# Immune Lag Is a Major Cost of Prokaryotic Adaptive Immunity During Viral Outbreaks

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**Abstract**

CRISPR adaptive immune systems enable bacteria and archaea to efficiently respond to viral pathogens by creating a genomic record of previous encounters. These systems are broadly distributed across prokaryotic taxa, yet are surprisingly absent in a majority of organisms, suggesting that the benefits of adaptive immunity frequently do not outweigh the costs. Here, combining experiments and models, we show that a delayed immune response which allows viruses to transiently redirect cellular resources to reproduction, which we call "immune lag", is extremely costly during viral outbreaks, even to completely immune hosts. Critically, the costs of lag are only revealed by examining the non-equilibrium dynamics of a host-virus system occurring immediately after viral challenge. Lag is a basic parameter of microbial defense, relevant to all intracellular, post-infection antiviral defense systems, that has to-date been largely ignored by theoretical and experimental treatments of host-phage systems.

## Introduction

CRISPR immune systems are the only known form of adaptive immunity found in prokaryotic organisms [38, 3]. These antiviral defense systems enable bacteria and archaea to incorporate short stretches of viral genetic material into specific loci on the host genome (the CRISPR "array") as individual immune memories, called "spacers" [3]. Spacers are later transcribed and processed into crRNA sequences that guide CRISPR-associated (Cas) proteins to degrade viral targets [8, 21, 12]. Thus, a genomic record of past infections is used to prevent future infection (see [41] for a recent review of the mechanisms of CRISPR immunity).

CRISPR systems are widely but sparsely distributed across the tree of life [29, 39, 33]. Their broad distribution among taxa is likely attributable to the fact that these systems are highly effective at clearing viral infections (e.g., [3]), extremely adaptable in a constantly shifting co-evolutionary arms race [16, 55], and easily and frequently horizontally transferred [51, 47, 58] (for reviews of various aspects of CRISPR biology see refs. [24, 34, 33, 63]). Yet, a majority of prokaroytes lack CRISPR immune systems [10], even as CRISPR can usually be found in a closely-related relative. To solve this apparent paradox various authors have proposed a number of costs and limitations of CRISPR immunity that may drive selection against this system in favor of alternative defense strategies (of which there are many [6]). These "cons of CRISPR" potentially include autoimmunity [53], the inhibition of beneficial horizontal gene transfer [30, 67], the inhibition of other cellular processes by the *cas* genes (specifically DNA repair; [5, 4]), incompatibility with lysogenic phage [49], and the possibility that CRISPR may be unable to keep up with extremely diverse pathogenic

environments [59, 28]. Nevertheless, experiments show that CRISPR can be essentially cost-free in phage-free culture conditions [64, 2, 35].

In contrast to results indicating that CRISPR generates little-to-no constitutive costs for the host in the absence of phage – at least in lab-reared *Pseudomonas aeruginosa* – a severe inducible cost of CRISPR immunity upon phage infection has been observed [64]. The source of the inducible cost of CRISPR immunity was, until recently, mysterious. Chabas et al. [13] suggested that the inducible cost is the result of transient expression of phage genes in the cell before CRISPR is able to clear an infection (figure 1). Recently Meaden et al. [35] have provided evidence confirming this lag hypothesis in experiments with *Pseudomonas aeruginosa* strain PA14 and its phage DMS3*vir*. When a virus infects a cell, it may reprogram that cell to become a "virus factory" (or "virocell"; [19, 18, 25]). At the same time, viral genes will be strongly upregulated. Intracellular defenses may take some time to find and degrade invading genetic material in the cell, and during that time transcription in an infected cell is transiently altered, potentially halting host growth and re-purposing cellular resources. This phenomenon, which we call "immune lag", was observed by Meaden et al. [35] in CRISPR immune cells, so that even when cells are able to effectively clear infections and prevent lysis they still pay a heavy growth cost associated with infection (figure 1). Could immune lag be a major cost of adaptive immunity, leading the host to sometimes favor alternative immune strategies?

Upon closer examination, the impact of immune lag on natural systems is less clear. Experiments that demonstrate the inducibile cost of CRISPR immunity require the host to be exposed to extraordinarily high viral titres (at least $10^8$-$10^{10}$ PFU/mL in our own experiments, described below) to see any effect. For lag to have any population-level impact on an immune host population, a substantial portion of the immune population must be exposed to phage. Thus, in the case of an already-immunized host population, lag is probably irrelevant because viruses have no way to reach sufficiently high titres to suppress the immune host.

Yet, in natural systems, host populations are rarely completely immune to their viral pathogens. CRISPR spacers are often lost [30, 60], and viral escape mutants frequently emerge [50, 7], both leading CRISPR to be a somewhat transient form of immunity [35]. In natural communities, entirely new species of virus, to which the host lacks preexisting immunity, may migrate into the system via dispersal [9]. Thus, to fully characterize the role of immune lag in natural systems, we must assess it's impact on non-equilibrium systems with viral coevolution or migration. We combined experiments and mathematical models to investigate how lag transiently affects the costs of CRISPR during a viral outbreak, and found that even completely-immune host cells face a strong fitness detriment when viruses invade a primarily susceptible host population. Importantly, the costs of lag are only revealed by examining the dynamics of the system that occur immediately after viral challenge.

# Models

## Replicon-Based Spacer Acquisition

We constructed a model of bacterial growth and evolution alongside a viral population, incorporating details about the CRISPR spacer acquisition process that are typically neglected (e.g., self versus non-self recognition). See S1 Table for parameter definitions. Specifically, we built on classical host-phage chemostat (or "virostat", see discussion below) models [54, 31, 62], where resources ($R$) are modeled explicitly as being supplied by some constant reservoir ($r_0$), and there is constant

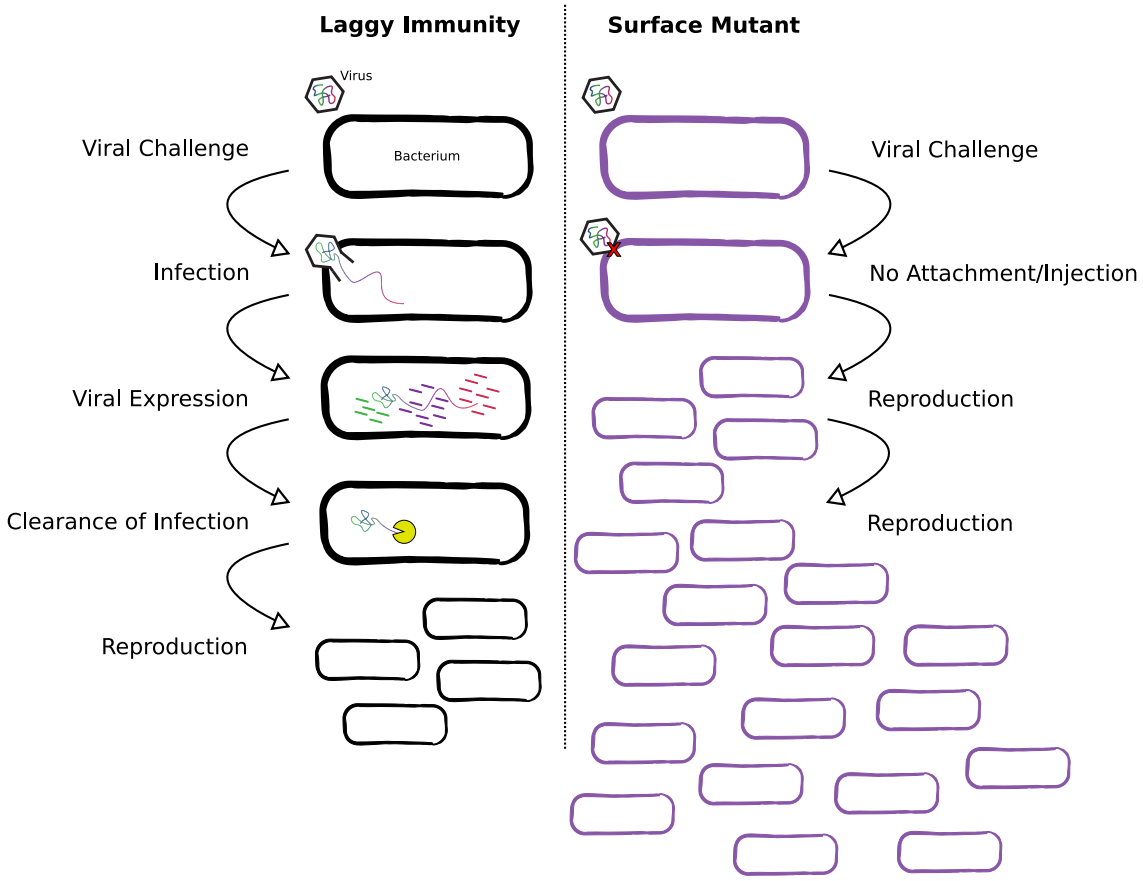


Figure 1: **During competition, immune lag can lead infected cells to face significant delays in reproduction while cellular resources are temporarily diverted to viral production before the infection can be cleared (left).** In contrast, an SM strategy does not allow viral genetic material to enter the cell in the first place, preventing any lag (right).

flow ($w$) through the system:

$$
\begin{aligned}
\overbrace{\dot{R}}^{\text{Resources}} &= \overbrace{w(r_0 - R)}^{\text{Flow}} - \overbrace{\frac{evR}{z+R}(S+C)}^{\text{Resource Uptake by Cells}} \\
\overbrace{\dot{S}}^{\text{Susceptible}} &= \left( \overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{\delta V}^{\text{Infection}} - \overbrace{w}^{\text{Washout}} - \overbrace{2\mu P}^{\text{Autoimmunity}} \right) S \\
\overbrace{\dot{C}}^{\text{Immune}} &= \left( \overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{w}^{\text{Washout}} - \overbrace{2\mu P}^{\text{Autoimmunity}} \right) C + \overbrace{\mu I_v}^{\text{Immunization}} \\
\overbrace{\dot{I}}^{\text{Infected}} &= \overbrace{\delta SV}^{\text{Infection}} - \overbrace{\gamma I}^{\text{Lysis}} - \overbrace{wI}^{\text{Washout}} - \overbrace{\mu I_v}^{\text{Immunization}} \\
\overbrace{\dot{I}_v}^{\text{Intracellular Virions}} &= \overbrace{\delta SV}^{\text{Infection}} + \overbrace{\gamma \log(\beta) I_v}^{\text{Reproduction}} - \overbrace{\left( \frac{\gamma e^{\xi \log \beta}}{\xi} \right) I}^{\text{Immunization Window}} - \overbrace{wI_v}^{\text{Washout}} - \overbrace{\mu \left( \frac{I_v^2}{I} \right)}^{\text{Immunization}} \\
\overbrace{\dot{V}}^{\text{Viruses}} &= \overbrace{w(v_0 - V)}^{\text{Flow}} + \overbrace{\beta \gamma I}^{\text{Lysis}} - \overbrace{\delta (S+C) V}^{\text{Adsorption}} .
\end{aligned}
\tag{1}
$$

We equipped our host population with a CRISPR system, so that there will be naive, undefended, but CRISPR-encoding host ($S$) that may become infected ($I$) by free virus ($V$) and may also undergo immunization to become defended (i.e., spacer-possessing) host ($C$). For some analyses we also included a costly-but-virus-resistant surface mutant (SM) strain in the model:

$$
\overbrace{\dot{M}}^{\text{SM}} = \left( \overbrace{\frac{(1-\kappa) vR}{z+R}}^{\text{Growth}} - \overbrace{w}^{\text{Washout}} \right) M \tag{2}
$$

with growth cost $\kappa$.

We explicitly modeled CRISPR's inherent capacity for self versus non-self recognition in our model. We did so by modeling the CRISPR spacer acquisition process as being "replicon-based", where the spacer acquisition machinery preferentially acquires spacers from double-strand breaks occurring at collapsed replication forks [32]. Accordingly, we tracked the number of intracellular viral replicons from which spacers can be acquired ($I_v$), which is a significant departure from previous models (see S1 Text for a discussion of how this equation was constructed). We modeled the immunization process as being specifically dependent on the number of viral replicons in a cell, assuming the majority of spacers are acquired from collapsed replication forks [32]. We assumed exponential growth of the intracellular viral population and defined a time-window ($\xi$) in which immunization is possible (accounting for the fact that spacer acquisition likely happens early on during infection [37]; changing the model to linear viral production makes little difference in our conclusions, S2 Text).

In addition to immunization, the process of auto-immunization will also be replicon-based. Bacterial cells may have high "effective ploidy" [11, 36], where cells require multiple active replication centers to achieve high growth rates given physical limitations on the rate of DNA replication [1, 40]. Let $P$ the minimum number of simultaneously occurring DNA replication cycles required to observe a given host growth rate and let $\alpha$ be the maximum rate of genome replication on a single strand (theoretically $\sim$ 600bp/sec [65, 62]). Assuming that $\alpha$ is constant across growth rates, $P$ can be expressed at the rate of host cell duplication divided by the rate of host genome duplication:

$$P = \frac{vR}{\alpha(z+R)}. \tag{3}$$

Thus, assuming bidirectional replication, and letting $\mu$ be the per-replicon-per-hour spacer acquisition rate, the rate at which self targeting spacers are acquired will be $2\mu P$. Note that at low growth rates $P$ can drop below one, corresponding to the proportion of time that the genome must be actively replicating to obtain the observed growth rate. In reality bacteria may have multiple ways of further differentiating self from non-self [61] (e.g., by the presence of Chi sites on the host genome [32, 37]) so that our model represents a "worst case scenario" for the host in terms of autoimmunity. Nevertheless, in agreement with experimental data that detects no constitutive cost of CRISPR immunity [64, 2, 35], our model predicted that there should be essentially no impact of autoimmunity on the hosts' fitness (S3 Text, S1 Figure).

Finally, observe that in a small departure from the classical chemostat model we allowed constant immigration of viruses into the system from some environmental pool ($v_0$). This is an entirely experimentally tractable modification (e.g., by adding set concentrations of virus to the resource reservoir), and better represents natural systems which are not closed and are likely face constant challenge by newly-arriving viruses. For traditional continuous culture without viral inflow simply let $v_0 = 0$.

## Immune Lag

Meaden et al. [35] provide strong evidence that the inducible cost of CRISPR is associated with transient expression of viral genes and possible virus-induced reprogramming of cellular transcriptional networks. Because CRISPR immunity does not remove viral genetic material from the cell instantaneously, even fully immune hosts face a temporary growth setback during infection while viruses transiently reprogram the cell (figure 1). Virus-resistant surface mutants do not experience this growth setback, as viruses are unable to adsorb to the cell in the first place. Consider the replicon-based acquisition model above. We added an equation for transiently infected but immune host ($L$):

$$\overbrace{\dot{L}}^{\text{Lagged}} = \overbrace{\delta DV}^{\text{Infection}} - \overbrace{\phi L}^{\text{Clearance}} - \overbrace{wL}^{\text{Washout}} \tag{4}$$

and modified the equation for immune host accordingly:

$$\dot{C} = \left( \overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{\delta V}^{\text{Infection}} - \overbrace{w}^{\text{Washout}} - \overbrace{2\mu P}^{\text{Self-Targeting}} \right) C + \overbrace{\mu I_v}^{\text{Immunization}} + \overbrace{\phi L}^{\text{Clearance}} . \tag{5}$$

We also found that lag can be modeled in a simple four-parameter system with qualitatively similar results (S4 Text and S2 Fig). Thus, for completeness and comparison with experimental results, we present a parameter-rich model, but our results can be replicated with minimal models of host-phage interactions.

## Upregulation of the CRISPR Locus

CRISPR can be strongly upregulated in response to infection [48, 46], or in situations where there is likely a high risk of infection [66, 44, 26, 45, 27]. The specific regulatory cues used by the CRISPR locus are diverse [46], and new methods are being developed to probe them [22]. Consider the case where the CRISPR locus is specifically upregulated in response to infection. We implemented this scenario by letting recently immunized and lagged cells pass into a "fast" immunity ($C_F$) state where the CRISPR system does not experience immune lag because the *cas* targeting genes are upregulated:

$$\dot{C}_F = \left( \overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{w}^{\text{Washout}} - \overbrace{2\mu P}^{\text{Self-Targeting}} \right) C_F + \overbrace{\mu I_v}^{\text{Immunization}} + \overbrace{\phi L}^{\text{Clearance}} - \overbrace{\zeta C_F \left( \frac{C_F}{C_F + \delta V} \right)}^{\text{Downregulation}} \tag{6}$$

and modified the equation for immune host accordingly:

$$\dot{C} = \left( \overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{\delta V}^{\text{Infection}} - \overbrace{w}^{\text{Washout}} - \overbrace{2\mu P}^{\text{Self-Targeting}} \right) C + \overbrace{\zeta C_F \left( \frac{C_F}{C_F + \delta V} \right)}^{\text{Downregulation}} \tag{7}$$

Note that we do not include any cost of increased transcription/translation in this model, as we have no empirical estimate or intuition for the scale of this cost, though one almost certainly exists (since in the absence of a cost the expression of CRISPR-Cas would be expected to be constitutively high). Also, observe that we modeled an upregulation of the *cas* targeting genes, which will reduce or eliminate immune lag (in our case eliminate), rather than the *cas* acquisition machinery, which would possibly increase autoimmunity (though both may be upregulated during infection since the *cas* genes are often transcribed as an operon). Finally, the return to a downregulated state will be prevented if there are still many viruses in the environment infecting our "fast" immune cells, hence our inclusion of a nonlinear downregulation rate that will approach zero at high multiplicity of infection (MOI).

# Results

## A Tipping Point between CRISPR and SM Strategies at High Viral Titres

We analyzed our model to find the long-term equilibrium outcome of competition between a laggy CRISPR-immune strain and a costly SM strain (S5 Text; $\kappa = 0.01$). Over a wide range of parameter values the model yielded a single CRISPR-immune equilibrium where the SM strain went extinct (figure 2). Only when there was extremely high flow of viruses into the system did we see an

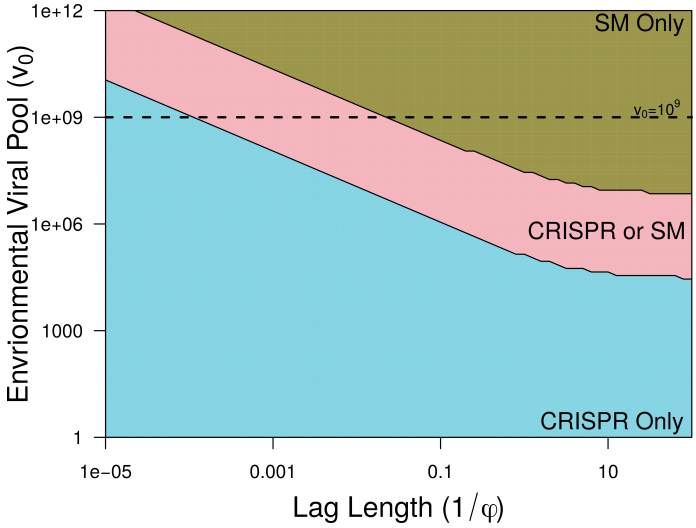


Figure 2: **Model equilibria show a transition from complete reliance on CRISPR immunity to complete reliance on surface modifications by the host at very high viral titres.** No parameter conditions leading to stable coexistence of the two host strains were observed. Blue region denotes region of parameter space in which the CRISPR-only equilibrium was the only stable equilibrium and brown denotes the region in which the SM-only equilibrium was the only stable equilibrium. Pink denotes the region in which both CRISPR-only and SM-only equilibria were stable, indicating that the final state of the system depends on initial conditions. See S5 Text for details of model analysis.

alternative outcome where the system settled in an SM-only state and the CRISPR immune strain went extinct. For short lag times ($\phi \geq 10^3$), the "tipping point" from an all-CRISPR to all-SM state occured as the external viral pool ($v_0$) exceeded concentrations of $10^9$ PFU/mL (figure 2). In no case did the two strains, CRISPR and SM, coexist stably over the parameter regimes considered. Thus our model predicts a sharp transition from a CRISPR strategy being favored to an SM strategy being favored at high viral titres. This is consistent with previous work on inducible immunity that saw a steep decrease in the relative fitness of a CRISPR immune strain when competed against an SM strain at very high viral titres [64].

Yet, previous experiments appear to disagree on the severity of the inducible cost of CRISPR immunity. In the original work on the topic, Westra et al. [64] observed a steep transition from high relative fitness ($> 1$) to a relative fitness of essentially zero for a CRISPR strategy competed against an SM strategy in competition experiments with increasing MOI, consistent with our model's predictions (S3 Figure). More recently, Alseth et al. [2], did not observe this steep fitness decrease while performing nearly identical experiments. We suspected that these later experiments failed to capture the transition from high to low relative fitness seen in our model because they were not carried out to a sufficiently high viral titre. Therefore, we replicated the Alseth at al. [2] experiments with the same host-phage system, *Pseudomonas aeruginosa* UCBPP-PA14 and its lytic phage DSM3*vir,* out to a higher viral titre ($10^{10}$ PFU/mL) and were able to capture the steep decrease in fitness of the CRISPR immune strain at high MOI (figure 3), confirming our model

predictions. We replicated these competition experiments *in silico* to more precisely illustrate this point (figure 3, red line). Our lag model captures the major shift from CRISPR-to-SM strategy that happens at high viral densities as seen by Westra et al. [64], consistent with the idea that immune lag causes the inducible cost of CRISPR immunity (figure 3, S3 Figure).

Finally, we note that while the qualitative results of these competition experiments are highly reproducible, with a steep decrease in the fitness of CRISPR immune strains occurring at high MOI, where exactly this transition occurs and the baseline relative fitness of the CRISPR immune strain in the absence of virus appear to be quite variable between replicates and experiments (figure 3, S3 Figure). Viruses and host cells were quantified using serial dilutions, introducing the possibility of multiplicative errors and perhaps making cross-experiment variability less surprising. This cross-experiment variability prevented us from obtaining precise lag estimates (we estimate that $10^{-3} \leq \frac{1}{\phi} \leq 0.1$). Initial host density in particular can strongly affect model expectations (S4 Figure). For model results reported below we include an analysis of both short ($\phi = 10^3$) and long ($\phi = 10$) lag times to capture the full range of experimental variability.

## Immune Lag Is Extremely Costly During an Outbreak of Novel Virus

We found that during an outbreak of a novel virus immune lag can be extremely costly, leading the host to potentially abandon a CRISPR strategy in favor of an SM strategy. This cost is only apparent when we examine the non-equilibrium dynamics of our model and is relevant to natural systems where outbreaks of novel or mutant viral strains may occur at moderate to high frequency. Unlike our results described above for systems at equilibrium, even a very low rate of viral inflow to the system can lead to a massive inducible cost of CRISPR immunity if most host are not already immunized.

We solved our lag system numerically starting from a dense resident susceptible population ($S = 10^8$), small populations of CRISPR immune and SM host ($C = M = 100$) and a small environmental viral pool ($v_0 = 100$). During the resulting outbreak, as the viral population spikes early it suppresses the initial growth of CRISPR-immune host, leading the SM population to dominate (figure 4a-c). This initial dominance of SM even occurs when the cost of an SM strategy is very high (S5 Figure). The only way for CRISPR immune host to dominate during an outbreak is for the duration of immune lag ($\frac{1}{\phi}$) to approach zero, but even short lags ($\phi = 10^3$) result in a substantial initial expansion of the SM population (figure 4b).

Even though an SM strategy will dominate immediately after an outbreak, if the SM strategy is sufficiently costly then the system will eventually return to a CRISPR-dominated equilibrium. How long will this return to CRISPR-dominance take, and what happens if the system is perturbed again before then? In natural communities, novel viral strains to which the host lacks preexisting immunity may emerge via mutation or immigrate into the system via dispersal. We found that even moderately frequent outbreaks can prevent the host from adopting a CRISPR-based defense strategy.

We simulated our system's dynamics under repeated outbreaks at set intervals (see Methods), corresponding to either the emergence of an escape mutant in the viral population or the arrival of a novel viral species into the system against which the host lacks a preexisting spacer. We observe that for long lags ($\phi = 0.1$), if outbreaks occur even with moderate (monthly) frequency, immune lag will prevent a CRISPR strategy from rising to dominance (figure 4d). These results agree with empirical observations that the repeated addition of susceptible host into a host-phage system promotes the evolution of an SM strain over a CRISPR immune strain [13].

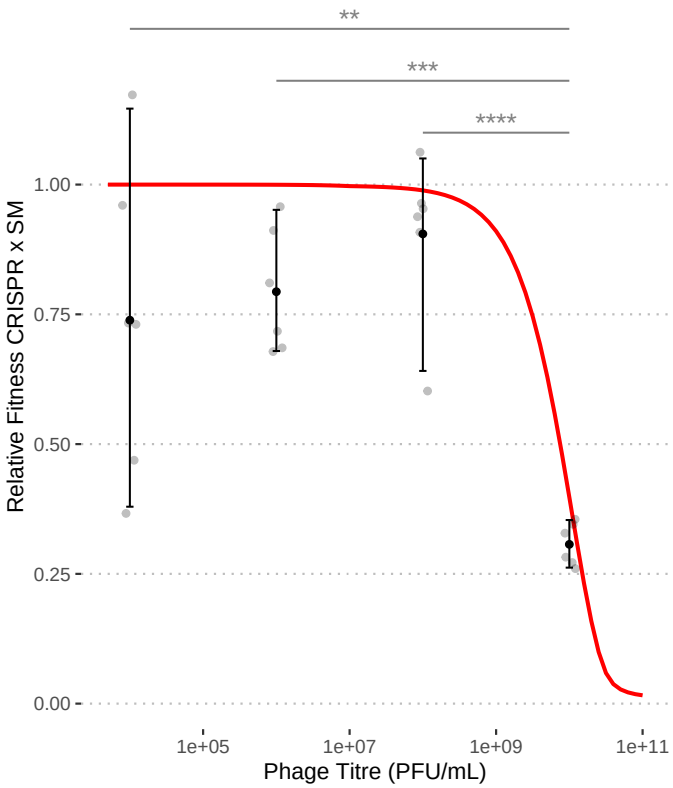


Figure 3: **A strongly inducible cost of CRISPR immunity at high viral titre is consistent with "laggy" CRISPR immunity.** (a) Competition experiments between BIM2 and SM strains in the presence of various viral titres. Fitness calculated based on densities one day post infection. Red line shows results from *in silico* competition experiements with no cost for SM ($\kappa = 0$) and a short lag time ($\phi = 10^3$) for comparison. Stars indicate significant differences between conditions (**, $p < 10^{-2}$; ***, $p < 10^{-3}$; ****, $p < 10^{-4}$; Tukey's Honest Significant Difference Test). These results qualitatively reflect the outcomes of similar previous experiments with the same strains (S3 Figure; [2, 64]), though that work suggests a much longer lag period ($\phi \approx 10$).

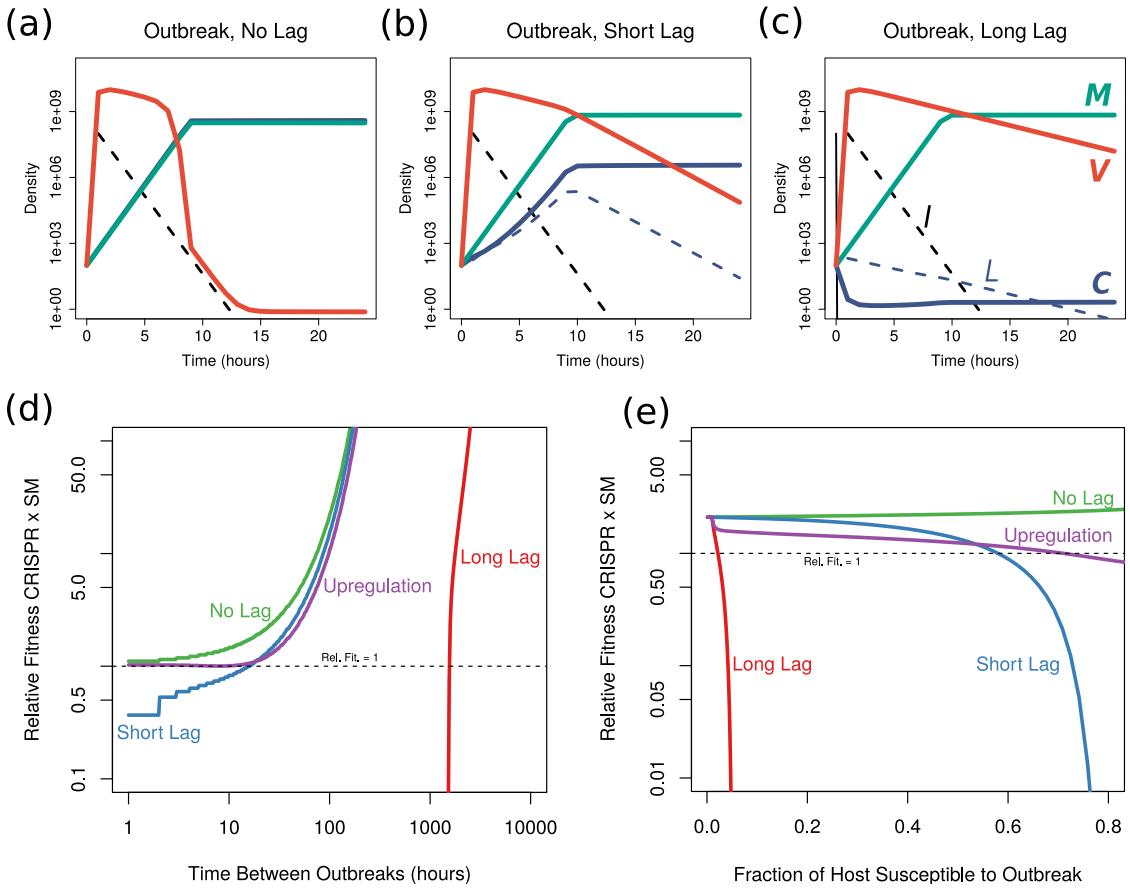


Figure 4: **Immune lag prevents the dominance of a CRISPR immune ($C$) strategy over a costly SM strategy ($M$) during repeated outbreaks.** (a-c) Immune lag prevents CRISPR from out-competing a costly SM strategy ($\kappa = 0.01$) early on in an outbreak (starting from a dense susceptible population, $S = 10^8$). Numerical solutions for lag model with CRISPR-immune (purple), SM (green), viral (orange), and susceptible (black) populations shown. Note that as the system approaches equilibrium CRISPR will eventually out-compete the SM population, regardless of lag. (d-e) Immune lag prevents CRISPR from out-competing an SM strategy when outbreaks are frequent. Results shown for iterated outbreak model with the size and frequency of outbreaks varied. Outbreaks of novel virus must be rare or affect a small fraction of the community for CRISPR to out-compete an SM strategy. "Short" and "long" lags correspond to the upper and lower bounds estimated for $\phi$ in figure 3 ($\phi = 10^3$ and $\phi = 10$, respectively). "Upregulation" refers to a system with a CRISPR immune strain with a long lag ($\phi = 10$), but that can be upregulated to a "fast" immune state ($\zeta = 0.1$). In (e) outbreaks affect 50% of the host population (interval between outbreaks varied) and in (d) outbreaks occur every 24 hours (fraction of host population affected varied).

CRISPR systems have one distinct advantage that our model neglects – different host may have different spacers, leading to a great deal of immune diversity in the population. This diversity is protective, as it makes it much more difficult for viral escape mutants to gain a foothold [15, 14, 57]. To account for host immune diversity, we varied the fraction of the host population susceptible to each novel viral outbreak (figure 4e). Even daily outbreaks affecting less than 10% of the host population will prevent the dominance of a CRISPR defense strategy when lags are long ($\phi = 10$). Given that viral outbreaks are generally expected to affect the most abundant host sub-populations [56], for CRISPR to dominate in the presence of small, frequent outbreaks the host population would need to be incredibly immunologically diverse. Thus, while spacer diversity can in part mitigate the effects of immune lag, that diversity must be extremely high to do so when outbreaks are even moderately frequent.

### Inducible Defenses Can Mitigate The Effects of Immune Lag

The *cas* operon, or a subset of *cas* genes, are often upregulated in response to infection or to conditions that indicate a high risk of infection [46]. We found that if strong upregulation occurs after infection, so that the resulting "fast immune" cells with an upregulated CRISPR locus do not experience lag, the overall effects of immune lag can largely be mitigated during an outbreak (figure 4d-e). This result is relatively robust to variations in the rate at which cells return to normal expression levels ($\zeta$), though high rates of return will ameliorate lag less than lower rates (S7 Figure).

### Laggy Immunity is Less Costly in Slow Growing Hosts

It has been suggested that slow growing and less dense host populations will favor CRISPR immunity whereas fast growing, dense populations will favor alternative defense strategies (e.g., SM; [59, 28]). Consistent with growth affecting the evolution of host defense strategy, Westra et al. [64] showed that high resource environments favored an SM strategy over a CRISPR strategy in direct competition experiments. We wondered if lag could partially explain this phenomenon. Using our lag model, we found that in host populations with a high maximal growth rate the cost of immune lag was much greater than in slower growing populations (figure 5). Thus, if CRISPR immunity is laggy, it is much more likely to be favored over an SM strategy if the host has a slow maximal growth rate. The opposite is true when CRISPR has no lag (figure 5). Intuitively, the temporary slow down in growth for host with a laggy immune systems is felt less strongly if growth is already slow, whereas the impact is much greater in a highly competitive system with fast growers.

## Discussion

We built a biologically-motivated model of CRISPR immunity that links population-scale host-virus dynamics to molecular-scale changes within the cell. In doing so, we were were able to demonstrate that immune lag can strongly impact the evolution of immune strategy in prokaryotes [35, 64]. Immune lag's effect is felt most severely during an outbreak of novel virus. We showed that for even moderately frequent outbreaks of novel viruses that are unrecognized by the CRISPR immune system, immune lag will lead to selection against CRISPR in favor of other defense strategies (e.g., surface modifications). Note that we are making an argument here about selective forces acting on immune strategy – the rate and mechanics of how CRISPR functionality might be lost in natural

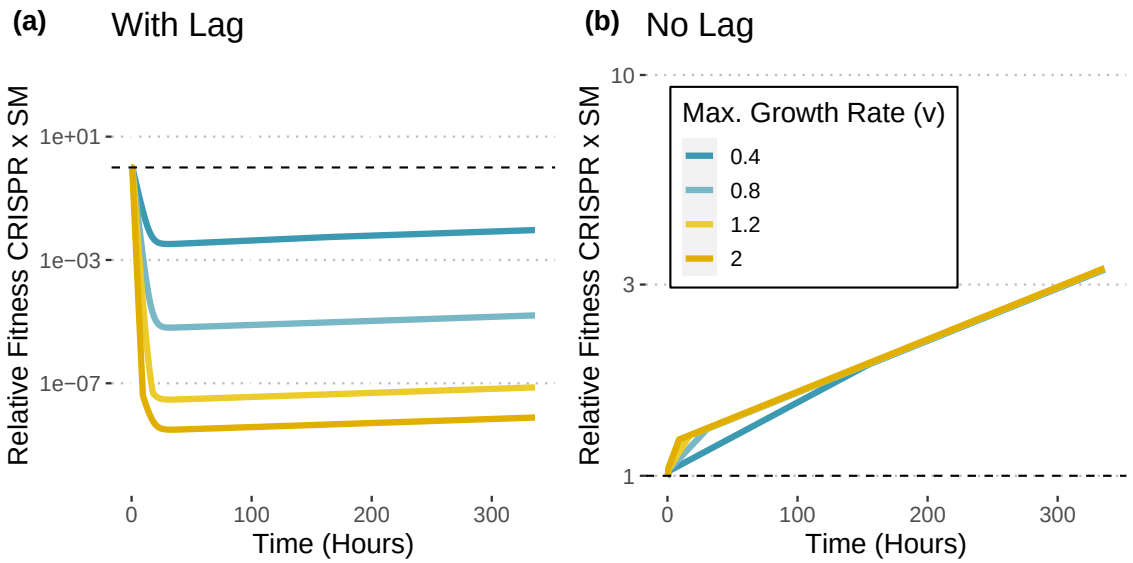


Figure 5: **Immune lag is less costly in systems with slow growth.** Change in relative fitness with respect to $t = 0$ during a viral outbreak in host populations with different maximal growth rates ($v$). (a) Relative fitness for the CRISPR immune strain competed against an SM strain drops off very steeply at the outset of an outbreak for faster growth rates (starting from a dense susceptible population, $S = 10^8$; long lag time, $\phi = 10$). The drop in relative fitness is less severe in slow growing host populations. (b) For a system without lag this pattern is flipped, with CRISPR immune strains having higher relative fitness when growth rates are high.

settings, as well as the implications of this loss, are outside the scope of this study and have been explored elsewhere [30, 60].

Even considering the beneficial effects of priming seen in many systems [16, 55], where partial matches allow host to "update" their immune memory against viral escape mutants, it is not unreasonable to expect wholly novel outbreaks on a daily or weekly timescale for natural systems with high viral migration. Our results emphasize the benefits of having multiple redundant spacers towards the same target, as even temporary loss of immunity in a subset of the population can lead to strongly negative fitness effects for the entire immune host population due to lag induced by the resulting high-density viral bloom.

Immune lag has strong negative fitness effects even when CRISPR immune strains are competed against very costly SM strains. In culture, SM strategies may occasionally be essentially cost-free, but in natural systems surface molecules often play an important role in host fitness which can prevent the emergence of SM strains [2, 23]. Nevertheless, during an outbreak in a primarily susceptible population very costly SM strains still rise to dominance (S6 Figure). Thus lag is likely to be relevant even in natural systems where surface modifications are very costly.

Some immune host strains may be able to partially avoid the effects of lag. The impact of lag can be mitigated by upregulation of the *cas* locus (figure 4). Thus lag may help explain both why expression of the cas genes is tightly regulated in many systems [2, 35]. Additionally, lag seems to have less of an impact on slow growing host populations, perhaps explaining in part the suggested, though not yet systematically demonstrated, pattern in which CRISPR is more common among slow growing and low density taxa [59, 28].

Finally, it is clear that the severity of immune lag is a shared trait determined by both host and virus. Different viruses reprogram the cell in different ways and to different degrees (e.g., [25]). Similarly, hosts will likely vary in their susceptibility to reprogramming. Our overall conclusions are relatively insensitive to this variability, as even short lags can have a severe impact on host fitness during an outbreak (S5 Figure). Nevertheless, this variability makes it difficult to put realistic bounds on our lag parameters, and as we show in figure 4, upregulation of the CRISPR locus may mitigate immune lag for the host [46]. In any case, we highlight an important parameter of virus-host dynamics, the recovery rate of defended cells to viral infection ($\phi$), that is not typically measured or considered in theoretical treatments of host-phage systems. This parameter is likely universal to all intracellularly-acting DNA- or RNA-degrading defense systems, including restriction-modification systems, which are nearly ubiquitous [43]. Immune lag is quite possibly a widespread phenomenon common to many classes of defense systems acting within the cell (e.g., [17, 20, 42]), and deserves consideration in any population-level study of prokaryotic antiviral defenses.

# Methods

All code and raw data necessary to run models and generate figures available at `https://github.com/jlw-ecoevo/immunelag`. See S5 Text for model analysis.

## Competition Experiments

For the competition experiment shown in figure 3, a *Pseudomonas aeruginosa* UCBPP-PA14 bacteriophage-insensitive mutant with two CRISPR spacers (referred to as BIM2), a surface mutant derived from the PA14 *csy::LacZ* strain, and the *P. aeruginosa* specific lytic phage DMS3*vir* were used. Prior to experiment start, bacteria were grown for 24 h in glass microcosms containing 6 ml

LB medium, being incubated at 37°C while shaking at 180 r.p.m. The two PA14 phenotypes were competed against each other for 24 hours in the presence of phage DMS3*vir* at either $10^4$, $10^6$, $10^8$, $10^{10}$ PFU/ml ($n = 6$ per treatment) while incubated under the same conditions as described above. Bacteria were sampled at timepoint 0 and 24 h post phage infection, before being diluted in M9 salts and plated out on LB agar. The agar was supplemented with ca. 50 µg $mL^{-1}$ X-gal to differentiate between the BIM2 and surface mutant. Bacterial colonies were counted, after which relative fitness was calculated as previously described [64].

## Modeling Competition Experiments

We modify our model to take place in serial transfer, where the change resource density is described by a constant decay over time until the next transfer

$$\dot{R} = -\frac{evR}{z+R}(S+C+M) \tag{8}$$

and the washout terms are omitted from equation (1). Based on an equilibrium host density in LB of $\sim 10^9$ CFU/mL, we estimate an initial resource density at each transfer of $R(0) \approx 500$ $\mu$g/mL. We initialize the experiment with with a density of $10^7$ CFU/mL of CRISPR-immune and and SM host (no susceptible) and solve our system numerically at 24 hours. Viruses are either added at varying initial densities up to $10^{11}$ PFU/mL. System was solved numerically using "lsoda" solver in the deSolve R package [52].

## Modeling Intermittent Outbreaks

To model the effects of intermittent outbreaks we solved our ode system with immune lag in a chemostat/virostat ($v_0 = 100$). The outbreak interval was set at some length $\tau$, and the system was solved numerically to this point (using the "lsoda" solver in the deSolve R package [52]). The solver was then paused, and the identity of the viral pathogen flowing into the system was changed to a novel strain (implemented by letting $U = f \times (C + L + M)$, $C = (1 - f) \times C$, $L = (1 - f) \times L$, and $M = (1 - f) \times M$). This was repeated for 10 iterations of length $\tau$ and relative fitness was calculated by comparing initial to final densities of CRISPR immune and SM populations. Importantly, we let both CRISPR immune and SM populations experience the outbreak in order to directly compare the ability of these strategies to deal with repeated infection. This setup is similar to the experiments performed by Chabas et al. [13] who found similar results in an empirical system. We also neglect initial immunization dynamics by starting the population in an immune state ($C(0) = M(0) = 5 \times 10^7$). For figure 4d we let $f = 0.5$ and for figure 4e we varied $f$.

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