Keep Your Frenemies Closer: Bacteriophage That Benefit Their Hosts Evolve to be More Temperate

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Abstract

Bacteriophages, also known as phages, are viruses that infect bacteria. They are found everywhere in nature, playing vital roles in microbiomes and bacterial evolution due to the selective pressure that they place on their hosts. As obligate endosymbionts, phages depend on bacteria for successful reproduction, and either destroy their hosts through *lysis* or are maintained within the host through *lysogeny*. Lysis involves reproduction within the host cell and ultimately results in the disruption or bursting of the cell to release phage progeny. Alternatively, lysogeny is the process by which phage DNA is incorporated into the host DNA or maintained alongside the host chromosome, and thus the phage reproduces when their host reproduces. Recent work has demonstrated that phages can exist along the parasitism-mutualism spectrum, prompting questions of how phage would evolve one reproductive strategy over the other, and in which conditions. In this work, we present an agent-based model of bacteriophage/bacterial co-evolution that enables lysogenized phage to directly impact their host's fitness by using the software platform Symbulation. We demonstrate that a viral population with beneficial lysogenic phage can select against lytic strategies. This result has implications for bottom-up control of vital ecosystems.

Introduction

Bacteriophages, viruses that specifically infect bacteria, are found everywhere in nature (Chevallereau et al., 2022). Phages play vital roles in the construction of their ecosystems as a result of selective pressure that they place on their hosts (Hobbs and Abedon, 2016; Chevallereau et al., 2022). The interactions between phage and bacterial hosts have demonstrated co-evolutionary dynamics such as the Red Queen hypothesis, Lotka-Volterra, arms races, bet hedging, etc. (Maslov and Sneppen, 2015; Stern and Sorek, 2011; Rohwer and Segall, 2015; Weitz and Dushoff, 2008) and they have particular importance in many microbial communities, including the human gut and digestive system (Subramanian et al., 2020; Bäckhed et al., 2005; Allison and Verma, 2000). The rise in antibiotic-resistant bacteria has led to renewed interest in phage therapy, the use of bacteriophages to combat bacterial infections, driving further research regarding phage-bacterial evolutionary dynamics (Lin et al., 2017; Lu and Koeris, 2011; Neu, 1992; Sulakvelidze et al., 2001). Additionally, bacterial populations can develop resistance to phage, and phage can increase bacterial cooperation in eukaryotic cells (Obeng et al., 2016). Finally, some bacteriophages can transfer genes between bacteria or disrupt genes upon integration into the chromosome, leading to phenotypic changes of the host (Miller, 2001). Because of these dynamics and their medical relevance, increasing our understanding of phage-bacteria co-evolution is crucial.

Phages have been historically considered strictly-harmful obligate endosymbionts because they depend on their bacterial hosts for successful reproduction and often cause the death of their host. However, there is growing evidence that phages can also confer beneficial traits to their hosts (Anderson et al., 2014; Harrison and Brockhurst, 2017; Owen et al., 2021; Obeng et al., 2016). As such, it is clear that phage have the potential to exist along a parasitism-mutualism spectrum.

Where a species lands on that parasitism-mutualism spectrum is largely influenced by its reproductive strategy (Yamamura, 1993; Ewald, 1987; Vostinar and Ofria, 2019). That is, endosymbionts that employ *horizontal transmission* are less likely to be mutualistic than their counterparts who rely on *vertical transmission*. Horizontal transmission is the process where an endosymbiont reproduces and its offspring is released into the world, independent of host reproduction. Vertical transmission, on the other hand, involves the transfer of a symbiont (or symbiont's offspring) from the parent host to the host offspring near the moment of reproduction. This method thereby provides selective pressure for the symbiont to increase their host's fitness in order to increase the likelihood of the symbiont's own reproduction. Thus vertical transmission is a mechanism that directly fosters mutualistic relationships between organisms, unlike horizontal transmission.

Bacteriophages mainly use two reproductive strategies, which can be considered approximate parallels to horizontal and vertical transmission: lysis and lysogeny (Hobbs and Abedon, 2016). Upon successful infection of a bacterial host, a bacteriophage will either enter the lytic cycle or be-

come a temperate lysogenic phage. Lytic phage redirect the bacterium's replication machinery and metabolic processes to mass produce phage particles, ultimately releasing those particles into the environment upon death of the host cell. This process is similar in dynamic to other methods of horizontal transmission, where endosymbiont offspring spread to other hosts in a population, and therefore the endosymbiont is not under selective pressure to increase host fitness. Lysogenic phage, however, can enter the host's genome and become an integrated prophage that may benefit or harm the bacterium's ability to function, or they may be completely inert (Anderson et al., 2014; Harrison and Brockhurst, 2017; Owen et al., 2021; Obeng et al., 2016). In this state, lysogenic phage do not produce virulent offspring but instead are maintained along with the bacterium's own reproductive process. Thus, lysogeny is a vertical transmission strategy, since endosymbiont offspring are transmitted vertically to host offspring: when the host reproduces, so too does the prophage. These are not the only two ways for phages to reproduce; however, the majority of phage reproduction likely exists along a continuum between these two methods (Mäntynen et al., 2021).

Phage replication through either the lytic or lysogenic cycle can dramatically affect host cells at both the individual and population levels. Evaluating this relationship between reproductive strategy and eco-evolutionary dynamics has been challenging, yet critical to our understanding of phage-host interactions. With phages now being intentionally used to treat antibiotic-resistant bacterial infections, the ability to predict potential outcomes of these interactions is becoming more crucial. Specifically, phage therapy relies on obligately lytic bacteriophage to kill bacteria, but their infection cycle is typically evaluated in controlled, pure culture systems. However, the environment in which they are introduced has multiple organisms, including temperate phages within the pathogenic hosts they may be targeting. It is unknown whether or how the obligately lytic phages may transition to a temperate infection cycle, or vice versa.

Previous modeling research shows that a regular influx of naive or uninfected hosts selects for phage to be lytic, because there are sufficient hosts for phage offspring to infect (Wahl et al., 2019; Sinha et al., 2017). Conversely, a lack of naive hosts selects for phage to be temperate, with a higher chance of lysogeny, because the number of available hosts for their offspring to infect is limited. However, most models assume that lysogenic phage are dormant and do not have an impact on their host. There is growing evidence that that is not necessarily the case, and that lysogenized phage can either harm or help the infected bacterium (Anderson et al., 2014; Harrison and Brockhurst, 2017; Owen et al., 2021; Obeng et al., 2016). The factors determining the switch or preference for phages to be lytic or lysogenic is therefore likely more complicated than host population density alone.

For example, many strains of the species *Shigella flexneri* harbor prophages and parasitic genes in their genomes. Although it is a close relative of *Escherichia coli*, *Shigella flexneri* is an intracellular human pathogen that proliferates in intestinal epithelial cells (Labrec et al., 1964). The genus *Shigella* causes approximately 167 million cases of bacillary dysentery annually (Troeger et al., 2018). Its virulence has been attributed to the fact that some of the prophages disrupt functional avirulence genes, while others contribute to virulence or immune evasion (Nakata et al., 1993; Maurelli, 2007). For example, part of what determines the virulence of *Shigella flexneri*, its survival in the intestinal environment, and its ability to evade the human immune system is its serotype West et al. (2005). A molecule on the outer surface of the bacterium–the O-antigen–determines the serotype, with at least 20 serotypes described thus far (Muthuirulandi Sethuvel et al., 2017). This property is highly evolvable, with new serotypes regularly emerging Livio et al. (2014); Muthuirulandi Sethuvel et al. (2017). Sequencing has revealed that a majority of genes involving serotype modification originated from bacteriophages (Knirel et al., 2015). While some of these phages are now defunct prophages, others still persist as functional viruses. Thus, phages can provide a benefit to the host through serotype modification or conversion, by facilitating immune evasion of the *Shigella flexneri* host.

While a phage may be able to provide a benefit to its host bacterium, lysogenized phage still have the potential to induce and enter the lytic cycle either spontaneously or when in stressful environmental conditions (Nanda et al., 2014; Bruneaux et al., 2022). This possibility leads to conflicting selective pressures for bacteria and phage, where prophage that benefit their hosts may be under increased selection towards lysogeny, but hosts that evolve to rely on such benefits are then highly susceptible to eventual induction and lysis. It is therefore an open question (1) under what environmental conditions temperate phage are under selective pressure towards more frequent lysogeny if they are able to impact host fitness, (2) whether more frequent lysogeny then selects for temperate phage to be more beneficial to their hosts, and (3) whether these phenomena might reinforce one another.

These questions are difficult to experimentally test in wet-lab systems due to the necessary control of the relevant traits, the time required to observe evolutionary timescales, and the cost of materials and labor. However, agent-based modeling enables us to overcome those challenges as well as investigate the general principles potentially governing these systems (Vostinar et al., 2021). Therefore, we expanded the open-source agent-based modeling platform Symbulation to simulate bacteria/phage coevolutionary dynamics and determine the effect of beneficial prophage on the evolution of lysogeny.

We determined that if the prophage population provides a benefit to hosts on average, the population will also evolve

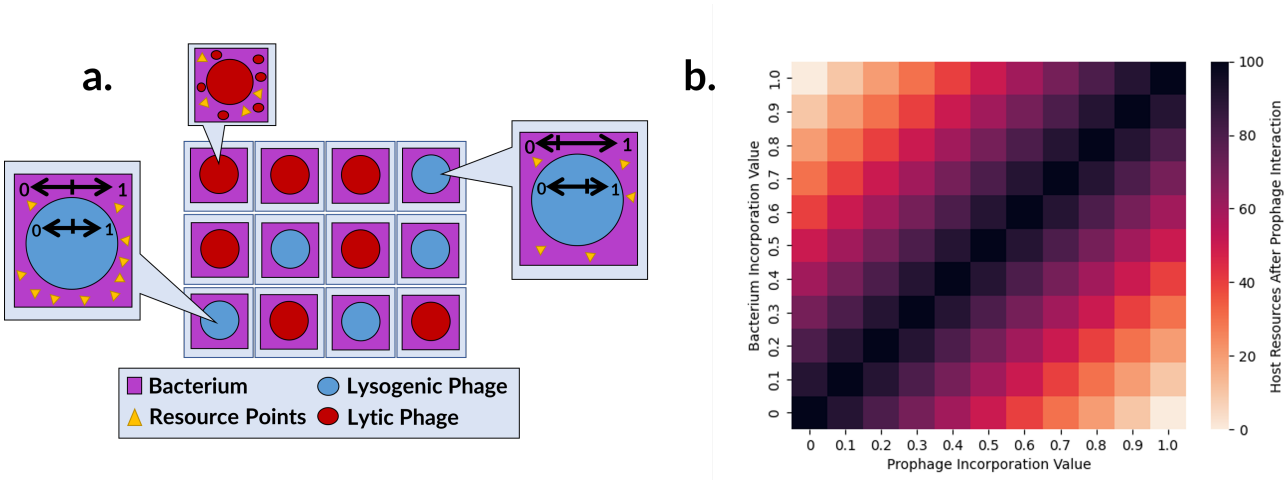

Figure 1: **Overview of the simulation.** (a) A population of hosts (purple squares) can be infected by up to one phage (blue or red circles). Phage and hosts each have an *incorporation value* (slider between 0 and 1). Lytic phage hijack host resources and produce viral particles, while lysogenic phage influence host resource levels depending on the similarity of incorporation values. (b) The amount of resources a host receives depends on the similarity between the host and prophage's incorporation value. The more closely they match, the more resources the host receives. The prophage influence ranges from depleting to doubling the 50 resources given to the hosts at each timestep.

higher rates of lysogeny, even to the point of evolving to be temperate when they otherwise would have evolved to be lytic. These results indicate that these co-evolutionary dynamics need to be explored in wetlab systems and their implications considered in phage therapy and related applications.

Methods

For this work, we expanded Symbulation, an agent-based modeling platform (Vostinar, 2021). Symbulation enables symbiotic relationships between agent populations and can track the evolution of genes and characteristics over time at an individual level. In Symbulation, a population of simulated bacteria are able to compete for resources, reproduce with mutation, and therefore evolve. In addition to bacterial hosts, the virtual world also supports a population of bacteriophage, as shown in Figure 1a. Each phage can survive outside of a host, but must infect a bacterial host to reproduce. Upon infection, a phage enters the lytic or lysogenic life cycle, depending on their genome.

Host/Bacterium Characteristics

Each host and symbiont in Symbulation has a set of characteristics, which can be viewed as the subset of interest of its genome. The bacterial host genome consists of an *interaction value* (between -1 and 1) and an *incorporation value* (between 0 and 1). The interaction value determines the degree to which it will defend against a potentially harmful symbiont. In this work, the interaction value is always negative, though able to evolve, due to the hostile interaction between bacterial hosts and phage symbionts. The incorporation value determines how successful a lysogenic bacteriophage is at incorporating itself into the bacterial host genome. At each simulated timestep, a bacterium collects 50 resources from the environment, and is able to reproduce when it accrues 600 resources. Thus, an uninfected bacterium will reproduce every 12th time step.

However, an infected bacterium may spend some of its resources on defense against its symbiont and will therefore reach the reproduction threshold at a slower rate. This is an abstraction of the many ways that bacteria defend against bacteriophage (Ofir and Sorek, 2018) by imposing a trade-off between reproductive speed and defensive capability. The amount of resources spent on defense is proportional to the host's interaction value. For example, a bacterium with an interaction value of -0.3 will spend 15 resources (30% of its collected 50 resources) on defense at each timestep. The resources remaining after defense spending can be further augmented or disrupted by the behaviors of its symbiont, leading to each infected bacterium reaching the reproduction threshold at its own rate.

When a bacterium reaches the reproduction threshold, it will create a copy of itself as its offspring (along with any lysogenized prophage), however the copying process is imperfect and mutations occur in both values of its genome. The size of a mutation is taken from a normal distribution with a mean of 0 and standard deviation of 0.02. The bacterial offspring (along with lysogenized prophage), after mutation, is then placed into a random position in the world. If the selected position is already occupied by another bac-

terium, the previous occupant is killed and replaced by the new offspring. By this mechanism, the organisms that accrue resources the quickest, and thus reproduce the fastest, will eventually dominate the population.

A bacterium that is infected with a bacteriophage may be helped or harmed by the phage and thus reproduce at a different rate, if at all. In particular, a host's resource amounts are influenced differently if the phage is lytic or lysogenic. Lytic phage typically steal incoming resources from (and eventually kill) their hosts, while the impact of a lysogenic phage varies depending on both phage and host genomes, and ranges from destroying all new resources to doubling them, as shown in Figure 1b.

Symbiont/Bacteriophage Characteristics

The bacteriophage genome consists of an *interaction value* (between -1 and 1), an *incorporation value* (between 0 and 1), a *chance of lysis* (between 0 and 1), and a *chance of induction* (between 0 and 1). What a phage does at each timestep differs for phage living outside of a host, lytic phage, and lysogenic phage.

Free-Living Phage At each time step, each freely-living phage (those that are outside of a host) attempts to infect a nearby host to begin the process of reproduction. If the targeted host is not already hosting a phage, the freely-living phage will infect said host with a 100% success rate. Upon infection of a host, the phage will then begin either the lytic or lysogenic life cycle, with a probability based on its chance of lysis. However, if the targeted host for infection is already hosting a phage, the freely-living phage will die upon attempted infection. The freely-living phage cannot collect resources, reproduce, or move their position in the world without an available susceptible host, making them obligate endosymbionts that can survive temporarily outside of a host. Thus the successful infection of a bacterial host is essential for their evolutionary survival.

Lytic Phage Lytic phage behavior follows the mechanics of the lytic cycle, where the phage redirects the bacterium's resources to produce phage offspring until the host cell is eventually burst during lysis. At each timestep, the lytic phage attempts to steal resources from its host. To be successful, the phage's interaction value must be more negative, or smaller, than its bacterial host's interaction value. Otherwise, the phage will be unable to steal any resources and therefore will be unable to reproduce. If the phage's genome will allow it to steal resources, then the amount stolen from its host will be proportional to the difference between the bacterium and bacteriophage interaction values. For example, if the bacterium interaction value is -0.3, then it will have 35 resources leftover after spending 15 resources on defense. Then if the phage interaction value is -0.7, it will successfully steal an additional 14 resources ($|(-0.3) - (-0.7)| * 35 = 14$). Therefore the lytic bacteriophage will be able to use 14 resources for phage reproduction, and its host is left with only 21 resources for bacterial reproduction.

Once the bacterium resources have been successfully redirected, the lytic phage will use these resources to create as many phage offspring as possible (each of which uses 10 resources) before bursting its host cell. Once offspring are created, they are dormant in the bacterial host until the cell bursts. The time at which a lytic phage will burst its host cell is determined by the phage's burst timer, which starts at 0 upon injection to their host. At each time step, the burst timer is incremented by a random number pulled from a normal distribution centered around 1 with a standard deviation of 1 (the addition of some noise to the timer is necessary to prevent artifacts from perfectly synchronized phage populations). Once the burst timer reaches a value of 100, the bacterial host cell will burst. Upon bursting, each phage offspring is released into the world where they become freely-living phage. The bacterial host and lytic phage then both die.

Lysogenized Prophage The lysogenic phage cycle includes 1) a potential interaction with the host resources, 2) a chance for the phage to induce and begin the lytic cycle, and 3) a chance that the phage is killed off - simulating the degradation of prophage DNA. The lysogenic phage in Symbulation have an active life cycle, which relaxes assumptions from previous work that prophage are dormant. In this model, lysogenic phage have the ability to interact with their host's resources, thereby affecting rates of reproduction and creating an environment in which lysogenic phage and bacterial hosts may develop a (tenuous) mutualistic relationship. The *incorporation* mechanism described here is an abstraction of the many ways that a lysogenized prophage can positively or negatively impact its host's fitness through the genetic material that the prophage contains and how that genetic material complements or interferes with the host's genes. In addition, the location that the prophage incorporates into the host's genome can directly change the host's gene expression, which can have varying impacts on the host fitness. The mechanism that we implemented in this work captures the core dynamics of this interaction, specifically the importance of how matched or mismatched the prophage and host genomes are to each other and the possibility that a phage type may have a positive impact on one host but a negative impact on another host, depending on chance and the hosts' genomes.

Incorporation Value The ability of prophage to influence host resources is controlled by a configuration setting and was enabled for only some experiments in this study. If the direct effects of lysogenic phage on host resources is enabled, it proceeds as follows.

At each time step, the lysogenic phage's host may have spent some of its resources on defense, as detailed previously. A lysogenic phage will then be able to influence the host's remaining resources, ranging from removing all resources to doubling host resources. The amount of resources left for the host after the lysogenic phage has interacted with them is proportional to the difference between the phage's incorporation value and the bacterial host's incorporation value. More specifically, the resources left for the host will be equal to $r * (1 - |i_b - i_p|) * s$, where r is the host resources remaining after defense spending, i_b is the bacterial host incorporation value, i_p is the phage incorporation value, and s is the synergy value (which is set to 2 for all experiments). Therefore, the closer together the incorporation values are, the more resources the host accrues. For example, if a bacterium's interaction value is -0.3, it will first use 15 resources on defense and have 35 remaining resources. Then, if the bacterium's incorporation value is 0.8 and the lysogenic phage's incorporation value is 0.6, the bacterium will have 56 resources ($35 * (1 - |0.8 - 0.6|) * 2 = 56$). Because 56 resources for the host is more than 35 resources, the presence of the prophage would lead to a faster rate of reproduction for the bacterial host, as well as the prophage by vertical transmission.

Induction After influencing their host's resources, lysogenic phage have a chance of inducing back to the lytic cycle, determined by the probability based on their inherited chance of induction. The ability for an incorporated lysogenic phage to induce back into the lytic cycle means that any mutualistic relationship between bacterial host and bacteriophage is unstable and could be exploited by the phage.

Prophage Loss Last in the process of a lysogenic phage is the possibility of prophage loss, or DNA degradation. The probability of prophage loss is based on a global setting and remains constant for the entirety of an experiment. If the prophage is lost, it is immediately removed from the bacterial host, leaving it uninfected.

Configuration Settings

Each experiment described below begins with 1000 bacterial hosts and 500 bacteriophages, leading to a multiplicity of infection of 0.5. The world has a carrying capacity of 10,000 bacteria and has no spatial structure - meaning offspring are placed randomly in the world upon birth. We assume that only one phage can infect each host at a time, and any subsequent infection attempts lead to the death of the second phage, following the assumptions made by previous models. All experiments were run for 10,000 timesteps, and were replicated 30 times with varying random seeds. For all statistical significance tests, we conducted Wilcoxon rank-sum tests and applied a Bonferroni correction for multiple comparisons to all p-values.

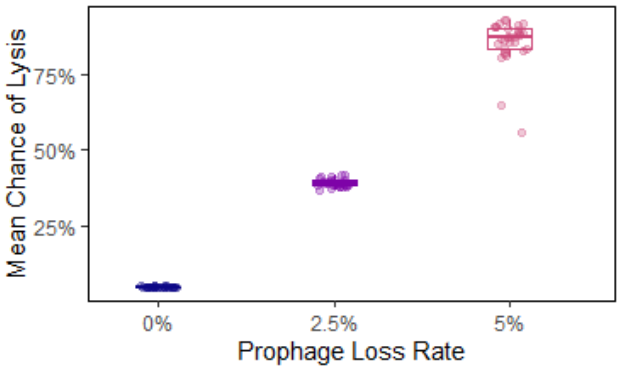

Figure 2: **Final average chance of lysis at three prophage loss rates.** Induction and prophage interaction were prevented to remain consistent with previous work.

Code for generating all the data and supplemental figures, including configuration settings, are available at `https://github.com/anyaevostinar/Evolution-of-Lysogeny-Paper`. All plots were created with R (R Core Team, 2020) and ggplot2 (Wickham, 2016) using the Viridis color library (Garnier et al., 2021). Symbulation is available at `http://www.symbulation.org` (Vostinar, 2021) and is built on the Empirical platform, which is available at `https://github.com/devosoft/Empirical` (Ofria et al., 2020).

Results and Discussion

To investigate the effect of beneficial or harmful prophage on the evolution of lysogeny, we conducted three sets of experiments: 1) verification that higher prophage loss rate selects for lysis when prophage are strictly dormant, 2) determination of the effect of a chance of induction on the evolution of lysogeny when prophage were still strictly dormant, and 3) determination of the effect of prophage that can be beneficial or harmful on the evolution of lysogeny.

Higher prophage loss rate selects for lysis

Previous work has shown that a higher density of uninfected hosts selects for phage with a higher propensity for lysis (Wahl et al., 2019; Sinha et al., 2017). In the previous work, the population of uninfected hosts came into the population through either migration or loss of prophage.

We first verified that our system was consistent with these previous findings by running experiments with varying amounts of prophage loss. Increased chance of prophage loss leads in turn to a higher proportion of naive/uninfected hosts, and so should select for increased chance of lysis in the phage population. We examined the resulting phage population for propensity to enter the lytic or lysogenic life cycle upon infection. In these experiments, induction and

prophage interaction were both prevented to match the assumptions of previous models.

As shown in Figure 2, when the prophage loss rate was 0%, bacteriophage populations evolved to have a final average of 4.87% chance of lysis. At a higher prophage loss rate of 5%, the chance of lysis evolved to a significantly higher rate of 85.64% ($p < 0.005$). At an intermediate prophage loss rate of 2.5%, the chance of lysis evolved to a value of 39.19%. These results agree with previous work that the density of naive hosts has a large impact on the evolution of phage reproductive strategies. Specifically, lysis is not beneficial when there are not enough uninfected hosts for the offspring to successfully infect and spread, because the offspring generally die. Therefore, when prophage loss rate is low, a lysogenic strategy is more successful. However, when prophage loss rate is higher, there are more uninfected hosts, making rapid spread through lysis a more fit strategy.

Effect of induction back into the lytic cycle

Most previous work modeling the evolution of the lysis/lysogeny switch has made the simplifying assumption that once a phage becomes lysogenic, it stays that way. However, in most bacteriophage, there is a small chance that a lysogenic phage will induce back into the lytic cycle when under certain kinds of stress, as discussed previously. Therefore, we determined whether the possibility of induction back to the lytic cycle would significantly change the evolution of lysis and lysogeny.

To determine the effect of induction, we enabled the possibility for lysogenic phage to induce and begin the lytic cycle, and repeated the same experiments with varying prophage loss rates. Because the starting chance of induction could influence the evolutionary trajectory, we conducted two separate treatments: one where the population of phage started with a 0% chance of induction and another where they started with a 10% chance of induction.

As Figure 3c shows, the final average chance of induction evolved to be at or below 10% regardless of prophage loss rate and the final average chance of induction was not meaningfully impacted by the starting chance of induction for all treatments. However, the final average chance of induction was impacted by different prophage loss rates. Specifically, when the chance of induction started at 0% and prophage loss rate (PLR) was either 2.5 or 5%, the chance of induction evolved to an average of 4.78% and 7.49%, respectively, both significantly above the rate of 1.56% when PLR was 0% (both $p < 0.005$). These results show that the common modeling assumption that prophage are not able to induce is not in agreement with selection pressures, however the impact may be small.

Further, as shown in Figure 3b, the possibility of the induction chance evolving significantly impacts the evolution of the lysis/lysogeny decision at only some prophage loss rates. When the prophage loss rate is 0%, the possibility of induction (starting at 0% or 10% probability) led to probabilities of lysis that are not meaningfully different than when induction is not possible. Specifically, with a prophage loss rate of 0% and without induction, the final average chance of lysis was 4.88%. With the possibility of induction, the final average chance of lysis was 4.92% and 4.93% with starting induction chances of 0% and 10% respectively. The similarity in these values demonstrate that the ability for prophage to induce and begin the lytic cycle does not have a meaningful effect on the lysis/lysogeny decision when the prophage loss rate is 0%, most likely because the lytic cycle is not beneficial.

However, the chance of lysis at a prophage loss rate of 2.5% is significantly impacted by the possibility of induction, with final average chance of lysis of 13.23% and 19.23% when chance of induction started at 0 and 10% respectively, compared to 39.19% when induction was not possible (both $p < 0.005$). In addition, the prophage loss rate of 5% showed meaningful difference in the evolution of the lysis/lysogeny decision when the chance of induction started at 10%, but not 0%. Without any induction to the lytic cycle, the bacteriophage evolved to have an average chance of lysis of 85.64% and when the chance of induction started at 0%, the chance of lysis evolved to 82.20%. This difference in the chance of lysis was also insignificant with $p = 0.302$. With induction starting at 10%, however, the chance of lysis evolved to 54.81%, significantly more than without the possibility of induction ($p < 0.005$).

These results indicate that the prophage inducing into the lytic cycle does have some effect on the evolution of the lysis/lysogeny decision, especially when other selection pressures are not as strong, such as when there are a limited number of uninfected hosts. Specifically, induction back into the lytic cycle has little impact in stable environmental conditions as it does not significantly affect the evolutionary dynamics between bacterial host and phage when there are other strong selective pressures. However, future work should investigate whether the ability to induce would have a significant impact on evolutionary dynamics in unstable environments, such as when there are varying numbers of uninfected hosts.

Beneficial Prophage Select for Increased Lysogeny

The historical assumption has been that lysogenic phage are dormant and have little to no effect on their bacterial hosts. However, recent work has shown that lysogenic phage have the potential to actively influence their hosts (Anderson et al., 2014; Harrison and Brockhurst, 2017; Owen et al., 2021; Obeng et al., 2016), and in some cases can confer beneficial traits such as serotype conversion (Knirel et al., 2015). This benefit largely depends on how well a phage can incorporate itself into the host DNA and how compatible these genomes are.

To determine the effects of active prophage, we expanded

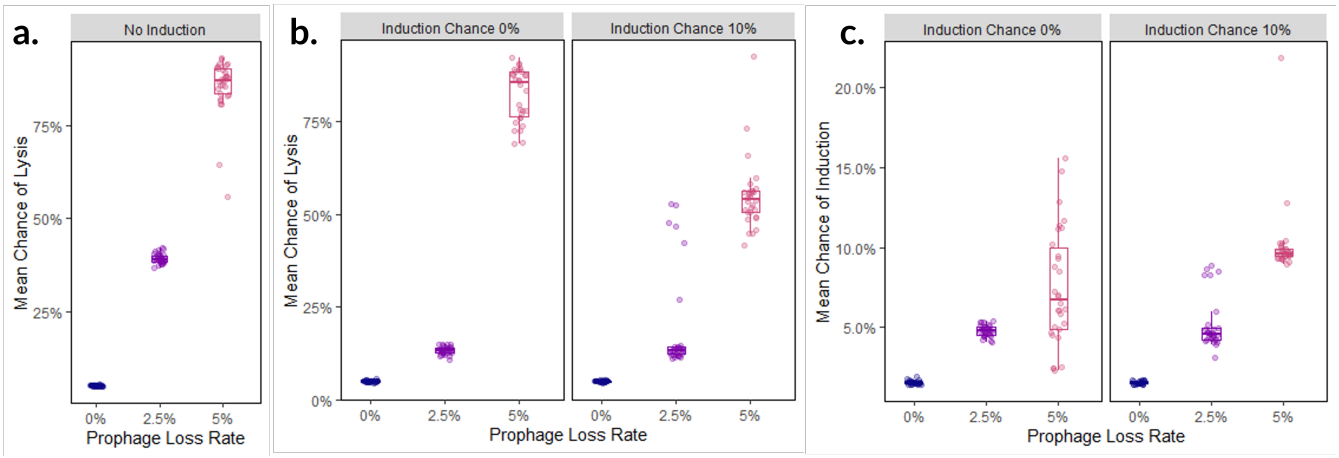

Figure 3: **Final average chance of lysis for (a) no induction enabled and (b) induction enabled. Final average chance of induction (c) when induction is enabled. All panels were tested across prophage loss rates.** Prophage were not able to impact host fitness. Induction chance in all phage for (b) and (c) was initialized to either 0% or 10% and then allowed to evolve. Note that the data in (a) is the same as Figure 2 and that the y-axis of (b) is from 0% to 25% chance.

upon Symbulation to allow lysogenic phage to influence host metabolism, as detailed in the methods. Based on the compatibility between a host and phage, the host resources can range from being eliminated to doubled (Figure 1b). In this experiment, we enabled this direct interaction between bacteria and phage and did not enable the lysogenic phage to induce back to the lytic cycle. We had three classifications of starting populations: beneficial phage (doubling host resource), neutral phage (no effect on host resources), and harmful phage (cancelling out host resources). Once these populations were initialized, their compatibility genes, or *incorporation values*, were permitted to evolve. Once again, we tested these conditions across varying levels of prophage loss rate to investigate how non-dormant prophage would influence the lysis/lysogeny decision in known environmental settings.

As shown in Figure 4b, in populations of harmful phage, the lytic reproductive strategy is highly conserved. At a prophage loss rate of 5%, the chance of lysis evolved similarly for populations of harmful phage and populations of dormant phage, with average probabilities of 90.76% and 85.64%, respectively. However, at a more intermediate PLR of 2.5%, a population of harmful phage led to a significantly higher propensity for lysis (94.59%) than dormant phage (39.19%) ($p < 0.005$).

Conversely, in populations of beneficial phage, the lysogenic reproductive strategy is highly conserved. That is, at a prophage loss rate of 5%, populations of beneficial phage evolved to have a 38.19% chance of lysis, while populations of dormant phage evolved to a significantly higher 85.64% chance of lysis ($p < 0.005$). A similar significance follows with an intermediate PLR of 2.5%, where the average chance of lysis for beneficial phage and dormant phage evolved to 5.54% and 39.19%, respectively ($p < 0.005$).

These results indicate that the active influence of prophage over host fitness (whether harmful or beneficial) has significant impacts on the lysis/lysogeny decision. Populations of harmful phage are far more likely to evolve to lysis, while populations of beneficial phage are far more likely to evolve to lysogeny. Notably, even in environmental conditions that would typically select for lysis (prophage loss rate of 5%), a starting population of beneficial phage leads to a primarily temperate and lysogenic phage population (Figure 4b). However, for a prophage loss rate of 0%, the lysogenic strategy was conserved for all populations of phage. This result indicates that a lack of naive hosts is a stronger selection pressure for lysogeny than the active influence of prophage over host fitness.

The relationship between the chance of lysis and the phage/host compatibility is likely to be a reinforcing dynamic. Figure 4c shows how phage/host compatibility evolved across varying starting phage populations. As explained in the methods, the closer the incorporation values between host and phage, the higher the host-phage compatibility, and thus the more beneficial the impact of prophage on host fitness. In environmental conditions that led to higher chances of lysis (harmful phage with PLR 2.5% and 5%), the incorporation values evolved to be far apart - leading to very low compatability. Note that a highly incompatible phage does not gain more benefit from the host. Conversely, in conditions that lead to lower chances of lysis and thus higher chances of lysogeny (beneficial phage at all PLRs), the incorporation values evolved to be quite similar - leading to very high compatibility.

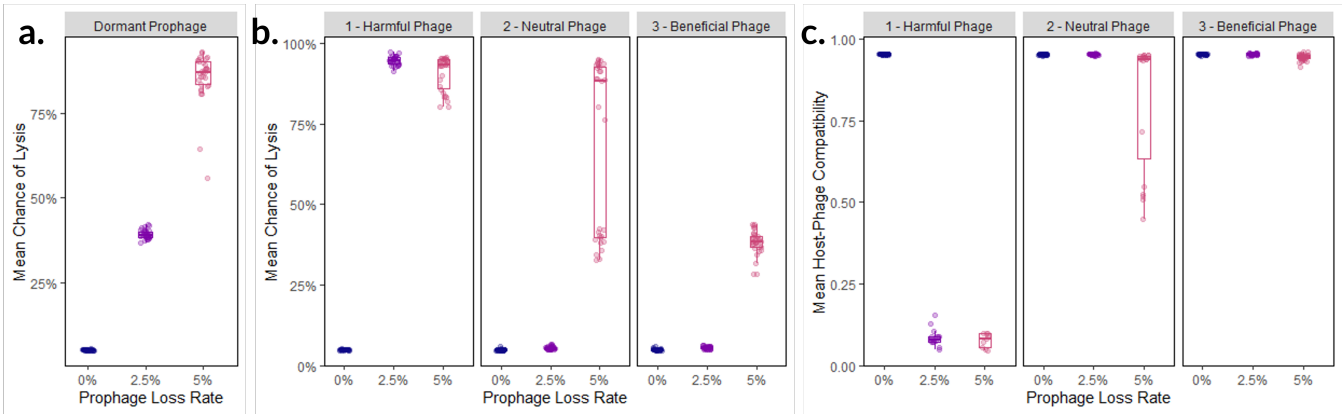

Figure 4: **Final average chance of lysis for (a) dormant prophage and (b) prophage impacting host resources. Final average host-phage compatibility (c) when prophage impact host resources. All panels were tested across prophage loss rates.** Prophage were not able to induce into the lytic cycle. Incorporation values for phage and host in (b) and (c) were initialized such that the phage were either harmful, neutral, or helpful, and then allowed to evolve. Note that the data in (a) is the same as Figure 2, and 100% compatibility indicates that the incorporation values were the same for a host-phage pair.

Therefore, environmental conditions and starting populations that select for lysis also select for low host-phage compatibility. Similarly, the conditions that select for lysogeny also select for high host-phage compatibility. These results imply that the lysis/lysogeny decision and compatibility between host and phage may be reinforcing phenomena. That is, a population of harmful phage may select for lysis and a population of lytic phage may select for incompatibility. Similarly, a population of beneficial phage may select for lysogeny and a population of lysogenic phage may select for strong compatibility. Furthermore, this implies that it is not evolutionarily advantageous for phage to be highly lytic, while maintaining traits that would allow it to be beneficially lysogenic, or vice versa.

Conclusion

In this work, we expanded upon the Symbulation software to investigate the effects on the lysis/lysogeny decision for (1) lysogenic induction into the lytic cycle and (2) non-dormant prophage. The system was first calibrated to match the assumptions of previous modeling work by showing that a regular influx of naive hosts leads to a more lytic population of phage. Then, we relaxed previous assumptions by allowing for induction. From these experiments, we concluded that induction has some effect on the lysis/lysogeny decision, particularly when other selection pressures are not as strong, such as the density of naive hosts. Next, we further relaxed previous assumptions by allowing prophage to have an active influence on host metabolism and therefore host fitness. We found that populations of harmful phage evolve towards lysis, while populations of beneficial phage more often evolve towards lysogeny. Furthermore, there is a negative relationship between the probability of lysis and host-phage compatibility in non-dormant phage. These results imply that both induction and the influence of prophage on host fitness play an active role in the evolution of the lysis/lysogeny decision, making it a vital area for further discovery.

Future work exploring these dynamics should be conducted both *in vitro* and *in silico*. Specifically, the above results and general evolutionary dynamics should be thoroughly tested in wetlab. Further computational research should address the limitations and assumptions of our work. First, the association demonstrated between the propensity for lysis and host-phage compatibility should be investigated to determine if there is a causal relationship in either direction. Second, the impact of multi-infection (more than one phage infecting a host) should be explored. And last, the model should be expanded to allow for more open-ended prophage incorporation dynamics.

A more complete understanding of the lysis/lysogeny decision is vital to our conceptions of evolutionary trajectories for both bacteriophage and bacteria. Because of the prevalence of phage and bacteria in the natural world, the co-evolutionary dynamics and potential mutualistic relationships between them has serious implications for human and environmental health as highlighted in this work.

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