages could drive the selection for increased mutation rates in natural environments[132], where the deleterious effects of hypermutation may outweigh its benefits. While high mutation rates increase the likelihood of beneficial (adaptive) mutations, they also continuously generate deleterious mutations, which may compromise the fitness of the population on the long-term. However, this trade-off may not occur if increased mutation rates arise only in a fraction of the population (thereby generating within-population diversity), for a limited amount of time and restricted to specific genomic loci[133]. One may consider that the CRISPR-Cas system follows such a pattern: the acquisition of insertion mutations (spacers) occurs in specific loci (CRISPR arrays) and is limited to stressful phases (phage infection). Reciprocally, phages also encode mechanisms that increase their local mutation rates, as exemplified by the diversity-generating retroelements that introduce mutations in a specific variable region (often located in tail genes[134]). Recent findings suggest an important role of DGRs in the adaptation of phages in complex environments, particularly in intestinal microbiomes[135].

Temperate phages can directly influence host evolution by inserting and disrupting bacterial genes (**Figure 3a**). While some temperate phages integrate into specific sites, causing little disruption, transposable phages randomly insert into the bacterial chromosome and replicate by copy-pasting into other sites. This mode of integration/replication can therefore lead to profound modifications of the bacterial genome and likely causes deleterious effects (i.e. disruption of essential genes). However, it may also enhance bacterial adaptability by increasing the mutation rate, and hence, the chances of acquiring beneficial mutations, which may favour both the host and the phage. For example, under laboratory conditions, transposable phages have been shown to impact the evolution of bacterial social strategies by favouring (or reducing) the production of public goods (e.g. siderophores)[136], adaptation to a biofilm environment[137] and drive the evolutionary loss of bacterial adaptive immunity[138], which in turn may have important consequences on the ecology of microbial communities.

Phages as sources of genetic innovation

Given the widespread high prevalence of prophages in microbial genomes[39], lysogenic conversion likely has a strong influence on the adaptation and evolution of microbial communities[139]. Prophages may encode beneficial traits (e.g. virulence factors, metabolic genes, etc.) which enable their host to colonise new ecological niches and survive in changing environments (**Figure 3b**). Indeed, prophage knock-out considerably reduces the ability of bacteria to colonize their animal host, as demonstrated with avian pathogenic *E. coli*[140], scarlet fever-causing *Streptococcus pyogenes*[141] or human commensal *Enterococcus faecalis*[142], amongst others. This phenomenon is highly dynamic as phages can be rapidly transferred from one strain to another[116,143]. For example, a study of gut colonisation in mice showed that an invading *E. coli* strain is attacked by (pro)phages induced from a resident strain, which in turn lysogenize the invading strain (which therefore became resistant to phage infection due to homoimmunity), and this constitutes a key step for gut colonisation[116]. Prophage transfer may be promoted by environmental conditions. For example, in a murine *Salmonella Typhimurium* diarrhea model, inflammation was found to trigger the induction and transfer of phage SopEϕ from one strain to another[143]. Prophages may also protect their hosts against other predatory phages (**Figure 3b and Box 1**). Such phage-encoded protective mechanisms are likely widespread in nature since many bacteria have prophages (sometimes many of them[39]) and we probably have identified only a small fraction of the diversity of these protective mechanisms. In addition to homoimmunity, other mechanisms that protect lysogens against superinfection have been described (**Box 1**) and recent data suggested that perhaps >50% of these mechanisms are directed against heterologous phages[90,91]. In addition, some prophages "captured" bacterial defence mechanisms, such as CRISPR-Cas[144] or abortive infection systems[145], that are specifically directed towards exogenous invaders, hence protecting the host against secondary infection.

Phages are vectors of horizontal gene transfer

Phages can impact the evolution of bacterial genomes by mediating HGT through generalised and specialised transduction, which have been extensively studied in the laboratory[146]. Novel mechanisms, known as lateral transduction[147] and auto-transduction[148], have been discovered recently and some involve phage-like particles[146,149] (phage-inducible chromosomal islands[146] and gene transfer agents[149]). Transduction is thought to be an important driver of the adaptation of bacterial communities to environmental changes. Recently, the identification of antibiotic-resistance genes (ARGs) in the genomes of isolated phages and in virome datasets suggested that phages may serve as reservoirs of ARGs, which they could transfer between bacteria[150] (**Figure 3c**). However, this view remains debated since the abundance of virome-associated ARGs may have been overestimated (due to bacterial contamination of sequenced viral samples or due to the low similarity thresholds used to predict ARGs[151]). The frequency of transduction in nature remains unclear. One study reported that generalized transduction occurred at high frequencies when using a freshwater bacterial community as a recipient[152]. The development of new approaches (e.g. transductomics[153]) which specifically detect signatures of the different transduction modes in environmental samples will likely provide a clearer picture. It will be especially interesting to estimate the frequency of lateral transduction in nature, as it can transfer bacterial DNA at frequencies 1000-fold higher than "classical" mechanisms *in vitro*[147]. Furthermore, understanding the dynamics and the impact of phage-mediated HGT within natural communities will require considering the influence of abiotic and biotic parameters. For

example, biofilms may be hotspots of HGT[154] (**Figure 3c**). While reconstruction of transduction events (based on phylogenetic analyses) suggested that they mostly occur between closely related donors and recipients[155], the discovery of broad host-range transducing phages, which can connect a wide range of bacterial strain/species, may challenge this view[156].

IV – Coevolution in (semi-)natural contexts

Phages are important drivers of bacterial evolution, which in turn select for reciprocal adaptation by phages. This coevolution has mainly been studied *in vitro* in well-mixed broth, using clonal bacterial and phage populations[121]. These studies have elucidated key principles of coevolution, but more studies in natural environments are needed to better understand how biotic and abiotic complexity shapes the coevolutionary dynamics of bacteria-phage interactions.

Impact of biotic and abiotic contexts

Recent lab studies have explored how biotic complexity impacts coevolutionary dynamics. For example, the presence of multiple phages was found to influence the mode of coevolution, switching from fluctuating-selection dynamics to a continuous increase in levels of resistance (bacteria) and in levels of infectivity (phages), a phenomenon known as arms race dynamic[128]. Outside the test tube, studies in semi-natural environments provided evidence that bacteria evolve in response to phages (and reciprocally) and, in some instances, ongoing coevolution has been observed. For example, phage P10, which originally infects *E. coli* strain LF82, evolved the ability to infect *E. coli* MG1655 in the mouse intestine (with its resident microbiota). This was not observed when LF82 and MG1655 were the only strains present (i.e. *in vitro* or in dixenic mice)[72] because the evolution of P10 host range required a step of amplification in an intermediate host from the murine microbiota (*E. coli* MEc1)[72]. In turn, this adapted phage P10 drove the evolution of resistant *E. coli* MG1655 (modification of phage receptor) and phages that restored the ability to infect these resistant clones were subsequently isolated[157]. While these studies demonstrated that bacterial biodiversity had a major impact on phage-bacteria coevolution[72,157], other studies reported only limited impact. For example, *Pseudomonas fluorescens* and its phage phi2 can coevolve in sterilized soil for several months, but the reintroduction of a natural soil bacterial community in this system did not affect their coevolutionary dynamics[158].

The presence of other bacteria is not the only factor that affects coevolution. For example, the coevolution mode of *P. fluorescens* and phi2 is consistent with a fluctuating-selection dynamic in a sterilized soil environment, while an arms race dynamic prevails in test tubes[159]. These contrasted dynamics may be due to differences in abiotic parameters (e.g. nutrient availability or spatial structure). Experiments in (semi-)natural contexts often report much lower levels (or absence) of phage-resistance evolution[158,160], compared to lab-based studies. This may be explained in part by reduced contact rates between bacteria and phages because of the structure of some environments, where sensitive bacteria can survive in 'spatial refuges[161], and in part by phenotypic resistance (*i.e.* non-genetic metabolic state impeding phage replication), which might be an important factor for bacterial survival in nature (**Box 3**). These lower levels of resistance in natural environments might have implications for phage-bacteria long-term persistence and their coevolutionary interaction (**Box 3**).

Coevolution in nature and long-term consequences

Few studies have also assessed coevolution using samples from natural environments. Cross infection between bacteria and phages collected at different times from horse chestnut leaves[121] or fish farming facilities[162], showed that bacteria are more resistant to past phages but less resistant to future phages and, reciprocally, phages are more infective toward past bacteria and less infective toward future bacteria. These results indicate that a coevolutionary arms race takes place over the course of several months[121] or years[162]. In contrast, similar experiments with *Vibrio cholerae* and phages isolated from patients over 3 years revealed that *V. cholerae* isolates were generally susceptible to contemporary phages, but resistant to phages from the past or the future[164]. Remarkably, the authors found that this fluctuating-selection pattern was (at least partly) due to the exchange of integrative and conjugative elements carrying anti-phage systems between bacterial isolates, which in turn, positively selected for the acquisition of counter-defence by phages[164].

On the short-term, coevolution between bacteria and phages often results from the accumulation of point mutations (or spacers in CRISPR loci) in the host genome to acquire resistance, and point mutations in the phage genome to overcome resistance[68,128] (**Figure 4a**). Over longer timescales, phages and bacteria have evolved more complex resistance and infectivity strategies (**Figure 4b**). Bacteria have developed a broad range of defence systems that are often clustered into "defence islands" in bacterial genomes[165]. These systems are possibly hierarchically organised, with primary and secondary lines of defence[166]. Recent findings showed that the inhibition of the anti-phage complex RecBCD by a phage-encoded protein is sensed by bacterial retrons, which trigger abortive infection (*i.e.*, bacterial suicide) to protect the bacterial population against phage epidemic[166]. The recent identification of many new defence systems uncovered parallels between eukaryotic and prokaryotic immune systems, suggesting that some immunological functions may have been conserved in bacteria, plants and animals[167–170]. Indeed, some components of eukaryotic anti-viral systems, namely RNA interference, cGAS-STING, viperins and Toll/interleukin-1 receptor domains, were found to have prokaryotic homologs which also mediate anti-viral defences using similar mechanisms[167–170] (**Figure 4c**).

In response, phages have evolved diverse counter-resistance measures[171] and a recent bioinformatic analysis found that, similar to bacteria, some anti-defence genes were grouped in several phage genomes, therefore hypothesising the existence of "anti-defence islands"[172]. These findings may fuel future discoveries of phage counter-resistance systems, which have been lagging behind so far.

Concluding remarks

Since their discovery a century ago, interactions between phages and bacteria were mainly studied in pairwise experiments. However, over the last years, growing numbers of studies focused on phage-bacteria (co)evolution in complex communities. As of today, there is still an important gap between reductionist studies (with a limited number of phage and bacteria) and observational environmental studies. Therefore, our understanding of phage-bacteria interactions in natural environments is far from being complete, and more studies are needed to fully appreciate how biodiversity and abiotic factors influence phage-bacteria ecological and evolutionary dynamics. Moreover, it is becoming increasingly clear that phage and bacterial communities can have a significant impact on their eukaryotic host (**Box 2**). There is an increasing interest in microbiome manipulation by addition of beneficial bacteria used as probiotics[173], addition of phages to remove pathogenic bacteria (phage therapy)[174] or microbiome reshaping through virome transplantation[175]. If we are to successfully apply phages for microbiome manipulations, we need to have a thorough understanding of the

evolutionary responses of bacteria, and the implications for their virulence[176] or pathogenicity[177]. Likewise, we need to better understand how phages interact with the human immune system and their consequences for clearing bacterial infections[31,178]. This requires studying bacteria-phage interactions in relevant models that incorporate biotic and abiotic complexities, which influence the outcome of phage therapy and coevolution. Therefore, studying the concepts of phage-bacteria interactions and evolution in complex communities and natural environments is pivotal, not only for fundamental knowledge, but also for developing phage-based applications.

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Author contributions

A.C. and E.R.W. conceived the manuscript. A.C. and B.J.P wrote the manuscript. E.R.W and S.V.H. reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests

Proposed display items:

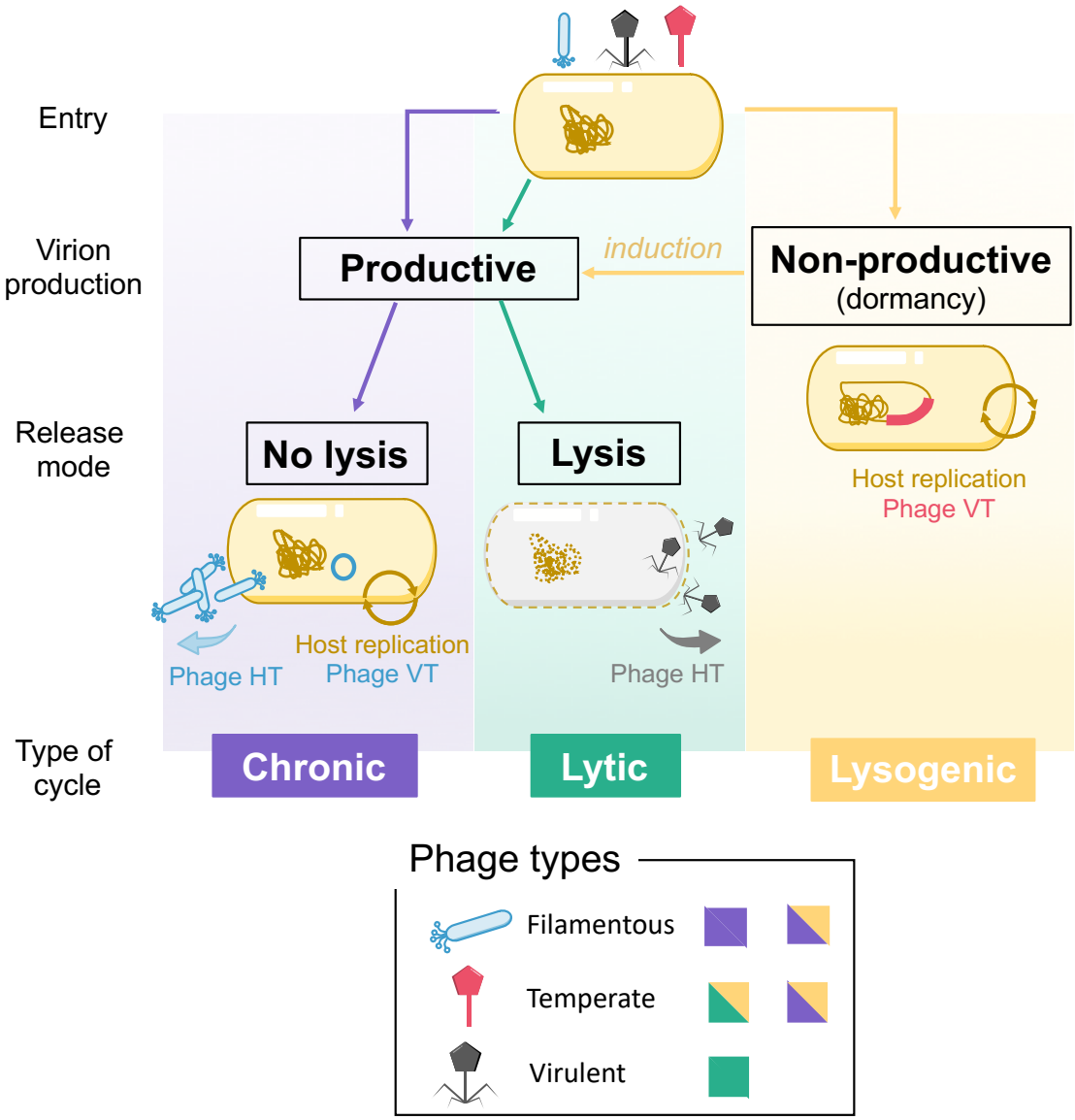


Figure 1: Canonical phage infection cycles

Upon entry into their host cell, phages can enter a productive replication cycle which results in the release of new virions either without impairing the host (chronic cycle) or upon host lysis (lytic cycle). Alternatively, phages may follow a non-productive cycle, where the phage genome (thick pink line) integrates into, and is replicated along with, the host chromosome (lysogenic cycle). Phage can exit this dormant state, either spontaneously or upon exogenous stimuli, and switch to one of the productive cycles. Depending on their infection cycle, phages can be categorised into different types: filamentous phages usually follow a productive chronic cycle, with some (but not all) having the capacity enter a non-productive lysogenic cycle. Temperate phages are characterised by their ability to be lysogenic and, upon induction, they can produce new virions either through a chronic or a lytic cycle. Virulent phages replicate only through a lytic cycle. VT: Vertical transmission, HT: Horizontal transmission

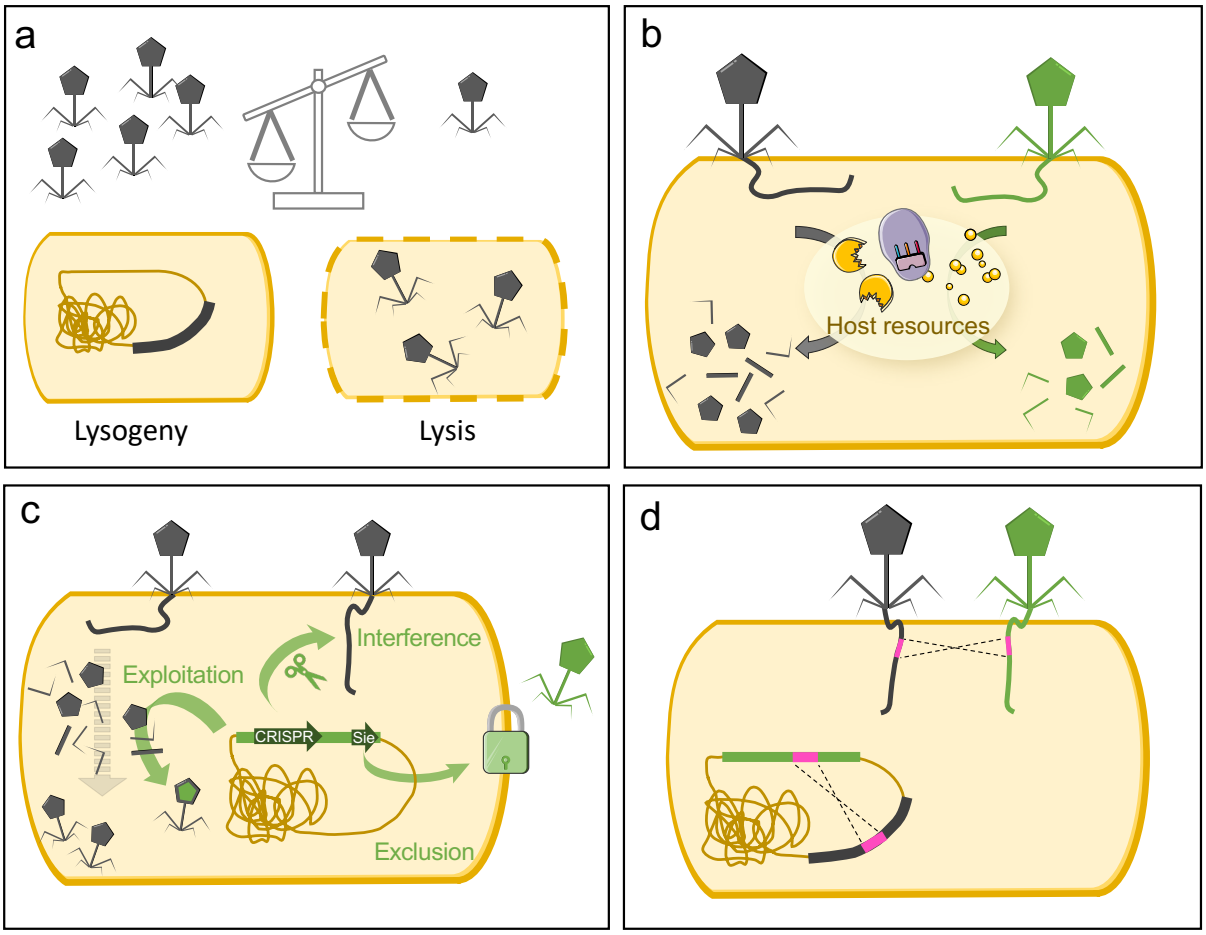


Figure 2: Consequences of coinfection on phage epidemiology and evolution.
a. The potential of coinfections is an important factor that influences decision to engage into a lytic or a lysogenic cycle. Typically, high phage densities promote lysogeny while low densities favour the lytic cycle. Other factors influence lysis/lysogeny decisions (not illustrated - reviewed in REF[102]), which can be host-associated (e.g. growth rate, physiological state and genetic background) or related to the environment (physicochemical stressors, bacterial population density and dynamics), b. Coinfecting phages (black and green) may compete for intra-cellular host resources (e.g. ribosomes, nucleotides, amino acids) necessary to produce phage progeny, c. Examples of phage social interactions: a prophage (depicted as a thick green line into the host chromosome) may exploit capsid proteins produced by a black coinfecting phage (e.g. case of satellite and helper phages). The green prophage may also provide protection against competing heterologous black phage (e.g. by encoding a CRISPR-Cas system, as described in *Vibrio* phages). Finally, many temperate phages encode mechanisms that prevent secondary infections by homologous phages (e.g. homoimmunity, Imm, depicted as a green lock) d. Coinfecting phages, whether they are in productive or non-productive state, may exchange genetic material inside their host cell.

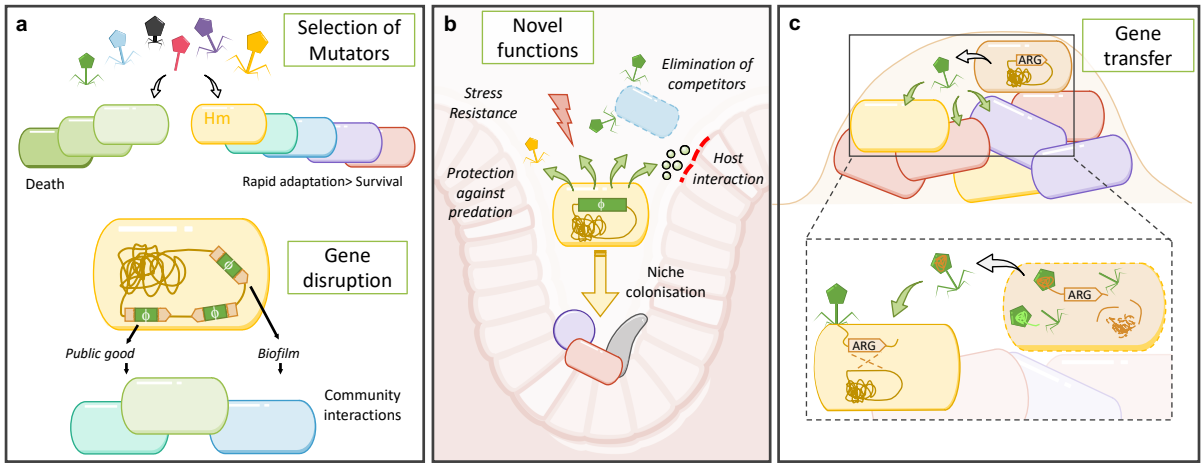


Figure 3: Phage-mediated evolution of bacterial communities

a. Phages may alter the mutational background of bacteria. Phage communities can positively select hypermutator (Hm) bacteria, which rapidly generates a population of diverse bacterial mutants (represented by different colours), ultimately enhancing population survival against phage infection. Transposable temperate phages randomly insert into the bacterial chromosome (green rectangle) which may result in gene disruption (broken orange arrows) and lead to the emergence of phenotypes that influence bacterial community behaviour (e.g. social interactions, biofilm formation). b. Prophage (green rectangle) may encode genes that provide their host with fitness advantages during colonisation of a new ecological niche. c. Phages can mediate horizontal gene transfer (HGT) via transduction. For example, erroneously encapsidated antibiotic resistance genes (ARG) may be transferred from one strain to another. The frequency of these events depends on the environment (e.g. biofilms likely favour HGT because different bacterial strains are in close proximity) and on phage host-range.

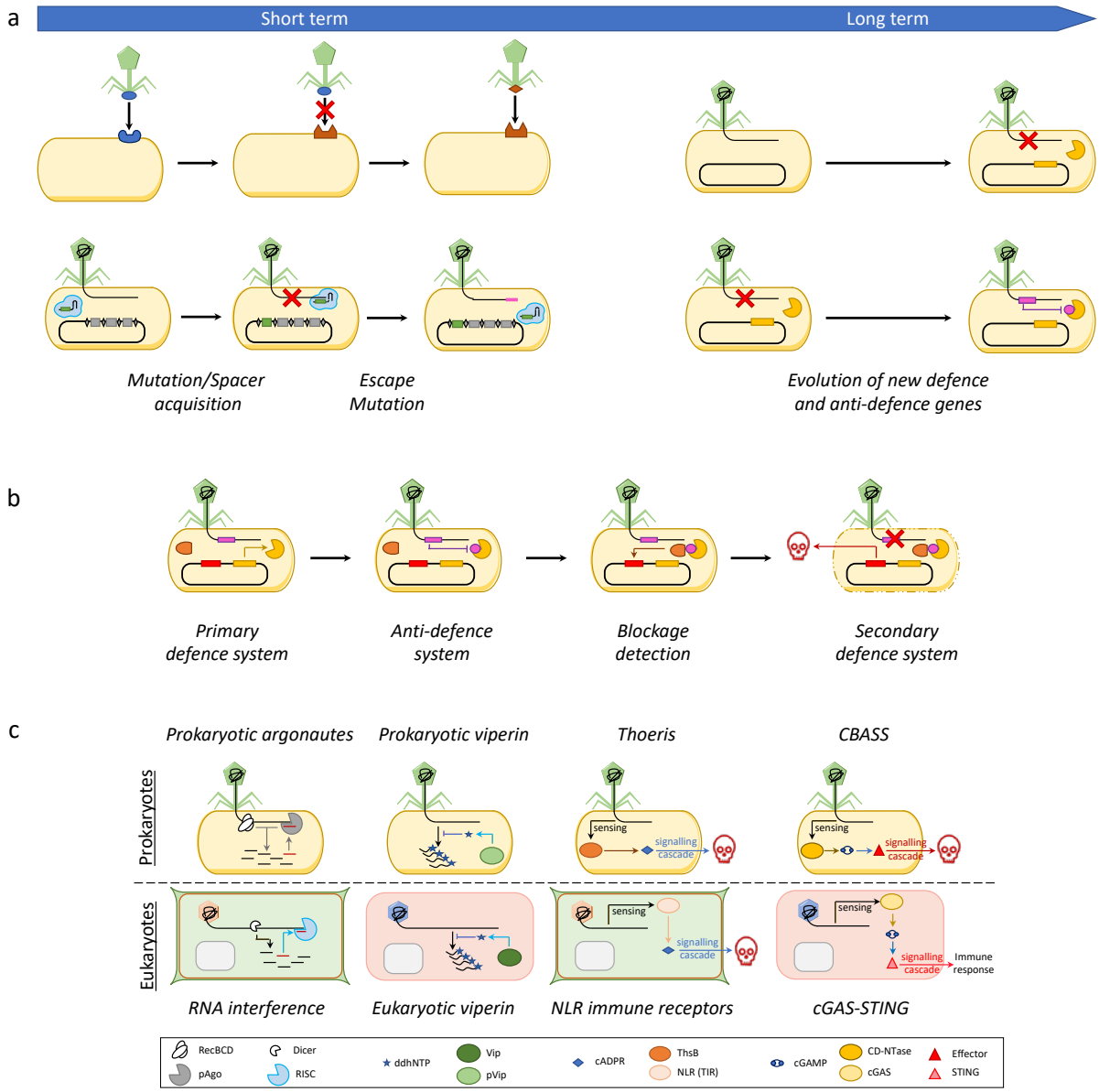


Figure 4: Short- and long-term consequences of phage-bacteria coevolution.
a. Short-term coevolution generally leads to the acquisition of receptor mutations or CRISPR spacers in bacteria and reciprocal escape mutations in phages. Long-term coevolution may lead to evolutionary innovations such as new bacterial defence or phage anti-defence systems, b. Accumulation of defence and anti-defence systems may lead to a complex multi-layered organisation. If a primary defence system is blocked by the phage anti-defence system, a second defence line (e.g. abortive infection) may be activated upon blockage detection. c. Conceptual and mechanistic parallel between prokaryotic and eukaryotic defence systems. Some eukaryotic defences might have ancient evolutionary origin that stems from bacteria. Prokaryotic argonautes (pAgo) and RNA interference rely on acquisition of genetic material from past infections (DNA and RNA, respectively), which is then used to recognise new infecting viruses. Viperin (Vip) and prokaryotic viperin (pVip) produce modified ribonucleotides (ddhNTP) which impair viral transcription. Thoeris system and plant NLR (Nucleotide binding Leucine-rich repeats immune Receptors) both involve proteins with TIR domains (Toll/Interleukin-1 Receptor) which sense virus infection and lead to the production of cADPR (cyclic adenine diphosphate ribose). This triggers a signalling cascade causing abortive infection. Plant cells and mammalian cells are represented in green and red, respectively.

Box 1: Phage co-infections can drive the evolution of social interactions

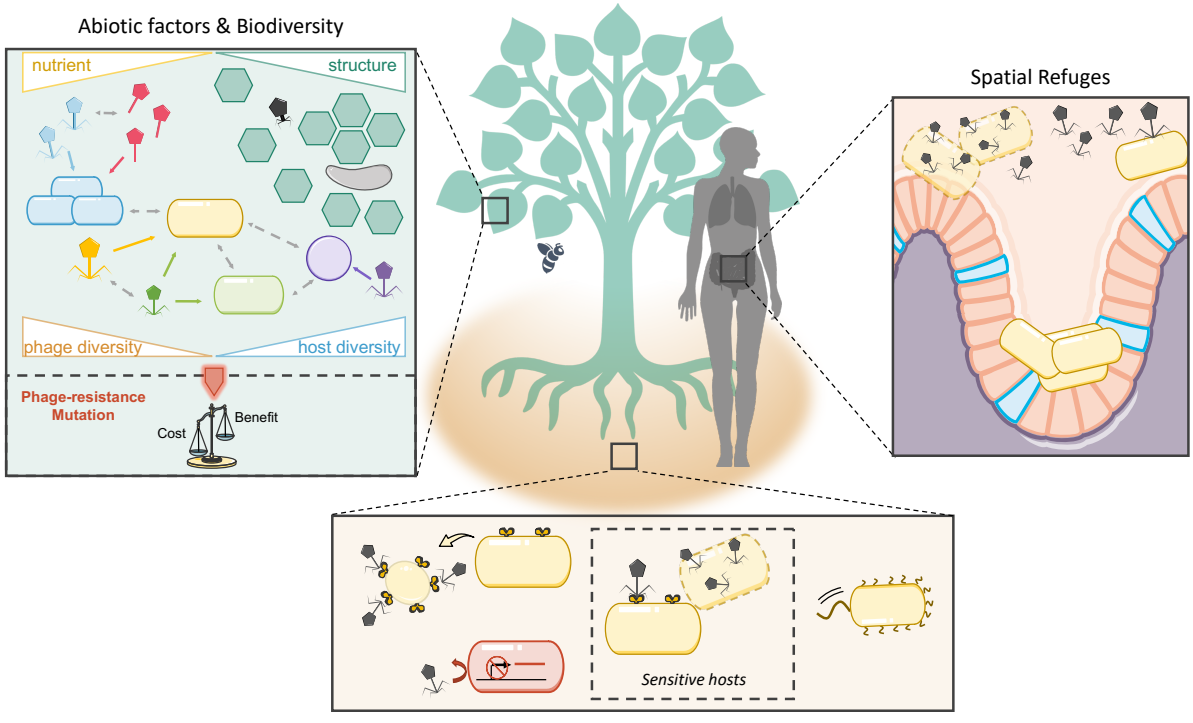
Phages that (co)infect the same host can access a common pool of phage-derived proteins, which can be viewed as intracellular public goods. For example, phages encoding anti-CRISPR proteins (Acr) initially fail to kill CRISPR-resistant hosts but turn them into an immunosuppressed state which can be successfully lysed upon re-infection by a second clonal Acr-phage[88,89]. Similarly, when frequencies of lytic infections are high, some temperate phages can produce small signalling peptides (*Arbitrium*) that promote a collective switch towards a more prudent lysogenic replication in the phage population[83]. Cheater phages that exploit these public goods - but do not participate to their production - might evolve in these populations, if production of the public good is costly. For example, some phages may lose a part of their genome, which provides them with a replication advantage[86,93], and consume proteins produced by coinfecting phages or develop high efficiency in consuming public goods[92] (e.g. by being preferentially encapsidated[179]). Different types of cheater phages may evolve within a given population and potentially compete with one another[179]. Such behaviours may also occur during mixed coinfection, as in the case of satellite phages that strictly depend on "helper" phages (e.g. coliphages P4 and P2[87]). Cooperation/exploitation may also occur indirectly, for example when phages encoding depolymerase enable other phages to adsorb to encapsulated hosts[75] or when Acr-negative phages exploit immunosuppressed bacteria generated by Acr-positive phages[180]. In contrast, coinfections can lead to competitive relationships[92]. Phages developed various strategies to prevent superinfection by other phages. For example, mechanisms that alter receptors, block DNA injection or inhibit intracellular replication of phage competitors are often found in prophages[90,91]. More sophisticated mechanisms have recently been uncovered, such as phage-encoded CRISPR-Cas systems targeting other phages[181,182] or systems triggering premature lysis of competing phages[183]. Mechanisms that allow phages to overcome these restrictions have also been identified, which can be thought of as a phage-phage arms race[91]. While these strategies can be directed towards genetically similar phages, it seems that they are more often directed against distinct phage genotypes[90].

Box 2: Phage impact on eukaryotic host

Phage-mediated control of microbial communities can have important impacts on their eukaryotic host. For example, correlations between gut virome composition and pathologies (e.g. inflammatory bowel diseases, Crohn's disease) have been reported, although causality remains often unclear[61]. Virulent phages can shape the microbial community composition by influencing the colonization success of bacteria[116] and by selecting for phenotypes with altered virulence[176]. For example, application of a phage cocktail targeting *Ralstonia solanacearum* to the plant rhizosphere not only reduced the prevalence of the pathogen but also selected for mutants with reduced virulence and globally affected the composition and diversity of the whole microbiome, all of which led to a suppression of plant disease[184]. However, when the bacterium has a mutualistic interaction with its eukaryotic host, the presence of virulent phages may be deleterious[185]. Temperate and filamentous phages are also important determinants of pathogen colonization success. Not only they may carry beneficial genes (e.g. virulence factors[139]), they can also directly contribute to biofilm formation. For example the filamentous virions of phage Pf produced during chronic infection of *Pseudomonas aeruginosa* constitute a key component of the bacterial biofilm structure, which provides protection against antibiotics[30]. Moreover, interactions between phages and the host immune system appear as important mediators of the infection process. A synergy between virulent phages and the host immune system can occur, where the joint action of phage-mediated lysis and immune cells improves clearance of bacterial infections[178]. Interestingly, some lytic phages can adhere on mucosal surfaces, which may benefit both the phage – by providing a better access to bacteria – and the host – by limiting bacterial proliferation in mucus[186,187]. In addition, phages may promote the expression of innate immunity genes[188], prevent activation and proliferation of immune cells[189], disturb phagocytosis by dendritic cells[190,191] or affect bacterial antigen expression, which might alter recognition of pathogens by the immune system[192].

Box 3: Why lower levels of phage-resistance evolve in nature

Abiotic factors (e.g. nutrient and structure) and biodiversity influence the amplitude of the fitness costs associated with anti-phage defence mechanisms, which are often higher in natural environments compared to the lab[122], explaining their frequent loss from prokaryotic genomes[193] (left panel; distinct phage and bacterial species are represented with different colours and their interactions are depicted with arrows). In many environments (e.g. soil, gut or biofilms), bacteria are heterogeneously distributed across space and hence unequally accessible to phages, especially when diffusion is limited (right panel). Phages can replicate on nearby hosts but cannot reach additional hosts located in so-called spatial refuges, leading to the decay of the phage population. However, sustainability of the phage population can be ensured by periodic immigration of susceptible hosts. Theoretical and *in vitro* studies showed that this non-evolutionary "source-sink dynamics" stabilize phage-host coexistence[194–196] and recent observations of the heterogeneous distribution of phages and bacteria in the mouse intestine revealed this is likely an important phenomenon *in vivo*[161]. Bacteria may also enter a transient non-heritable phage-refractory state, known as phenotypic resistance, which generally relies on metabolic or transcriptional changes that slow down or prevent phage infection[197] (bottom panel). This typically occurs in nutrient-limited conditions when bacteria are in stationary phase[198]. Several mechanisms have been identified, including phase variable expression[199] or quorum sensing-mediated down-regulation[200,201] of phage receptors (red bacterium), production of outer membrane vesicles containing phage receptors which act as decoys (yellow bubble)[202,203] or modification of swarming behaviours allowing bacteria to "run away" from infected areas (flagellated bacterium)[204]. While the importance of phenotypic changes has been well studied in the context of antibiotic tolerance, their role in phage-bacteria dynamics remains unclear. Single-cell approaches have propelled the exploration of phenotypic heterogeneity in bacterial populations and their application to phage-bacteria systems will provide valuable insights into their eco-evolutionary dynamics[205].



Glossary

Auxiliary metabolic genes: Phage-encoded genes which originate from bacterial cells and can modulate host cell metabolism likely resulting in improved phage replication.

Arms Race Dynamics: Type of phage-bacteria coevolutionary dynamics where phage infectivity and host resistance generally increase over time.

Coinfection: Infection where two phages are simultaneously present within the same host cell. This definition therefore widely includes phenomena such as superinfection or polylysogeny.

Fluctuating Selection Dynamics: Type of phage-bacteria coevolutionary dynamics where phage and host genotype frequencies oscillate over time because of negative frequency-dependent selection.

Homoimmunity: Mechanism by which a prophage inhibits the secondary infection of its host by a closely related phage. The repressor of lytic genes (enabling the maintenance of lysogeny) encoded by the resident prophage can bind the operator sequence carried by the superinfecting phage, preventing it from initiating an infection cycle.

Host range: The range of genetically distinct bacteria in which a phage can replicate (i.e. productive or non-productive infection). Usually, phages that infect a low diversity of hosts (i.e. a single or few genetically close strains) are referred to as specialists whereas phages that are able to infect many strains of the same species, or even multiple different species or genera tend to be referred to as generalists.

Lysis inhibition: Delay of the lysis of an infecting phage (this extension of phage latent period results in an increased burst-size) induced by secondary adsorptions of additional phages. This mechanism has originally been described for T-even phages and has recently been discovered in *Vibrio* phages.

Lysogenic conversion: Phenotypic changes that result from the acquisition and expression of prophage-encoded genes.

Phase variation: Switch of gene expression from an ON to an OFF phase, generally caused by the introduction of a mutation into a hypermutable DNA region. A secondary mutation in the same region can reactivate gene expression, rendering this phenomenon reversible. Phase variation generates phenotypic diversity in bacterial populations.

(Poly)lysogeny: Phenomenon where one (several) temperate phage(s) parasitizes a host bacterium (hence called a lysogen), often by integrating their genetic material into the host DNA, but without producing phage particles nor triggering host lysis.

Mosaicism: The description of a phage genome as composed of gene blocks similar to other phage genomes and flanked by dissimilar sequences. This mosaic genome architecture is due to horizontal transfer of genetic material.

Phage-inducible chromosomal islands (PICIs): Class of mobile genetic elements that specifically exploit (and interfere with) temperate phages for their horizontal transfer.

Prophage: Latent form of a phage in which lytic functions are repressed and that is often integrated into the host chromosome.

Superinfection: Infection of a host that already accommodates a phage from an earlier infection, which can be engaged either in a lytic or in a lysogenic cycle and integrated as a prophage.

Satellite phage: Phage that lacks the ability to replicate autonomously and requires the products of infection generated by a helper phage (e.g. capsid proteins).

Viral Dark Matter: Refers to a viral species (or genome) that has not yet been characterised, and which thr existence had been revealed by environmental metagenomic sequencing. More restrictively, viral dark matter can also refer to phage genes that have no assigned functions.

Virocell: Concept stating that the reproductive, living form of a virus is the infected cell (virocell) as opposed to its dissemination form, the virion.

Virome: Ensemble of viral genomes found in an organism or within a given environment. In this review, when we use the term “virome”, we refer to the phage fraction of viromes (also called phageome). Phages are generally the main constituents of viromes.

Virulent phage: Phage that replicates exclusively through a lytic cycle, which ultimately triggers the destruction of the host cell to release new phage particles.

Temperate phage: Phage that replicates either through a lytic cycle or through a lysogenic cycle.

Transduction: Transfer of bacterial DNA from one bacterium to another, mediated through phage infection. Transferred DNA may result from the erroneous packaging of host genome fragments into a phage capsid (generalized transduction) or from the imperfect excision of a prophage genome, which takes along a portion of the bacterial DNA flanking its integration site. This hybrid phage-bacterial DNA is then replicated and packaged into phage capsids (**specialized** transduction). **Lateral** transduction occurs when the induction of the lytic cycle, which triggers phage replication and gene expression, occurs prior to prophage excision. Packaging of phage DNA starts while it is still integrated, taking along adjacent bacterial DNA, and ends after several capsids have been filled (which corresponds to several hundred kilobases of packed bacterial DNA).