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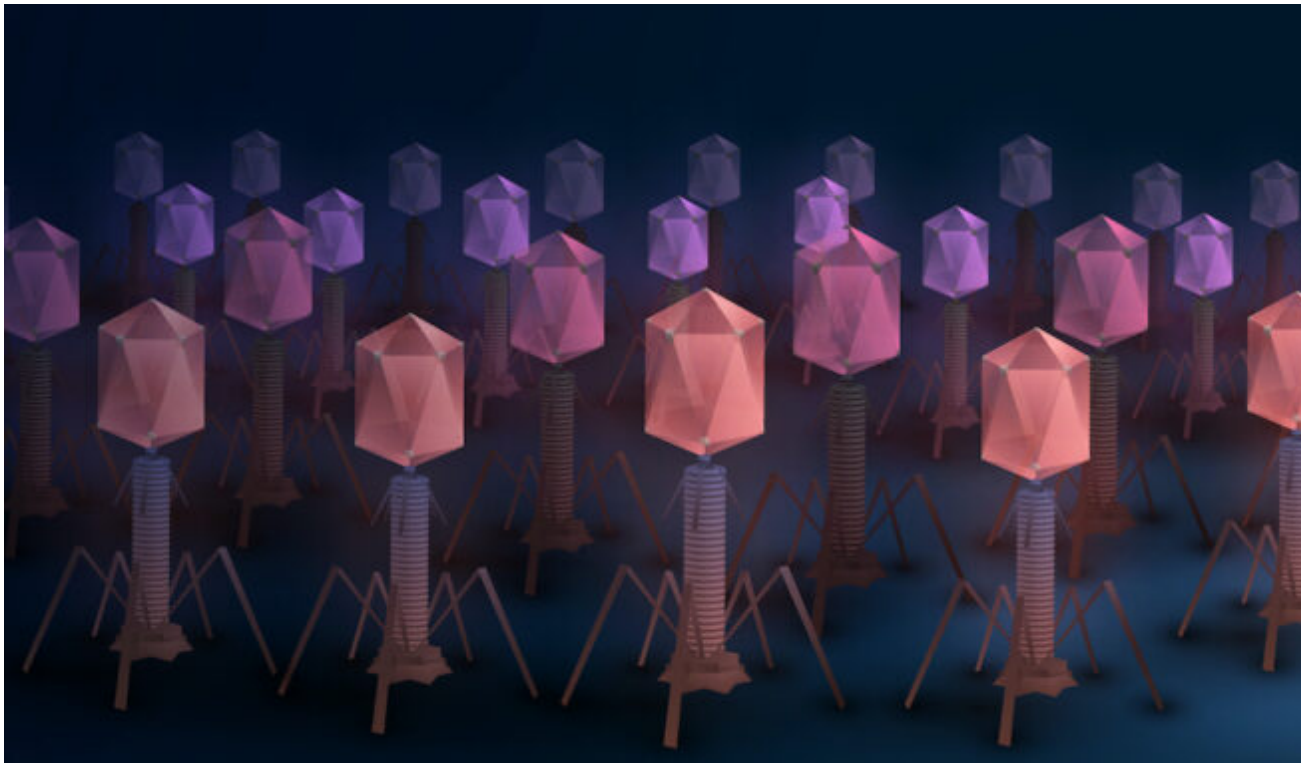

Master thesis

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MSc in Physics

Modelling Bacteriophage Communication Strategies

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May 2022

Abstract

Molecular communication mechanisms are found to be in the essential decision-making roles in temperate bacteriophages to choose entering lysis or lysogeny. Experimental studies have shown high concentration of signaling molecules triggers lysogenic infection while lytic response is triggered with low signal concentration.

Evolutionary and game theoretic models were made to attempt explaining their behaviors as selection pressures in environments where multiple phage variants compete for the same host. Previous studies have modelled similar systems as well as provided insights for bet-hedging strategy among temperate phages. However, the strategies for temperate phages in both well mixed and spatially structured systems are still not fully understood.

This quorum sensing system among phages enable them to sense the abundance of lysogens in the population. With involvement of diffusions of both arbitrium and phages in structured systems, it is expected to identify changes in strategies of phages as encoded information is changed from globally spread to semi-localized. It is likely that the strategies of phages adapt changes in accordance to different goals with such communication by avoiding lysis when lysogens have been established.

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Acknowledgements

I would first like to thank the tremendous help and guidance by my supervisor Namiko Mitarai. She's has been very nice and helpful, and been assisting me through the discussion and reviews. I feel lucky to work with her. I would then like to thank my best friends Petar, Dorothea, Pavel, Santiago and Zichan, who have been supportive to me in the very days with cheers and laughters. I am also thankful to my partner Nicole for her encouragement and advice. She has been always taking care of me so I have nothing else to worry about. Last but not least, I am utmost grateful for my parent's endless love and support.

1 Introduction

What are bacteriophages?

Bacteriophages (phages in short) are a class of viruses that specifically infect bacterial cells and proliferate inside the host cells. [1] Phages are estimated to be the most abundant class of organisms on earth and they play important roles in basic sciences and applications, such as microbial evolution, ecosystems, to medical treatments. [1] [2] [3]

The operation of phages as viruses can be generalized into 5 steps[4]:

1.Attachment: Phages come in contact with host cells and bind with the molecular receptors on the cell surface.
2.Entry: Phages inject the viral DNAs into the host cells
3.Synthesis: Host cells copy viral DNAs that hijack the molecular machinery for viral protein production
4.Assembly: Host cells assemble the viral protein into virions and form new phages
5.Release: Newly formed phages are being released

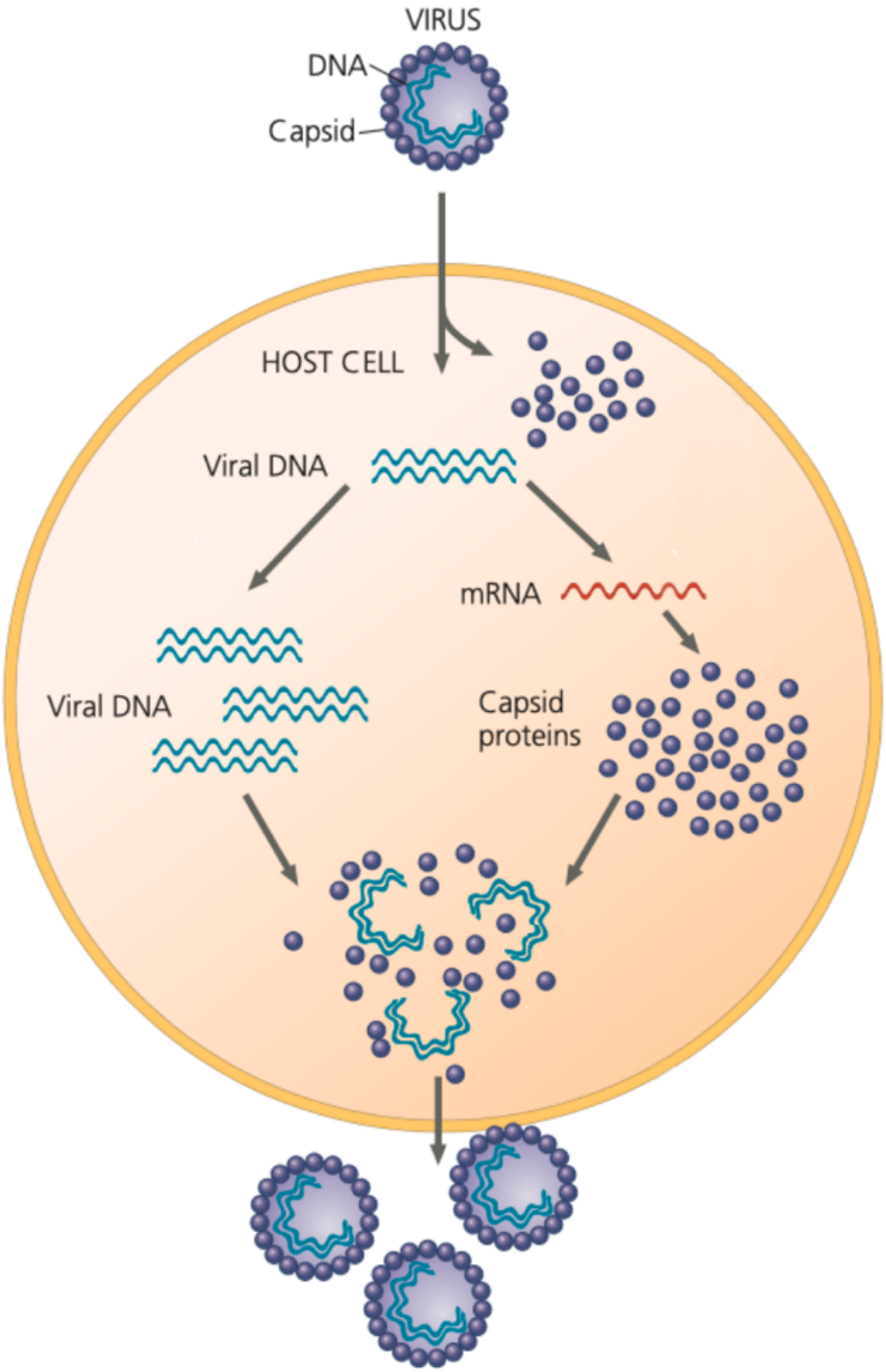


Figure 1.1: Simplified viral cycle. Virus attaches, enter the host and releases genome(attachment and entry). Host replicates and make new parts (synthesis). Self-assembly of new virus particles then exits the host(assembly and release). Credit to [5] for the illustration.

Phages decision making

There are many subclasses of phages, that are mainly classified in terms of their replication strategies, often the use of two key processes, lytic and lysogenic, as detailed below and in figure 1.2.

Virulent phages only replicate by entering lytic cycles, that is, phage DNA immediate circularize upon infection and hijack the host cells into phage production mode such that virions are quickly formed and released by bursting the host cell (lysing). In this way, virulent phages are able to reproduce rapidly, while risking killing the host cells which they rely. For instance, T4 phage that infects E. Coli bacterium is a virulent phage.[8]

Chronic phages don't lyse, but their phage DNAs stay in the host cells and be replicated while allowing host cells to divide indefinitely. This way, host cells produce and extrude new phages every cycle in a more commensal manner for long period of time without substantial disruption. Chronic phages reproduce slower by following the reproduction rate of host cells, but they can propagate without killing the host cells for longer term. M13 phage is an example of chronic phage that infects E. Coli.[8]

Temperate phages can enter both lytic or lysogenic cycle. When in lytic cycles, temperate phages behave like virulent phages and reproducing and bursting host cells. During lysogenic cycles, temperate phages incorporate their phage genomes into bacterial chromosomes, as a form called prophage. Prophages are replicated as every time the host cells divide themselves such that the daughter cells also inherit the phage genomes as part of the prophages. Individually, temperate

phages may switch such lysogenic state to lytic state through induction and produce more phages instantly. In this way, temperate phages resemble the combination of the aforementioned strategies. Example of typical temperate phage is λ phage, which also infects E. coli.[7] [8]

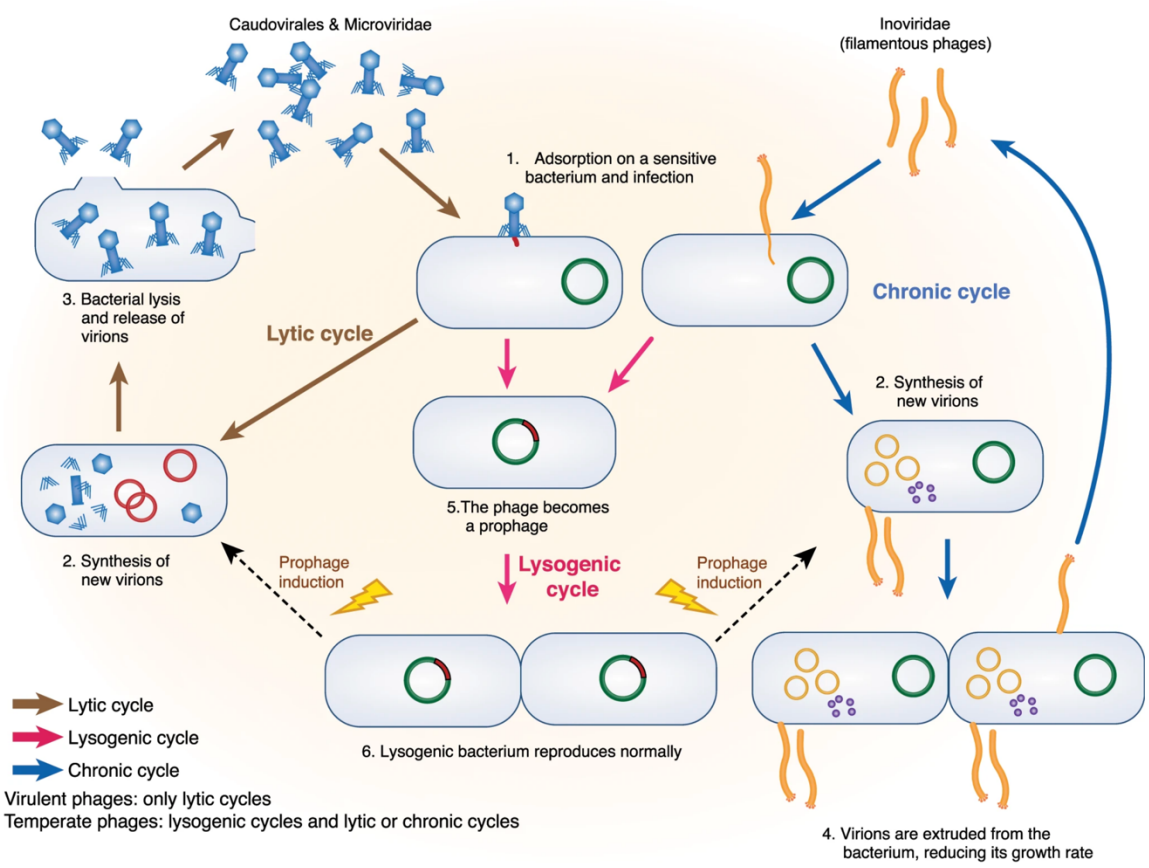


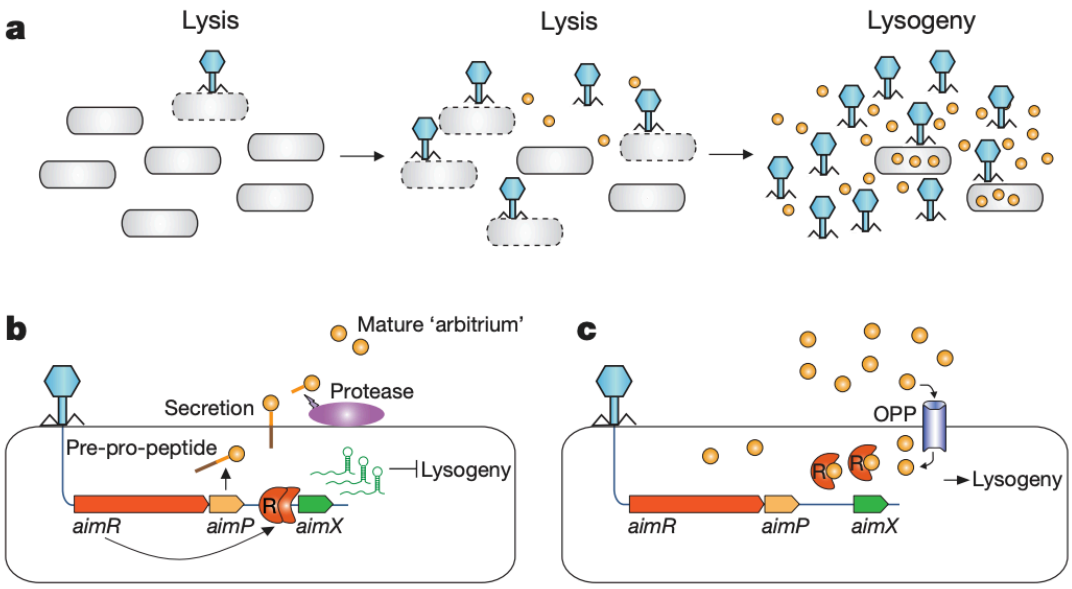
Figure 1.2: Example of typical phage cycles. The production of new virions is realized either through lytic cycles (left side of the figure, brown arrows) or through chronic infection (blue arrows). Credit to [6] for the illustration.

Quorum sensing mechanism

Recently, there is a group of SPbeta temperate phages, phi3T that infect Bacillus, can also control the propensity of lysogenic cycle through molecular communications to control proportion lytic lysogenic in a population level. Their genome contains a specific sequence allowing secretion of signaling molecule "arbitrium", which upregulates the propensisty of lysogeny.[9]

Immediately after a phage comes in contact with the bacteria cells, the early gene sequence aimR and aimP activates during infection. AimR is a dimer protein promoter that upregulates AimX expression. Here, AimX is a regulatory non-coding RNA that acts as a lysogenic inhibitor that directs phages into lytic cycles. AimP on the other hand, can derive a short peptide sequence GMPRGA (i.e. aribitrium) extracellularly that promotes lysogeny in SPBeta phages.[9]

At the early stage of infection, only lytic cycles are activated as aimX pathway is fast. Later in the infection dynamics, arbitrium accumulates in the system transfer into the bacteria by OPP transporter. After internalization, arbitrium bind to AimR such that AimR cannot function as AimX promoters, hence promoting preference for lysogeny.[9]



Aribitrium is the first demonstration of actual small-molecule communication between viruses. It is found this communication to be manifested through the arbitrium system in a large group of Bacillus phages; we envision that such a strategy will also be discovered for other phages using different communication systems. Moreover, this strategy may not be limited to phages, and possibly also guide dormancy and/or lysis decisions in viruses infecting eukaryotes.[10]

Note that quorum sensing in Bacilli based on short peptides are known to regulate horizontal transfer of plasmids. The bacterial quorum sensing systems on phage genomes have also been previously found. However, whether they are linked to interference of host bacterial communication is still not clear.[11][12]

Motivation of the thesis

As antibiotic resistance rises around the globe, phages are increasingly considered as a new way to fight off harmful bacteria. Deciphering the way these viruses communicate could help to understand how they could be harnessed to control the spread of bacteria.

Previous studies proposed that the arbitrium system may have evolved to allow phages to cope with the changing environment during an epidemic, allowing the phages to exploit available susceptible bacteria through the lytic cycle when few infections have so far taken place and hence the concentration of arbitrium is low, while entering the lysogenic cycle when many infections have taken place and the arbitrium concentration has hence increased.[13][14][15]

Modelling work has shown that the effect of the number of phage adsorptions on an infection can be selected as a phage adaptation to host-cell density, as it allows phages to switch from a virulent infection strategy when the phage/host-cell ratio is low to a less virulent strategy when the phage/host-cell ratio is high.[13][14][15]

Often, phages are not infecting bacterial communities in a premixed manner, but as scattered concentrated points of contacts in real life. In this way, infections and communications become diffusive processes. It is anticipated to identify alterations in behaviors of phages as encoded information is changed from globally spread to semi-localized.

We intend to further investigate the theoretical models in spatially structured systems to address how the optimal strategies change. Upon finding the

quantitative difference and qualitative difference of well mixed systems and spatially structured systems, one is able to identify the biological significance of such communication strategies.

Secondly, we investigate the significance and role of lysogen-induced arbitrium. It is recently found found that the small-molecule signalling can continue from the lysogenic state, allowing prophages to inform other phages of their presence, a function that could not be achieved by coinfection sensing. The frequency of lysogens in the population is valuable information from the phage perspective.[16]

Next, it was also found that in the temperate phage l, the propensity towards lysogeny increases with the number of co-infecting virions, called the multiplicity of infection (MOI). Instead of communication through arbitrium, decision making is based on co-infection. Previous studies have shown that this MOI dependence is to minimize the chance of extinction and that the shift from determinism to stochasticity is due to a shift from a single-player to a multiplayer game.[13][17] The dependence of diffusion in such strategy differs greatly hence we expect to see the survival advantages and disadvantages in these cases.

Thesis outline

In this thesis, we are to elucidate the phage-bacteria interaction and how phages achieve optima. We have made various assumptions concerning both models and parameters, as the exact values and mechanisms are yet fully understood. In this way, we aim to capture the general dynamics of systems with help of reasonable simulations, while being informative for the contexts.

The thesis is divided into 3 parts. Each part aims to compare different systems:

1. Well-mixed vs Structured Environments

We first describe a well-mixed system consisting of susceptible bacteria, free phages, lysogenic phages and signaling molecules by modelling them based on different hypothesized communication strategies, then move on to structured environments and investigate both quantitative and qualitative differences from well mixed settings using reaction-diffusion type partial differential equations.

2. Systems with or without lysogen-induced arbitrium

We investigate the significance and role of recently found lysogen-induced arbitrium in both well-mixed systems and structured environments, as well as the dependence of behaviors including strategic optimum on the unknown exact values of production/uptake ratio.

3. Arbitrium sensing vs multiplicity of infection dependent mechanism

Finally, we discuss the biological advantages and disadvantages of quorum sensing mechanism by comparing it with multiplicity of infection dependence mechanism found in wide range of viruses, both quantitatively and qualitatively.

2 Communications in Well-mixed and Structured Environments

Description of the elements in the model and their interactions

To establish the interaction between phages and bacteria, we first consider the simplest system by ordinary differential equations. In this case, phages are non-communicating and are in well-mixed setting. The system consists of susceptible bacteria, lysogens (i.e. infected bacteria in lysogenic state) and free phages.

The population dynamics can be generally described as Lotka-Volterra type systems. The population densities of susceptible bacteria S, phage particles P and corresponding lysogens L. When the lytic pathway is chosen upon infection, the free phages are produced after intermediate steps. This model can then be described by:

$$\frac{dS}{dt} = \overbrace{rS\left(1-\frac{N}{K}\right)}^{logistic\ growth} - \overbrace{aSP}^{infection}$$

$$\frac{dL}{dt} = \overbrace{rL\left(1-\frac{N}{K}\right)}^{logistic\ growth} + \overbrace{\varphi aSP}^{lysogenic\ infection} - \overbrace{\alpha L}^{induction}$$

$$\overbrace{\frac{dI^{(1)}}{dt} = [1-\varphi]aSP - \frac{M}{\tau}I^{(1)}}^{induction\ delay}$$

$$\frac{dI^{(n)}}{dt} = \frac{M}{\tau}(I^{(n-1)} - I^{(n)})$$

$$\frac{dP}{dt} = \overbrace{B\alpha L}^{induction\ burst} + \overbrace{B\frac{M}{\tau}I^{(M)}}^{infection\ burst} - \overbrace{(\delta + aN)P}^{decay\&absorption}$$

Here we assume the growth rate r of host cells are not affected by infection of phages, while growing logistically with carrying capacity K. Lysogens can be induced at rate α, and then lyse and release with burst rate B. Free phages can decay at rate δ, or infect bacteria at rate a. The propensity of phages change to lysogeny is φ. τ is the latency time needed for lysis after infection. We assume the latency time and the burst rate remain the same for all pathways. All simulation here terminates at T = 12hr as a reasonable experimental timescale. All quantities here are non-dimensionalized, such that they are in reference to the maximum capacity.

Figure 2.1 shows that without arbitrium communication, bet-hedging phages are selected with low constant lysogeny propensity. For the case of phage distribution, it is intuitive that early phages favor lytic strategy as they are mostly exposed to environments with a high density of susceptible cells. However, it is interesting that the evolutionarily steady state for lysogen is non-trivial at $\varphi = 0.06$ which shows that indeed, the lysis-lysogeny decision is indeed under selection.

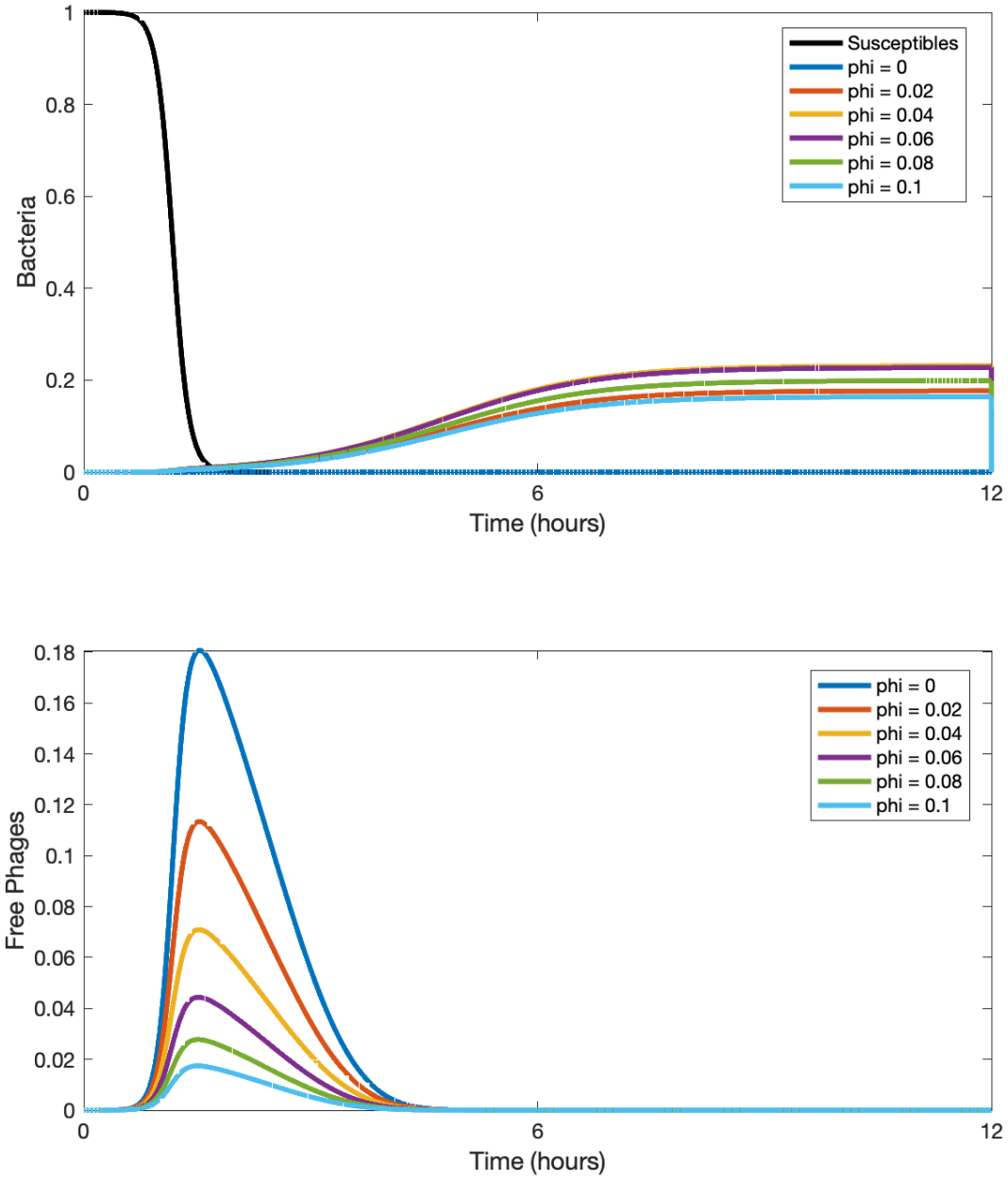


Figure 2.1: Simulation of bet-hedging phages in well-mixed environment.

Communicating phages vs bet-hedging phages

For non-communicating phages, the propensity of lysogeny φ is fixed, such that in population level, the φ portion of new infections are always pushed into the reserve to face environmental changes as a diversified bet-hedging strategy. For communicating phages, the propensity of lysogeny changes in response to the number infected hosts in the environment, by actively receiving arbitrium signals. It is found that the arbitrium response $\varphi(A)$ is switch like, that is, sensitive to threshold concentration. This response can be modelled by a step function

$$\varphi(A) = \begin{cases} \varphi_{max}\ if\ A \geq \theta \\ 0\ if\ A < \theta \end{cases}$$

where θ is the threshold concentration, φ_{max} is lysogeny propensity when the arbitrium concentration exceeds the response threshold.

With the possibility of arbitrium communication, the dynamics of arbitrium can be described by an ODE

$$\frac{dA}{dt} = \overbrace{cbaSP}^{production\ upon\ infection} - \overbrace{uNA}^{uptake\&degradation}$$

Example of communicating phages with $\varphi_{max} = 1, \theta = 0.5$ is shown in figure 2.2.

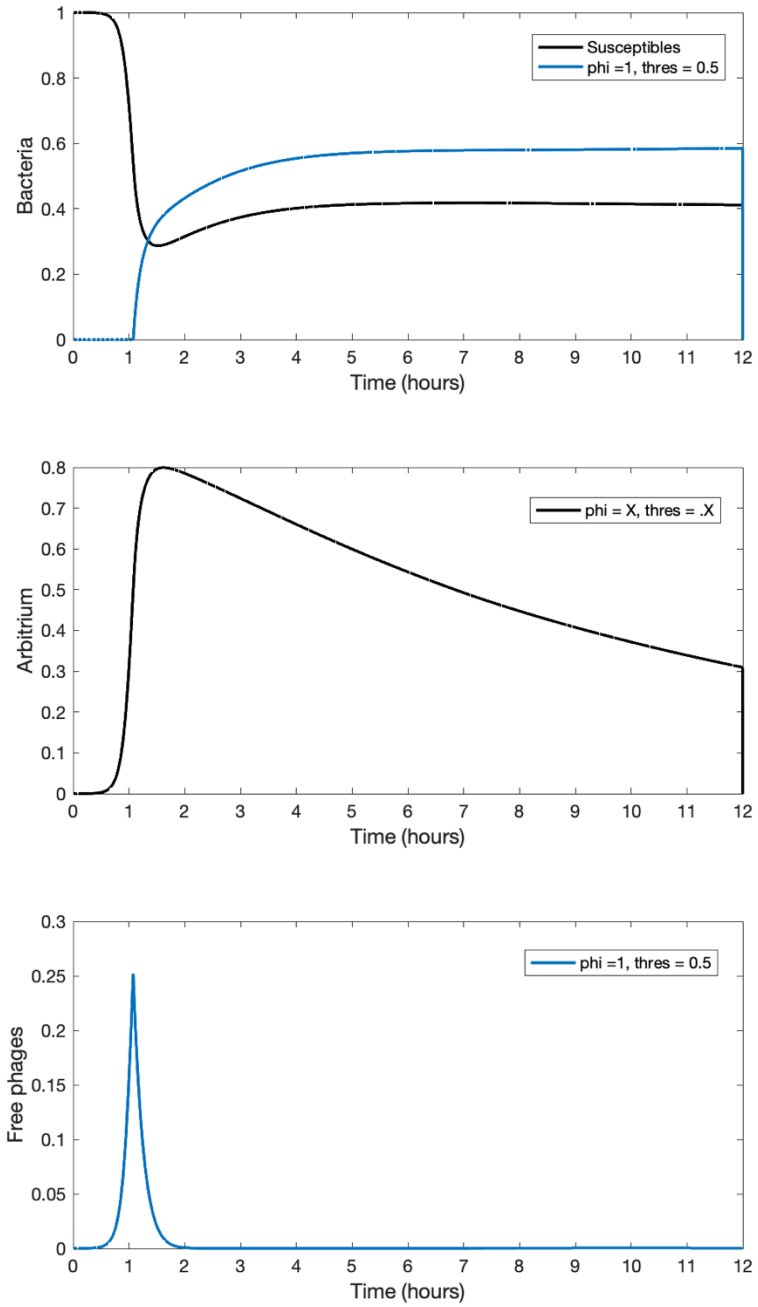


Figure 2.2: Simulation of communicating phages in well-mixed environment.

Presence of variants

One can also consider evolution of phages by adding mutation on top of the above formulations, that is, allowing phages to have variants with different propensities of lysogeny φ_i with different underlying threshold concentrations θ_i, corresponding to variants P_i, L_i.

The implementation allows phages to mutate when lytic phages P_j bursts to form new progeny free phages variant P_i with probability μ_{ij}:

$$\frac{dP_i}{dt} = \overbrace{B \sum_j \mu_{ij} (\alpha L_i + \frac{M}{\tau} I_i^{(M)})}^{infection\ burst} - \overbrace{(\delta + aN) P_i}^{decay\&absorption}$$

With the presence of mutation, one can simulate the internal competitions between variants. We can ask the following questions: (1) Is there an evolutionarily steady state variant? (2) If yes, is it completely dominant? (3) Which strategy is more evolutionary advantageous?

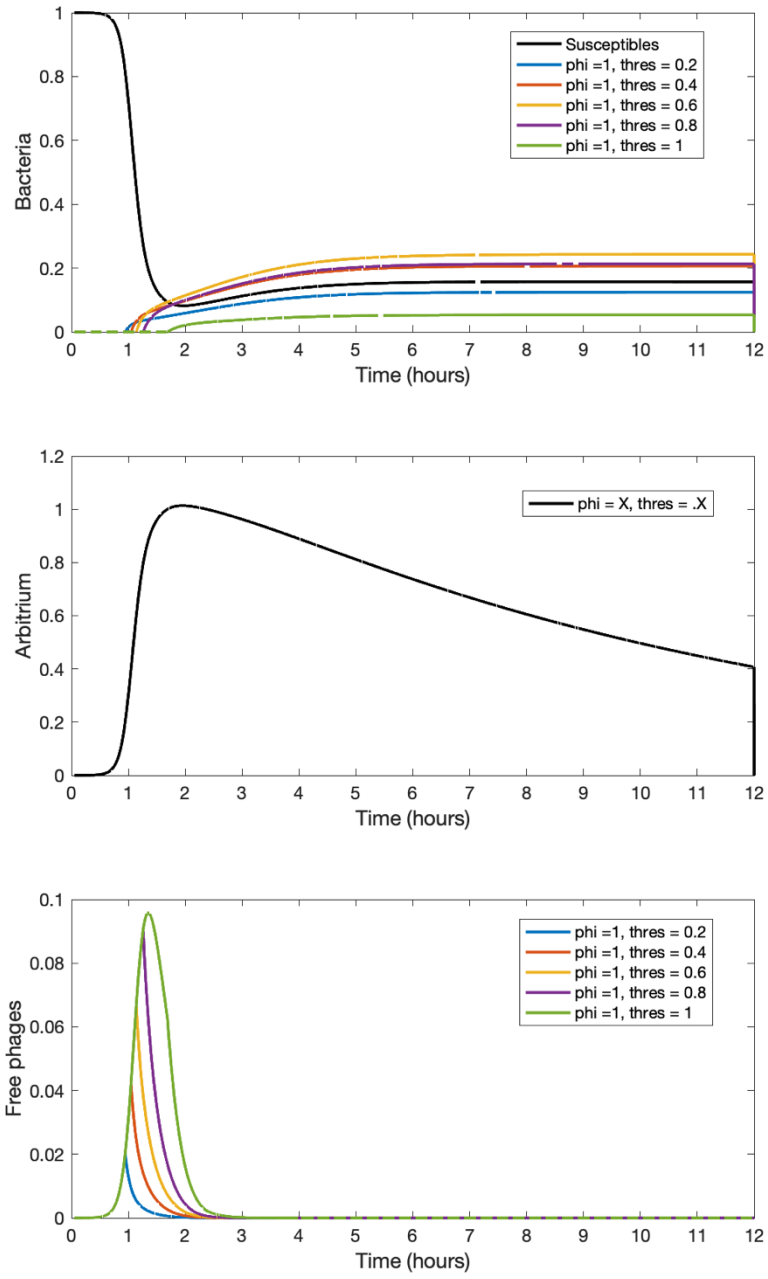


Figure 2.3: Simulation of communicating phages with multiple variants in well-mixed environment.

In Figure 2.3, dynamics are shown for multiple competing phage variants, with different response thresholds. The arbitrium concentration builds up at the beginning of outbreak. As phages variant have different response thresholds, they switch from lytic to lysogenic at different time. Similar to bet-hedging phages, there exists a non-trivial evolutionarily steady state for lysogen at $\theta = 0.6$ that the lysis-lysogeny decision with communication is also under selection.

Noticeably, diversity is maintained. This is the result of mutation-selection balance as mutation continuously proceeds among similar thresholds, selection against the mutants is not strong. There is a balance between gain in mutant variants by mutation and loss by selection such that this gradient of optimum is resulted instead of a single dominant variant.

Here, to compare communicating phages and bet-hedging phages, we can simply set bet-hedging phages as $\theta = 0$ with $\varphi = 0.06$. Optima in individual case are mix together. We can see that the lysogeny production of communicating phage quickly surpass that of bet-hedging phages and takes over, confirming that communication is more favorable over bet-hedging (Figure 2.4).

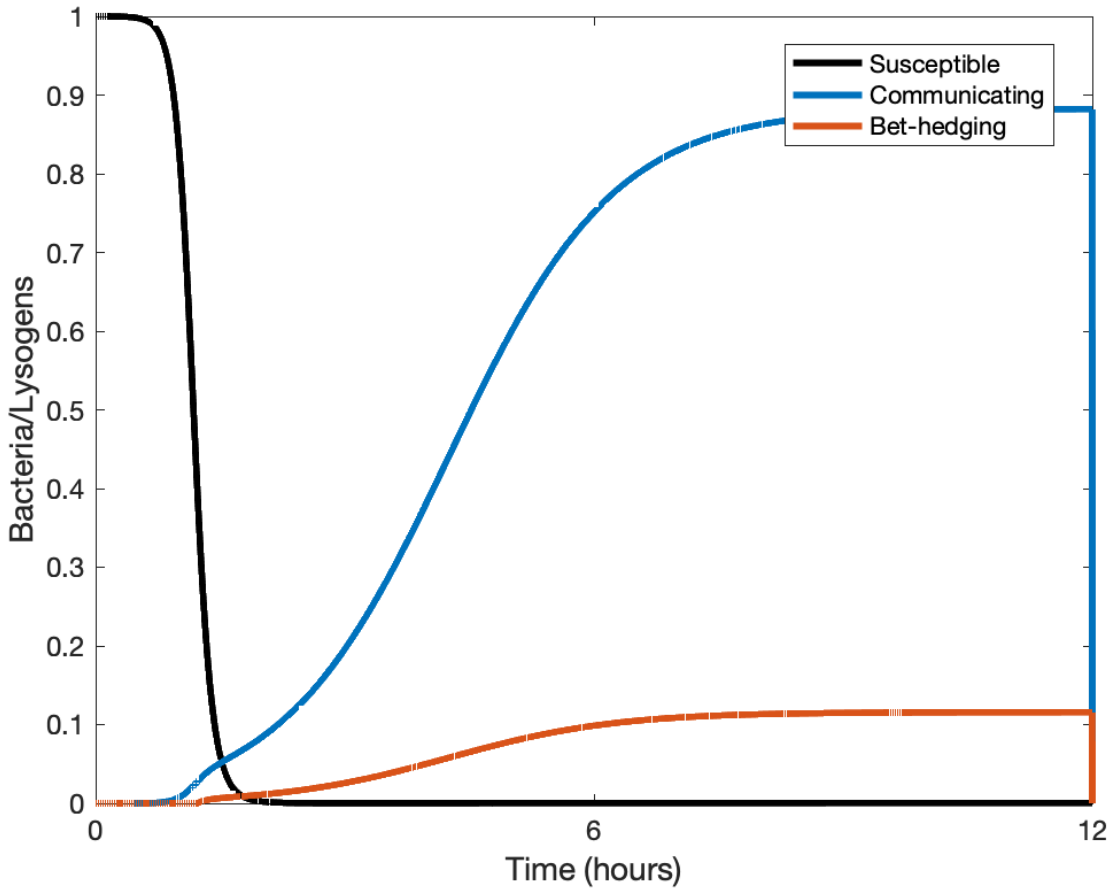


Figure 2.4: Simulation of competition between communicating and bet-heding phages.

Communicating populations in structured environments

Another major factor that can be suggested to be advantageous for phages is that in spatial structure, even though susceptible bacteria may be depleted in local environments, a global lysogeny level may be maintained as a diversification strategy. On the other hand, in well-mixed systems, the call to lysogeny is global while it is diffusion limited in spatial settings. We anticipate that the responsibilities to phage level change also differ, both spatially and temporally.

To describe the spatial model as in figure 2.5, we use two-dimensional partial differential equations by adding diffusion terms to free phages and arbitrium. We assume that the system is uniform in the depth direction of the substrate. We assume that the bacterial cells spread over the whole substrate uniformly and do not move or diffuse. We assume that arbitrium as small molecule diffuses in rate D_A with an order of magnitude faster than of free phages D_P. The set up simulates a small drop of free phages being introduced to the middle of circular disc of bacteria, starting with maximum capacity.

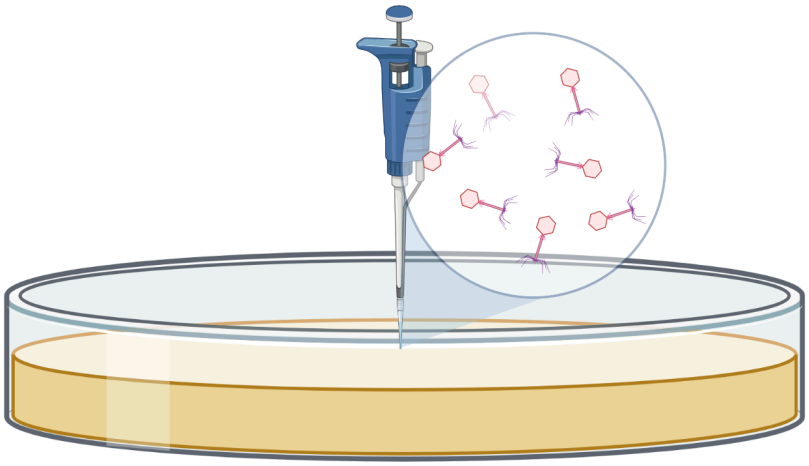


Figure 2.5: Illustration of the set-up mimicking the experiment to be done

This can be seen as a partial differential equations to be solve in polar coordinate with the origin at the center. We consider it to be homogeneous angularly and only variates along the radial direction. Reflecting boundary conditions are imposed at the outer boundary.

Here in figure 2.6 we present the communicating population with same population components as in last section. In the previous sections, evolutionarily steady state is assumed to be the optimum. However, in spatial setting, such dominance is not global, therefore we can only assume that the variant with the highest concentration at the location to be the local optimum. We can see transitions of local optimal variant along the spreading of epidemic as diffusion, burst delay and communication create delays and filter the best lysogen-reproducing variant locally. Especially, the highest arbitrium level is always at the phage wave front while the production is faster than removal, the wave front gradually turns the local environment to favor higher threshold variants. Therefore, the local dominance shift from lower thresholds to higher thresholds. On the other hand, the population of free phages is always dominated by higher threshold variants as they are always in the lytic cycle for longer term before arbitrium builds up to the switch level.

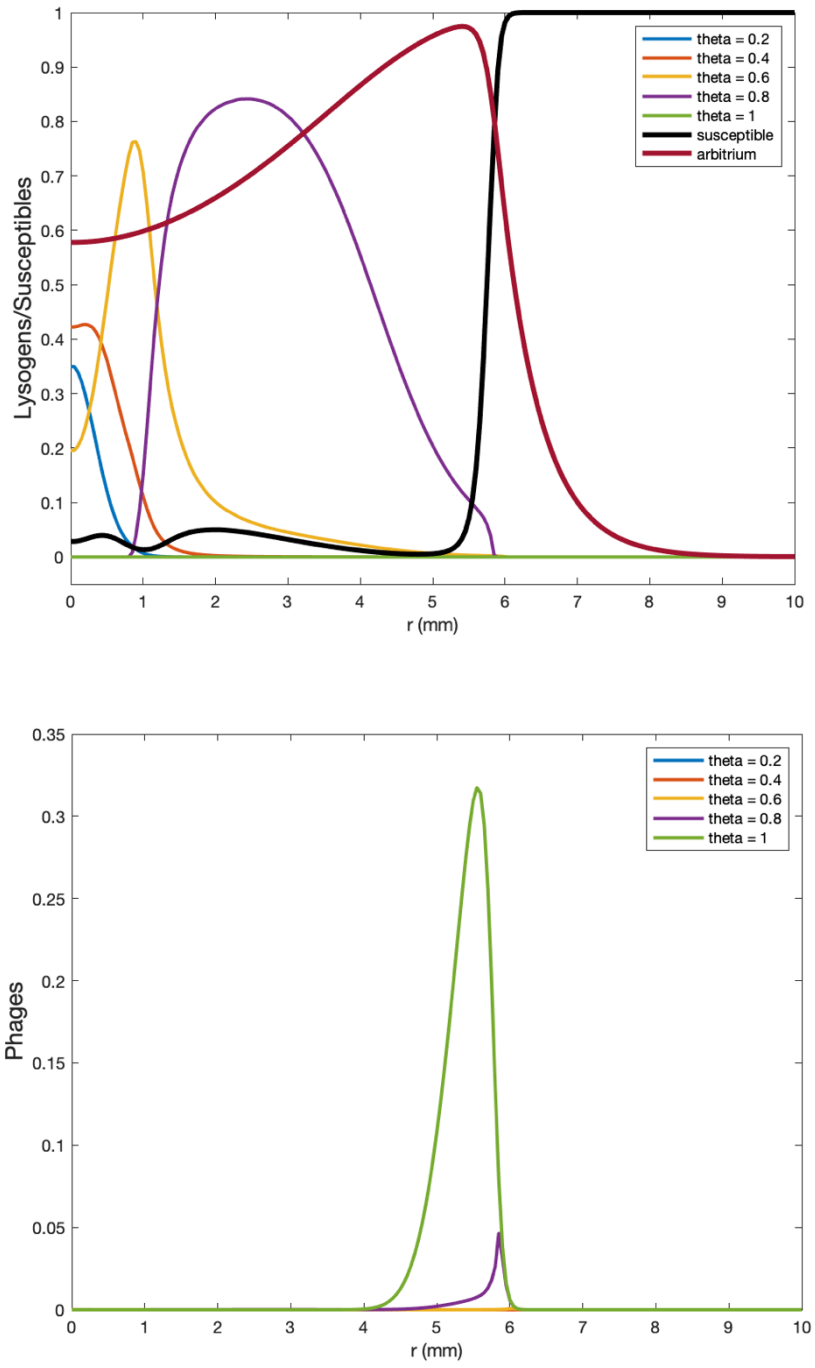


Figure 2.6: Spatio-temporal simulation of interacting phage dynamics on 2D plate

Optimal lysogenic production shifts

One important change when switching from well-mixed to structured environments is the global optimal variant. To directly compare the global optimum with well-mixed environment, we compute the integral of lysogen in space over time.

The global optimum in structured environment is higher than that of well-mixed environment as the interactions among species play important roles in building up arbitrium. It is obvious that the dominant variant is no in steady state as the system is always nonequilibrium. However the lower threshold variants are all almost in steady state, implying that their actions end upon occupying the center of plaque. Also, such global dominance for higher threshold variant is time dependent. This is because the global dominance for them is inevitable with the build-up of arbitrium with aid of lower threshold variants as susceptible cells are always abundant at the wave front. This is not true for well-mixed system as susceptible cells are shared by all variant at the same time without transitions, the system tries to reach steady state early on.

To find the threshold value and how sensitive the optimum is, here we iterate two variants competitions as following:
Step 1. Competitor 1 and competitor 2 randomly choose threshold in [0,1]
Step 2. Two competitors put together in variant model
Step 3. The competitor with more total lysogens wins and is kept, the loser chooses a new random threshold within proximity
Step 4. Repeat step 2.

In this way, if there exists a global optimum, the iteration allows convergence to the threshold that ties, that is, no competitor can get more total lysogens. The competition result can be shown as a ratio map showing the total lysogen ratio between winner and loser. The simulation result in figure 2.7 shows a matching global optimum to the previous result. Selection pressure well below global optimum is high where there can always be a better choice of threshold for more total lysogen production. Close to the Pareto optimum, the selection pressure is lower, as it may not be favorable to change much when having a close tie. This stability at the optimum explains the lower diversity at given space, as well as single variant dominance that are not seen in the well-mixed systems in figure 2.8.

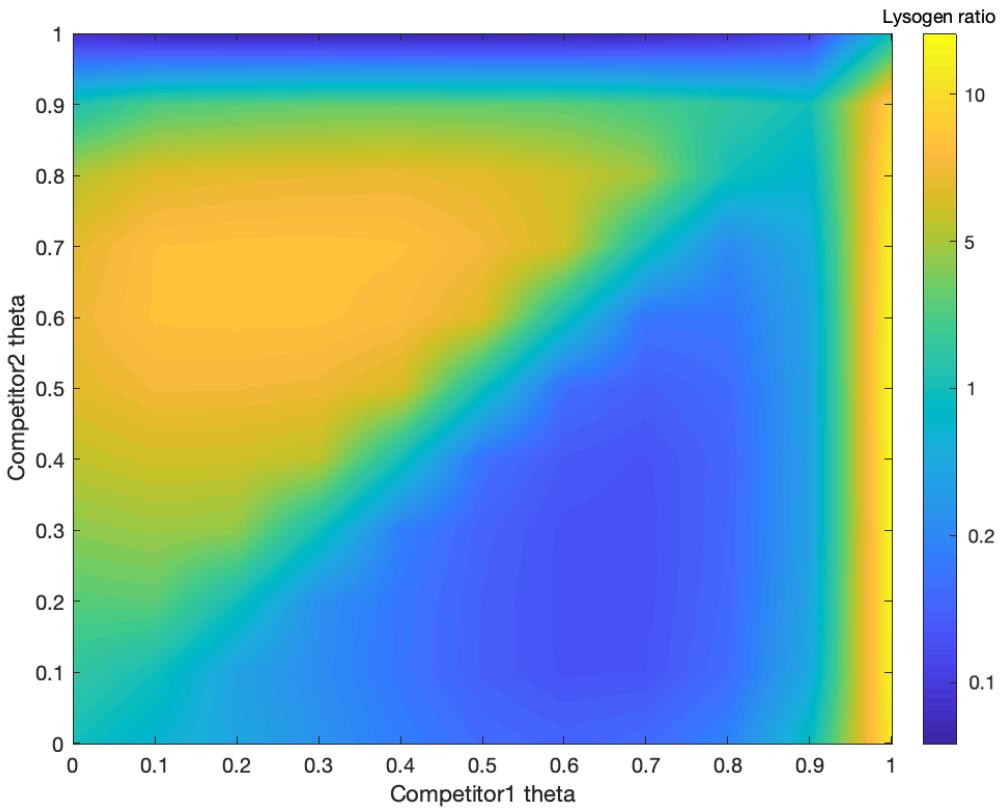


Figure 2.7: Ratio map for global optimum: value of each pixel is the lysogeny ratio, corresponds to that total lysogens in Competitor2 divided by total lysogens in Competitor1 with their corresponding thresholds

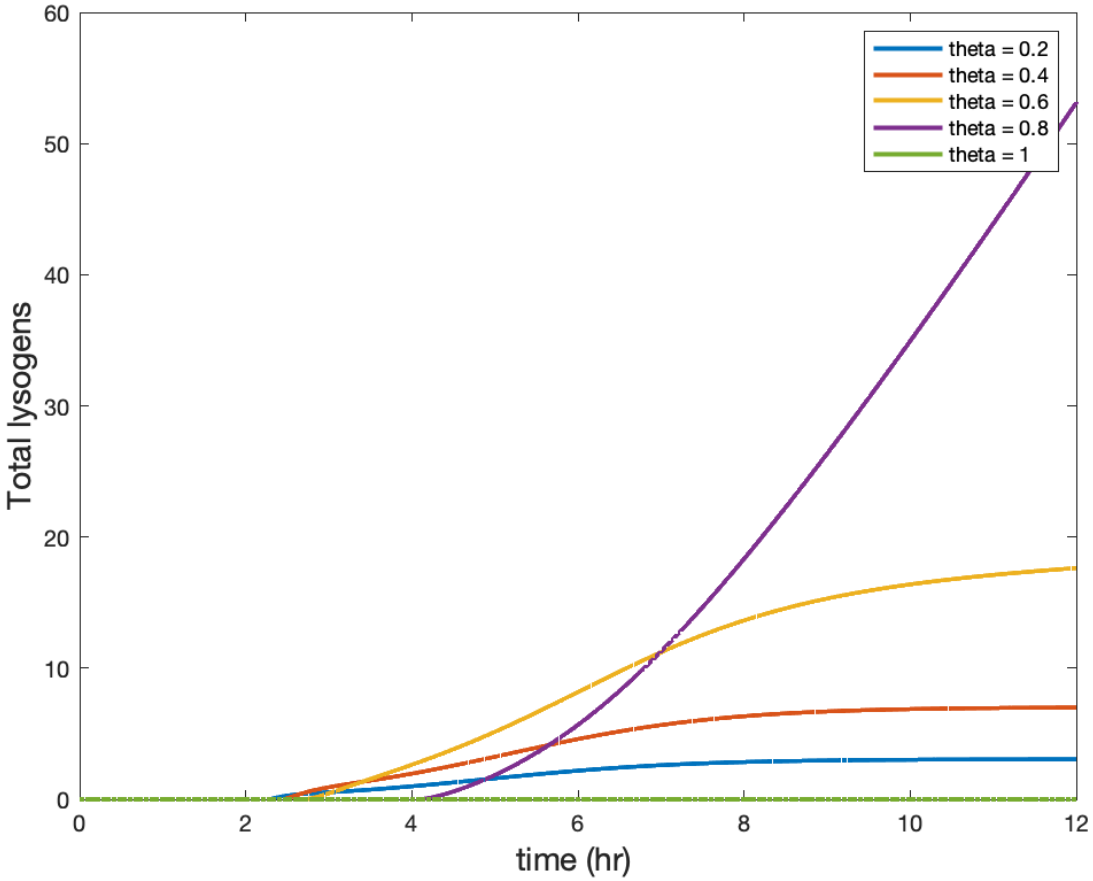


Figure 2.8: Total lysogens as integral over space

Significance of interactions among variants

It is important to note that the effects of co-existence are due to the interactions among variants. In figure 2.9, without interactions, the system only autoregulates and responses to own phage level, which ignores the importance evolutionary dynamics. In particular, since co-existence of species collectively share arbitrium production, the upper limit of concentrated arbitrium is higher, and the time needed to achieve the upper limit is shorter. This is because the local population always tends to mutate to the optimal variant until balance, such that the most

dominant variant is responsible to lysogenic growth. Also, the competition among species allow each variant reproduce the most phage/lysogeny in the region that matches its threshold the most. This favors the growth of higher threshold variant in longer term as overall arbitrium level developed as lower threshold variants have lysed in early stage thus more likely to achievement higher total lysogens, faster spreading dynamics and better spreading diversity.

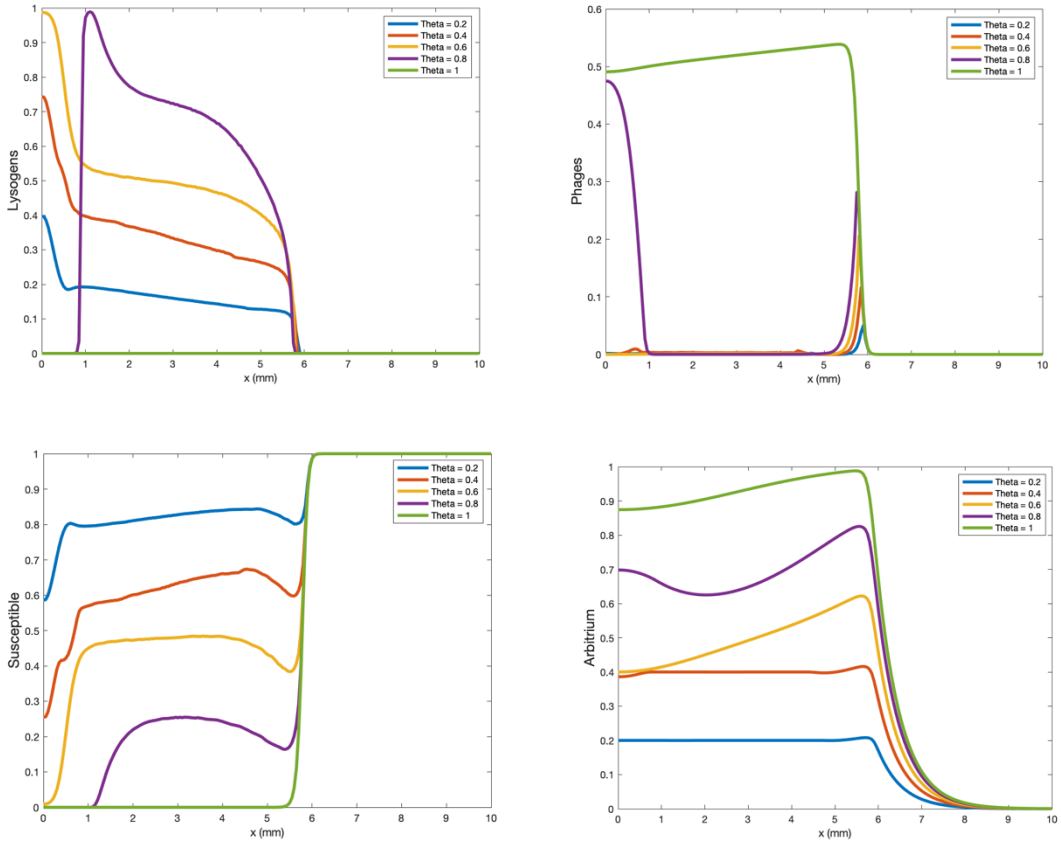


Figure 2.9: Spatio-temporal simulation of non-interacting phage dynamics on 2D plate

Discussion

We have explored the evolutionary advantage of communication in phage survival and reproduction over bet-hedging strategy. On the other hand, we found distinctive spreading dynamics with the existence of quorum sensing, as well as that optimal survival strategy for phages varies in the structured environments. Qualitatively, these features can be addressed by the presence of delays, due to feedbacks, and diffusive processes. One can simplified the molecular signaling into two feedback loops: one faster positive feedback loop inhibiting lysogeny, and one slower negative feedback promoting lysogeny. (Figure 2.10)

This representation is useful as it provides the qualitative insight of how the system behave and the advantage. The faster positive feedback loop allows phages to reproduce quickly even with small amounts of phages, amplifying the burst of phages in short time. While the slower negative feedback, with a delayed response, slows down the burst of phages such that the host population is not completely killed and can keep populating with the hosts. In such way, phages can both reproduce quickly through lytic cycles, and reproduce slowly through lysogenic cycles. On the other hand, combined loops better prevent sudden change in environment. If the amounts of either hosts or free phages are reduced, the communication system can re-ratio the lysogeny/free phages from/to the lysogen reserve, such that the phage population doesn't go extinct, in order to achieve commensalism. This separation of time scales allows lower threshold variants to win the early game, while later be taken over by the variant with close to equilibrium threshold as the optimum.

In addition to the feedbacks, signaling as a diffusive process also plays an important role in the spatial dynamics. As the diffusion of arbitrium is much faster than the spread of phages. Although the center of the system is mainly

lysogenized by the lower threshold variants, higher threshold variants have produced high concentration of arbitrium that diffuses faster than they do. Therefore, the wave front of infection is always covered by arbitrium that changes the local environment and provides different selection pressure to attain new threshold. One can observe a transition of optimal variant along the infectious pathway. Separation of time scale due to diffusion effectively acts as filter and allows the to-be-optimal variant that requires time to optimize to travel for longer distance, such that the spatially structured system can also diversify the investments under space limited competition. This effect also turns the decision based on surrounding from global to semi-local as arbitrium is not evenly distributed in the system, the optimal of the system is also dynamic and non-global, compared to its well-mixed counterpart.

In the nature, it is often thought that bacteria tend to grow as dense spatially structured communities. In dense spatially structured communities, rather than overall population density, molecular based communication reports the local information of signal producers. [18][19] As the local density of lysogen is a better predictor of potential future infections, rather than global distribution, it may be more advantageous to employ such short-range communication.

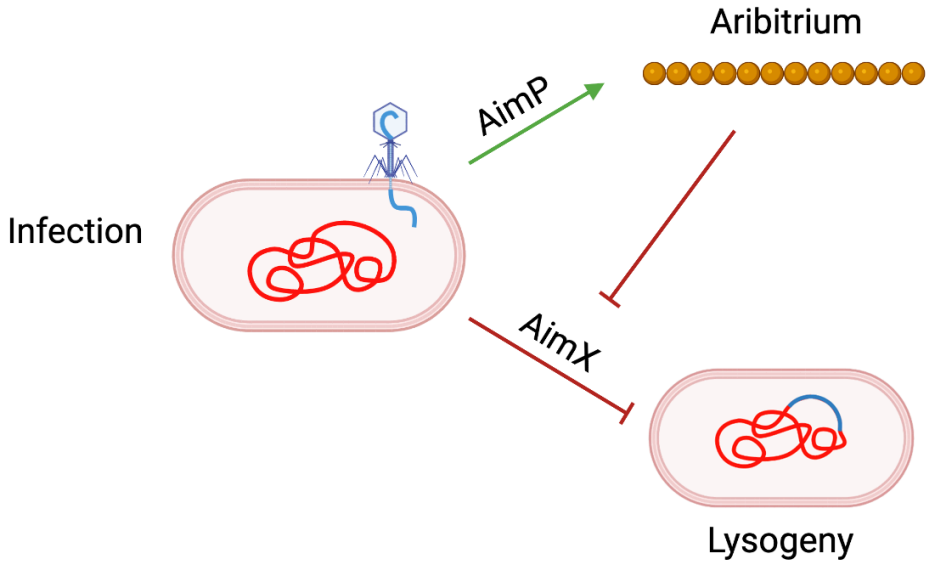


Figure 2.10: Illustration of feedbacks based on molecular regulations

3 Significance of Lysogen-induced Arbitrium

Possibility of lysogen-induced arbitrium

Recent studies suggest that apart from communication using a peptide signal from infection, arbitrium-coding prophages may continue to communicate from the lysogenic state by secreting and sensing the arbitrium signal [16]. This means arbitrium instead of announcing only the infection frequency, it can announce the total potential prophage candidate, from possible lysogenic inductions and from new infections. In short, phages are prompted to lysogenize when surrounded by infected cells. The total production can be described by

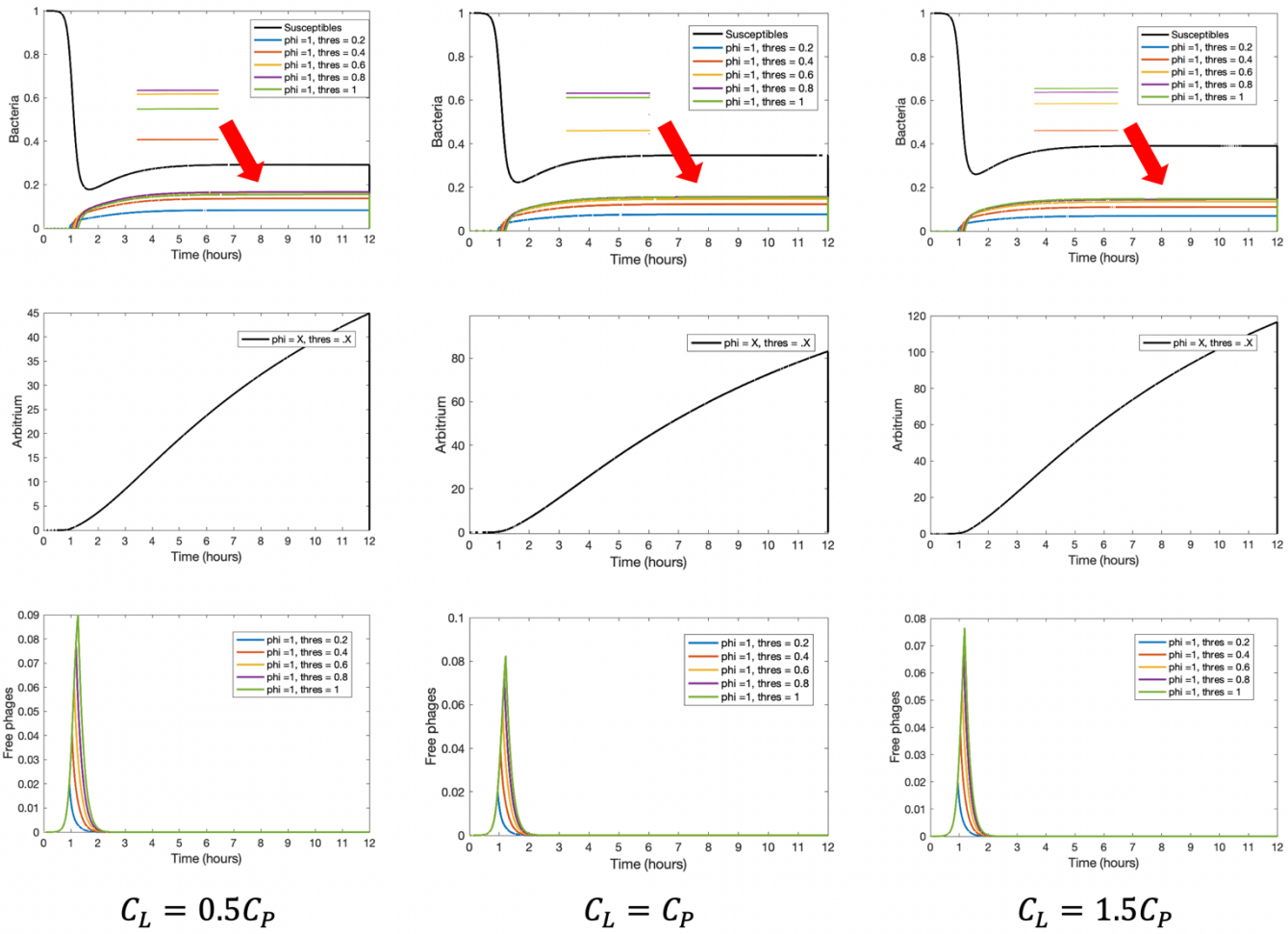
$$\overbrace{c_P \sum_{\substack{n=1\ to\ M \\ i}} {I_i}^{(n)} + c_L \sum_i L_i}^{production\ from\ infection\ and\ lysogen}$$

where c_L is the lysogen-induced production rate.

Effects of lysogen-induced arbitrium in well-mixed systems

Because phages thereby would be informed about the presence of neighbouring lysogens, it has been suggested that the arbitrium system would be benefited by lysogen-induced arbitrium.[20] However, the quantitative details of regulation level is yet to be found. As experiments conducted only suggest the existence of lysogen-induced arbitrium, not the relative production level between that from infection and lysogen induction. Therefore, it is meaningful to assume different lysogen-induced production rates: equal, lower or higher than that of production from infection.

Figure 3.1 presents the well-mixed result for $C_L = 0.5C_P, 1C_P$ and $1.5C_P$. Indeed, there exists a change evolutionarily steady state. With introduction of lysogen-induced arbitrium, the optimal thresholds increase from 0.6 ($C_L = 0$), to $0.8(C_L = 0.5C_P, C_L = 1C_P)$ and 1 ($C_L = 1C_P$) respectively. We can see the ranking of steady state slowly shifts to higher thresholds while the time needed to achieve the steady state is also shorter. The optimal threshold shift showing that the lysis-lysogeny decision with communication is under stronger selection near the previous steady state. The profile of arbitrium indicates the minute difference in arbitrium build-up time surpassing the overall threshold is the cause of such optimal threshold shift.



$C_L = 0.5C_P$ $C_L = C_P$ $C_L = 1.5C_P$

Figure 3.1: Simulation of communicating phages with lysogeny-induced arbitrium. Red arrows denote the magnified image to identify variants' abundance

It is intuitive to suggest that the minute difference in arbitrium build-up time is originated from the total arbitrium production from C_P and C_L against the uptake rate u. To show the dependence of of c/u, we consider cases of $c_P = c_L$ and scan through the combinations of different c and u to map the optimal thresholds corresponding to the combinations. We can see that, there is effective communication driven selection pressure towards lysogenic only if the c/u ratio is high. Qualitatively, this is because the higher the c/u, the more arbitrium stays in the system for a longer time, favoring the growth of higher threshold variants. The case for $c_P \neq c_L$ is not as straight forward as the effective production c is now in between the two. However, the scaling still exists that allows us to choose any threshold ranges as long as c/u is reasonable, which justifies our choice of parameters. Here, we choose c/u such that the optimal threshold is in [0,1] for the sake of convenience.

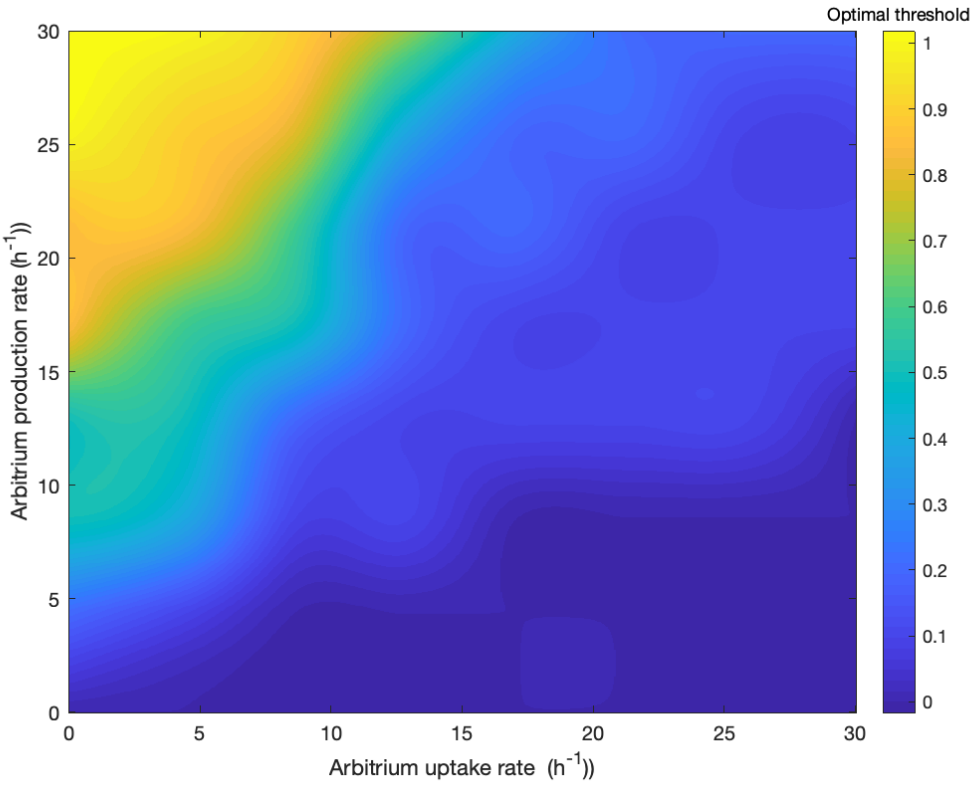
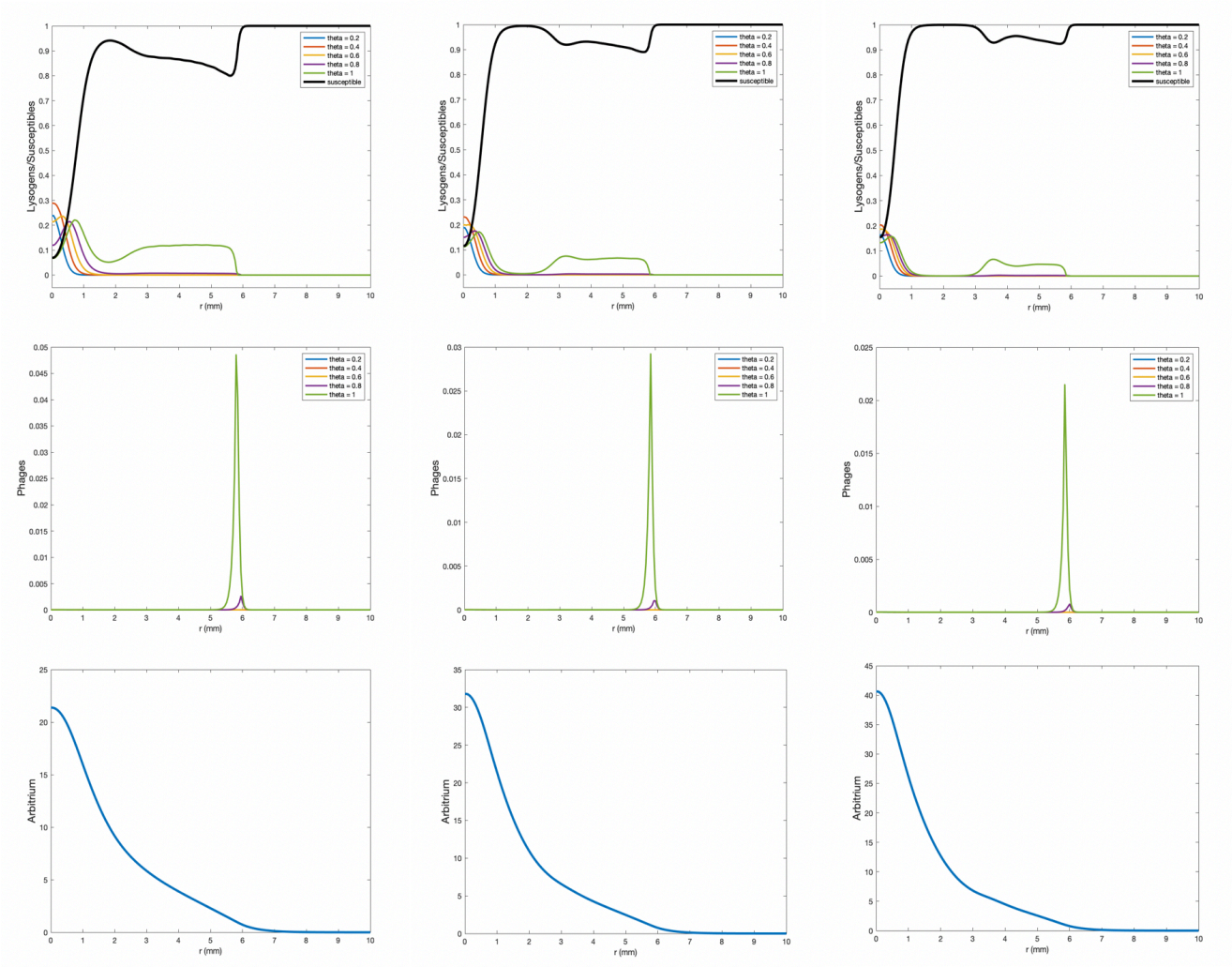


Figure 3.2: For each pair of c/u, a well-mixed simulation is performed. Value denotes the optimal variants in the competition.

Effects of lysogen-induced arbitrium in structured systems

In the case of structured environment in figure 3.3, one can observe that the qualitative behavior of infection also changes drastically where transition of local optimum is much faster such that the lysogens are more confined in the origin. The global optimum at also have shifted to higher threshold (figure 3.4). The portion of outgoing free phages is also much reduced, compared to the minute change in well mixed system. This is because with abundance of susceptible bacteria in sptial model, free phages are sequentially switch to lysogenic. The switch is gradual without lysogen induction. However, with lysogen induction, all variants are switched to lysogen almost simultaneously, as the arbitrium production is much amplified by lysogens. Moreover, there is a "leak" in lysogen, that only high threshold variant can propagate in longer range, this is discussed in more details later.

One may argue that the change in behavior is due to limited threshold. Indeed, if we scale the thresholds accordingly, this allow us to restore the behavioral change due to extra arbitrium production (figure 3.5). However, for a given starting pool of variants, it still means an extra selection pressure to restore the behavior if needed. On the other hand, as it is still unclear what is the available range for threshold in nature, it is reasonable to claim that different expression levels of arbitrium induction do change the system behavior.



$C_L = 0.5C_P$ $C_L = C_P$ $C_L = 1.5C_P$

Figure 3.3: Simulation of communicating phages with lysogeny-induced arbitrium in space.

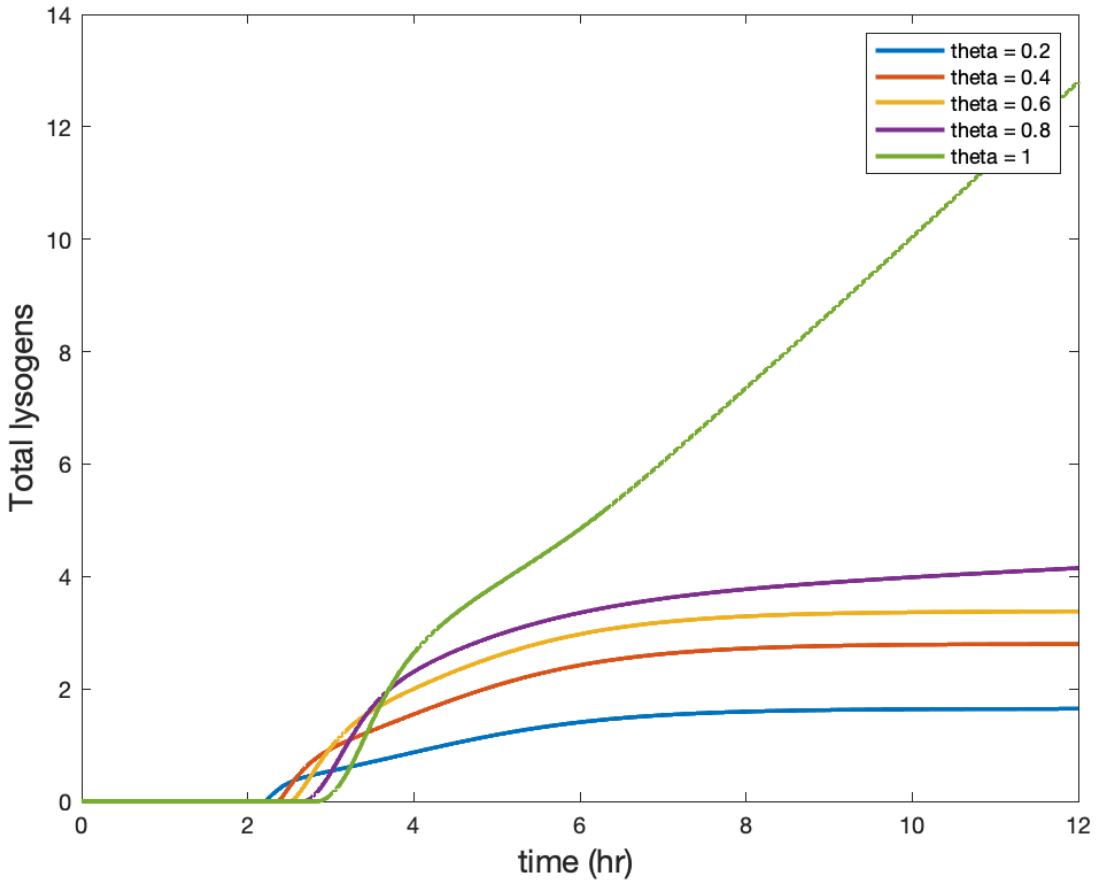


Figure 3.4: Total lysogens as integral over space where optimum shifts to theta=1

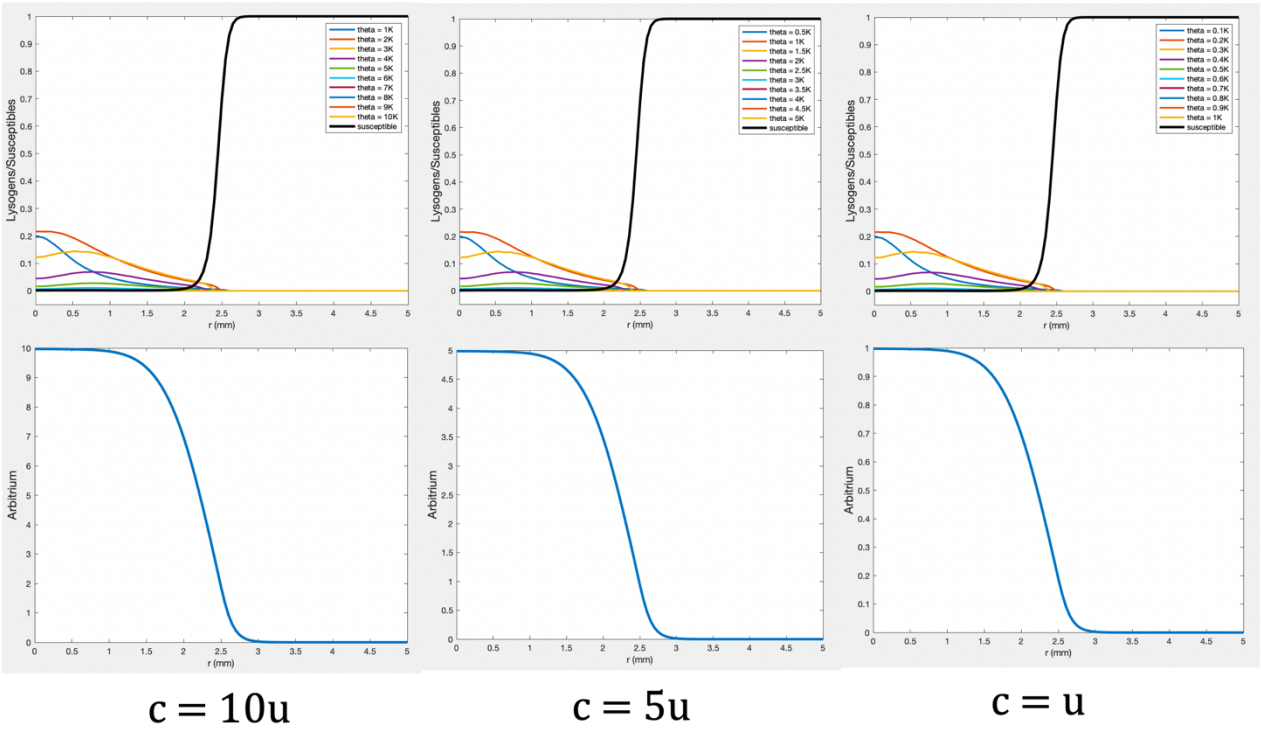


Figure 3.5: Even with different c/u ratios, the spatial structure is consistent when the thresholds are scaled accordingly.

On top of that, we can also observe the change in optimal threshold from spatial effect as well as the shift due to lysogen induced arbitrium. It is interesting to note that, the spatial system's behaviors scale linearly on the ratio between arbitrium production and arbitrium uptake. This coincides with the case of well-mixed system, when C_P and C_L are chosen in the reasonable range. This includes the optimal threshold, and the spatial temporal distribution of lysogens.

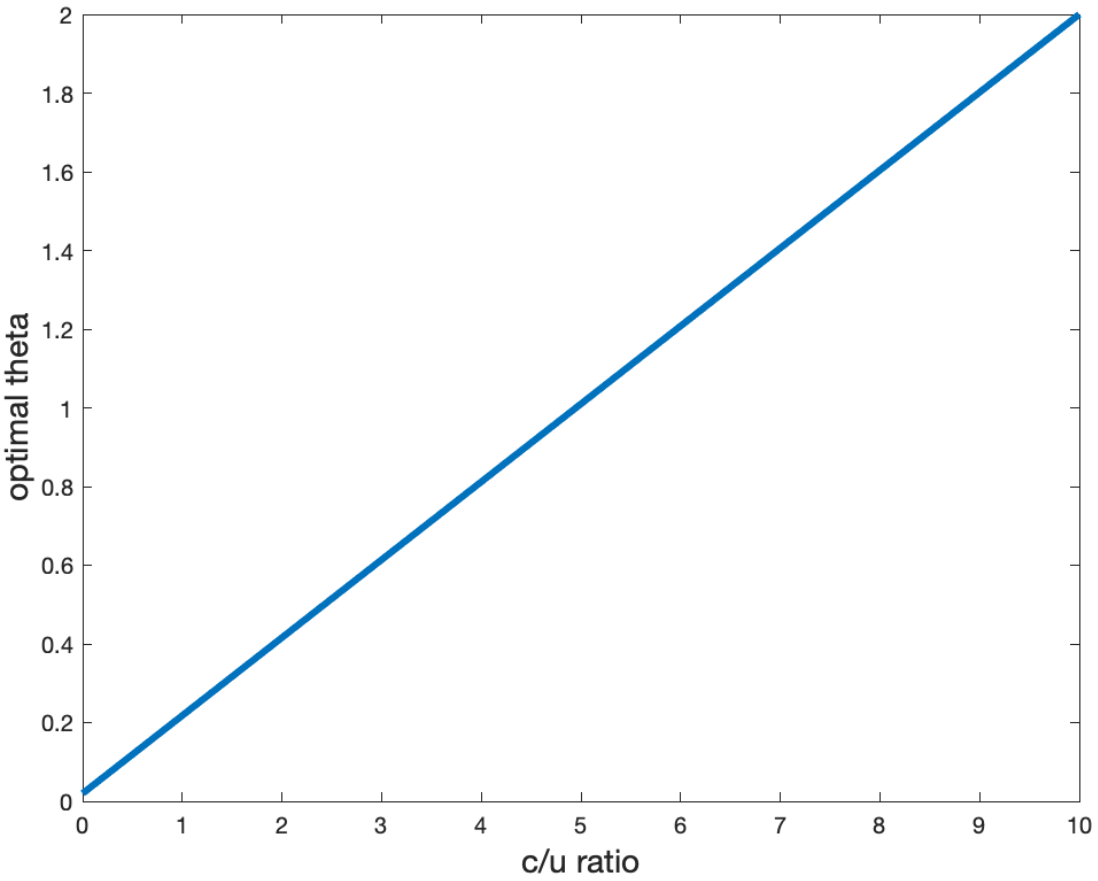


Figure 3.6: Linear scaling is found when different c/u pair is simulated in space

Discussion

The lysogen-induced arbitrium essentially created a positive feedback for lysogenic states (figure 3.7). Therefore, arbitrium concentration increases much more rapidly upon infection, hence further increases the selection pressure.

In spatial settings, the transition of optimum is much more abrupt with higher the rate of lysogeny-induced arbitrium production. This is because the accumulation of arbitrium is amplified such that the early game for lower threshold variants is much shortened. On the other hand, as lysogens continue to produce arbitrium, the center of the system acts a source stopping any phage production nearby. Only small amount of more virulent variants can outrun the arbitrium source, filtering all the lower threshold variants, dividing the system into confined lysogenic region and outgrowing virulent region. In the virulent region, there is a balance among backward diffusion of phages, forward diffusion of arbitrium and local arbitrium production, leaving a quasi-steady trace of lysogens behind the travelling phage peak.

Lysogen-induced arbitrium restrict the phages spread at a limited rate. This ensure the core lysogenic region is stable while outgoing phages can have a regular exposure to susceptible cells. It is reported that bacteria tend to stay in dense spatially structured communities to control horizontal gene transfers with short range communication. This suggests the localness and diversity of lysogens maybe better predictors in a small and dense structured community. The former prevents over-infecting bacteria, in case of limited resource for hosts. While the latter can preserve a local bet-hedging strategy, allowing the local population to be more adaptive to changes. On the other hand, a small amount of variants escape while the arbitrium concentration is high. The low level of travelling phages may be favoured in finding new colonies, as the density of bacteria

between colonies are much lower than that inside the colonies, forward diffusion of arbitrium keep travelling phages from depleting until new dense hosts are found. Hence, such lysogen-induced arbitrium as an active confinement maybe a potential alternative strategy, or an adaptive strategy by changing the expression level thus the production rate.

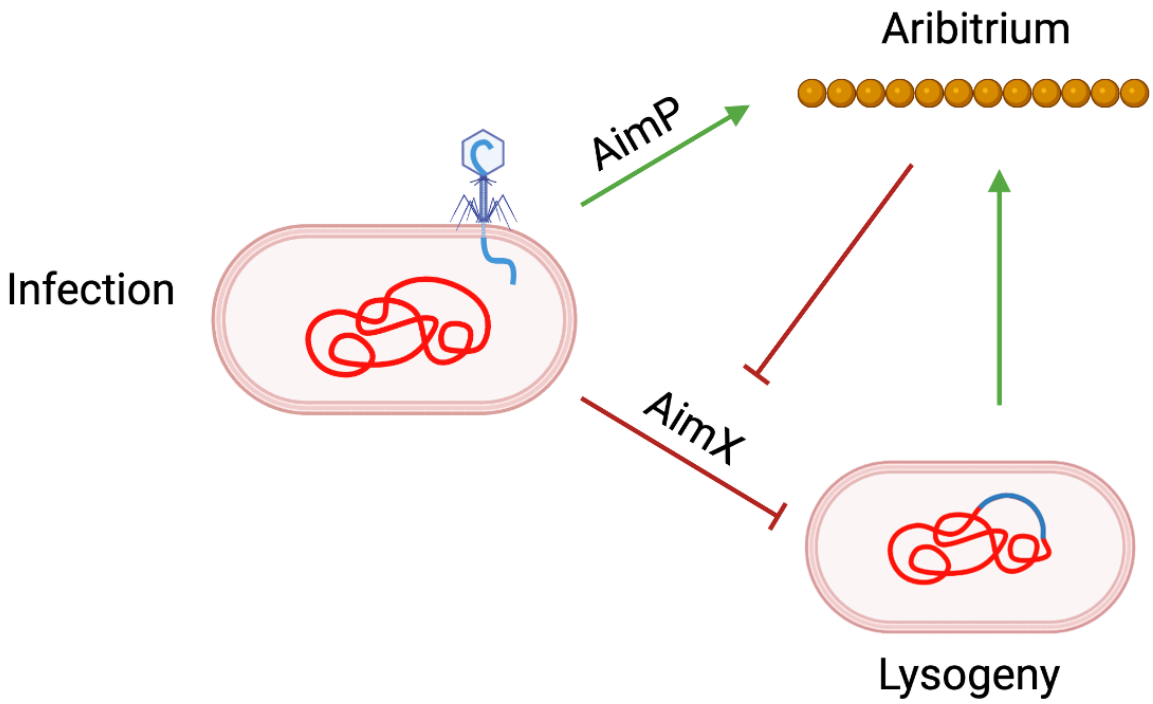


Figure 3.7: Illustration of feedbacks based on molecular regulations with lysogen-induced arbitrium

2 Comparisons with Multiplicity of Infection Dependent Mechanism

Multiplicity of Infection Dependent Mechanism

Bacteriophage λ, also a temperate phage, makes use of another strategy to make lysis/lysogeny decision where the propensity of lysogeny is dependent on the multiplicity of infection (MOI), that is, how many concurrent phages are infecting the same host. It is found that when MOI is low, it hints that surrounding phage concentration is low, prompting the infecting phage to lyse and reproduce. Conversely, when MOI is high, infecting phages tend to enter lysogeny, to prevent overkill of hosts (figure 4.1).[21]

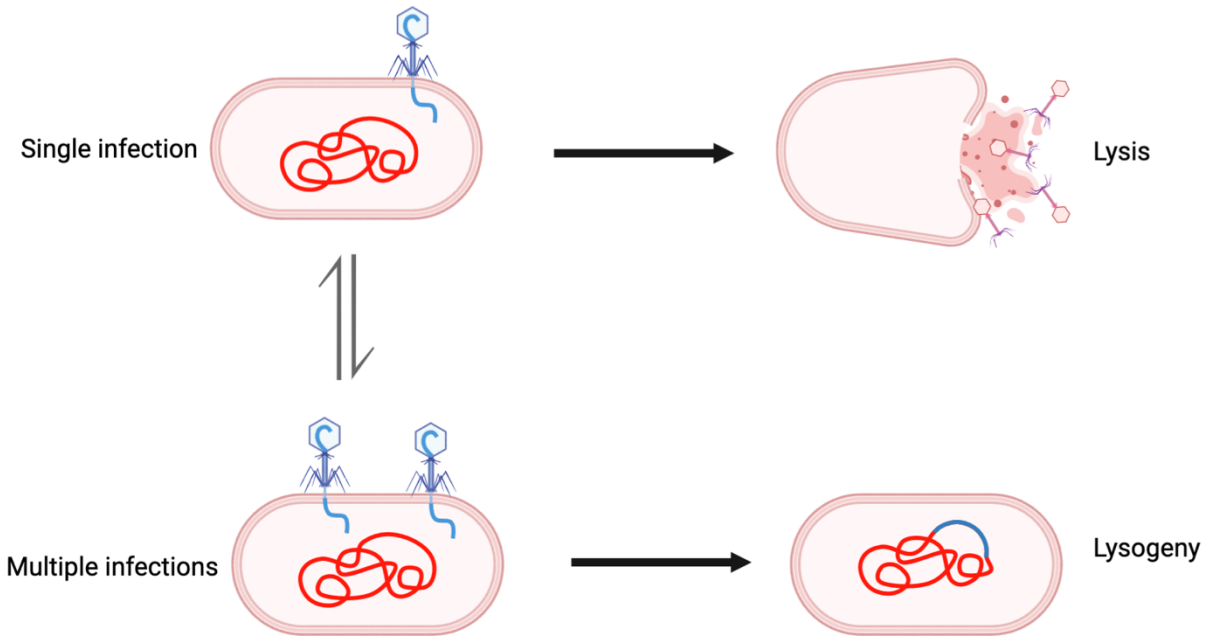


Figure 4.1: Illustration of phages' lytic-lysogenic decision based on multiplicity of infection

The model to describe MOI dependence is similar to that of before,

$$\frac{dS}{dt} = \overbrace{rS\left(1-\frac{N}{K}\right)}^{logistic\ growth} - \overbrace{\eta SP}^{infection}$$

$$\frac{dL}{dt} = \overbrace{rL\left(1-\frac{N}{K}\right)}^{logistic\ growth} + \overbrace{\delta\sum_{m=1}^{\infty}\varphi(m)S_m}^{lysogenic\ infection} - \overbrace{\alpha L}^{induction}$$

$$\overbrace{\frac{dS_1}{dt} = \eta SP - \eta S_1 P - \delta S_1}^{multiple\ infections}$$

$$\frac{dS_m}{dt} = \eta S_{m-1}P - \eta S_m P - \delta S_m$$

$$\frac{dP}{dt} = \overbrace{B\alpha L}^{induction\ burst} + \overbrace{\beta\delta\sum_{m=1}^{\infty}(1-\varphi(m))S_m}^{lytic} - \overbrace{(\delta + aN)P}^{decay\&absorption}$$

except that the propensity is now a function of MOI. $\varphi(m)$ indicates the lysogeny propensity for hosts being infected by m concurrent phages. Here, we assume that the propensity of lysogeny is the same for all m≥3 for practical purpose.

Following previous work where evolutionary optimum is given where optimal in well-mixed system has propensity $\varphi(1) = 0, \varphi(m > 1) = 1$ [13]. We can compare it with the non-MOI-sensitive case where $\varphi(m) = 0.5$ for all m, essentially a bet-hedging phage with φ =0.5. We can see the significant advantage of employing MOI dependent strategy in terms of both phage and lysogen productions, while the depletion of bacteria is also maximized in MOI optimum.

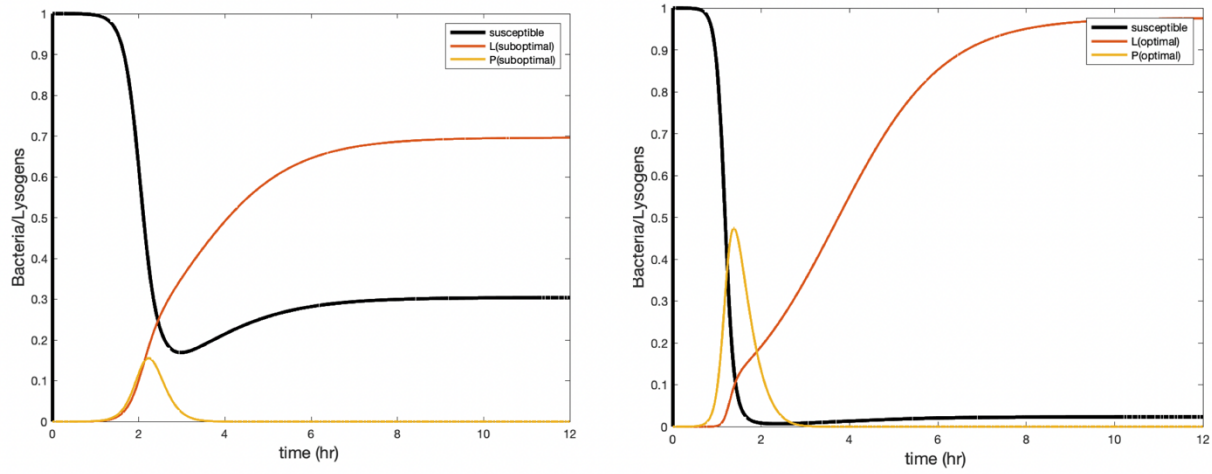


Figure 4.2: Simulation of well-mixed MOI systems with suboptimum(left) and optimum(right)

Comparisons in structured environments

We can then explore the behavior of MOI dependent phages in structured environments. We aim to compare optimal and suboptimal cases for arbitrium systems without lysogen-induced arbitrium($\theta = 0.6, \theta = 1$), arbitrium systems with lysogen-induced arbitrium ($\theta = 0.8, \theta = 1$), and MOI systems (figure 4.3). Surprisingly, the suboptimal virulent variants in arbitrium systems always produce most phages, as virulent variants are only sensitive to arbitrium if the arbitrium concentration is high enough. If they are outran by diffusion of arbitrium, they are essentially never entering lysogeny, neglecting the negative feedback. On the other hand, even in suboptimum, MOI systems still act as bet-hedging phages, such that there are always portions of phages entering lysogeny. Consider the optima, we can see switch like behaviors in arbitrium systems where phages are sensitive to the wavefront and almost fully commit to one decision after the arbitrium flux approaches steady state. Whereas for MOI optimum, the information is not governed by diffusion, but the history of infections. Therefore

the adaptive processs is more gradual, there are always production in both lysogen and phages, which in turn produce more in both.

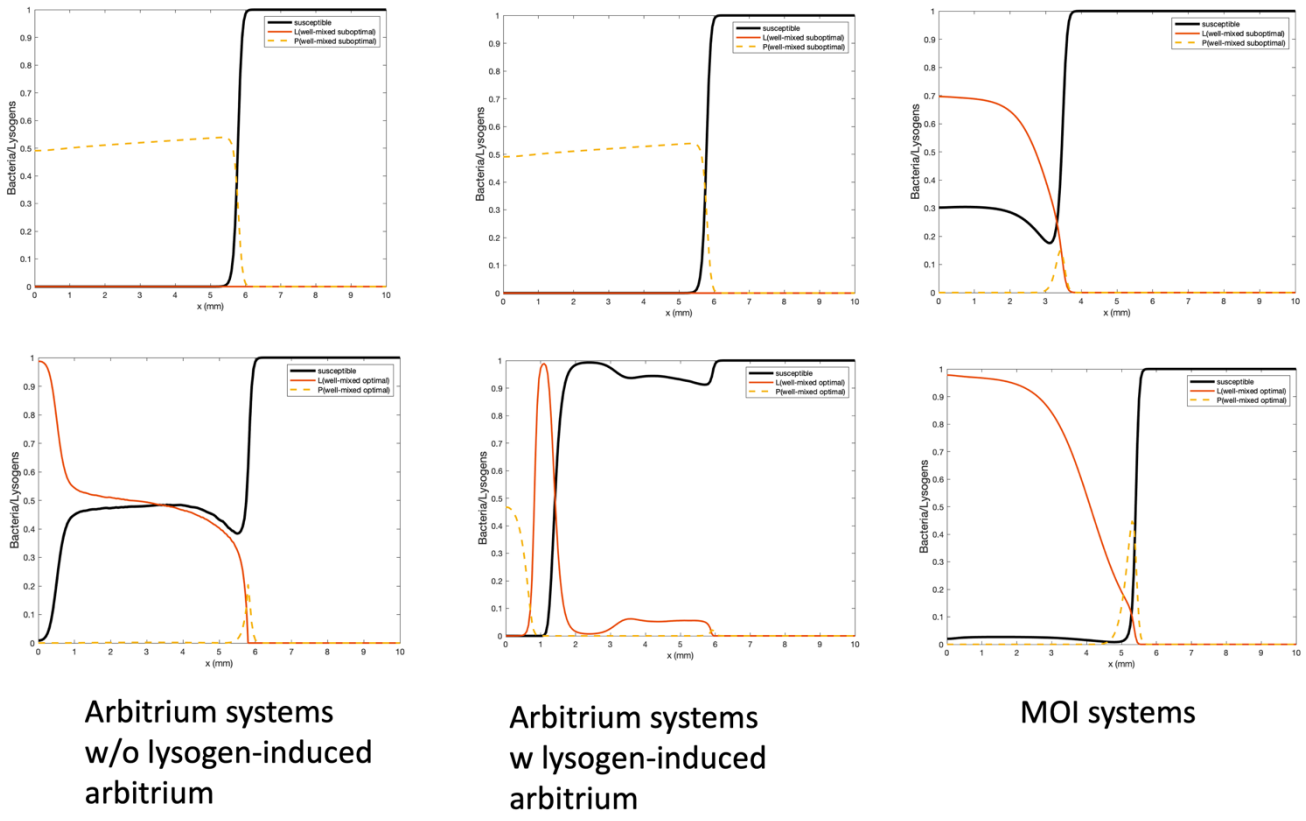


Figure 4.3: Comparisons of spatial momdels. Top row: suboptimum settings, bottom row: optimum settings. First column: Arbitrium systems with $C_L = 0$, second column: Arbitrium systems with $C_L = C_P$, third column: MOI systems

Discussion

It is also worthwhile to look at the "feedback" perspective again. Although it is not in the way of direct molecular regulation, the same concept may apply for the multiple states in MOI systems. Consider the case with $\varphi(1) = 0, \varphi(m > 1) = 1$, it is very similar to that of arbitrium based communication, that it essentially contains a fast positive feedback (single infection with lysis makes more phage

and increase MOI) and a slow negative feedback (delayed lysogeny from multiple infection based). This creates similar dynamics compared to arbitrium based communication. Indeed, the use of propensity as a function of MOI is spontaneous adaptation within phage communities, as a communication of population level without medium. However, the absence of communication medium is significant in spatially structured environments. As aforementioned, the direct communication as a diffusive process plays an important role in creating delay for local optimal variant to grow in late games. In the case of MOI spatial model, with only diffusion of phages, the wave front is always having both single infection and multiple infection working together in growth. In other words, the behavior of MOI dependent phages is the same everywhere in MOI systems. Whereas in the case of arbitrium based communication, arbitrium acts as a diffusive switch, that changes the behavior locally, in an abrupt manner.

Although it appears that MOI based strategy can produce more lysogens and phages at its optimum, as previously discussed, the reservation using abitrium communication might be more beneficial for certain scenario such as influx of susceptible cells due to cell migration, or loss of superinfection immunity by lysogens. As opposite to MOI based strategy, abitrium communication is more favorable for diversity, while the individuals have more extreme properties, their optima are not completely dominant, allowing coexistence of variants, and division of labor if needed. For example, more virulent variants can reproduce more free phages and travel further, while more lysogenic variants can reproduce more lysogens that peak high and within a confined region, making mutatable abitrium based phages versatile to changes. As the winning condition in real life can be situational. Unlike arbitrium vs bet-hedging, it is difficult to say which strategy is more superior in all the way with the plasticity of both strategies.

3 Conclusion

The investigation here elucidates thee effects and advantages of communication among phages. We also have shown the significance of spatial effect to communication properties that temperate phages use the arbitrium molecular signaling system to optimize lysis-lysogeny decisions. By responding to the concentration of signal in the environment by using different response thresholds for lysogeny, they are able to that optimize the lysogen distribution or other goals in the system. With several other recent examples of temperate phages interact with signaling molecules, our results add to growing evidence that temperate viruses are able to utilize both biotic and abiotic signal to adapt their infection strategies and the evolutionary advantage of such plasticity. The model used also allows us to test hypotheses about the arbitrium system and extend upon it. For instance, phages' lysogeny propensity response is not fully known. Here we only implement the arbitrium response as a stepwise function. In nature, phages might respond more gradually, with potential prophage reactivate mechanisms. By altering the arbitrium equation, the model can also be adapted to capture other regulation mechanisms by similar quorum sensing signals in other temperate phages affected by small signalling molecules. While the details of arbitrium systems are not well explored, the models here are to capture important features of communications in general. Once more data become, these can be incorporated in the model by adjusting the parameters, response and missing mechanisms, in order to refine a more specific model of the arbitrium system. Last but not least, the actual ecology of temperate phages using arbitrium communication is to be explored, as the evolutionary advantages of using arbitrium are only suggestive here. Mathematical modelling and experiments in parallel help to better understand the regulation behind these ecology and evolution.

Table of parameters

Parameter	Description	Value	Reference
a	Infection rate	$1000h^{-1}$	[22]
r	Growth rate	$1h^{-1}$	[23]
τ	Induction delay	$0.67h$	[17]
B	Burst size	20	[22]
K	Carrying capacity	$10^9\ ml^{-1}$	[23]
α	Induction rate	$0.001h^{-1}$	[23]
C_P	Signal production	$20h^{-1}$	[16]
δ	Phage decay rate	$0.01h^{-1}$	[22]
u	Signal uptake rate	$10h^{-1}$	Estimated
D_P	Diffusion of phages	$0.01mm^2h^{-1}$	[24]
D_A	Diffusion of signal	$0.4mm^2h^{-1}$	[25]

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