**Distribution Agreement**

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Andrew Worthy April 14, 2020

# Spiteful Weapons and the Environmental Dependence of Phage

by

Andrew Worthy

Nic Vega
Adviser

Department of Biology

Nic Vega

Adviser

Kathleen Campbell

Committee Member

Matthew Weinschenk

Committee Member

2020

# Spiteful Weapons and the Environmental Dependence of Phage

By

Andrew Worthy

Nic Vega

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Department of Biology

2020

Abstract

Spiteful Weapons and the Environmental Dependence of Phage
By Andrew Worthy

The assembly of bacteria-phage communities occurs in an environmental context. To delineate how temperate phage-bacteria dynamics are affected by their environment, phage infection parameters were estimated in varying nutrient and temperature conditions. Models of lysogen versus phage-sensitive bacterial competitions in these same conditions were then formed from the parameter estimates and were used to predict competition outcomes. The empirical models accurately described qualitative competition outcomes and predicted a phage-resistant subpopulation in one of the putatively phage-sensitive strains. Moreover, this study shows environmentally dependent phage-bacteria dynamics can direct competition outcomes when given enough time before the bacterial populations reach stationary phase.

# Spiteful Weapons and the Environmental Dependence of Phage

By

Andrew Worthy

Nic Vega

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Department of Biology

2020

Acknowledgements

I am deeply grateful for the guidance and support of my research advisor Dr. Nic Vega throughout this research project and my time at Emory. I offer my sincere appreciation to my honors committee for their encouragement and helpful advice: Dr. Nic Vega, committee chair; Dr. Kathleen Campbell; and Dr. Matthew Weinschenk. I am also appreciative for the help of my colleagues in the Vega Lab.

Finally, I wish to express my gratitude to my parents and best friend Mitchell for providing me with unfailing support throughout my years of undergraduate study. My achievements would not have been possible without them. Thank you all.

Table of Contents

# Spiteful Weapons and the Environmental Dependence of Phage

## Introduction

The assembly of microbial communities does not happen in a vacuum—the environmental context of the interactions between competing bacterial lineages influences competition outcomes. Environmental parameters, such as nutrient availability and temperature, alter the metabolism and growth dynamics of competing bacteria, potentially favoring the prevalence of one bacterial lineage over another.

Other factors, such as the presence of temperate bacteriophages (phages), can also alter bacterial competition. Phages, the viruses that infect bacteria, are classified as either lytic or lysogenic (temperate) depending on the lifecycle they follow. In the lytic lifecycle, a phage adsorbs to its bacterial host's membrane, injects its genetic payload (DNA or RNA) into the host, and uses the host's infrastructure to manufacture new phage particles before lysing the host so that the phage's progeny can diffuse into the environment to infect new hosts [1]. Lysogenic (or temperate) phages follow the same lytic process, but sometimes the virus's genetic material integrates into the host's chromosome [2]. The embedded prophage waits in this latent state, replicating with the host chromosome during cell division, until environmental conditions are appropriate for the phage to enter the lytic cycle and kill its host, thus continuing the reproductive process [1].

The presence of temperate phages in bacterial communities can generate spiteful weapon dynamics in conspecific bacterial competition [3]. Lysogenized lineages "spitefully" sacrifice their own fitness when lysed, releasing new viral particles that can kill phage-sensitive cells. Rapid formation of new lysogens from formerly susceptible lineages, however, renders those cells resistant to further infection by the temperate phage. Consequently, the killing by phage is rapidly reduced and the fitness benefit to lysogens rendered null. The efficacy of a temperate phage as a

spiteful weapon is therefore expected to depend on how quickly the phage can replicate and kill sensitive cells, and how quickly the sensitive cell population can evolve resistance of any form—the quickest method being lysogenization.

Phages are essentially bacterial parasites. Therefore, all phage infection and production parameters are expected to be intimately tied to bacterial metabolism and thus the environment in which bacteria live. The rate of lysogen formation is related to bacterial growth rate and multiplicity of infection (MOI) [4]—the number of phage virions infecting a single cell. Local phage density dictates MOI and is predominantly determined by the phage's production parameters (burst size and lytic induction rate). Phage production is tied to bacterial growth rate and metabolism, so it is therefore expected to change in response to altered environmental parameters. These interconnecting parameters are all linked in some way to the environment in which phage-bacteria interactions are occurring. Delineating how these parameters interrelate is necessary to understand phage-bacteria interactions.

The environmental contexts under which spiteful weapon dynamics are expected to either lead to exclusion of sensitive bacteria, allow the spread of phages to new host lineages, or fail to affect the dynamics entirely are not fully understood. Here, I will seek to disentangle these opposing forces to better predict outcomes of spatially structured bacterial competitions in the presence of phages. Furthermore, I seek to identify the environmental "knobs" that can be turned to shift phage-bacteria dynamics and drive bacterial competition to predicted outcomes.

## Methods

*Bacteria, phage and growth media*

The *E. coli* strains used in our experiments were derivatives of the parent strain K-12 MG1655 or K-12 DH5α (Table 1). Bacterial cultures were grown at 25ºC or 37˚C in either LB broth (LB) *[1M $MgSO_4$ • $5H_2O$ (Fisher, AC124900010) 10mL/L, tryptone (Fisher, BP1421-500) 10g/L, yeast extract (Fisher, BP1422-500) 5g/L, NaCl (Fisher, BP358-1) 10g/L]* or S-medium + glucose (SM+GLC) *[S-Basal 1L, 1M potassium citrate buffer pH 6 10mL/L, trace metals solution 10mL/L, 1M $CaCl_2$ • $2H_2O$ (Fisher, AC423525000) 3mL/L, 1M $MgSO_4$ • $5H_2O$ 10mL/L (Fisher AC124900010), 20% w/v glucose (Fisher AC410950010) 5 mL/L, 20% w/v $NH_4Cl$ (Fisher AC199975000) 5 mL/L, 5% w/v thiamine (Fisher BP892100) 2mL/L, 20% w/v casamino acids (Fisher BP1424100) 10mL/L]*. The S-medium was supplemented with thiamine and casamino acids to compensate for the thiamine and arginine auxotrophy of DH5α. Plating was performed on LB agar for bacterial CFU/mL counts or phage agar for PFU/mL titers *[tryptone (Fisher, BP1421-500) 10g/L, yeast extract (Fisher, BP1422-500) 5g/L, NaCl (Fisher, BP358-1) 10g/L, glucose 1g/L, agar (Fisher 443570050) 10g/L, 1M $CaCl_2$ 2mL/L]*. Potassium citrate *(Fisher AC611755000)* was added to media and plates as needed to inhibit λ phage activity.

Phage lysates were prepared from single phage plaques inoculated into mid-log phase cultures of wild type *E. coli* MG1655 grown at 37˚C in LB medium. After incubation, chloroform was added to each lysate before centrifuging to remove any remaining bacterial debris. The $\lambda_{vir}$ strain used in these experiments was obtained from Sylvain Moineau. The procedure for construction of the temperate phage $\lambda_{KmR}$ is described by Pleška et al. [5].

**Table 1: Bacterial strains and phage used in this study.**

| # | Bacterial Strain | Properties | Reference |
|---|---|---|---|
| 1 | *E. coli* MG1655 (K-12) | Wild Type | Ole Skovgaard at Roskilde University in Denmark |
| 2 | *E. coli* DH5α | $F^-$ φ80*lac*Z ΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 ($r_K^-$, $m_K^+$) *pho*A *sup*E44 $\lambda^-$ *thi*-1 *gyr*A96 *rel*A1 | Bruce Levin Lab |
| 3 | MG1655-$\lambda_{KmR}$ | Lysogen of $\lambda_{KmR}$ | Bruce Levin Lab |
| 4 | MG1655-GmR-RFP | Chromosomal insertion of miniTn7 GmR-RFP plasmid | |
| 5 | DH5α-$\lambda_{KmR}$ | Lysogen of $\lambda_{KmR}$ | Bruce Levin Lab |
| 6 | DH5α-GmR-GFP | Chromosomal insertion of miniTn7 GmR-GFP plasmid | |

| # | Phage | Properties | Reference |
|---|---|---|---|
| 1 | $\lambda_{KmR}$ | Wild type, temperate λ with Kanamycin antibiotic resistance cassette | [5] |
| 2 | $\lambda_{vir}$ | Virulent mutant of λ | Sylvain Moineau Lab |

*Phage Parameter Assays*

*Latent period, adsorption rate, and burst size.* Culture tubes containing 1mL LB or SM+GLC were inoculated 1:1000 with *E. coli* MG1655 overnight culture and grown for 4 hours at 37ºC or for 16 hours at 25ºC with 300rpm orbital shaking to reach mid-log phase of growth. Once the cultures reached mid-log phase, enough $\lambda_{vir}$ (cI protein mutant that is unable to form lysogens) phage lysate was added to reach a low MOI (0.01–0.1). At 10-minute intervals for LB cultures and 20-minute intervals for SM+GLC cultures, 20µL was sampled.

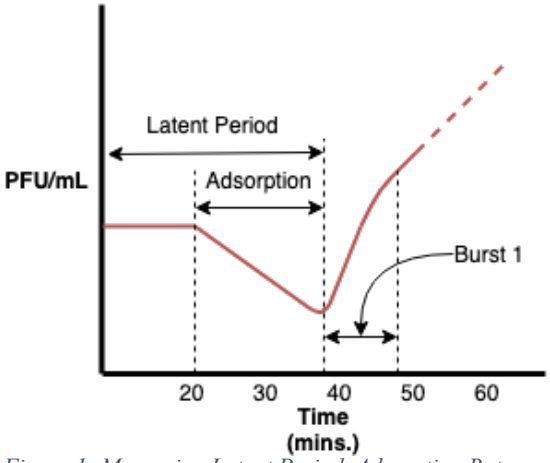


*Figure 1: Measuring Latent Period, Adsorption Rate, and Burst Size.*
Adsorption rate estimated from the rate of change that occurs before the first burst event. Burst size is estimated by dividing phage produced during the burst by the number of infecting phages (~number adsorbed).

Samples were serially diluted ten-fold in PBS for selective plating on agar to determine population sizes in culture: 5μL spots were plated onto LB agar + 20mM potassium citrate plates (LB+Cit20) to measure CFU/mL, and 20μL spots were plated onto Phage Agar with MG1655 soft agar lawns to measure PFU/mL. Latent period, adsorption rate, and burst size are derived from PFU/mL versus time (Fig. 1). The latent period is the duration of time from inoculation with phage lysate to the first observed free phage production and bursting. The adsorption rate is estimated at a point along the PFU/ml curve when the free phage concentration drops below the inoculation concentration:

$$\delta = \frac{|PFU_f - PFU_i|}{CFU_f \cdot PFU_f}, \qquad [1]$$

where $PFU_f$ is the free phage concentration at the timepoint *f* where phage concentration is steadily decreasing, $CFU_f$ is the concentration of bacteria present at timepoint *f*, and $PFU_i$ is the PFU/mL at the timepoint that precedes *f*. Burst size was calculated for λ phage by dividing the number of free phage that lysing cells produce by the number of initially infecting phage (the number of phage adsorbed, or roughly the number of free phage removed from culture before the first bursting event):

$$\beta = \frac{PFU_f - PFU_a}{PFU_i - PFU_a}, \qquad [2]$$

where $PFU_f$ is the final free phage concentration after bursting, $PFU_a$ is the decreased phage concentration after adsorption, and $PFU_i$ represents the initial phage concentration before adsorption or bursting.

*Lysogen formation probability.* 96-well plates containing 150μL LB or SM+GLC were inoculated 1:1000 with overnight cultures of MG1655 or DH5α and incubated at 37ºC or 25ºC

with 300rpm orbital shaking. After cells reached early-log phase of growth, enough $\lambda_{KmR}$ phage lysate was added to each well to reach MOIs of 0.1, 1, or 10. Plates were incubated for 1 hour, then 3µL 1M potassium citrate was added to reach 20mM—citrate prevents adsorption of any remaining free-phage to cells. Plates were incubated for one more hour before dilution plating the resulting cultures onto LB+Cit20 plates to get total CFU/mL counts, and onto LB+Cit20 plates containing 25µg/mL kanamycin (Km25) to select for newly lysogenized cells. Lysogenization frequency is calculated by dividing the number of lysogens produced by the total number of cells. This procedure was repeated for cells grown to mid-log phase before inoculation with phage lysate to assay their lysogenization rates as well.

*Lytic induction rate.* Culture tubes containing 2mL LB or SM+GLC were inoculated 1:1000 with an overnight MG1655- $\lambda_{KmR}$ resistant lysogen culture ($\lambda_{KmR}$ lysogens that are envelope resistant—λ phage cannot adsorb to them and therefore accumulate in the medium after lytic induction). Cultures were incubated at 25ºC or 37ºC with 300rpm orbital shaking until cells reached mid-log phase growth. Cultures were dilution plated every 30-60 minutes to measure phage induction: 5µL spots onto LB+Cit20+Km25 for CFU/mL counts, and 10µL spots onto phage agar with 100µg/mL ampicillin (Amp100) that has a soft agar lawn of *E. coli* MC4100-AmpR for PFU/mL counts. The prophage's rate of lytic induction was calculated with the following formula:

$$I = \frac{(PFU_f - PFU_i) \div (CFU_f)}{\beta}, \qquad [3]$$

where $PFU_f$ and $PFU_i$ are free phage concentrations at the final and initial timepoints respectively, $CFU_f$ is the bacterial concentration at the final timepoint, and β is the burst size estimate for the

phage at the experimental conditions matching those of this experiment. The induction rates are reported in units of [$CFU^{-1} \bullet hour^{-1}$].

*Lysogen vs. Sensitive Competition Experiments*

Growth rates and lag times were estimated for all bacterial strains (MG1655-$\lambda_{KmR}$, DH5α-$\lambda_{KmR}$, DH5α-GmR-GFP), and these data were used to predict the outcomes of competition in the absence of a spiteful-weapon advantage. To measure growth kinetics, overnight cultures of each strain were inoculated into translucent 96-well plates containing LB or SM+GLC with appropriate antibiotic selection. Plates were grown in a microplate photometer (BioTek Synergy HTX) for 24 hours at 37ºC and for 48 hours at 25ºC with 300rpm orbital shaking, taking $OD_{600}$ measurements every 10 minutes. Optical Density measurements were plotted against time, yielding growth curves. These curves informed the expected outcomes of bacterial competition: the faster-growing strain being expected to outcompete the slower-growing strain.

Next, an *E. coli* sensitive strain was paired in competition assays with each of the lysogens in liquid culture under varied environmental conditions (37°C vs 25°C, LB vs SM+GLC) to observe the effects of spiteful-weapon dynamics on competition between strains. In 96-well plates containing 150µL medium (LB or SM+GLC), equal amounts of overnight cultures of a lysogen strain, MG1655-$\lambda_{KmR}$ (MKM) or DH5α-$\lambda_{KmR}$ (DKM), and the sensitive strain DH5α-GmR-GFP (DGG) were inoculated $1{:}10^3$ into each well. Plates were incubated at either 37ºC or 25ºC with 300rpm orbital shaking. At 24-hour intervals for 37ºC plates and 48-hour intervals for 25ºC plates, new 96-well plates containing fresh medium were inoculated $1{:}10^3$ with culture from the previous plates. At every propagation, cultures were dilution plated onto LB+Cit20 plates [total CFUs], LB+Cit20+Gm25 (25µg/mL gentamicin) plates [sensitive + new lysogen], LB+Cit20+Km25 [original lysogen + new lysogen], and LB+Cit20+Gm25+Km25 [new lysogen].

These results were compared across conditions to determine whether altering the environmental conditions of these interactions affects the outcomes of phage-mediated competition between strains. Competition results in given environmental conditions were compared to the expected outcomes informed by the corresponding growth curve data. If the competition results defied our expectations, we determined if the environment-imposed phage parameters were sufficient to explain the inconsistency with expectations by comparing the competition data with mathematical models of phage-bacteria interactions at the given environmental parameters.

**Results**

*Phage Parameter Assays*

*Latent period.* The latent period for λ phage infection was estimated by calculating the interval of time (in minutes) from phage inoculation until the first bursting events—roughly, the first increase in free phage concentration (Fig. 1). The resulting latent period estimates (Table 2) derived from the first parameter estimate assay show that latent periods change if cultures are grown at the different temperatures (25ºC or 37ºC). Conversely, the latent period does not change meaningfully when the liquid medium is changed (LB or SM+GLC). Cultures grown at 37ºC, the optimal temperature for *E. coli* growth, have an order of magnitude lower phage latent period than do identical cultures grown at a more stressful 25ºC.

*Burst size.* The burst size is the average number of phage particles released from an infected cell that is lysed (Equation 2). The resulting burst size estimates show an order of magnitude difference yielded by changing the temperature or by changing the liquid medium used (Table 2). More phage were produced per cell lysed in the nutrient-rich LB medium compared to the minimal SM+GLC, and likewise more at 37ºC compared to 25ºC.

*Adsorption rate.* The adsorption rate for λ phage was calculated by dividing the change in free phage concentration by the final CFU/mL and by the final PFU/mL (Equation 1). This value is a point estimate of the number of phage adsorbed by a single CFU during the period of adsorption before the first bursting (Fig. 1). The adsorption rate estimates for all conditions were effectively equivalent (Table 2).

**Table 2: Latent Period, Adsorption Rate, and Burst Size Estimates**

| | | Burst Size | | Adsorption Rate | |
|---|---|---|---|---|---|
| Condition | Latent Period | Mean | SD | Mean | SD |
| LB 37ºC | 40 min. | 234.5 | 49.13 | 3.00E-08 | 8.69E-09 |
| SM+GLC 37ºC | 40 min. | 15.14 | 2.48 | 2.54E-08 | 2.23E-08 |
| LB 25ºC | 120 min. | 13.94 | 2.24 | 4.14E-08 | 9.76E-09 |
| SM+GLC 25ºC | 130 min. | 7.05 | 1.8 | 3.92E-08 | 2.44E-08 |

*Lysogen formation probability.* The frequency of lysogen formation after one hour of phage exposure was calculated by dividing the CFU/mL of lysogens produced (cells that grew on LB+Cit20+Km25 plates) by the total CFU count (cells that grew on LB+Cit20 plates). This value was estimated across environmental conditions, and for three different MOIs (0.1, 1, and 10) (Fig. 2). The resulting lysogen frequencies were highly variable across experiments. Because of this, it is hard to distinguish substantial differences between environmental conditions, but

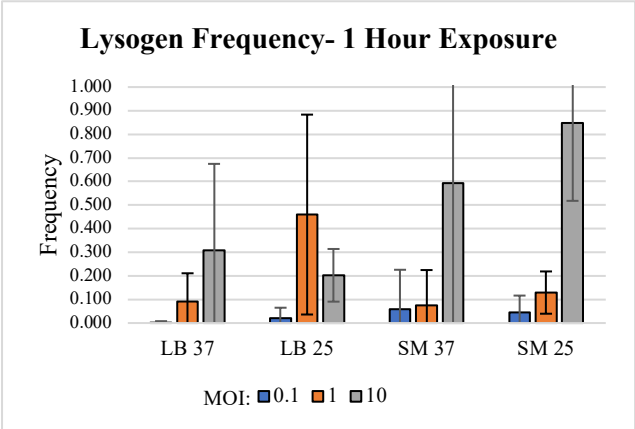


*Figure 2: Probability of Lysogen Formation.*
Log-phase LB or SM+GLC cultures of MG1655 grown at 37ºC or 25ºC were inoculated at MOI 0.1, 1, or 10 with $\lambda_{vir}$. The following are averages of the CFU/mL of lysogens formed after one hour of exposure divided by the total CFU/mL. Error bars are a 95% confidence interval.

there seems to be some correlation with MOI. There is an apparent increase in the frequency of lysogen formation at higher MOIs.

*Lytic induction rate.* Lytic induction rate was calculated for MG1655--$\lambda_{KmR}$ lysogens under all environmental conditions as described in Methods (Equation 3). The resulting data show orders of magnitude faster induction rates for λ prophage in SM+GLC than in LB across temperatures, and about an order of magnitude faster rates at 37ºC than at 25ºC in both LB and SM+GLC cultures (Table 3).

**Table 3: Lytic Induction Rate ($CFU^{-1} \bullet hour^{-1}$)**

| Condition | Mean | SD |
|---|---|---|
| LB 37ºC | 1.08E-07 | 3.21E-08 |
| SM+GLC 37ºC | 1.03E-04 | 4.90E-05 |
| LB 25ºC | 5.37E-08 | 2.16E-06 |
| SM+GLC 25ºC | 4.26E-06 | 9.02E-07 |

*Lysogen vs. Sensitive Competition Experiments*

From estimates of the bacterial growth rates, lag times, and carrying capacities derived from growth curves, and from phage infection parameters that were estimated in the above assays for given environmental conditions, non-resource-explicit growth models were produced (Fig. 3B and 3C). These models informed the expected outcomes for the lysogen versus phage-sensitive competition experiments. Throughout the competitions, the CFU/mL for each population subtype was tracked with antibiotic selective plating. The potential population constituents resulting from the competitions include the original phage-sensitive strain (S), the original lysogenic strain (L), the newly formed lysogen from the sensitive lineage (NL), and the free phage concentration measured in PFU/mL (P).

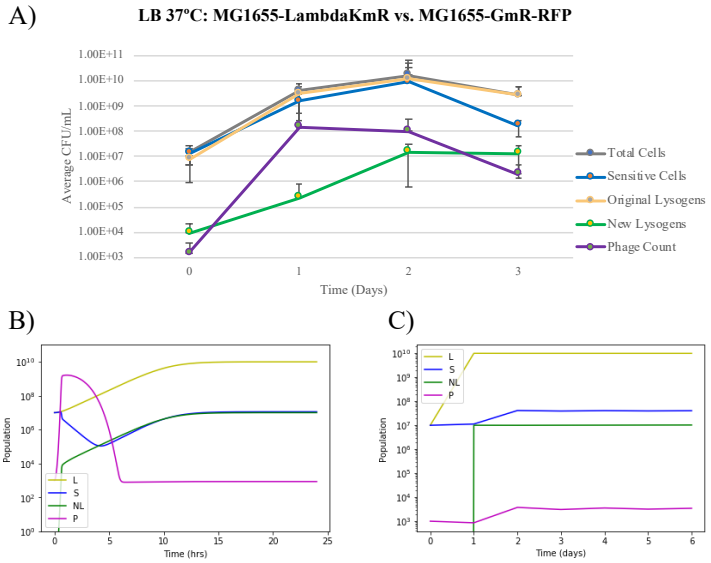


*Figure 3: Competition Models and Experimental Data.* (**a**) Experimental competitions data. Starting 1:1 mixture of the MKM lysogen and MGR sensitive strains grown in LB at 37ºC and reinoculated daily at 1:$10^3$ dilution factor. (**b,c**) Models based on phage parameter estimates and bacterial growth characteristics in LB at 37ºC on scales of hours (b) and days (c). (**c**) This simulation displays similar growth patterns to those in the experimental data, but the model predicts a faster emergence of new lysogens and fewer sensitive cells and phage at equilibrium.

For competitions between either lysogen (MKM or DKM) and the sensitive strain MG1655-GmR-RFP (MGR), the model fell short of accurately predicting the quantities of phage and bacteria; there were too many sensitive cells and phage in the experimental data, and the lysogens emerged slower than predicted (Fig. 3). Moreover, competitions containing the sensitive strain DGG deviated even further from the model and had far higher concentrations of phage-sensitive cells at each sampling than were projected by the models (Fig. 4). To assess if the putatively sensitive starting DGG population was truly sensitive to phage infection, single colony

picks from the strain's frozen glycerol stock were grown alongside single picks of the parent DH5α strain in a clear 96-well plate in the microplate photometer at 37ºC with 300rpm shaking in the presence of $\lambda_{vir}$ lysate. The resulting growth curves showed growth at a similar rate to the parent strain of DGG, but also revealed that DGG grew to a much higher density in the presence of lytic phage (Fig. 5A).

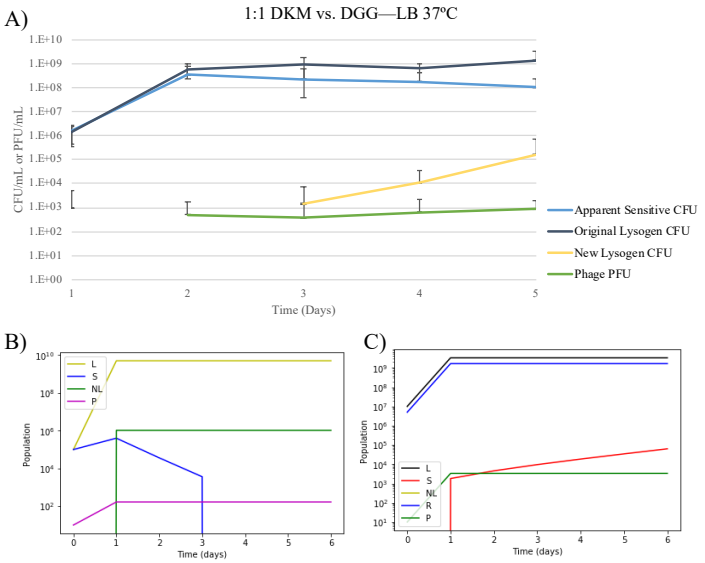


*Figure 4: Modelling a Phage-Resistant Subpopulation.* (**a**) Experimental data for a 1:1 competition of DKM lysogen versus DGG phage-sensitive bacteria grown in LB at 37ºC. (**b**) Model for LB 37ºC competition between DKM and DGG, but assumes sensitive strain is actually phage-sensitive. Compared to the model, the experimental data had too high of a putatively sensitive population. (**c**) The model was refined to include a phage-resistant population that reverts to sensitive at a rate of $10^{-7}$. This new model mirrors the trends of the experimental data.

To attempt to recover a sensitive stock out of the largely resistant DGG population, cultures of DGG were passaged over 3 days in the minimal medium SM that utilized maltose as its only carbon source. Even after this procedure, the DGG population was still predominantly phage-resistant (Fig. 5B).

## Discussion

### *Phage Parameter Assays*

Changing the environmental conditions in which bacteria grow can change the parameters of phage infection and reproduction. This series of experiments delineates how environmental conditions affect specific phage parameters, and then uses that information to model how phage-bacteria dynamics will unfold in each environment.

Since phage growth is dependent on bacterial metabolism while bacterial metabolism is governed by environmental conditions, phage production, reflected in burst size and latent period, is expected to be environmentally determined. The resulting data seem to support this hypothesis.

Burst size is higher and latent period is lower in the rich medium and at an optimal temperature of 37ºC compared to the minimal medium and stressful temperatures (Table 2). These order of magnitude differences confirm strong associations between bacterial metabolism and the production rate of their phage parasites.

Adsorption rate, however, did not change noticeably across conditions. In order to adsorb to *E. coli*, the λ phage must bind to the *lamB* receptor [6]. The *lamB* maltoporin receptor allows cells to take in the sugar maltose and the starch maltodextrin [7]. Since neither medium used in these experiments contains maltose, only a baseline level of the *lamB* receptor is expected to be expressed on the cells' surfaces. The lack of maltose explains the low but steady rate of adsorption across media (Table 2). Adsorption is minimally dependent on temperature, which may negligibly alter binding kinetics.

Phage infection parameters like lysogen frequency and lytic induction rate are also environmentally dependent. The frequency of lysogen formation has previously been linked to the phage multiplicity of infection (MOI), or the number of phage infecting a single bacterial cell [8]. Though the resulting lysogen frequencies of this assay were highly variable, that general trend of MOI-dependence holds here (Fig. 2). It is unclear from this data, however, if medium or temperature have any effect on the probability of lysogen formation. These assays would need to be repeated with a greater sample size, more consistent inoculation with phage lysate, and greater sensitivity when measuring phage and total CFU counts in order to distinguish any clear differences across environmental conditions.

Unlike with lysogen formation, there is clearly environmental dependence for lytic induction rates. Almost counterintuitively, lytic induction occurred at higher rates in the less-rich SM+GLC compared to LB medium (Table 3). Studies with other phages have shown temperature

and carbon source availability to affect prophage stability once they reach sufficiently stressful levels [9]. The limited nutrition offered by SM+GLC causes orders of magnitude faster rates of lytic induction than LB, corroborating the findings of Lunde et. al. [9] (Table 3). Furthermore, the trend that higher temperatures increases lytic induction holds in this experiment too (Table 3).

*Lysogen vs. Sensitive Competition Experiments*

With the above phage parameter estimates for each set of environmental conditions, simulations of lysogen versus phage-sensitive bacterial competitions were created to predict how each population in the competition would change over time. The resulting population trends of DKM-DGG competition, however, did not match the predictions of the model: sensitive bacteria grew in number rather than dying out as was expected (Fig. 4A, 4B). The simplest explanation for this irregularity would be the presence of a sizable phage-resistant fraction in the initial, putatively phage-sensitive, inoculum. Adding to the simulated competition a fully phage-resistant population that can revert to sensitive resulted in a model that more closely aligned with the experimental data (Fig. 4A, 4C). To assay the λ-resistance of DGG and confirm the new model's accuracy, the optical density of cultures in the presence of the virulent $\lambda_{vir}$ phage was recorded over time (Fig. 5). The uninhibited growth of DGG in the presence of $\lambda_{vir}$ suggests some

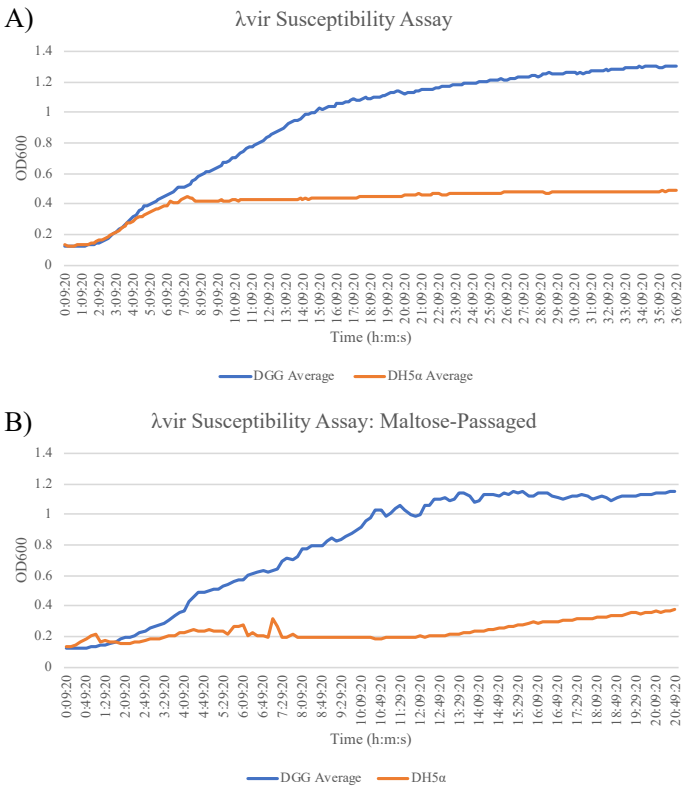


*Figure 5: Assaying Phage-Resistance and Attempted Recovery.* (**a**,**b**) Growth curves of putatively sensitive DGG and the parent strain DH5α in the presence of $\lambda_{vir}$ lysate at a MOI of 1. (**a**) The stock DGG grows to stationary phase without inhibition. (**b**) DGG was passaged twice through a minimal maltose medium to select for sensitive individuals. After this procedure, DGG still grew without visible effects from the

form of λ phage resistance emerged during the process of creating the DGG strain. If phage contaminated the post-transformation cultures as they recovered, envelope resistance—reduction in expression or mutation of the *lamB* maltoporin and λ phage receptor—could have easily surfaced and proliferated after positive selection by the contaminating λ phage.

To attempt a recovery of sensitive cells from the λ-resistant DGG stocks, overnight LB cultures made from single colony picks of stock DGG were centrifuged, washed and resuspended in a minimal S-medium containing maltose as its sole carbon source. Cells were grown overnight at 37ºC and were passaged in this medium the following day. In theory, this procedure would select for DGG variants that possess functional lamB maltoporins because they would have increased fitness relative to those individuals bearing non-functional maltose receptors. In practice, however, the resulting maltose-passaged DGG cultures appeared to be at least equally resistant (Fig. 5B). The failure to recover phage-sensitive DGG variants is perhaps due to the low number of maltose-passages—perhaps there was just not enough time for sensitive variants to overtake their resistant counterparts. Alternatively, the resistant subpopulation may have been subsisting on residual nutrients from the starting LB culture; more passages through maltose would have diluted off some of those remaining carbon sources.

Should a truly phage-sensitive DGG strain be established in the future, the model of competition between DGG and DKM suggests simply changing the starting inoculum concentration and the dilution used for inoculation could yield the conditions necessary to generate qualitatively different competition outcomes by changing environmental conditions (Fig. 6). The original competition experiments (e.g. Fig. 3) were performed with a starting concentration around $10^7$ CFU/ml and were reinoculated daily at a 1:1000 dilution factor. By instead starting the experiments at roughly $10^4$ CFU/ml and reinoculating daily with a $1{:}10^4$ dilution factor, the time

from inoculation to stationary phase is increased (Fig. 6A, 6C). When bacterial cultures approach the stationary phase of growth, bacterial metabolism (and therefore viral production) slows to a standstill. At that point phage DNA may still accumulate in infected bacteria, but no more cells lyse [10]. Increasing the time until stationary phase increases the time for phage-bacterial dynamics to make their mark. By changing the starting and reinoculating cell concentrations, a decrease of viral burst size from 200 to around 10 can determine whether sensitive cells persist or are eradicated (Fig. 6D, 6E). This shift in burst size can be elicited by simply swapping a rich medium like LB with SM+GLC or by decreasing the temperature of a competition in LB from 37ºC to 25ºC (Table 2). By adjusting such environmental knobs, the qualitative outcomes of competition may be altered under special circumstances.

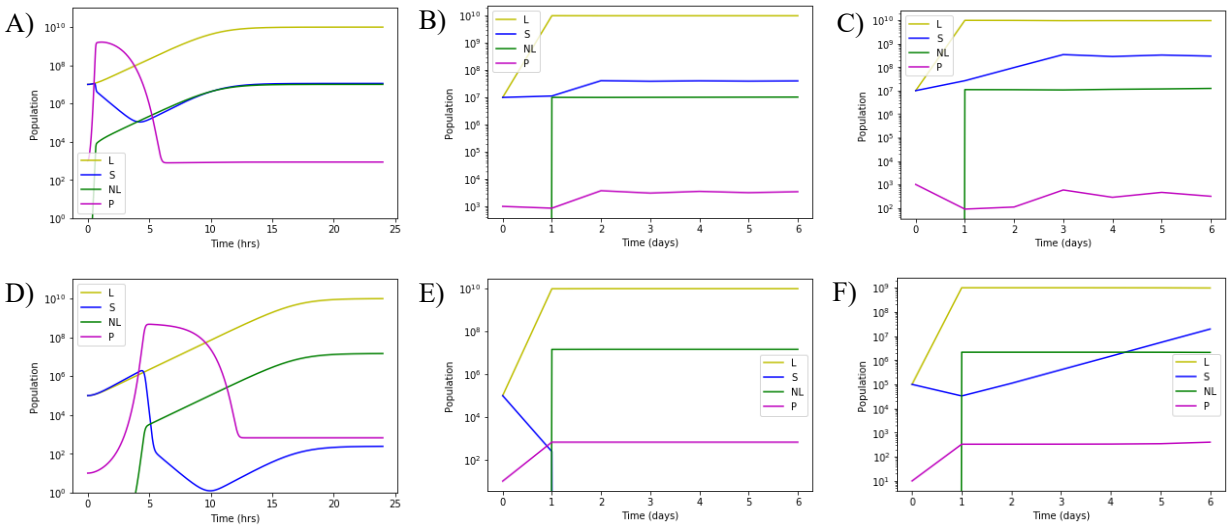


*Figure 6: Improving the Competitions Based on the Model.*
(**a,b**) The original 1:1 competition models for MKM versus MGR in LB at 37ºC. This assumes $10^7$ CFU starting inoculum and a 1:1000 dilution upon reinoculation. (**a**) There is a short duration of time from inoculation to stationary phase, (**b,c**) preventing bacteria-phage dynamics from qualitatively changing competition outcomes when burst size is decreased. (**d**) Under the same conditions, except with $10^5$ CFU starting inoculum and $1{:}10^4$ reinoculation dilution, there is a larger time to stationary phase. (**e,f**) Decreasing the burst size (β=200 to β=10) in the new competition conditions yields a qualitative outcome difference.

*Future Directions*

One environmental factor's impact on phage-bacteria dynamics has largely been unexplored: spatial structure. Most investigations of phage-bacteria interactions have been performed in well-mixed liquid media cultures. Many of these bacteria-phage communities, however, typically live in spatially structured environments like soil, or the integumentary and digestive systems of multicellular hosts. Consequently, it is important to translate experiments to structured environments. Unstructured environments, such as well-mixed media, are essentially at a uniform concentration for all diffusible particles (e.g. phage) and everything collides with everything (all-versus-all). Conversely, spatial structure imposes physical separation between individuals, and diffusion of phages and nutrients produces spatial gradients. This means individuals can only collide and interact with neighbors—local interactions are therefore intensified [11]. Recent simulations predict that spatial structure may provide refuges for sensitive bacteria [12] and reduce phage diffusivity [13], thereby neutralizing the fitness benefit lysogens attain in homogenous media. Conversely, spatial structure can increase local phage densities, potentially causing phage to act as a "replicating toxin," thereby increasing killing of sensitive cells over short timescales [3]. Investigating the effects of spatial structure is the next step in understanding phage-bacteria interactions in natural environments.

**References**


1. Stewart, F. M. & Levin, B. R. The population biology of bacterial viruses: Why be temperate. *Theoretical Population Biology* **26**, 93–117 (1984).
2. Gandon, S. Why Be Temperate: Lessons from Bacteriophage λ. *Trends in Microbiology* **24**, 356–365 (2016).
3. Gama, J. A. *et al.* Temperate Bacterial Viruses as Double-Edged Swords in Bacterial Warfare. *PLoS One* **8**, (2013).
4. Robb, M. L. & Shahrezaei, V. Stochastic Cellular Fate Decision Making by Multiple Infecting Lambda Phage. *PLoS One* **9**, (2014).
5. Pleška, M., Lang, M., Refardt, D., Levin, B. R. & Guet, C. C. Phage–host population dynamics promotes prophage acquisition in bacteria with innate immunity. *Nature Ecology & Evolution* **2**, 359–366 (2018).
6. Werts, C., Michel, V., Hofnung, M. & Charbit, A. Adsorption of bacteriophage lambda on the LamB protein of Escherichia coli K-12: point mutations in gene J of lambda responsible for extended host range. *Journal of Bacteriology* **176**, 941–947 (1994).
7. Charbit, A., Gehring, K., Nikaido, H., Ferenci, T. & Hofnung, M. Maltose transport and starch binding in phage-resistant point mutants of maltoporin: Functional and topological implications. *Journal of Molecular Biology* **201**, 487–493 (1988).
8. Blotnick, J. A., Vargas-García, C. A., Dennehy, J. J., Zurakowski, R. & Singh, A. The effect of multiplicity of infection on the temperateness of a bacteriophage: Implications for viral fitness. in *2017 IEEE 56th Annual Conference on Decision and Control (CDC)* 1641–1645 (2017). doi:10.1109/CDC.2017.8263885.
9. Lunde, M., Aastveit, A. H., Blatny, J. M. & Nes, I. F. Effects of Diverse Environmental Conditions on φLC3 Prophage Stability in Lactococcus lactis. *Appl. Environ. Microbiol.* **71**, 721–727 (2005).
10. Testa, S. *et al.* Spatial structure affects phage efficacy in infecting dual-strain biofilms of Pseudomonas aeruginosa. *Communications Biology* **2**, 1–12 (2019).
11. Stewart, P. S. & Franklin, M. J. Physiological heterogeneity in biofilms. *Nat. Rev. Microbiol.* **6**, 199–210 (2008).
12. Heilmann, S., Sneppen, K. & Krishna, S. Coexistence of phage and bacteria on the boundary of self-organized refuges. *PNAS* **109**, 12828–12833 (2012).
13. Simmons, M., Drescher, K., Nadell, C. D. & Bucci, V. Phage mobility is a core determinant of phage–bacteria coexistence in biofilms. *ISME J* **12**, 531–543 (2018).