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Ecological and Evolutionary Benefits of Temperate Phage: What Does or Doesn't Kill You Makes You Stronger

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Infection by a temperate phage can lead to death of the bacterial cell, but sometimes these phages integrate into the bacterial chromosome, offering the potential for a more long-lasting relationship to be established. Here we define three major ecological and evolutionary benefits of temperate phage for bacteria: as agents of horizontal gene transfer (HGT), as sources of genetic variation for evolutionary innovation, and as weapons of bacterial competition. We suggest that a coevolutionary perspective is required to understand the roles of temperate phages in bacterial populations.

1. Introduction

Temperate phages lead a double life. Like purely lytic phages, they are capable of infecting and killing bacterial cells to release phage progeny. But on occasion, rather than lysing the cell, temperate phages can integrate into the host chromosome. Here they become prophages and are replicated along with the rest of the bacterial genome when the cell divides. Prophage carriage is clearly accompanied by some jeopardy for the bacterium. Temperate phage can re-enter the lytic cycle spontaneously[1] or in response to cues from changing host or environmental conditions,[2,3] killing (with some exceptions)[4] the bacterial cell in the process. Since temperate phages are very abundant,[5–7] their ecological and evolutionary impacts on bacteria are likely to be important. It is notable that temperance is expected to be favored under conditions where host population sizes are likely to fluctuate.[8,9] This may explain comparative genomic evidence that prophages are especially common in pathogenic bacteria, which undergo small population bottlenecks when establishing infections.[7] However, population size fluctuations are also common for environmental bacteria experiencing seasonal growth rates, and correspondingly temperate phages are more abundant compared to lytic phages in high latitude, seasonal marine systems than more stable tropical regions.[10]

The relationship between temperate phages and bacteria is therefore multifaceted, comprising both costs and benefits. The nature of the bacteria-temperate phage relationship and whether prophage elements are maintained in bacterial genomes will depend on the balance of these costs and benefits. The costs of prophage carriage include, most obviously, the risk of cell death if lysis is induced,[11] the physiological costs of maintaining additional genetic material, and potential for disruption of cellular homeostasis via regulatory interference or the cytotoxic effects of prophage genes.[12] A number of ecological and evolutionary benefits of temperate phages for bacteria have been suggested. Here we define three major potential benefits of temperate phages: as agents of horizontal gene transfer (HGT), as sources of genetic variation for evolutionary innovation, and as weapons of bacterial competition (**Figure 1**). We briefly review the evidence for each of these effects and then consider the need for a coevolutionary perspective for studying bacteria-temperate phage relationships that accounts for the conflict and cooperation inherent to their interaction.

2. Temperate Phage as Agents of Horizontal Gene Transfer

HGT can accelerate bacterial evolution by providing new functional genes. Temperate phages contribute to HGT by two mechanisms, transduction, and lysogenic conversion (reviewed more comprehensively here).[13] Transduction involves the packaging bacterial DNA into the viral capsule and transferring this to new hosts via infection, and comes in two varieties: Generalized transduction is the packaging random fragments of bacterial DNA instead of the viral genome, whereas in specialized transduction, the phage take flanking bacterial DNA with them when they excise from the chromosome.[13] It is still unclear whether transduction is

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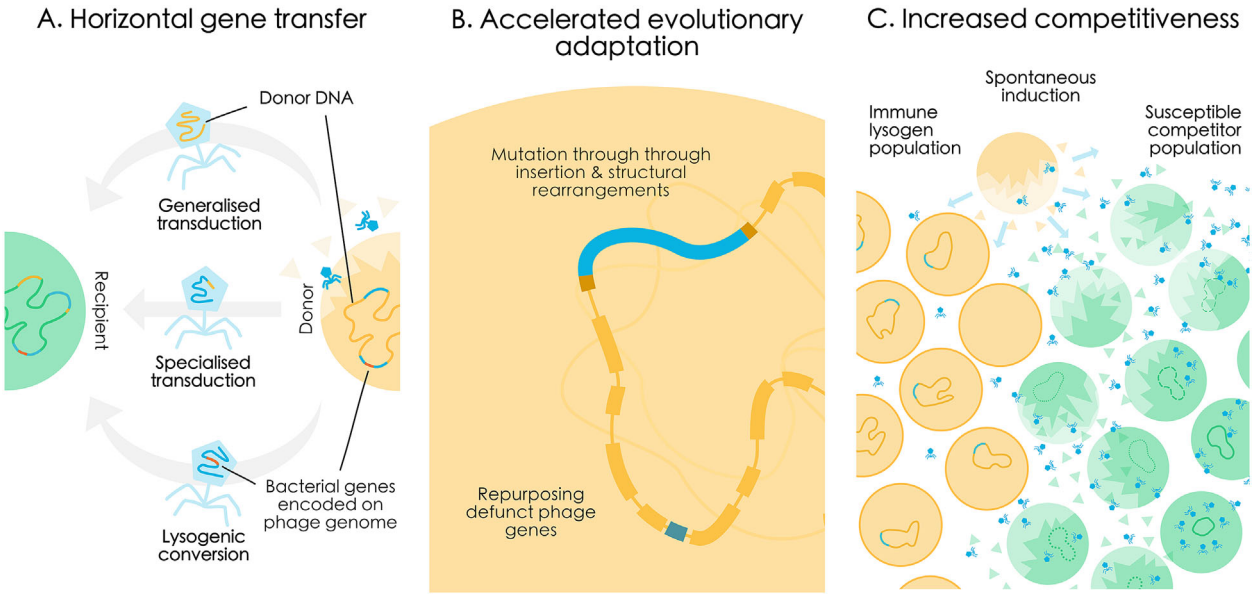

Figure 1. The potential benefits of the bacteria-temperate phage interaction. A) Temperate phages can mediate horizontal gene transfer of bacteria through transduction, where DNA of the most recent host is packaged into the viral capsule (either generalized – random fragments – or specialized – flanking DNA excised along with the phage genome). Alternatively phages can carry bacterial genes encoded on their own genome, called lysogenic conversion. B) Prophages can accelerate the rate of bacterial adaptation through insertion and transposition which generate novel mutations and rearrangements, or through the repurposing of phage genetic raw materials. C) Temperate phages can be deployed as weapons of bacterial warfare, as low rates of induction create infectious phage particles which go on to kill susceptible competitors in a self-sustaining wave of infection while fellow lysogens are immune.

simply a by-product of the DNA packing process, or an adaptation. While all phages are likely to have the potential for transduction through errors in excision or DNA packaging here is significant variation in transduction rate between phages.[14] Phages employing the "headful" DNA packaging mechanism for example have very high rates of transduction[15,16] while it is uncommon in others.[17] For the transducing phage there can be serious costs, particularly if some or all of the phage's own genome is excluded from the virus capsid.[18] There can also be biases in the DNA that is transferred, since in some cases other mobile genetic elements such as pathogenicity islands are disproportionately mobilized by transduction relative to the genome as a whole.[19] This may be because mobile elements have adapted to hijack transduction for their own transmission.

The contribution of transduction to bacterial evolution is difficult to estimate but some studies indicate that it is likely to be significant: the vast majority of prophages within *Escherichia coli* are thought to be capable of generalized transduction[20] and fine-scale sequencing of environmental samples reveals that a large proportion of phage particles carry bacterial DNA.[21] There is evidence however that transduction can confer indirect benefits to both phage and lysogen.[22] Spontaneous entry into lysis of prophages from a small number of lysogens generates lytic phage particles which will infect and kill bacteria susceptible to infection. As prophages confer immunity to their lysogenic hosts – called superinfection immunity – susceptible bacteria will be non-lysogens and potentially genetically distinct from the lysogenic host. While a small number of phages originating from lysogenic hosts may package DNA from the lysogenic host, the subsequent wave of lysis within the susceptible population will produce vastly more lytic phages creating a high likelihood that they will package novel DNA sequences from the susceptible population. Transducing phages can then transfer this DNA back to the lysogens. This process has been observed in *Staphylococcus aureus* infections as a mechanism for acquiring antibacterial resistance genes from the community, a process termed "auto-transduction."[22]

As with other mobile genetic elements, temperate phages can themselves encode useful bacterial accessory genes in their own genomes, which then become available for use by the lysogenized bacterial host cell in a process called lysogenic conversion. Lysogenic conversion is best known for its role in pathogen virulence. Prophage-encoded genes are responsible for virulence of a number of well-known human diseases (e.g., cholera,[2] scarlet fever,[23] shigella,[24] diphtheria,[25] and botulism[26]) where prophage encode toxins or proteins that modulate host-pathogen interaction, such as antigens and effector proteins.[27] Often the production of toxins – botulism and shiga toxin for example – requires lysis of the cell to release the toxin, such that benefits accrue to related bacterial cells within the infection rather than the producing cells themselves.[28] Carriage of multiple prophages – polylysogeny – is common among pathogens, allowing acquisition of multiple traits and contributing to diversity in disease pathology.[29–31]

Prophage acquisition plays an important role in the contemporary evolution of a range of pathogens. More than half of the genome variation in group A *Streptococcus* (GAS) strains, each of which is responsible for a characteristic array of syndromes from toxic shock syndrome and necrotizing fasciitis,

is due to variation in their prophage cargo.[30] Shifts in disease serotypes associated with acquisition of new prophages have been observed in real time in *Streptococcus*[32,33] and *S. aureus*.[29] Temperate phages frequently encode antibiotic resistance genes and lysis induced by exposure to antibiotics has been shown to lead to mobilization of antibiotic resistance genes between bacterial hosts in the animal gut.[34] It is possible that the high proportion of prophages linked to pathogen associated traits represents a bias in the literature rather than a real biological phenomenon, indeed greater sequencing of environmental samples has revealed some surprising examples of phage-encoded traits, including photosynthetic genes carried by a cyanophage.[35]

3. Temperate Phages as Sources of Genetic Variation for Evolutionary Innovation

Many temperate phages have evolved elegant mechanisms to minimize the disruption caused by prophage insertions, targeting highly conserved insertion sites in regions unlikely to disrupt essential host functions,[36] or even carrying sections of the genes into which they insert allowing coding sequence to be maintained.[37] By contrast, the transposable phages have turned genome disruption into a fine art, having evolved to insert as prophage at seemingly random sites in the chromosome.[38] Thereafter, the transposable phage can copy and paste itself to other sites in the chromosome, and in doing so cause high rates of gene knockouts as well as large scale structural changes.[38,39] The first of the transposable phages to be identified, Mu, transposes at random within the genome, generating inversions, deletions, and integration between copies of itself, the chromosome and other mobile elements.[38] Mu also performs transduction and typically mobilizes at least 2KB of flanking DNA during excision.[38] Mu-like phages have now been shown to infect many bacterial clades,[40–42] although many of these phages are in fact mosaics made up of fragments of transposable and non-transposable phages.[41]

As with any mutational process, the majority of mutations caused by transposable phage insertions will have deleterious effects for host cell fitness by disrupting useful gene functions. However, the additional mutational load caused by transposable phages is likely to be beneficial during adaptation to new environments because it also increases the supply of rare beneficial mutations, accelerating evolutionary innovation.[43] In a recent experimental evolution study, populations of *Pseudomonas aeruginosa* adapted more rapidly when cocultured with temperate phages including the transposable phage, ϕ4 (related to *Pseudomonas* phage D3112).[44] *P. aeruginosa* is the most common pathogen infecting the lungs of cystic fibrosis (CF) sufferers, where it must rapidly adapt to the lung environment. These experiments modeled the CF lung environment by using a sputum-like growth medium that recapitulates the physiochemical properties of CF lung sputum. Bacterial evolution was more highly parallel in the presence of temperate phages, suggesting stronger selection driven by phages. Moreover, in populations cocultured with phages, the observed adaptive mutations in bacteria were often caused by ϕ4 insertions, which disrupted a range of functions including type-IV pilus dependent motility and quorum sensing regulators.[44] The temperate phages used in the study were all derived from the Liverpool Epidemic Strain (LES) of *P. aeruginosa*, a common transmissible strain infecting CF patients that is associated with higher morbidity and mortality.[45] Interestingly, these LES temperate phages remain active in the bacterial genome and capable of lysis many years after colonizing the CF airway,[46] and appear to directly contribute to the fitness and competitiveness of LES in the lung environment.[47]

In addition to causing mutations, prophage sequences can also provide the raw genetic material for bacterial innovation. Bacterial genomes are littered with copies of deactivated prophages that through mutation accumulation have lost the ability to reproduce by lysis.[48] However, far from being "junk" DNA, many deactivated prophage regions are conserved[48] and experimental deletions of these cryptic prophage genes in *E. coli* has revealed that they positively contribute to bacterial fitness.[49] Prophage sequences are frequently successfully repurposed by bacteria. Numerous major bacterial innovations stem from the co-option of viral hardware including gene transfer agents (virus-like particles that transfer bacterial DNA only)[50] and type VI secretion systems (phage-derived syringes to inject or puncture neighboring cells).[51] Deactivated prophages can also act as regulatory switches – a process called "active lysogeny."[52] Here, prophages sit within the coding region of the gene disrupting gene function. Excision of the prophage restores function, but the excised phage is unable to perform lysis. Because once excised the prophage may be lost, such switches are often unidirectional and thus useful for developmental processes. For example, in some Cyanobacteria, phage excision repairs nitrogen fixation genes during differentiation of vegetative cells into heterocysts.[53] However, in other cases active lysogeny switches have been shown to be reversible, where excised phages persist in the cell as a plasmid. In *Streptococcus pyogenes* a defective prophage is used to fine tune the bacterial mutation rate, by insertion/excision of the mismatch repair gene *mutL*.[54]

4. Temperate Phages as Weapons of Bacterial Competition

In contrast to deactivated prophages, in many other cases prophages retain the ability to switch from lysogenic quiescence into the lytic cycle even after 1000 s of generations of bacterial evolution, however, counter-intuitively, this process can be beneficial for bacteria. Induction of the lytic cycle can be triggered by cellular or environmental cues[2,3] but also occurs spontaneously[1] at low levels within the bacterial population. While this is clearly bad news for the individual bacterium (now dead), low rates of phage lysis have been shown to increase the competitiveness of the surviving bacterial population.[47,55–57] Infectious phage particles are released into the environment, where they infect and kill susceptible bacteria in a self-sustaining wave of infection. Because temperate phages usually provide their host cells with super-infection immunity,[58] bacteria that are lysogenized by the same temperate phage – most likely to be the clone mates of the now deceased individual cell – are

immune to infection. Non-lysogens – likely to be less closely related and more likely to be competitors – are susceptible. Temperate phage-mediated competition allows lysogens to invade from rare, because of the amplifying nature of viral infection where each subsequent infection yields yet more infectious phages.[55]Consequently, this process is likely to enhance the fitness of colonizing bacteria, facilitating invasion of a resident community. Consistent with this, lysogens have been shown to invade a population of non-lysogens of *P. aeruginosa* in rat lungs.[47]

The benefits of temperate phage-mediated competition appear to diminish over time. In a small fraction of lytic phage infections phages will become integrated into the genome, resulting in lysogeny and immunity within the initially susceptible competitor population.[59] Moreover, because phages often have narrow host ranges, each individual temperate phage is likely to be effective against a narrow spectrum of competitor genotypes. Interestingly, both these limitations can be overcome by polylysogeny – carrying multiple different prophages. Because the different temperate phages are likely to have different host ranges, this expands the range of bacterial competitors that a lysogen's arsenal of phage weapons[60] is effective against. Moreover, resistance against multiple phage weapons is harder to evolve since this requires that competitors either gain multiple resistance mutations or insertion of multiple prophages. Consistent with this, polylysogenic *P. aeruginosa* are fitter than single lysogens when competing against non-lysogens during experimental infections of insects.[47,56]

5. Future Directions – Unraveling the Evolutionary Ecology of Temperate Phages

While temperate phages have been well-studied in terms of their genetics and molecular mechanisms, understanding of their evolutionary and ecological roles is by comparison much more limited. Because temperate phages have their own fitness interests distinct from those of the bacterial host it is essential to take a coevolutionary perspective. Antagonistic coevolution between lytic phage and bacteria has been well characterized[61] but unlike the purely antagonistic relationship with lytic phage, the relationship between bacteria and temperate phage is a continuum between parasitism and mutualism. As such the coevolutionary process is expected to be more complex, involve more traits and be highly dependent on the environmental context. We highlight three areas for future study:

1. *Life History Evolution: Should I stay or should I go?* The more complex life-histories of temperate phages compared to lytic phages offer opportunities for adaptation in a wider range of traits. Key among these are the insertion/excision events. The benefits of lysogeny versus lysis are likely to depend on the prevailing environmental conditions, for example, prophage insertion is more likely when the multiplicity of infection is high[62] (when the availability of hosts is limited), whereas excision is induced by the bacterial SOS response[63] (when the probability of vertical transmission in that host is low). The molecular switches controlling these life-history decisions are likely to be important targets for selection, indeed the lysis/lysogeny switch of phage lambda has been shown to evolve in the lab,[64] future work should focus on how key environmental drivers shape adaption of temperate phage life-history.
2. *Does intracellular conflict between temperate phages drive phage-phage coevolution?* While polylysogeny is common, interactions between coinfecting prophages are likely to strongly influence the life history decisions of phages and potentially shape how these evolve. As we have described, polylysogeny can increase the vertical transmission of all phages if it increases host fitness by enhancing host competitiveness[47,56] or by providing complimentary fitness-enhancing bacterial traits (e.g., toxins and effector proteins). However, during induction prophages which excise earliest can suppress the reproduction of co-infecting prophages, lysing the cell before their counterpart's lytic phage particles are fully assembled.[65] Thus, more trigger-happy prophages gain a reproductive advantage during lysis.[66,67] Such conflict over lysis timing could potentially drive a coevolutionary arms race for ever-more sensitive induction among co-infecting temperate phages.
3. *Domestication of prophages is common but is it "game over" for the phage?* Active prophages, able to replicate through lysis, are vastly outweighed by defective prophage fragments within bacterial genomes demonstrating that domestication is a prominent process in prophage evolution. While phage genes may remain active within the genome, loss of replicative autonomy profoundly alters that evolutionary relationship between phage and bacterial host. With no replicative advantage over the rest of the chromosome, phage genomes will be subject to selection which maximizes the fitness of the bacterial genome as a whole. This would be expected to lead to the loss of phage genes which do not benefit the bacteria, although high rate of conservation suggest that many are beneficial.[48] The extent to which host evolution plays a role in this process is unknown, for example in commandeering control of phage lysis decisions or altering expression of cues that induce lysis. How defective and active temperate phage evolutionary trajectories interact is also currently unclear. Defective prophages may confer immunity to lytic copies[31] altering the ecological dynamics of related phages. Alternatively, the genetic material of defective prophages could be a tool-box for other prophages to be repaired through recombination,[68] potentially accelerating temperate phage evolution.

6. Conclusions

Given the ecological importance of temperate phage-bacteria interactions in a wide range of environments and the contribution of temperate phages to bacterial genome evolution, there is clearly an urgent need to better understand the ecology and evolution of these relationships. While temperate phages are most well-studied in the context of bacterial pathogens, they are widespread in environmental microbial communities[5,69] where

their role is yet to be fully explored. However this is an exciting time for the study of temperate phages. The rise of accessible genomics as well as increasingly sophisticated analytical tools has begun to yield incredible insights into the macroevolution of these complex genetic elements. Recent genomic studies have, for example, provided insights into the structure of phage taxa, addressing the role of gene exchange,[70] and host range[71] in shaping phage genome evolution. In concert with this comparative approach, experimental (although see Ref. [72]) and theoretical (although see Ref. [73]) work will be required to unpick the ecology and evolution of these complex and intriguing interactions at finer scales.

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