# Revisiting the rules of life for viruses of microorganisms

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Abstract | Viruses that infect microbial hosts have traditionally been studied in laboratory settings with a focus on either obligate lysis or persistent lysogeny. In the environment, these infection archetypes are part of a continuum that spans antagonistic to beneficial modes. In this Review, we advance a framework to accommodate the context-dependent nature of virus–microorganism interactions in ecological communities by synthesizing knowledge from decades of virology research, eco-evolutionary theory and recent technological advances. We discuss that nuanced outcomes, rather than the extremes of the continuum, are particularly likely in natural communities given variability in abiotic factors, the availability of suboptimal hosts and the relevance of multitrophic partnerships. We revisit the 'rules of life' in terms of how long-term infections shape the fate of viruses and microbial cells, populations and ecosystems.

Viral shunt
Prevention of dissolved and particulate carbon from being incorporated into consumers at higher trophic levels due to the release of this carbon from infected host cells via viral lysis.

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https://doi.org/10.1038/s41579-021-00530-x

Interactions among microorganisms shape population dynamics, evolutionary trajectories and ecosystem functioning across plant-associated and animal-associated systems, as well as in built environments. In turn, populations and communities of these microorganisms are shaped by top-down (that is, viral infection and grazing) and bottom-up (abiotic) forces. Initially revealed in foundational studies reporting total counts of virus-like particles in the oceans[1,2], the high abundance of viruses that are predicted to infect microorganisms, and their ubiquitous distribution, is staggering. The pervasiveness of viruses across systems, from deep subseafloor sediments[3] to the human gut[4], is now well established, and viral ecologists are increasingly motivated to quantify ecosystem-level impacts that are triggered by viral activity (see, for example, REFS[5–8]), including modulation of community structure and function, and the release of organic matter and nutrients back into the environment via the 'viral shunt'[5,9]. Initial efforts to characterize the ecosystem-level impacts of viruses focused on lytic infections — in part because it was infeasible to distinguish and track other modes of infection. The first evidence of diverse viral infection strategies became available in the early twentieth century to mid-twentieth century, based on isolation and characterization of model laboratory systems (for example, T bacteriophages (or phages) for lytic infections and phage λ for lysogenic infections[10]). Further progress in characterizing viral diversity has been enabled by ongoing developments in molecular biology and in environmental sequencing technologies, as well as by the discovery of novel viral lineages. Together, these advances suggest the need to revisit the molecular mechanisms and eco-evolutionary consequences of virus–microorganism interactions.

As environmental virology comes of age[11], new challenges to the early paradigms that defined the 'rules of life' for viruses (BOX 1) have arisen. Culture-independent approaches have helped to expand[12–17] and taxonomically organize[18–20] catalogues of viral sequences. Yet, it remains difficult to link a virus with its microbial host (BOX 2). Concurrently, diverse model systems have helped reveal that the most commonly studied canonical 'lytic' and persistent 'lysogenic' infection modes (FIG. 1) are not representative of all virus–microorganism interactions[21] and may not be the most common infection modes in nature. For viruses in complex communities, a key research focus is now identifying the community-level and ecosystem-level conditions that favour lysis, lysogeny and more fluid (and less well-studied) interactions, including chronic infections (reviewed in REFS[22,23]) and inefficient lytic infections[24–29].

In this Review, we revisit the rules of life for viruses by embracing a conceptual framework that recognizes virus–host interactions across a continuum of infection modalities (FIG. 1), and we examine the influence of these modalities on viruses, their hosts and ecosystems. Intracellular infection mechanisms at the 'middle' of the continuum are recognized by many phage biologists (see, for example, REFS[21,30,31]), but the idea that these mechanisms may be both common and ecologically relevant has not yet broadly taken hold. Most examples in this Review are drawn from a century of seminal studies on viruses of bacteria (phages)

Box 1 | **Revisiting the rules of life for viruses of microorganisms**

For decades, studies of viruses that infect microorganisms have mainly focused on antagonistic interactions, in which infection leads to the death of the microbial host cell and the release of new viruses. Yet, recent evidence from experimental model systems, modern viromics and new theoretical advances suggests that virus–microorganism interactions often differ along a continuum of infection, which ranges from lysis to persistent lysogeny. In practice, infections are fluid and dynamic across a continuum of infection strategies that respond to host and environment cues (FIG. 1); viral fitness can be understood by tracking infecting cells (instead of free viral particles) (FIG. 2); viral infections can reshape cells and their functions (for example, metabolism, regulatory elements and communication systems) in ways that could benefit both the virus and the host; inefficient lytic interactions are likely to be common; viruses can infect other viruses (for example, Sputnik); and viruses also affect higher organisms that harbour their hosts (Russian doll symbioses). These findings provide inspiration for expansion of the rules of life (see the table), integrating knowledge gained in the past few years with that from seminal studies of viruses of microorganisms.

| Paradigmatic rules of life for viruses of microorganisms | Expanded rules of life for viruses of microorganisms |
|---|---|
| The number of virus particles produced from an infection is a metric of viral fitness | The number of infected cells produced is the basis for measuring the feasibility of viral invasion in the short term and viral fitness in the long term |
| Viruses are strict antagonistic parasites of their hosts | Viral infections can trigger various outcomes for their microbial hosts, ranging from harmful to beneficial |
| Viruses cannot communicate | Viruses co-opt host communication systems |
| Temperate phages are dormant and do not interfere with host regulation | Temperate phages can actively regulate host genomes |
| A viral type is capable of infecting its one target host species efficiently | A viral type can infect various potential hosts, but with various degrees of efficiency |
| A temperate virus is generally static in its use of either a lytic infection mode or a latent infection mode upon entry | A temperate virus modifies its initial infection mode (lytic versus lysogenic) on the basis of the context of the environment and host cell condition |
| Once successful receptor contact has been established and any antivirus defences have been overcome, a virulent viral infection will be successful | Environmental factors, intracellular host compatibility and virus–virus crosstalk, in addition to binding site characteristics, influence the outcomes of virus–microorganism interactions |
| Viral infection of a microbial partner within a Russian doll symbiosis (BOX 3) affects only the direct host of the virus (that is, the microorganism) | Viral infection within a Russian doll symbiosis can affect various partners (for example, the host of the viral host) |
| Viruses cannot infect other viruses | Viruses can infect other viruses (for example, virophages) |

with double-stranded DNA genomes (see, for example, REFS[30,32–34]), which provide the foundation for our current understanding of the rules of life for viruses. This Review aims to inspire further work to identify principles governing viral infections of other microorganisms (including archaea and microbial eukaryotes). Towards this effort, we first consider what it means to be temperate (disentangling the evolutionary strategy from the state of lysogeny) and how temperate phages 'decide' between lysis or lysogeny. We then describe the potential cellular benefit of viral infections in an eco-evolutionary context, and seek to formalize a cell-centric unit for measuring and comparing viral fitness across the continuum (FIG. 2). We next evaluate current understanding of the nature of mutualistic and antagonistic relationships between viruses and microbial hosts, as well as how inefficient lytic infections can arise. Finally, we examine how bioinformatic approaches can help identify diverse viral infection strategies from environmental sequence data, potentially revealing new principles by which viruses modulate microbial fates at scales from cells to ecosystems.

Lytic infections
Infections involving the reproduction of viral genetic material, packing of viral genetic material into capsids and release of virus particles into the environment following the lysis (that is, rupture) and death of the host cell.

Bacteriophages (or phages)
Viruses that exclusively infect bacterial cells.

## What it takes to be temperate

Temperate phages can initiate lytic or lysogenic infections. In the lytic mode, infection leads to the production of new virions, the lysis of host cells and the release of virions back into the environment (FIG. 1). In the lysogenic mode, temperate phage genomes persist as a prophage within the host, where they are integrated into the host chromosome (and replicate in conjunction with the host genome) or maintained extrachromosomally (FIG. 1). Switching between lysis and lysogeny is a 'decision' shaped by genetic switches encoded within phage genomes[20], and is strongly influenced by host-associated and external factors. Examples of host factors can be drawn from seminal work on phage λ, whereby the probability of the phage to initiate lysis versus lysogeny is related to the cellular multiplicity of infection[35] (FIG. 1). More recently, single-cell measurements using phage-encoded fate reporters have confirmed that the probability of lysogeny for phage λ increases with increasing multiplicity of infection, as well as with decreasing cell volume[36,37]. Hence, the well-studied example of phage λ represents an example of how a temperate phage strategy exists along a continuum in which the probability of initiating lysogeny as well as induction represent mutable traits.

Other, non-host factors also influence the outcome of infection by temperate viruses (FIGS 1,2). The probability of establishing lysogeny can vary across the same[37–39] or different[40] temperate phage lineages, and also varies with the environmental context in which it occurs (reviewed in REF.[31]; see later). Likewise, after lysogeny has been established, induction to initiate the lytic cycle is also influenced by cell state and environmental conditions (FIG. 1), although many prophages can also spontaneously induce and initiate the lytic cycle. Prophage induction often occurs at low frequencies as in the case of the Shiga toxin-encoding prophage that resides within pathogenic *Escherichia coli*[41] or a mycosphere prophage associated with *Paraburkholderia* species[42]. Environmental context can also influence temperate phage dynamics[43]. For example, in marine polar waters, lysogeny was shown to be favoured during periods of low ecosystem productivity; lysis was shown to be favoured during high-productivity periods[44]. Diverse environmental stressors, such as anomalously high temperatures[45] or nutrient levels[46], or anomalously low salinity[47], are hypothesized to trigger lysogenic switching in viral infections of microeukaryote (dinoflagellate) symbionts of stony corals (see, for example, REFS[48–50]). In the coral system, viral lysis of dinoflagellates may contribute to coral death and reef ecosystem decline[7].

Model systems, such as *E. coli* and its phages, have been instrumental in understanding the link between host cell stress and temperate phage behaviour.

Lysogenic infections
A viral infection state in which the viral genome is integrated into that of the host cellular genome and can be replicated during division without lysing the cell.

Under stress, the host's SOS response system cleaves a phage-encoded repressor molecule (CI), initiating genomic phage DNA excision out of the host genome, followed by replication, ultimately resulting in lysis of the cell[51]. Beyond responding to host signals, temperate phages can also manipulate host physiology (reviewed in REF.[52]). For example, phages can co-opt host 'communication' systems in striking ways. The *Vibrio cholerae* CTXΦ prophage encodes a quorum sensing receptor protein that binds a host cell density-dependent factor (DPO), triggering the lytic pathway in the prophage. The phage hijacks the host's communication system and mediates the lysogenic–lytic decision (that is, favouring lysogeny at low host cell densities and initiating lysis at high host cell densities)[53]. In a more extreme example of appropriation, bacillus phage Φ3T encodes a small peptide (arbitrium) that is released extracellularly and taken up by other cells[54–56]. The concentration of arbitrium thus influences the lysis–lysogeny switch and represents a form of viral phenotypic plasticity, see REF.[57]), such that lysogeny is positively correlated with arbitrium levels. As Φ3T phages proliferate, infected cells generate peptides, potentially signalling the local depletion of susceptible hosts. If the phage continued to lyse increasingly scarce host cells, then viral progeny from lytic infection would be unlikely to encounter a susceptible host to infect (FIG. 2). Instead, lysogenic infections in cells with high peptide signals enable viral genomes to persist at the population scale as lysogens (that is, inside cells) rather than be lost or degraded as particles without a host (that is, outside cells). These communication strategies may be widespread across phage–host systems[55,58]. These findings suggest that temperate viruses have evolved to modulate their infection mode on the basis of environmental and/or cellular cues. Similar findings also exist for the responsiveness of temperate phage infection states to restriction modification systems[59].

In sum, temperate phages commonly modulate whether they preferentially lyse host microorganisms or initiate lysogeny following initial infection, and how long they remain as prophages within their host. Hence, to be temperate does not mean to automatically initiate lysogeny. Instead, viral infections exhibit phenotypic plasticity such that feedback from the environment, including other viruses and the host cellular state, modulates the infection mode of a given virus–host interaction.

## Fitness and viral life cycles

Phage genomes persist within cells for various lengths of time, which raises an important question: why be temperate rather than lytic? The evolutionary advantage or fitness of a virus has been traditionally equated with the number of virus particles in the environment[60]. The lysogenic mode does not produce any virus particles (at least not in the short term), yet evidently being temperate is evolutionarily adaptive. Thus, a different approach for measuring viral fitness is needed. Early work[61] hypothesized that a temperate strategy is advantageous at low host cell densities and represents an adaptation to fluctuating environments. Building on studies of epidemiological dynamics of horizontally and vertically transmitted pathogens[62,63], recent theory[64–67] has revisited this hypothesis and proposed to measure viral fitness in a cell-centric (rather than a particle-centric) fashion by examining the life cycle of temperate phages, both inside and outside cells (FIG. 2).

Inside cells, if a viral genome does not kill its host, it can be integrated into microbial genomes as a prophage, persist as extrachromosomal elements such as episomes or via carrier infections, and also persist transiently as genomes to be packaged into virus particles preceding lysis (which critically could be stalled under suboptimal conditions in nature[68]). Outside cells, viral genomes may

### Box 2 | Who is the host?

Culture-independent techniques have revealed substantial diversity of DNA and RNA viral genomes (from metagenomes and metatranscriptomes) and virus-like particle morphologies and sizes (from electron microscopy) within environmental samples[134,135]. Yet, determining which hosts these viruses infect remains challenging in complex communities[136]. The host range of viruses can be experimentally determined when host cells are in culture, either via traditional lysis in liquid or solid media or using high-throughput approaches such as adsorption sequencing[137] or viral tagging[138,139] (when high-end flow cytometry and ultraclean techniques are available). However, most microorganisms in natural settings are not yet cultivated, and for these, strategies have ranged from inferring viruses detected in single-cell amplified genomes as belonging to that host (see, for example, REFS[124,140]) to in silico host predictions for newly available viral genomes discovered in metagenomes (reviewed in REFS[19,141]). Currently, in silico strategies include linking viruses and hosts on the basis of the presence of sequence composition, statistical co-occurrence analyses, CRISPR spacers and shared non-viral genes. Although no consensus yet exists in the literature, these metrics seem to predict hosts at various levels of confidence. For example, a sequence composition approach that assessed tetranucleotide frequency to predict hosts from a large-scale dataset (nearly 15,000 microbial genomes and their 12,498 detected viruses) suggested host prediction at the genus-level could achieve up to 99% accuracy when optimally representative host data were available. Yet, accuracy could be as low as 30% when there was not at least a genus-level representative genome available for the known host[12]. Predictions made using statistical co-occurrence analyses are also relatively lower confidence, at least when host or virus abundances and/or sample sizes are small[129,130,141,142]. By contrast, CRISPR spacer matches of 100% identity are thought to be nearly certain signatures of a past virus–host interaction. Shared non-viral genes, such as photosynthesis auxiliary metabolic genes, which are interpreted as key metabolic manipulations specific for a particular host, are also considered indicative of long-time virus–host associations. Problematically, in silico metrics are inherently database limited: undersampled taxa that have yet to be genomically documented are unlikely to be predicted as hosts. This limitation is rapidly being ameliorated as hundreds of thousands of metagenome-assembled genomes are emerging across diverse environments[143–147]. As such approaches gain power, viral ecology will be able to advance from community-wide averaged patterns towards lineage-specific inferences that more closely mimic the ecological interactions that drive ecosystem impacts (see, for example, REF.[14]). Such efforts will benefit immensely from centralized resources that collect host prediction information, such as Virus-Host Database[148], HoloVir[149] and iVirus[150].

As new viruses are being discovered via increasingly scaled metagenomics studies (see, for example, REF.[16]), complementary scalable experimental methods are needed. Viral tagging approaches could be adapted and optimized for new 'bait' hosts in laboratories interested in viruses for a particular host strain that is in culture. Viruses may also be linked to hosts via adaptations of solid-phase PCR to generate 'polonies' that can enable quantification of virus particles and intracellular infections[151,152]. Beyond cultivated hosts, another strategy would be to enrich single-cell genomic sequencing for virus-infected cells by hybridizing the amplified single-cell genomic DNA against purified virus DNA from a natural sample[153]. Additional approaches that borrow technologies from other disciplines will undoubtedly emerge as well. For example, viruses could be linked to their hosts by adapting epicPCR[154], a fused PCR primer approach that 'links' barcode genes to genes of interest. Another promising direction is proximity ligation that uses fixation-based sulfur-bridging chemistry to assess when two genomes are within the same cell[155,156]. Conceptually, both approaches should work, although as with any new technology, their output requires experimental assessment to determine false-positive rates using mock communities of virus–host pairs with known linkages and non-linkages before any ecologically meaningful linkage inferences can be drawn.

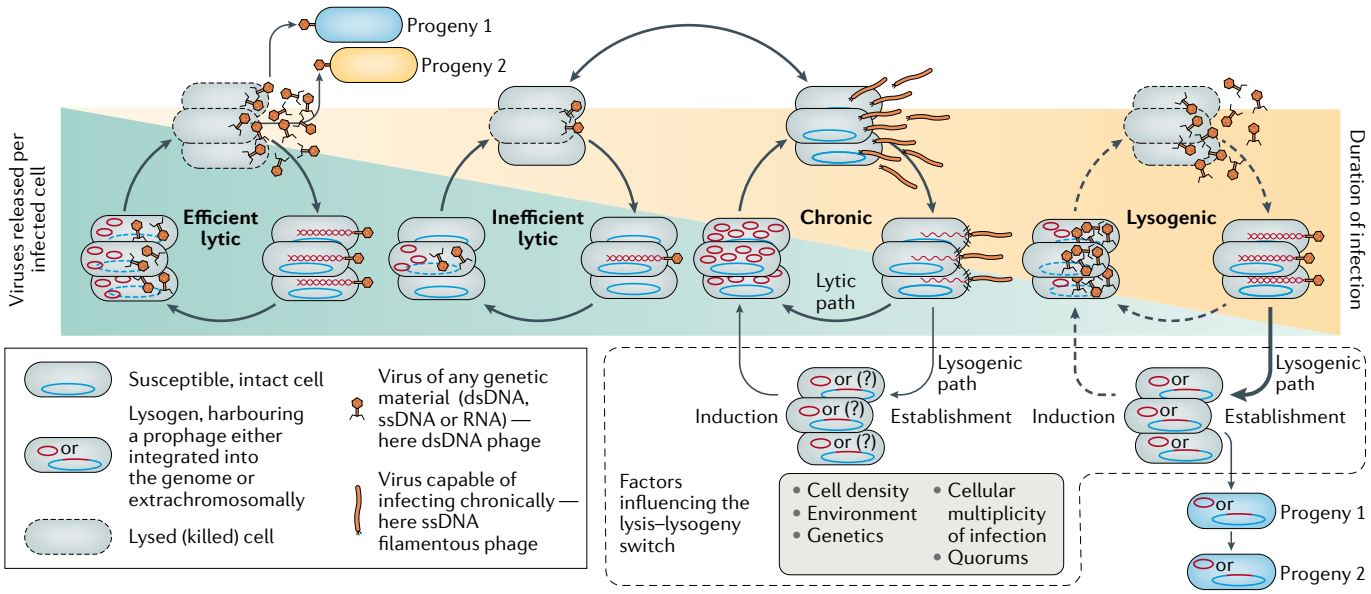



Fig. 1 | **The viral infection continuum.** The modes of viral infection exist in a continuum that ranges from efficient infections by virulent phages (left side of the figure) to persistent lysogenic infections by temperate phages (right side of the figure; where phages in the lysogenic cycle, denoted as 'prophages', have integrated their genomes into the host). Other infections along this continuum include inefficient lytic by virulent phages and chronic infections. The position of a given infection along the continuum is determined both by how many virus particles are released per infected cell and by the duration of one infection cycle. Specifically, efficient lytic infections are the shortest cycles that produce the most virus particles per infected cell (darkest green shading), whereas lysogenic infections last the longest (after establishment) but release no virus particles (as long as there is no induction; darkest yellow shading). Many factors can influence both the establishment of lysogeny and the induction of the lytic cycle in temperate phages. These factors relate to the host cell and the virus (genetics, cell density and cellular multiplicity of infection), the environment (for example, temperature, pH and other stressors) and other external signals (for example, quorum sensing signalling molecules). There is also fluidity in the position of inefficient infections and chronic infections along the continuum (depicted by the double-headed arrow); this depends on how long each infection cycle lasts and on how many viruses are produced per infected cell. dsDNA, double-stranded DNA; ssDNA, single-stranded DNA.

Chronic infections
Infections in which viral progeny are released from the host cell into the environment but lysis and death of that infected cell do not necessarily occur.

Inefficient lytic infections
Infections by a virulent virus that may be stalled or terminated at one or multiple stages of the infection cycle, from adsorption to the host cell through to cell lysis.

Latent infection
A state of reduced lytic activity, which includes lysogeny (that is, the viral genome is integrated into the host genome), chronic infection and other infection states (including otherwise lytic viruses that infect hosts during non-optimal conditions).

Temperate phages
Viruses that can establish a lytic cycle or a lysogenic cycle.

Prophage
An integrated genome of a temperate phage inside a lysogen.

be 'naked' (although the lifespan of such DNA or RNA is likely to be short) or persist inside virus particles. As a temperate phage can persist both inside and outside host cells, a particular lineage can potentially increase its near-term fitness by dynamically sliding along the continuum of infection on the basis of its environmental context (FIGS 1,2).

Analysing the evolutionary benefit of the temperate strategy is possible when one formally considers a 'virus life cycle' that begins and ends inside cells[69]. Such a formalization accommodates both the 'virocell' concept[70–73] and conventional definitions of lysogeny. In this framework, the utility of a given viral strategy in a host population can be determined by quantifying how many newly virus-infected microbial cells are generated, on average, by a single infected cell during its lifetime and the lifetime of its progeny virus particles (FIG. 2). This metric is equivalent to the epidemiological concept of the basic reproduction number, albeit adapted to the infections of microorganisms by viruses, in which infected cells (rather than virus particles) are used to measure proliferation[74,75]. When this 'reproduction number' is greater than 1, each infected cell generates at least one infected cell, which in turn generates more infected cells, and the viral lineage is able to proliferate[74–77]. When this number is less than 1, each infected cell generates less than one infected cell (on average), leading to fewer infections over time, until the virus is lost from the population. Thus, although lysis may produce many virions, the individual-level viability of the lytic strategy must be calculated in terms of those few virions that initiate new lytic infections in cells (FIG. 2a,c). Lysis therefore represents a form of horizontal transmission of a parasite at the microbial scale. Even though latency or infection of a suboptimal host may produce no virions, the individual-level viability of a temperate strategy can be calculated in terms of new infected daughter cells, which contain the viral genome (FIG. 2b,d). Hence, lysogeny represents a form of vertical transmission of a parasite at the microbial scale. Formalizing the viability of transmission routes requires expressing the interplay between nutrients and other external factors that influence viral strategy in terms of non-linear population dynamic models[61,64,66,67,78].

To move from the individual to the population, and ultimately into an eco-evolutionary framework, requires a dynamic perspective. In that sense, use of the basic reproduction number as the threshold criterion for invasion is the first step in efforts to quantify and understand the proliferation of viruses in a microbial population. In the event that susceptible host cell densities are low (FIG. 2cI,dIII), then a virulent strategy that favours horizontal transmission may not be successful. For example, the lysis of a host cell may generate 100 virions, but if on average at least 99 decay (or are rendered non-infectious)

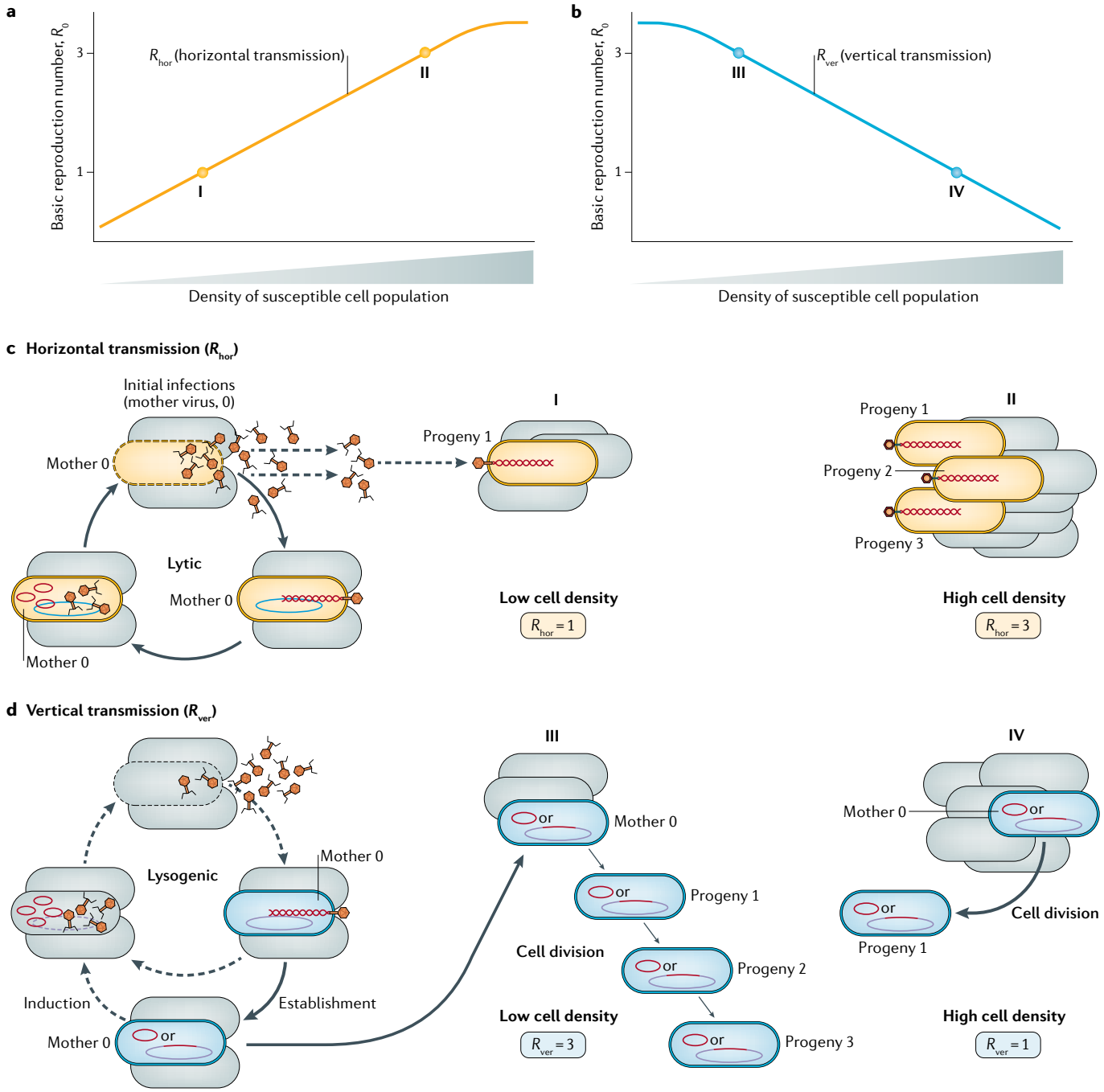



Fig. 2 | **Viral transmission strategies and ecological context. a**,**b** | The basic reproduction number ($R_0$; *y* axis) as a function of susceptible host density (*x* axis) increases for horizontal transmission ($R_{hor}$) (part **a**) and decreases for vertical transmission ($R_{ver}$) (part **b**). $R_0$ denotes the average number of new infected cells produced by a single infected cell (and its progeny virions) in an otherwise susceptible population. **c**,**d** | The lytic and lysogenic pathways, in which the 'mother' virus is denoted by the label 'mother 0', where 0 denotes that this is the focal infected cell. Using illustrative examples, each of the panels shows how variation in susceptible cell population density affects viral transmission strategies. When there are few susceptible hosts, only one of the virions produced in the burst of the mother virus leads to a progeny virus; hence, $R_0$ of this horizontal transmission strategy is 1, denoted by $R_{hor} = 1$ (corresponding to point I in the graph shown in part **a**). By contrast, when there are more susceptible hosts, three of the virions produced in the burst of the mother virus lead to a progeny virus; hence, $R_{hor} = 3$ (corresponding to point II in the graph shown in part **a**). When there are few susceptible hosts, a single infected cell faces less competition and undergoes three divisions before loss and/or decay; hence, $R_0$ for this vertical transmission strategy is 3, denoted by $R_{ver} = 3$ (corresponding to point III in the graph show in part **b**). By contrast when there are more susceptible hosts, a single infected cell faces more competition and undergoes one division before loss and/or decay; hence, $R_{ver} = 1$ (corresponding to point IV in the graph show in part **b**). The numbers of new infected cells (for example, one or three) are illustrative and will depend on quantitative life history parameters and the ecological context.

Cellular multiplicity of infection
The discrete number of viruses that have infected a given cell. 'Cellular multiplicity of infection' is distinct from the commonly used term 'multiplicity of infection' (that is, the population-level ratio of the number of virus particles to the number of cells).

Adsorption
Viral attachment to a host cell.

Lysogens
Cells with a prophage, which is either integrated into the cellular genome or is extrachromosomal.

Virocell
A cell infected by a virus that reshapes cellular physiology so that it is controlled by viral genetic programmes.

Heteroimmunity
Denoting when two phages have heterotypic (unrelated) genetic elements (that is, repressor and cognate operator) to control the lytic cycle and, as a consequence, neither prophage is able to prevent infection of the host by the other virus.

Superinfection
Viral infection of a cell harbouring another virus.

Cytoplasmic incompatibility
Caused by maternally inherited bacteria, a situation in which factors in the cytoplasm of two gametes are not compatible, preventing the formation of viable offspring.

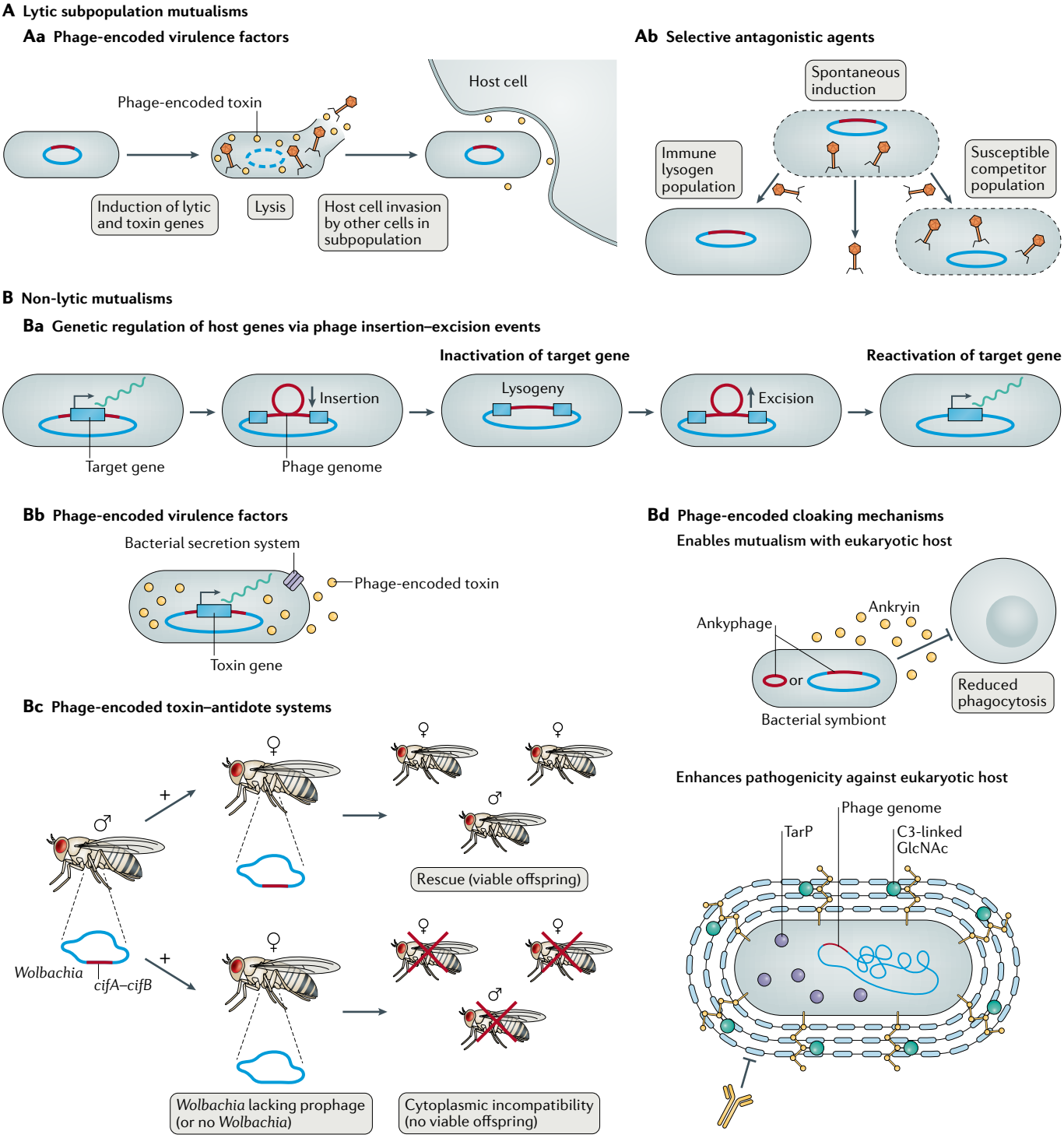
before they encounter and infect another host, then the invasion will fail at the population level, even if the infection was successful at the scale of an individual cell (FIG. 2cI). This gap between intracellular and intercellular dynamics reveals the potential benefits of vertical transmission under some conditions (FIG. 2dIII), as opposed to horizontal transmission by virulent infections in other conditions (FIG. 2cII). In an environment where there are few hosts but sufficient resources for cell growth, a phage that manages to encounter and infect a host cell (and integrate its genome) may still be able to proliferate. As long as the lysogen typically divides at least once, on average, then a temperate strategy can provide the viral lineage with an evolutionary benefit (FIG. 2dIII). The proliferation of a virus depends on processes of encounter, infection, establishment, induction and efficiency of lysis, which themselves depend on host cell number, cellular status and other factors. Vertical transmission requires that cells survive long enough to divide and that prophages (integrated or extrachromosomal) are passed on with high fidelity to progeny cells (FIG. 2d). Hence, phage-associated traits that increase either cell division or host cell survival (due to, for example, defence, improved stress response or heteroimmunity) can lead to direct fitness benefits for both the virus and the host, and even the establishment of mutualistic partnerships. By contrast, horizontal transmission requires that viruses are able to infect cells efficiently and release new infectious particles and that virus particles survive in the extracellular environment long enough to infect new cells (FIG. 2c). Therefore, modifications to the genetic architecture of switches could lead to differences in the ways that a phage genome responds to the intracellular environment, potentially leading to changes (over long timescales) in the stochastic switch to integrate or lyse, as well as the stochastic switch to initiate lysis after integration. The analysis of eco-evolutionary dynamics over long timescales requires bridging of the gap between thresholds for near-term invasion (for example, via the basic reproduction number) and metrics of viral growth rate and long-term fitness. Indeed, in sufficiently long associations, the fate of viruses may become entangled with that of hosts, leading to fundamental changes in the nature of virus–microorganism relationships.

## Virus–microorganism mutualisms

Given the intertwined nature of virus–host fitness trajectories, it is unsurprising that a variety of mutualistic virus–host interactions have been identified (*sensu* REFS[79–82]; select examples are presented in FIG. 3). Beneficial viral infections of microorganisms can be broadly described as protecting hosts from new viral infections (for example, via superinfection immunity and/or exclusion), enabling hosts to expand their fundamental or realized ecological niche[83] (that is, 'making winners') and/or enhancing host competitiveness through phage-mediated weaponry (for example, wielding phages as selective, antagonistic weapons). Although many of these ideas are known and embraced by phage ecologists (see, for example, REFS[84–88]), they are not necessarily recognized across the broad field of biology. Mutualistic interactions affect microbial population and community-level characteristics, as well as individual cell fates, and generally extend the time frame in which viruses are in close physical association, or symbiosis, with hosts.

Fig. 3 | **Examples of temperate phage–bacterium mutualisms. A** | Mutualistic phage–host interactions may depend upon initiation of lysogenic–lytic switching in a subpopulation of the host. **Aa** | Some phage-encoded virulence factors (for example, Shiga toxins[104]) are expressed upon induction of the lytic cycle. **Ab** | Phage progeny released from a subpopulation of spontaneously induced host cells can function as selective antagonistic agents against non-immune competitors[111–113]. In this example, some fraction of host cells succumb to lytic infection, but the remaining non-induced cells benefit from the sacrifice of their lysed siblings. **B** | Alternatively, phage–host mutualisms can manifest themselves when prophages are in lysogenic states. **Ba** | Phages can function as part of the regulatory machinery of their microbial hosts via active lysogeny, where reversible phage insertion–excision events influence expression of host genes (reviewed in REF.[109]). **Bb** | In contrast to panel **Aa**, other phage-encoded virulence factors (for example, cholera or diphtheria toxins[102,103]) are expressed while the phage is in a lysogenic state. **Bc** | Phage-encoded toxin–antidote systems have been implicated in microorganism–insect interactions. Phage-derived genes (*cifA*–*cifB*) in parasitic *Wolbachia* promote insect host reproductive manipulation (cytoplasmic incompatibility)[132]; for additional examples of virus–microorganism interactions within metazoan hosts, see BOX 3). Female insect hosts that are infected with *Wolbachia* bacteria that lack a homologous phage-encoded *cifA* gene are incapable of producing viable offspring when mating with a male infected with a *Wolbachia* (*cifA*–*cifB*+) strain. The *cifA* gene product rescues cytoplasmic incompatibility. **Bd** | Temperate phages can encode factors that aid their microbial hosts in evading eukaryotic immune systems. These factors can cloak the foreign microorganism, enabling the microorganism to persist in its host as a mutualist (for example, sponge symbionts[106]; top panel), or increase the pathogenicity of an invading bacterium (for example, methicillin-resistant *Staphylococcus aureus*[105]; bottom panel). In the top panel, a bacterial symbiont of a sponge contains a prophage (termed an 'ankyphage') encoding an ankryin domain-containing protein. Expression of this protein modulates the sponge immune response, facilitating sponge–bacterium coexistence. In the bottom panel, *S. aureus* prophages encode an alternative wall teichoic acid glycosyltransferase (TarP) that modifies the positioning of *N*-acetylglucosamine (GlcNAc) on the bacterial host cell surface. *S. aureus* harbouring this modification is able to evade the host's immune system. Figures **Aa**, **Ba** and **Bb** adapted from REF.[109], Springer Nature Limited. Part **Ab** is adapted from REF.[88], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **Bd** (top) adapted with permission from REF.[106], Elsevier. Part **Bd** (bottom) adapted from REF.[133], Springer Nature Limited.

First, superinfection immunity arises when infection of a host with one virus prevents secondary infection of that cell by similar virus types. Mechanistically, a repressor protein is typically capable of ensuring stable establishment of lysogeny and defending against new viral infections either through prevention of or interference with incoming viral nucleic acids (for example, binding and degrading similar nucleic acid sequences).




Additional immunity and/or resistance mechanisms may arise when prophage establishment leads to changes in host cell receptor components necessary for viral recognition and/or attachment[89–92]. Although superinfection immunity has been observed for decades[30], it is not yet feasible to predict whether prophages can confer protection against another virus on the basis of sequence data alone. This poses a challenge as we try to understand 'who infects whom' in complex communities or at ecological scales (BOX 2): a virus that is not observed to infect a particular host strain in a given assay may not be capable of doing so, or an infection may have been impeded if the host already had superinfection immunity. A recent analysis of single-cell amplified genomes of SUP05 marine bacteria suggests lysogens are more immune to co-infection with other prophages and, albeit to a lesser extent, extrachromosomal viruses[93]. The generation of additional model systems and genome sequence space may ultimately make genomic identification of immunity tractable.

Second, temperate phages can help a host lineage become a 'winner' if genomes of this temperate phage

Co-infection
The simultaneous infection of a cell by more than one virus; the viruses need not be closely related.

Box 3 | **Towards understanding virus–microorganism–metazoan associations**

Virtually all metazoans harbour microbial symbionts within their tissues; viruses that infect these microbiotas are also likely to be ubiquitous within metazoan tissues. Analysis of so-called 'Russian doll' symbioses, named after nested wooden dolls that sit one inside the other (see, for example, REFS[79,157–159]), reveals that viruses can manipulate their microbial hosts, but also the eukaryotic hosts of their microbial hosts[160]. Few Russian doll symbioses have been comprehensively characterized, but representative examples argue for viewing these interactions through a holobiont lens (that is, the collective of the host and all its symbionts). For example, an obligate and ubiquitous bacterial symbiont (*Wolbachia*) was originally thought to encode a toxin that provides antipredatory defences for its insect host against a parasitizing wasp. However, the toxin is actually encoded by a prophage (WO) in the highly-reduced *Wolbachia* genome (FIG. 3Bc); this prophage has now been documented in many insect hosts of *Wolbachia*[161,162] (reviewed in REF.[163]). In other cases, subversion of host immunity is accomplished via maladaptive viral pattern recognition receptors and suppression of phagocytosis, such as in chronic wound-forming *Pseudomonas aeruginosa*. It was also recently reported that when *P. aeruginosa* was infected by phage Pf, the immune system of a mouse model host was suppressed, reducing the clearance of chronic wounds containing *P. aeruginosa* in humans[164]. In the virus–fungal endophyte–tropical panic grass symbiosis, the fungus (*Curvularia protuberata*) is more thermotolerant when it is positive for *Curvularia* thermal tolerance virus[165]. Viral infections enable the fungal endophyte (inside its grass host, *Dichanthelium lanuginosum*) to survive in soil temperatures of up to 65 °C at Yellowstone National Park (USA). The virus–dinoflagellate (family Symbiodiniaceae)–stony coral system may be ripe for similar enquiries (see, for example, REF.[166]), as Symbiodiniaceae members are known to influence host thermotolerance by up to 1.5 °C, and preliminary studies suggest that Symbiodiniaceae-infecting viruses influence the thermotolerance of the dinoflagellate cells they infect[45]. These interactions may have important ecological implications for coral bleaching resistance and/or susceptibility, and ultimately the survival of coral reefs in warming seas. Finally, such nested symbioses may extend beyond three levels, as in virus–bacterium–protozoan–termite systems[167]. Although the study of the roles of viruses in multipartite symbioses remains in its nascent stage, continued work should reveal the influence of these symbionts on metazoans, particularly when there are not bright lines demarcating where one organism's life cycle begins and another's ends.

integrate into genomes of their host, forming lysogens, and these lysogens then outcompete other cells, leading to expansion of the host's niche. Mechanistically, expansion of the host's realized or fundamental niches may occur through improved competitive ability via superinfection immunity (see earlier, realized niche only), or through conferral of new traits (for example, cellular regulation or virulence factors; see later). Making winners (as 'puppet masters', *sensu* REF.[94]) contrasts with piggybacking on 'winning' hosts (*sensu* REF.[95], and see the discussion in REFS[96,97]), but they are not mutually exclusive. It is possible that some viral lineages respond to environmental cues, whereas others modify the trajectories of their hosts through infection. Virus–microorganism associations may evolve towards mutualisms when viral infections confer new cellular-level traits that enable both the virus and the host microorganism to proliferate in additional environments (analogous to the expansion of a fundamental niche). For instance, virus–microorganism interactions can enhance the invasion, establishment and distribution of microbial pathogens within and among mammalian hosts[98]. It was recently discovered that certain strains of enteric bacteria differentially bind multiple polioviruses to their cell surface, increasing the viral contact rate with human host cells, resulting in higher co-infection and recombination, even under low multiplicity of infection[99].

Third, lysogens can exhibit enhanced competitiveness via the acquisition of new viral-encoded traits, including virulence, toxin and/or antibiotic resistance genes, and modulation of regulatory machinery. Enhanced competitiveness can also be achieved through the occasional release of phages as selective, antagonistic weapons. These traits may be acquired in multiple ways, including transduction[40,100], as well as the expression of virus-encoded genes (reviewed in REFS[31,87,101]). Virulence factors may be expressed by prophages during lysogeny (for example, cholera toxin of *V. cholerae*[102] or diphtheria toxin of *Corynebacterium diphtheriae*[103]; FIG. 3Bb) or may be co-expressed with viral lytic genes upon prophage switching to the lytic pathway (for example, Shiga toxin in *E. coli* O157:H7 (REF.[104]); FIG. 3Aa). Indeed, most 'bacterial' toxins are encoded by the toxic prophage of the bacteria (BOX 3). These different strategies are related to the fitness of a given virus–microorganism pair in a particular niche[25] and involve lysogenic switching, principally through a global host stress regulon (that is, the SOS response). More recently, it has been revealed that some temperate phages can encode factors that aid their microbial hosts in directly subverting the eukaryotic immune system. This has been demonstrated to enhance microbial pathogenicity (for example, methicillin-resistant *Staphylococcus aureus*[105]; FIG. 3Bd, bottom panel) and also to facilitate mutualistic interactions between microorganisms and their eukaryotic partners (for example, sponge microbial symbionts[106]; FIG. 3Bd, top panel). Intriguingly, SOS-mediated expression of phage-encoded genes may also be relevant in metazoans that harbour symbiotic microorganisms. For example, in silico analyses suggest prophages may similarly shift *Vibrio coralliilyticus* towards pathogenic or antagonistic interactions with their host when residing on coral colonies[107].

Prophages can also help hosts to become winners by modulating their regulatory machinery. This type of cooperative virus–microorganism behaviour, termed 'active lysogeny', results when virus insertion–excision events engineer the cellular genome[108] or control a host's gene expression by interrupting host genes or regulatory regions (reviewed in REF.[109]; FIG. 3Ba). In the few characterized examples, these phage regulatory switches mediate conditional expression of virulence factors in microbial pathogens (for example, in *Listeria* and *Streptococcus* strains). These interactions are beneficial for infected hosts on the basis of the logic of 'the enemy of my enemy is my friend': virus-mediated expression of toxins or host virulence factors assists the host cell in competitive interactions with other microorganisms. These virus-mediated responses can potentially lead to community-level shifts in environmental microbial communities or in the microbiotas residing in metazoans (that is, in virus–microorganism–metazoan 'Russian doll' symbioses; BOX 3).

Finally, viruses may also function as selective antagonistic weapons by coexisting with one host but killing off different hosts through occasional lysis events that affect a small fraction of the first host population (for an example see REF.[81]; reviewed in REF.[88]). In these instances, the lytic pathway is activated in a subpopulation of lysogenized cells in a nutrient-dependent manner, resulting in the release of viral progeny that attack non-immune

competitors[110] (FIG. 3Ab). Although the fitness advantage for the host microorganism may be limited if the principal outcome is lysogenization of previously phage-free populations, this mechanism of competition enables the invasion and establishment of new niches[111–113]. This strategy was recently documented in a two phage–single bacterium system, which demonstrates the possibility of reciprocal attacks by genetically similar, but heteroimmune, phages that share an integration site in their common host, a species of the marine *Roseobacter* lineage[114]. This type of biological warfare has also been proposed to modulate competition among members of the microbiota of the freshwater cnidarian *Hydra vulgaris* (another example of a Russian doll symbiosis; BOX 3). In this instance, one microbiota member, a *Curvibacter* species, has an inducible prophage that lytically infects another microbiota member, a *Duganella* strain[115]. As microbial symbionts influence the emergent physiological properties of their metazoan hosts in many systems, including ecological engineering species such as corals, virus-mediated shifts in metazoan microbiotas can potentially impact communities of macroorganisms and ecosystem functions (for example, nutrient cycling or productivity).

## Viruses infecting alternative hosts

In natural environments, virus–microorganism interactions occur in the context of diverse communities, where many suboptimal microorganisms may be potential virus targets[116]. The consequences and relevance of suboptimal infections have only begun to be the subject of inquiry, primarily in marine systems. Cyanophages, viruses that infect cyanobacteria, nicely illustrate variation in infection efficiency among virus types. Single-cell measurements of virulence (44–82%) and burst sizes (21–43 infective viruses per cell) differed substantially in the evaluation of two phages during infection of two *Synechococcus* strains, with the single-cell variation ranging from 2 to 100 virions produced per cell[26]. Notably, average burst sizes for one phage (Syn9) differed substantially from one host to the other, whereas the average burst size for the second phage (S-TIM5) was relatively invariant across the same two hosts. Cyanophage isolates within the same population can differ in infection efficiency given the same host over orders of magnitude[27], which suggests that host range is a highly evolvable trait. A mechanistic illustration of struggling or stalling at each infection stage was shown in a detailed study of phage infection of *Prochlorococcus* and *Synechococcus* strains[29]. When a phage could enter the target host, infections failed at different stages of typical lytic pathways: after injection, after transcription and even after virus particles had started to assemble[29]. This study revealed that inefficient infections may arise due to changes in host cell physiology that are not necessarily detectable via extracellular changes alone.

The prevalence and status of suboptimal host infections has also been investigated for marine heterotroph–phage interactions. Via large-scale (38 phages and 19 hosts) quantitative host range assays in strains of *Cellulophaga* (a genus of the bacterial phylum Bacteroidetes), phages were shown to infect as many as 17 of 19 tested host strains and, remarkably, infection efficiency of a phage within a single population (approximately species-level designation) could vary by up to 10 orders of magnitude[28]. Subsequent investigation of a single *Cellulophaga* phage (Φ38:1) on both an efficiently infected host (a more 'optimal host') and an inefficiently infected host (a 'suboptimal host') showed that the suboptimal infection was inefficient at many steps of the infection cycle, from attachment to the cell surface through to cell lysis[24,25,117,118]. This indicates that phages in nature can encounter closely related hosts, and their infection efficiency can be drastically different on each of those hosts, whereby the phage will still reproduce, but only after overcoming one or multiple inefficiencies during the infection cycle of its host[24,25]. Finally, even efficient infections by two viruses that infect the same host (for example, in separate experiments) can differentially metabolically reprogramme these cells. In this case, the resultant host metabolic differences are driven by one virus being genetically well complemented by the host genome and efficiently infecting it with minimal reprogramming, whereas the other virus must more drastically reprogramme the cell and access its intracellular resources to achieve efficient infection[69]. Together, these findings illustrate that the same cell can potentially be transformed into virocells that are metabolically distinct depending on the infecting phage, with consequently distinct ecosystem-level impacts[72]. Scaling such measurements across diverse virus–host model systems (encompassing generalist and specialist viruses[119]) and conditions is critical to uncovering generalizable 'rules' for the roles of viruses in ecosystems, particularly those containing diverse communities.

## Omics and the infection continuum

Viral genomes are being discovered and catalogued at unprecedented rates. These genomes can now routinely be used to map the ecological distributions of viruses and their drivers, to make in silico predictions about the hosts being infected (BOX 2) and to assess the ways in which viruses might directly modulate microbial metabolisms through virus-encoded auxiliary metabolic genes or regulators. However, these ecogenomic inferences are just a first critical step in assessing the ecosystem-level impacts of viruses. Studies to date have specifically focused on highly efficient lytic or persistent lysogenic infection states — the extremes of the infection continuum — in single virus–host pairings. This leaves virus–host interactions at the middle of the infection continuum (for example, those that dynamically switch between lytic and non-lytic infections) largely unstudied.

Omics-based inferences (reviewed in REF.[120]) suggest there is a critical need for the analysis of lysogeny (and latency), inefficient lytic infections and viral interactions during co-infections in cultured organisms and in natural systems. First, in a survey of cultured bacterial and archaeal genomes, non-integrated lysogenic infections were found to be more common (nearly one in six viruses) than previously estimated. A substantial number of viruses (1,756 of 12,498, 14%) identified from these ~15,000 microbial isolate genomes were found to exist as 'extrachromosomal prophages'[11].

Homoimmunity
Denoting when a prophage confers immunity against infection by similar viruses because both the resident prophage and the incoming virus contain homotypic (identical or nearly identical) genetic elements (that is, repressor and cognate operator) to control the lytic cycle.

Carrier states
Cells that are considered to have a chronic infection.

Although these sequences were derived from microbial genomes generated from bacterial and archaeal isolates that were maintained in culture for years to decades, the impacts of these infections on host cells are relatively undescribed. Second, experiments with marine phage–host model systems reveal that regulation of infection efficiency is multilayered and extends beyond 'surface'-based resistance[24,25]; microbial defence against viral attack differs depending on whether a phage uses a generalist strategy or a specialist strategy (for example, in cyanobacteria, resistance to generalist phage attack tends to be intracellular, whereas resistance to specialist phage attack tends to be extracellular[29]); and a single host cell can have vastly different ecosystem impacts depending on whether it is uninfected, infected by an efficient virus or infected by a less efficient virus[72]. Third, growing evidence suggests co-infection by viruses is predominant in microorganisms in natural systems[12,93,119,121–124]; in some instances half of infected cultures or single cells are infected with two to three identifiable viruses[93,125]. Such viruses are likely to manipulate a host cell's readiness for infection via heteroimmunity and/or homoimmunity[30,89], which implies that virus–virus interactions may also drive microbial physiology in diverse communities[126]. For the most part, our understanding of virus–host interactions and their physiological impacts is traditionally constrained to single virus–host pairings, which misses virus–virus interactions (including those cases where viruses may interact to overcome host CRISPR immunity, as in anti-CRISPR phage counterdefence[127]).

The continued expansion of virus–microorganism sequence data raises the question of whether there are sequence-based indicators to predict lysogeny, variably lytic infection modes and virus–virus interactions in the environment. Bioinformatics methods are available to infer the prevalence of lysogens[128] (reviewed in REF.[31]). These methods focus on identifying proxy genes (for example, excisionase, integrase and virulence) that are associated with lysogenic lifestyles[31,95,129]. Although such lists of proxy genes are currently constrained to those proxy genes known from canonical model systems, they might be expanded more broadly through virome-enabled induction experiments (see, for example, REF.[44]), and they must also be used with caution when one is trying to link patterns of indicator gene abundance to underlying infection mechanisms[95–97,129,130].

However, beyond lysogenic indicator genes, there is a dearth of diagnostic genes available for inferring chronic infections from omics data. For double-stranded DNA viruses, there are now clear examples in which a virus can infect one host well and another host poorly (for example, infections of the abundant marine phage Φ38:1 in different hosts[31,117]). However, such diagnostics might not be possible at the level of the genome; infection dynamics may need to be evaluated at the transcript level. Furthermore, if eukaryotic viruses are any indication, then RNA and single-stranded DNA viruses may be ideal targets for inferring chronic infections from omics data as they are known to more commonly adopt chronic infection modes, at least in cultivated isolates[131]. Identifying genetic markers for such persistent infection modes constitutes a ripe area for future research and may help to resolve non-standard phage types (for example, filamentous phages) and/or carrier states for phages. Omics approaches have advanced the field of environmental virology by helping to structure viral genome sequence space, and revealing that virus–host interactions that frequently switch between lytic and non-lytic infection modes are common in diverse systems. The development of further tools and analytical approaches that can detect virus–host associations, including co-infections, and illuminate dynamic lysogenic switching along a continuum of infection will critically inform developing theoretical frameworks for when it is (and is not) beneficial for viruses to lyse their hosts.

## Conclusions and outlook

More than a century of viral research, primarily on laboratory model systems, established a solid basis for defining the rules of life for viruses of microorganisms; these rules have also been applied or extrapolated to phages in nature, with caveats. Rapid sequencing of environmental samples, technological developments, isolation and characterization of new model systems and growing interest in the diversity of Earth's virosphere have propelled expansion of the horizons of our knowledge of viruses in nature. This Review has focused on seminal work in phage biology and the rules of life principles that emerged from it, while highlighting that these rules are being revised and rewritten. As we have shown, there is increasing awareness that infections are not static but are fluid along an infection continuum; there is utility in quantifying viral fitness from a cell-centric perspective; and infections in nature (unlike those typically used in laboratory systems) may occur more commonly on suboptimal, rather than on optimal, hosts (BOX 1). This Review has centred on principles derived from phages with double-stranded DNA genomes. We anticipate that future work will reveal additional nuances in the rules of life for viruses of microorganisms (particularly for viruses with other genomic and morphologic architectures) and continue to solidify understanding of how diverse viruses can shape microbial hosts, ecosystems and the biosphere.

Published online: 24 March 2021


1. Proctor, L. M. & Fuhrman, J. A. Viral mortality of marine bacteria and cyanobacteria. *Nature* **343**, 60–62 (1990).
2. Bergh, O., Børsheim, K. Y., Bratbak, G. & Heldal, M. High abundance of viruses found in aquatic environments. *Nature* **340**, 467–468 (1989).
3. Cai, L. et al. Active and diverse viruses persist in the deep sub-seafloor sediments over thousands of years. *ISME J.* **13**, 1857–1864 (2019).
4. Reyes, A., Semenkovich, N. P., Whiteson, K., Rohwer, F. & Gordon, J. I. Going viral: next-generation sequencing applied to phage populations in the human gut. *Nat. Rev. Microbiol.* **10**, 607–617 (2012).
5. Fuhrman, J. A. Marine viruses and their biogeochemical and ecological effects. *Nature* **399**, 541–548 (1999).
6. Weinbauer, M. G. Ecology of prokaryotic viruses. *FEMS Microbiol. Rev.* **28**, 127–181 (2004).
7. Vega Thurber, R. L., Payet, J. P., Thurber, A. R. & Correa, A. M. S. Virus-host interactions and their roles in coral reef health and disease. *Nat. Rev. Microbiol.* **15**, 205–216 (2017).
8. Zimmerman, A. E. et al. Metabolic and biogeochemical consequences of viral infection in aquatic ecosystems. *Nat. Rev. Microbiol.* **18**, 21–34 (2020).
9. Wilhelm, S. W. & Suttle, C. A. Viruses and nutrient cycles in the sea. *Bioscience* **49**, 781–788 (1999). **Seminal work modelling how viral activity in the oceans prevents up to a quarter of organic matter from being exported to higher trophic levels; instead, this matter is recycled (by viral lysis) into a form that can be assimilated by microorganisms**.

10. Calendar, R. L. *The Bacteriophages* 2nd edn (Oxford University Press, 2005).
11. Sullivan, M. B., Weitz, J. S. & Wilhelm, S. W. Viral ecology comes of age. *Environ. Microbiol. Rep.* **9**, 33–35 (2017).
12. Roux, S., Hallam, S. J., Woyke, T. & Sullivan, M. B. Viral dark matter and virus–host interactions resolved from publicly available microbial genomes. *eLife* **4**, e08490 (2015).
13. Paez-Espino, D. et al. Uncovering Earth's virome. *Nature* **536**, 425–430 (2016).
**An in silico catalogue of the diversity of viruses on Earth that serves as the foundation for the Joint Genome Institute's growing IMG/VR database**.
14. Emerson, J. B. et al. Host-linked soil viral ecology along a permafrost thaw gradient. *Nat. Microbiol.* **3**, 870–880 (2018).
15. Stough, J. M. A. et al. Diversity of active viral infections within the *Sphagnum* microbiome. *Applied Environ. Microbiol.* https://doi.org/10.1128/AEM.01124-18 (2018).
16. Gregory, A. C. et al. Marine DNA viral macro- and microdiversity from pole to pole. *Cell* **177**, 1109–1123.e1114 (2019).
17. De Corte, D. et al. Viral communities in the global deep ocean conveyor belt assessed by targeted viromics. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2019.01801 (2019).
18. Jang, H. B. et al. Taxonomic assignment of uncultivated prokaryotic virus genomes is enabled by gene-sharing networks. *Nat. Biotechnol.* **37**, 632–639 (2019).
19. Roux, S. A viral ecogenomics framework to uncover the secrets of nature's "microbe whisperers". *mSystems* **4**, e00111–e00119 (2019).
20. Roux, S. et al. Minimum information about an uncultivated virus genome (MIUViG). *Nat. Biotechnol.* **37**, 29 (2018).
21. Hobbs, Z. & Abedon, S. T. Diversity of phage infection types and associated terminology: the problem with 'lytic or lysogenic'. *FEMS Microbiol. Lett.* https://doi.org/10.1093/femsle/fnw047 (2016).
22. Hay, I. D. & Lithgow, T. Filamentous phages: masters of a microbial sharing economy. *EMBO Reports* **20**, e47427 (2019).
23. McLeod, S. M., Kimsey, H. H., Davis, B. M. & Waldor, M. K. CTXphi and Vibrio cholerae: exploring a newly recognized type of phage-host cell relationship. *Mol. Microbiol.* **57**, 347–356 (2005).
24. Howard-Varona, C. et al. Regulation of infection efficiency in a globally abundant marine Bacteriodetes virus. *ISME J.* **11**, 284–295 (2017).
25. Howard-Varona, C. et al. Multiple mechanisms drive phage infection efficiency in nearly identical hosts. *ISME J.* **12**, 1605–1618 (2018).
26. Kirzner, S., Barak, E. & Lindell, D. Variability in progeny production and virulence of cyanophages determined at the single-cell level. *Environ. Microbiol. Rep.* **8**, 605–613 (2016).
27. Gregory, A. C. et al. Genomic differentiation among wild cyanophages despite widespread horizontal gene transfer. *BMC Genomics* **17**, 930 (2016).
28. Holmfeldt, K. et al. Large-scale maps of variable infection efficiencies in aquatic *Bacteroidetes* phage-host model systems. *Environ. Microbiol.* **18**, 3949–3961 (2016).
29. Zborowsky, S. & Lindell, D. Resistance in marine cyanobacteria differs against specialist and generalist cyanophages. *Proc. Natl Acad. Sci. USA* https://doi.org/10.1073/pnas.1906897116 (2019).
**A meticulous investigation revealing that cyanobacteria defend against specialist phages by blocking their entry, whereas generalist phage infections are arrested intracellularly; thus generalist phages may be more common agents of horizontal gene transfer and co-infection**.
30. Lwoff, A. Lysogeny. *Bacteriol. Rev.* **17**, 269–337 (1953).
31. Howard-Varona, C., Hargreaves, K. R., Abedon, S. T. & Sullivan, M. B. Lysogeny in nature: mechanisms, impact and ecology of temperate phages. *ISME J.* **11**, 1511 (2017).
32. Abedon, S. T. The murky origin of Snow White and her T-even dwarfs. *Genetics* **155**, 481–486 (2000).
33. Demerec, M. & Fano, U. Bacteriophage-resistant mutants in Escherichia coli. *Genetics* **30**, 119–136 (1945).
34. Bronfenbrenner, J. J. & Korb, C. Studies on the bacteriophage of d'Herelle: I. Is the lytic principle volatile? *J. Exp. Med.* **41**, 73–79 (1925).
35. Kourilsky, P. & Knapp, A. Lysogenization by bacteriophage lambda: III. - Multiplicity dependent phenomena occuring upon infection by lambda. *Biochimie* **56**, 1517–1523 (1975).
36. St-Pierre, F. & Endy, D. Determination of cell fate selection during phage lambda infection. *Proc. Natl Acad. Sci. USA* **105**, 20705 (2008).
37. Zeng, L. et al. Decision making at a subcellular level determines the outcome of bacteriophage infection. *Cell* **141**, 682–691 (2010).
**Re-examination of the phage λ decision switch via single-cell tracking of infection fates, revealing how increasing cellular multiplicity of infection increases the stochastic tendency towards lysogeny after infection**.
38. Trinh, J. T., Székely, T., Shao, Q., Balázsi, G. & Zeng, L. Cell fate decisions emerge as phages cooperate or compete inside their host. *Nat. Commun.* **8**, 14341 (2017).
39. Joh, R. I. & Weitz, J. S. To lyse or not to lyse: Transient-mediated stochastic fate determination in cells infected by bacteriophages. *PLOS Comput. Biol.* **7**, e1002006 (2011).
40. Fillol-Salom, A. et al. Bacteriophages benefit from generalized transduction. *PLOS Pathog.* **15**, e1007888 (2019).
41. Howard-Varona, C. et al. Fighting fire with fire: phage potential for the treatment of E. coli O157 infection. *Antibiotics* **7**, 101 (2018).
42. Pratama, A. A. & van Elsas, J. D. A novel inducible prophage from the mycosphere inhabitant Paraburkholderia terrae BS437. *Sci. Rep.* **7**, 9156 (2017).
43. Jiang, S. C. & Paul, J. H. Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. *Mar. Ecol. Prog. Ser.* **104**, 163–172 (1994).
44. Brum, J. R., Hurwitz, B. L., Schofield, O., Ducklow, H. W. & Sullivan, M. B. Seasonal time bombs: dominant temperate viruses affect Southern Ocean microbial dynamics. *ISME J.* **10**, 437–449 (2016).
**Demonstration that lysogenic activity is favoured in low-productivity polar months (and lytic activity is favoured in high-productivity months), providing support for decades-old ecological hypotheses on the link between abiotic factors and viral strategies**.
45. Levin, R. A., Voolstra, C. R., Weynberg, K. D. & van Oppen, M. J. H. Evidence for a role of viruses in the thermal sensitivity of coral photosymbionts. *ISME J.* **11**, 808–812 (2017).
46. Vega Thurber, R. L. et al. Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral *Porites compressa*. *Proc. Natl Acad. Sci. USA* **105**, 18413–18418 (2008).
47. Correa, A. M. S. et al. Viral outbreak in corals associated with an *in situ* bleaching event: atypical herpes-like viruses and a new megavirus infecting *Symbiodinium*. *Front. Microbiol.* **7**, 127 (2016).
48. Lawrence, S. A., Davy, J. E., Aeby, G. S., Wilson, W. H. & Davy, S. K. Quantification of virus-like particles suggests viral infection in corals affected by *Porites* tissue loss. *Coral Reefs* **33**, 687–691 (2014).
49. Lawrence, S. A., Floge, S. A., Davy, J. E., Davy, S. K. & Wilson, W. H. Exploratory analysis of *Symbiodinium* transcriptomes reveals potential latent infection by large dsDNA viruses. *Environ. Microbiol.* **19**, 3909–3919 (2017).
50. Weynberg, K. D. et al. Prevalent and persistent viral infection in cultures of the coral algal endosymbiont *Symbiodinium*. *Coral Reefs* **36**, 773–784 (2017).
51. Ptashne, M. et al. How the λ repressor and cro work. *Cell* **19**, 1–11 (1980).
52. Warwick-Dugdale, J., Buchholz, H. H., Allen, M. J. & Temperton, B. Host-hijacking and planktonic piracy: how phages command the microbial high seas. *Virol.* **16**, 15 (2019).
53. Silpe, J. E. & Bassler, B. L. A host-produced quorum-sensing autoinducer controls a phage lysis-lysogeny decision. *Cell* **176**, 268–280.e213 (2019).
54. Erez, Z. et al. Communication between viruses guides lysis–lysogeny decisions. *Nature* **541**, 488 (2017).
**Demonstration that viruses can 'communicate' to decide between lysis and lysogeny by co-opting a host system: extracellular release of small peptides**.
55. Stokar-Avihail, A., Tal, N., Erez, Z., Lopatina, A. & Sorek, R. Widespread utilization of peptide communication in phages infecting soil and pathogenic bacteria. *Cell Host Microbe* **25**, 746–755 (2019).
56. Ofir, G. & Sorek, R. Contemporary phage biology: from classic models to new insights. *Cell* **172**, 1260–1270 (2018).
57. McNamara, J. M. & Houston, A. I. State-dependent life histories. *Nature* **380**, 215–221 (1996).
58. Tan, D. et al. High cell densities favor lysogeny: induction of an H20 prophage is repressed by quorum sensing and enhances biofilm formation in *Vibrio anguillarum*. *ISME J.* **14**, 1731–1742 (2020).
59. Pleška, M., Lang, M., Refardt, D., Levin, B. R. & Guet, C. C. Phage–host population dynamics promotes prophage acquisition in bacteria with innate immunity. *Nat. Ecol. Evol* **2**, 359–366 (2018).
60. Güemes, A. G. C. et al. Viruses as winners in the game of life. *Annu. Rev. Virol.* **3**, 197–214 (2016).
61. Stewart, F. M. & Levin, B. R. The population biology of bacterial viruses: why be temperate. *Theor. Popul. Biol.* **26**, 93–117 (1984).
**A seminal article that lays out key pressure points that should dictate temperate phage biology**.
62. Lipsitch, M., Siller, S. & Nowak, M. A. The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* **50**, 1729–1741 (1996).
63. Frank, S. A. Models of parasite virulence. *Q. Rev. Biol.* **71**, 37–78 (1996).
64. Weitz, J. S., Li, G., Gulbudak, H., Cortez, M. H. & Whitaker, R. J. Viral invasion fitness across a continuum from lysis to latency. *Virus Evol.* https://doi.org/10.1093/ve/vez006 (2019).
**Theoretical study that examines the impact of ecological factors on the proliferation of viruses, enabled by a cell-centric (rather than a particle-centric) view of viral invasion fitness**.
65. Li, G., Cortez, M. H., Dushoff, J. & Weitz, J. S. When to be temperate: on the fitness benefits of lysis vs. lysogeny. *Virus Evol.* https://doi.org/10.1093/ve/veaa042 (2020).
66. Berngruber, T. W., Froissart, R., Choisy, M. & Gandon, S. Evolution of virulence in emerging epidemics. *PLOS Pathog.* **9**, e1003209 (2013).
67. Wahl, L. M., Betti, M. I., Dick, D. W., Pattenden, T. & Puccini, A. J. Evolutionary stability of the lysis-lysogeny decision: Why be virulent? *Evolution* **73**, 92–98 (2019).
68. Coy, S. R., Alsante, A. N., Van Etten, J. L. & Wilhelm, S. W. Cryopreservation of Paramecium bursaria Chlorella virus-1 during an active infection cycle of its host. *PLoS ONE* **14**, e0211755 (2019).
69. Godfrey-Smith, P. in *Individuals Across the Sciences* (eds Guay, A. & T. Pradeu, T.) (Oxford University Press, 2015).
70. Forterre, P. The virocell concept and environmental microbiology. *ISME J.* **7**, 233 (2013).
**Proposes the virocell concept, which argues that a given cell represents distinct entities when infected versus uninfected by a virus, providing a non-lytic mechanism by which viruses can significantly alter biogeochemical cycles**.
71. Rosenwasser, S., Ziv, C., van Creveld, S. G. & Vardi, A. Virocell metabolism: metabolic innovations during host-virus interactions in the ocean. *Trends Microbiol.* **24**, 821–832 (2016).
72. Howard-Varona, C. et al. Phage-specific metabolic reprogramming of virocells. *ISME J.* **14**, 881–895 (2020).
73. Forterre, P. (ed.) Virocell Concept, The. In *eLS* https://doi.org/10.1002/9780470015902.a0023264 (2012).
74. Diekmann, O., Heesterbeek, H. & Britton, T. *Mathematical Tools for Understanding Infectious Disease Dynamics*. 1st edn, 517 (Princeton University Press, 2012).
75. Diekmann, O., Heesterbeek, J. A. P. & Metz, J. A. J. On the definition and the computation of the basic reproduction ratio R0 in models for infectious diseases in heterogeneous populations. *J. Math. Biol.* **28**, 365–382 (1990).
76. Diekmann, O., Heesterbeek, J. A. P. & Roberts, M. G. The construction of next-generation matrices for compartmental epidemic models. *J. R. Soc. Interface* **7**, 873–885 (2010).
77. van den Driessche, P. & Watmough, J. in *Mathematical Epidemiology. Lecture Notes in Mathematics* Vol. 1945 (eds Brauer, F., van den Driessche, P. & Wu, J.) 159–178 (Springer, 2008).
78. Gandon, S., Day, T., Metcalf, C. J. E. & Grenfell, B. T. Forecasting epidemiological and evolutionary dynamics of infectious diseases. *Trends Ecol. Evol.* **31**, 776–788 (2016).
79. Roossinck, M. J. The good viruses: viral mutualistic symbioses. *Nat. Rev. Microbiol.* **9**, 99–108 (2011).
80. Bondy-Denomy, J. & Davidson, A. R. When a virus is not a parasite: the beneficial effects of prophages on bacterial fitness. *J. Microbiol.* **52**, 235–242 (2014).
81. Nanda, A. M., Thormann, K. & Frunzke, J. Impact of spontaneous prophage induction on the fitness

of bacterial populations and host-microbe interactions. *J. Bacteriol.* **197**, 410 (2015).
82. Obeng, N., Pratama, A. A. & Elsas, J. D. V. The significance of mutualistic phages for bacterial ecology and evolution. *Trends Microbiol.* **24**, 440–449 (2016).
83. Hutchinson, G. E. Concluding remarks. *Cold Spring Harb. Symposia Quant. Biol.* **22**, 415–427 (1957).
84. Taylor, V. L., Fitzpatrick, A. D., Islam, Z. & Maxwell, K. L. The diverse impacts of phage morons on bacterial fitness and virulence. *Adv. Virus Res.* **103**, 1–31 (2019).
85. Hendrix, R. W., Lawrence, J. G., Hatfull, G. F. & Casjens, S. The origins and ongoing evolution of viruses. *Trends Microbiol.* **8**, 504–508 (2000).
86. Casjens, S. R. & Hendrix, R. W. Bacteriophage lambda: early pioneer and still relevant. *Virology* **479-480**, 310–330 (2015).
87. Fortier, L. C. & Sekulovic, O. Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence* **4**, 354–365 (2013).
88. Harrison, E. & Brockhurst, M. A. Ecological and evolutionary benefits of temperate phage: what does or doesn't kill you makes you stronger. *BioEssays* **39**, 1700112 (2017).
89. Berngruber, T. W., Weissing, F. J. & Gandon, S. Inhibition of superinfection and the evolution of viral latency. *J. Virol.* **4**, 10200–10208 (2010).
90. Susskind, M. M., Botstein, D. & Wright, A. Superinfection exclusion by P22 prophage in lysogens of Salmonella typhimurium: III. Failure of superinfecting phage DNA to enter sieA$^+$ lysogens. *Virology* **62**, 350–366 (1974).
91. van Houte, S., Buckling, A. & Westra, E. R. Evolutionary ecology of prokaryotic immune mechanisms. *Microbiol. Mol. Biol. Rev.* **80**, 745 (2016).
92. Dodd, I. B., Shearwin, K. E. & Egan, J. B. Revisited gene regulation in bacteriophage lambda. *Curr. Opin. Genet. Dev.* **15**, 145–152 (2005).
93. Díaz-Muñoz, S. L. Viral coinfection is shaped by host ecology and virus-virus interactions across diverse microbial taxa and environments. *Virus Evol.* **3**, vex011 (2017).
94. Breitbart, M., Bonnain, C., Malki, K. & Sawaya, N. A. Phage puppet masters of the marine microbial realm. *Nat. Microbiol.* **3**, 754–766 (2018).
95. Knowles, B. et al. Lytic to temperate switching of viral communities. *Nature* **531**, 466–470 (2016).
96. Weitz, J. S., Beckett, S. J., Brum, J. R., Cael, B. B. & Dushoff, J. Lysis, lysogeny and virus-microbe ratios. *Nature* **549**, E1–E3 (2017).
97. Knowles, B. & Rohwer, F. Knowles & Rohwer reply. *Nature* **549**, E3–E4 (2017).
98. Wagner, P. L. & Waldor, M. K. Bacteriophage control of bacterial virulence. *Infect. Immun.* **70**, 3985–3993 (2002).
99. Erickson, A. K. et al. Bacteria facilitate enteric virus co-infection of mammalian cells and promote genetic recombination. *Cell Host Microbe* **23**, 77–88.e75 (2018).
100. Davies, E. V., Winstanley, C., Fothergill, J. L. & James, C. E. The role of temperate bacteriophages in bacterial infection. *FEMS Microbiol. Lett.* https://doi.org/10.1093/femsle/fnw015 (2016).
101. Schroven, K., Aertsen, A. & Lavigne, R. Bacteriophages as drivers of bacterial virulence and their potential for biotechnological exploitation. *FEMS Microbiol. Rev.* https://doi.org/10.1093/femsre/fuaa041 (2020).
102. Waldor, M. K. & Mekalanos, J. J. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* **272**, 1910–1914 (1996).
103. Matsuda, M. & Barksdale, L. Phage-directed synthesis of diphtherial toxin in non-toxinogenic *Corynebacterium diphtheriae*. *Nature* **210**, 911–913 (1966).
104. O'Brien, A. D. et al. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science* **226**, 694 (1984).
105. Gerlach, D. et al. Methicillin-resistant *Staphylococcus aureus* alters cell wall glycosylation to evade immunity. *Nature* **563**, 705–709 (2018).
106. Jahn, M. T. et al. A phage protein aids bacterial symbionts in eukaryote immune evasion. *Cell Host Microbe* **26**, 542–550.e545 (2019).
107. Weynberg, K. D., Voolstra, C. R., Neave, M. J., Buerger, P. & Van Oppen, M. J. H. From cholera to corals: Viruses as drivers of virulence in a major coral bacterial pathogen. *Sci. Rep.* **5**, 17889 (2015).
108. Menouni, R., Hutinet, G., Petit, M. A. & Ansaldi, M. Bacterial genome remodeling through bacteriophage recombination. *FEMS Microbiol. Lett.* **362**, 1–10 (2015).
109. Feiner, R. et al. A new perspective on lysogeny: prophages as active regulatory switches of bacteria. *Nat. Rev. Microbiol.* **13**, 641–650 (2015).
110. Duerkop, B. A., Clements, C. V., Rollins, D., Rodrigues, J. L. M. & Hooper, L. V. A composite bacteriophage alters colonization by an intestinal commensal bacterium. *Proc. Natl Acad. Sci. USA* **109**, 17621–17626 (2012).
**Demonstrates that temperate virus infections (including those derived from distinct, spatially separated prophage elements) can 'make winners' out of their hosts by providing the hosts with competitive advantages.**
111. Gama, J. A. et al. Temperate bacterial viruses as double-edged swords in bacterial warfare. *PLoS ONE* https://doi.org/10.1371/journal.pone.0059043 (2013).
112. Davies, E. V. et al. Temperate phages enhance pathogen fitness in chronic lung infection. *ISME J.* **10**, 2553–2555 (2016).
113. Bossi, L., Fuentes, J. A., Mora, G. & Figueroa-Bossi, N. Prophage contribution to bacterial population dynamics. *J. Bacteriol.* **185**, 6467–6471 (2003).
114. Basso, J. T. R. et al. Genetically similar temperate phages form coalitions with their shared host that lead to niche-specific fitness effects. *ISME J.* **14**, 1688–1700 (2020).
**Demonstrates that two genetically similar, but incompatible, temperate phages that lysogenize the same *Roseobacter* host can impart distinct physiological traits on that host; thus, each makes its host 'the winner' under different environmental conditions.**
115. Li, X. Y. et al. Temperate phages as self-replicating weapons in bacterial competition. *J. R. Soc. Interface* https://doi.org/10.1098/rsif.2017.0563 (2017).
116. Weitz, J. S. et al. Phage-bacteria infection networks. *Trends Microbiol.* **21**, 82–91 (2013).
117. Dang, V., Howard-Varona, C., Schwenck, S. & Sullivan, M. B. Variably lytic infection dynamics of large Bacteroidetes podovirus phi38:1 against two Cellulophaga baltica host strains. *Environ. Microbiol.* **17**, 4659–4671 (2015).
118. Holmfeldt, K., Howard-Varona, C., Solonenko, N. & Sullivan, M. B. Contrasting genomic patterns and infection strategies of two co-existing Bacteroidetes podovirus genera. *Environ. Microbiol.* **16**, 2501–2513 (2014).
119. Flores, C. O., Meyer, J. R., Valverde, S., Farr, L. & Weitz, J. S. Statistical structure of host–phage interactions. *Proc. Natl Acad. Sci. USA* **108**, E288–E297 (2011).
120. Parmar, K. M., Gaikwad, S. L., Dhakephalkar, P. K., Kothari, R. & Singh, R. P. Intriguing interaction of bacteriophage-host association: an understanding in the era of omics. *Front. Microbiol.* **8**, 559 (2017).
121. Flores, C. O., Valverde, S. & Weitz, J. S. Multi-scale structure and geographic drivers of cross-infection within marine bacteria and phages. *ISME J.* **7**, 520 (2012).
122. Koskella, B. & Meaden, S. Understanding bacteriophage specificity in natural microbial communities. *Viruses* **5**, 806–823 (2013).
123. Roux, S. et al. Ecology and evolution of viruses infecting uncultivated SUP05 bacteria as revealed by single-cell- and meta- genomics. *eLife* https://doi.org/10.7554/eLife.03125 (2014).
124. Labonte, J. M. et al. Single-cell genomics-based analysis of virus-host interactions in marine surface bacterioplankton. *ISME J.* **9**, 2386–2399 (2015).
125. Munson-McGee, J. H. et al. A virus or more in (nearly) every cell: ubiquitous networks of virus-host interactions in extreme environments. *ISME J.* **12**, 1706–1714 (2018).
126. Díaz-Muñoz, S. L., Sanjuán, R. & West, S. Sociovirology: conflict, cooperation, and communication among viruses. *Cell Host Microbe* **22**, 437–441 (2017).
127. Landsberger, M. et al. Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity. *Cell* **174**, 908 (2018).
128. Kieft, K., Zhou, Z. & Anantharaman, K. VIBRANT: automated recovery, annotation and curation of microbial viruses, and evaluation of viral community function from genomic sequences. *Microbiome* **8**, 90 (2020).
129. Coutinho, F. H. et al. Marine viruses discovered via metagenomics shed light on viral strategies throughout the oceans. *Nat. Commun.* https://doi.org/10.1038/ncomms15955 (2017).
130. Alrasheed, H., Jin, R. & Weitz, J. S. Caution in inferring viral strategies from abundance correlations in marine metagenomes. *Nat. Commun.* **10**, 501 (2019).
131. Roossinck, M. J. Metagenomics of plant and fungal viruses reveals an abundance of persistent lifestyles. *Front.Microbiol.* https://doi.org/10.3389/fmicb.2014.00767 (2015).
132. Bordenstein, S. R. & Bordenstein, S. R. Eukaryotic association module in phage WO genomes from *Wolbachia*. *Nat. Commun.* **7**, 13155 (2016).
133. Gilmore, M. S. & Miller, O. K. A bacterium's enemy isn't your friend. *Nature* **563**, 637–638 (2018).
134. Callanan, J. et al. RNA phage biology in a metagenomic era. *Viruses* **10**, 386 (2018).
135. Dion, M. B., Oechslin, F. & Moineau, S. Phage diversity, genomics and phylogeny. *Nat. Rev. Microbiol.* **18**, 125–138 (2020).
136. Ross, A., Ward, S. & Hyman, P. More is better: Selecting for broad host range bacteriophages. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2016.01352 (2016).
137. de Jonge, P. A. et al. Adsorption sequencing as a rapid method to link environmental bacteriophages to hosts. *iScience* https://doi.org/10.1016/j.isci.2020.101439 (2020).
138. Deng, L. et al. Viral tagging reveals discrete populations in *Synechococcus* viral genome sequence space. *Nature* **513**, 242–245 (2014).
139. Džunková, M. et al. Defining the human gut host–phage network through single-cell viral tagging. *Nat. Microbiol.* https://doi.org/10.1038/s41564-019-0526-2 (2019).
140. Labonte, J. M. et al. Single cell genomics-based analysis of gene content and expression of prophages in a diffuse-flow deep-sea hydrothermal system. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2019.01262 (2019).
141. Edwards, R. A., McNair, K., Faust, K., Raes, J. & Dutilh, B. E. Computational approaches to predict bacteriophage-host relationships. *FEMS Microbiol. Rev.* **40**, 258–272 (2016).
142. Jover, L. F., Romberg, J. & Weitz, J. S. Inferring phage–bacteria infection networks from time-series data. *R. Soc. Open Sci.* **3**, 160654 (2016).
143. Woodcroft, B. J. et al. Genome-centric view of carbon processing in thawing permafrost. *Nature* **560**, 49–54 (2018).
144. Nayfach, S., Shi, Z. J., Seshadri, R., Pollard, K. S. & Kyrpides, N. C. New insights from uncultivated genomes of the global human gut microbiome. *Nature* **568**, 505–510 (2019).
145. Almeida, A. et al. A new genomic blueprint of the human gut microbiota. *Nature* **568**, 499–504 (2019).
146. Pasolli, E. et al. Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell* **176**, 649–662.e620 (2019).
147. Tully, B. J., Graham, E. D. & Heidelberg, J. F. The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans. *Sci. Data* **5**, 170203 (2018).
148. Mihara, T. et al. Linking virus genomes with host taxonomy. *Viruses* **8**, 66 (2016).
149. Laffy, P. W. et al. HoloVir: a workflow for investigating the diversity and function of viruses in invertebrate holobionts. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2016.00822 (2016).
150. Bolduc, B., Youens-Clark, K., Roux, S., Hurwitz, B. L. & Sullivan, M. B. iVirus: facilitating new insights into viral ecology with software and community datasets imbedded in a cyberinfrastructure. *ISME J.* **11**, 7–14 (2017).
151. Baran, N., Goldin, S., Maidanik, I. & Lindell, D. Quantification of diverse virus populations in the environment using the polony method. *Nat. Microbiol.* **3**, 62–72 (2018).
152. Mruwat, N. et al. A single-cell polony method reveals low levels of infected *Prochlorococcus* in oligotrophic waters despite high cyanophage abundances. *ISME J.* (2020).
153. Martínez-García, M., Santos, F., Moreno-Paz, M., Parro, V. & Antón, J. Unveiling viral–host interactions within the 'microbial dark matter'. *Nat. Commun.* **5**, 4542 (2014).
154. Spencer, S. J. et al. Massively parallel sequencing of single cells by epicPCR links functional genes with phylogenetic markers. *ISME J.* **10**, 427–436 (2016).
155. Bickhart, D. M. et al. Assignment of virus and antimicrobial resistance genes to microbial hosts in a complex microbial community by combined long-read assembly and proximity ligation. *Genome Biol.* **20**, 153 (2019).

156. Marbouty, M., Baudry, L., Cournac, A. & Koszul, R. Scaffolding bacterial genomes and probing host-virus interactions in gut microbiome by proximity ligation (chromosome capture) assay. *Sci. Adv.* **3**, e1602105 (2017).
157. Lopez-Madrigal, S., Latorre, A., Porcar, M., Moya, A. & Gil, R. Mealybugs nested endosymbiosis: going into the 'matryoshka' system in *Planococcus citri* in depth. *BMC Microbiol.* https://doi.org/10.1186/1471-2180-13-74 (2013).
158. Noda, S. et al. Cospeciation in the triplex symbiosis of termite gut protists (*Pseudotrichonympha* spp.), their hosts, and their bacterial endosymbionts. *Mol. Ecol.* **16**, 1257–1266 (2007).
159. Woyke, T. & Schulz, F. Entities inside one another - a matryoshka doll in biology? *Environ. Microbiol. Rep.* **11**, 26–28 (2019).
160. Chatterjee, A. & Duerkop, B. A. Beyond bacteria: Bacteriophage-eukaryotic host interactions reveal emerging paradigms of health and disease. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2018.01394 (2018).
161. Bordenstein, S. R., Marshall, M. L., Fry, A. J., Kim, U. & Wernegreen, J. J. The tripartite associations between bacteriophage, *Wolbachia*, and arthropods. *PLOS Pathog.* **2**, e43 (2006).
162. Shropshire, J. D., On, J., Layton, E. M., Zhou, H. & Bordenstein, S. R. One prophage WO gene rescues cytoplasmic incompatibility in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **115**, 4987 (2018).
**One of the genes in *Wolbachia*-infecting prophage WO that was previously shown to induce cytoplasmic incompatibility (in combination with a second gene) in insect gametes is demonstrated to also independently rescue cytoplasmic incompatibility and nullify associated embryonic defects**.
163. Beckmann, J. F. et al. The toxin–antidote model of cytoplasmic incompatibility: Genetics and evolutionary implications. *Trends Genet.* **35**, 175–185 (2019).
164. Sweere, J. M. et al. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science* **363**, eaat9691 (2019).
165. Marquez, L. M., Redman, R. S., Rodriguez, R. J. & Roossinck, M. J. A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance. *Science* **315**, 513–515 (2007).
**An early example of a mutualistic 'nested' symbiosis involving viruses; in this case, the direct fungal host of a virus as well as the plant host of the fungus benefitted from viral infection**.
166. van Oppen, M. J. H., Leong, J.-A. & Gates, R. D. Coral-virus interactions: a double-edged sword? *Symbiosis* **47**, 1–8 (2009).
167. Tikhe, C. V. & Husseneder, C. Metavirome sequencing of the termite gut reveals the presence of an unexplored bacteriophage community. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2017.02548 (2018).

## Acknowledgements
Stimulating discussions at a Canadian Institute for Advanced Research–Gordon and Betty Moore Foundation workshop entitled "Continuum of Persistence – Ecology and Function of Persistent Virus Infections" contributed to the initial motivation for and direction of this work. An American Association for the Advancement of Science (AAAS) special session entitled "Viruses, Microbes and Their Entangled Fates" facilitated discussions and ultimately led to this Review, and AAAS is acknowledged for travel support provided to A.M.S.C. Funding that supported this synthesis was provided by the NSF Biological Oceanography Program (OCE#1635798 and OCE#1928609 to A.M.S.C., OCE#1829831 to M.B.S., OCE#1829640 to M.B.S. and OCE#1829636 to J.S.W. and OCE#1737237 to A.B.), an Early-Career Research Fellowship (#2000009651) from the Gulf Research Program of the US National Academies of Sciences to A.M.S.C., the Gordon and Betty Moore Foundation (#3790 to M.B.S.) and the Simons Foundation (SCOPE Award ID 329108 and Award ID 722153 to J.S.W and Award ID 735083 to A.B.).

## Author Contributions
A.M.S.C., A.B., M.B.S. and J.S.W. conceptualized the Review. All authors contributed to all aspects of manuscript development, including research, writing and editing.

## Competing interests
The authors declare no competing interests.

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