How many ways are there to be a phage?

Rachel E. Szabo*[1,2], Martin Ackermann[1,2,3]

Author affiliations

[1]Department of Environmental Microbiology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[2]Institute of Biogeochemistry and Pollutant Dynamics, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland
[3]Laboratory of Microbial Systems Ecology, School of Architecture, Civil and Environmental Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

*Corresponding author. Email: rachel.szabo@eawag.ch

Abstract

The search for predictive rules for how microbiomes assemble and function rarely include their most abundant members: phages. Nearly a century of phage research has yielded few ecological principles for the impacts of phages within microbial communities – sequencing surveys resolve phage presence but not phage activity, while mechanistic studies of relatively few model phages provide insights that are deep but narrow. The same challenge of connecting observations between complex communities and diverse individuals also exists in bacterial ecology, where this gap is being bridged by coarse-graining species into functional groups defined by their metabolic activities. Phages, having no metabolism of their own, require a different approach. Here, we argue for building a quantitative, trait-based framework for phage ecology based on the life history traits that determine their reproduction and survival. Creating such a framework has become possible only now due to recent advances that make high-throughput phage phenotyping feasible at an unprecedented scale. We propose that an extensive, systematic map of phage trait space has the potential to reveal phage functional groups arising from physiological or evolutionary trade-offs. Because phage traits are not fixed properties across conditions, this map must contain functions describing the dependence of phage traits on host physiology and the chemical environment. Comparing these functions across phages can generate testable predictions about which phage types succeed, coexist, or exclude one another across conditions and can provide a basis for linking complex virome compositions to the ecological processes that shape them. Ultimately, ecological frameworks for phages would improve models of microbiome dynamics, unlock greater interpretability of phage sequence data, and support the rational design of phage-based microbiome interventions.

Introduction

The study of complex systems can often be summarized by the question: what are the right variables for describing how a system assembles, functions, and adapts? Answering this question involves identifying organizing principles that distill the complexity of a system with an enormous number of interacting parts into a smaller set of dimensions, revealing general trends and enabling predictions of future behavior. For microbial communities composed of thousands of types of organisms and orders of magnitude more genes and metabolites, delineating such principles remains a key challenge, and doing so is fundamental to transforming microbial ecology from a descriptive science into a predictive one[1]. To date, however, the search for principles in microbial ecology has focused almost exclusively on the microbes that carry out metabolic transformations – bacteria, fungi, and protists – overlooking the most abundant biological entities on the planet: viruses.

The viruses infecting bacteria – known as bacteriophages, or phages – are pervasive forces in microbial ecosystems, yet they are rarely incorporated into studies or models of microbiome development. Omitting

phages, while a tempting simplification, is not a neutral choice: ignoring them amounts to assuming that phages exert a uniform pressure on bacterial communities (*e.g.*, a constant death rate for all community members) that is implicitly already accounted for in models of microbial ecosystems. However, not all phage-bacteria encounters have equivalent consequences at a community level. Phages preferentially target bacteria with particular life history traits[2–4] and are often most active under certain conditions, such as high ecosystem productivity and rapid host growth[5,6]. The influence phages have on microbial community development also extends beyond mortality, with phages driving the evolution of their hosts through the selective pressures they impose and the genetic cargo carried within them[7]. As a result, phages shape the dynamics and emergent functions of microbiomes in ways that cannot be inferred or predicted from measuring or modeling the properties of bacterial populations alone.

In this perspective, we argue that we cannot treat phages as hidden variables and must instead include them in the development of organizing principles for how the parts of microbial communities give rise to their whole. We outline an approach for identifying such principles grounded in trait-based ecology and life history theory. Trait-based approaches are the current frontier for identifying ecological principles for bacterial communities, but they have not yet been established for phages. This is, in part, because the data required to build generalizable frameworks for phages could not yet be generated, which is a constraint that recent advances in high-throughput phage phenotyping have begun to lift. We propose that quantitatively mapping the space of possible phage traits, and how a phage's position within this space shifts with its host and environment, would enable us to explain and ultimately predict the distributions of diverse phages and their influence on microbiome development. Ultimately, such a framework would offer phage ecology a means of finding generality from the phages we can measure to the vastly greater number that we cannot, opening a path towards predicting their impacts in complex microbial systems.

Why we have so much data on phages and yet so few principles

Our current knowledge about phages is primarily derived from two forms of inquiry that operate at very different scales: meta-omics profiling of natural communities over the past decade, and molecular investigations of individual phages dating back nearly a century. Despite the wealth of knowledge that each approach has produced, the disconnect between these scales has so far precluded the development of robust principles for phage ecology.

Most of our information about phages in complex communities comes from sequencing surveys that have revealed which phages are present across an enormous range of environments and how their distributions shift across space and time. However, it is very difficult to make interpretations about phages' activities within microbiomes from this type of data, much more so than for bacteria. Bacterial sequence space can be condensed into ecologically meaningful units: taxa can be identified with universal marker genes; many genes with known metabolic functions can be reliably annotated; and phylogenetically conserved traits[8,9] can directly link community compositions to certain metabolic processes. Despite the inherent limitations of (meta)genomes as static snapshots, these factors all support valuable inferences about the organization and function of a bacterial community from sequence data alone. By contrast, phage sequence space, which by some estimates exceeds that of bacteria[10,11], is not nearly as interpretable. Phage phylogeny and taxonomy are difficult to resolve[12–14] and only weakly informative about phages' activities[15,16]; most phages cannot be reliably linked to their hosts from sequence information alone[17]; and nearly half of phage genes have no reliable annotations[18]. Importantly, these challenges are manifestations of the rapid rates of gene gain, loss, and divergence that are hallmarks of phage genome evolution[19,20] – as such, more sequencing data is not likely to resolve them. As a result, we can catalog the phages in a community but cannot determine how those phages are reshaping the metabolic functions of the bacteria around them.

Mechanistic information about phages' activities and impacts on bacteria comes from detailed characterizations of model phages. For these phages, we have mapped the intricate cascades of how a phage recognizes and enters a host, redirects its metabolic program, releases intracellular resources, and produces the progeny that go on to infect others. However, this information does not extrapolate readily to understanding the impacts of phages on bacterial dynamics and functions in complex communities. Many

model phages (such as the T-even phages) are exceptionally experimentally tractable; their tendency to adsorb strongly, replicate quickly, and persist well outside a host makes them more likely to represent the tails of the distributions of phage phenotypes found in nature than their means. They are also most often characterized under conditions unlike those encountered in nature (*e.g.*, in rich media, in which many host defense systems are not expressed[21]), making it unclear to what extent insights from many laboratory measurements are transferable to natural communities. Finally, phage-host systems are also almost always characterized as single pairs, which excludes from consideration interactions with the surrounding community – including with bacteria and co-infecting phages alike[22–24]– that shape infection outcomes. Our mechanistic knowledge of phages is therefore deep but narrow and, as a whole, insufficient for generalizing beyond measured systems to more complex scenarios.

The question of how to connect observations across scales remains a challenge across microbial ecology as a field. For bacteria, a successful strategy has emerged in recent years: coarse-graining the immense diversity of bacterial species into groups based on the functions they perform, rather than their identities. This process creates an intermediate level of description that has enabled us to bridge the divide between the physiology of individual species and the collective metabolic functions of microbiomes[25–28]. Phages, however, have no metabolism of their own. For phages, how can we leverage our mechanistic knowledge to identify principles for how phages assemble within and shape microbial communities?

Quantitative trait-based ecology for phages

We propose a similar coarse-graining program to identify potential functional groups for phages by building a map of phage trait space (Fig. 1a), *i.e.*, a quantitative account of phage traits across hosts and conditions. Statistical structure in this map points towards general strategies that phages might employ to propagate across different conditions. Firstly, not all regions of phage trait space are likely to be realized in nature: physiological or evolutionary constraints may render some trait combinations impossible (*e.g.,* a generalist phage with high adsorption rates across all hosts), such that occupied regions of trait space are bounded by a multidimensional surface. Secondly, correlations between traits, arising from physiological or evolutionary trade-offs, would constrain the distributions of realized phages within this space. Finally, clusters within this space represent functional groups for phages, similar to how functional groups for bacteria can be defined as taxa with similar metabolic capabilities. The existence of these groups would indicate that phage communities can be described in terms of a small number of core strategies rather than a vast number of unique genomes.

Which phage traits can best explain the variation between phage types and form the basis for a phage-specific theory remains a fundamental open question. We propose starting to build a map of phage trait space with the key life history traits[29] that determine a phage's reproduction and survival – namely, adsorption rate, latent period, burst size, decay rate, and host range. Phage life history traits determine not only which phages persist under a given set conditions but also the specificity and rate at which hosts are lysed, making these traits a mechanistic link between the functional profile of a phage and its impact on the bacteria around it.

Crucially, a phage's position in trait space is not fixed. Phage traits are not properties of phages alone but of phage-host pairs in particular environments, since a phage's niche is set jointly by host physiology[30], the external chemical environment[31,32], and ecological factors such as host density[33]. As a result, the same phage in different hosts[34] or in the same host under two nutrient regimes[35] may express different trait values, such as in burst size or latent period. Therefore, building a single map of phage trait space would obscure a meaningful association between a phage, its host, and the environment that could illuminate the underlying strategies that make phage fitness so context-dependent. This dependency of particular phage's trait on the environment can be described by a function (referred to as a "reaction norm" in evolutionary ecology), and we propose mapping phage trait space not in terms of static trait values but instead in terms of these functions (Fig. 1b,c). The dependence of phage traits on host physiology and the environment has been appreciated for individual phages for decades[36–38], but these functions have not been measured across enough diverse phages, hosts, and conditions to define general or quantitative rules. Identifying

these functions systematically rather than cataloging a patchwork of trait values is therefore what would enable us to extrapolate from the phages and conditions we test in the lab to those we cannot.

We propose that the functional groups or strategies revealed by a systematic mapping of phage trait space should be empirically discovered rather than imported from other branches of ecology, as it is entirely possible that the organizing dimensions of phage trait space do not resemble those of other organisms. One current existing phage-specific framework for organizing phage diversity – the division of phages into those with virulent vs. temperate lifestyles – is also insufficient for identifying principles for phage ecology, similar to how a classification of bacteria as aerobes vs. anaerobes is meaningful but lacks the context-dependent resolution needed to explain or predict bacterial activity. Thus, we need to go beyond purely qualitatively distinguishing phages by their infection lifestyle and instead identify which properties of phages are predictive of their effects on microbiomes.

Does phage trait space have structure?

Given existing data, can we already begin to hypothesize what the structure of phage trait space might be? Detailed characterizations of the life history traits of model phages suggests that there might be several trade-offs constraining phage trait space, such as between latent period and burst size[39,40], or between reproduction and extracellular persistence[41]. While these trends provide a compelling initial picture of the structure of phage trait space, they have not been tested across a broad enough diversity of phages to determine whether they are universal or not.

The positive relationship between latent period and burst size, representing a trade-off between phage generation time and reproduction, is an illustrative case. There is a clear mechanistic basis for this trade-off, as longer latent periods enable more virions to be assembled before the host cell lyses. While this relationship appears among evolved variants of the same phage[39] and at the level of single-cell infections by isogenic phages[40], latent period does not account for variation in burst sizes across different phages infecting the same host under the same conditions[41]. Therefore, a trade-off that exists at very fine phylogenetic scales may not hold at even slightly broader ones. Determining the resolution at which phage trait space has structure thus requires systematic, high-throughput phenotyping of diverse, non-model phages and hosts across many conditions.

At a fundamental level, resolving the structure of phage trait space would reveal which kinds of phages can exist in nature and which do not. Identifying trade-offs in trait space could inspire the search for the molecular and evolutionary mechanisms underlying those constraints, and those mechanisms would in turn allow us to further generate hypotheses about phage-host systems we have yet to measure.

Entering a new era of high-throughput phage phenotyping

The primary barrier to mapping phage trait space is a lack of suitable data. A meta-analysis of reported phage traits from the existing literature would provide a view of trait space that is both sparse and unreliable: traits are rarely measured systematically across conditions, and traditional phage assays often produce poorly reproducible results[42], either because host physiology is rarely precisely controlled or because standards for measuring key phage traits remain ambiguously defined[6].

Excitingly, the field is now poised to be able to generate the systematic, quantitative phage phenotyping data required for this effort. A set of recent conceptual and methodological advances (Box 1) enables many key phage traits to be inferred in high throughput rather than through laborious assays that remain the gold standard yet are limited in their scalability. Although some phage traits are still not measurable at scale, these advancements from the past three years have made the goal of establishing a trait-based framework for phages feasible now.

To start building the foundational datasets for this goal, we recommend that these new approaches be implemented for phenotyping phage-host pairs across environmental conditions and beyond current model systems, including the greater diversity of phages being made more accessible through novel isolation techniques[43]. Given the high sensitivity of phage traits on the host's physiological state, guidelines should be developed for reporting phage trait data as a function of host growth (*e.g.*, growth rate) and media composition to make observations across systems and labs more directly comparable.

Connecting phage trait space to phage ecology

How can we use the structure of phage trait space to make inferences about the distributions of phages across ecological contexts? We propose that the arrangement of the functions defining the dependence of phage traits on the environment contains critical information to connect these scales of information – namely, the fitness of a phage across conditions relative to that of other phages. Comparing trait functions across phages (Fig. 1b) would allow us to generate hypotheses about which phage types succeed under various conditions, and which combinations of phages may coexist or exclude one another, that are testable *in vitro*.

These hypotheses can extend beyond laboratory systems to also enable inferences about phage community assembly in natural environments. While metaviromes and metagenomes reveal differences between phage communities across locations, seasons, and treatments, the environmental filters generating those differences are often unknown. Once the functions mapping changes in phage traits along chemical gradients are established, their geometry can be compared to changes in virome composition along natural gradients or across *in situ* perturbations, providing a starting point for linking the community-level patterns we observe to the underlying processes generating them.

Future impacts of a trait-based framework for phages

There are three major future impacts we foresee from building a quantitative trait-based framework for phages: (i) greater predictive power for microbial ecology as a field, (ii) improved interpretability of phage sequence space, and (iii) more effective application of phages for targeted microbiome engineering.

Integrating phages into the search for a predictive microbial ecology

Mapping the structure of phage trait space would constitute a key step towards integrating omnipresent yet often-overlooked phage-bacteria interactions into our frameworks for how microbial communities assemble and function. We believe that this has the potential to significantly increase our predictive power for microbial ecology as a whole in two ways in the near term.

Firstly, quantitative data on the distributions of phage trait values, how those values co-vary, and how they depend on host physiology and the environment would improve our mathematical models for phage-bacteria dynamics. Parameterizing models with trait values measured only for model phages poses the risk of representing phages as having not only extreme individual traits but also extreme combinations of traits corresponding to sparsely populated regions of phage trait space. With systematic phage trait data, models could instead be run based on empirically derived distributions of trait values measured for phages from an ecosystem of interest. The goal with parameterizing models in this way would not be to precisely predict the outcome each phage-bacterium interaction – our ability to scale directly from pairwise interactions to complex communities remains questionable for bacteria[44], and emerging evidence indicates the same may be true for phages[45]. Instead, such models would enable us to make statistical statements at the community level, such as which combinations of phage traits are likely to coexist under a given condition, or how phages alter the dynamics of bacterial functional groups rather than individual hosts. These predictions

would thus describe the likelihood of potential community assembly outcomes rather than forecasts of species-level trajectories, paralleling approaches in plant ecology that explain variation in community-level trait distributions as a function of environmental conditions[46].

Secondly, identifying functional groups for phages would provide an intermediate level of organization between the species level and the community level that would allow us to use lower-dimensional representations of phage diversity in models of complex microbiomes than currently possible. Just as coarse-grained groups have improved structure-function prediction for bacterial communities[26,47], we anticipate that simplifying phage diversity through functional groups will enable more robust predictions of microbiome states and functions, including at the ecosystem scale.

Unlocking interpretability for phage sequence space

Our current inability to interpret the ever-increasing volume of 'omics data for phages is rooted in part in the fact that phage phenotypes are thus far not predictable from phage genotypes. Genotype-phenotype mapping is a frontier in bacterial ecology that is opening new possibilities for predicting the metabolic functions of individual microbial community members[48] – and ultimately the entire community[28] – from sequence data. For phages, genotype-phenotype mapping is inherently much more difficult than for bacteria, where many traits (*e.g.*, metabolic transformations, motility) can be directly linked to characterized genes. The feasibility of such a mapping for phages will depend on how strongly phage strategies are determined by phage genetics alone vs. in combination with host genetics and physiology, higher-order ecological interactions, and the abiotic environment. These dependencies are not yet known but are encoded in the context-dependent maps of phage trait space we propose developing. Despite the inherent difficulty of this goal, recent evidence indicates that large-scale phenotyping of phage collections can support genome-based predictions of whether a particular phage will successfully infect a given host[49,50], suggesting that systematic, high-throughput measurements may ultimately reveal genomic correlates for certain phage properties.

If genomic signatures for phage functional groups could be identified, this would make the existing wealth of phage genomic and metagenomic data interpretable in ecological terms, enabling us to infer from sequences the distributions of phage types and their potential strategies in complex microbiomes. It may also become possible to bypass our limited understanding of "viral dark matter" using machine learning approaches to genomically distinguish one phage strategy from another without needing to characterize what every phage gene encodes. This would enable designing phages for applications with specified combinations of properties *de novo*[51], rather than needing to isolate or evolve them.

Designing microbiome interventions with phages

The fundamental insights into phage biology and ecology gained from mapping the structure of phage trait space would bring us closer to realizing the tremendous promise of leveraging phages within a microbiome engineering toolkit. Phages could serve not only as therapeutic agents for eliminating undesirable strains but also as precise modulators of the bacterial dynamics that determine a community's collective metabolic output. A trait-based framework for phages would improve how we design phage inoculants (termed "cocktails" in the clinic) for this purpose. Qualitatively, a map of phage trait space revealing which combinations of traits are possible to find in individual phages and which are precluded by trade-offs could serve as the basis for designing cocktails with desired overall properties or effects on a community. Quantitatively, mathematical models of phage-bacteria community assembly, improved through empirical phage trait distributions, could aid in the design of stable and effective cocktails. Rather than precisely forecasting the community-level effects of any particular cocktail when deployed, such models would enable us to scan large phage collections for their properties and generate recommendations for candidate cocktails based on likelihoods of outcomes, thereby narrowing the possible search space enough to make empirical testing tractable. Finally, even microbiome engineering efforts intended to manipulate bacteria alone (*e.g.*, through resource supply) could be shaped by feedbacks with phages (*e.g.*, through Kill-the-

Winner dynamics)[52]. Anticipating how phages will influence a community's response to a perturbation is therefore essential for designing interventions with their intended effects.

Concluding remarks

The search for organizing principles in microbial ecology has advanced in recent years by coarse-graining the diversity of bacterial species according to the functions they perform. In this perspective, we have argued for taking the same approach with phages, to describe their strategies by the life history traits that determine their reproduction, survival, and impact on bacterial populations. Realizing this vision requires quantitative phage phenotyping at a scale the field has not previously attempted but that is now becoming possible to perform. While we have focused here on the need and means to collect large-scale phage trait data, we also advocate for developing new theory to generate hypotheses about the mechanisms driving the phenotypic patterns we find and how those patterns translate to community outcomes. Establishing ecological frameworks for phages will transform them from a source of unexplained variation in microbial communities to an integral component whose behavior we can predict and harness in the future.

Acknowledgements

We thank Matti Gralka for helpful discussions and feedback on this manuscript. R.E.S. was supported by an EMBO Postdoctoral Fellowship (ALTF 867-2024) and an ETH Zürich Postdoctoral Fellowship (24-2 FEL-068). M.A. was supported by the NCCR Microbiomes, a National Centre of Competence in Research, funded by the Swiss National Science Foundation (SNSF) (51NF40_180575), as well as by the Simons Foundation through the collaboration on Principles of Microbial Ecosystems (PriME, 542395).

Competing interests

The authors declare no competing interests.

Box 1: *Recent advancements in high-throughput phage phenotyping.*

The plaque assay – although laborious, low-throughput, and often poorly reproducible – has remained the gold standard technique for quantifying phage traits for nearly a century. Two recent studies[16,53] have vastly increased the scale at which we can now infer certain phage traits using straightforward plate reader-based growth assays. The fundamental insight underlying both studies is that coupling measurements of bacterial population dynamics with mathematical models of phage-bacteria dynamics enables us to infer a phage's growth rate in a particular condition, where growth rate is defined as an unconstrained composite of adsorption rate, burst size, and/or latent period. Critically, this approach provides a quantitative parameter to describe a phage's activity that is directly linked to its life history traits, providing far greater mechanistic insight than commonly used analyses for plate reader-based phage assays (*e.g.*, comparing endpoint bacterial densities or areas under the curve for cultures with vs. without phages). Furthermore, after creating a standard curve of the timing of bacterial population collapse as a function of input phage titer, this approach can be used to quantify phage concentrations in any sample, enabling rapid inferences of phage adsorption rate, burst size, latent period, and decay rate.

In these two studies, bacterial population dynamics were measured through optical density (OD) or a reporter signal. While fluorescent or luminescent reporters can increase the sensitivity of these assays relative to the coarseness of OD measurements, many bacterial strains are not genetically tractable.

Alternatively, phage lysis kinetics can also be inferred for non-engineered strains with greater precision than from OD using recently developed high-throughput assays that detect the release of intracellular host products. These include: (i) plate reader-compatible fluorescent dyes that bind host DNA[54]; (ii) plate reader-compatible luminescence-based assays for detecting host ATP[55]; and (iii) universal 16S-based ddPCR probes for detecting host DNA[56].

While there is an emerging recognition of the ecological relevance of variability in phage traits at the level of individual infections[40,57,58], most techniques enable measurements only of population-level trait values. Two novel techniques – for inferring adsorption rates through microscopy[59] and adsorption rates and latent periods through droplet microfluidics[60] – not only increase the speed at which these traits can be measured but also estimate individual-scale trait variability.

The traditional plaque assay will continue to be a valuable tool for phage researchers as it provides a direct read-out of the number of infectious virions. Importantly, the plaque assay was recently converted into a nanoliter-scale format, dramatically increasing the number of assays that can be performed in parallel[61]. This modernized version of a classic technique will enable much higher throughput screening of phages' host ranges as well as efficiency of plaquing measurements.

Figures

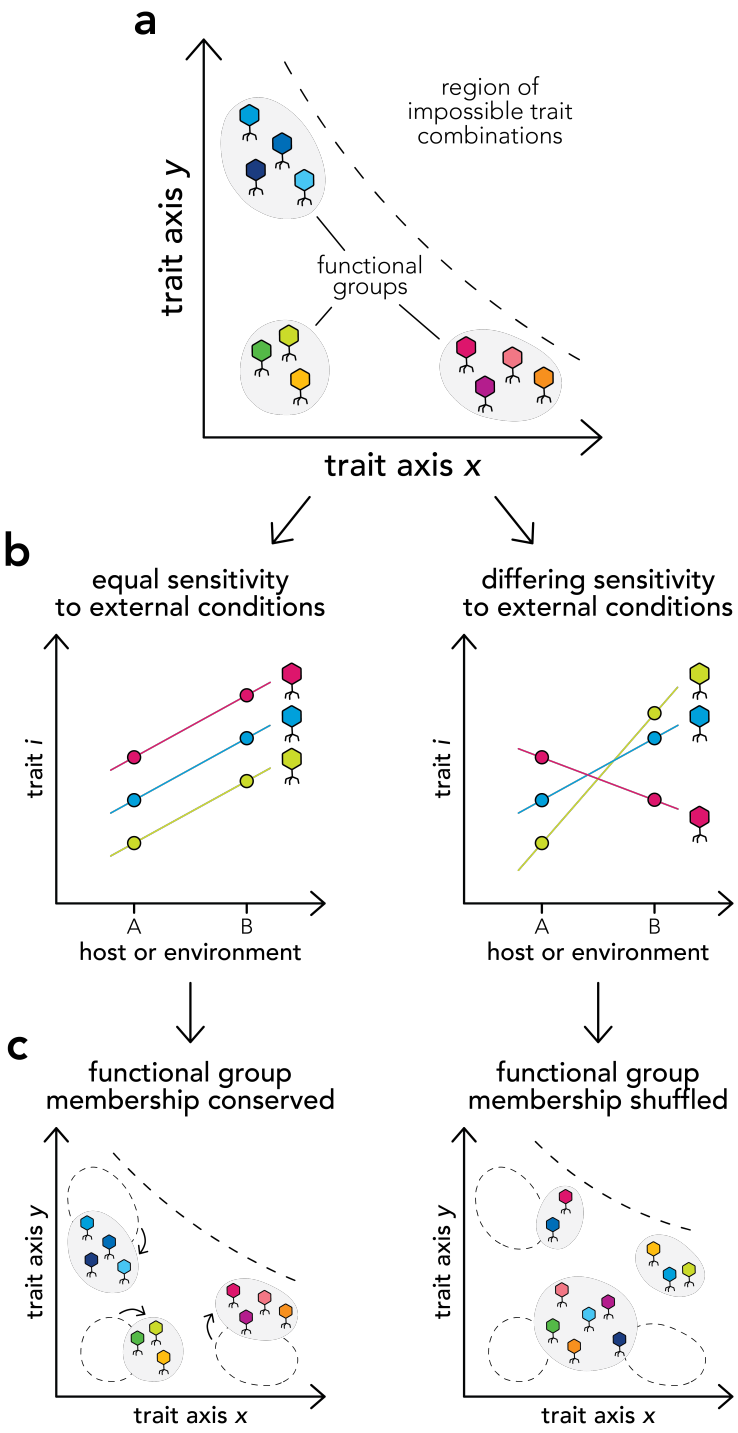

Fig. 1: *Mapping the structure of phage trait space.* **(a)** Phage trait space is a multidimensional representation of phage trait profiles whose axes are individual traits or composites of traits that capture a shared underlying property (*e.g.*, summarizing reproductive output through both burst size and latent period). Each phage, infecting a given host and in a given environment, occupies a point in this trait space. Any clusters within this space represent the equivalent of functional groups for phages. Not all regions of phage trait space are likely to be realized in nature: physiological or evolutionary constraints may render

some trait combinations impossible, such that occupied regions of trait space are bounded by what amounts to a multidimensional trade-off surface. **(b)** Phage traits are not fixed but instead vary as a function of external factors such as host identity, host physiology, and the chemical environment. *Left:* If the functions describing the dependence of a phage trait on the external conditions run parallel to one another, then a strict competitiveness hierarchy is maintained across these phages in different contexts. If fitness depends strongly on this trait, then competitive exclusion among these phages is a likely outcome (in the absence of other factors such as spatial structuring). *Right:* If the functions describing these dependencies cross, then fitness rankings flip, making coexistence among many phage types expected under fluctuating conditions. **(c)** Because phage traits are context-dependent, trait space is not a static map but instead shifts with external conditions. *Left:* If key traits structuring phage trait space have equal sensitivities to external conditions, then phages' positions in trait space shift in a similar way, maintaining cohesion among functional groups across conditions. *Right:* If key traits have different sensitivities to external conditions, then phages' positions in trait space shift unevenly, potentially creating an entirely distinct map with different functional groups. Similarly to bacteria, where functional group membership depends on the function of interest (*e.g.*, coarse-graining species by carbon vs. nitrogen metabolism would result in different groupings), definitions of phage functional groups may also be context-dependent and orthogonal (*e.g.*, host range breadth vs. extracellular persistence).

References

1. Gralka, M. Searching for Principles of Microbial Ecology Across Levels of Biological Organization. *Integr. Comp. Biol.* **63**, 1520–1531 (2023).
2. Touchon, M., Bernheim, A. & Rocha, E. P. C. Genetic and life-history traits associated with the distribution of prophages in bacteria. *ISME J.* **10**, 2744–2754 (2016).
3. Pattenden, T., Eagles, C. & Wahl, L. M. Host life-history traits influence the distribution of prophages and the genes they carry. *Philos. Trans. R. Soc. B Biol. Sci.* **377**, 20200465 (2022).
4. Wu, C. *et al.* Host life-history strategy is a critical determinant of virulent phage infection propensity. *ISME J.* **20**, wrag168 (2026).
5. Warwick-Dugdale, J. *et al*. Host-hijacking and planktonic piracy: How phages command the microbial high seas. *Virol. J.* **16**, 1–13 (2019).
6. Zimmerman, A. E. *et al.* Metabolic and biogeochemical consequences of viral infection in aquatic ecosystems. *Nat. Rev. Microbiol.* **18**, 21–34 (2020).
7. Chevallereau, A. *et al*. Interactions between bacterial and phage communities in natural environments. *Nat. Rev. Microbiol.* **20**, 49–62 (2022).
8. Martiny, A. C., Treseder, K. & Pusch, G. Phylogenetic conservatism of functional traits in microorganisms. *ISME J.* **7**, 830–838 (2013).
9. Hoffert, M. *et al*. Constructing a "periodic table" of bacteria to map diversity in trait space. *ISME J.* **20**, wraf289 (2026).
10. Koonin, E. V., Krupovic, M. & Dolja, V. V. The global virome: How much diversity and how many independent origins? *Environ. Microbiol.* **25**, 40–44 (2023).
11. Anthony, M. A., Bender, S. F. & Van Der Heijden, M. G. A. Enumerating soil biodiversity. *Proc. Natl. Acad. Sci.* **120**, e2304663120 (2023).
12. Turner, D. *et al.* Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. *Arch. Virol.* **168**, 74 (2023).
13. Weinheimer, A. R., Ha, A. D. & Aylward, F. O. Towards a unifying phylogenomic framework for tailed phages. *PLOS Genet.* **21**, e1011595 (2025).
14. Dion, M. B., Oechslin, F. & Moineau, S. Phage diversity, genomics and phylogeny. *Nat. Rev. Microbiol.* **18**, 125–138 (2020).
15. Kauffman, K. M. *et al.* Resolving the structure of phage–bacteria interactions in the context of natural diversity. *Nat. Commun.* **13**, 372 (2022).
16. Mulla, Y. *et al*. Extreme diversity of phage amplification rates and phage–antibiotic interactions revealed by PHORCE. *PLOS Biol.* **23**, e3003065 (2025).
17. Roux, S. *et al.* iPHoP: An integrated machine learning framework to maximize host prediction for metagenome-derived viruses of archaea and bacteria. *PLOS Biol.* **21**, e3002083 (2023).

18. Bouras, G. *et al.* Protein structure-informed bacteriophage genome annotation with Phold. *Nucleic Acids Res.* **54**, gkaf1448 (2026).
19. Mavrich, T. N. & Hatfull, G. F. Bacteriophage evolution differs by host, lifestyle and genome. *Nat. Microbiol.* **2**, 17112 (2017).
20. Minot, S. *et al.* Rapid evolution of the human gut virome. *Proc. Natl. Acad. Sci.* **110**, 12450–12455 (2013).
21. Brenes, L. R. & Laub, M. T. E. coli prophages encode an arsenal of defense systems to protect against temperate phages. *Cell Host Microbe* **33**, 1004-1018.e5 (2025).
22. Bisesi, A. T. *et al.* Bacteriophage specificity is impacted by interactions between bacteria. *mSystems* **9**, e01177-23 (2024).
23. Nguyen, T. V. P. *et al.* Coinfecting phages impede each other's entry into the cell. *Curr. Biol.* **34**, 2841-2853.e18 (2024).
24. Bürkle, M. *et al.* Phage–phage competition and biofilms affect interactions between two virulent bacteriophages and *Pseudomonas aeruginosa*. *ISME J.* **19**, wraf065 (2025).
25. Gralka, M. *et al*. Trophic Interactions and the Drivers of Microbial Community Assembly. *Curr. Biol.* **30**, R1176–R1188 (2020).
26. Shan, X. *et al*. Annotation-free discovery of functional groups in microbial communities. *Nat. Ecol. Evol.* **7**, 716–724 (2023).
27. Schmitt, M. S. *et al*. Learning functional groups in complex microbiomes. *bioRxiv*, doi: 10.64898/2026.03.03.709366 (2026).
28. Gowda, K. *et al*. Genomic structure predicts metabolite dynamics in microbial communities. *Cell* **185**, 530-546.e25 (2022).
29. Goldhill, D. H. & Turner, P. E. The evolution of life history trade-offs in viruses. *Curr. Opin. Virol.* **8**, 79–84 (2014).
30. Pyenson, N. C. *et al.* Diverse phage communities are maintained stably on a clonal bacterial host. *Science* **386**, 1294–1300 (2024).
31. Carlson, H. K. *et al.* Geochemical constraints on bacteriophage infectivity in terrestrial environments. *ISME Commun.* **3**, 78 (2023).
32. Blazanin, M. *et al*. Decay and damage of therapeutic phage OMKO1 by environmental stressors. *PLOS ONE* **17**, e0263887 (2022).
33. Laguna-Castro, M. & Lázaro, E. Propagation of an RNA Bacteriophage at Low Host Density Leads to a More Efficient Virus Entry. *Front. Virol.* **2**, 858227 (2022).
34. Yu, P. *et al*. Isolation of Polyvalent Bacteriophages by Sequential Multiple-Host Approaches. *Appl. Environ. Microbiol.* **82**, 808–815 (2016).
35. Howard-Varona, C. *et al.* Environment-specific virocell metabolic reprogramming. *ISME J.* **18**, wrae055 (2024).
36. Hadas, H. *et al*. Bacteriophage T4 Development Depends on the Physiology of its Host Escherichia Coli. *Microbiology* **143**, 179–185 (1997).
37. You, L., Suthers, P. F. & Yin, J. Effects of *Escherichia coli* Physiology on Growth of Phage T7 In Vivo and In Silico. *J. Bacteriol.* **184**, 1888–1894 (2002).
38. Nabergoj, D., Modic, P. & Podgornik, A. Effect of bacterial growth rate on bacteriophage population growth rate. *MicrobiologyOpen* **7**, 7:e558 (2018).
39. Wang, I.-N. Lysis Timing and Bacteriophage Fitness. *Genetics* **172**, 17–26 (2006).
40. Dominguez-Mirazo, M. *et al*. Inferring single-cell heterogeneity of bacteriophage lysis-associated life-history traits from population-scale dynamics. *Sci. Adv.* **12**, eaed6456 (2026).
41. De Paepe, M. & Taddei, F. Viruses' Life History: Towards a Mechanistic Basis of a Trade-Off between Survival and Reproduction among Phages. *PLoS Biol.* **4**, e193 (2006).
42. Anderson, B. *et al.* Enumeration of bacteriophage particles: Comparative analysis of the traditional plaque assay and real-time QPCR- and NanoSight-based assays. *Bacteriophage* **1**, 86–93 (2011).
43. Zhang, H. *et al.* PRISM: A unified platform for phage isolation and characterization from single-droplet microenvironments. *Sci. Adv.* **12**, eaeb2362 (2026).
44. Aguadé-Gorgorió, G. & Kéfi, S. Emergent coexistence and the limits of reductionism in ecological communities. *PLOS Comput. Biol.* **22**, e1014116 (2026).
45. Dey, R. *et al.* Density-dependent feedback and higher-order interactions enable coexistence in phage–bacteria community dynamics. *ISME J.* **20**, wrag041 (2026).

46. Cornwell, W. K. & Ackerly, D. D. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecol. Monogr.* **79**, (2009).
47. Zakem, E. J. *et al.* Functional biogeography of marine microbial heterotrophs. *Science* **388**, eado5323 (2025).
48. Gralka, M., Pollak, S. & Cordero, O. X. Genome content predicts the carbon catabolic preferences of heterotrophic bacteria. *Nat. Microbiol.* **8**, 1799–1808 (2023).
49. Gaborieau, B. *et al.* Prediction of strain level phage–host interactions across the Escherichia genus using only genomic information. *Nat. Microbiol.* **9**, 2847–2861 (2024).
50. Piya, D. *et al.* Comprehensive interaction profiling and machine learning prediction of bacteriophage infectivity across clinically diverse Pseudomonas aeruginosa. *bioRxiv,* doi: 10.64898/2026.05.19.726084 (2026).
51. King, S. H. *et al.* Generative design of bacteriophages with genome language models. *Science* **393**, eaec2657 (2026).
52. Voigt, E. *et al*. Phage strategies facilitate bacterial coexistence under environmental variability. *PeerJ* **9**, e12194 (2021).
53. Geng, Y. *et al*. Using bacterial population dynamics to count phages and their lysogens. *Nat. Commun.* **15**, 7814 (2024).
54. Egido, J. E. *et al.* Monitoring phage-induced lysis of gram-negatives in real time using a fluorescent DNA dye. *Sci. Rep.* **13**, 856 (2023).
55. Bayat, F. *et al.* High throughput platform technology for rapid target identification in personalized phage therapy. *Nat. Commun.* **15**, 5626 (2024).
56. Liu, Y. *et al.* Rapid and quantitative measurement of bacteriophage infectivity via fully automated droplet digital PCR. *Nat. Commun.* **17**, 7584 (2026).
57. Dominguez-Mirazo, M. *et al*. Accounting for cellular-level variation in lysis: implications for virus–host dynamics. *mBio* **15**, e01376-24 (2024).
58. Antani, J. D. & Turner, P. E. Rage against the mean: a perspective on measuring fitness of individual phage particles. *npj Viruses* **4**, 21 (2026).
59. Antani, J. D. *et al*. Microscopic phage adsorption assay: High-throughput quantification of virus particle attachment to host bacterial cells. *Proc. Natl. Acad. Sci.* **121**, e2410905121 (2024).
60. Givelet, L. *et al*. Quantifying phage-host dynamics using droplet microfluidics. *Nat. Commun.* **17**, 3857 (2026).
61. Nair, G. *et al*. Micro-plaque assays: A high-throughput method to detect, isolate, and characterize bacteriophages. *bioRxiv*, doi: 10.1101/2024.06.20.599855 (2024).