Bacteriophage-mediated competition in *Bordetella* bacteria

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**ABSTRACT** Apparent competition between species is believed to be one of the principle driving forces that structure ecological communities, although the precise mechanisms have yet to be characterized. Here we develop a model system that isolates phage-mediated interactions by neutralizing resource competition using two genetically identical *B. bronchiseptica* strains that differ only in that one is the carrier of a phage and the other is susceptible to the phage. We observe and quantify the competitive advantage of the bacterial strain bearing the prophage in both invading and in resisting invasion by bacteria susceptible to the phage, and use our measurements to develop a mathematical model of phage-mediated competition. The model predicts, and experimental evidence confirms, that the competitive advantage conferred by the phage depends only on the relative phage pathology and is independent of other phage and host parameters. This work combines experimental and mathematical approaches to the study of phage-driven competition, and provides an experimentally tested framework for evaluation of the effects of pathogens/parasites on interspecific competition.

**INTRODUCTION** Pathogen-induced damage to hosts, commonly observed as infectious disease, has been extensively investigated in humans and other animals [1, 2]. A pathogen can also confer a competitive advantage to one of two competing species through the process known as apparent competition [3, 4, 5, 6]. In both scenarios pathogens appear to drive the evolution of their hosts, exerting a selection pressure toward greater resistance [2, 3, 4, 5]. Increased resistance mechanisms of the hosts, including those as complex as the adaptive immune response of higher eukaryotes, does not seem to confer freedom from infection, but offers substantial advantage against other hosts more susceptible to the pathogen [2, 3]. Pathogens are also driven to maximize their fitness (a function of virulence and transmission) although this is often a consequence of balancing virulence with transmission [7, 8]. Pathogen-mediated competition is an outcome of this ever-escalating arms race between the co-evolving hosts and pathogens.

There are a few excellent illustrative examples from the laboratory and field studies of ecological assembly [9, 10] and even from the history of human diseases [11, 12]. However, due to the complexities originating from dynamical interactions among multiple-hosts and multiple-pathogens, it is not always easy to single out and quantitatively measure the effect of pathogen-mediated competition in nature. In a system of bacteria and bacteriophage it is relatively easy to manipulate both host resistance mechanisms and pathogen virulence and thus this is one of the most suitable systems for the exploration of pathogen-mediated competition. In fact in recent studies [11, 12] laboratory communities of bacteria and lytic bacteriophage have been used as model systems for phage-mediated competition between phage-sensitive and phage-resistant bacteria. However, in these studies resource- and phage-mediated competitions were strongly intertwined and the role of one of the most important players in phage-mediated competition, lysogens (carriers of the phage), was not investigated.

To address these concerns in an examination of pathogen-mediated competition, we established an infection system using bacteriophage, neutralized resource competition by having two genetically identical strains and using large nutrient excess, and then examined competition between bacterial strains that differ only in their sensitivity to this phage. Particularly we used *Bordetella bronchiseptica*, a causative bacterium of mammalian respiratory disease, and its natural virus (BPP-1), a temperate phage that can either incorporate its DNA into the genome of *B. bronchiseptica* (lysogeny) or replicate itself and lyse the host bacterium (lysis). Here we demonstrate, both experimentally and theoretically, the existence of a competitive advantage conferred by this virus in a bacterial population, using a system in which this effect can be measured and quantified. Our results suggest that the lysogens are not only the source of phage during an infection process but can lead to fundamentally different dynamics in phage-mediated competition. We observe that the bacterial strain bearing the phage has an advantage in both invading and resisting invasion by bacteria susceptible to the phage and that the differential pathology on the two hosts is the sole variable that determines the quantitative value of phage-mediated competitive advantage. The theoretical representation of these interactions should have broad application to pathogen-mediated competition at many levels.

**RESULTS** The quantitative assessment of pathogen-conferred competitive advantage is usually hampered by the existence of direct competition over resources [2, 3, 4]. To address this concern we use as our host populations *B. bronchiseptica* strains that are genetically identical except for defined genetic changes. The wild type parental *B. bronchiseptica* strain RB50 (Bb) was used to generate a strain (BbGm) that carries a gentamycin resistance marker shown not to affect expression of nearby genes and another (Bb::φ) that is the carrier of, and is therefore resistant to, the temperate phage BPP-1 (φ) [10, 11]. To examine possible nutrient-dependent competition among
these bacterial strains, we observed their in vitro growth rates. All three strains grew in nutrient rich medium with identical doubling time (77 minutes) over a range of cell densities from less than 1000 CFU/ml to over 10^9 CFU/ml (see Fig. 1). Bb and BbGm grew at the same rate when co-cultured, indicating that there is no effect of direct resource competition on their growth rates. The proportion of the final density of each strain was identical to the proportion at the start of the co-culture, indicating neutral competition between these two strains under these conditions.

We then examined phage-mediated competition using these B. bronchiseptica strains and the temperate bacteriophage BPP-1. The interactions involved in this system are schematically represented in Fig. 2. All bacterial strains divide with a constant rate a and bacterial populations grow with a density-dependent rate r. Susceptible bacteria (BbGm) become infected with a rate \( \kappa \), defined as the number of contacts between a phage particle and a host bacterium per unit time multiplied by the probability of the host being infected upon contact. Upon infection the phage can take one of two pathways. In a fraction \( P \) of infected BbGm, the phage replicate and then lyse the host after an incubation period \( 1/\lambda \), during which the bacteria do not divide. Alternatively the phage lysogenize a fraction \( 1-P \) of their hosts, incorporating their genome into that of the host. Thus the parameter P characterizes the pathogenicity of the phage, incorporating multiple aspects of phage-host interactions resulting in damage to host fitness. The lysogens (Bb::\( \phi \) and BbGm::\( \phi \)) carrying the prophage grow, replicating prophage as a part of the host chromosome, and are \( \phi \)-resistant. Even though these lysogens are very stable without external perturbations, spontaneous induction can occur at a low rate \( \delta \), consequently replicating the phage and lysing the host bacteria. In general, both the number of phage produced (burst size) and the phage pathology P depend on the culture conditions. This model predicts that when co-cultured, these two strains should compete through the phage, giving advantage to the lysogens.

To experimentally observe phage-mediated competition between two strains, Bb::\( \phi \) and BbGm were co-cultured in vitro. Since spontaneous induction of the phage from Bb::\( \phi \) is inevitable but would occur at variable time points (see Appendix B), we added a small number (1,000 PFU/ml) of exogenous phage to synchronously initiate phage-mediated competition. 1,000 CFU/ml of Bb::\( \phi \) and 1,000 PFU/ml of phage were added to a culture containing 10,000 CFU/ml of BbGm. As shown on Fig. 2a, the total BbGm concentration (both susceptible BbGm and BbGm::\( \phi \)) increased with the same initial growth rate as Bb::\( \phi \), but suddenly fell at about 8-12 hours postinfection. Within 24 hours the initial 1:10 ratio of Bb::\( \phi \) to BbGm was reversed to approximately 10:1, indicating a 100 fold relative increase in the proportion of Bb::\( \phi \).

To observe the advantage of the lysogens in resisting invasion by the strain susceptible to the phage, 1,000 CFU/ml of BbGm was added to a culture containing
on the dynamics of multiple hosts \cite{19, 20, 21, 22, 23, 24}.

We directly measured the values of five parameters, \(a, \delta, \lambda, \chi\) and \(N_{\text{max}}\), and estimate the others. The dimensions and relevant ranges of all parameters are given in Table I.

We compared the numerical simulation results with our experimental results for invasion and protection of the lysogens (Bb::\(\phi\)) carrying the phage in Fig. 3. The results validate our choice of theoretical model and parameters. We find that the experimentally determined and estimated values of parameters (\(\chi = 50, \alpha \equiv \delta/a = 0.1, \beta \equiv \lambda/a = 0.15, P = 0.98, \gamma \equiv S_B(0)\kappa/a = 0.01\) for (a) and \(\gamma = 0.002\) for (b) where \(S_B(0)\) is the initial concentration of BbGm.

\begin{align}
\frac{dI_A(t)}{dt} &= (r(t) - \delta)I_A(t) \\
\frac{dS_B(t)}{dt} &= (r(t) - \kappa \Phi(t))S_B(t) \\
\frac{dL_B(t)}{dt} &= P \kappa \Phi(t)S_B(t) - \lambda L_B(t) \\
\frac{dI_B(t)}{dt} &= (1 - P)\kappa \Phi(t)S_B(t) + (r(t) - \delta)I_B(t) \\
\frac{d\Phi(t)}{dt} &= \chi(\delta I_A(t) + I_B(t) + \lambda L_B(t)) - \kappa \Phi(t)S_B(t)
\end{align}

where \(I(t), L(t)\) and \(S(t)\) are the concentrations of the infected, latent and susceptible bacteria at time \(t\), \(N(t) = I_A(t) + I_B(t) + S_B(t) + L_B(t), r(t) = a(1 - N(t)/N_{\text{max}})\) is the density-dependent growth rate of all bacteria, and \(N_{\text{max}}\) is the holding capacity, i.e., the concentration of bacteria supported by the nutrient broth environment.

The success of our model in capturing the dynamic behavior of the bacteria-phage system in two different scenarios enables us to use it to determine the condition of a successful phage-mediated invasion and explore cases that are not addressed by experiments. We first investigated the invasion criterion, the choice of parameters in Table I which makes the invading strain A dominant in number over the invaded strain B. The condition of becoming the predominant organism depends only on the phage pathology \(P\) and on the initial ratio of the concentrations of bacterial strains A and B. We find that the final population ratio is approximately

\begin{equation}
r_{AB}(\infty) \simeq \frac{r_{AB}(0)}{1 - P},
\end{equation}

where \(0 \leq P < 1, r_{AB}(\infty) = N_A(\infty)/N_B(\infty), r_{AB}(0) = N_A(0)/N_B(0),\) and \(N_A[N_B]\) is the total concentration of bacteria A[B] and \(N_A(\infty) + N_B(\infty) = N_{\text{max}}\). In conclusion the invasion criterion (that is, the condition of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{In vitro experimental results for invasion and protection of the lysogens (Bb::\(\phi\)) carrying the phage in Fig. 3. (a) The initial 10:1 ratio of Bb::\(\phi\) to the total BbGm was amplified to approximately 1,000:1, indicating again a 100 fold increase in the proportion of Bb::\(\phi\) to the total BbGm. (b) The initial 10:1 ratio of Bb::\(\phi\) to the total BbGm was amplified to approximately 1,000:1, indicating again a 100 fold increase in the proportion of Bb::\(\phi\) to the total BbGm.}
\end{figure}
TABLE I: Parameters used for the numerical simulation of the phage-mediated competition in B. bronchiseptica. a
[hours⁻¹] is determined from the measured doubling time (77
minutes) of Bb bacteria in mid-log phase in Fig. 4. λ [hours⁻¹]
is determined from the observed phage incubation period (6-
12 hours), which is the time-interval between initial contact
of the phage particles with susceptible bacteria and bacterial
lysis. χ is measured from the difference in the phage concen-
trations between 0 and 12 hours of φ and Bb co-culture. δ
[hours⁻¹] is set to be 0.054 in our simulations. Note that we
verified in Supporting Information that the invasion criterion in
Eq. 6 remains valid for any 0 ≤ δ/a < 0.5. The two
undetermined parameters P and κ [[hours-CFU/ml]⁻¹] are
estimated by comparing the experimental results with those of
the theoretical model and by minimizing discrepancies.

| Parameter | Name          | Range | Resources |
|-----------|---------------|-------|-----------|
| a         | (Free) growth rate | 0.54  | measured  |
| δ         | Spontaneous lysis rate | 0 ≤ δ < a | measured |
| λ         | φ-induced lysis rate | 0.08 - 0.17 | measured |
| χ         | Burst size     | 10 - 50 | measured  |
| P         | Phage pathology | 0 ≤ P ≤ 1 | estimated |
| κ         | Contact rate   | κ > 0  | estimated |
| Nmax      | Holding capacity | ∼ 10⁹ | measured  |

\[
r_{AB}(\infty) > 1 \quad \text{is}
\]

\[
r_{AB}(0) > 1 - P.
\] (3)

The derivation of Eq. (2) was provided in Appendix in the
limit γ ≫ 1 (κS_B(0) ≫ a) and β ≫ 1 (λ ≫ a), that is,
when susceptible bacteria are rapidly infected by phage
particles and lysed immediately after infection. We also
verified by extensive numerical simulations the validity of
Eq. (2) in a much broader parameter range, especially
for small γ and β.

The theoretical model predicts that different initial
phage concentrations or different contact rates have no
effect on the steady state outcome of the phage-mediated
competition while either can modify the kinetics of the
interactions (see the inset of Fig. 4). To validate its predic-
tion, we performed time course experiments
with three different phage concentrations (see Fig. 4).
The trajectories of the total BbGm population depend
sensitively on the initial phage concentrations during the
intermediate time-steps (between 0 and 24 hours), show-
ing a larger fall of the total BbGm for higher phage concen-
tration between 8 and 12 hours of co-culture. However
the trajectories of Bb::φ and the total BbGm converge to
the same steady states within statistical errors after 48
hours regardless of initial phage concentration, indicat-
ing that the final outcome of phage-mediated competition
does not depend on the initial phage concentration.
This verifies the independence of the invasion criterion in
Eq. 6 on the initial phage concentration and provides
justification for the addition of exogenous phage to the
system in Figs. 5(a) and 5(b).

We investigated the effect of the phage pathology on
the amount of the phage-mediated competition. We ex-
perimentally manipulated the phage pathology using a
lytic phage (φΔcI) with an in-frame deletion in the cI
repressor gene required for lysogeny [16, 17] that always
lyses the host bacterium (and thus has P = 1).

Finally we investigated one of several possible resis-
tance mechanisms of bacteria against pathogenicity of
the phage. A simple mutation can take place in the re-
ceptor complex of the host bacteria that renders them no
longer susceptible to phage lacking the tropism switch-
ning mechanism. We used a mutant phage (φΔorf5) with
in-frame deletion in the gene, orf5, encoding the reverse
transcriptase necessary for the tropism switching mecha-
nism. This phage can only infect bacteria bearing the
protein pertactin. In Fig. 4(b) we co-cultured a bacterial
strain with an in-frame deletion in the gene encoding per-
tactin (Bb ∆prnGm) with Bb::φΔorf5 in the presence
of 1000 PFU/ml of the mutant phage (φΔorf5) [16, 17, 25].
Both strains grow without any sign of phage-mediated

FIG. 4: Independence of the steady state outcome of
phage-mediated competition on the initial phage concen-
tration. Main: In vitro experiments of the time evolution of
Bb::φ (open symbols connected by a solid line) and the
total BbGm (filled symbols, dotted lines) with initial exogenous
phage concentrations of 10 (filled circles), 10³ (filled squares)
and 10⁵ (filled diamonds) PFU/ml. Inset: Numerical simu-
lations of the time evolution of Bb::φ (solid line) and the
total BbGm with three different initial phage concentrations of
10⁴ (long-dashed line), 10³ (dashed line) and 10⁵ (dot-dashed
line) PFU/ml. The parameters are χ = 50, α = 0.1, β = 0.15,
γ = 0.02 and P = 0.98. Note that the numerical simulation
results of the time-evolution of Bb::φ and the total BbGm at
different contact rates γ are similar to the pattern in the inset.

The trajectories of Bb::φ and the total BbGm for higher phage concentrations or different contact rates have no
effect on the steady state outcome of the phage-mediated
competition while either can modify the kinetics of the
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different contact rates γ are similar to the pattern in the inset.
FIG. 5: Dependence of phage-mediated competition on the phage pathology $P$ is observed in in vitro experiments (symbols) and numerical simulations (lines). Presented are time-evolutions of (a) the co-cultured Bb::$\phi$ (open squares, solid line) and total BbGm (filled circles connected, dashed line) in the presence of exogenous lytic phage ($\phi\Delta elf$), and (b) the co-cultured Bb$\Delta prn$Gm (filled circles, solid line) and Bb::$\phi$orf5 (open rectangles, dashed line) in the presence of mutant phage ($\phi\Delta or f5$). The parameters used for the numerical simulations are the same as in Fig. 3(a) except $r = 0.01$ in (a) and $r = 0$ and $P = 0$ in (b).

competition and maintain the initial ratio for more than 24 hours.

**DISCUSSION** In most of the systems where pathogen-mediated competition has been observed, there have been confounding factors, such as different growth and reproductive rates of hosts, and intertwined resource and phage-mediated competitions, that have limited quantitative assessments of pathogen-mediated competition. The bacteria-phage system described here overcomes these difficulties to allow the accurate estimation of the parameters, in most cases directly measuring them experimentally. Understanding the impact of the pathogen pathology (the product of co-evolving pathogen virulence and host resistances against pathogens) on pathogen-mediated competition requires a system in which both host defense mechanisms and pathogen pathology can be manipulated. Again bacteria and phage are suitable systems for these purposes.

We utilized in vitro experiments, analytical and numerical analysis to show the existence of bacteriophage-mediated competition between two host bacterial strains. In our in vitro experiments direct competition between host strains was minimized by using genetically identical strains and culture conditions that allow for unrestricted, exponential growth over many generations. The two bacterial strains differ only in the pathology of phage infecting them: the phage pathology ($P$) of the strain bearing the prophage (Bb::$\phi$) is almost zero while that of the susceptible BbGm is close to one. As we demonstrated here, the strain bearing the prophage has advantage in both invading and resisting invasion by the phage-susceptible bacteria.

Our competition studies have revealed a single determining factor for competitive advantage. First, when the phage was manipulated to increase its pathology in the susceptible strain, the amount of competitive advantage conferred to the host carrying the prophage dramatically increased. Second, when both strains (Bb::$\phi$orf5 and Bb$\Delta prn$Gm) are resistant to phage ($\phi\Delta or f5$) infection, the phage didn’t confer any competitive advantage to either strain. Third, we conjectured that none of the other details of the system contribute to the steady state outcome of phage-mediated competition between two strains, and we demonstrated experimentally the independence of the competition outcome on the initial concentration of the phage. Although different initial phage concentrations modified the kinetics of the interactions, they did not affect the final ratio of bacterial strains.

Our theoretical model captures the dynamical behavior of the bacteria-phage system and can be extended to predict the steady state outcome of phage-mediated competition under more general conditions. We demonstrated that when there is a quantitative difference in the resistance of the two bacterial strains, the success of the competing strains depends only on the ratio of the initial concentrations of two strains and the relative phage pathology. This conclusion leads to the following predictions for general pathogen-mediated invasion beyond bacteria-phage systems: a) For a pathogen to contribute to the ability of a host to invade an ecological niche requires that the pathogen pathology is lower in the invading population than in the invaded population. This is generally true when a disease endemic to one population is carried to populations that are naive to the disease. b) In the case of differential host resistance, one can conjecture that the final ratio of the two populations and the success of the invasion are determined by the following fraction,

$$r_{AB}(\infty) = r_{AB}(0) \frac{(1 - P_A)}{(1 - P_B)}$$

where $P_A[P_B]$ is the pathogen pathology. The condition for the success of the invasion of population A to population B is that $r_{AB}(\infty) \geq 1$. In Appendix we have verified the validity of the generalized invasion criterion by analytical and numerical analysis.

The above predictions can be naturally extended to pathogen-mediated invasion in nature if all the details of individual pathogen-host interactions can be condensed into a single parameter describing pathogen pathology. There are certain limitations to extrapolating the above conclusions to pathogen-mediated invasion in ecology and humans. First, the assumption that host populations and pathogens are well-mixed may be better suited in vitro than in nature. However, the theoretical models and experimental manipulation of phage concentrations suggest that the ultimate effect of pathogen-mediated
competition is not dependent on the rate of contact, and successful pathogens are, by their nature, very efficient at transmission (infection-causing contact). Second, the infection processes are discrete, stochastic and spatial in nature, and might not be completely described by differential equations. Third, pathogens can be transmitted without killing hosts in general infection processes, and the birth and death events in animal and human populations can be markedly different from bacterial growth. We expect that these differences will not have a major impact, though.

In conclusion our studies have important implications in relation to the long-term advantage of bearing multiple pathogens. An a priori view might have been that the heavier pathogen load might reduce fitness relative to a competitor. This view is directly contradicted by the findings of this study, in which the advantages of bearing a pathogen are clear and related to its relative pathology on the hosts. One further implication is that the relative advantage conferred by the pathogen to the host should be related to the length of time during which they have coexisted and co-evolved. Over time the pathogen will select for more resistant hosts, but will alter its own virulence to maintain optimal transmission and overall fitness. When that pathogen is introduced into a host population that has not been under that selection it will exhibit inappropriately high virulence. This effect is observed in, and potentially explains, zoonotic diseases that often cause more pathology in humans than in their naturally co-evolved host. Although each pathogen may moderate pathology to optimize reproduction in their new host, humans will continue to be assaulted by new pathogens with greater virulence.

APPENDIX A: EXPERIMENTAL METHODS

A temperate bacteriophage BPP-1 and a mutant, which lacks the reverse transcriptase necessary for the tropism switching mechanisms and can only infect B. bronchiseptica bacteria bearing the protein pertactin BPP-1Δorf5, were the kind gift of Jeff Miller [16, 17, 25].

B. bronchiseptica strains were grown on Bordet-Gengou (BG) agar plate, and incubated for three days at 37°C. Then 2-3 colonies of each strain were inoculated in 4 ml of Stainer Sholte media with supplements, and grown at 37°C overnight to mid log phase. For co-culture experiments, two B. bronchiseptica strains were subcultured together into 10ml of Stainer Sholte media at the appropriate concentrations. The co-culture was incubated at 37°C with continuous agitation. To determine the concentration of each strain in the co-culture at various time points, 100µl of the co-culture was serially diluted in PBS and spread on six Bordet-Gengou agar plates, half of which were treated with gentamycin. After two days of incubation at 37°C, the colony forming units (CFU) on each plate were counted to determine the concentrations of each strain at consecutive time points.

The phage-sensitivity of a single BbGm colony was tested by using the standard protocol [16]. The lack of host diversity mechanisms of φΔorf5 was tested at 0 and 48 hours postinoculation by using the standard phage-titering protocol on the lawns of susceptible Bb and BbΔprnGm.

APPENDIX B: SPONTANEOUS PHAGE INDUCTION

In vitro evidence of the spontaneous release of phage is provided in Fig. 6. The strain (Bb::φ) carrying the phage and the susceptible strain (BbGm) are co-cultured without exogenous phage. The initial ratio of the strain Bb::φ to the strain BbGm is reversed around 8-10 hours, which is mediated by the phage spontaneously released from the strain Bb::φ. Based on this result, we use spontaneous lysis rate δ = 0.054 for the numerical simulations.

APPENDIX C: DERIVATION OF THE INVASION CRITERIA IN EQS. (2) AND (4)

Our primary model of phage-mediated competition depicted in Fig. 2 is limited to the case where one host is perfectly phage-resistant and the other is phage-susceptible. However in general cases both invading and resident hosts can be susceptible to phage infection but with differential susceptibilities. Here we model the invasion of a host A endogenously and exogenously carrying the phage to another host B. The hosts are characterized by the differential susceptibilities κ_A and κ_B against the phage, and the phage pathology P_A and P_B. We rescale and non-dimensionalize the variables, i_j =

![Figure 6](image-url)
\( I_j/S_B(0), s_j = S_j/S_B(0), I_j = L_j/S_S(0), \phi = \Phi/S_B(0), \)
\( n_{max} = N_{max}/S_B(0), \tau = at, \alpha = \delta/a, \beta = \lambda/a, \)
\( \gamma_j = \kappa_j S_B(0)/a \) where \( j = A, B \). Then we obtain
\[
\frac{ds_j}{d\tau} = (1 - n/n_{max} - \gamma_j \phi)s_j
\]  
(C1)
\[
\frac{di_j}{d\tau} = (1 - P_j)\gamma_j \phi s_j + (1 - n/n_{max} - \alpha)i_j
\]
\[
\frac{dl_j}{d\tau} = P_j \gamma_j \phi s_j - \beta l_j
\]
\[
\frac{d\phi}{d\tau} = \chi(\alpha \sum i_j + \beta \sum l_j) - \gamma_j \phi s_j
\]
where \( n = \sum (i_j + s_j + l_j) \) and \( j = A, B \). The initial conditions for Eq. (C1) are \( i_B(0) = I_A(0) = \beta_S(0) = 0, s_B(0) = 1, s_A(0) > 0, i_A(0) > 0 \) and \( \phi(0) > 0 \). The above general model and Eq. (C1) are reduced to the primary model depicted in Fig. 2 and Eq. (1) when \( P_A = 0, \phi_A = 0 \) and \( S_A(0) = 0 \).

**CASE I:** If \( \phi(0) = 0 \) and \( \alpha = 0 \), then the 7-dimensional ODE system reduces to
\[
\frac{ds_j}{d\tau} = (1 - (\sum s_j + i_A)/n_{max})s_j,
\]
\[
\frac{di_A}{d\tau} = (1 - (\sum s_j + i_A)/n_{max})i_A.
\]
where \( j = A, B \) and \( i_B(\tau) = I_A(\tau) = l_B(\tau) = 0 \) for \( \tau > 0 \). All populations \( s_j \) and \( i_A \) will grow with the same growth rate and the initial ratio \( i_A(0) : s_A(0) : s_B(0) \) remains unchanged to be \( i_A(\tau) : s_A(\tau) : s_B(\tau) \) for all \( \tau > 0 \). In other words, there will be no pathogen-mediated competition.

**CASE II:** When \( \phi(0) > 0 \), we can derive the invasion criteria in Eqs. (2) and (4) in the limit \( \gamma_j \to \infty \) and \( \beta \to \infty \).

**CASE II-A:** \( \tau = 0 \) Limit.
An appropriate timescale near \( \tau = 0 \) is \( \sigma = \tau/\epsilon \) where \( \epsilon = 1/\beta \). The effect of the transformation is \( \tau/\epsilon = \tau/\epsilon \) to magnify the neighborhood of \( \tau = 0 \), i.e., for a fixed \( 0 < \tau \ll 1 \), we have \( \sigma \gg 1 \) as \( \epsilon \to 0 \). With the transformations \( \sigma = \tau/\epsilon \), \( s_j(\tau; \epsilon) = s_j(\sigma; \epsilon), i_j(\tau; \epsilon) = \hat{i}_j(\sigma; \epsilon), l_j(\tau; \epsilon) = \hat{l}_j(\sigma; \epsilon), \phi_j(\tau; \epsilon) = \hat{\phi}_j(\sigma; \epsilon), \xi_j = \gamma_j/\beta, \) Eq. (C1) become
\[
\frac{d\hat{s}_j}{d\sigma} = \epsilon(1 - n/n_{\text{max}})\hat{s}_j - \xi_j \hat{\phi}\hat{s}_j
\]  
(C2)
\[
\frac{d\hat{i}_j}{d\sigma} = (1 - P_j)\hat{\phi}\hat{s}_j + \epsilon(1 - n/n_{\text{max}} - \alpha)\hat{i}_j
\]
\[
\frac{d\hat{l}_j}{d\sigma} = P_j \hat{\phi}\hat{s}_j - \hat{l}_j
\]
\[
\frac{d\hat{\phi}}{d\sigma} = \chi(\alpha \sum \hat{i}_j + \beta \sum \hat{l}_j) - \xi_j \hat{\phi}\hat{s}_j + \epsilon \chi \alpha \sum \hat{i}_j
\]

In a regular perturbation theory, the solutions are expanded in order of \( \epsilon \), \( \hat{s}_j(\sigma; \epsilon) = \sum_{n=0}^{\infty} e^{\epsilon n} \hat{s}_{j,n}(\sigma) \), \( \hat{i}_j(\sigma; \epsilon) = \sum_{n=0}^{\infty} e^{\epsilon n} \hat{i}_{j,n}(\sigma) \), \( \hat{\phi}(\sigma; \epsilon) = \sum_{n=0}^{\infty} e^{\epsilon n} \hat{\phi}_n(\sigma) \). We now set \( \epsilon = 0 \) to get \( 0(1) \)
\[
\frac{ds_{j,0}}{d\sigma} = -\xi_j \hat{\phi}_0 \hat{s}_{j,0}
\]  
(C3)
\[
\frac{di_{j,0}}{d\sigma} = (1 - P_j)\xi_j \hat{\phi}_0 \hat{s}_{j,0}
\]
\[
\frac{dl_{j,0}}{d\sigma} = P_j \xi_j \hat{\phi}_0 \hat{s}_{j,0} - \hat{l}_{j,0}
\]
\[
\frac{d\phi_0}{d\sigma} = \chi \sum \hat{i}_{j,0} - \sum \xi_j \hat{\phi}_0 \hat{s}_{j,0}
\]
where the initial conditions \( \hat{s}_{B,0}(0) = \hat{i}_{A,0}(0) = \hat{l}_{B,0}(0) = 0, \)
\( \hat{s}_{B,0}(0) = 1, \hat{s}_{A,0}(0) > 0, \hat{i}_{A,0}(0) > 0 \) and \( \hat{\phi}_0(0) > 0 \).

By integrating Eqs. (C3) and (C4), we obtain
\[
\hat{s}_{j,0}(\sigma) = \hat{s}_{j,0}(0) \exp(-\int_0^\sigma \xi_j \hat{\phi}_0(x) dx),
\]
\[
\hat{i}_{j,0}(\sigma) = \hat{i}_{j,0}(0) + (1 - P_j) \hat{s}_{j,0}(0) \int_0^\sigma F_j(y) dy
\]
where \( F_j(y) = \xi_j \hat{\phi}_0(y) \exp(-\int_0^y \xi_j \hat{\phi}_0(x) dx) \). Eqs. (C5) and (C6) can be rewritten
\[
\frac{d\hat{i}_{j,0}}{d\sigma} = P_j \hat{s}_{j,0}(0) F_j(\sigma) - \hat{l}_{j,0}(\sigma)
\]  
(C9)
\[
\frac{d\hat{\phi}_0}{d\sigma} = \chi \sum \hat{i}_{j,0}(\sigma) - \sum \hat{s}_{j,0}(\sigma) F_j(\sigma)
\]  
(C10)

**Lemma 1.** \( \hat{\phi}_0(\sigma) \) is strictly positive for \( \sigma \geq 0 \) if \( \hat{\phi}_0(0) > 0 \) and \( \chi P_j > 1 \).

**Proof.** Let \( Z(\sigma) = \hat{\phi}_0(\sigma) + \chi \sum \hat{i}_{j,0}(\sigma) \). \( Z(0) > 0 \) because \( \hat{\phi}_0(0) > 0 \). Because \( \hat{i}_{j,0}(\sigma), \hat{s}_{j,0}(\sigma), \hat{l}_{j,0}(\sigma) \) and \( \hat{\phi}_0(\sigma) \) are non-negative for \( \sigma \geq 0, \frac{dZ}{d\sigma} = \chi \sum (P_j - 1) \hat{s}_{j,0}(\sigma) F_j(\sigma) \geq 0 \) if \( \chi P_j > 1 \). Therefore Z(\sigma) is strictly positive and non-decreasing for all \( \sigma \geq 0 \). Suppose now that there exists \( \sigma_o > 0 \) such that \( \hat{\phi}_0(\sigma) = 0 \) for \( \sigma > \sigma_o \). Then both \( F_j(\sigma) \) and \( \hat{l}_{j,0}(\sigma) \) will become zero for \( \sigma > \sigma_o \), resulting in \( Z(\sigma) = 0 \) for \( \sigma > \sigma_o \). This contradicts that \( Z(\sigma) \) is strictly positive for all \( \sigma \geq 0 \). Therefore \( \hat{\phi}_0(\sigma) > 0 \) for all \( \sigma \geq 0 \).

**Lemma 2.** \( F_j(y) \) is strictly positive for \( y > 0 \) and \( F_j(y) \) asymptotically approaches zero as \( y \to \infty \).

**Proof.** Strict positiveness of \( F_j(y) \) for \( y > 0 \) follows from lemma 1. For the second part, we divide the integration in the exponent into two parts,
\[
\int_0^y dx \xi_j \hat{\phi}_0(x) = \int_0^{y-w} dx \xi_j \hat{\phi}_0(x) + \int_{y-w}^y dx \xi_j \hat{\phi}_0(x)
\]
where \( y \gg 1 \) and \( w \in (0, y) \) must be such that \( \hat{\phi}_0(x) \) is either non-decreasing or non-increasing in the interval \( x \in [y-w, y] \). Then there exists \( \lambda \in [0,1] \) such that
\[
\int_{y-w}^y dx \xi_j \hat{\phi}_0(x) = \lambda [\xi_j \hat{\phi}_0(y) + (1-\lambda) \xi_j \hat{\phi}_0(y-w)]w.
\]
By defining \( \hat{\phi}_{min} = \min_{x \geq \hat{\phi}_0(x)} \), \( \int_{y-w}^y dx \xi_j \hat{\phi}_0(x) \geq (y-w)\xi_j \hat{\phi}_{min} \) and \( \int_{y-w}^y dx \xi_j \hat{\phi}_0(x) \geq [\lambda \xi_j \hat{\phi}_0(y) + (1-\lambda) \xi_j \hat{\phi}_{min}]w \). Then we can obtain
\[
F_j(y) = \xi_j \hat{\phi}_0(y)Exp(-\int_{0}^{y} dx \xi_j \hat{\phi}_0(x)) \leq \xi_j \hat{\phi}_0(y)e^{-\lambda \nu x \xi_j \hat{\phi}_0(y)}e^{-(y-w)\xi_j \hat{\phi}_{min}} \leq \frac{1}{\lambda w}e^{-(y-w)\xi_j \hat{\phi}_{min}-1}
\]
where in the third line we used \( xe^{-x} \leq e^{-1} \) for all \( x > 0 \).

As \( y \to \infty \), \( F_j(y) \to 0 \).

**Lemma 3.** Let \( G_j(\sigma) = \int_{0}^{\sigma} F_j(y)dy \). \( G_j(\sigma) \) asymptotically approaches 1 as \( \sigma \to \infty \).

**Proof.**
\[
G_j(\sigma) = \int_{0}^{\sigma} dy \xi_j \hat{\phi}_0(y)Exp(-\int_{0}^{y} dx \xi_j \hat{\phi}_0(x)) = \int_{0}^{\sigma} dy H_j(y) e^{-H_j(y)} = 1 - e^{-H_j(\sigma)}
\]
where \( H_j(y) = \int_{0}^{y} dx \xi_j \hat{\phi}_0(x) \) and \( H_j(0) = 0 \). Using \( H_j(\sigma) \geq \sigma \xi_j \hat{\phi}_{min} \), \( e^{-H_j(\sigma)} \leq e^{-\sigma \xi_j \hat{\phi}_{min}} \) for \( \sigma > 0 \) as \( \sigma \to \infty \), \( e^{-H_j(\sigma)} \to 0 \) and \( G_j(\sigma) \to 1 \).

Using the above lemmas, we know that both \( \hat{s}_j(\sigma) \) and \( \hat{l}_j(\sigma) \) approaches zero as \( \sigma \to \infty \) while keeping \( 0 < \sigma < \tau \). In the limit of \( \sigma \to \infty \) we obtain, using Eq. (C8) and initial conditions, \( \hat{s}_{B,0}(0) = 1, \hat{i}_{B,0}(0) = \hat{l}_{A,0}(0) = 0 \), and
\[
r_{AB}(\sigma) = \frac{\hat{i}_{A,0}(\sigma) + \hat{s}_{A,0}(\sigma) + \hat{i}_{A,0}(\sigma)}{\hat{i}_{B,0}(\sigma) + \hat{s}_{B,0}(\sigma) + \hat{l}_{B,0}(\sigma)} = \frac{(1 - P_A)\hat{s}_{A,0}(0) + \hat{i}_{A,0}(0)}{1 - P_B} \] (C11)

where \( r_{AB}(0) = \hat{i}_{A,0}(0) + \hat{s}_{A,0}(0) \). When \( \hat{s}_{A,0}(0) \gg \hat{i}_{A,0}(0) \), Eq. (4) is recovered, in the limit of \( \gamma_j \to \infty, \beta \to \infty \) and \( \sigma \to \infty \) while keeping \( 0 < \sigma < \tau \) (C12)
\[
r_{AB}(\sigma) = r_{AB}(0)(1 - P_A)/(1 - P_B)
\]

Moreover when \( P_A = 0, \gamma_A = 0 \) and \( \hat{s}_{A,0}(0) = 0 \), Eq. (2) is recovered, in the limit of \( \gamma_B \to \infty, \beta \to \infty \) and \( \sigma \to \infty \) while keeping \( 0 < \sigma < \tau \) (C13)
\[
r_{AB}(\sigma) = r_{AB}(0)/(1 - P_B)
\]

In case II-B we will prove that these ratios in Eqs. (C12) and (C13) remain unchanged in the limit of \( \tau = \infty \).

**APPENDIX D: NUMERICAL INVESTIGATION OF THE INVASION CRITERIA FROM EQUATIONS (2) AND (4)**

The invasion criteria in Eqs. (2) and (4) are exact in the limit of large infection-induced lysis rate \( \beta \) and contact rate \( \gamma \) with restrictions on \( P_B > 1 \) and on the spontaneous lysis rate \( \sigma \to \infty \). In order to investigate their validity for small \( \beta \) and \( \gamma \), we performed numerical simulations. First, the linear relationship in Eq. (2) between the phage patholgy \( P \) and \( r_{AB}(0)/r_{AB}(\infty) \) is validated by extensive numerical calculations with 2000 parameter sets where all parameters are selected uniformly from the biologically relevant intervals (see Fig. 4 for detailed information). Note that \( \chi P > 1 \) is used for numerical calculations. When \( \gamma \) and \( \beta \) are relatively large, i.e., \( 0.1 < \gamma, \beta < 10 \), all data points fall into the linear range \( r_{AB}(0)/r_{AB}(\infty) = 1 - P \) as illustrated in Fig. 4. When \( 0 < \gamma, \beta < 0.1 \), the deviation from the linear relationship increases for small phage pathology \( P \). Thus we conclude that the linear relationship in Eq. (2), \( r_{AB}(0)/r_{AB}(\infty) = 1 - P \), is robust to parameter variations and valid for small \( \gamma \) and \( \beta \).
Second, we also validate the generalized invasion criterion from Eq. (4) numerically with diverse sets of parameters. Fig. 8 shows that the linear relationship in Eq. (4) between $r_{AB}(0)/r_{AB}(\infty)$ and $(1-P_A)/(1-P_B)$ is robust against parameter variations. Note that we impose restrictions on $\chi_j > 1$ and $s_A(0) \gg i_A(0)$ in the numerical calculations. However the linear relationship in Eq. (4) becomes inaccurate when the pathogen is more virulent on the invading population A than on the resident population B, i.e., when $P_A$ is large and $P_B$ is small.

FIG. 7: Numerical verification of the invasion criterion in Eq. (2). A thick solid line is the prediction from Eq. (2), $r_{AB}(0)/r_{AB}(\infty)$ was numerically evaluated by solving Eq. (C1) with 2000 sets of parameters chosen uniformly in the intervals $0 < P < 1$ for phage pathology, $1/P < \chi < 100$ for burst size, $0 < \alpha < 0.5$ for normalized spontaneous induction rate, $0 < I_A(0), \phi(0) < 10S_B(0)$ for the initial concentrations of infected bacteria A and phage with respect to the initial concentration of susceptible bacteria B. Filled circles represent the data from 1000 sets of parameters with relatively large $\gamma$ and $\beta$ ($0.1 < \gamma, \beta < 10$). Open circles are from another 1000 sets of parameters with small $\gamma$ and $\beta$ ($0 < \gamma, \beta < 0.1$).

FIG. 8: Numerical verification of the generalized invasion criterion in Eq. (4). $r_{AB}(0)/r_{AB}(\infty)$ was numerically evaluated by solving Eq. (4) with 2000 sets of parameters chosen uniformly in the intervals $0 < P_A, P_B < 1$ for phage pathologies on the host A and B, $1/min\{P_A, P_B\} < \chi < 100$ for burst size, $0 < \alpha < 0.5$ for normalized spontaneous induction rate, $10^{-1}S_B(0) < S_A(0) < 10S_B(0)$ and $0 < I_A(0), \phi(0) < 10^{-2}S_B(0)$ for the initial concentrations of susceptible and infected bacteria A and phage. Filled circles represent the data from 1000 sets of parameters with relatively large $\gamma_j$ and $\beta$ ($0.1 < \gamma_j, \beta < 10$). Open circles are from another 1000 sets of parameters with small $\gamma_j$ and $\beta$ ($0 < \gamma_j, \beta < 0.1$).

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