

Supplementary Analysis

Degradation of the generalist phage in minimal media cannot be clearly attributed to any particular media component

We investigated the cause of phage degradation in our minimal media by incubating 10^3 particles of EH7, P22*vir*, or a combination of both phage per well at 37°C with shaking at 432 rotations per minute in glucose minimal media for 48 hours. We found that EH7 degraded below the limit of detection within 24 hours whether incubated alone or with P22*vir*, half the time period of our standard phage competition assays (Supplemental Figure 2A). However, phage recovery was possible following the addition of cells in some cases (Figure 5B), suggesting that infectious phage particles remained. P22*vir* titer was unchanged over the course of 48 hours whether incubated alone or with EH7 (Supplemental Figure 2A, $p = 1.0$).

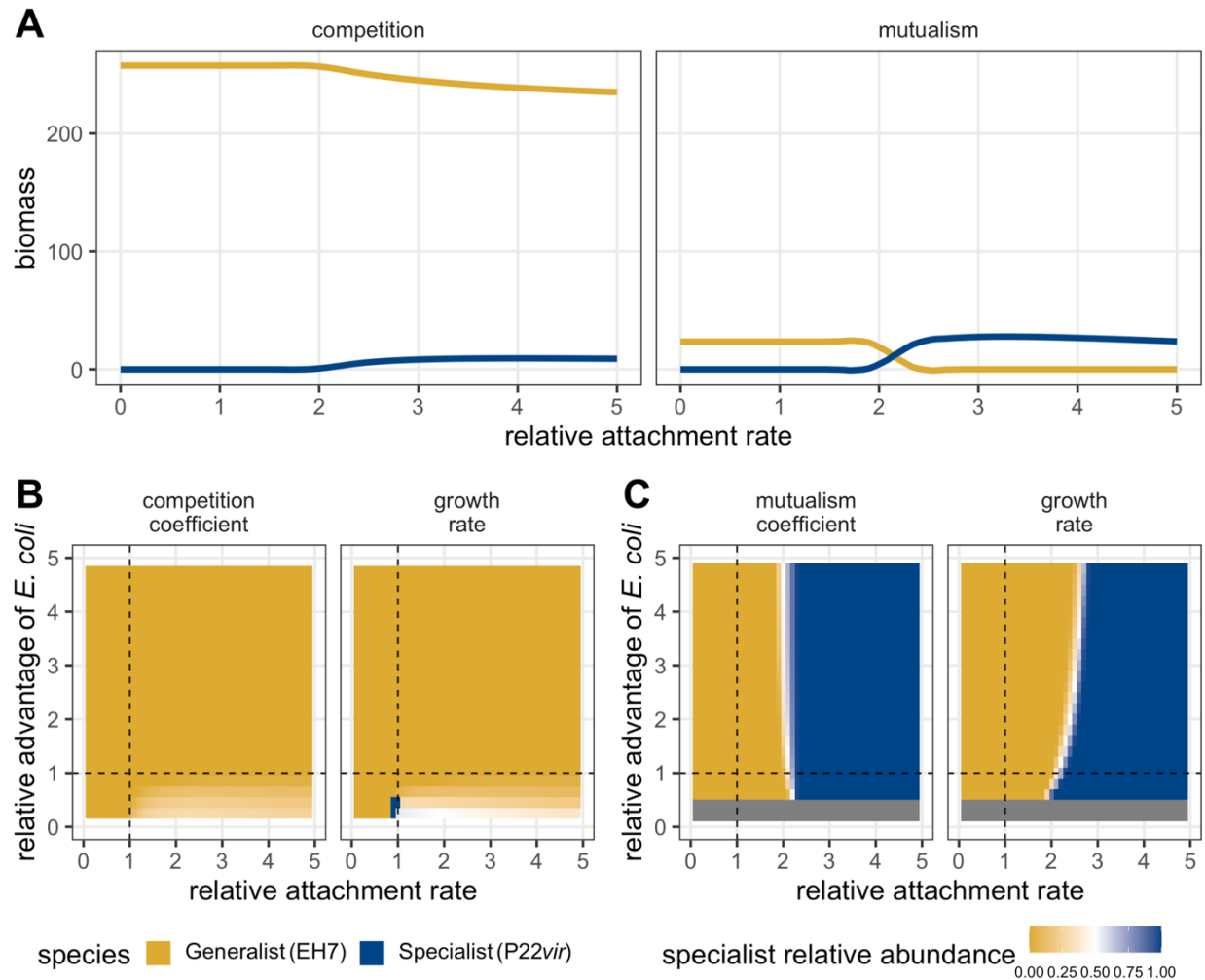
Given previous observations that environmental factors such as salinity [63, 64, 65, 66], pH [61, 62, 63], and metal presence can have significant impacts on phage titer [67, 68, 69], we attempted to identify the cause of degradation in our minimal media by removing one component at a time. We created minimal media without metals, without sulfur, without phosphorus, or some combination thereof. We incubated only 10^3 EH7 particles per well at 37°C with shaking at 432 rotations per minute in each minimal media type for 48 hours. However, phage degraded below the limit of detection regardless of which media component was absent, suggesting that metals and osmolarity were not driving the reduction in titer (Supplemental Figure 2C).

Additionally, because phage titer can be reduced as the result of adsorption of viral particles to plastic surfaces, we tested our phage by incubating it in LB [70, 71, 72]. We determined that adsorption of phage particles to the plastic was likely not driving the decrease in phage titer, given that EH7 density was unchanged following 48 hours of incubation in LB in the same 96-well plate where degradation in minimal media was observed (Supplemental Figure 2B, $p =$

0.00035). This was true despite the low starting phage density, which has been shown to increase the likelihood of rapid phage degradation due to adsorption to plastic [61, 70].

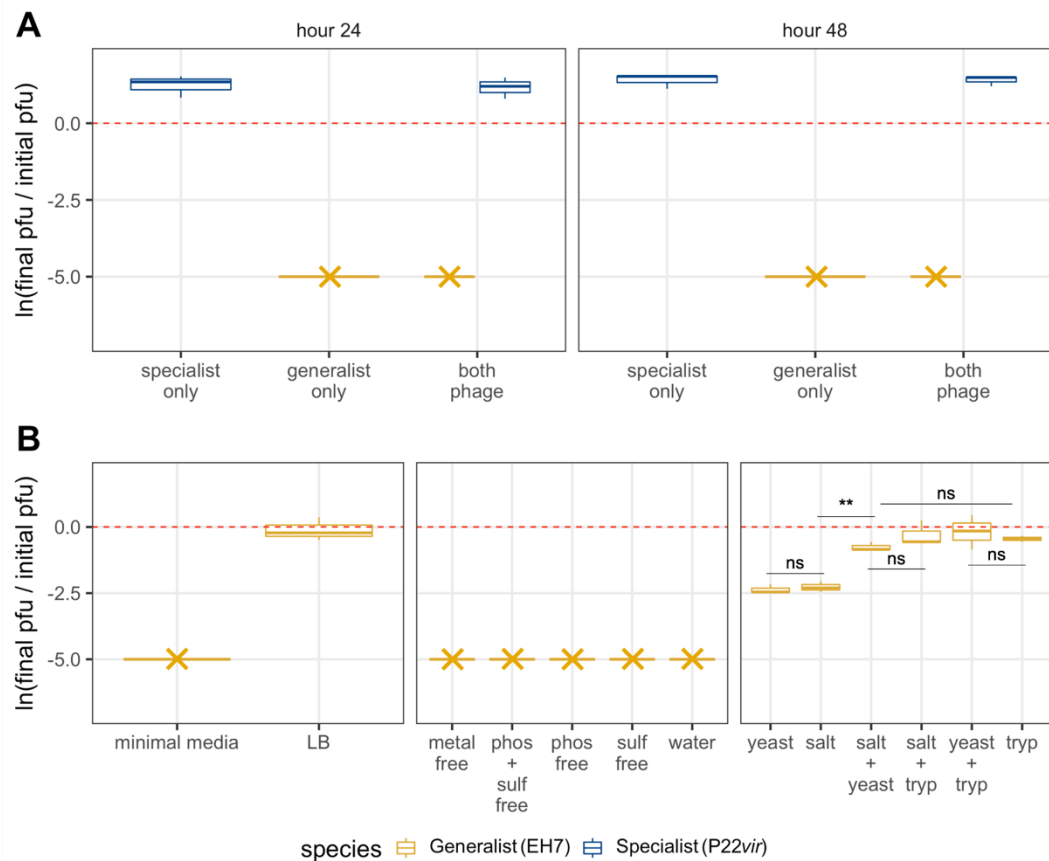
Finally, we investigated which component of LB was responsible for the preservation of phage titer by removing one component at a time. We repeated our previous experiments by incubating only 10^3 EH7 particles per well at 37°C with shaking at 432 rotations per minute in each LB type for 48 hours. The titer of phage incubated in solutions containing tryptone were unchanged over the window of the 48 hour experiment (Supplementary Figure 2D). When incubated in a solution of yeast and salt, the density of EH7 particles was also not significantly changed (Supplementary Figure 2D). However, titer did decrease significantly relative to other conditions when phage were incubated in only yeast or only salt (Supplementary Figure 2D, yeast: $p < 0.001$ for all multiple comparisons, salt: $p < 0.001$ for all multiple comparisons). These results suggest that tryptone may play a role in the stability of EH7 titer in LB.

Supplemental Figures



Supplemental Figure 1. 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 attachment rate. When prey are mutualistic, a cost of generalism exists that favors specialists (blue line) over generalists (yellow line). When prey compete, no such cost exists; this is true even as the specialist's attachment rate 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) 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). Note that there are benefit

and growth rate values for *E. coli* below which the system cannot be supported, indicated by the grey bar.



Supplemental Figure 2. Generalist phage degradation differs in minimal media versus LB.

A: EH7 is undetectable after 24 and 48 hours, as indicated by the yellow asterisks, when incubated in minimal media without cells. However, because replication is still possible when cells are added at 24 hours (see Figure 5B, where EH7 titer increases on competitive co-culture even when the addition of cells is delayed), these results suggest that phage are below the limit of detection but some infectious particles remain. Statistical significance was determined using a one-way ANOVA with Tukey's HSD multiple comparison test. **B:** Change in EH7 titer across media conditions. In LB without cells, EH7 densities are unchanged over a 48 hour period, while the phage disappears below the limit of detection in minimal media ($p = 0.00035$), as indicated by the yellow asterisk. Statistical significance for the first facet was determined using a two-tailed t-test. EH7 degradation is not impacted when different components of the minimal media are removed - it is always undetectable after 48 hours, as indicated by the yellow asterisks. Regardless of what component is removed, the phage is undetectable after 48 hours. Change in EH7 titer does differ when different components of LB are removed. Phage is always detectable when at least one component of LB is present, while solutions containing tryptone or both yeast and salt best preserve phage titer over 48 hours of incubation (Supplemental Analysis 2). Statistical significance for the third facet was determined using a one-way ANOVA with Tukey's HSD multiple comparison test. **Note:** in part B, facet panels indicate experiments were completed on different days. **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.

Supplemental Tables

Strain ancestor	Antibiotic marker	Flourescent marker	Source	Relevant phenotype
Salmonella enterica LT2 metA* metJ*	kanR	YFP	Harcombe 2010	Methionine hypersecreter
E. coli K-12 BW25113 ΔmetB	kanR	CFP	Harcombe 2010	Methionine auxotroph
E. coli K-12 BW25113 ΔtrxA	kanR	NA	Baba et al. 2006	Sensitive to EH7, resistant to P22vir
S. enterica serovar Typhimurium NCTC 74 ΔbtuB	NA	NA	S. Bowden	Sensitive to P22vir, resistant to EH7
P22vir	NA	NA	I. J. Molineaux	S. enterica-specific phage
EH7	NA	NA	E. Hansen	Generalist phage

Supplemental Table 1. Phage and bacterial strains. Strains used for experiments. See materials and methods for additional details.

Type	Component	metric	S monoculture	E monoculture	ES mutualism	ES competition
C source	lactose	mM	0	0	2.9	0
	glucose	mM	5.6	5.6	0	5.6
auxotroph amendments	methionine	mM	0	0.5	0	0.5
SO ₄ source	(NH ₄) ₂ SO ₄	mM	3.7	3.7	3.7	3.7
	MgSO ₄	mM	0.814	0.814	0.814	0.814
P source	K ₂ HPO ₄	mM	14.5	14.5	14.5	14.5
	NaH ₂ PO ₄	mM	16.3	16.3	16.3	16.3
Metals	ZnSO ₄	μM	1.2	1.2	1.2	1.2
	MnCl ₂	μM	1	1	1	1
	FeSO ₄	μM	18	18	18	18
	(NH ₄) ₆ Mo ₇ O ₂₄	μM	2	2	2	2
	CuSO ₄	μM	1	1	1	1
	CoCl ₂	μM	2	2	2	2
	Na ₂ WO ₄	μM	0.33	0.33	0.33	0.33
	CaCl ₂	μM	20	20	20	20

Supplemental Table 2. Hypho minimal media composition. Media composition for mutualistic and competitive communities, as well as bacterial monocultures. Note that phage degradation assays on starved cells were completed in ES mutualism media.

Parameter	Minimum	Maximum
$\alpha_{j,i}$	0.1	2.5
$\beta_{j,i}$	0.1	2.5
μ_i	0.1	2.5
$\gamma_{i,x}$	15	65
$\zeta_{i,x}$	0.0009	0.01
δ_i	0.0009	0.1
κ_i	0.1	10
R	0	5

Supplemental Table 3. Sobol' sensitivity analysis and Morris screening parameter ranges. Minimum and maximum uniform distribution values used for each ODE parameter in Morris screening and Sobol' sensitivity analyses.

Predicted mutations					
position	mutation	freq	annotation	gene	description
30,899	G→T	100%	intergenic (-240/+114)	24 ← / ← c2	unknown/prophage repressor
31,700	C→G	100%	intergenic (-37/-44)	c2 ← / → cro	prophage repressor/repressor
31,716	A→G	100%	intergenic (-53/-28)	c2 ← / → cro	prophage repressor/repressor

Supplemental Table 6. Breseq predictions of point mutations responsible for repressing lysogeny in P22_{vir}. Breseq predictions of point mutations in P22_{vir} strain used for all experiments relative to the ancestral, lysogenic version of P22 (GenBank accession NC_002371.2). Sequencing of the lab phage strain was completed by the Microbial Genome Sequencing Center (<https://www.seqcenter.com/>).