CHAPTER FOUR

Bacteria–Phage Interactions in Natural Environments

Samuel L. Díaz-Muñoz*,†,‡, Britt Koskella§,1

*Department of Biology, Center for Genomics and Systems Biology, New York University, New York, New York, USA
†Department of Integrative Biology, University of California, Berkeley, California, USA
‡Department of Plant and Microbial Biology, University of California, Berkeley, California, USA
§Department of Biosciences, University of Exeter, Penryn Campus, Tremough, Cornwall, United Kingdom
1Corresponding author: e-mail address: b.l.koskella@exeter.ac.uk

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Abstract

Phages are considered the most abundant and diverse biological entities on Earth and are notable not only for their sheer abundance, but also for their influence on bacterial hosts. In nature, bacteria–phage relationships are complex and have far-reaching consequences beyond particular pairwise interactions, influencing everything from bacterial virulence to eukaryotic fitness to the carbon cycle. In this review, we examine bacteria and phage distributions in nature first by highlighting biogeographic patterns and nonhost environmental influences on phage distribution, then by considering the ways in which phages and bacteria interact, emphasizing phage life cycles, bacterial responses to phage infection, and the complex patterns of phage host specificity. Finally, we discuss phage impacts on bacterial abundance, genetics, and physiology, and further aim to clarify distinctions between current theoretical models and point out areas in need of future research.

1. INTRODUCTION

Microbial interactions are now known to shape biological and geochemical processes at a global scale. The historical trajectory to this view, however, has not been straightforward and the study of microbes was traditionally much more narrow in context, primarily within the germ theory of disease (reviewed in Lederberg, 2000). Despite an early tradition of environmental microbiology, the appreciation of microbes as fundamental parts of every ecosystem did not gain mainstream scientific acceptance until recently, when culture-independent methods of detecting microorganisms were developed (Hobbie, Daley, & Jasper, 1977), notably those based on nucleic acid sequencing (Lane et al., 1985). A combination of microscopy and sequence-based methods, in particular the development of high-throughput, primerless sequencing, has provided ample data on the suspected ubiquity and diversity of microorganisms (Pace, 1997).

Bacteria, in particular, are prominent players in the microbial world and shape biogeochemical cycles that ultimately enabled the rise of eukaryotes (Karl et al., 1997). Bacteria serve as prey, fix nitrogen, generate oxygen, and may even be involved in mass extinctions of animals (Rothman et al., 2014). The burgeoning field of microbial ecology is cataloging the distribution of microbes in every imaginable biome and gaining insight into the processes that govern these distributions (Martiny et al., 2006). The realization of the ubiquity of microbial life, stemming mainly from environmental microbiology studies, has come back full circle to medical applications, as research into the human microbiome has revealed that we depend on microbes for normal...
bodily function, even prior to birth (Funkhouser & Bordenstein, 2013). Thus, the importance of microbes in shaping many of the Earth’s processes, and human health in particular, has led to a growing interest in the microbial world.

More recently, attention has turned to the viruses of bacteria, the bacteriophages (Abedon, 2009). A series of technological improvements in bacteriophage detection have provided evidence that phages are ubiquitous and more numerous and diverse than any other microbial entity (Engelhardt, Kallmeyer, Cypionka, & Engelen, 2014; Suttle, 2005, 2007; Williamson et al., 2013). However, we do not yet have a clear picture of the role that natural bacteriophage populations might play in shaping bacterial populations and communities as obligate parasites, vectors of horizontal gene transfer, drivers of bacterial evolution, and mediators of competition among species. The reciprocal selection of bacteria on phage populations and phages on bacterial populations has been demonstrated in both the lab and the field, and there is increasing evidence that this process can maintain bacterial diversity, influence bacterial virulence, increase bacterial evolvability, and even shape the stability of ecosystems (recently reviewed in Koskella & Brockhurst, 2014). The goal of this chapter is to summarize current knowledge of natural bacteria–phage interactions and highlight current gaps in our understanding. First, we set the stage by discussing bacterial and phage distributions in nature and examining their interactions in space and time: Where are bacteria and phages found? Under which circumstances do they come into contact? Which particular phage and bacteria will interact and how sustained are these interactions likely to be? Next, we review bacteria–phage dynamics in nature and explore the potential impacts of phages on bacterial populations: Do phages impact bacterial population dynamics? Do bacteria and phages coevolve? Does phage-mediated selection influence bacterial diversity? Finally, we discuss the effects of phage and bacteria interactions on other organisms and ecosystem processes and outline areas for future research. We limit our discussion herein to interactions between bacteria and phages, but point readers to a recent review of coevolution between marine viruses and their hosts, including bacteria (Martiny, Riemann, Marston, & Middelboe, 2014), as well as one examining primarily molecular interactions between archaea and their viruses (Held et al., 2013).

2. SETTING THE STAGE: BACTERIA AND PHAGE DISTRIBUTION IN NATURE

Phages typically outnumber their bacterial hosts (Engelhardt et al., 2014; Suttle, 2007; Williamson et al., 2013), but perhaps more important is their
diversity and distribution across nearly every niche on the planet (Clokie, Millard, Letarov, & Heaphy, 2011; Fuhrman, 1999). The ability to make predictions about bacteria–phage interactions in nature requires an understanding of where each are found, when and where the ranges overlap, and what processes shape those patterns. We aim to address these topics in turn, and introduce concepts that may be useful tools for examining the distribution of phages and bacteria and their overlap.

2.1. Bacterial and phage range limits

Bacteria are distributed across most niches on the planet and, although less well examined, phages are expected to be similarly distributed (Clokie et al., 2011). Bacteria are found in environments experiencing extreme temperature, pressure, and salinity, as well as milder, intermediate environments (reviewed in Sogin et al., 2006; Martiny et al., 2006). In fact, it seems a greater challenge to find niches on Earth without bacterial colonists. Even intraorganismal fluids protected by immune systems and thus often considered sterile can show signs of thriving bacterial communities (Funkhouser & Bordenstein, 2013). In the free environment, the most likely niche without bacterial life are water deposits that are concentrated MgCl₂ brines, such as Discovery Basin in the bottom of the Mediterranean Sea and the Don Juan pond in McMurdo Antarctica (M. Fox-Powell, personal communication). Magnesium chloride is highly soluble, reduces water activity, and is chaotropic (i.e., disrupts hydrogen bonds in water), imposing a potential limit to life on Earth (Hallsworth et al., 2007).

The ubiquity of bacteria in the environment was underestimated from the beginning of the discipline of microbiology. In particular, the ability of bacteria to grow in a very narrow range of environmental conditions in the lab (i.e., culturability) greatly reduced the representation of all the bacteria in the environment. Advances in culture techniques expanded the number of bacteria discovered, but this number would not increase dramatically until advances in direct-count methods (staining followed by microscopy: Hobbie et al., 1977), which increased the resolution of environmental quantification of bacteria. Another significant leap came from environmental sequencing, employing nucleic acid sequencing of the 16s ribosomal gene, which is common to all bacteria (Torsvik & Øvreås, 2002). Even this restriction would be lifted when massively parallel sequencing enabled sequencing of environmental nucleic acids, even in the absence of primers (i.e., known sequence) (Xu, 2006).
The prevailing hypothesis until recently was that bacteria had global dispersal capabilities and the Bass Becking hypothesis stated: “everything is everywhere, but the environment selects” (Baas Becking, 1934; De Wit & Bouvier, 2006). Debate regarding the omnipresence of bacteria has continued until recently, as geographic and microgeographic structure, influenced both by the current environment and historical events, has become evident in an increasing number of studies (reviewed in Martiny et al., 2006). In sum, while bacteria as a whole inhabit nearly every niche on the planet, most species or groups of bacteria show heterogeneous geographic distributions.

In contrast to bacterial ecology, knowledge about phage distribution in natural environments is in its infancy. Collectively, bacteriophages and viruses of archaea are found in a variety of environments across the biosphere. Phages are abundant in sea and freshwater throughout the globe (Fuhrman, 1999), including hypersaline environments (Atanasova, Roine, Oren, Bamford, & Oksanen, 2011), the soil (Salifu, Casey, & Foley, 2013), deserts (Fancello et al., 2013; Prestel, Regear, Salamitou, Neveu, & Dubow, 2013), polar regions (Säwström et al., 2008), and on (Iriarte et al., 2007; Koskella, Thompson, Preston, & Buckling, 2011) as well as within other organisms (Bordenstein, Marshall, Fry, Kim, & Wernegreen, 2006; Buée, Boer, Martin, Overbeek, & Jurkevitch, 2009; Reyes, Wu, McNulty, Rohwer, & Gordon, 2013). What remains somewhat of a mystery is whether phages exhibit any geographic structuring. Unlike free-living microbial life, the idea that “everything is everywhere” has still not been discarded as a possibility for viruses (Thurber, 2009). However, given that the distribution of bacteria across the Earth is heterogeneous and phages rely entirely on their bacterial hosts for reproduction, it is likely that geographic structure of phage populations will also be uncovered. Indeed, research into phage distributions has led some investigators to conclude that phages are widespread in the environment, but selection may lead to distinct assemblages at particular locations (Breitbart & Rohwer, 2005). Therefore, the presence of biogeographic patterns in phage distributions remains a question under active debate (Thurber, 2009).

Knowledge about the distribution of phages mirrors that of bacteria in that a series of technological advances increased appreciation, interest, and study of phages. These advances parallel those in bacteria, but have lagged somewhat behind. Culture-dependent techniques greatly limited phage detection; not only did the bacterial host necessarily have to be culturable, but phage also had to infect the bacteria in question under those particular
lab conditions. As occurred for bacteria, but more than a decade later, advances in microscopy and specific staining (Bergh, Børshheim, Bratbak, & Heldal, 1989) led to the realization that phage abundance had been dramatically underestimated. Because phages, unlike bacteria, do not share a common gene, the culture-independent sequence identification performed for bacteria (Lane et al., 1985) could not be easily conducted for phages (Rohwer & Edwards, 2002).

At present, the question of the distribution of phages and the existence of patterns across space and time is still open (see review by Clokie et al., 2011). Much of the research regarding phage distributions comes from aquatic environments, and several sequence-based surveys have provided evidence of widespread distribution of phages. For instance, studies examining conserved phage genes have found similar sequences across diverse aquatic environments for cyanophage structural genes (Short & Suttle, 2005) and DNA polymerase genes of podophages (Breitbart, 2004). A global survey of marine viruses using metagenomics found that most viral species are shared between oceans (Angly et al., 2006). However, this same survey also found evidence for a latitudinal gradient in species richness and dominance of particular phages in some locations. Taken together, these studies have led to the conclusion that phages are widespread, but that selection favors enrichment at particular locations (Angly et al., 2006). Similarly, a number of recent studies using sequence data have also revealed clear biogeographic patterns in phages. A study examining stromatolites and thrombolites in marine and freshwater sediments revealed evidence of biogeographic structure and endemism (Desnues et al., 2008). The use of such sequence data to uncover patterns of phage distribution across space is not without problems. Diverse phages are known to share many conserved genes, and rampant horizontal gene transfer among phages with diverse host ranges has been suggested (Hendrix, Smith, Burns, Ford, & Hatfull, 1999).

Knowledge of host distributions is also critical for determining biogeographic patterns of their phages (Held & Whitaker, 2009). Biogeographic studies of Pseudomonas phages have found evidence for genetic exchange on a continental scale (Silander et al., 2005), but also geographic structure in some areas within North America (O’Keefe et al., 2010). Clear geographic patterns were also uncovered in a study of Sulfolobus islandicus archaean viruses, that investigated both the archaea and virus genotypes to find signatures of encounters in host genomes (Held & Whitaker, 2009). Another study examined abundances of bacteria, archaea, and viruses in Arctic waters, revealing spatial patterns of community composition
The rapid turnover of viruses in concert with their apparently ubiquitous distribution implies a role for migration. The presence of similar genotypes in different biomes uncovered from biogeographic studies (Breitbart & Rohwer, 2005; O’Keefe et al., 2010; Silander et al., 2005) suggests movement between environments, and in some cases contemporary genetic exchange between viruses may be responsible for biogeographic patterns (Breitbart, 2004; Díaz-Muñoz et al., 2013; Snyder et al., 2007). The suggestions of viral migration then beg the question of physical dispersal by virus particles. The small size of viral particles suggests that limitations on dispersal should be few, and there is evidence that phages can move via aerosols (Tanner, Brooks, Haas, Gerba, & Pepper, 2005; Verreault, Moineau, & Duchaine, 2008; Wang & Brion, 2007), animal vectors (Dennehy, Friedenberg, Yang, & Turner, 2006), and water currents. For instance, transfer via nematodes for bacteriophage Φ6 was mediated by the host (Dennehy et al., 2006).

In sum, we are in the early stages of documenting phage diversity and distributions. The most recent studies revealing biogeographic patterns suggest that examining phages in concert with their hosts, expanding the spatial and temporal breadth of sampling, and employing a variety of techniques will be necessary to grasp the patterns phage distributions in nature. Current studies of phages in the environment routinely increase the number of known phages by dramatic amounts (Labonté & Suttle, 2013), find abundant, undescribed families of phages (Zhao et al., 2013), or suggest that perhaps half the viruses in the ocean go undetected (Steward et al., 2013). Thus, it is pertinent to exercise some restraint in describing patterns of phage distributions when such a small portion of global diversity has been discovered (Suttle, 2007). The challenge of documenting phage distributions is daunting, but also an incredibly exciting phase of biology.

2.2. Phage-mediated selection of bacterial distributions

Although both phage fitness and range limits will necessarily be influenced by the presence of their hosts, it is unclear how ubiquitous phage-mediated selection of bacteria may be in nature. Indeed, there is likely a strongly asymmetric selection pressure acting on phages to find and infect a host cell (without which the phage could not reproduce) versus that acting on bacteria to resist phages (given that there is always a chance a susceptible bacterial cell
can reproduce without ever being challenged by a phage particle). Although it is logical to look at the distribution of bacteria for clues into phage distribution patterns, phage distribution may be more widespread than particular host species precisely because of this asymmetric selection pressure. Ogbunugafor, McBride, and Turner (2010) propose that viruses with greater niche breadth may be more likely to survive extinctions, similar to patterns put forward for macroorganisms (Jablonski, 1986). Phages capable of infecting multiple bacterial genotypes or species should experience a survival advantage and thus a plausible expectation is that phages overlap their distribution with multiple susceptible hosts, a conjecture supported by the fact that bacteria can often be infected with several different types of phage (Paterson, Nayar, Mitchell, & Seuront, 2012). As such, rather than being limited by the range of a particular bacterial host, phages may be under selection to increase host range in order to increase geographic range.

Phages may also expand their distribution beyond that of a particular host bacterium by increasing their tolerance to environmental degradation. Although phage particles found outside of host cells are not alive, i.e., they do not exhibit physiological processes, traits involved in survival outside the host are still potential targets of selection. This is because the environment can inactivate phage particles that would have otherwise infected cells (thus “killing” them), removing these from the gene pool. Phages are generally stable under optimal conditions for long periods of time (Clokie et al., 2011) and can tolerate a range of environmental stress. Crucially, the tolerance of phages to environmental stress is greater than that of bacteria (Drees, Abbaszadegan, & Maier, 2003; Moce-Llivina, Muniesa, Pimenta-Vale, Lucena, & Jofre, 2003; Muniesa, Lucena, & Jofre, 1999; Sano, Carlson, Wegley, & Rohwer, 2004) and phages can adapt to increased environmental stress independently of adaptation to their hosts. For instance, bacteriophage Φ6 exposed to heat shock can evolve tolerance to a 50 °C heat shock (Dessau, Goldhill, McBride, Turner, & Modis, 2012), well above the optimal growth temperature of the host species (Dessau et al., 2012; Young, Luketina, & Marshall, 1977). Increased environmental tolerance may enhance the ability to find different susceptible hosts or alternatively, survive temporal cycles in host abundance fluctuations (Ram, Boucher, Sime Ngando, Debroas, & Romagoux, 2005).

The high turnover rate of phages in the environment and implied migration suggests high temporal variability of phage distributions. The corollary is that considering both the spatial and temporal dimensions of phage is necessary to reveal biogeographic patterns, as temporal variability may limit the
ability to detect spatial patterns (Marston et al., 2012). Thus, sequence-only
studies that sample at only one time point and do not examine host distri-
butions are likely to miss important biogeographic patterns. Thus, increased
spatial and temporal sampling of specific phages along with information on
their hosts will greatly improve the ability to detect biogeographic patterns,
as seen in a number of recent studies (Held & Whitaker, 2009; Labonté &
Suttle, 2013; Marston et al., 2012; Zhao et al., 2013). These studies also
highlight the strength of using multiple techniques, including culturing,
whole genome sequences of cultured phages, and bioinformatic analyses
of existing sequences. Supporting this point, older, but updated techniques
can be of utility as shown by Brum, Schenck, and Sullivan (2013) who used
quantitative TEM to show little regional variation in phages, but structuring
across abiotic gradients.

Delimiting the distribution of bacteria and phage will be a challenging
endeavor, as outlined above, and determining their overlap may be a still
more formidable challenge. The bacterial environment and the abiotic envi-
ronment outside—and independent of—bacteria can shape phage distribu-
tions and consequently their interactions with bacteria. However,
borrowing concepts and methods from macroorganismal biogeography,
such as mechanistic environmental niche modeling (Kearney & Porter,
2009; Monahan, 2009), may facilitate our understanding of the distributions
of both phage and bacteria in the natural environment.

3. INTERACTIONS AMONG BACTERIA AND PHAGE

Beyond the overlap in distributions of phage and bacteria, a critical
parameter of bacteria and phage interactions is their ability to interact bio-
logically. Certainly in some environments bacteria and phage will overlap
but not interact. This is due to the dynamic nature of susceptibility to infec-
tion. This section will detail potential interactions between phage and bac-
teria at the organismal level by reviewing phage life cycles, bacterial
responses to phage encounters, phage countermeasures in reaction to spe-
cific bacterial responses, and phage host range. The impact of phages on bac-
terial populations and the coevolutionary dynamics of phage and bacteria are
treated in separate sections (Sections 4 and 5, respectively).

3.1. Phage life cycles

Phages depend on bacterial hosts to complete their life cycle, and there are
several life cycles employed by phages to achieve this purpose. Despite their
usual moniker, phages do not always “eat” their bacterial hosts. Although some phage life cycles are destructive and involve lysing the bacterial cell, other strategies can be neutral or even beneficial to bacteria. In particular, temperate phages integrate stably into bacterial genomes, only to enter the lytic cycle at a future time under certain environmental triggers. Below, we provide a brief overview of the variety of phage life cycles.

Lytic phages infect their host by adsorbing to host cells and then introducing their genetic material into the cell via ejection (Molineux & Panja, 2013) or entering the cell via endocytosis-like mechanisms (Romantschuk, Olkkonen, & Bamford, 1988). Adsorption requires that phages recognize bacterial receptors on the cell surface, including lipopolysaccharides, flagella, or pili (Lindberg, 1973; Mattick, 2002; Samuel et al., 1999). The bacterial cell machinery then produces the viral proteins encoded in the foreign nucleic acids and replicates the viral genetic material, after which viral proteins generally self-assemble, packaging their genetic material into capsids (Aksyuk & Rossmann, 2011). After enough virions have been produced, the cell lyses, usually through the production of lytic enzymes (Bernhardt, Wang, Struck, & Young, 2002; Young, 2013), thereby releasing progeny viruses and killing the host cell. The cycle then starts anew upon contact with another susceptible host. Unlike lytic phages, temperate phages are capable of integrating their genetic material into the bacterial genome, as so-called prophages, and can be transmitted to daughter cells during bacterial reproduction. Temperate phages can, under certain—typically stressful—conditions, reenter the lytic cycle, thus killing the cell and resetting the cycle. However, over evolutionary time, prophages can also lose the ability to excise from the host genome and form new virions, at which point they can be considered “cryptic prophages.” Many such prophages and cryptic prophages can have beneficial effects on the fitness of their host cells, for example, by increasing resilience under stressful conditions (Wang et al., 2010; Section 4.3) or influencing virulence to eukaryotic hosts (Fortier & Sekulovic, 2013; Section 4.4).

Although the lytic and lysogenic cycles are the most commonly described phage life cycles, there are other, less well-understood phage lifestyles, including pseudolysogeny and the carrier state. Pseudolysogeny is a term that has several different interpretations in the literature (Hyman & Abedon, 2010; Siringan, Connerton, Cummings, & Connerton, 2014). The most used definition is a state where the phage neither integrates into the host genome nor enters the lytic cycle (Los & Wegrzyn, 2012; Ripp & Miller, 1997). This state is associated with conditions of cell starvation, and usually
“resolves” with phages entering the lytic or lysogenic cycles (MarcinŁos & Wegrzyn, 2012). However, it is not clear whether pseudolysogeny represents an actual life cycle or a necessary “pause” in any phage activity as a consequence of the cell’s reduced activity due to starvation. Regardless, this state has important implications for bacteria–phage interactions in nature. If nutrients are transiently limited in a given environment, phage may not be eliminated from a population by a lack of bacterial growth, but may be able to “pick up where they left off” once cell growth resumes.

The carrier state is another life cycle of phages in which viruses establish a chronic infection of bacteria. In the carrier state, the phage neither integrates into the host genome nor induces lysis. Instead, the virus forms a persistent infection where progeny are routinely budded off the cell or passed down to daughter cells asymmetrically after division (Cenens et al., 2013). Unlike pseudolysogeny, which is thought to be induced by conditions of cell starvation, the carrier state can be reached and maintained in rich nutrient conditions and persist during exponential growth. For this reason, the carrier state has been described as a host resistance mechanism, as has been observed for Pseudomonas syringae interactions with bacteriophage Φ6 (Cuppels, Vidaver, & Van Etten, 1979). Perhaps more accurately, the carrier state represents a mechanism of coexistence of bacteria and phage. However, this need not mean that phage production does not alter bacterial physiology or fitness. For instance, although much is unknown about filamentous phage life cycles (Rakonjac, Bennett, Spagnuolo, Gagic, & Russel, 2011), Escherichia coli filamentous phages can form carrier states producing up to $10^{13}$ phage/mL and slowing down host growth rates (Rakonjac et al., 2011). The carrier state has the potential to affect bacteria–phage interactions in nature, but the population implications of this life cycle have been rarely characterized. In a recent study, Siringan et al. (2014) outline the potential of the carrier state to affect the population dynamics of Campylobacter jejuni. Biofilms of C. jejuni exposed to phages led to the appearance of the carrier state in some cells. These persistently infected bacteria had increased environmental tolerance outside of the chicken gut, but were deficient in its colonization. Furthermore, infection with carrier state cells reduced resident C. jejuni populations in the gut as efficiently as pure phage preparations (Siringan et al., 2014), providing compelling evidence of the potential of carrier state cells as dispersal vehicles for phage in the environment. Thus, the carrier state is an understudied phage lifestyle that may have significant ecological and evolutionary consequences for bacteria–phage interactions in the environment.
3.2. Bacterial responses to phage infection

Just as phage fitness is ultimately determined by transmission of genetic material to the next generation, bacterial fitness requires survival and reproduction, which can both be affected by phage infection. Most obvious is the case of lytic phage infection, whereby the cell host dies upon lysis. However, lysogenic and persistent infections (e.g., by filamentous and carrier state phages) can also carry costs to their bacterial hosts, as described above. Correspondingly, bacteria have evolved a variety of mechanisms to gain resistance to phages (reviewed in Labrie, Samson, & Moineau, 2010; Westra et al., 2012). These mechanisms can be grouped into three categories (Hyman & Abedon, 2010): adsorption reduction, restriction (post-infection blocks on cell takeover), and abortive infections (both cell and phage die). Strategies for eliminating or reducing phage adsorption are the most commonly cited resistance mechanism, as these are the most readily observed under laboratory conditions (Bull, Vegge, Schmerer, Chaudhry, & Levin, 2014). However, bacteria can have multiple mechanisms to resist phage, even in the case of a single bacterial and phage strain pairing. *P. syringae* develops resistance to bacteriophage Φ6 through a variety of mechanisms including pilus loss, pilus modification, hyperpiliation, and the establishment of a carrier state (persistent infection) (Cuppels et al., 1979). Although bacteria may have a number of ways to successfully evade phage infection in the lab, it is likely that resistance mechanisms will be constrained in some natural environments. This is because in nature phage attachment sites are often important for bacterial survival, reproduction, and pathogenesis (Bohannan & Lenski, 2000).

Bacteria can also employ mechanisms that degrade phage nucleic acids upon entry into the cell, as is the case for restriction enzymes (Hyman & Abedon, 2010). More sophisticated bacterial defenses which degrade phage genomic material have been discovered more recently: an “adaptive immune system” for bacteria named the CRISPR-Cas system (Jansen, Embden, Gaastra, & Schouls, 2002). Given that this newly described bacterial defense system has been found in approximately half of all bacterial species studied, resistance mechanisms that target foreign genetic material in the cell may be less constrained than mechanisms targeting phage adsorption. Furthermore, these molecular defense mechanisms can now be productively studied using sequence-based technologies (Tyson & Banfield, 2008) to establish an idea of the relative importance of the different categories of bacterial resistance mechanisms. The prevalence of these mechanisms will undoubtedly shed light into bacteria–phage interactions in the environment.
3.3. Phage responses to bacterial defenses

Phages have their own set of tools to respond in kind to bacterial defenses (reviewed in Samson, Magadán, Sabri, & Moineau, 2013; Young, 2013). Phage response to the most commonly described mechanism of bacterial resistance, changes in phage attachment sites, can involve matching modifications in phage proteins. However, phages also have the ability to respond in completely orthogonal ways to preserve transmission, for example, by completely switching attachment sites (Meyer et al., 2012). Phages may also form a carrier state, which does not destroy the bacterium but ensures transfer of phage genetic material. Phages can go to dramatic lengths to counter bacterial defenses, including importing an entire bacterial nucleic acid degradation system into the genome, as was recently discovered for ICP1 vibriophages that encode a CRISPR–Cas system in their genome (Seed et al., 2013). Similar to bacterial defense mechanisms, the prevalence of phage countermeasures will have important effects on bacteria–phage interactions. In particular, the ability of phage strains to respond to bacterial defense with a number of different countermeasures may buffer coevolutionary interactions between phage and bacteria.

3.4. Phage host range

Phages have traditionally been considered as highly specific to individual bacterial species or even strains, but increasing experimental and observational evidence from natural and laboratory systems suggests that susceptibility and resistance may not be as tidy as previously supposed (reviewed in Hyman & Abedon, 2010; Koskella & Meaden, 2013). In particular, phage susceptibility may not be a binary value, but instead there can be a continuum of bacterial sensitivity (Koskella & Meaden, 2013). This is important, as phage in the environment may alter the density of two susceptible host populations to differing degrees based on their relative degree of quantitative, rather than qualitative, resistance.

As discussed above, phages have several tasks to accomplish when infecting a bacterium: adsorb, deliver nucleic acids, and produce virions or integrate into the host genome. Bacterial resistance mechanisms may target one or more of these steps, making elucidation of a phage’s “true” host range a very complicated task. Laboratory methods for determining host ranges usually do not distinguish among these steps and can vary depending on assay conditions (Hyman & Abedon, 2010). For instance, a bacteriophage may not form plaques (localized absence of bacterial growth on a lawn due to lysis),
but establish productive infections in liquid culture. **Hyman and Abedon (2010)** suggest that host range should be qualified with the method used to determine it (e.g., plaquing host range, productive host range). In nature, the implication is that environmental conditions may determine not only which bacteria a phage can infect at any given time, but also the effects on bacteria and phage productivity of that specific interaction (Poisot, Lepennetier, Martinez, Ramsayer, & Hochberg, 2011; Wilson, Carr, & Mann, 1996). An additional complication to determinations of phage specificity is the possibility that phenotypic plasticity in bacterial populations may affect phage productivity (Pearl, Gabay, Kishony, Oppenheim, & Balaban, 2008).

The patterns of phage specificity are not well understood, but are crucial to our understanding bacteria–phage interactions. Whereas textbook accounts of phage host ranges indicate that they are highly specific to particular bacterial species and sometimes particular strains, it is clear that some phages have broad host ranges (Koskella & Meaden, 2013) and that many have the ability to readily infect new hosts across species, genera, and—in some cases—greater taxonomic barriers (Kaiser & Dworkin, 1975). A novel approach to ascertain phage specificity patterns, the study of the statistical structure of phage and bacteria infection networks (Weitz et al., 2013), has revealed new insights. A major meta-analysis of the infection networks of lytic phages in nature reveals a signature of nested infection networks (Flores, Meyer, Valverde, Farr, & Weitz, 2011), wherein phages with broad host range infect a larger proportion of bacterial hosts and are also more likely to infect highly resistant hosts, whereas phages with narrow host ranges are only able to infect the most susceptible hosts. Note that this pattern is in opposition to the idea that phages face a trade-off between being a “generalist” and being a specialist on more resistant strains, although the dataset do not take into account quantitative differences in infectivity such as growth rate within the host (Keen, 2014). This general result of nestedness contrasts with another possible expectation, modular interactions, where phages are most infective to the subset of bacteria from the same module, relative to those from other modules. This type of interaction network can include, for example, two phages with relatively large host ranges but which do not overlap in the hosts they can infect. While the meta-analysis revealed a general pattern of nestedness across most studies, there were some taxa that clearly had modular interactions (Flores et al., 2011). Furthermore, Flores et al. (2011) highlight that networks of bacterial and phage infection will take different shapes at different phylogenetic scales.
The development of an approach to determine patterns of host specificity is a critical first step, but several challenges remain. First, the initial network analyses have focused on lytic phage (Flores et al., 2011, Flores, Valverde, & Weitz, 2013); the infection networks of temperate phages are unexplored (Weitz et al., 2013). Second, as discussed above, lab techniques for determining host range have important limitations (Hyman & Abedon, 2010). Third, studies of phage–bacteria networks (Flores et al., 2011; 2013) have revealed contrasting patterns according to taxonomic group, habitat, and biogeographical scale, warranting caution in inferring general patterns based on currently known phages. In sum, determining the patterns of phage specificity in nature is remarkably complex: phage infection may be context and environment dependent, different infection patterns may predominate at different spatial scales or depending on the mode of infection, and we have characterized but a sliver of existing phage diversity. But intriguingly, the complex patterns in infection networks can be generated by relatively simple biological processes (Beckett & Williams, 2013).

The determination of the level of phage specificity will allow predictions regarding the response of host populations and communities to phage-mediated selection. Since bacteria are typically found within very diverse microbial communities, including dense biofilms and the human gut, the influence of a given phage within these bacterial communities will depend on whether it is highly specific to a given strain of a single species or whether it can infect multiple strains and species. This specificity is supported by evidence that phages are often “locally adapted” to their bacterial hosts across space (Koskella et al., 2011; Vos, Birkett, Birch, Griffiths, & Buckling, 2009), indicating a degree of specialization to common bacterial strains or species in a given population. However, the emergence of evolved “generalist” phenotypes of both bacteria and phages during experimental coevolution has been demonstrated in a marine cyanobacteria–cyanophage system (Marston et al., 2012). Similarly, when the lytic phage SBW25Φ2 was coevolved with its host bacterium, Pseudomonas fluorescens SBW25 (Buckling & Rainey, 2002; Scanlan et al., 2013), it continually increased its host range against previously resistant strains. However, this increase in local host range was not correlated with any general increase in infectivity against a panel of novel P. fluorescens strains.

In these cases, selection by a single phage clone that can infect multiple strains of bacteria leads to the evolution of similar resistance profiles, and perhaps even mutations, in multiple bacterial lineages. In addition, the evolution of resistance by one host genotype may lead to fission of the phage
population into two types with now different host ranges (i.e., reproductive isolation). Similarly, bacteria–phage infection networks indicate that a single bacterial genotype can often be infected by multiple phages in the local environment. As such, the evolution of resistance (in particular, the evolution of a more general resistance mechanism) may lead to changes in multiple phage lineages simultaneously, having potential knock-on effects to the phages, impact on other bacterial hosts in the community.

The patterns of phage host range have several important implications. First, current theoretical models have assumptions about phage host range that may be outdated or oversimplified in a way that colors our interpretations of natural patterns. Second, the nature of phage host range can expand or limit the potential for ecological and coevolutionary interactions between bacteria and phage. Both ecological and coevolutionary models have assumed a tight, specific fit with phages and bacteria (see Table 4.1). The explanatory power of these models can fade as deviations from the specificity assumption occur. Finally, the importance of examining phage beyond its association with a putative host species becomes greater as phage may be under selection by other unknown hosts as well as the abiotic environment. Theoretical and laboratory models will be useful as we try to make sense of the enormous diversity of phage, bacteria, and their interactions, but these should be guided by more data on natural infection networks (Forde et al., 2008) to elucidate the range of interactions in nature.

### 4. IMPACT OF PHAGES ON BACTERIAL POPULATIONS AND COMMUNITIES

Phages can have a variety of impacts on their bacterial hosts, including changes in bacterial physiology, competitive ability, and virulence (Rohwer & Thurber, 2009). These impacts may follow logically from phage life cycles. For example, lytic phages have the potential to decrease host population density. However, they may also have unexpected consequences on individual bacteria and populations. For example, prophages can encode critical toxins or virulence factors that feedback to shape bacterial fitness. In this section, we discuss the variety of impacts phages may have on their bacterial host cells and populations.

#### 4.1. Abundance

The first expected impact that phages might have on bacteria, especially if one considers lytic phage, is decreased abundance. In fact, the notion that
Table 4.1 Assumptions underlying phage-mediated negative frequency-dependent selection and the Kill the Winner hypothesis, and examples of evidence in support of or against each.

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Key finding</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. High prevalence of infective phage in the environment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ashelford, Day, and Fry (2003)</td>
<td>Estimates from sugar beet rhizosphere were found to be $1.5 \times 10^7$ per gram of soil</td>
<td>X</td>
</tr>
<tr>
<td>Sullivan, Waterbury, and Chisholm (2003)</td>
<td>Estimated abundance of cyanophages in open ocean to be two orders of magnitude lower than their hosts</td>
<td>X</td>
</tr>
<tr>
<td>Kim et al. (2008)</td>
<td>Estimated viral abundance from the soil of rice paddies were approximately $3 \times 10^8$ viruses from 1 g of soil</td>
<td>X</td>
</tr>
<tr>
<td>Kang, Oh, Kang, and Cho (2013)</td>
<td>Discovered marine phage (HMO-2011) accounting for up to 25% of all viral genome reads in the ocean</td>
<td>X</td>
</tr>
<tr>
<td>Engelhardt et al. (2014)</td>
<td>Viral density in sediment cores always exceeded cell counts, sometimes up to a ratio of 225:1</td>
<td>X</td>
</tr>
<tr>
<td><strong>II. Phage specificity at the strain and/or species level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jensen et al. (1998)</td>
<td>Found 9/10 phages in collection were broad-host-range phages, infecting &gt;1 genus of bacterial hosts</td>
<td>X</td>
</tr>
<tr>
<td>Langley, Kenna, Vandamme, Ure, and Govan (2003)</td>
<td>Evidence for broad-host-range lytic and temperate phages, infecting multiple species and genera</td>
<td>X</td>
</tr>
<tr>
<td>Holmfeldt, Middelboe, Nybroe, and Riemann (2007)</td>
<td>Found phage host range spanning 1 to 20 out of 23 Flavobacterium hosts</td>
<td>X</td>
</tr>
<tr>
<td>Flores et al. (2013)</td>
<td>Meta-analysis of 215 phages and 286 hosts from Atlantic Ocean shows variation and geographic structure in phage host range</td>
<td>X</td>
</tr>
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</table>

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<table>
<thead>
<tr>
<th>Assumption</th>
<th>Key finding</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assumption Key finding Evidence</strong></td>
<td><strong>Supporting</strong></td>
<td><strong>Opposing</strong></td>
</tr>
<tr>
<td><strong>III. Impact of phages on bacterial abundance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantastico-Caldas, Duncan, Istock, and Bell (1992)</td>
<td>In experimental microcosms, temperate phages had no effect but lytic phages reduced host density by a factor of 10</td>
<td>X</td>
</tr>
<tr>
<td>Waterbury and Valois (1993)</td>
<td>Found most bacterial hosts resistant to co-occurring phages, despite high phage abundance and diversity</td>
<td>X</td>
</tr>
<tr>
<td>Hennes and Simon (1995)</td>
<td>Found &lt;11% of total bacterial mortality explained by phage during phytoplankton bloom and 18–21% following the bloom</td>
<td>X</td>
</tr>
<tr>
<td>Fuhrman and Noble (1995)</td>
<td>Experimental mesocosm data indicating that 24–66% of bacterial mortality is due to infection by phage</td>
<td>X</td>
</tr>
<tr>
<td>Mathias, Kirschner, and Velimirov (1995)</td>
<td>Evidence that phage-induced death rates range from 10.8% to 43.2% of bacteria in a freshwater system</td>
<td>X</td>
</tr>
<tr>
<td>Weinbauer and Suttle (1996)</td>
<td>Found that only ≈1.5% of natural, marine bacterial community were lysogenic</td>
<td>X</td>
</tr>
<tr>
<td>Jiang and Paul (1998)</td>
<td>Assays suggest that &gt;40% of marine bacterial isolates contained inducible prophage</td>
<td>X</td>
</tr>
<tr>
<td>Middelboe et al. (2001)</td>
<td>Experimental manipulation revealed short, but not long-term effects of phage on bacterial population dynamics</td>
<td>X</td>
</tr>
<tr>
<td>Bettarel et al. (2004)</td>
<td>Found phage-induced mortality was less important than flagellate or ciliate grazing, except under low productivity</td>
<td>X</td>
</tr>
</tbody>
</table>
### Table 4.1 Assumptions underlying phage-mediated negative frequency-dependent selection and the Kill the Winner hypothesis, and examples of evidence in support of or against each.

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Key finding</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Harcombe and Bull (2005)</strong></td>
<td>Incorporation of lytic phage into two species bacterial community strongly influences abundance of susceptible host</td>
<td>X</td>
</tr>
<tr>
<td><strong>Allen, Wilner, Oechel, and Lipson (2010)</strong></td>
<td>Evidence from lab and field studies that removal of phages can increase microbial biomass and respiration</td>
<td>X</td>
</tr>
<tr>
<td><strong>Shapiro, Kushmaro, and Brenner (2010)</strong></td>
<td>Phage densities were found to correlate with bacterial abundance in wastewater</td>
<td>X</td>
</tr>
</tbody>
</table>

### IV. Variation in resistance among bacteria

<table>
<thead>
<tr>
<th>Study</th>
<th>Key finding</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterbury and Valois (1993)</td>
<td>Found evidence for widespread resistance to co-occurring phages in seawater</td>
<td>X</td>
</tr>
<tr>
<td>Andersson and Banfield (2008)</td>
<td>Variation in CRISPR spacers associated with local phages in natural acidophilic biofilms</td>
<td>X</td>
</tr>
<tr>
<td>Koskella and Meaden (2013)</td>
<td>Quantitative variation in resistance among susceptible host strains from phyllosphere</td>
<td>X</td>
</tr>
<tr>
<td>Flores et al. (2013)</td>
<td>Meta-analysis of bacteria–phage interaction network shows great variation in susceptibility across bacterial strains</td>
<td>X</td>
</tr>
<tr>
<td>Koskella et al. (2011)</td>
<td>Bacterial species from the horse chestnut tree phyllosphere differ in sensitivity to local phages</td>
<td>X</td>
</tr>
<tr>
<td>Holmfeldt et al. (2007)</td>
<td>Of 23 bacterial strains examined, all showed unique phage susceptibility patterns and up to six orders of magnitude differences in phage sensitivity</td>
<td>X</td>
</tr>
</tbody>
</table>
bacteria have too few predators paved the way for the realization of the importance of phages in the biosphere (Breitbart & Rohwer, 2005). As researchers began uncovering the immense numbers and diversity of bacteria, it became clear that grazing by protozoans and microbial eukaryotes was not sufficient to explain bacterial mortality. After culture-independent methods (direct counts) revealed that viruses outnumbered bacteria by a factor of 5–10 (Fuhrman & Noble, 1995), a number of studies showed that viruses were significant factors explaining bacterial mortality, although the magnitude of their importance varies considerably (Table 4.1; Hennes & Simon, 1995; Mathias et al., 1995). By some estimates, viruses are responsible for up to 50% of bacterial mortality, roughly equal the rate of the other major bacterial predators, protists (Fuhrman & Noble, 1995), and in some

Table 4.1 Assumptions underlying phage-mediated negative frequency-dependent selection and the Kill the Winner hypothesis, and examples of evidence in support of or against each.—cont’d

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Key finding</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenski (1988)</td>
<td>Evidence for pleiotropic effects of resistance mutations which influenced bacterial fitness and competitive ability</td>
<td>X</td>
</tr>
<tr>
<td>Brockhurst, Buckling, and Rainey (2005)</td>
<td>Costs of resistance to phage emerged over the course of experimental evolution</td>
<td>X</td>
</tr>
<tr>
<td>Lennon, Khatana, Marston, and Martiny (2007)</td>
<td>Found ≈20% reduction in relative fitness for about half of resistant strains of cyanobacteria</td>
<td>X X</td>
</tr>
<tr>
<td>Quance and Travisano (2009)</td>
<td>Uncovered reduction in competitive fitness for resistant mutants which generally increased with temperature</td>
<td>X</td>
</tr>
<tr>
<td>Koskella et al. (2011)</td>
<td>No evidence for reduced motility of resistant bacterial strains from the phyllosphere</td>
<td>X</td>
</tr>
<tr>
<td>Koskella, Lin, Buckling, and Thompson (2012)</td>
<td>Costs of resistance only found for bacteria evolving in the presence of multiple phage types, not for single phages</td>
<td>X X</td>
</tr>
</tbody>
</table>
environments phage predation can be even greater than grazing pressure (Weinbauer & Peduzzi, 1995). Bacteria and phages were hypothesized to emulate predator–prey interactions (Campbell, 1961) and subsequent laboratory experiments confirmed the broad stroke applicability of these models (Levin, Stewart, & Chao, 1977). These oscillating cycles of bacterial and viral abundance have been found in certain planktonic environments (Rodriguez-Brito et al., 2010). However, in other environments, the impact of phages on bacterial abundance can be considerably more complex. For instance, within the same environment phages may affect bacterial density in some bacterial species but not in other members of the community (Parsons, Breitbart, Lomas, & Carlson, 2012). Additionally, the evolution of bacterial resistance is likely to buffer the effects of phage predation (Harcombe & Bull, 2005; Waterbury & Valois, 1993).

Phages are not limited to decreasing bacterial abundances by lysis. Prophages can increase the abundance of lysogenic bacterial strains by conferring an advantage over other bacterial community members, for example via induction of temperate phages that infect competing bacteria in vitro (Bossi, Fuentes, Mora, & Figueroa-Bossi, 2003) and in the mouse gut (Duerkop, Clements, Rollins, Rodrigues, & Hooper, 2012). In this way, phage induction can be viewed as an altruistic strategy by a subset of the bacterial population, which carries indirect fitness benefits to the host by reducing competition for nearby hosts of the same genotype. Although bacterial cells that induce prophage are typically lysed in the process (except cells with viruses in the carrier state, such as filamentous phages), the rest of the population—which is protected from further infection by virtue of sharing the prophages—benefits from the elimination of the competing bacterial population (Bossi et al., 2003). Although the competitive advantage provided by induced prophage is greatly reduced after some time (a few days in laboratory conditions) due to lysogenization of the attacked bacteria (Bossi et al., 2003; Gama et al., 2013), in natural conditions this may be a sufficient advantage to allow bacteria to colonize a new habitat (Brown, Le Chat, De Paepe, & Taddei, 2006). In support of the applicability of this conjecture, lysogenic bacteria in a natural aquatic environment show large seasonal fluctuations in the amount of bacteria carrying inducible prophages (Cochran & Paul, 1998).

### 4.2. Genetic innovation and phage-mediated bacterial gene transfer

Phages are gene traffickers, so it should not be surprising that they affect their hosts’ genetics in a number of ways (reviewed in Briissow, Canchaya, & Hardt,
In some cases, phages reproduce with seemingly little interaction between the host and viral genome. For instance, lytic phages or phages in a carrier state merely use the cell’s genetic machinery to reproduce their own genetic material. However, in some cases, phage capsids package cell-derived nucleic acids that are then transported to other bacterial cells, in a process called generalized transduction. Through transduction, phages can facilitate the transfer of antibiotic resistance genes among bacterial strains (Goh et al., 2013) or genera (Evans et al., 2009), and as a population serve as a reservoir for these genes (Marti, Variatza, & Balcazar, 2013). Selection can act against these foreign nucleic acids once inside the bacterial cell, or they may be transferred consistently if they are selectively neutral or beneficial. At the other end of a continuum lie phages that perform specialized transduction, where specific regions of the bacterial genome are targeted for incorporation into the phage genome. These acquired bacterial nucleic acids may confer an advantage to phages by changing host physiology in a way that enhances phage infection (Section 4.3), or may aid host bacterial fitness by increasing dispersal, as occurs with phage-mediated changes in bacterial virulence (Section 4.4). Phage-mediated selection can also indirectly influence the bacterial genome. For instance, in experimental coevolution in the presence of phage SBW25Φ2, P. fluorescens mutator strains are advantaged in the population (Pal, Macia, Oliver, Schachar, & Buckling, 2007). These strains obtain a mutation that impairs DNA repair mechanisms, accelerating the mutation rate, which may aid in acquiring phenotypes that are resistant to phage infection. In other cases, bacteria may go well beyond a single mutation. C. jejuni inverts a third of its genome as a result of phage selective pressure (Scott et al., 2007).

### 4.3. Changes in physiology

Phages can affect host metabolism by encoding genes which are homologous to host genes. In some cases, these “auxiliary metabolic genes” (Clokie et al., 2011) may be used to enhance cell physiology in a way that advantages the infecting virions. For instance, cyanophages carry essential photosynthesis genes (Mann, Cook, Millard, Bailey, & Clokie, 2003) that may be used to maintain energy generation after the host cell has ceased to manufacture essential photosynthesis proteins (Clokie et al., 2006), thus allowing phages to continue reproduction. Furthermore, prophage elements in the bacterial genome can alter cell physiology in ways that enable bacteria to cope with different environmental conditions. A recent study that experimentally removed prophage elements from the E. coli K–12 strain’s genome (Wang
et al., 2010) found that these elements provided a wider tolerance of environmental stressors despite not being inducible. Thus, phages may impart genetic diversity that can be harnessed to the bacterium’s advantage even after the phages are no longer active.

4.4. Virulence

Phage infection can alter a number of bacterial traits (Rohwer & Thurber, 2009), as discussed above. Many of these phage-mediated changes facilitate host survival or bacterial host range expansion that generally improves phages’ chances of successful reproduction. Sometimes these phage-mediated traits increase the virulence or pathogenicity of host bacteria, which from the phage’s “perspective” are merely side effects (Boyd & Brüssow, 2002; Levin & Bull, 1996). However, these changes in virulence are of interest in their own right as many human diseases (or diseases in species of importance to humans) involve phages as crucial actors. Wagner and Waldor (2002) have reviewed the involvement of phages in bacterial pathogenesis, so we will only briefly summarize and then extend our discussion to the impact of phage-mediated bacterial pathogenicity in nature.

Phages can alter bacterial pathogenicity in a number of different ways (Wagner & Waldor, 2002). As is most commonly appreciated, phages may transfer genes encoding toxins to bacteria, but they can also increase expression of “native” bacterial genes by encoding regulatory elements (Spanier & Cleary, 1980) or increase pathogenicity by virtue of elements of phage particles that are themselves toxic (Benchetrit, Gray, Edstrom, & Wannamaker, 1978).

Phages are well known for their role in transferring toxins or other factors that bestow virulence upon their host bacteria, as is illustrated by the acquisition of toxin genes from phage by *Vibrio cholerae* (Waldor & Mekalanos, 1996), which causes this otherwise harmless (to humans) and common bacteria to cause intense diarrheal discharge. The symptoms caused by the phage-encoded toxin likely increase transmission of the bacterial host and phage between humans; the disease and the significant mortality it causes are a side effect of this strategy. Other exotoxins encoded by phage that enhance bacterial virulence are found in *Corynebacterium diphtheriae* (Holmes & Barksdale, 1969), *Vibrio harveyi* (Munro, Oakey, Bromage, & Owens, 2003), *Pseudomonas aeruginosa* (Hayashi, Baba, Matsumoto, & Terawaki, 1990), and Shiga toxins in *E. coli* (Newland, Strockbine, & Neill, 1987) and *Shigella dysenteriae* (McDonough &
Butterton, 1999), Although phages are not thought to play a role in the regulation of toxins, there is evidence that phage-encoded regions can play a role in transcriptional control of Shiga-like toxins (Neely & Friedman, 1998). In addition, beyond toxins, phages may have impact on a suite of bacterial traits that increase virulence, such as host adhesion, entry into host cells, immune resistance, biofilm formation (Rice et al., 2008), and susceptibility to antibiotics (reviewed in Wagner & Waldor, 2002; see also Section 7.2).

Increased virulence mediated by phages can have several consequences on phage and bacterial interactions in nature. Phages can expand their niches or facilitate migration between different habitats via the newly acquired capacity for bacteria to infect a new host. In turn, the newly acquired virulence by bacteria can have other effects on the wider phage community. For example, *V. cholerae* virulence in humans is enhanced by a number of phage-encoded traits, most importantly the CTX toxin (Nelson, Harris, Morris, Calderwood, & Camilli, 2009). Because the O antigen on the LPS of *V. cholerae* is required for both infection of the phage and to cause human disease, this antigen has become a target of a lytic phage, ICP1, which is the dominant strain in human *V. cholerae* outbreaks (Seed et al., 2010). The abundance of this particular phage type is thus dramatically altered by the presence of cholera outbreaks, presumably with concomitant effects on the rest of the phage community infecting *Vibrio* bacteria. Additionally, the genetic makeup of the ICP1 phage is altered by a coevolutionary arms race with *V. cholerae*, in which the phage has acquired a CRISPR–Cas system into its genome (but apparently not acquired from *V. cholerae*, which has no CRISPR–Cas system) to serve as a counter to bacterial defenses encoded in a pathogenicity island (Seed et al., 2013). This example of the effect of a phage in the microbial community is well documented due to our interest in the impact on human populations. However, it is reasonable to suppose that the phage-mediated alterations of hosts that (happen to) lead to increased virulence are commonplace and may have similarly dramatic effects on microbial and other communities. We discuss these cascading effects, though not exclusively related to virulence, further in Section 6.

5. BACTERIA AND PHAGE DYNAMICS IN NATURE

What is clear from the current body of work on interacting networks of bacteria and phages is that there exists great variation in who infects whom. That is, bacterial strains in a population often differ in the subset
of phages to which they are susceptible and vice versa (e.g., Flores et al., 2013). Similarly, different species within microbial communities often show differing levels of susceptibility to local phages (e.g., Koskella et al., 2011). This specificity raises the possibility that phages capable of specializing on common strains or species of bacteria can maintain diversity within bacterial populations or communities. The idea that one species can select against common types of the other has a long history, and may have originated with the observation that predators are likely to preferentially attack common prey species (Poulton, 1884). In this section, we will review the theory and evidence behind the idea that parasite-mediated selection can shape the evolution and ecology of bacterial populations and communities in predictable ways.

5.1. Phage-mediated frequency-dependent selection

In the most simple case, if phages are able to evolve to infect highly abundant bacterial clones and impose a fitness disadvantage to these hosts (e.g., by lysing the cell or reducing its reproductive rate), they can mediate a rare host advantage and ultimately slow the dominance of the otherwise most fit clonal genotype. The idea that the realized fitness of a given genotype is a function of the frequency of similar genotypes in the population, rather than simply the match between the phenotype and the local environment—whether it be abiotic environment or other interacting species—was first formalized by Fisher (1927, 1958). Negative frequency-dependent selection (FDS), in particular, has been put forward as a hypothesis to help explain the great genotypic and phenotypic diversity found within natural populations (Clarke, 1979; Endler, 1986; Fisher, 1927; Hedrick, 1972), and is now supported by a large body of experimental evidence from nonmicrobial systems (e.g., Allen, 1972; Harpole & Suding, 2007; Koskella & Lively, 2009; Olendorf et al., 2006).

Negative FDS can result from either direct interactions among genotypes in a population (e.g., bacteriocin production by bacteria [Levin, Antonovics, & Sharma, 1988] or sexual color morphs in lizards [Sinervo & Lively, 1996]) or through indirect interactions mediated by other species (such as predators [Allen & Greenwood, 1988] or parasites [Koskella & Lively, 2009]). In regard to the latter, predators or parasites that specialize on common types of prey or hosts will confer an advantage to rare phenotypes and therefore hinder the fixation of particular alleles or, in the case of asexual organisms, lineages. Negative FDS resulting from predation...
will occur when, for example, predators utilize a specific search image and therefore are able to rapidly identify and catch common prey phenotypes (Tinbergen, 1960). This rare prey advantage, where individuals that deviate from the norm attain a selective advantage, can lead to the maintenance of polymorphism in prey populations (Clarke, 1962; Paine, 1966; Power et al., 1996) and, for predators that switch prey species, this idea can be extended to explain diversity in prey communities (Murdoch, 1969). In their metapopulation model incorporating competition, predation, and dispersal, Shurin & Allen (2001) show that predators can increase the abundance of otherwise inferior competitors, although the exact outcome depends on the relative dispersal of predator and prey species. Despite the intuitive nature of these predictions, however, the impact of shared predators on prey communities is often unpredictable (Almany & Webster, 2004). For example, in a model exploring the impact of a shared predator on prey species abundance, Holt (1977) found that the impact of predator-mediated selection on prey diversity will be influenced by how productivity affects intrinsic growth rate of each prey species.

In order for phages to play a role in shaping bacterial diversity via negative FDS, they must be able to adapt to dominant host strains/species in a specific manner and have a significant impact upon their fitness (Table 4.1). Evidence for such adaptation is building, for example, by data indicating that phages are well-adapted to their local hosts relative to those from other populations (e.g., Koshella et al., 2011; Vos et al., 2009) or that changes in a particular phage genotype’s abundance is correlated with changes in abundance of its associated host (Parsons et al., 2012; Rodriguez-Brito et al., 2010; Shapiro et al., 2010). For example, cyanobacteria and five associated cyanophage genotypes were found to be highly dynamic over both 5-year and 1-day sampling periods (Kimura, Sako, & Yoshida, 2013) and correlated changes over 3 months were found between populations of Sphingomonas sp. and its lytic phage from a freshwater lake (Jost & Wiese, 2013). Furthermore, artificial removal of phages has been shown to lead to increases in frequency of previously rare bacterial species (Bouvier & Del Giorgio, 2007), and introduction of phages has been shown to increase bacterial richness (Weinbauer et al., 2007). Finally, the relative fitness of lysogenic strains of E. coli in experimental microcosms was found to be highest when the lysogen was rare, as the high number of susceptible (i.e., nonlysogenic) strains in the environment lead to amplification of the phage and subsequent decreases in the susceptible host population (Brown et al., 2006).
The potential role of phage-mediated selection in shaping genomic diversity of microbial populations has been reviewed recently (Cordero & Polz, 2014). The authors suggest that FDS mediated by phages, as well as by other ecological factors such as competition and predation, can lead to both stable diversity and rapid turnover of genes in the population. Furthermore, the authors point out that “because of the low linkage between phage receptors and the rest of the genome, the negative FDS that affects these receptors could have little effect on the diversity of other genes in the genome” (Cordero & Polz, 2014). In particular, they argue that due to the lack of linkage between genes encoding phage receptors and the core genome, phage predation is unlikely to correlate with genotype abundance. Instead, fitness will depend on the specific phage receptor expressed by a given bacterial host and the frequency of that receptor in the rest of the population or community.

5.2. The Kill the Winner hypothesis

In its conception, the Kill the Winner (KTW) hypothesis put forward a mechanism by which host-specific viral lysis and nonselective predation by protozoa might interact to maintain microbial diversity using classical Lotka–Volterra equations (Thingstad, 2000; Thingstad & Lignell, 1997). The model uses the simple assumption that host growth rate is positively correlated with viral fitness, and therefore abundance, to demonstrate that phage infection in proportion to host relative abundance in the population will negate any inherent fitness advantage of particular bacterial hosts. This idea that viruses could help maintain coexistence of bacterial strains/species with differing intrinsic growth rate was a major step forward in explaining microbial diversity, and there is now good evidence that phages can indeed act in this manner. As pointed out by Winter, Bouvier, Weinbauer, and Thingstad (2010), phage-mediated negative FDS is only one component of the KTW hypothesis. Under phage-mediated negative FDS, rare host genotypes for which no infective phages exist in the local environment can increase in frequency in the population until the evolution or immigration of an associated phage occurs, at which point the phage will be amplified by the relatively high abundance of susceptible hosts and will subsequently drive down the frequency of its host type. At this point, further rare and temporarily resistant host types can increase in frequency due to their higher relative fitness. This time-lagged phage-mediated selection can lead to oscillations among associated phage genotypes and their bacterial hosts,
whether at the strain or species level. On the other hand, the “winner” being referred to in the KTW hypothesis is that bacterial strain or species which is the better competitor in the absence of phage. This intrinsic growth rate advantage could be due to, for example, increased competitive ability via adaptation to the abiotic environment or via toxin production. The KTW hypothesis therefore predicts coexistence of good competitors (which are regulated by their associated phages) and bacterial strains which instead invest in defense against phages. The recent review by Winter et al. (2010) outlines in great detail the current evidence in support of the KTW theory, and therefore these data will only briefly be examined below.

Våge et al. (2013) recently extended this model to include a cost of resistance to phages. By assuming that bacteria face a trade-off between defense against phages and growth rate, the authors show that communities should be stably dominated by slow growing, resistant strains/species and phages that infect the rapidly growing competitors. This extended model further explains the inverse rank-abundance distributions often found in marine microbial communities and the finding that a large portion of the rare microbial populations are highly active (Campbell, Yu, Heidelberg, & Kirchman, 2011). There now exists clear evidence for costs associated with phage resistance (Table 4.1), and these costs can extend to increased sensitivity to antibiotics (e.g., Hagens, Habel, & Bläsi, 2006; Jalasvuori, Friman, Nieminen, Bamford, & Buckling, 2011) and increased susceptibility to other phages (Avrani, Wurtzel, Sharon, Sorek & Lindell, 2011; Marston et al., 2012). Together, the evidence to date suggests a clear role for phages in shaping microbial diversity. The KTW model has also been recently extended through the incorporation of realistic bacteria–phage infection networks (Jover, Cortez, & Weitz, 2013) and through inclusion of bottom-up regulation of diversity by substrate composition (Miki, Nakazawa, Yokokawa, & Nagata, 2008; Töpper et al., 2013). In the future, the KTW hypothesis could be further extended to include eco-evolutionary feedbacks, whereby the local density of susceptible hosts determines the strength of phage-mediated selection and therefore the payoff for investing in costly resistance (Boots, Best, Miller, & White, 2009).

5.3. Phage-mediated apparent competition

As outlined above, the ability of phages to shape relative fitnesses of bacterial strains and species in a community depends on the specificity of the interaction. However, this specificity does not have to be complete in order for
phages to influence the abundance of one host relative to another. When two bacterial host strains or species share an infective phage, there may well be differences in the phage adsorption rate, time to cell lysis, or burst size across the hosts. In this case, the phage would mediate apparent competition among the hosts, as one strain/species would be at a competitive disadvantage relative to the other in the presence, but not absence, of the phage (Fig. 4.1; Bohannan & Lenski, 2000a, 2000b). Alternatively, if the two hosts are able to evolve resistance to the phage but pay differential costs for any evolved resistance, phage-mediated selection may again alter competition among strains/species, but this time the effect would be sustained even in the absence of the phage (Koskella et al., 2012). This apparent competition could be further pronounced when one host species can tolerate, or is less sensitive to, a shared phage than the other. In this case, one bacterial host species would be better off overall in an environment with only the phage and its own type (as the other host leads to a higher phage density overall

Figure 4.1 Simple illustration of the potential impact of either narrow or broad host range lytic phages (triangle) on the apparent competition among bacterial hosts (circles) with otherwise similar intrinsic growth rates. (A) In the case where a phage infects only one of two hosts (either strains or species), the resistant host should become dominant in any environment in which the phage is present. (B) When two bacterial hosts share a phage, but differ in how sensitive they are to the phage (either in terms of adsorption rate or time to cell lysis), we would expect the more resistant strain to again become dominant in any environment in which the phage is present. (C) Finally, if bacterial hosts are able to evolve resistance to a shared phage, their relative fitness may still differ depending on the cost each has paid for resistance (realized both in the presence and absence of phage in the environment).
than the first host could sustain). In a model built to explain apparent com-
petition among prey species with a shared predator, Holt, Grover, and 
Tilman (1994) show that the dominant prey species will be the one which 
suppresses resources to the lower level and which sustains the higher pred-
ator density.

In sum, it is now clear that phages can drive and maintain bacterial diver-
sity, from the genome level through the population level and up to microbial 
communities. The underlying mechanisms likely include frequency-
dependent host-specific phage adaptation, correlations between bacterial 
abundance in a given environment and associated phage density, and 
trade-offs between growth and defense against phages. For example, 
Middelboe, Holmfeldt, Riemann, Nybroe, & Haaber (2009) evolved rep-
licate populations of marine *Flavobacteria* in the presence of two lytic phages 
for 3 weeks and found both increased resistance and diversification of met-
abolic activity of various carbon sources. This observed cost and variation in 
breadth of resistance among the strains suggests that phage-mediated selec-
tion could play a critical role in shaping competition among bacterial hosts, 
and perhaps even drive niche diversification. Many of these mechanisms are 
similar to theory put forward in classic ecological theory for predator- and 
parasite-mediated selection, as outlined above, but bacteria–phage-specific 
models have allowed for the incorporation of additional bottom-up and 
top-down regulators of bacterial abundance that work in concert with phages 
to maintain bacterial diversity. Further inclusion of known trade-offs in phage 
life history evolution (e.g., Keen, 2014) and spatial structure (Best, Webb, 
White, & Boots, 2011) will likely be helpful in further extending these models 
to explain both spatial and temporal variation in microbial diversity.

## 6. CASCADING EFFECTS OF BACTERIA AND PHAGE 
INTERACTIONS

Any given interaction between a phage and bacteria pair can affect 
other bacteria beyond that particular interaction. As discussed through-
out this article, phages can change a bacterial community through bac-
terial mortality (Section 4.1), ecological and coevolutionary dynamics 
(Section 5), transfer of genes that influence bacterial physiology and fitness 
(Sections 4.2–4.4). The interactions of phage and bacteria have potential 
consequences, not only for the interacting pair but also for other micro 
and macroorganisms and the ecosystem at large, including nutrient cycling 
and other biogeochemical processes.
6.1. Impact of phages on other nonbacterial species

The complex, interconnected nature of biotic interactions can cause phages to have dramatic impacts not only on their bacterial hosts but on other organisms as well.

Recently, it has become common knowledge that the microbiota of humans, particularly in the gut, plays a central role in human nutrition and even behavior (Cryan & Dinan, 2012). Much of the recent research on the human microbiome has focused on bacterial types inhabiting the gut, although viruses were known to be components of the gut microbiota for quite some time. In fact, the co-discovery of bacteriophages by Twort (1915) and d’Herelle (1917) was made by examining the microbiota of humans and other animals. Recently, experimental approaches have attempted to elucidate the role of phages on that bacterial community. Surveys of the viruses in human fecal material (Breitbart et al., 2003) suggest that individuals have distinct viral compositions, even among monozygotic twins inhabiting the same environment and despite distinct bacterial gut profiles (Reyes et al., 2010). Using a mouse model, Reyes et al. (2013) provided evidence that viruses of the human gut, predominantly phages, have measurable effects on the abundance and diversity of human gut microbes.

Another striking example of the reach of phage–bacteria interactions is that of phage affecting population fluctuations of the flamingos in east African lakes. Flamingos are filter feeders relying on photosynthetic cyanobacteria and their population numbers dramatically decline when the cyanobacterial population dwindles. Cyanophages were traced as sources of bacterial population reductions with concomitant flamingo population crashes (Peduzzi, Gruber, Gruber, & Schagerl, 2014).

Bacteria have well-known symbioses with a number of animals, notably insects. Recent studies have led to the realization that many insect–bacteria symbioses may actually be nested symbioses with three actors: phage, bacteria, and animal (Bordenstein et al., 2006; Kent, Funkhouser, Setia, & Bordenstein, 2011; Moran, Degnan, Santos, Dunbar, & Ochman, 2005; Oliver, Degnan, Hunter, & Moran, 2009; Weldon, Strand, & Oliver, 2012). Phages can encode virulence factors that are necessary for bacterial protection of wasps from parasitism (Oliver et al., 2009; Weldon et al., 2012). In other cases, lytic phage can reduce the detrimental effects of an endosymbiont causing cytoplasmic incompatibility in insects (Bordenstein et al., 2006). The examination of phages in association with other organisms promises many similar surprises.
6.2. Role in the ecosystem

Bacterial viruses can affect the abiotic environment, as well as the biotic environment, by altering biogeochemical cycles (Suttle, 2007). One of the main ways nutrient and biogeochemical cycles are affected by bacteria–virus interactions is via bacterial mortality (Wilhelm & Suttle, 1999). After lysis, bacterial organic material is released including nucleic acids, proteins, and lipids. Because viruses are such a major cause of bacterial mortality (50% or greater in some environments), the components released by lysis are suspected to represent a significant contribution to nutrient pools including the carbon, nitrogen, and phosphorus cycles. Experimental evidence from a marine food web microcosm suggests that phages have a strong effect on available phosphorus and can change the ratios of carbon, nitrogen, and phosphorus (Lennon & Martiny, 2008). Additionally, bacterial mortality can also increase the availability of trace elements, notably iron, that limit primary productivity. Primary productivity may also be directly affected by phages as bacteria can be important primary producers, especially in the aquatic environment where they represent a significant portion of the phytoplankton. Experimental evidence suggests that increasing virus abundance in seawater, can reduce primary productivity by up to 78% (Suttle, Chan, & Cottrell, 1990). Given that 50% of global primary productivity can be attributed to phytoplankton, it is likely that miniscule viruses actually have an impact on the global food web.

7. FUTURE DIRECTIONS

We now have a clear glimpse into the abundance, diversity, and ecology of phages in nature and their impacts on their bacterial hosts and as a consequence other species, communities, and global biogeochemical cycles. If recent research is any indication, the study of phage–bacteria interactions will yield many unexpected findings. Furthermore, not only are potential discoveries in basic biology exciting, but it is reasonable to suppose that many of these findings will fuel applications to societal problems as diverse as human health, drug discovery, agricultural safety, energy production, and biocontrol. Below we highlight under-appreciated and under-studied areas in bacteria–phage interactions, both in basic biology and potential applications.
7.1. Phage–phage interactions

As reviewed above, phage and bacterial communities are large and diverse throughout most of the biosphere and in most studied habitats phage outnumber bacteria by a large margin. Therefore, it is likely that there are ample opportunities for multiple phage to interact with a single host. Much of our knowledge of phage and bacterial relationships is based on laboratory systems where strains are largely examined in single infections. However, there is ample evidence that viral coinfection occurs commonly in nature across all life forms (Dapalma, Doonan, Trager, & Kasman, 2010). Bacteria are not the exception and there are several lines of evidence suggesting that phage–phage interactions occur frequently in nature. Genome sequences of bacteria commonly harbor the signature of past phage infections. Although past infections leading to integration do not necessarily suggest that phages interacted simultaneously, there is evidence that prophage sequences from lysogenic bacteria can interact. For instance, Refárdt (2011) showed that the reproductive success of temperate bacteriophages—upon lysis induction in *E. coli*—was adversely affected when a second prophage was present. Another line of evidence comes from the phenomenon of superinfection exclusion (Dulbecco, 1952), which suggests that phages have mechanisms to mediate interactions with other viruses (Turner, Burch, Hanley, & Chao, 1999). Phages can also exchange genes via homologous recombination or reassortment that occurs via coinfection (Worobey & Holmes, 1999). In the cystoviruses, there is biogeographical (O’Keefe et al., 2010; Silander et al., 2005) and experimental (Díaz-Muñoz et al., 2013) evidence suggesting that genetic exchange, and thus, phage interactions, are a frequent occurrence in natural settings. Phages also exhibit complex, evolved interactions with other phages. Satellite phages are defective in some aspect of the viral cycle and are dependent on a “helper virus” to reproduce. For example, coliphage P4 is deficient for capsid and tail production and successful lysis, and depends on P2 to provide these functions (Liu, Renberg, & Haggård-Ljungquist, 1997; Six & Klug, 1973), yet P4 inhibits its helper virus reproduction to gain a replicative advantage upon induction of lysis (Christie & Calendar, 1990; Liu et al., 1997).

Phage–phage interactions are not only widespread, but have the potential to affect the course of bacteria–phage interactions, with implications for the microbial community and associated macroorganisms. In the human gut, lysogenic *Enterococcus faecalis* has two distinct prophage elements that combine to produce a “composite phage” that is capable of entering the lytic
cycle (Duerkop et al., 2012). This composite phage provides the lysogenic strain with a competitive advantage over closely related strains that do not harbor prophages, both in vitro and in a mouse model (Duerkop et al., 2012).

In sum, several lines of evidence suggest that social interactions among phages are common and alter viral fitness. Moreover, coinfection is not only a selection pressure on phages, but also affects bacterial abundance with the potential for changes in community composition. The impact of phage–phage interactions is rarely considered in the context of microbial ecology (Refardt, 2011) and available evidence suggests that these interactions may be an important, but overlooked component of bacteria and phage interactions.

7.2. Potential role for phages in immunology and mediated epidemiology

Phages can alter the progression of disease, both at a population level and within-individual (macro) organisms, with consequences for epidemiology and immunology.

Phage and bacteria interactions inside animals happen on an intra-organisinal stage: the body. While it is well known that the body can interact with bacteria via the immune system, it is less appreciated that phages also have the potential to interact with the immune system (Duerkop & Hooper, 2013). A handful of studies have provided evidence that phages can prompt antibody responses (Inchley & Howard, 1969), alter the immune gene expression (Eriksson et al., 2009), and inhibit T cells (Górski et al., 2006). However, the role of naturally occurring phage in the microbiota in affecting the immune system is less well known (Duerkop & Hooper, 2013). For example, phages promote the growth of commensals either directly, by shaping the evolution of the microbe, or indirectly, by altering apparent competition in the bacterial community (Bohannan & Lenski, 2000a, 2000b). Bacteriophages have been suggested to play a role in human inflammatory bowel disease (Lepage et al., 2008), via association with host intestinal mucosa. An interesting, but somewhat contradictory, finding is for a preferential association of bacteriophages with mucosal surfaces across animal taxa (Barr, Auro, et al., 2013) and the suggestion that phages may be a nonhost adaptive immune system (Barr, Youle, & Rohwer, 2013). Another line of evidence that suggests phages interact with the immune system is the presence of hypervariable loci in viruses isolated from the gut, which are predicted to encode proteins in the immunoglobin superfamily (Minot, Grunberg, Wu,
Lewis, & Bushman, 2012). However, it is not known whether these pro-
teins are a response to immune system antigens or some other factor.
Finally, the possibility that phages, which after all are viruses, elicit antiviral
responses from mammalian cells seems possible given potential phage
uptake, but remains hypothetical (Duerkop & Hooper, 2013). The role
of the immune system in shaping phage evolution is just beginning to
be contemplated and many discoveries surely await.

Phages can also dictate the progression of disease at a population level
by exerting selection pressure on bacteria, which in turn evolve under
the pressure of the (macroorganism) host, thus altering disease epidemi-
ology. A well-studied example in humans is the dynamics of cholera epi-
demics. As discussed above (Section 4.4), phages enabled pathogenicity of
*V. cholerae* in human populations via phage-encoded toxins. Phages are also
involved in shaping the epidemiology of the cholera disease. Environmental
phage abundances have been related to seasonal cholera epidemics (Faruque,
Naser, et al., 2005), and phage reproduction may limit the continued spread
of epidemic cholera (Faruque, Islam, et al., 2005). In addition, selection
pressure from phage infection can alter *V. cholerae* receptors that are critical

Phages are also involved in the epidemiology of other macroorganisms.
For instance, in apple and pear plants, the fire blight is caused by *Erwinia
amylovora*. Lab studies suggest that prophages in lysogenic bacteria often asso-
ciated with *E. amylovora* may be induced and lyse the causative agent, affect-
ing the epidemiology of disease (Erskine, 1973).

The interactions among phage, bacteria, and macroorganisms are likely
to be complex and multidirectional and affect disease in macroorganisms at
an individual and population level. As the studies above have already shown,
the potential for bacteria and phage interactions to affect disease is apparent
and studies will likely continue to reveal the potential of microorganisms to
affect the appearance and spread of disease in complex ways.

### 7.3. Impact of phage biocontrol on environmental microbes

In recent years, there has been renewed interest in employing bacterio-
phages as antibacterial agents (Knoll & Mylonakis, 2014). This interest
has been driven in part by the increasing awareness of the abundance and
diversity of bacteriophages but primarily by the widespread antibiotic resis-
tance now apparent among bacteria (Kährström, 2013). The hope is that
phages, which in contrast to antibiotics evolve in response to bacterial
resistance and are abundant and diverse in the environment, can be employed as targeted therapies. Modern applications of phage therapy are proving increasingly successful due to, for example, the incorporation of costs associated with bacterial resistance in treatment design (Hall, De Vos, Friman, Pirnay, & Buckling, 2012; Koskella et al., 2012), engineering of phages (Lu & Collins, 2009), further examination of timing of application (Iriarte et al., 2007), co-application of carrier bacterial hosts to maintain phage densities (Azegami, 2013), the combined use of multiple phages (Schnabel, Fernando, Meyer, Jones, & Jackson, 1998), and the combination of phages and chemical control (Borah, Jindal, & Verma, 2000).

However, despite the potential of phages to meet this promise, significant hurdles remain (reviewed in Meaden & Koskella, 2013). For instance, we still have very little data on the potential for phage host range expansion and, therefore, knock-on effects to the local microbial community as well as increased risk of horizontal gene transfer of antibiotic resistance and toxin genes among bacterial strains (e.g., Colomer-Lluch, Jofre, & Muniesa, 2011). As such, the study of bacteria–phage interactions in nature, as outlined here, will be essential to understand the impact that phage biocontrol will have on target species and the environmental microbial community.

8. CONCLUSIONS

Although the role of phages in killing their bacterial host cells was first uncovered in the early 1900s, the impact that phages have on microbial populations and communities in nature has still received relatively little attention. As we have highlighted throughout this chapter, however, the elegant work that has been done so far is coming together to reveal that interactions between bacteria and phages can shape bacterial genomes, drive and maintain diversity within and among bacterial populations, stabilize and influence microbial communities, and can have important cascading effects beyond the microbial community itself. Although this field remains in its infancy relative to microbial ecology writ large, it is clear that the further development of modeling, experimental, and comparative approaches will continue to unveil both the magnitude and complexity of phage-mediated selection within microbial communities. These further advances will allow for more clear predictions regarding bacterial evolution, and therefore will lend important insight to issues of human health, disease dynamics within and evolution of natural eukaryotic populations, and ecosystem function.
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