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The Effect of Multiplicity of Infection on the Temperateness of a Bacteriophage: Implications for Viral Fitness

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Abstract— **Since phage-bacteria interactions are among the most abundant in nature, they have become in the object of intensive study. In particular, how phages evolved temperateness – the propensity of the phage to enter lysogeny – and how temparateness has been optimized, remains poorly understood. In this work, we study the advantages of the lysogeny over the lytic path in phages under fluctuating environments. Also we explore how multiplicity of infection (MOI) drives the decision made by the phage. We found that temperate phages might use the lysogenic path to protect themselves from long periods of bad conditions. Additionally, phages with MOI strategies might have better chances of survive long periods of detrimental conditions.**

I. Introduction

Since the evolution of parasites and pathogens is important to human [1], agricultural, and wildlife systems, there is a mature theory that focuses on how infection mechanisms may evolve. Given that viruses are the most abundant and simple entities on the planet, they are frequently used as models for studying parasite-pathogen evolution. In particular, parameters like replication, lysis time, adsorption among others have been suggested as possible knobs used by viruses to drive infection [2]–[5].

Bacteriophages are viruses that infect bacteria. Their natural environment is challenging, characterized by fluctuating host cell populations and other sources of stress to the phage [6]. In this situation, a phage has two courses of reproductive action: lysogenization or initiation of the lytic cycle (see Fig. 1). Lysogenization is a means for the phage to lie dormant inside of a bacterial host by integrating viral nucleic acid into the genome of the host cell [7], [8]. A phage thus integrated is called a prophage. While the prophage remains latent, it does not impede the host cell in any way. The bacterium will continue thriving and propagating, copying and transmitting the prophage into its progeny. This allows the phage to reproduce without exposing itself to the detrimental effects of the outside environment. The lysogen has the ability to maintain its current state of latency or undergo induction. When induction occurs, prophage DNA is cut off from the bacterial genome and coat proteins are produced via transcription and translation of the phage DNA for the regulation of lytic growth.

In the lytic cycle, the genome of the phage is inserted into the cytoplasm of the bacterium. The DNA resides separately from that of the genetic material of the host. Replication of the phage begins and, once many phage components have been created, new phage are produced. Over time, the phage will begin to accumulate within the host cell. This eventually results in the lysing of the bacterium and the release of the free phages. A phage that has the ability to enter lysogeny is called a temperate phage, and its temperateness can be understood as the propensity of the phage to enter a lysogeny. The idea of temperateness has been greatly deliberated and analyzed [9], [10]. However, the main questions still stands: what advantage does a phage derive by being able to switch between the lysogenic cycle and the lytic cycle?

One mechanism phages might use to regulate temperateness is by means of the multiplicity of infection (MOI) – the ability of a phage to infect an already infected cell. To gain a more concrete understanding of the behavior of the temperate phage, we analyzed how MOI may affect the probability of entering the lysogenic cycle. Mathematical modeling of a multitude of fluctuating environments [11]–[18] allow us to theoretically and quantitatively understand the adaptive nature of fixed and plastic latency.

In this paper, we explore the advantages of choosing the lysogenic path under fluctuating environments. Additionally, we explore the extra advantages provided by MOI. The paper is organized as follows: first we describe the modeling

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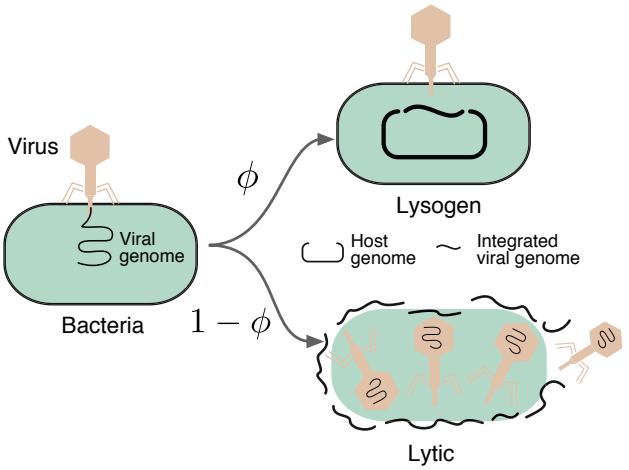

Fig. 1. Plasticity of the temperate phage. Under the lysogenic cycle, the cell can either undergo the lytic cycle with a probability of $1-\phi$ or integrate the prophage into the genome of the host cell with a probability of ϕ.

approach used to represent the dynamics of the different species under fluctuating environments. Then we explore the lysogeny-lytic decision made by the phages. We study the impact of MOI on the overall fitness of the phage and present some conclusions.

II. Model

We modeled the fluctuating environments using a similar approach as in [19]. Consider a situation where the environment fluctuates between "good" and "bad" conditions. The "good" conditions allow for an environment in which the phage is proliferating efficiently through a replenishing population of bacteria. The "bad" conditions result in the entirety of the free phage population decreasing to 0. This would be consistent with an environment of extreme host scarcity. The phage has no control over its environment. However, it does have the ability to infect a host with a probability $k > 0$ assuming the "good" conditions. Then, with a probability of ϕ, the lysogenic cycle can be induced. This allows the phage to reproduce after a period of dormancy by incorporating it's own DNA into the genome of the host. Furthermore, the phage has a probability of $(1-\phi)$ to enter the lytic cycle. This causes the bacteria to lyse and, in turn, create new free phage. These new phage are represented by BV where B is the phage burst size per infection. Fluctuations in the free phage and lysogen populations during the "good" condition are represented by

$$\mathrm{V} \xrightarrow{(1\text{-}\phi)\,\mathrm{k}} \mathrm{B\,V}, \quad \mathrm{V} \xrightarrow{\phi\mathrm{k}} \mathrm{L}, \tag{1}$$

During the "bad" condition, the free phage die out completely and the lysogen population decreases at a fixed rate. The change of lysogen population under "bad" conditions is given by

$$\mathrm{L} \xrightarrow{\alpha} \emptyset, \tag{2}$$

where $\alpha > 0$ is the degradation rate of the lysogens. Once the "bad" condition ends, all lysogens release their phage (BL), and a new "good" condition starts. This does not imply the existence of a sensor in the lysogens that recognize the good condition. This is a simplification, without loss of generality, since the contribution of new free phage will be proportional to the lysogen population at the beginning of the good time. Additionally, the exponential growth via the lytic pathway rapidly overwhelms any residual contribution of remaining lysogens from the previous round.

The dynamics of such phenomena can be represented by the hybrid system depicted in Fig. 2a. The ellipses represent the good and bad environment situations. Arrows represent birth, death or environmental switching events. Free phage is created in bursts of fixed size B at a rate $(1-\phi)k\,v$. Alternatively, new infections might choose the lysogenization path, which happens at a rate $\phi\,k\,v$, adding an extra lysogen to the system. A timer variable keeps track of the elapsed time in the good condition until time τ_g (event rate is $\delta(\tau-\tau_g)$). After this time, the environment switches to bad conditions, resetting the free phage population and the timer τ to zero. In bad conditions, death solely occurs at a rate αl. Once ($\tau = \tau_b$), the environment returns to good conditions,τ is reset to zero, and initial conditions are set to a free phage population proportional to the lysogen population at the end of the bad time, with lysogen population set to zero.

III. Why do Bacteriophages display Temperateness?

To study the above hybrid system, an equivalent model can be constructed by assuming the deterministic counterpart (see Fig. 2b). Let τ_g and τ_b be the time spent in the good and bad environment, respectively. Starting with a single copy, the free phage and lysogen count at the end of the good environment are given by

$$v\left(\tau_g\right) = e^{kB(1-\phi)\tau_g},\; l\left(\tau_g\right) = \frac{\phi\left(e^{kB(1-\phi)\tau_g}-1\right)}{B(1-\phi)}. \tag{3}$$

Note, that when the probability ϕ of becoming a lysogen is 0, the lysogen count is zero. For example, a completely lytic virus will produce zero lysogens. Although, if ($\phi = 1$), the average lysogen count is $k\,\tau_g$. Fig. 3 shows the lysogen population at the probability range of $\phi \in [0,1]$. Note that phage fitness is optimized for a value between 0 and 100% chance of lysogeny.

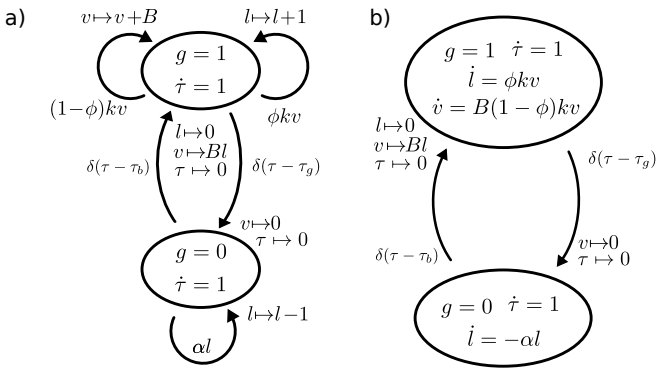

Fig. 2. Hybrid systems describing the fluctuating environment that lysogenic capable phage might face. a) The environment dynamics is described by one birth-death processes and a pure death process. Under good environment situations ($g = 1$), phage might produce bursts of B free phage particles at a rate $(1-\phi)\,k\,v$. Alternatively, the virus might go lysogenic. A timer τ keeps track of the elapsed time in each condition. Once time spend under good conditions is τ_g, the environment switches to bad conditions by resetting τ, g, and v to zero. Under bad conditions, lysogenic cells die at a rate αl. When the time spent in the bad conditions is complete ($\tau = \tau_b$), the environment switches back to good conditions by resetting $g = 1$, $v = Bl$, $l = 0$, and $\tau = 0$. b) Deterministic version of the hybrid system on part a).

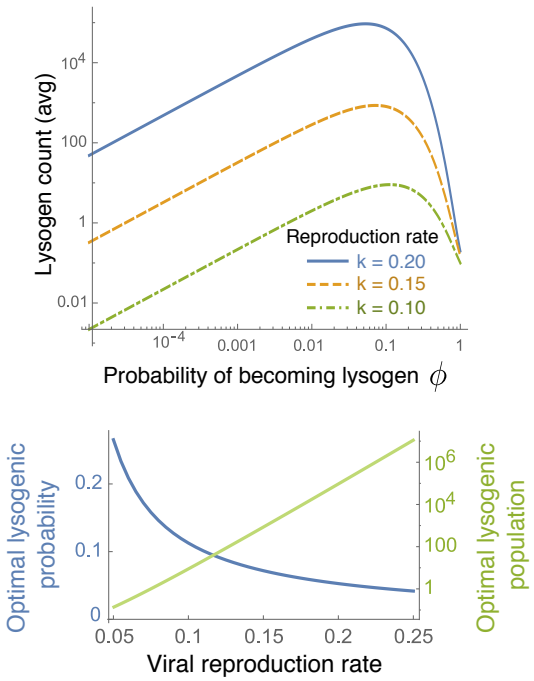

Fig. 3. Effects of lysogenic probability on phage infection. a) Population count vs lysogenic probability for several infection rates. Note that average lysogenic count is optimized for a value between 0 and 100% chance of lysogeny. b) The optimal chance of lysogeny decreases as the phage reproduction rate increases.

Next, we speculate how a phage can spread over multiple rounds of good-bad conditions. Since the free phage is wiped out completely during bad conditions, the only way for the phage to propagate is through the lysogenic cell. Therefore, we are interested in the conditions that allow for the lysogenic cells to thrive. At the end of the first round, the lysogenic count is given by the equation 3

$$l_0 = \frac{\phi\left(e^{kB(1-\phi)\tau_g}-1\right)}{B(1-\phi)}e^{-\alpha\tau_b}. \tag{4}$$

Subsequent rounds ($n > 1$) are described by

$$l_n = \frac{\phi\left(e^{kB(1-\phi)\tau_g}-1\right)}{(1-\phi)}l_{n-1}e^{-\alpha\tau_b} \tag{5}$$

$$= \frac{1}{B}\left(\frac{\phi\left(e^{kB(1-\phi)\tau_g}-1\right)}{(1-\phi)}e^{-\alpha\tau_b}\right)^n. \tag{6}$$

The lysogenic population will grow unbounded when

$$\frac{\phi\left(e^{kB(1-\phi)\tau_g}-1\right)}{(1-\phi)} > e^{\alpha\tau_b}, \tag{7}$$

i.e., when the amount of phage (in form of lysogens) per infection during the good condition is larger than the average lost per infection during the bad condition.

Now, the question is how this optimal lysogen count reflects on the phage population in subsequent rounds of good-bad environments.

Since the free phage count is zero during the bad conditions, the lysogen concentration at the end of this cycle is given by

$$l\left(\tau_g+\tau_b\right) = \frac{\phi\left(e^{kB(1-\phi)\tau_g}-1\right)}{B(1-\phi)}e^{-\alpha\tau_b}. \tag{8}$$

At the end of the next good condition, the free phage concentration can be written as

$$v\left(\tau_g+\tau_b+\tau_g\right) = \frac{\phi\left(e^{kB(1-\phi)\tau_g}-1\right)}{(1-\phi)}e^{-\alpha\tau_b}e^{kB(1-\phi)\tau_g}. \tag{9}$$

Fig. 4 shows the free phage concentration profile for the nominal parameter values. Note that, similar to the lysogen count, intermediate lysogen probabilities produce maximum free phage counts.

IV. Probability of Survival of a Lysogen

Is there an optimal lysogen count that characterizes the survivability of the lysogen cells after the end of a good-bad environment sequence? To answer this question we model the dynamics of free phage and lysogens as deterministic. Under bad conditions, we assumed that lysogens dynamics obey a pure death process. Let the hybrid system in Fig. 5a represent the dynamics of the free phage and lysogen species at the end of the bad conditions. Since the dynamics of the lysogen species during the bad conditions results in the degradation of the lysogen count, the probability of having

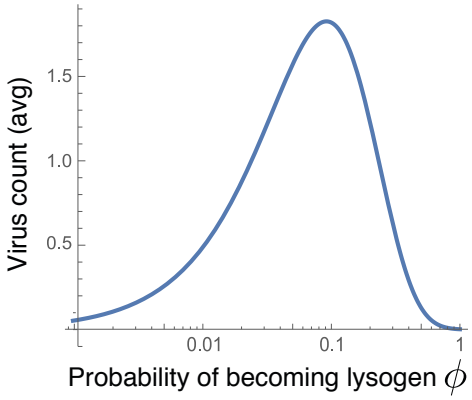

Fig. 4. Free phage population count vs chance of lysogeny. Population count is optimized at a value between 0 and 100 % chance of lysogeny.

x lysogens can be written as

$$P\left(x,\tau_b\right)=\left(\begin{array}{c}x_0\\x\end{array}\right)e^{-\alpha\tau_b x}\left(1-e^{-\alpha\tau_b}\right)^{x_0-x}, \qquad (10)$$

where $x0$ is the lysogen count at the end of the good condition. The probability that at least one lysogen survives the bad conditions is then

$$P_s=1-P\left(0,\tau_b\right). \qquad (11)$$

The term $h=1-e^{-\alpha\tau_b}$ in equation (10) represents the probability of extinction for one single lysogen during bad conditions. This probability is dictated by the ratio between the average lifetime of the lysogen and the length of the bad environment. The larger the ratio between these two, the larger the probability of extinction for one single copy.

From equation (10) when the probability of extinction is close to 0, the survival of one single lysogen will suffice to preserve this virus across multiple rounds of good and bad conditions. For extinction probabilities close to 1, virus should compensate by getting the maximum profit out of the good conditions, which is only achieved at moderated lysogenic probabilities. Note that when viral reproduction rate is large enough to produce lysogen counts $>>1$, the probability that at least one lysogen survives the bad conditions is 1 for $\phi<1$. Fig. 5b shows the probability distribution P_s.

V. The Effect of Multiplicity of Infection (MOI)

Consider the expanded version of the model in Fig. 2b where the dynamics of the lytic cell are slow. We represent the transient dynamics by the set of ODEs

$$\dot{l}=\phi\,k\,v+\phi\,a\,i\,v \qquad (12)$$

$$\dot{i}=(1-\phi)\,k\,v-d_i i-\phi\,a\,i\,v \qquad (13)$$

$$\dot{v}=B\,d_i\,i \qquad (14)$$

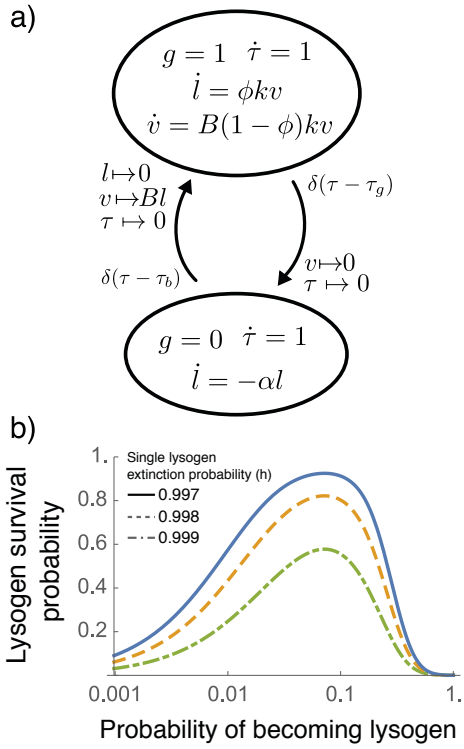

Fig. 5. Hybrid system describing the switching dynamics of the good and bad environment. a) Population dynamics under good conditions are modeled as a deterministic system of ODEs. Population dynamics under bad conditions are modeled as a pure death process. b) Survival probability of lysogenic cells at the end of the bad conditions. Note that this probability is optimal when the phage is tempered. Additionally, the larger the extinction probability of a single lysogen, the lower the survival probability.

where i describes the rate at which the infection undergoes the lytic pathway. The death rate of the lytic cells is given by d_i. Parameter a determines the adsorption rate of the phage to the already infected cells. These cells might become lysogens with the same probability as the new infections (ϕ). Note that the phage reproduction rate k is defined in terms of a, i.e., $k=ac$ where c is a fixed number of healthy bacteria cells.

We explored situations with and without MOI effects. To this end, when the phage is unable to re-infect an already infected lysogen, the term $\phi\,a\,i\,v$ disappears from equations (12) and (13). For MOI-enabled phage we varied the adsorption rate (a) and kept constant the phage reproduction rate (k). Since $k=a\,c$, an increase in phage adsorption implies a reduction in the constant number of bacteria in the environment.

Fig. 6 shows the effects of MOI for different adsorption rates. The blue solid curve represents the non-MOI version of each phage adsorption rate. Note that by adding MOI, the survival probability increases. Additionally, the optimal lysogeny probability decreases. As the phage becomes aggressive, the optimal lysogenic probability reduces and the

optimal survival probability increases.

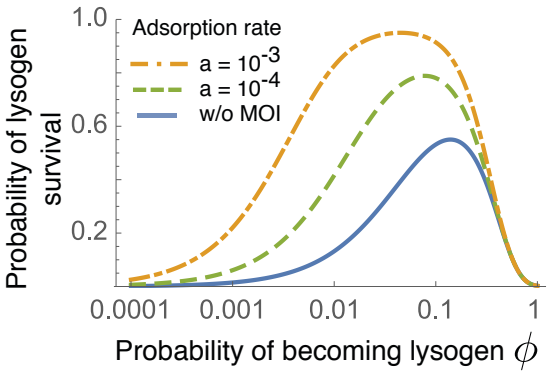

Fig. 6. Effects of the multiplicity of infection (MOI) on the survival probability. The optimal chance of lysogeny decreases for MOI enabled phages compared with phages without MOI. The blue solid curve represents the non-MOI version of both MOI enabled phage ($a = 10^{-4}$,$a = 10^{-3}$)

VI. Conclusions

When the availability of the bacterial population is held constant, classical theory of parasite evolution states that aggressive phage strains will be selected for in their environment. In this case, the probability of becoming lysogenic should be close to zero. However, the role of lysogeny on viral fitness under these conditions has yet to be fully uncovered. In this paper, we observed a series of dynamic environments with constantly changing conditions. These conditions oscillate between those where the bacterial populations is readily available to the phage, followed by periods where there is no bacterial population to infect. During such periods, the free phage has a null chance of survival.

We found that under such scenarios, lysogenic cells play a key role in the preservation of the phage for subsequent generations. Moreover, only temperate phages (those with intermediate lysogenic probabilities) maximize phage population in good conditions, lysogenic population at the end of bad conditions, and virus survival probability. How temperate the phage should be depends on the phage reproduction rate, which depends on how aggressive the phage is (adsorption rate). The more aggressive the phage is, the lower the required probability of becoming lysogenic and therefore, the larger the optimal survival probability.

Using this framework, we also studied the impact of the multiplicity of infection mechanism implemented by some phage species. We found that in some cases, MOI might double the probability of lysogen survival, and reduce the optimal probability of becoming a lysogen (ϕ) by a factor of 10.

Acknowledgment

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