**Bacteriophage diet breadth is impacted by interactions between bacteria.**

**Authors:**

Ave T. Bisesi1, Wolfram Möbius2,3, Carey Nadell4, Eleanore G. Hansen5, Steven D. Bowden5, William R. Harcombe1,6

1 Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN, USA.

2 Living Systems Institute, University of Exeter, Exeter, UK.

3 Department of Physics and Astronomy, University of Exeter, Exeter, UK.

4 Department of Biological Sciences, Dartmouth College, Hanover, NH, USA.

5 Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA.

6 BioTechnology Institute, University of Minnesota, St. Paul, MN, USA.

**Corresponding Author**

[harcombe@umn.edu](mailto:harcombe@umn.edu)

(0000-0001-8445-2052)

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# **Abstract**

Predators play a central role in shaping community structure, function, and stability. The degree to which bacteriophage predators (viruses that infect bacteria) evolve to be specialists with a single bacterial prey species versus generalists able to consume multiple types of prey has implications for their effect on microbial communities. The presence and abundance of multiple bacterial prey types can alter selection for phage generalists, but less is known about how interactions between prey shapes diet breadth in microbial systems. Using a phenomenological mathematical model of phage and bacterial populations, we find that the dominant phage strategy depends on prey ecology. Given a fitness cost for generalism, generalist predators maintain an advantage when prey species compete, while specialists dominate when prey are obligately engaged in cross-feeding interactions. We test these predictions in a synthetic microbial community with interacting strains of *Escherichia coli* and *Salmonella enterica* by competing a generalist T5-like phage able to infect both prey against P22*vir*, an *S. enterica*-specific phage. Our experimental data conform to our modeling expectations when prey are competing or obligately mutualistic, although our results suggest that the *in vitro* cost of generalism is caused by a biological mechanism not represented in our model. Our work demonstrates that interactions between bacteria play a role in shaping ecological selection on diet breadth in bacteriophage and emphasizes the diversity of ways in which fitness trade-offs can manifest.

# **Introduction**

Predators often impose top-down control of ecosystems, impacting species abundances, community structure, and community function [1, 2]. For example, in marine environments, lytic bacteriophages (phages), the viral predators of bacteria, are critical drivers of microbial populations and nutrient cycling, lysing up to 40% of phytoplankton biomass per day [3]. The diet breadth of predators – how many different prey species they can consume – is an important component of how top-down control shapes an environment (Figure 1A) [4, 5, 6, 7, 8, 9, 10, 94]. Specialist predators often drive limit cycles with their prey, while generalists are more likely to stabilize prey populations through the emergence of apparent mutualisms, in which the presence of one prey species reduces the burden of predation on the other [13, 14, 15, 16, 17, 57]. Diversity in specificity is widespread in microbial communities, where some phages are generalists that can prey upon bacterial species across multiple genera, while others specialize on a single serovar [11, 12]. Identifying the forces shaping predator diet breath would therefore have substantial consequences for our ability to predict the long-term dynamics of multitrophic microbial communities.

The composition of the prey community is one force known to impact predator diet breadth. The evolution of generalist predators often requires prey heterogeneity to provide opportunities for diversification [18, 19, 20, 21, 22, 23, 34, 35, 100]. While it has been suggested that diversity in prey types could reduce the incidence of predator generalism, given the demands of engaging in coevolutionary arms races with more than one species [27], recent microbial studies have shown that the presence of multiple bacterial species was sufficient to select for generalists [18, 28]. Assuming a heterogenous prey environment, optimal foraging theory provides several additional predictions of how the structure of prey communities might shape predator diet breadth. First, it suggests that absolute prey densities alter selection on predator specificity by impacting foraging time. Generalism is predicted to be favored at low prey densities when foraging time is high, while specialism is favored at high prey densities; this prediction has been validated in a microbial system [26]. Optimal foraging theory also emphasizes the importance of relative prey abundances, such that predators should experience selection to exploit the most abundant prey types, even if those prey are low-quality or intraspecific competition between predators is strong [25, 32, 34, 35]. However, even when relative abundances are considered, most studies on diet breadth assume a static ratio of prey types over time, omitting a critical dimension of natural communities.

In particular, interactions between prey complicate the assumption of static ratios by generating correlations between prey abundances [29, 30, 31]. Competition for nutrients between bacteria tends to generate anti-correlated abundances between species, while positive interactions such as obligate mutualism tend to generate positively correlated abundances [29, 30, 31, 32]. When predators are consuming prey species with anti-correlated abundances, a generalist strategy is likely to be favored, because predation on one species should lead to an increase in the abundance of the alternative prey through competitive release (Figure 1B). Theoretical work provides some support for the notion that there are situations in which competing prey should favor generalist predators [32, 33]. When prey compete, mathematical modeling suggests that competitive dominance by a novel prey type is generally required for broadened predator diet breadth when fitness trade-offs for generalism are present [32]. Comparatively little work has been done investigating the impact of prey engaged in direct positive interactions on predator diet breadth. Positively correlated prey abundances are likely to favor specialist predators because predation by a specialist would also lead to a reduction in abundance of the alternative prey (Figure 1B). The interdependence between prey species may also increase the likelihood of overexploitation by predators [37, 38, 85]. However, these hypotheses - that competition between prey should favor predator generalism and that obligate mutualism between prey should favor predator specialism - have not been validated empirically.

Here we use a mathematical model and an *in vitro* system to investigate how interactions between prey species govern ecological selection on predator diet breadth. Our Lotka-Volterra type model incorporates two bacterial prey which either compete or engage in obligate mutualism, along with two phage predators, a specialist and a generalist (Figure 1C). The experimental system upon which our model is based is composed of *Escherichia coli* and *Salmonella enterica,* as well as two types of obligately lytic phage, a specialist and a generalist (Figure 1C). Our *S. enterica* strain secretes methionine and the *E. coli* strain used hereis a methionine auxotroph [42]. These bacteria form an obligate mutualism in lactose minimal media, where *S. enterica* provides methionine to *E. coli* and *E. coli* secretes carbon byproducts that can be used by *S. enterica*. They compete in glucose minimal media when exogenous methionine is added to the media, because both species can use glucose and *E. coli* is not limited by methionine. P22*vir* is an obligately lytic phage specific to *S. enterica*. EH7 is an obligately lytic phage able to infect both *S. enterica* and *E. coli*.

We found that, in our model, obligately mutualistic interactions between microbial prey were more likely to favor a specialist phage predator, while competition between prey was more likely to favor a generalist phage predator. These findings were in accordance with our initial hypotheses and are broadly relevant to systems where interactions between prey drive correlations in their abundance. Our experiments recapitulated our ecological modeling results, although the biological mechanisms underpinning our observations were different than those considered in our model. Our work provides insight into how interactions between microbial prey species alter ecological selection on phage predator diet breadth and provides a foundation for predicting the evolution and maintenance of diet breath in bacteriophage, with implications for designing and managing microbial communities.

# **Materials and Methods**

## Model description

We constructed a model of the concentrations of two interacting bacterial species, a generalist phage, and a specialist phage. Bacteria (dimensionless biomass denoted by E or S) can either compete for resources or engage in obligate mutualism [adapted from Hoek et al., 2016; 44]. We assumed that prey interactions follow Lotka-Volterra-like dynamics, defining as the maximum intrinsic growth rate of prey species *i*. We also considered a generalist predator (G) that could consume both prey species and a specialist predator (P) that could consume only one. Biomass of prey changes through growth (depending on the interaction with other prey species) and decreases due to predation and death/dilution:

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| --- | --- |
|  | Equation (1) |
|  | Equation (2) |

Biomass of predators increases through predation and decreases through death/dilution:

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| --- | --- |
|  | Equation (3) |
|  | Equation (4) |

Our model was constructed such that three parameters determine prey interactions. Mutualism is determined by the saturation constant and the mutualism coefficient which reflects the beneficial effect of prey species *i* on the per capita growth of prey species *j*. If all and values are positive, bacterial species grow faster together and cannot grow alone. Competition is driven by the coefficient , where determines the competitive effect of prey species *i* on the per capita growth rate of prey species *j*. Phage reproduction is modeled via adsorption with attachment rate which directly leads to lysis with burst size . The default natural death rate was initially identical for all four species, as in a chemostat (Table 1).

Using these equations, we investigated the extremes of pure mutualism ( and > 0, = 0), and pure competition ( = 0, = 1, > 0) (Table 1). defined the carrying capacity of the system in each interaction type. The value of was increased when prey competed relative to the value used when prey were mutualistic so that both phage types could be maintained across at least some parameter domains in each ecological condition.

## Model analyses

To compare how obligate mutualism and competition between prey affected relative abundance of the two predator types, we completed fixed point analysis to examine the fundamental behavior of the model and to determine the life history parameters of the predators expected to favor one phenotype over the other. Fixed point analysis of the model was performed in Mathematica 13.2.1.

We also investigated the equilibrium dynamics of the system by solving the ODEs until species abundances no longer changed between timepoints. All numerical simulations were completed in R v. 4.2.1 with the DeSolve package v. 1.32, using the LSODA solver with an absolute tolerance of 10-14. These results were verified by fixed point stability analysis. We evaluated equilibrium abundances of the phage predators under three different scenarios: 1) imposing a trade-off for expanded diet breadth by penalizing the burst size of the generalist phage, 2) altering the intrinsic growth rates or interaction coefficients of the bacterial prey, or 3) some combination of scenarios 1 and 2 (Table 2). To quantify phage coexistence, we used the equilibrium abundance of both phage. Relative abundance was calculated as the equilibrium density of the specialist divided by the sum of the equilibrium density of the specialist plus the equilibrium density of the generalist. Values greater than 0.5 indicated that the specialist was more abundant. Initial densities were the same across numerical simulations; all four species were always initialized at a density of 0.1. To confirm the significance of the parameters tested, we conducted two types of sensitivity analyses on our ODE system: the Morris screening method and the variance-based Sobol’ test [45, 46, 47, 48]. Morris screening and Sobol sensitivity analyses were performed in R with the ODESensitivity package v. 1.1.2 using the same parameter distribution ranges for each test type (Supplemental Table 3).

Finally, following the *in vitro* finding of high rates of degradation of the generalist phage, we amended our model to impose a cost of generalism by increasing the death rate of the generalist relative to the three other species.

## Bacterial co-culture system and phage strains

The bacterial strains have been previously described [42]. Strains are listed in Supplemental Table 1. The *Salmonella enterica* serovar Typhimurium LT2 strain secretes methionine as a result of mutations in *metA* and *metJ* [51]. The *Escherichia coli* is a methionine auxotroph due to a deletion of *metB* [42]. To track bacterial abundances and relative ratios during growth, *E. coli* was tagged with a cyan fluorescent protein and *S. enterica* was tagged with a yellow fluorescent protein [52].

The specialist phage used was P22*vir*. It is an obligately lytic version of the lysogenic *S. enterica*-specific phage P22, created through several point mutations in its prophage repressor gene (Supplemental Table 6). P22*vir* was provided by I.J. Molineux. A generalist phage strain, EH7, was isolated and provided by E. Hansen and S. Bowden. EH7 is an obligately lytic T5-like siphovirus that uses BtuB, a differentially expressed outer membrane protein for vitamin B12 uptake, as a receptor. It is similar to T5-like coliphages described in Kim and Ryu (2011) [12] and Switt et al (2015) [53] (Supplemental Table 7).

Two additional bacterial strains were used for plaque assays (Supplemental Table 1). They were chosen so that, in mixed cultures of phage, phage types could be quantified independently of each other. The *E. coli* K-12 BW25113 ∆*trxA* from the Keio collection was used to quantify EH7 densities [96]. An *S. enterica* serovar Typhimurium NCTC 74 strain with *btuB* knocked out through a transposon insertion (EZ-Tn5 <Kan-2>, Lucigen) was used to quantify P22*vir*. The ∆*btuB* *S. enterica* strain was provided by S. Bowden.

## Media

Minimal hypho liquid media for experiments was prepared as previously described, with each component sterilized prior to mixing [99] (Supplemental Table 2). In addition to the appropriate carbon source, solutions containing sulfur, nitrogen, phosphorus, and metals were supplemented into each media type (Supplemental Table 2). Routine culturing of all bacterial strains was carried out on Miller Lysogeny Broth (LB) unless otherwise indicated. Working stocks of both phage types were grown on log-phase *S. enterica* LT2 cultures in LB and stored at 4°C. Stock titer was determined by plaque overlay assay on the appropriate strains as described above.

## Phage competition assays

Phage competition assays were performed in 96-well flat bottom plates on a Tecan Infinite Pro200 plate reader for 48 hours at 37°C with shaking at 432 rotations per minute. Experiment duration was chosen to allow batch culture experiments to reach a final state (stationary phase, phage densities unchanging), thus allowing us to compare to our chemostatic model. Overnight stationary phase cultures in LB started from single colonies were washed three times in saline, adjusted to a density of 107 cells per mL, and used to inoculate 200µL of appropriate medium with 2.0 x 105 total cells per well (i.e. 2.0 x 105 total *S. enterica* cells in monoculture, 1.0 x 105 total *S. enterica* cells and 1.0 x 105 total *E. coli* cells in co-cultures). Phage stocks were diluted in saline to 105 plaque-forming units per mL and inoculated into the appropriate wells to an MOI between 0.005 and 0.01, depending on the fraction of infectable cells for each phage type. Phage strains were added either in isolation (103 total phage particles of either P22*vir* or EH7) or in a one-to-one ratio (103 total phage particles of P22*vir* and 103 total phage particles of EH7 for a final density of 2 x 103 total phage particles). Phage densities were confirmed by plaque overlay assay at the start of the experiment on the appropriate strains as described below.

OD600, *E. coli*-specific CFP (Ex: 430 nm; Em: 480 nm), and *S. enterica*-specific YFP (Ex: 500 nm; Em: 530 nm) fluorescence were read every 20 minutes. Fluorescent protein signals were converted to species-specific OD equivalents using an experimentally determined conversion factor as previously described [54]. A single initial experiment was completed to confirm the reproductive ability of each phage on *S. enterica* monoculture or *E. coli* monoculture. Full factorial experiments testing all three phage conditions (P22*vir*, EH7 or EH7 + P22*vir*) on either *S. enterica* monoculture, mutualistic co-culture, or competitive co-culture were then completed with four biological replicates per condition, plus three biological replicates for no-phage controls per condition. Three experiments were set up and completed during different weeks to confirm the repeatability of the results; one representative run was chosen for display in this paper. We plated for PFUs for each replicate from half of the total 200µL volume using plaque overlay assays on LB plates with 0.7% LB top agar at the end of the 48 hour growth period. All replicates were quantified using plaque assays on both ∆*trxA* *E. coli* and ∆*btuB* *S. enterica.* ∆*trxA* *E. coli* and ∆*btuB* *S. enterica* were prepared for use in plaque assays through overnight culture growth in LB, prior to being diluted 1:10 (∆*btuB* *S. enterica*) or 1:5 (∆*trxA* *E. coli*) and allowed to grow for 30 minutes. Plaque assays were otherwise performed as previously described using 2µL of phage spot dilutions from 100 to 10-7 with three technical replicates per dilution per sample [55, 56]. The lower limit of detection was 500 PFU/mL. Change in phage titer was represented as the natural log of the final phage density divided by the starting phage density (ln(final PFU/mL / initial PFU/mL)). All plates were incubated overnight at 37°C.

To impose a cost of generalism in our system, we repeated the phage competition assays as previously described, incubating the phage in minimal media at 37°C with shaking for 24 hours prior to the addition of cells in either *S. enterica* monoculture or competitive co-culture. Mutualistic co-culture was not tested. The experiment was completed once following preliminary trials to confirm that EH7 did not degrade below the limit of recovery after 24 hours. Once cells were added, cultures were grown for an additional 24 hours. Phage densities were quantified at the beginning and end of the 48 hour experiment.

## Phage degradation assays

We examined the impact of cell starvation on the formation of new EH7 particles using a full factorial design of both phage types and *E. coli* or *S. enterica* monoculture in lactose hypho minimal media. Neither bacterial strain could grow, as each was starved of essential nutrients. Bacteria were inoculated in lactose hypho monoculture at a density of 105 cells per 200µL of medium in a 96-well plate and treated with either EH7 or P22*vir* at a total density of 103 PFU (MOI = 0.01) or incubated in a no-phage control. Additionally, we tested each phage in isolation in lactose minimal media without cells to determine phage decay rates. Each condition consisted of three replicates. Both experiments were completed once at 37°C with shaking at 432 rotations per minute. Phage density in each well was determined by plaque overlay assay at the beginning and end of the 48 hour experiment. Change in phage titer was again represented as the natural log of the final phage density divided by the starting phage density (ln(final PFU/mL / initial PFU/mL)).

## Scripts and data availability

Numerical simulations, sensitivity analyses, data analysis, statistics, and figure generation were performed using R v. 4.2.1 using custom scripts available at <https://github.com/bisesi/Host-Ecology-and-Host-Range>. Raw experimental data and Mathematica notebooks for fixed point analysis are available at the same link.

# **Results**

## In a phenomenological model, phage relative abundance depends on prey interactions and fitness trade-offs

We used a phenomenological model to predict how communities of two interacting prey species respond to attack by predatory lytic phage during chemostatic growth. We predicted that competition between prey species was likely to favor predator generalism by increasing temporal heterogeneity in resource availability [36, 98], while obligate mutualism between prey species would result in less temporal heterogeneity, as bacterial species would either occur together or not at all, likely favoring specialization [36].

We first investigated the behavior of the model with a single parameter set. Using our default parameters (Table 1), we examined cases in which phage were not present, or when only one phage type was present. When phage were not modeled in our system, prey species reached a 50:50 ratio at equilibrium (Figure 2A, left panel). The introduction of a specialist resulted in competitive release of *E. coli* when prey competed and correlated reductions in bacterial abundances when prey were mutualistic. This behavior was consistent with our conceptual model (Figure 2A, middle panel; see also, Figure 1B). In comparison, the introduction of a generalist phage predator reduced the density of both prey species at equilibrium, keeping their abundances at a 50:50 ratio (Figure 2A, right panel). We also considered the behavior of the model when phage phenotypes competed against one another. In alignment with our conceptual model, when the specialist had a burst size five times greater than that of the generalist, the generalist dominated when prey competed (Figure 2B), while the specialist dominated when prey were mutualistic (Figure 2C).

To further understand the behavior of the model when phage phenotypes competed against one another, we then sought to simplify the system, focusing on Eqs 3-4. theory [58] suggests that, when two species compete for the same resource, the species with the lower - that is, the species that can sustain zero net population growth on fewer resources - will be able to competitively exclude the other species. To apply this to our system, we treated the shared prey, *S. enterica*, as a resource, and identified domains in which the amount of *S. enterica* required by the specialist to hold its net population growth at zero, , would be expected to be less than the amount of *S. enterica* required for zero net growth of the generalist population, [58]. To do so, we assumed that the generalist phage had an equivalent burst size and attachment rate on both prey types and that the intrinsic mortality rate was not species-specific, such that described the burst size of the generalist on both prey, described the attachment rate of the generalist on both prey, described the burst size of the specialist on *S. enterica*,  described the attachment rate of the specialist on *S. enterica*, and described the intrinsic mortality rate of all four species.

and were obtained by setting the left-hand side of Eqs 3-4 equal to 0, and solving for . Using these equations, in accordance with R\* theory, requiring that

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| --- | --- |
|  | Equation (5) |

leads to:

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|  | Equation (6) |

This analysis therefore suggested that the specialist phage could dominate (i.e. have the lower ) when the alternative prey source *E. coli* was rare, or when the generalist suffered a fitness trade-off for expanded diet breadth in the form of reduced burst size or attachment rate. This inequality is broadly consistent with the “jack of all trades, master of none” hypothesis for the predominance of specialization [24, 59, 74, 75, 76, 77, 78, 79, 92, 95, 97].

To verify the intuition of our inequality, we used our default parameters (Table 1) to consider various domains in which the specialist phage predator was favored when prey species converged to a 50:50 ratio at equilibrium in the absence of predators. To do so, we systematically changed the burst size (Figure 3; for attachment rate , see Supplemental Figure 1) of the specialist phage on the shared prey *S. enterica* ()and considered the relative abundance of each phage type at equilibrium. We found that even as the cost of phage generalism increased, the generalist phage was always favored when prey competed (Figure 2B; Figure 3A, left panel). Fixed point analysis demonstrated that if or , i.e., specialist adsorption rate or burst size was twice as large as that of the generalist, then the only stable fixed point was a four-species equilibrium. No domain existed within relevant parameters where only the specialist phage could stably coexist with the two prey species. These results suggested that, even in those cases where the generalist was not dominant, it could not be driven extinct by a specialist when prey competed. Conversely, the specialist was favored on mutualistic prey (Figure 2C; Figure 3A, right panel) given a minimum cost of generalism. If or , i.e. the specialist’s burst size or attachment rate was 2.83 times as large as that of the generalist, then the only stable fixed point was that of the two bacterial species coexisting with the specialist. As such, given a threshold cost of generalism, only the specialist could coexist with mutualistic bacterial species, regardless of initial conditions.

To visualize the next prediction of our inequality - that the relative availability of the alternative prey source *E. coli* mattered for the competitive outcome of phage diet breadth strategies - we loosened the previous assumption that bacterial species should reach a 50:50 ratio without predators. In addition to varying the ratio of prey growth rates (in both competition and mutualism) or the ratio of interaction coefficients ( for mutualism, for competition), we systematically altered the cost of generalism (as both burst size and attachment rate; for attachment rate, see Supplemental Figure 1). Our analyses demonstrated that when prey were competing, relative growth rates and competition coefficients mediated the abundance of the specialist such that it had a fitness equivalent to or greater than that of the generalist only in those cases where the alternative prey *E. coli* was competitively excluded by the shared prey *S. enterica* (Figure 3B). However, even in these cases, only the four-species equilibrium point was stable, suggesting that even when the specialist dominated due to the competitive superiority of its prey, the generalist was able to coexist. In contrast, when prey were mutualistic, neither relative growth rates nor relative mutualistic benefit coefficients altered phage relative abundance greatly. Instead, ecological dominance of the specialist depended mainly on the cost of generalism, such that the specialist tended to proliferate given a sufficient cost regardless of biased bacterial abundances driven by unequal mutualistic benefit (Figure 3C).

Finally, to ensure that our tested parameters captured the fundamental behavior of the model, we performed two sensitivity analyses - a Morris screening and a Sobol variance analysis - on our ODE equations to determine which parameters had the largest impact on the final biomass of each phage type. In the case of both obligate mutualism and competition, Morris screening methods suggested that the death/dilution rate, burst size and attachment rates of both phage, and the interaction parameters for the microbial species were of greatest impact (Supplemental Table 5). The variance-based Sobol method reinforced the importance of dilution rate (Supplemental Table 6). These results were consistent both with the parameters identified by fixed point analysis, our inequality, and the basic construction of the model, which requires that both phage types have reproductive parameters sufficient to offset the chemostat-induced mortality rate.

## Phage relative abundance *in vitro* aligns with modeling results in co-culture

Using our wet-lab experimental cross-feeding system, we tested the mathematical prediction that generalist predators would be favored on competing prey and specialist predators would be favored on mutualistic prey. We first verified that over 48 hours our specialist phage (P22*vir*) could only replicate on *S. enterica* and that our generalist phage (EH7) could replicate on both prey species (Figure 4A). Each phage alone also grew well on competitive and mutualistic bacterial co-cultures. The final density of EH7 appeared similar when replicating on both interaction types (Figure 4A, p = 0.999). This was true for P22*vir,* as well, such that type of interaction did not result in significantly different final titers (Figure 4A, p = 0.909). However, when phage were added in isolation, EH7 reached a higher titer than P22*vir* on both competitive (Figure 4A, p < 0.0001) and mutualistic co-culture (Figure 4A, p < 0.0001). Consistent with our model, P22*vir* increased *E. coli* frequency relative to the no-phage control in competitive co-culture (Figure 4C, p < 0.0001). Applying the specialist phage also suppressed, but did not eliminate, both bacterial species in mutualism (Figure 4D). In contrast, the generalist phage more effectively suppressed *E. coli* in competitive co-culture, resulting in a higher relative density of *S. enterica* compared to the no-phage control (Figure 4C, p = 0.0035). Unlike P22*vir*, the application of EH7 to mutualistic co-culture did not suppress co-culture growth for the duration of the experiment (Figure 4C).

We also evaluated population dynamics when the two phages competed against one another (Figure 4B, 4C and 4D). When both phage were present in competitive co-culture, the generalist reached a higher final density than the specialist (Figure 4B, p < 0.0001), as predicted by our modeling results. Bacterial dynamics were also consistent with our model, with *E. coli* dominating through competitive release (Figure 4C, p < 0.0001). When both phage were present in mutualistic co-culture, the specialist reached a higher final titer (Figure 4B, p < 0.0001) and co-culture densities were suppressed for the duration of the experiment (Figure 4D). Curiously, however, the presence of the specialist phage reduced EH7 below the limit of detection when prey were mutualistic (Figure 4B), a result that we interrogated further.

## *In vitro,* cost of generalism manifests as increased rate of degradation and reduced infectivity of starved cells

To understand our inability to detect the generalist phage in mutualistic co-culture at the end of our experimental window, we investigated the ability of each phage to reproduce on starved cells by adding phage to monocultures in lactose minimal media as previously described (see Materials and Methods). With *E. coli* or when placed in wells without bacterial cells, P22*vir* titer remained unchanged over the 48 hour growth period (Figure 5A). When placed in wells with starved *S. enterica*, P22*vir* was able to reproduce despite the expected physiological inaccessibility of its prey in a nutrient-deprived state, with its titer increasing relative to the condition without cells (Figure 5A, p = 0.001) or with *E. coli* (Figure 5A, p = 0.0001).

In comparison, the generalist phage EH7 decreased in abundance in all conditions without growing cells after the 48 hour growth period. There were no detectable phage particles in wells without cells or with starved *E. coli.* In wells with only starved *S. enterica*, some phage were still detectable, although phage titer was greatly reduced, suggesting that *S. enterica* may be more physiologically accessible to EH7 than *E. coli* in a starved state (Figure 5A, p = 0.012). Taken together, these results indicate that the generalist phage suffers a cost that manifests in two distinct ways: first, a rapid rate of degradation in minimal media, and, second, a reduced capacity to infect and reproduce inside starved cells relative to the specialist phage P22*vir*. As a result, when competing with P22*vir* on mutualistic co-culture, the starved physiology of the interdependent cells and the generalist’s degradation can explain our inability to detect it at the end of previous experiments.

Interestingly, these results are specific to minimal media, as EH7 does not degrade in LB (Supplemental Analysis 2; Supplemental Figure 2). We were not able to identify which component of our minimal media was responsible for the degradation of the phage, though it does not appear to be related to the presence of metals or the result of osmolarity (Supplemental Analysis 2; Supplemental Figure 2).

## The generalist is favored in competition even when a cost is imposed *in vitro*

While our *in vitro* experiments replicated our modeling results when bacterial prey were mutualistic or competing, we did not observe a cost of generalism when both phage types were competing for the shared prey *S. enterica*, with each phage reaching titers that did not differ significantly from one another(Figure 5B, p = 0.999). In our system, a trade-off may thus only manifest in the mutualistic treatment where the generalist phage degrades due to bacterial growth delayed by P22*vir* predation on *S. enterica*. To impose a cost of generalism across treatments, we repeated our phage competition assay experiments, incubating the phage for 24 hours prior to the addition of cells, anticipating that some degradation of the generalist EH7 would occur, while the titer of P22*vir* would remain unchanged. Previous preliminary experiments had indicated that while EH7 titer is often below the limit of detection after 24 hours (Supplemental Figure 2), the phage can be recovered following the addition of cells, suggesting that some infectious phage particles remain. We therefore expected that the reduced titer of EH7 when cells were applied would mirror the conditions of mutualism when phage competed. Given that the presence of P22*vir* in competitive co-culture increases *E. coli* frequency, we hypothesized that a lower titer of EH7 when cells are added may still result in a higher final titer of the generalist phage relative to the specialist, because any remaining EH7 would be able to utilize *E. coli*.

When a cost was imposed and phage were competed on *S. enterica monoculture,* the generalist disappeared below the limit of detection, while P22*vir* final titer was not significantly different than in the condition without cost (Figure 5B, p = 0.982). In contrast, we found that when bacteria competed, final titers of both EH7 (Figure 5B, p = 0.723) and P22*vir* (Figure 5B, p = 0.999) were comparable to the no cost condition, as we had expected. These results are consistent with our modeling results, suggesting that, even with a cost of generalism, a generalist predator should be favored on competing prey.

## In a phenomenological model, prey interactions determine the intrinsic death rate needed to favor specialism

Finally, we amended our model to see if we could replicate the results of our *in vitro* experiment, given the known constraints of high rates of degradation of the generalist phage when cultured in minimal media. To do so, we imposed a cost of generalism not as burst size (or attachment rate), but instead as an increased intrinsic mortality rate for the generalist phage, while keeping the mortality rate the same for the three other species.

We observed that, when fitness cost was modeled as intrinsic mortality, our qualitative results matched those when fitness cost was measured as burst size or attachment rate (Figure 6; for burst size, see Figure 3A; for attachment rate, see Supplemental Figure 1A). In this case, the generalist phage could be maintained on competing prey even as its intrinsic mortality rate increased; competition with the specialist phage increased its rate of loss, but was insufficient to drive the generalist extinct across a wide range of parameters (Figure 6). However, if the intrinsic mortality rate of the generalist exceeds 5.7 times that of the specialist, it will be lost when bacteria compete. In contrast, comparable intrinsic mortality rates resulted in the early loss of the generalist when prey were mutualistic (when the intrinsic mortality rate of the generalist exceeds 2.6 times that of the specialist), with the specialist able to persist (Figure 6). However, it is worth noting that, because degradation rates *in vitro* are impacted by the physiological accessibility of the available prey and the differential abilities of the two phage to replicate on starved cells, our model is not currently designed to capture changes in intrinsic mortality rate as a function of prey density or physiological state. It is thus unlikely to perfectly replicate our experimental results. Regardless, in its current form, it reinforces our previous qualitative modeling predictions and our *in vitro* findings that suggest that competition between prey favors generalist predators across a wider range of fitness trade-offs than mutualism between prey.

# **Discussion**

We aimed to determine whether ecological interactions between bacterial prey species impacted the abundance of phage with different prey specificity. We developed a simple four-species phenomenological model composed of two interacting bacterial species, a specialist phage, and a generalist phage. Using this chemostatic model, we found that specialist phage were favored when prey are mutualistic, while a generalist diet breadth was favored when prey compete. These results were robust to initial conditions across a range of parameter values, suggesting that they may be both ecologically and evolutionarily informative. We found that our modeling predictions were well-matched by the outcome of batch culture experimental phage competition assays, but that biological and environmental details not considered in our model contributed to these results. *In vitro*, phage degradation and inability to infect inactive bacteria drove ecological outcomes for phage. Our results highlight that ecological interactions between prey can alter predator specificity through a range of mechanisms.

Our modeling results suggest that interactions between bacterial prey impact the prevalence of phage specificity phenotypes. Experimental evolution has previously shown that the presence of different types of resources can select for generalism [9, 18, 73]. Both absolute and relative prey densities are relevant predictors of phage diet breadth [25, 26, 34, 35]. However, while much of the previous work done on diet breadth has assumed a constant relative abundance of available prey, our model upended that assumption by allowing relative prey abundances to vary as a function of prey ecology. Previous theoretical modeling has demonstrated that resource competition between prey species can select for expanded predator diet breadth even when trade-offs for generalism exist, although this result generally required the competitive dominance of the novel prey source [32, 33]. Our results align with these findings, underscoring that resource competition should favor a generalist strategy in most cases, even when a trade-off is present. Additionally, we expanded previous findings to include mutualistic interactions between prey, showing that a specialist predator strategy dominated assuming a minimal trade-off for generalism. Our model demonstrates that ecological interactions between prey species favor different predator diet strategies because switching from competition to mutualism changes relative prey abundances from being anti-correlated to being positively correlated. We anticipate that our modeling result will apply broadly to systems when interactions between prey generate correlations in their abundance.

The experimental results of our study largely align with our modeling predictions, although they also highlight two important aspects of our microbial system. First, *in vitro*, we did not observe a reproductive fitness cost of generalism on the shared prey species as we had anticipated in our model. Instead, we found that the trade-off manifested as an increased intrinsic mortality rate in minimal media and reduced replication rates on starved cells. Our results contribute to the body of work suggesting that pleiotropic costs are often context dependent [24, 36, 74, 75, 76, 77, 78, 79, 97]. Additionally, our experiments emphasize that in addition to altering population dynamics, interactions between bacteria can also impact phage diet breadth by altering prey physiology. Bacterial sensitivity to phage is rarely a binary trait and can change between physiological states due to differences in growth rate, metabolism, transcription and translational activity, and the availability of intracellular components [39, 40, 41]. Phage reproduction on slow-growing or stationary phase cells is often more difficult due to reduced cell size and lower densities of the receptors phage use to adsorb [25, 56]. Because microbial physiology is driven strongly by resource availability and interactions between bacterial species [39], our work suggests that ecological selection on phage specificity is likely impacted by how interactions between bacterial prey shift prey abundances and physiological states over time.

There are several limitations to the study we performed which impact the generality of the results. The two phage types tested differ significantly in traits in addition to diet breadth. They utilize different receptors for viral entry, with P22*vir* attaching to the O-antigen of *S. enterica*’s lipopolysaccharides (LPS) and EH7 using BtuB, a vitamin B12 uptake receptor. Previous work has demonstrated that BtuB expression is highly context dependent, while the LPS is constitutively expressed[80, 81, 82, 83, 84, 86, 87, 88, 89, 90, 91]. Additionally, our phages are vastly different sizes, with EH7 consisting of 110kb and significant similarity to phage strains with as many as 166 putative proteins, while P22*vir* is much smaller at 44kb and 74 proteins [53]. These differences are likely to accentuate the role of bacterial physiology in determining selective outcomes, as the intracellular demands for producing EH7 will be much higher and thus more limited by slow growth or low nutrient availability. Future work should test the competitive ability of phages that use the same or similar receptors and with greater stoichiometric similarity to disentangle these confounding factors. Additionally, we have not completed evolution experiments in this study to see if longer term selective outcomes can be well-predicted by ecological dynamics. However, in the context of microbiological engineering and biomedical applications, we expect that ecological selection will be a useful metric for predicting community outcomes.

Our results suggest numerous directions for future study. In the context of phage therapy, the performance of EH7 in minimal media emphasizes the necessity of testing how different environments affect phages and whether phage characteristics such as specificity tend to correlate with susceptibility to degradation [63]. These data also suggest that the ways bacteria modify their environments through alteration to local pH or vitamin concentrations may have consequences for the evolution and persistence of their viral predators. For example, human gut microbes often directly compete with their hosts for vitamin B12 [93]; the resultant availability of B12 in the human gut may alter the efficacy of BtuB-specific phages in phage therapy applications. Continued characterization of phage-bacteria interactions in the complex community contexts in which they are found will improve our ability to use phage for engineering and biomedical purposes. Finally, we note that the spatial structure of interacting bacterial species, as in a biofilm, will alter local prey availability in natural environments in ways that might complicate or invalidate the results we have presented here [36].

We took a simple modeling approach, paired with an ecological experiment, to gain insight into the role of prey ecology on the competitive ability of bacteriophage with different diet breadths. We found that, in both our model and *in vitro* experiments, prey interactions shape the prevalence of phage diet breadth phenotypes, though the mechanisms differ between our modeling approach and synthetic community. Management and design of microbial communities is contingent upon our ability to predict the evolutionary outcomes and higher order ecological effects of multitrophic interactions. Understanding the complex biotic factors driving ecological and evolutionary outcomes for bacteriophage is a critical step to effectively harnessing microbes for industrial and biomedical applications.

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# **Competing Interests**

The authors declare no conflicts of interest.

# **References**

[1] Friman VP, Teppo H, Jouni L, Veijo K. Availability of Prey Resources Drives Evolution of Predator–Prey Interaction. *Proceedings of the Royal Society B: Biological Sciences.* 2008; 1643(275): 1625–33.

[2] Chesson P, Kuang J. The interaction between predation and competition. *Nature*. 2008; 456(1): 235–238. [h](https://doi.org/10.1038/nature07248)

[3] Suttle C. Viruses in the sea. *Nature. 2005;* 437(1): 356–36.

[4] Bohannan BJM, Lenski RE. Effect of Prey Heterogeneity on the Response of a Model Food Chain to Resource Enrichment. *Am Nat.* 1999; 153(1): 73-82.

[5] Worsfold, NT, Warren PH, Petchey OL. Context-Dependent Effects of Predator Removal from Experimental Microcosm Communities. *Oikos.* 2009; 118(1): 1319–26.

[6] Imrie RM, Roberts, KE, Longdon B. Between Virus Correlations in the Outcome of Infection across Host Species: Evidence of Virus by Host Species Interactions. *Evolution Letters*. 2021; 5(5): 472-483.

[7] Forister ML, Jenkins SH. A Neutral Model for the Evolution of Diet Breadth. *The American Naturalist*. 2017; 190(2): E40–54.

[8] Loxdale HD, Lushai G, Harvey JA. The Evolutionary Improbability of ‘Generalism’ in Nature, with Special Reference to Insects. *Biological Journal of the Linnean Society*. 2011; 103(1):1–18.

[9] McLeish M, Sacristán S, Fraile A, García-Arenal F. Scale Dependencies and Generalism in Host Use Shape Virus Prevalence. *Proceedings of the Royal Society B: Biological Sciences.* 2017; 284(1869): 20172066.

[10] Dennis RLH, Dapporto L, Fattorini S, Cook LM. The Generalism–Specialism Debate: The Role of Generalists in the Life and Death of Species. *Biological Journal of the Linnean Society.* 2011; 104(4):725–37.

[11] Kortright KE, Chaan BK, Koff JL, Turner PE. Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria. *Cell Host & Microbe*. 2019; 25(2): 219–32.

[12] Kim M, Ryu S. Characterization of a T5-Like Coliphage, SPC35, and Differential Development of Resistance to SPC35 in Salmonella Enterica Serovar Typhimurium and Escherichia Coli. *Applied and Environmental Microbiology*. 2011; 77(6): 2042–50.

[13] Abrams PA, Holt RD, Roth JD. Apparent Competition or Apparent Mutualism? Shared Predation When Populations Cycle. *Ecology*. 1998; 79(1): 201–12.

[14] Hanski I, Hansson L, Henttonen H. Specialist Predators, Generalist Predators, and the Microtine Rodent Cycle. *Journal of Animal Ecology*. 1991; 60(1): 353–67.

[15] May RM. (1981). Models for two interacting populations. *Theoretical Ecology* (Ed. by R.M. May), pp. 78-104. Blackwell Scientific Publications, Oxford.

[16] Schreiber SJ. Generalist and Specialist Predators That Mediate Permanence in Ecological Communities. *Journal of Mathematical Biology*. 1997; 36(2): 133–48.

[17] Schreiber SJ, Křivan V. Holt (1977) and Apparent Competition. *Theoretical Population Biology.* 2020; 133: 17–18.

[18] Sant DG, Woods LC, Barr JJ, McDonald MJ. Host Diversity Slows Bacteriophage Adaptation by Selecting Generalists over Specialists. *Nature Ecology & Evolution*. 2021; 5: 350-359.

[19] Woolhouse ME, Taylor LH, Haydon DT. Population biology of multihost pathogens. *Science.* 2001; 292: 1109–1112.

[20] Betts A, Gray C, Zelek M, MacLean RC, King KC. High parasite diversity accelerates host adaptation and diversification. *Science.* 2018; 360: 907–911.

[21] Gause GF. (1934). *The struggle for existence*. Baltimore, MD: Williams and Wilkins.

[22] Duffy S, Turner PE. (2009). Phage evolutionary biology. In S. T. Abedon (Ed.), *Bacteriophage Ecology* (pp. 147–176). Cambridge, UK: Cambridge University Press.

[23] Via S. Ecological genetics and host adaptation in herbivorous insects: The Experimental Study of Evolution in Natural and Agricultural Systems. *Annual Review of Entomology*. 1990; 35: 421–446.

[24] Duffy S, Turner PE, Burch CL. Pleiotropic costs of niche expansion in the RNA bacteriophage φ6. *Genetics.* 2006; 172:751–757.

[25] Heineman RH, Springman R, Bull JJ. Optimal Foraging by Bacteriophages through Host Avoidance. *The American Naturalist*, 2008; 171(4): E149–57.

[26] Guyader S, Burch CL. Optimal Foraging Predicts the Ecology but Not the Evolution of Host Specialization in Bacteriophages. *PLoS ONE*. 2008; 3(4): e1946.

[27] Chappell TM, Rausher, MD. Evolution of Host Range in Coleosporium Ipomoeae, a Plant Pathogen with Multiple Hosts. *Proceedings of the National Academy of Sciences.* 2016; 113(19): 5346–51.

[28] Betts A, Rafaluk C, King, KC. Host and parasite evolution in a tangled bank. *Trends Parasitol.* 2016; 32: 863–873.

[29] Mizrahi P, Sivan AG, Gore J. Community Interactions Drive the Evolution of Antibiotic Tolerance in Bacteria. *Proceedings of the National Academy of Sciences*. 2023; 120(3): e2209043119.

[30] Adamowicz EM, Muza M, Chacón JM, Harcombe WR. Cross-feeding modulates the rate and mechanism of antibiotic resistance evolution in a model microbial community of Escherichia coli and salmonella enterica. *PLoS Pathog.* 2020; 16: e1008700.

[31] Pauli B, Oña L, Hermann M, Kost C. Obligate mutualistic cooperation limits evolvability. *Nat. Commun.* 2022; 13: 1–11.

[32] Okamoto KW, Amarasekare P, Post DP, Vasseur DA, Turner PE. The Interplay between Host Community Structure and Pathogen Life‐history Constraints in Driving the Evolution of Host‐range Shifts. *Functional Ecology* 2019; 33(12): 2338–53.

[33] Velzen E, Etienne RS. The evolution and coexistence of generalist and specialist herbivores under between-plant competition. *Theoretical Ecology*. 2012; 6: 87–98.

[34] Bono LM, Gensel CL, Pfennig DW, Burch CL. Evolutionary Rescue and the Coexistence of Generalist and Specialist Competitors: An Experimental Test. *Proceedings of the Royal Society B: Biological Sciences*. 2015; 282(1821).

[35] Bono LM, Gensel CL, Pfennig DW, Burch CL. Competition and the Origins of Novelty: Experimental Evolution of Niche-Width Expansion in a Virus. *Biology Letters.* 2013; 9(1): 20120616.

[36] Kassen, R. The Experimental Evolution of Specialists, Generalists, and the Maintenance of Diversity: Experimental Evolution in Variable Environments. *Journal of Evolutionary Biology.* 2002; 15(2): 173–90.

[37] Pels B, Sabelis MW. Local Dynamics, Overexploitation and Predator Dispersal in an Acarine Predator-Prey System. *Oikos*. 1999; 86(3) 573–83.

[38] Murdoch WW. Switching in General Predators: Experiments on Predator Specificity and Stability of Prey Populations. *Ecological Monographs*. 1969; 39(4): 335–54.

[39] Zimmerman AE, Howard-Varona C, Needham DM, John SG, Worden AZ, Sullivan MB, Waldbauer JR, Coleman ML. Metabolic and Biogeochemical Consequences of Viral Infection in Aquatic Ecosystems. *Nature Reviews Microbiology*. 2020; 18(1): 21–34.

[40] Voigt E, Rall BC, Chatzinotas A, Brose U, Rosenbaum B. Phage Strategies Facilitate Bacterial Coexistence under Environmental Variability. *PeerJ*. 2021; 9: e12194.

[41] Netter Z, Dunham DT, Seed K. Adaptation to Bile and Anaerobicity Limits Vibrio Cholerae Phage Adsorption. *bioRxiv.* 2023.<https://doi.org/10.1101/2023.04.22.537938>.

[42] Harcombe W. Novel Cooperation Experimentally Evolved Between Species. *Evolution*. 2010; 64(7): 2166–72.

[43] Longdon B, Brockhurst MA, Russell CA, Welch JJ, Jiggins FM. The Evolution and Genetics of Virus Host Shifts. *PLOS Pathogens*. 2014; 10(11): e1004395.

[44] Hoek TA, Axelrod K, Biancalani T, Yurtsev EA, Liu J, Gore J. Resource Availability Modulates the Cooperative and Competitive Nature of a Microbial Cross-Feeding Mutualism. *PLOS Biology.* 2016; 14(8): e1002540.

[45] Renardy M, Joslyn LR, Millar JA, Kirschner DE. To Sobol or Not to Sobol? The Effects of Sampling Schemes in Systems Biology Applications. *Mathematical Biosciences*. 2021; 337:108593.

[46] Qian G, Mahdi A. Sensitivity Analysis Methods in the Biomedical Sciences. *Mathematical Biosciences*. 2020; 323:108306.

[47] Weber F, Theers S, Surmann D, Ligges U, Weihs C. Sensitivity Analysis of Ordinary Diﬀerential Equation Models: Methods by Morris and Sobol’ and Application in R. (2018)

[48] Liu D, Li L, Rostami-Hodjegan A, Bois FY, Jamei M. Considerations and Caveats When Applying Global Sensitivity Analysis Methods to Physiologically Based Pharmacokinetic Models. *The AAPS Journal*. 2020; 22(5): 93.

[49] Otto SP, Day T. *A Biologist’s Guide to Mathematical Modeling in Ecology and Evolution*. (2007)

[50] Smith JM, Price GR. The Logic of Animal Conflict. *Nature*. 1976; 246(5427): 15–18.

[51] Douglas SM, Chubiz LM, Harcombe WR, Marx CJ. Identification of the Potentiating Mutations and Synergistic Epistasis That Enabled the Evolution of Inter-Species Cooperation. *PLOS ONE.* 2017; 12(5): e0174345.

[52] Harcombe WR, Riehl WJ, Dukovski, I Granger DR, Betts A, Lang AH, Bonilla G, Amrita K, Leiby N, Mehta P, Marx CJ, Segre D. Metabolic Resource Allocation in Individual Microbes Determines Ecosystem Interactions and Spatial Dynamics. *Cell Reports.* 2014; 7(4): 1104–15.

[53] Switt AIM, Sulakvelidze A, Wiedmann M, Kropinski AM, Wishart DS, Poppe C, Liang Y. Salmonella Phages and Prophages: Genomics, Taxonomy, and Applied Aspects. In *Salmonella: Methods and Protocols*, edited by Heide Schatten and Abraham Eisenstark, 237–87. Methods in Molecular Biology. New York, NY: Springer, 2015.

[54] Adamowicz EM, Flynn J, Hunter RC, Harcombe WR. Cross-Feeding Modulates Antibiotic Tolerance in Bacterial Communities. *The ISME Journal.* 2018; 12(11): 2723–35.

[55] Kutter E. Phage Host Range and Efficiency of Plating. In *Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions*, edited by Martha R.J. Clokie and Andrew M. Kropinski, 141–49. Methods in Molecular BiologyTM. Totowa, NJ: Humana Press, 2009.

[56] Adams MH. 1959. Bacteriophages. Interscience, New York.

[57] Abrams PA, Matsuda H. Positive Indirect Effects Between Prey Species That Share Predators. *Ecology.* 1996; 77(2): 610–16.

[58] Tilman D. Resource Competition and Community Structure. *Monographs in Population Biology*, December 1982.

[59] MacArthur RH. 1972. Geographical ecology. Princeton University Press, Princeton, NJ.

[60] Fazzino LF, Anisman J, Chacón JM, Heineman RH, Harcombe WR. Lytic Bacteriophage Have Diverse Indirect Effects in a Synthetic Cross-Feeding Community. *The ISME Journal*. 2020; 14(1): 123–34.

[61] Duyvejonck H, Merabishvili M, Vaneechoutte M, de Soir S, Wright R, Friman VP, Verbeken G, De Vos D, Pirnay JP, Van Melchelen E, Vermeulen SJT. Evaluation of the Stability of Bacteriophages in Different Solutions Suitable for the Production of Magistral Preparations in Belgium. *Viruses*. 2021;13(5): 865.

[62] Jonczyk E, Kłak M, Miedzybrodzki R, Górski A. The influence of external factors on bacteriophages—Review. *Folia Microbiol.* 2011; 56: 191–200.

[63] Blazanin M, Lam WT, Vasen E, Chan BK, Turner PE. Decay and Damage of Therapeutic Phage OMKO1 by Environmental Stressors. *PLOS ONE*. 2022; 17(2): e0263887.

[64] Anderson TF. The reactions of bacterial viruses with their host cells. *The Botanical Review.* 1949; 15 (7):464–505.

[65] Seaman PF, Day MJ. Isolation and characterization of a bacteriophage with an unusually large genome from the Great Salt Plains National Wildlife Refuge, Oklahoma,USA. *FEMS microbiology ecology. 2007*; 60(1):1–13.Epub 2007/01/26.

[66] Whitman PA, Marshall RT. Characterization of two psychrophilic Pseudomonas bacteriophages isolated from ground beef. *Applied microbiology.* 1971; 22(3):463–8.Epub 1971/09/01.

[67] Molan K, Rahmani R, Krklec D, Brojan M, Stopar D. Phi 6 Bacteriophage Inactivation by Metal Salts, Metal Powders, and Metal Surfaces. *Viruses.* 2022; 14(2): 204.

[68] Yeargin T, Buckley D, Fraser A, Jiang X. The survival and inactivation of enteric viruses on soft surfaces: A systematic review of the literature. *Am. J. Infect. Control.* 2016; 44: 1365–1373.

[69] Lin Q, Lim JYC, Xue K, Yew PYM, Owh C, Chee PL, Loh XJ. Sanitizing agents for virus inactivation and disinfection. *View* 2020; 24: e16.

[70] Richter Ł, Księżarczyk K, Paszkowska K, Janczuk-Richter M, Niedziółka-Jönsson J, Gapiński J, Łoś M, Hołyst R, Paczesny J. Adsorption of Bacteriophages on Polypropylene Labware Affects the Reproducibility of Phage Research. *Scientific Reports*. 2021; 11(1): 7387.

[71] Nakanishi K, Sakiyama T, Imamura K. On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. J*. Biosci. Bioeng.* 2011; 91: 233–244.

[72] Rabe M, Verdes D, Seeger S. Understanding protein adsorption phenomena at solid surfaces. *Adv. Colloid Interface Sci.* 2011; 162: 87–106.

[73] Wilson DS, Yoshimura J. On the coexistence of specialists and generalists. *American Naturalist*. 1994; 144:692–707.

[74] Visher E, Boots M. The Problem of Mediocre Generalists: Population Genetics and Eco-Evolutionary Perspectives on Host Breadth Evolution in Pathogens.”*Proceedings of the Royal Society B: Biological Sciences.* 2020; 287(1933): 20201230.

[75] Ferris MT, Joyce P, Burch CL. High frequency of mutations that expand the host range of an RNA virus. *Genetics.* 2007; 176: 1013-1022.

[76] Remold SK. Understanding specialism when the jack of all trades can be the master of all. *Proc. R. Soc. B*. 2012; 279: 4861-4869

[77] Satterwhite RS, Cooper TF. Constraints on adaptation of *Escherichia coli* to mixed-resource environments increase over time. *Evolution*. 2015; 69: 2067-2078.

[78] Kawecki TJ. Accumulation of deleterious mutations and the evolutionary cost of being a generalist. *Am. Nat.* 1994; 144: 833-838.

[79] Whitlock MC. The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *Am. Nat.* 1996; 148, S65-S77.

[80] Aufrere R, Tempete M, Bohin JP. Regulation of Expression of the Gene for Vitamin B12 Receptor Cloned on a Multicopy Plasmid in Escherichia Cob". *Mol Gen Genet*. 1986; 205:358-365.

[81] Kadner RJ, McElhaney G. Outer membrane-dependent transport systems in Escherichia coli: effect of repression or cessation of colicin receptor synthesis on colicin receptor activities. *J Bacteriol*. 1980; 143:135-141.

[82] Bradbeer C, Woodrow ML, Khalifah LI. Transport of vitamin B12 in Escherichia coli: common receptor system for vitamin B12 and bacteriophage BF23 on the outer membrane of the cell envelope. *J Bacteriol.* 1976; 125:1032-1039.

[83] Lundrigan, MD, Köster W, Kadner RJ. Transcribed Sequences of the Escherichia coli BtuB Gene Control Its Expression and Regulation by Vitamin B12. *Proceedings of the National Academy of Sciences* 1991; 88(4): 1479–83.

[84] Ravnum S, Andersson DI. Vitamin B12 Repression of the BtuB Gene in Salmonella Typhimurium Is Mediated via a Translational Control Which Requires Leader and Coding Sequences. *Molecular Microbiology*. 1997; 23(1): 35–42.

[85] Knight TM, Chase JM, Hillebrand H, Holt RD. Predation on Mutualists Can Reduce the Strength of Trophic Cascades. *Ecology Letters* 2006; 9(11): 1173–78.

[86] Holroyd CD, Bradbeer C. Cobalamin transport in Escherichia coli. In: Leive L, Schlessinger D (eds) *Microbiology* 1984. American Society for Microbiology, Washington, DC. p21.

[87] Wang J, Jiang Y, Vincent M, Sun Y, Yu H, Wang J, Bao Q, Kong H, Hu S. Complete genome sequence of bacteriophage T5. *Virology.* 2005;332:45–65.

[88] Niu YD, Stanford K, Kropinski AM, Ackermann HW, Johnson RP, She YM, Ahmed R, Villegas A, McAllister TA. Genomic, proteomic and physiological characterization of a T5-like bacteriophage for control of Shiga toxin-producing Escherichia coli O157:H7. *PLoS One*. 2012; 7:e34585.

[89] Hong J, Kim KP, Heu S, Lee SJ, Adhya S, Ryu S. Identification of host receptor and receptor-binding module of a newly sequenced T5-like phage EPS7. *FEMS Microbiol Lett.* 2008; 289:202–209.

[90] Venza Colon CJ, Vasquez Leon AY, Villafane RJ. Initial interaction of the P22 phage with the Salmonella typhimurium surface. P R Health Sci J 23:95–101

[91] Steinbacher S, Miller S, Baxa U, Weintraub A, Seckler R. Interaction of Salmonella phage P22 with its O-antigen receptor studied by X-ray crystallography. *Biol Chem*. 1997; 378:337–34.

[92] Abrams PA. The Prerequisites for and Likelihood of Generalist‐Specialist Coexistence. *The American Naturalist*. 2006; 167(3): 329–42.

[93] Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a Modulator of Gut Microbial Ecology. *Cell Metabolism.* 2014; 20(5)769–78.

[94] Kawecki TJ. Red Queen meets Santa Rosalia: Arms races and the evolution of host specialization in organisms with parasitic lifestyles. *American Naturalist*. 1998; 152: 635–651.

[95] Agrawal AA. Host-range evolution: adaptation and tradeoffs in fitness of mites on alternative hosts. *Ecology.* 2000; 81:500–508.

[96] Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. Construction of Escherichia Coli K-12 in-Frame, Single-Gene Knockout Mutants: The Keio Collection. *Molecular Systems Biology*. 2006; 2: 2006.0008.

[97] Fry JD. The evolution of host specialization: are trade-offs overrated? *American Naturalist.* 1996; 148(suppl.):S84–S107.

[98] Buckling A, Brockhurst MA, Travisano M, Rainey PB. Experimental Adaptation to High and Low Quality Environments under Different Scales of Temporal Variation. *Journal of Evolutionary Biology*. 2007; 8(1): 296–300.

[99] Delaney NF, Kaczmarek ME, Ward LM, Swanson PK, Lee MC, Marx CJ. Development of an Optimized Medium, Strain and High-Throughput Culturing Methods for Methylobacterium Extorquens. *PLOS ONE.* 2013: 8(4): e62957.

[100] Benmayor R, Hodgson DJ, Perron GG, Buckling A. Host Mixing and Disease Emergence.”*Current Biology.* 2009; 19(9):764–67.

**Figure 1. Diet breadth in a microbial synthetic community. A:** Schematic of diet breadth. Species with generalist diets have wide diet breadths spanning multiple resources, while specialists have narrower diet breadths, sometimes specific to a single resource. **B**: Expected community dynamics when mutualistic or competing prey species are challenged by a specialist predator. When prey are mutualistic, predation will reduce abundances of both species. When prey compete, predation will reduce the abundance of one species and result in an increase in the abundance of the other species through competitive release. **C:** Schematic of cross-feeding system consisting of an *E. coli* methionine auxotroph and *S. enterica* methionine secreter. In lactose minimal media, *E. coli* provides carbon byproducts to *S. enterica* and *S. enterica* provides methionine to *E. coli*. In glucose minimal media with methionine, the two bacteria compete. The phage P22*vir* is a specialist on *S. enterica,* while the phage EH7 is a generalist that can attack both bacterial species. **For A, B, and C:** *Figure created with BioRender.*

**Figure 2. Numerically-simulated bacterial dynamics demonstrate that competing prey provide a different selective environment for phage than mutualistic prey.** **A:** In the absence of phage, both prey species reach an equilibrium ratio of 50:50. In the presence of only a specialist phage, the prey attacked by the specialist (pink line, *S. enterica*) decreases. The prey that is not attacked (black line, *E. coli*) reaches a higher equilibrium frequency if the prey are competing and a lower equilibrium frequency if the prey are mutualists. When only the generalist predator is present, regardless of prey interactions, prey remain at a 50:50 ratio throughout the simulated growth period. **B:** When prey compete and both phage types are present, the generalist (yellow line, EH7) dominates over the specialist (blue line, P22*vir*). The fraction of the bacterial population consisting of *S. enterica* decreases significantly, while *E. coli* proliferates (inset plot). **C:** When prey are mutualistic and both phage types are present, the specialist (blue line, P22*vir*) dominates over the generalist (yellow line, EH7). The fraction of the bacterial population consisting of *S. enterica* decreases substantially less than in the case of competing prey, though the overall bacterial population is suppressed relative to the no-phage condition (inset plot). **For B and C:** System dynamics represent parameter space in which the specialist’s (blue line, P22*vir*) burst size is five times that of the generalist (yellow line, EH7).

**Figure 3. Numerically-simulated phage dynamics given a variety of parameter trade-offs demonstrate that prey interactions result in different patterns of predator abundance. A:** The final density of each phage type as a function of bacterial interactions and increasing cost of generalism modeled as increasing specialist burst size. When prey are mutualistic, a cost of generalism exists that favors specialists (blue line, P22*vir*) over generalists (yellow line, EH7). When prey compete, no such cost exists; this is true even as the specialist’s burst size increases well beyond the values displayed here. **B:** The relative abundance of the specialist phage on competing prey as a function of increasing cost of generalism and relative growth advantage of the alternative prey *E. coli*. Whether prey growth advantage is modeled through growth rate or competitive coefficients (beta), the generalist is favored (yellow, EH7) except in a small subset of cases where the alternative prey is competitively excluded. **C:** The relative abundance of the specialist phage on mutualistic prey as a function of increasing cost of generalism and relative growth advantage of the alternative prey *E. coli*. Whether prey growth advantage is modeled through growth rate or mutualistic benefit (alpha), a cost of generalism exists above which specialism is favored (blue, P22*vir*). Note that there are benefit and growth rate values for *E. coli* below which the mutualistic system cannot be supported, indicated by the grey bar.

**Figure 4. Phage and bacterial dynamics *in vitro* align with modeling expectations. A:** Change in individual phage titer on bacterial monocultures and co-cultures over a 48 hour time period. P22*vir* replicates effectively on *S. enterica* but not *E. coli*, while EH7 grows on both bacterial strains. EH7 reaches approximately equivalent densities on both interaction types (p = 0.999), as does P22*vir* (p = 0.909)*,* although its final density is reduced compared to EH7 (competition: p < 0.0001, mutualism: p < 0.0001). **B:** Change in phage titer when phage compete on different bacterial interaction types over a 48 hour time period. When competing against the specialist, EH7 dominates when prey compete (p < 0.0001), doubling more times than in isolation (p < 0.0001). P22*vir* dominates when prey are mutualistic (p < 0.0001), while EH7 disappears below the limit of detection, as indicated by the yellow asterisk. **For A and B:** The dotted red line indicates no change in titer from the start of the experiment to the end. Values greater than zero indicate an increase in titer, while values below zero indicate a decrease in titer. Statistical significance was determined using a two-way ANOVA with Tukey’s HSD multiple comparison test. **C:** Species-specific ODs over time across treatment conditions (top) and final fraction of bacterial co-culture composed of each strain across treatment conditions (bottom) when bacteria compete. The fraction of *E. coli* increases in those cases in which *S. enterica* is predominantly suppressed by phage (specialist only and both phage treatments). *E. coli* frequency is reduced relative to no-phage controls when only EH7 is applied (p = 0.0035). **D:** Species-specific ODs over time across treatment conditions (top) and final fraction of bacterial population composed of each strain across treatment conditions (bottom) when bacteria are mutualistic. *E. coli* dominates at similar levels in all treatment conditions, even in cases where overall growth is suppressed. **For C and D:** Statistical significance for bar graphs was determined using a two-way ANOVA with Tukey’s HSD multiple comparison test. OD600 traces represent 4 biological replicates for conditions with phage and 3 biological replicates for conditions without phage.

**Figure 5. Imposing a cost of generalism *in vitro* as phage intrinsic mortality recapitulates modeling results on *S. enterica* monoculture.** **A:** Change in phage titer across interaction types and phage treatments when cells are starved or not present in cultures. P22*vir* does not degrade over the 48 hour growth period, even in cases when there are no cells present that it can productively infect. Additionally, it can reproduce even on starved *S. enterica*. EH7 degrades below the limit of detection in all conditions, as indicated by the yellow asterisk, but does not disappear completely when *S. enterica* is present, suggesting *S. enterica* may be somewhat more physiologically accessible to it than *E. coli* in a starved state. **B:** Change in phage titer across interaction types when cells are either added to minimal media at the same time as phage (no cost) or 24 hours later (with cost). Results are shown for phage competition assays, when both phage types are present in wells at the start of the experiment. In the no cost condition, EH7 reaches comparable titer to P22*vir* on *S. enterica* monoculture (p = 0.999) and wins when prey compete (p < 0.0001). In the condition where cells are added after a period of phage incubation, EH7 degrades below the limit of detection on *S. enterica* monoculture but dominates when prey are competing (p < 0.0001). **For A and B:** The dotted red line indicates no change in titer from the start of the experiment to the end. Values greater than zero indicate an increase in titer, while values below zero indicate a decrease in titer. Statistical significance was determined using a two-way ANOVA with Tukey’s HSD multiple comparison test.

**Figure 6. When a cost of generalism is modeled as intrinsic mortality, qualitative patterns of ecological selection on predator specificity match findings when cost of generalism is modeled as burst size (Figure 3) or attachment rate (Supplemental Figure 1).** As the intrinsic mortality rate of the generalist phage increases, it maintains its advantage longer when prey compete and is driven extinct at an intermediate cost when prey are mutualistic. These qualitative results align with previous modeling findings when cost of generalism is imposed as burst size or attachment rate.