Should I stay or should I go: Transmission trade-offs in mobile genetic elements

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Abstract

Mobile genetic elements (MGEs), including temperate bacteriophages and conjugative plasmids, are major vectors of virulence and antibiotic resistance in bacterial populations. To maximize reproductive fitness, MGEs have to optimize horizontal and vertical transmission. Yet, the cost of horizontal transmission (e.g. phage lysis) puts these transmission modes at odds. Using virulence-transmission trade-off theory, we identify three groups of environmental variables affecting the balance between horizontal and vertical transmission: host density, host physiology, and competitors. We find that general theoretical predictions of the optimal response to environmental cues closely align with experimental evidence on the regulation of transmission by MGEs. We further highlight gaps between theory and experiments, differences between phage and plasmids, and suggest areas for future research.

Keywords: prophage induction, plasmid conjugation, virulence-transmission trade-off, HGT, environmental cues

Highlights:

- Temperate phages and conjugative plasmids have different lifestyles yet remarkably similar transmission trade-offs.
- To maximize their fitness these mobile genetic elements (MGEs) need to balance horizontal and vertical transmission. The optimal balance depends on the environment.
- Both conjugative plasmids and temperate phages can respond to host cell density to regulate their transmission.
- Differences are found in the response to host metabolism, stressors and competing MGEs.
- The fields of phage and plasmid biology can help each other identify gaps in the literature, such as shared regulatory cues, and reveal general rules of transmission regulation in MGEs.

1 Optimal transmission of MGEs is environment-dependent

Mobile genetic elements (MGEs) such as phages and plasmids are ubiquitous and highly abundant genetic symbionts of bacteria. To optimize their overall reproductive fitness, MGEs need to maximize the total number of offspring resulting from both horizontal and vertical transmission. However, the cost to host fitness imposed by horizontal transmission leads to a trade-off with vertical transmission (the horizontal-vertical transmission trade-off, HVTT) [1]. The optimal balance between both transmission modes will depend on the (relative) success rate of each mode in a given environment: e.g. when few hosts are available for infection, reproductive fitness is dominated by vertical transmission, while the contribution of horizontal transmission increases with the number of susceptible hosts.

Understanding which environments select for horizontal over vertical transmission is essential in antibiotic resistance epidemiology. It can help identify hotspots for the transmission of antibiotic resistance determinants by MGEs, and predict the evolution of clinically relevant vectors of resistance. To investigate which ecological conditions favor which MGE transmission mode, we take inspiration from existing theory on the virulence-transmission trade-off for parasites (Box 1) [2]. Here, we focus on conjugative plasmids and temperate phages, two types of MGEs that exhibit both horizontal and vertical mobility [3, 4, 5]. Based on their horizontal transmission mode they can be seen as distinct ends of a parasitism-mutualism continuum: most phages kill their original host to spread horizontally, whereas plasmids keep their host alive and transmit a copy of themselves. It is not clear whether these lifestyle differences translate to differences in the HVTT and resulting optimal transmission strategies.

Testing theoretical predictions of parasite evolution is generally difficult: experiments struggle to measure theory-relevant parameters and comparative studies across organisms suffer from confounding factors [6]. Here, we propose a new way to test such predictions: we investigate the mechanisms that MGEs have evolved to regulate their transmission modes to reveal selection from the HVTT. Specifically, we review and discuss experimental evidence for three key groups of environmental factors that MGEs use for transmission regulation in the light of predictions from transmission trade-off theory: (i) availability of susceptible hosts, (ii) host physiology, and (iii) competitors.

Box 1: Transmission trade-off theory

The fitness of a parasite is dictated by its ability to spread (produce offspring) in a population of hosts. Mathematically, this is described by the basic reproductive number R_0, the average number of secondary infections produced by a single parasite in a fully susceptible population S. For parasites that are transmitted horizontally and vertically, both modes add to the total number of offspring: $R_0 = H_0 + V_0$ [7]. Assuming density-dependent infection of susceptible hosts S at a constant rate β (e.g. for modeling plasmid transmission), R_0 is given by [7]:

$$R_0 = \underbrace{H_0}_{\text{horizontal } R_0} + \underbrace{V_0}_{\text{vertical } R_0} \tag{1}$$

$$= \underbrace{\beta S}_{\text{horizontal rate}} \cdot \Delta t + \underbrace{\lambda'}_{\text{vertical rate}} \cdot \Delta t = \frac{\beta S + \lambda'}{\mu + \alpha}. \tag{2}$$

Here $\lambda' = \lambda - c$ designates the growth rate of the infected host (with c as growth cost of infection),

μ the background death rate of the host, and α the surplus lethality due to the infection. Parasite fitness (R_0) is thus given by the rate at which new infections are created, horizontally (βS) or vertically (λ'), times the average duration spent within a host before it dies $\Delta t = 1/(\mu + \alpha)$. Parasite virulence, i.e. the harm a parasite causes its host due to increased death α or reduced growth λ' (increased c), generally reduces R_0 [2].

The virulence-transmission trade-off (VTT) is typically formulated for purely horizontally transmitted parasites ($R_0 = H_0$) and posits that the parasite transmission rate β and its virulence α cannot be varied independently to maximize R_0 [2, 6]. Experimental observations support that increased horizontal transmission tends to come at a cost to the host [8], leading to a trade-off between a high rate of horizontal transmission βS or a longer duration of infection Δt [6]. Hence, the (a)biotic environment and biological characteristics of host and parasite will determine the optimal evolutionary strategy in the face of this trade-off [9].

For parasites that are both horizontally and vertically transmitted (eq. 2), virulence associated with (increased) horizontal transmission (increased α or c) will also negatively impact the parasite's vertical transmission (V_0). The virulence-transmission trade-off is thus embedded in a horizontal-vertical transmission trade-off (HVTT). In cases where the VTT predicts selection for decreased virulence, we can assume that the HVTT will favor vertical transmission (increased V_0). The converse is not necessarily given, and will depend on the specific parasite. This requires system-specific forms of eq. 2 to allow more detailed predictions of population dynamics [10].

2 Phages and plasmids exhibit a horizontal-vertical transmission trade-off

We briefly outline how the horizontal-vertical transmission trade-off (HVTT) manifests for temperate phages and conjugative plasmids (Fig. 1). While we focus on two types of MGEs here, these trade-offs extend to a much wider diversity of MGEs (see Box 2).

Temperate bacteriophages transmit horizontally via free phage virions, which infect bacterial hosts through adsorption to specific cell surface receptors (Fig. 1A) [11]. After injecting their genome into the host, they commit to horizontal (lytic) or vertical transmission (lysogenic lifecycle). During the lytic cycle, the phage uses host machinery to replicate and produce the proteins needed for further transmission. After a characteristic 'latent period', the accumulated lysis proteins disrupt the host membrane to release a number of assembled virions (burst size). The virions then diffuse into the surrounding environment and start new rounds of infection in susceptible cells [12]. Adsorption to non-susceptible hosts and unfavorable environments can lead to virion decay without reproduction. If a phage instead initiates the lysogenic pathway, it integrates into the host chromosome as a prophage and is transmitted vertically with the host (which becomes a 'lysogen') [13]. Triggers can induce the prophage into the lytic cycle, switching transmission to horizontal [13]. This leads to a clear trade-off for temperate phages: horizontal transmission kills the host and precludes vertical transmission [9].

Conjugative plasmids reside in the bacterial cytoplasm as independent DNA molecules, and use the host machinery to replicate (Fig. 1B) [4, 5]. The plasmid is maintained in the cell at a tightly regulated 'copy number'. Plasmids are vertically transmitted to daughter cells at division through stochastic segregation or active partitioning [14]. Conjugative plasmids also transmit horizontally in a contact-dependent manner. During conjugation, the plasmid-carrying donor attaches to a recipient cell via a pilus and transfers a plasmid copy into the recipient (which becomes a 'transconjugant'). Conjugation is energetically expensive and opens the plasmid-carrying bacterium up to predation by pilus-specific phages. Conjugation may also decrease the growth rate of transconjugants due to membrane destabilization and activation of the SOS response by incoming single-stranded DNA [4]. Most conjugative plasmids reduce their fitness cost by tightly regulating and repressing conjugation.

This strongly suggests a trade-off between horizontal and vertical transmission [4, 15].

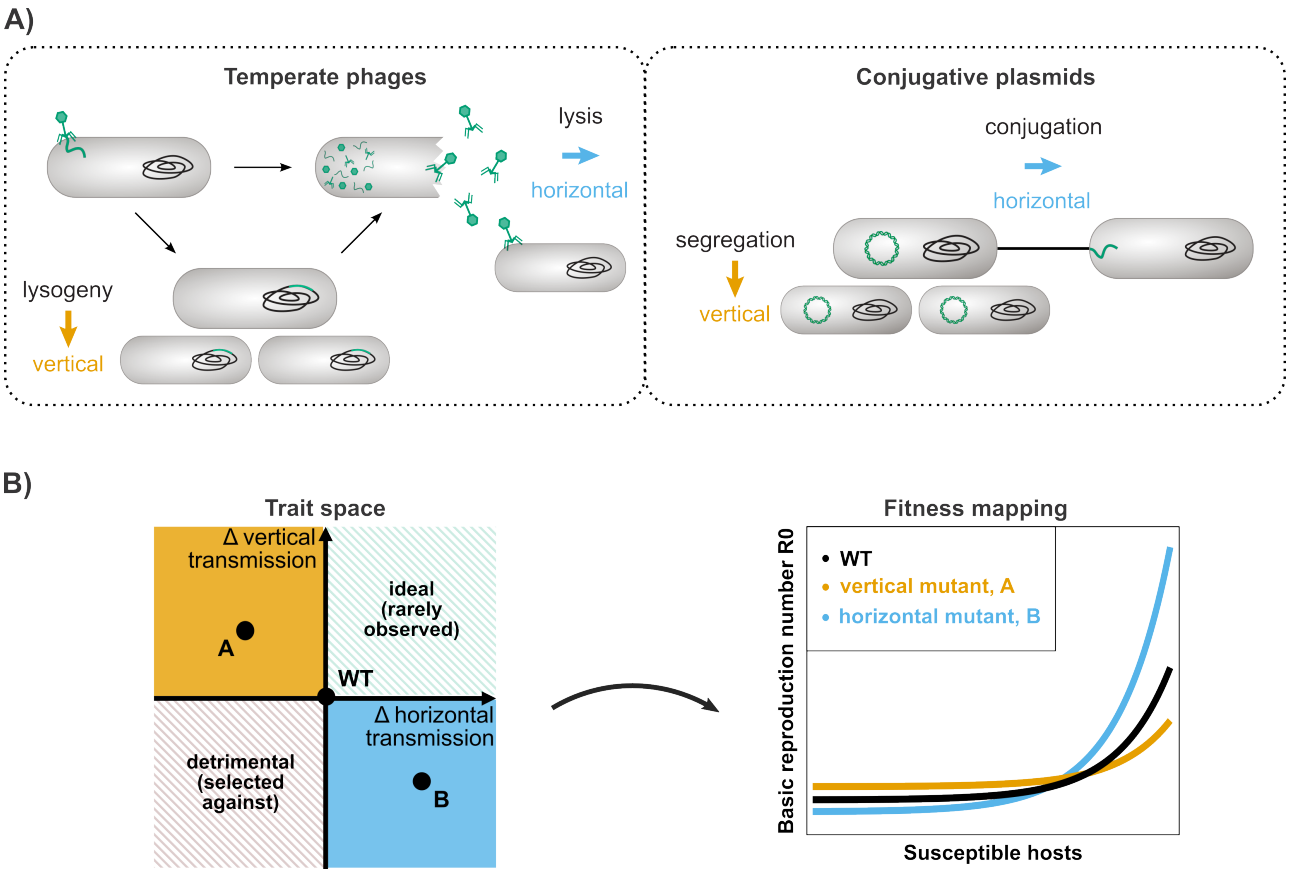

Figure 1: A) Lifecycles of temperate bacteriophages and conjugative plasmids. Temperate phages (left) can transmit either vertically, by integrating into the bacterial host genome (lysogeny), or horizontally, by replicating within the cell and lysing it to release new phage virions (lysis). Conjugative plasmids (right) can transmit vertically with their host cells through segregation, or horizontally via a conjugative pilus that transmits a plasmid copy to another bacterial cell. **B) The horizontal-vertical transmission trade-off.** The fitness of a parasite is determined by the total number of offspring it produces via both horizontal and vertical transmission (the basic reproduction number, R_0). Experiments show that mutations enhancing vertical transmission often come at the cost of horizontal transmission (orange quadrant, left), or vice versa (blue quadrant). Environmental factors, such as the availability of susceptible hosts (right), determine how shifts in transmission traits impact fitness. In principle, either mutant strategy may become more advantageous than the wildtype.

3 Experimental evidence for transmission regulation

The horizontal-vertical transmission trade-off in temperate phages and conjugative plasmids extends the classical virulence-transmission trade-off (VTT; Box 1). We take inspiration from existing predictions of the VTT to understand the regulation of horizontal vs. vertical transmission in MGEs in response to different environmental factors. In the following, we will compare theoretical predictions of parasite transmission optimization to experimental evidence for MGE transmission regulation to investigate the eco-evolutionary factors driving MGE transmission strategies. We apply this approach to three main categories of environmental factors (Fig. 2A): (i) availability of susceptible hosts, (ii) host physiology, and (iii) presence of MGE competitors (Table 1).

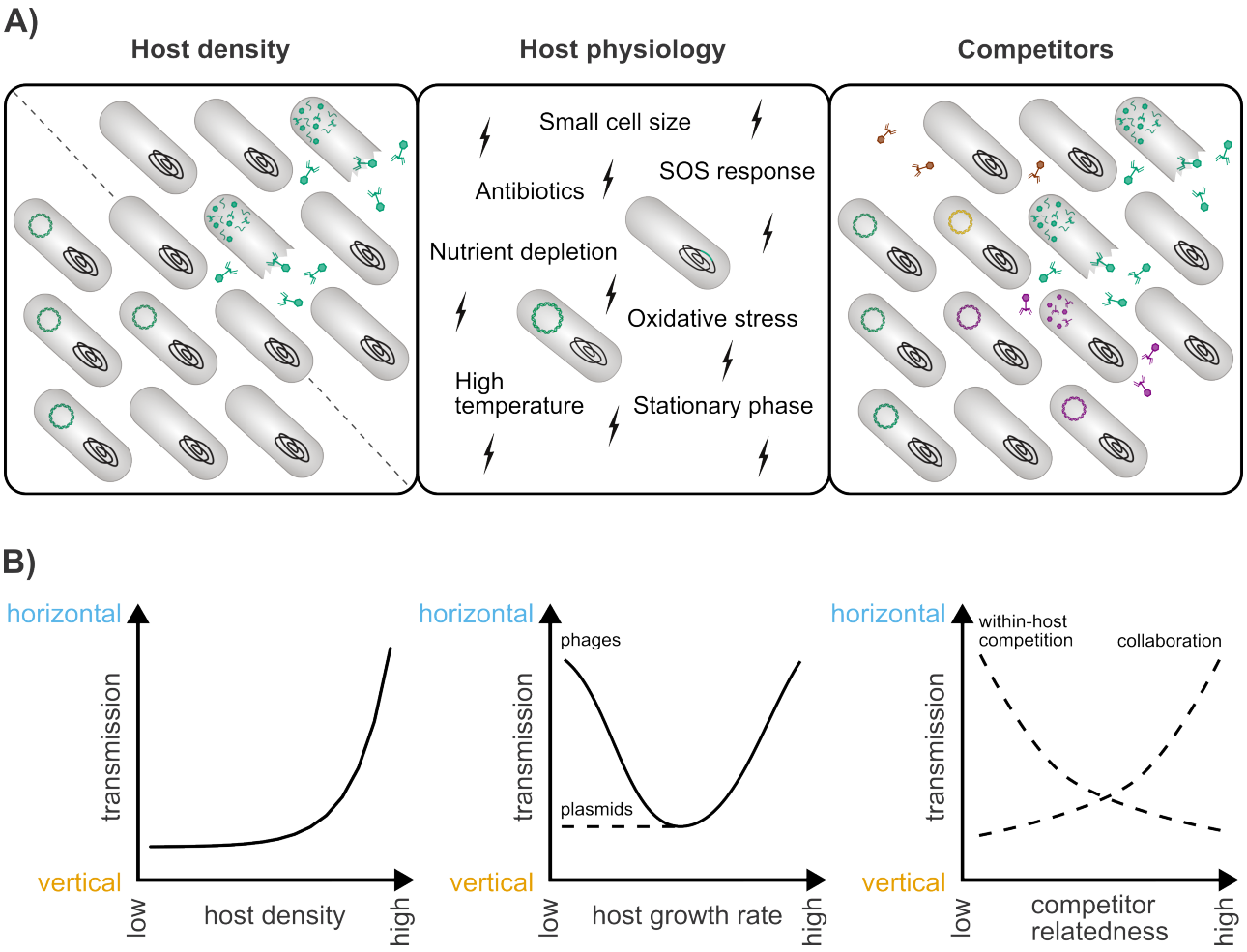

Figure 2: A) Phages and plasmids listen to three main types of cues: host density, host physiology, and competitors. **B) The theoretically optimal transmission strategy changes with the environment.** HVTT predicts that higher host densities select for increased horizontal transmission in phages and plasmids. The effect of host physiology is nonlinear: High host growth rates favor horizontal transmission. 'Medium' growth rates favor vertical transmission but very low growth (or high death) can show different trends, potentially depending on the MGE characteristics. Lastly, the optimal regulation of transmission in the presence of competitors depends on their relatedness and whether the competitors display collaboration or within-host competition.

3.1 Host cell density

Theoretical predictions Availability of susceptible hosts is a prerequisite for horizontal transmission. Theory shows that horizontal transmission is favored when susceptible hosts are abundant, and vertical transmission is favored when they are rare [2, 16]. When parasites are newly introduced into a population, horizontal transmission is selected for, as the abundance of susceptible hosts is still high. However, as the parasite becomes endemic, more vertically transmitted parasites will be favored [2].

Host availability is further tied to host mobility, as this determines the rate at which new hosts are encountered. Structured environments with limited cell mobility select for increased vertical transmission, while horizontal transmission is favored in more connected and well-mixed host populations [2, 17]. However, selection for vertical transmission will be weakened the longer a parasite can survive outside the host (e.g. phage virions) [2].

Phages Some temperate phages use density-related cues to modulate their transmission. A temperate *Vibrio* phage was shown to wiretap bacterial quorum sensing (QS), the density-dependent

communication system between bacterial cells, to induce the lytic cycle at high host densities [18]. This is in agreement with the HVTT predictions. Indeed, theory predicts that phages, which adjust their transmission strategy based on information about host availability have a distinct benefit over phages with a constant probability of switching between lysis and lysogeny [19].

Relying on host QS allows phages to gauge host density, but not susceptibility. When surrounded by lysogens induction into the lytic cycle is unfavorable [20], making the increase in phage numbers and decrease in susceptible hosts better cues to listen to. Indeed, some phages – especially those infecting the genus *Bacillus* – encode their own communication systems [21, 22]. An example is the Arbitrium system, which allows phages to modify the likelihood of lysogenization in infecting phages and the induction rate of prophages [23, 24]. The decision is driven by high peptide concentrations, which indicate that uninfected hosts are becoming rare. Remarkably, phages can detect the influx of non-lysogens based on the density-dependent degradation of phage signalling peptides by bacterial cells and induce into the lytic cycle accordingly [25]. The degradation and diffusion of quorum peptides could even provide information about the structure and composition of the environment [26], which is important for the success of phage spread via diffusion.

Another cue temperate phages use to gauge the availability of susceptible hosts is the number of infecting phage particles. If the number of related phages infecting a cell is high (multiplicity of infection, MOI), this indicates that the ratio of free phage virions to bacterial hosts is high and the density of susceptible hosts is low. Detection of co-infecting phages typically occurs through the phage regulator of the lysis-lysogeny decision (e.g. the CI repressor in phage λ) that increasingly favors lysogeny at high MOI [3, 27]. Single-virus studies have shown that individual phage genomes within the cell can interact with each other and make individual lysis-lysogeny decisions, potentially leading to mixed decisions and delayed lysis or mixed phage DNA integration into the host chromosome [28]. Delayed lysis due to secondary adsorption of related phages can even be seen in an obligately lytic coliphage, called lysis inhibition, which is selected for under conditions with restricted host access [29, 30, 31].

The density-dependence of transmission strategies is also seen in evolution experiments, where faster horizontal transmission evolves in obligately lytic phages when propagated with high densities of susceptible hosts [32, 33, 34]. Similarly, in epidemic settings, obligately lytic phages are transiently favored in the early, high-host density phase of an epidemic, but temperate phages, which rely more on vertical transmission, take over as susceptible hosts get depleted [35]. The HVTT thus changes over the course of the epidemic.

Not all experimental evidence fits neatly into the HVTT. For example, some prophages of fish pathogen *Vibrio anguillarum* use QS to increase lysogeny at high host densities [36]. Since increased lysogeny is associated with decreased biofilm formation in this system, the host may manipulate the lysis-lysogeny decision to promote its own fitness. Bioinformatic analyses and abundance counts of phages in natural environments have also produced conflicting evidence for ('Kill-the-winner') [37, 38] or against ('Piggyback-the-winner') [39, 40] the HVTT.

Plasmids Some conjugative plasmids also regulate conjugation by actively sensing high host density. The best described systems are pheromone-sensitive plasmids in *Enterococcus*, rep-phr QS in *Bacillus*, and N-acylhomoserine lactone (AHL) QS in *Rhizobium* and *Agrobacterium* [41, 15, 42, 43]. In Enterococci, several plasmids encode a secreted inhibitor to a host-encoded signalling peptide (pheromone) expressed by both plasmid-free and plasmid-carrying cells. When the ratio of host cells (pheromone) to plasmids (inhibitor) is high, transfer becomes derepressed [41]. Instead, *Bacillus subtilis* has a number of conjugative elements that encode a secreted signalling peptide which directly down-regulates transfer at high MGE numbers [44, 43]. In *Rhizobiales* plasmid conjugation is regulated through QS of host-produced AHLs [15, 42]. The plasmid represses AHL production, while plasmid-free recipients will produce AHLs that lead to plasmid transfer at high density [42]. These systems are in line with the HVTT predictions.

A notable exception is the Ti plasmid of *Agrobacterium tumefaciens*, which uses AHL-sensing and

-producing genes similar to *Rhizobium*, to transfer at high densities of plasmid-carrying cells [42]. Transfer further requires the presence of opines, a carbon source produced by plants after transfer of Ti plasmid-DNA into plant root cells. Ti plasmids further encode genes for opine metabolism. This important role in the interaction of *Agrobacterium* with plant hosts likely explains why Ti transfer departs from the expectation of the HVTT.

Most other plasmids repress conjugation through autoregulatory networks with strong negative feedback loops, leading to expression of transfer genes in only 1:1000 to 1:10^6 cells [15, 45, 46]. Such regulatory networks lead to a transient phase of derepression in the transconjugant after conjugation. Transient derepression might have evolved to use the fact that recent successful conjugation is a good indicator of available susceptible hosts [46].

Despite mechanistic evidence supporting the importance of plasmid-free recipients, experimental evolution studies have yielded more ambiguous results [5, 47]. Increasing susceptible host density did not affect the prevalence of conjugation-increasing mutations in an IncI1 plasmid [5], but rapidly increased conjugation rate in an IncFII plasmid [47]. Some of this disparity may stem from the experimental design and indirect selection for growth. Nonetheless, both studies support the existence of a HVTT as most mutations that occurred increased conjugation and decreased host growth, or vice versa.

3.2 Host physiology:

Theoretical predictions The environment-dependent physiological state of current and potential future hosts also affects the HVTT. For purely horizontally transmitted parasites, high metabolic activity of the host favors horizontal transmission as the benefits of fast replication and increased transmission outweigh the cost of decreasing host growth [2]. In this case, low growth rates favor reduced virulence, but as the stress increases towards deadly, horizontal transmission is favored again to allow escape from the dying host [2]. Few studies have modeled the effect of host metabolism and stress on parasites with both horizontal and vertical transmission, especially for conditions where these factors co-vary with susceptible host density.

Phages Nutrient depletion of the host typically increases lysogeny [3, 48]. This switch occurs through direct regulation of the lysis-lysogeny decision by host enzymes across a broad range of phages [3, 49]. For coliphage λ, lysogeny was further found to be more prevalent in smaller cells, which often indicates low nutrient availability [50]. A corresponding increase in lysis induction was observed upon an influx of nutrients, mediated by the host cAMP signal [51, 52].

The potential for dormancy allows temperate coliphages to use host resources more efficiently in spatially-structured and nutrient-limited environments than obligately lytic coliphages [53]. Integrated prophages can persist inside stationary phase hosts, while free phage virions will not manage to establish new productive infections without an influx of new hosts or nutrients.

Most known prophages respond to the cell's SOS response, a cellular cascade signalling DNA damage. The SOS response causes (self-)cleavage of the phage repressor controlling the lysis-lysogeny decision and triggers induction [3, 54]. Antimicrobials, antifungals, antiseptics, DNA damaging agents (including UV light, pollutants) and metabolites [55, 56, 57, 58] may all prompt induction via the SOS response.

However, not all prophages are induced by the SOS response indiscriminately. A *Salmonella* prophage was found to prevent phage production and lysis specifically if the SOS response is caused by oxidative stress, potentially to avoid lysis within the hostile environment of phagocytes [59]. This is particularly interesting because oxidative stress has been found to promote horizontal transfer in another *Salmonella* prophage SopEΦ [60] and the *Salmonella* virulence plasmid pSLT [15].

Box 2: The continuum of mobile genetic elements

Temperate bacteriophages and conjugative plasmids are two examples of a much richer pool of genetic symbionts called mobile genetic elements (MGEs). Advances in genome sequencing are increasingly revealing the abundance and diversity of MGEs. Different types of MGEs exhibit a variety of transmission strategies: only horizontally, only vertically, or both, either independently or with the help of other MGEs. In general, these MGEs exhibit a virulence-transmission trade-off, yet the biological differences may lead to alternative solutions.

Various MGEs mix characteristics of plasmids and phages. *Integrative and conjugative elements (ICEs)* integrate into the chromosome like a lysogen, but transmit through a conjugative pilus. *'Phage-Plasmids' (P-Ps)* take a plasmid-like circular form in the cytoplasm, yet transmit horizontally through cell lysis [61, 62, 63]. *Filamentous phage* are bacteriophages that do not lyse the host cell, but extrude from the cell in a continuous fashion. The nature of the HVTT will differ according to the virulence associated with increased horizontal transmission (decreased host growth or killing) and the fidelity of vertical transmission (chromosomally integrated or as a plasmid).

In addition, there are many MGEs that require a helper for their transmission [64]. *Mobilizable plasmids* hitchhike co-resident conjugative plasmids [62], and *phage satellites* hitchhike phage genomes and/or capsids. *Insertion sequences* (ISs) and *transposons* move between different DNA molecules within the same cell, and can move between different hosts by inserting into other MGEs. Much less is known about the timing and regulation of transmission for such non-autonomous MGEs. In some cases an incoming 'helper' phage is sensed through the SOS-response or complex formation between a protein from the incoming phage and the regulatory repressor of the resident prophage or pathogenic island [65, 66]. This triggers induction of the resident MGE, who 'steals' the helper phage virions for their own subsequent horizontal transmission [65]. These complex multi-level interactions will affect the HVTT of both resident and helper phage.

Plasmids The effect of host physiology on conjugation is best studied for F-like plasmids of enteric bacteria, which are tightly linked to their host's metabolic state [15]. These plasmids are repressed by Dam methylation [45]. Plasmid replication creates a hemi-methylated state, which leads to a higher probability of conjugation in the current host and transiently in transconjugants [45]. High host growth rates thus lead to higher horizontal transfer. Other metabolism-related cues have differing effects on conjugation across F-like plasmids. The presence of nutrients upregulates conjugation in F (through the cAMP receptor protein) [45, 15], but represses it in R100 and pSLT (through the leucine responsive regulatory protein Lrp) [67, 15]. Nucleoid protein H-NS represses transfer in F during entry into stationary phase, but activates plasmid pRK100 [45, 15]. These outcomes result from interplay between host and plasmid regulatory pathways, and likely represent the product of coevolution.

The effect of temperature similarly depends on the plasmid. H-NS represses conjugation of IncHI1 plasmid R27 at temperatures above 30 $^\circ$ [68], while it upregulates transfer of IncX3 plasmids in warm temperatures [69]. This suggests optimization for transfer in either the environment or warm-blooded hosts.

In contrast to phages, there does not seem to be a universal effect of host stress (as signalled by the bacterial SOS response) on plasmid conjugation. In F-like plasmids, the bacterial membrane stress protein (Cpx) and Hfq – a global regulator involved in RNA stress response – decrease conjugation, but the heat shock response (RpoH) promotes it [15]. Oxygen stress increases conjugation in pSLT (through ArcAB and SdhABCD) [15].

It has been widely debated whether the presence of antibiotics, non-antibiotic pharmaceuticals, or

disinfectants actively upregulates conjugation (directly or indirectly via the stress response). Some experimental studies report higher conjugation frequencies in the presence of these substances, but the estimation methods are often biased, conflating an effect on transconjugant growth with conjugation [70, 71]. Studies relating exposure levels, SOS response, transfer gene expression [72], and appropriate conjugation assays [73] are sorely needed.

3.3 Presence of competitors

Theoretical predictions The dynamics of MGEs are not only affected by their hosts, but also by co-infecting MGEs. MGEs interact both within and between hosts: they vie for the same susceptible cells, and influence each other's survival and onward transmission. Theory predicts that the effect of competition and cooperation on transmission strongly depends on the biology of the system [2]. Interactions occur between closely related elements ('self'), leading e.g. to phage MOI sensing, or more distantly related elements ('non-self'), which will be the focus here.

Both temperate phages and conjugative plasmids often encode mechanisms to prevent attachment ('surface exclusion'), entry ('entry exclusion'), or establishment (e.g. CRISPR-Cas, replication interference) of MGEs into cells they occupy [74, 75, 76]. This effectively reduces the pool of susceptible hosts for their competitors, and will favor increased vertical transmission [2]. Instead, if competitors can superinfect and displace other MGEs in a host, this may drive the evolution of higher horizontal transmission.

The competitive ability within a host and horizontal transmission can also be linked. For instance, a phage that lyses the cell earlier than its competitors will have a higher competitive ability at the cost of higher virulence. As this is deleterious for co-infecting competitors, this will result in selection for increased horizontal transmission as the number of unrelated competitors increases [2]. If horizontal transmission depends on the production of public goods (e.g. holins or phage capsids), increased relatedness of co-infecting strains will select for increased horizontal transmission. Instead, increased within-host competition reduces relatedness (selects for cheaters) and thus reduces horizontal transmission [2].

Phages Temperate phages have developed a range of strategies to protect themselves (and their hosts) from other phages while inserted in the genome [75, 77, 78]. To improve vertical transmission, prophages encode a range of superinfection exclusion, induction inhibition and defense systems [75, 79, 77]. Some systems, e.g. adsorption prevention of competitors, work preemptively, while others like CRISPR-Cas defense are reactive [77].

If a co-resident prophage induces the lytic cycle, switching to horizontal transfer becomes the best survival strategy for all resident prophages. Since the SOS response cleaves the lysogeny-regulating repressors of most prophages, it is a general but unspecific signal for competitors. Additionally, some lambdoid prophages are induced by the same 'antirepressor': a protein inactivating the lysogeny-regulating repressor [80]. Antirepressors produced by one prophage can induce related phages [80]. However, shared induction promotes within-host resource competition that all phages suffer from [81]. This might lead to a race for more sensitive or faster induction to avoid the resource competition. The discovery of phage-specific induction modules – independent of the SOS response – supports this theory, but their induction cues are not yet known [82].

Plasmids Like phages, conjugative plasmids encode superinfection exclusion and defense systems to prevent incoming plasmids from establishing in the same host. Nearly all conjugative plasmid families contain surface or entry exclusion systems [76, 83, 84], roughly 20% carry restriction-modification systems and some carry CRISPR-Cas [85, 86]. New defense and exclusion systems are being discovered continuously, painting a picture of widespread competition between plasmids (and other MGEs). However, there is less evidence that plasmids actively use the presence of competitors to regulate their conjugation.

Co-infecting plasmids affect each other's realized vertical and horizontal transmission rates. Vertical transmission is reduced if the competitor plasmid reduces host growth, and if replication or partitioning incompatibility reduces faithful plasmid segregation. Such incompatibility leads to within-host competition, which may also modulate horizontal transfer due to its effect on the copy number (and corresponding transfer gene expression) of both plasmids [47]. To repress horizontal transmission, some plasmids encode fertility inhibition systems that interfere with the derepression of unrelated, co-resident plasmids [87]. However, co-resident plasmids may also increase each other's horizontal transmission, as the simultaneous expression of two T4SSs could stabilize mating pairs and increase efficiency of transfer [88].

For equally fit plasmids, theory predicts that the population dynamics of two co-circulating plasmids depend on the fitness effects of co-infection [89]. If co-infection confers a fitness benefit (e.g. lower host death rate), the plasmid variants will be under negative frequency dependent selection, promoting invasion of rare variants. If co-infection is more costly than carriage of a single plasmid, there will be positive frequency dependent selection, promoting stability of the dominant variant. This remains to be tested in experiments or observational data.

Table 1: Environmental cues that affect the optimal balance between vertical and horizontal transmission in temperate phages and conjugative plasmids. We compare predictions from virulence-transmission trade-off theory [2] to experimental evidence for phages and plasmids. Symbols indicate whether a cue was predicted or observed to increase vertical (↓) or horizontal transmission (→). Cases where we did not find evidence in either direction are marked with "-".

Cue	Theory	Phages	Plasmids
Host cell density			
High host density	→	→ [18, 32, 33, 34], ↓ [36]	-
High MGE to host ratio	↓	↓ [3, 22, 21, 23, 24, 25, 27, 29, 30, 31, 35]	↓ [41, 42, 43, 44], → [42]
Recent transfer	→	-	→ [45, 46]
Host physiology			
Nutrient depletion	↓	↓ [3, 48, 49, 51, 52]	↓ [15, 45], → [15, 67]
Small cell size	↓	↓ [50]	-
Oxidative stress	↓ →	↓ [59], → [60]	→ [15]
Antibiotics and compounds with antimicrobial effect	-	→ [57, 56, 90, 55, 58]	-
DNA damage (e.g. UV)	-	→ [54]	-
High temperature	↓ →	→ [48, 91]	→ [15, 69], ↓ [68]
Stationary phase	-	-	↓ [15]
Increased survival outside host	→	-	-
Competitors			
External attachment of competitors	-	↓ [30]	-
Entry of competitors	-	→ [65, 66, 78]	-
Coresident competitor switches to horizontal transfer	-	→[80]	-

4 Concluding Remarks

We discussed how MGEs use environmental cues to regulate their transmission, and how this compares to theoretical predictions of the optimal transmission strategy in those environments. These cues group into three main categories: host availability, host physiology, and competitors. We find experimental evidence that MGE transmission regulation follows optimal transmission strategies predicted by theory. For example, horizontal transfer is favored at high host density and systems that sense host or MGE density are widespread [18, 21].

In contrast, host physiology encompasses a range of environmental stressors and metabolic cues, which trigger diverse responses in plasmids and phages. These responses do not always align with simple predictions from the HVTT. Stress reliably induces horizontal transfer in prophages, but less in plasmids. Because phages can survive independently outside host cells, they can bet on future infection. Instead, plasmid fate is tied to the host, and host survival is a priority. This seems to be a general trend, where plasmids are more strongly adapted to the host cell environment and lifestyle than phage. Perhaps the high cost of death by lysis enforces a stricter HVTT in phages, leading to better alignment with theoretical predictions. Further, domestication by the host may interfere with MGE transmission. Future work should consider how the host constrains MGE-driven strategies to optimize transmission and modulates the framework presented here.

The last category of cues, competitors, is characterized by a wealth of theoretical predictions but scarce experimental evidence. It is becoming increasingly clear that competition between MGEs is widespread, reflected in e.g. the diversity and ubiquity of mechanisms to prevent entry and establishment of competing elements. However, more work is needed to understand whether and how phages and plasmids actively regulate their transmission in response to competitors.

Many questions remain regarding the (a)biotic factors that affect the HVTT (see 'Outstanding Questions'). Comparison of MGEs is needed to disentangle the trade-offs set up by different aspects of the MGE lifestyle (e.g. replication and transmission mode). This will improve our understanding of the virulence-transmission trade-off, MGE-host co-evolution, and the MGE continuum. Comparisons can also identify gaps in the literature: for instance, QS systems are better studied in phages than plasmids, while they are likely equally relevant [22]. Beyond the dynamics of the MGEs, more study is needed to determine the influence of ecological interactions between hosts on transmission.

Understanding whether an environment favors more horizontal or vertical transmission is fundamental to predicting the eco-evolutionary dynamics of MGEs and the bacterial populations they infect. The transmission dynamics of temperate phages and conjugative plasmids are directly medically relevant, as limiting their horizontal transmission can reduce bacterial pathogenicity [92] and the spread of antibiotic resistance [93]. This knowledge can inform antimicrobial treatments like phage therapy [94], or bioremediation efforts using MGEs to promote degradation of organic pollutants [95]. More broadly, MGEs are ideal models for studying the evolution of virulence and transmission experimentally, given their simplicity, short generation times and high reproduction rates [13, 94, 47]. Insights derived from MGEs will aid in understanding and preventing disease transmission more broadly.

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Outstanding Questions

- MGEs use different sensing modalities to listen to host/MGE density or environmental conditions. How does MGE or bacterial host lifestyle determine which type of sensing system is optimal?
- Do plasmids not listen to the SOS response or has it just not been described yet? Modeling is needed to determine how the optimal response to stress may depend on MGE characteristics. Experiments and observational data are needed to determine the mechanisms and prevalence of stress-related regulation in MGEs.
- Surprisingly few experimental studies have investigated how MGEs respond to their competitors. Do plasmids have a way to sense MOI? How does the response to competitors depend on characteristics of the focal and competing MGE?
- Why do different phages and plasmids have opposite responses to host metabolic cues? Modeling could be used to understand the evolutionary pressures driving these differences, and experiments to investigate regulation in a wider diversity of MGEs.
- To which extent can MGEs optimize their transmission independent from host regulation? Which role does host domestication play in the evolution of MGEs?
- How does the HVTT manifest across the wider MGE continuum? How do these trade-offs lead to alternative life history strategies and speciation?

6 References

[1] Ing-Nang Wang. Lysis timing and bacteriophage fitness. *Genetics*, 172(1):17–26, jan 2006.

[2] Clayton E. Cressler, David V. Mcleod, Carly Rozins, Josée Van Den Hoogen, and Troy Day. The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. *Parasitology*, 143(7):915–930, aug 2015.

[3] Amos B. Oppenheim, Oren Kobiler, Joel Stavans, Donald L. Court, and Sankar Adhya. Switches in bacteriophage lambda development. *Annual Review ofGenetics*, 39:409–429, 2005.

[4] Alvaro San Millan and R. Craig MacLean. Fitness costs of plasmids: a limit to plasmid transmission. *Microbiology Spectrum*, 5(5), oct 2017.

[5] Paul E. Turner, Vaughn S. Cooper, and Richard E. Lenski. Tradeoff between horizontal and vertical modes of transmission in bacterial plasmids. *Evolution*, 52(2):315–329, apr 1998.

[6] S. Alizon, A. Hurford, N. Mideo, and M. Van Baalen. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology*, 22(2):245–259, dec 2008.

[7] M. Lipsitch, M. A. Nowak, D. Ebert, and R. M. May. The population dynamics of vertically and horizontally transmitted parasites. *Proceedings of the Royal Society B: Biological Sciences*, 260:321–327, 1995.

[8] R. M. Anderson and R. M. May. Coevolution of hosts and parasites. *Parasitology*, 85(2):411–426, oct 1982.

[9] Adrienne M. S. Correa, Cristina Howard-Varona, Samantha R. Coy, Alison Buchan, Matthew B. Sullivan, and Joshua S. Weitz. Revisiting the rules of life for viruses of microorganisms. *Nature Reviews Microbiology*, 19(8):501–513, aug 2021.

[10] Joshua S. Weitz, Guanlin Li, Hayriye Gulbudak, Michael H. Cortez, and Rachel J. Whitaker. Viral invasion fitness across a continuum from lysis to latency. *Virus Evolution*, 5, 1 2019.

[11] John J. Dennehy and Stephen T. Abedon. Adsorption: Phage Acquisition of Bacteria. *Bacteriophages*, pages 93–117, 2021.

[12] John J. Dennehy and Stephen T. Abedon. Phage Infection and Lysis. In *Bacteriophages*, pages 341–383. Springer International Publishing, Cham, 2021.

[13] Sylvain Gandon. Why be temperate: lessons from bacteriophage λ. *Trends in Microbiology*, 24(5):356–365, may 2016.

[14] Maria A. Schumacher. Bacterial plasmid partition machinery: a minimalist approach to survival. *Current Opinion in Structural Biology*, 22(1):72–79, feb 2012.

[15] Laura S. Frost and Günther Koraimann. Regulation of bacterial conjugation: balancing opportunity with adversity. *Future Microbiology*, 5(7):1057–1071, jul 2010.

[16] Lindi M. Wahl, Matthew I. Betti, David W. Dick, Tyler Pattenden, and Aaryn J. Puccini. Evolutionary stability of the lysis-lysogeny decision: why be virulent? *Evolution*, nov 2018.

[17] Sergei Maslov and Kim Sneppen. Well-temperate phage: optimal bet-hedging against local environmental collapses. *Scientific Reports*, 5(1), jun 2015.

[18] Justin E. Silpe and Bonnie L. Bassler. A host-produced quorum-sensing autoinducer controls a phage lysis-lysogeny decision. *Cell*, 176(1-2):268–280.e13, jan 2019.

[19] Hilje M Doekes, Glenn A Mulder, and Rutger Hermsen. Repeated outbreaks drive the evolution of bacteriophage communication. *eLife*, 10:e58410, jan 2021.

[20] Claudia Igler and Stephen T. Abedon. Commentary: a host-produced quorum-sensing autoinducer controls a phage lysis-lysogeny decision. *Frontiers in Microbiology*, 10, jun 2019.

[21] Avigail Stokar-Avihail, Nitzan Tal, Zohar Erez, Anna Lopatina, and Rotem Sorek. Widespread utilization of peptide communication in phages infecting soil and pathogenic bacteria. *Cell Host & Microbe*, 25(5):746–755.e5, may 2019.

[22] Charles Bernard, Yanyan Li, Philippe Lopez, and Eric Bapteste. Large-Scale Identification of Known and Novel RRNPP Quorum-Sensing Systems by RRNPP Detector Captures Novel Features of Bacterial, Plasmidic, and Viral Coevolution. *Molecular Biology and Evolution*, 40(4):1–19, 2023.

[23] Zohar Erez, Ida Steinberger-Levy, Maya Shamir, Shany Doron, Avigail Stokar-Avihail, Yoav Peleg, Sarah Melamed, Azita Leavitt, Alon Savidor, Shira Albeck, Gil Amitai, and Rotem Sorek. Communication between viruses guides lysis–lysogeny decisions. *Nature*, 541(7638):488–493, jan 2017.

[24] Nitzan Aframian, Shira Omer Bendori, Stav Kabel, Polina Guler, Avigail Stokar-Avihail, Erica Manor, Kholod Msaeed, Valeria Lipsman, Ilana Grinberg, Alaa Mahagna, and Avigdor Eldar. Dormant phages communicate via arbitrium to control exit from lysogeny. *Nature Microbiology*, 7(1):145–153, jan 2022.

[25] John B. Bruce, Sébastien Lion, Angus Buckling, Edze R. Westra, and Sylvain Gandon. Regulation of prophage induction and lysogenization by phage communication systems. *Current Biology*, 31(22):5046–5051.e7, 2021.

[26] D. M. Cornforth, R. Popat, L. McNally, J. Gurney, T. C. Scott-Phillips, A. Ivens, S. P. Diggle, and S. P. Brown. Combinatorial quorum sensing allows bacteria to resolve their social and physical environment. *Proceedings of the National Academy of Sciences*, 111(11):4280–4284, 2014.

[27] Vaibhhav Sinha, Akshit Goyal, Sine L. Svenningsen, Szabolcs Semsey, and Sandeep Krishna. In silico evolution of lysis-lysogeny strategies reproduces observed lysogeny propensities in temperate bacteriophages. *Frontiers in Microbiology*, 8, jul 2017.

[28] Jimmy T. Trinh, Tamás Székely, Qiuyan Shao, Gábor Balázsi, and Lanying Zeng. Cell fate decisions emerge as phages cooperate or compete inside their host. *Nature Communications*, 8(1), feb 2017.

[29] Inna V. Krieger, Vladimir Kuznetsov, Jeng Yih Chang, Junjie Zhang, Samir H. Moussa, Ryland F. Young, and James C. Sacchettini. The Structural Basis of T4 Phage Lysis Control: DNA as the Signal for Lysis Inhibition. *Journal of Molecular Biology*, 432(16):4623–4636, 2020.

[30] Stephen Abedon. Look Who's Talking: T-Even Phage Lysis Inhibition, the Granddaddy of Virus-Virus Intercellular Communication Research. *Viruses*, 11(10):951, oct 2019.

[31] Benjamin Kerr, Claudia Neuhauser, Brendan J. M. Bohannan, and Antony M. Dean. Local migration promotes competitive restraint in a host–pathogen 'tragedy of the commons'. *Nature*, 442(7098):75–78, jul 2006.

[32] S. T. Abedon, P. Hyman, and C. Thomas. Experimental examination of bacteriophage latent-period evolution as a response to bacterial availability. *Applied and Environmental Microbiology*, 69(12):7499–7506, dec 2003.

[33] Richard H Heineman and James J Bull. Testing optimality with experimental evolution: lysis time in a bacteriophage. *Evolution; international journal of organic evolution*, 61(7):1695–709, jul 2007.

[34] Christal M. Eshelman, Roxanne Vouk, Jodi L. Stewart, Elizabeth Halsne, Haley A. Lindsey, Stacy Schneider, Miliyard Gualu, Antony M. Dean, and Benjamin Kerr. Unrestricted migration favours virulent pathogens in experimental metapopulations: evolutionary genetics of a rapacious life history. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1552):2503–2513, aug 2010.

[35] Thomas W. Berngruber, Rémy Froissart, Marc Choisy, and Sylvain Gandon. Evolution of virulence in emerging epidemics. *PLoS Pathogens*, 9(3):e1003209, mar 2013.

[36] Demeng Tan, Mads Frederik Hansen, Luís Nunes de Carvalho, Henriette Lyng Røder, Mette Burmølle, Mathias Middelboe, and Sine Lo Svenningsen. High cell densities favor lysogeny: induction of an h20 prophage is repressed by quorum sensing and enhances biofilm formation in vibrio anguillarum. *The ISME Journal*, 14(7):1731–1742, apr 2020.

[37] Christian Winter, Thierry Bouvier, Markus G. Weinbauer, and T. Frede Thingstad. Trade-offs between competition and defense specialists among unicellular planktonic organisms: the "killing the winner" hypothesis revisited. *Microbiology and Molecular Biology Reviews*, 74(1):42–57, 2010.

[38] Jennifer R. Brum, Bonnie L. Hurwitz, Oscar Schofield, Hugh W. Ducklow, and Matthew B. Sullivan. Seasonal time bombs: Dominant temperate viruses affect Southern Ocean microbial dynamics. *ISME Journal*, 10(2):437–449, 2016.

[39] B. Knowles, C. B. Silveira, B. A. Bailey, K. Barott, V. A. Cantu, A. G. Cobian-Guëmes, F. H. Coutinho, E. A. Dinsdale, B. Felts, K. A. Furby, E. E. George, K. T. Green, G. B. Gregoracci, A. F. Haas, J. M. Haggerty, E. R. Hester, N. Hisakawa, L. W. Kelly, Y. W. Lim, M. Little, A. Luque, T. McDole-Somera, K. McNair, L. S. De Oliveira, S. D. Quistad, N. L. Robinett, E. Sala, P. Salamon, S. E. Sanchez, S. Sandin, G. G.Z. Silva, J. Smith, C. Sullivan, C. Thompson, M. J.A. Vermeij, M. Youle, C. Young, B. Zgliczynski, R. Brainard, R. A. Edwards, J. Nulton, F. Thompson, and F. Rohwer. Lytic to temperate switching of viral communities. *Nature*, 531(7595):466–470, 2016.

[40] Steven G. Sutcliffe, Alejandro Reyes, and Corinne F. Maurice. Bacteriophages playing nice: Lysogenic bacteriophage replication stable in the human gut microbiota. *iScience*, 26(2):106007, feb 2023.

[41] Gary M. Dunny. Enterococcal sex pheromones: Signaling, social behavior, and evolution. *Annual Review of Genetics*, 47:457–482, 11 2013.

[42] Vittoria E. Danino, Adam Wilkinson, Anne Edwards, and J. Allan Downie. Recipient-induced transfer of the symbiotic plasmid prl1ji in rhizobium leguminosarum bv. viciae is regulated by a quorum-sensing relay. *Molecular Microbiology*, 50:511–525, 10 2003.

[43] Praveen K. Singh, Gayetri Ramachandran, Ricardo Ramos-Ruiz, Ramón Peiró-Pastor, David Abia, Ling J. Wu, and Wilfried J.J. Meijer. Mobility of the native bacillus subtilis conjugative plasmid pls20 is regulated by intercellular signaling. *PLoS Genetics*, 9, 10 2013.

[44] Jennifer M. Auchtung, Catherine A. Lee, Rita E. Monson, Alisa P. Lehman, and Alan D. Grossman. Regulation of a bacillus subtilismobile genetic element by intercellular signaling and the global dna damage response. *Proceedings of the National Academy of Sciences*, 102:12554–12559, 8 2005.

[45] Eva M Camacho and Josep Casadesús. Regulation of traj transcription in the salmonella virulence plasmid by strand-specific dna adenine hemimethylation. *Molecular microbiology*, 57(6):1700–1718, 2005.

[46] Raul Fernandez-Lopez, Irene del Campo, Carlos Revilla, Ana Cuevas, and Fernando de la Cruz. Negative feedback and transcriptional overshooting in a regulatory network for horizontal gene transfer. *PLoS Genetics*, 10, 2 2014.

[47] Tatiana Dimitriu, Andrew C. Matthews, and Angus Buckling. Increased copy number couples the evolution of plasmid horizontal transmission and plasmid-encoded antibiotic resistance. *Proceedings of the National Academy of Sciences*, 118(31):1–7, aug 2021.

[48] Menghui Zhang, Tianyou Zhang, Meishun Yu, Yu-Lei Chen, and Min Jin. The Life Cycle Transitions of Temperate Phages: Regulating Factors and Potential Ecological Implications. *Viruses*, 14(9):1904, aug 2022.

[49] Gregory W. Broussard, Lauren M. Oldfield, Valerie M. Villanueva, Bryce L. Lunt, Emilee E. Shine, and Graham F. Hatfull. Integration-Dependent Bacteriophage Immunity Provides Insights into the Evolution of Genetic Switches. *Molecular Cell*, 49(2):237–248, jan 2013.

[50] F. St-Pierre and D. Endy. Determination of cell fate selection during phage lambda infection. *Proceedings of the National Academy of Sciences*, 105(52):20705–20710, 2008.

[51] Leanid Laganenka, Timur Sander, Alexander Lagonenko, Yu Chen, Hannes Link, and Victor Sourjik. Quorum sensing and metabolic state of the host control lysogeny-lysis switch of bacteriophage T1. *mBio*, 10(5), 2019.

[52] Agata Czyz, Marcin Los, Borys Wrobel, and Grzegorz Wegrzyn. Inhibition of spontaneous induction of lambdoid prophages in Escherichia coli cultures: simple procedures with possible biotechnological applications. *BMC biotechnology*, 1:1, 2001.

[53] Namiko Mitarai, Stanley Brown, and Kim Sneppen. Population dynamics of phage and bacteria in spatially structured habitats using phage λ and Escherichia coli. *Journal of Bacteriology*, 198(12):1783–1793, 2016.

[54] Arun M Nanda, Kai Thormann, and Julia Frunzke. Impact of Spontaneous Prophage Induction on the Fitness of Bacterial Populations and Host-Microbe Interactions. *Journal of Bacteriology*, 197(3):410–419, feb 2015.

[55] Jee-Hwan Oh, Laura M. Alexander, Meichen Pan, Kathryn L. Schueler, Mark P. Keller, Alan D. Attie, Jens Walter, and Jan-Peter van Pijkeren. Dietary Fructose and Microbiota-Derived Short-Chain Fatty Acids Promote Bacteriophage Production in the Gut Symbiont Lactobacillus reuteri. *Cell Host & Microbe*, 25(2):273–284.e6, feb 2019.

[56] Caroline Henrot and Marie Agnès Petit. Signals triggering prophage induction in the gut microbiota. *Molecular Microbiology*, 118(5):494–502, 2022.

[57] Elizabeth Tompkins, Brigitte Cadieux, Margot Amitrano, and Lawrence Goodridge. High-throughput screening of natural compounds for prophage induction in controlling pathogenic bacteria in food. *Frontiers in Food Science and Technology*, 3(November):1–10, nov 2023.

[58] Steven G. Sutcliffe, Michael Shamash, Alexander P. Hynes, and Corinne F. Maurice. Common oral medications lead to prophage induction in bacterial isolates from the human gut. *Viruses*, 13(3):1–14, 2021.

[59] Siva Uppalapati, Sashi Kant, Lin Liu, Ju Sim Kim, David Orlicky, Michael McClelland, and Andres Vazquez-Torres. Prophage terminase with tRNase activity sensitizes Salmonella enterica to oxidative stress. *Science*, 384(6691):100–105, 2024.

[60] Médéric Diard, Erik Bakkeren, Jeffrey K. Cornuault, Kathrin Moor, Annika Hausmann, Mikael E. Sellin, Claude Loverdo, Abram Aertsen, Martin Ackermann, Marianne De Paepe, Emma Slack, and Wolf-Dietrich Hardt. Inflammation boosts bacteriophage transfer between Salmonella spp. *Science*, 355(6330):1211–1215, mar 2017.

[61] A. V. Mardanov and N. V. Ravin. The antirepressor needed for induction of linear plasmid-prophage n15 belongs to the SOS regulon. *Journal of Bacteriology*, 189(17):6333–6338, jun 2007.

[62] Chris Smillie, M. Pilar Garcilla n Barcia, M. Victoria Francia, Eduardo P. C. Rocha, and Fernando de la Cruz. Mobility of plasmids. *Microbiology*

and Molecular Biology Reviews, 74(3):434–452, sep 2010.

[63] Eugen Pfeifer, Jorge A. Moura De Sousa, Marie Touchon, and Eduardo P.C. Rocha. Bacteria have numerous distinctive groups of phage-plasmids with conserved phage and variable plasmid gene repertoires. *Nucleic Acids Research*, 49(5):2655–2673, 2021.

[64] Manuel Ares-Arroyo, Charles Coluzzi, Jorge A Moura de Sousa, and Eduardo PC Rocha. Hijackers, hitchhikers, or co-drivers? the mysteries of mobilizable genetic elements. *PLoS biology*, 22(8):e3002796, 2024.

[65] Andreas F Haag, Magdalena Podkowik, Rodrigo Ibarra-Chávez, Francisca Gallego del Sol, Geeta Ram, John Chen, Alberto Marina, Richard P Novick, and José R. Penadés. A regulatory cascade controls Staphylococcus aureus pathogenicity island activation. *Nature Microbiology*, 6(10):1300–1308, sep 2021.

[66] J. Rafael Ciges-Tomas, Christian Alite, Suzanne Humphrey, J. Donderis, Janine Bowring, Xavier Salvatella, José R. Penadés, and Alberto Marina. The structure of a polygamous repressor reveals how phage-inducible chromosomal islands spread in nature. *Nature Communications*, 10(1), 2019.

[67] Eva M Camacho and Josep Casadesús. Conjugal transfer of the virulence plasmid of salmonella enterica is regulated by the leucine-responsive regulatory protein and dna adenine methylation. *Molecular microbiology*, 44(6):1589–1598, 2002.

[68] M. Gibert, A. Juárez, E. L. Zechner, C. Madrid, and C. Balsalobre. Trhr, trhy and htda, a novel regulatory circuit that modulates conjugation of the inchi plasmids. *Molecular Microbiology*, 94:1146–1161, 12 2014.

[69] Liu Baomo, Shui Lili, Robert A. Moran, Willem van Schaik, and Zhuo Chao. Temperature-regulated incx3 plasmid characteristics and the role of plasmid-encoded h-ns in thermoregulation. *Frontiers in Microbiology*, 12, 1 2022.

[70] Jana S. Huisman, Fabienne Benz, Sarah J.N. Duxbury, J. Arjan G.M. de Visser, Alex R. Hall, Egil A.J. Fischer, and Sebastian Bonhoeffer. Estimating plasmid conjugation rates: A new computational tool and a critical comparison of methods. *Plasmid*, 121:102627, 5 2022.

[71] Allison J. Lopatkin, Shuqiang Huang, Robert P. Smith, Jaydeep K. Srimani, Tatyana A. Sysoeva, Sharon Bewick, David K. Karig, and Lingchong You. Antibiotics as a selective driver for conjugation dynamics. *Nature Microbiology*, 1:16044, 4 2016.

[72] Yue Wang, Ji Lu, Shuai Zhang, Jie Li, Likai Mao, Zhiguo Yuan, Philip L. Bond, and Jianhua Guo. Non-antibiotic pharmaceuticals promote the transmission of multidrug resistance plasmids through intra- and intergenera conjugation. *ISME Journal*, 15:2493–2508, 9 2021.

[73] Olivia Kosterlitz and Jana S. Huisman. Guidelines for the estimation and reporting of plasmid conjugation rates. *Plasmid*, 126, 5 2023.

[74] Michael Hunter and Diana Fusco. Superinfection exclusion: A viral strategy with short-term benefits and long-term drawbacks. *PLoS Computational Biology*, 18(5):1–18, 2022.

[75] Joseph Bondy-Denomy, Jason Qian, Edze R. Westra, Angus Buckling, David S. Guttman, Alan R. Davidson, and Karen L. Maxwell. Prophages mediate defense against phage infection through diverse mechanisms. *ISME Journal*, 10(12):2854–2866, 2016.

[76] M. Pilar Garcillán-Barcia and Fernando de la Cruz. Why is entry exclusion an essential feature of conjugative plasmids? *Plasmid*, 60:1–18, 7 2008.

[77] Pramalkumar H. Patel and Karen L. Maxwell. Prophages provide a rich source of antiphage defense systems. *Current Opinion in Microbiology*, 73:102321, 2023.

[78] Jorge A Moura de Sousa, Alfred Fillol-Salom, José R. Penadés, and Eduardo P C Rocha. Identification and characterization of thousands of bacteriophage satellites across bacteria. *Nucleic Acids Research*, 51(6):2759–2777, apr 2023.

[79] Renata C. Matos, Nicolas Lapaque, Lionel Rigottier-Gois, Laurent Debarbieux, Thierry Meylheuc, Bruno Gonzalez-Zorn, Francis Repoila, Maria Fatima de Lopes, and Pascale Serror. Enterococcus faecalis Prophage Dynamics and Contributions to Pathogenic Traits. *PLoS Genetics*, 9(6), 2013.

[80] Sébastien Lemire, Nara Figueroa-Bossi, and Lionello Bossi. Bacteriophage Crosstalk: Coordination of Prophage Induction by Trans-Acting Antirepressors. *PLoS Genetics*, 7(6):e1002149, jun 2011.

[81] Dominik Refardt. Within-host competition determines reproductive success of temperate bacteriophages. *The ISME Journal*, 5(9):1451–1460, sep 2011.

[82] Justin E. Silpe, Olivia P. Duddy, and Bonnie L. Bassler. Induction mechanisms and strategies underlying interprophage competition during polylysogeny. *PLoS pathogens*, 19(5):e1011363, 2023.

[83] Malika Humbert, Kévin T. Huguet, Frédéric

Coulombe, and Vincent Burrus. Entry Exclusion of Conjugative Plasmids of the IncA, IncC, and Related Untyped Incompatibility Groups. *Journal of Bacteriology*, 201(10), may 2019.

[84] Nicolas Rivard, Malika Humbert, Kévin T. Huguet, Aurélien Fauconnier, César Pérez Bucio, Eve Quirion, and Vincent Burrus. Surface exclusion of incc conjugative plasmids and their relatives. *bioRxiv*, 2024.

[85] Pedro H. Oliveira, Marie Touchon, and Eduardo P.C. Rocha. The interplay of restriction-modification systems with mobile genetic elements and their prokaryotic hosts. *Nucleic Acids Research*, 42:10618–10631, 9 2014.

[86] Fabienne Benz, Sarah Camara-Wilpert, Jakob Russel, Katharina G Wandera, Rimvydė Čepaitė, Manuel Ares-Arroyo, José Vicente Gomes-Filho, Frank Englert, Johannes A Kuehn, Silvana Gloor, et al. Type iv-a3 crispr-cas systems drive inter-plasmid conflicts by acquiring spacers in trans. *Cell Host & Microbe*, 32(6):875–886, 2024.

[87] María Getino and Fernando de la Cruz. Natural and artificial strategies to control the conjugative transmission of plasmids. *Microbiology Spectrum*, 6:33–64, 1 2018.

[88] João Alves Gama, Rita Zilhão, and Francisco Dionisio. Co-resident plasmids travel together. *Plasmid*, 93:24–29, sep 2017.

[89] Claudia Igler, Jana S. Huisman, Berit Siedentop, Sebastian Bonhoeffer, and Sonja Lehtinen. Plasmid co-infection: linking biological mechanisms to ecological and evolutionary dynamics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 377, 1 2022.

[90] Lance Boling, Daniel A. Cuevas, Juris A. Grasis, Han Suh Kang, Ben Knowles, Kyle Levi, Heather Maughan, Katelyn McNair, Maria Isabel Rojas, Savannah E. Sanchez, Cameron Smurthwaite, and Forest Rohwer. Dietary prophage inducers and antimicrobials: toward landscaping the human gut microbiome. *Gut Microbes*, 11(4):721–734, 2020.

[91] Svetlana Alexeeva, Jesús Adrián Guerra Martínez, Maciej Spus, and Eddy J Smid. Spontaneously induced prophages are abundant in a naturally evolved bacterial starter culture and deliver competitive advantage to the host. *BMC Microbiology*, 18(1):120, dec 2018.

[92] E.Fidelma Boyd and Harald Brüssow. Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. *Trends in Microbiology*, 10(11):521–529, nov 2002.

[93] Andreas Porse, Kristian Schønning, Christian Munck, and Morten O. A. Sommer. Survival and evolution of a large multidrug resistance plasmid in new clinical bacterial hosts. *Molecular Biology and Evolution*, 33(11):2860–2873, aug 2016.

[94] Eric C. Keen. Tradeoffs in bacteriophage life histories. *Bacteriophage*, 4(2):e28365, feb 2014.

[95] Eva M. Top, Dirk Springael, and Nico Boon. Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters. *FEMS Microbiology Ecology*, 42(2):199–208, nov 2002.