Optimal strategies for pirate phage spreading

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Abstract

Pirate phages use the structural proteins encoded by unrelated helper phages to propagate. The best-studied example is the pirate P4 and helper P2 of coli-phages, and it has been known that the *Staphylococcus aureus* pathogenicity islands (SaPIs) that can encode virulence factors act as pirate phages, too. Understanding pirate phage spreading at population level is important in order to control spreading of pathogenic genes among host bacteria. Interestingly, the SaPI-helper system inhibits spreading of pirate/helper phages in a lawn of helper/pirate lysogens, while in the P4-P2 system, pirate/helper phages can spread in a lawn of helper/pirate lysogens. In order to study which behavior is advantageous in spreading among uninfected host, we analyzed the plaque formation with pirate-helper co-infection by using a mathematical model. In spite of the difference in the strategies, we found that both are relatively close to a local optimum for pirate phage spreading as prophage. The analysis highlights the complex strategy space in pirate-helper phage interaction.

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INTRODUCTION

Spreading of pathogenic genes including antibiotic-resistance genes and toxin genes among bacteria is a real threat to the human society [1, 2]. One of the well-known examples of the mobile genetic elements that contribute the spreading process is the *Staphylococcus aureus* pathogenicity islands (SaPIs). SaPIs spread among the host bacteria by using bacteriophages as their "helper" [1–5]. Interestingly, the mechanism of SaPIs spreading has many aspects in common with that of the well-studied pirate phage P4, that uses the helper phage P2 to spread among the host *Escherichia coli* [3, 5, 6].

The pirate phages and helper phages we consider here all belong to the order *Caudovirales* or the tailed bacteriophages [5, 7], hence a phage particle consists of a head that contains the double stranded DNA genomes and a tail. However, SaPI or pirate phage P4 does not have the structural genes that encode head and tail proteins, thus it cannot produce its progeny when infecting a host cell alone, and typically it becomes a prophage (Fig. 1a). Helper phages for them (e.g., ϕ11 for SaPIbov1, P2 for P4) are temperate phages. When infecting a sensitive host cell, it can enter either the lysogenic pathway with probability α_H where the phage genome is integrated to the host chromosome as a prophage, provide immunity to the same phage, and replicate with the host, or the lytic pathway with probability $1 - \alpha_H$ to produce multiple copies of the phage particles and come out by lysing the host cell (Fig. 1b). Interestingly, pirate phages encode genes that allow them to interfere with the helper phage burst if it happens in the same host cell, including a factor to modify the size of helper head capsid so that it becomes too small to carry the helper genome. As a result, pirate phages take over the structural proteins produced from helper genes and produce pirate phages. This phenomenon was termed as "molecular piracy" [5].

The fascinating mechanisms of the molecular piracy have been intensively studied, first for P4 which was found in 1963 [8]. SaPIs have attracted attention as a cause of the outbreak of toxic shock syndrome [9] and on-going studies are revealing the importance of them in *S. aureus* [2]. The pirate phages are ubiquitous; it has been found that the P4-type sequences are widespread among *E. coli* strains [10], while on average one SaPI was found per natural *S. aureus* strain [2]. These findings demonstrate the importance of the pirate phages as a source of horizontal gene transfer in bacteria.

When looking closer, however, SaPIs and P4 appear to have chosen very different strategies in their behavior. In the case of SaPI, when it infect a helper lysogen, it simply integrates

its genome to the host genome to form a double lysogen [11]. However, if a helper phage infect a SaPI lysogen and goes lytic (or the helper prophage in a double lysogen is induced), majority (fraction $f_P^{(H)} \geq 0.9$) of the produced phage particles are SaPI particles [12]. The interference is so severe that helper phages are normally not able to make a visible plaque on a pirate lysogen lawn [4] (Fig. 1c). On the contrary, when infecting a helper P2 lysogen, a pirate P4 phage chooses lysogenic pathway with probability $\alpha_P \sim 0.4$, otherwise it chooses lysis [13]. Upon bust, almost all the produced phages are P4 (fraction $f_P \sim 1$) [14], i.e., P4 phage can spread on a lawn of P2 lysgoen. When a P2 phage infects a P4 lysogen, the interference is weak, and upon lysis only small part (fraction $f_P^{(H)} \sim 0.001$) of the produced phages are P4 [15], enabling P2 phage to spread on a lawn of P4 lysogens (Fig. 1d).

Since both SaPIs and P4 are widespread, both choices seems to be viable. However, at least when looking at spreading on lysogens, the SaPI-helper system is inhibiting each other, while the P4-P2 system is supporting each other. A nontrivial question is how they spread when both pirate phage and helper phage encounter a population of host cells without any prophage. Can they spread, or inhibit each other? Is one of the strategy clearly better than the other, or do they do equally well?

In order to answer this question, we study the spreading of pirate phage and helper phage among uninfected host cells at population level by using a mathematical model. Previous studies on phage-bacteria systems have shown that the Lotka-Volterra type population dynamics model can reproduce many of the important phage-bacteria dynamics reasonably well in various length and time scales [16–26]. As a simple and well-defined set up, we here analyze the plaque formation in a lawn of uninfected cells initially spotted by a droplet of pirate-helper phage mixture, using the Lotka-Volterra type equations with diffusion of phage and nutrient [24]. We vary the key parameters of the phage strategies, α_H, $f_P^{(H)}$, α_P, and f_P, to reveal their effect on phage spreading.

MATERIALS AND METHODS

Model equations

We model a plaque formation experiment setup called the spot assay [27], where large number of bacteria in a soft agar is cast in a thin layer on a hard agar plate that contains nutrient, a droplet of liquid that contains phages is placed, and subsequently incubated

overnight. With this method, it is easy to co-infect the bacteria with both helper and pirate phages in a well-controlled manner. Bacteria cannot swim visibly in the high viscosity of soft agar, hence each initially casted bacterium grow and divide locally to form a microcolony [28, 29]. As time goes, phages produced upon lysis of infected bacteria reach neighbor bacteria by diffusion to infect them and continue spreading. When the nutrients run out, both bacteria and phage growth ceases, leaving the plaque in the final configuration. When the phage is temperate, lysogens can grow in the infected area, resulting in a turbid plaque.

We model this plaque formation dynamics by modifying the model proposed in [24], where the infection of bacteria by phage is modelled by using Lotka-Volterra type equation. The latency time for the phage burst is taken into account by considering the intermediate states, and bacteria grows locally while the phage and the nutrient diffuse in space. Even though the local growth of bacteria into microcolonies may provide extra protection against phage attack [25, 29], for simplicity we here assume interaction in a locally mixed population. To extend the model to the pirate-helper phage system described in Fig. 1, we consider the following variables that are function of position $\mathbf{r}$ and time t; uninfected bacteria $B(\mathbf{r}, t)$, the pirate phage lysogen $L_P(\mathbf{r}, t)$, the helper phage lysogen $L_H(\mathbf{r}, t)$, the double lysogen of the pirate and helper phages $L_{PH}(\mathbf{r}, t)$, the pirate phage $P(\mathbf{r}, t)$, the helper phage $H(\mathbf{r}, t)$, intermediate states I with subscripts that express each infection pathway, and the nutrient level $n(\mathbf{r}, t)$. Note that, in the case of P4, 99% of the case the genome is integrated to the chromosome, but 1% go into the plasmid state [13]. We here ignore these distinctions for simplicity and treat them as lysogens. We express these populations in the unit of number per area, summing up the population over the thin soft-agar thickness Δa.

When the lytic pathway is chosen upon infection, the free phages are produced after intermediate steps. Naturally, when a helper phage infect a uninfected bacterium and choose lysis, it will produce helper phages only as long as there is no super infection by a pirate phage in early part of lytic pathway. The production of the pirate phage (or mixture of the helper and the pirate phages) can happen in different ways: (i) When a pirate phage infects a helper lysogen and force the lytic pathway with the probability $1 - \alpha_P$. (ii) When a helper phage infects the pirate lysogen and choose the lytic pathway with probability $1 - \alpha_H$. Furthermore, co-infection experiment at different timing [30] suggests that some pirate phages can be produced (iii) when a pirate phage infects a bacterium in an early stage of the helper lytic pathway. The model reflects these different possibilities for production of phages, that affect the outcome upon burst.

This gives a set of the equations as follows:

$$B_{tot} = B + L_P + L_{PH} + L_H + \sum_{i=1}^{M} \left(I_H^{(i)} + I_{HP}^{(i)} + I_P^{(i)} \right), \tag{1}$$

$$g(n) = g_{max} \frac{n}{K_s + n}, \tag{2}$$

$$\frac{\partial n}{\partial t} = -g(n) B_{tot} + D_n \nabla^2 n, \tag{3}$$

$$\frac{\partial B}{\partial t} = g(n) B - \frac{\eta_P}{\Delta a} P \cdot B - \frac{\eta_H}{\Delta a} H \cdot B, \tag{4}$$

$$\frac{\partial L_P}{\partial t} = g(n) L_P + \frac{\eta_P}{\Delta a} P \cdot B - \frac{\eta_H}{\Delta a} H \cdot L_P, \tag{5}$$

$$\frac{\partial L_H}{\partial t} = g(n) L_H + \alpha_H \frac{\eta_H}{\Delta a} H \cdot B - \frac{\eta_P}{\Delta a} P \cdot L_H, \tag{6}$$

$$\frac{\partial L_{PH}}{\partial t} = g(n) L_{PH} + \alpha_H \frac{\eta_H}{\Delta a} H \cdot L_P + \alpha_P \frac{\eta_P}{\Delta a} P \cdot L_H \tag{7}$$

$$\frac{\partial I_H^{(1)}}{\partial t} = (1 - \alpha_H) \frac{\eta_H}{\Delta a} H \cdot B - \frac{\eta_P}{\Delta a} P \cdot I_H^{(1)} - \frac{g(n)}{g_{max}} \frac{M}{\tau} I_H^{(1)}, \tag{8}$$

$$\frac{\partial I_H^{(i)}}{\partial t} = \frac{g(n)}{g_{max}} \frac{M}{\tau} (I_H^{(i-)1} - I_H^{(i)}) \qquad (\text{for } i = 2, \cdots, M), \tag{9}$$

$$\frac{\partial I_{HP}^{(1)}}{\partial t} = (1 - \alpha_H) \frac{\eta_H}{\Delta a} H \cdot L_P + \frac{\eta_P}{\Delta a} P \cdot I_H^{(1)} - \frac{g(n)}{g_{max}} \frac{M}{\tau} I_{HP}^{(1)}, \tag{10}$$

$$\frac{\partial I_{HP}^{(i)}}{\partial t} = \frac{g(n)}{g_{max}} \frac{M}{\tau} (I_{HP}^{(i-)1} - I_{HP}^{(i)}) \qquad (\text{for } i = 2, \cdots, M), \tag{11}$$

$$\frac{\partial I_P^{(1)}}{\partial t} = (1 - \alpha_P) \frac{\eta_P}{\Delta a} P \cdot L_H - \frac{g(n)}{g_{max}} \frac{M}{\tau} I_P^{(1)}, \tag{12}$$

$$\frac{\partial I_P^{(i)}}{\partial t} = \frac{g(n)}{g_{max}} \frac{M}{\tau} (I_P^{(i-)1} - I_P^{(i)}) \qquad (\text{for } i = 2, \cdots, M). \tag{13}$$

$$\frac{\partial H}{\partial t} = \beta \frac{M}{\tau} \left(I_H^{(M)} + (1 - f_P^{(H)}) I_{HP}^{(M)} + (1 - f_P) I_P^{(M)} \right) - \frac{\eta_H}{\Delta a} H \cdot B_{tot} - \delta H + D_H \nabla^2 H, \tag{14}$$

$$\frac{\partial P}{\partial t} = \beta \frac{M}{\tau} \left(f_P^{(H)} I_{HP}^{(M)} + f_P I_P^{(M)} \right) - \frac{\eta_P}{\Delta a} P \cdot B_{tot} - \delta P + D_P \nabla^2 P. \tag{15}$$

Here, we assume Monod's growth law [31] with the maximum growth rate g_{max} and the Michaels constant K_s. Nutrient is measured in a unit where the yield for bacteria growth is one. We assume that the bacteria that are in intermediate steps of lysis consume the nutrient at the same rate as the growing bacteria. The pirate (helper) phage adsorption rate η_P (η_H) is divided by Δa reflecting that we are summing up the populations over this depth. The latency time τ and the total number of phage particles produced per burst β are assumed to be the same for all infection pathways for simplicity. The intermediate infection steps are divided into $M = 10$ steps, that gives about 30% fluctuation in the latency time

[24]. We assume that the latency time becomes longer proportional to the doubling time, ensuring that the phage production stops when the bacteria growth stops due to the lack of nutrient. Free phages decay at the rate δ, but it is in general small [32] that does not affect the present result. The diffusion constants for the helper phage, the pirate phage, and the nutrients are introduced as D_H, D_P, and D_n, respectively.

Parameters

Table I shows the list of the default parameters used, with the references for each value when available. Most of the values are chosen from the well-studied P4-P2 system, but when known the value for SaPI and a relatively well-studied helper phage for SaPIs, ϕ11, are also stated with references. To compare the difference of the outcomes due to the difference in the strategies described in Fig. 1, we vary the parameters α_H, $f_P^{(H)}$, α_P, and f_P, with keeping the rest of the parameters.

Initial condition and Numerical integration

We solved the model partial differential equations in polar coordinates with the origin at the center of the initially placed droplet. We assume the symmetry in circular direction, and the spatial variation is only considered along the radial direction r. At a distant outer boundary $r = R_{max}$, which is set to be 9 mm, we impose reflecting boundary condition for both phage and nutrient concentrations.

We analyze the plaque spreading in a lawn of uninfected bacteria. This is represented by initially distributing uninfected bacteria and nutrient uniformly as $B(r, 0) = B_0$ and $n(r, 0) = N_0$, with the rest of the variables set to zero, except for the phages placed in the middle where the phages are spotted. The average phage inputs in the initial spot of helper phage API_H and pirate phage API_P determine the inital phage concentrations. The initial spot of radius $R_s = 1$mm is given by setting $H(r, 0) = B_0 \cdot API_H \cdot \Theta(R_s - r)$ and $P(r, 0) = B_0 \cdot API_P \cdot \Theta(R_s - r)$, where $\Theta(x)$ is the Heaviside step function. We set $B_0 = 1/(18\mu\text{m})^2$ throughout this paper [24].

The integration was done by the finite difference method, and time integration was done by the 4-th order Runge-Kutta method. The size of the spatial discretization, Δr, was set to $18\mu m$, hence at the initial condition there is one bacterium per Δr^2 ($B_0 = 1/\Delta r^2$). Time

step of integration was set to $\Delta t = 10^{-4}h$.

The visualization of the plaque from the simulation data was done as described in [24]. In short, the random distribution of initially dispersed bacteria and the stochasticity of decision to take lysogenic pathway is taken into account in the visualization. This is to reflect that, especially at the early stage of the plaque formation, bacteria may often locally eliminated but a few survive by being lysogen, hence microcolony can be less in number but the surviving colony can grow bigger.

RESULTS

Comparison of a plaque in SaPI-helper system and a plaque in P4-P2 system

We analyze the plaque spreading in a lawn of uninfected bacteria. The simulation mimics the plaque formation by the spot assay [27], where soft agar with bacteria is cast over hard agar with nutrient, a droplet with mixture of phages is placed in the center, and incubated overnight. In the setup, bacteria cannot swim visibly and the local growth results in formation of a microcolony at the position where a bacterium was initially dispersed [28, 29]. Phages grow and diffuse to infect new bacteria, resulting in a circular zone of killing around the initial spotted area. When the phage is temperate, lysogens will survive and grow, making the plaque turbid. The simulation was started by placing a droplet of mixture of pirate and helper phage with radius 1mm. The concentration of phages in the droplet is defined by the average phage input per initially placed bacterium for the helper phage, API_H, and the pirate phage, API_P. The detail of the simulation is given in the method section.

In order to compare the SaPI-helper strategy and the P4-P2 strategy, we set most of the parameters same for both systems as described in Table I. The difference in strategy is reflected by choice of the following parameters: the helper frequency of lysogeny α_H, fraction of the pirate phage production upon a helper phage lysisng a pirate lysogen $f_P^{(H)}$, the pirate frequency of lysogeny upon infection of helper lysogen α_P, and the fraction of the pirate phage production upon a pirate phage lysing a helper lysogen f_P. For the SaPI-helper system, we set $\alpha_H = 0.15$, $f_P^{(H)} = 0.9$, and $\alpha_P = 1$ (f_P is meaningless in this case), and for the P4-P2 system, we set $\alpha_H = 0.15$, $f_P^{(H)} = 0.001$, $\alpha_P = 0.4$, and $f_P = 1$.

The resulting plaques when $API_H = API_P = 1$ (i.e., on average there is one of each phage

per initially placed bacterium under the initial spot) are visualized in Fig. 2 for the SaPI-helper system (a) and the P4-P2 system (h). Each filled circle represents a microcolony that had started from a single bacterium distributed at time zero. The radius of a microcolony was calculated based on the final population size, assuming that volume of a microcolony is given by number of bacteria times $1\mu m^3$, which is the typical volume of an *E. coli* cell. The microcolonies are colored darker in proportion to the fraction of the lysogen with pirate prophage $L_P + L_{PH}$ per microcolony. Note that the pirate phage only lysogen L_P is quite small in the final plaque (Fig. 2fm), hence L_{PH} represents the host cells with a pirate phage genome. The outer edge of the plaque is dominated by helper lysogen L_H, which can be distinguished from the uninfected lawn of B based on the smaller microcolony size. We see that the SaPI-helper system and the P4-P2 system has roughly the same plaque radius, but the pirate prophage spread more in the SaPI-helper system.

The time course of the plaque formation are shown in Fig. 2(b-g) for the SaPI-helper system and in Fig. 2(i-n) for the P4-P2 system, where populations at each location are shown as a function of distance from the infection origin, r. The profile for the uninfected bacteria and the helper phage at the front of the plaque spreading looks very similar between the two system (Fig 2bc vs. ij). This shows that the helper phage can start growth at the front of the plaque propagation, without feeling the pirate phage. This explains that the overall plaque size are the same since we set the helper phage property to be identical when there is no pirate phage.

The difference is in how the pirate phage can propagate. By comparing Fig. 2d and k, we see that pirate phage can come up much later and close to the center in the P4-P2 system, where pirate phages can be produced only when the helper lysogen L_H is lysed by the pirate phage infection. In the SaPI-helper case, the wave of pirate phage follows the front propagation of helper phage by taking over the part of the helper phage lysis, making it possible for the pirate phage to spread a lot faster and further away, making larger amount of the double lysogen in the final state (Fig. 2g vs. n).

These results depicts that the SaPI-helper strategy is indeed beneficial for the spreading of the pirate phage gene in spreading in space, because the strong interference does not inhibit helper spreading as long as the helper phage takes over the front of the plaque propagation. The P4-P2 strategy, on the other hand, does allow the helper phages to spread, but the pirate phage gene does not spread much because pirate phage can grow only later, after the helper lysogens are established.

Initial phage input dependence of the phage gene spreading

The spreading of the phage gene should depends on the initial concentration of the each phage, API_H and API_P, in the spot. When the initial pirate phage input API_P is too high compared to API_H, the strong interference of SaPI-helper strategy may inhibit spreading of both of the phages, while when $API_H \gg API_P$, the P4-P2 strategy may better to spread since it can lyse the helper lysogens. For systematic understanding, we investigated how the outcome changes when varying the initial concentration of the phages in the spot, API_H and API_P. The results are summarized in Fig. 3 for (a) the SaPI-helper strategy and (b) the P4-P2 strategy by the final population with the helper phage gene $L_H + L_{PH}$ and the pirate phage gene $L_P + L_{PH}$ integrated over space. The integration is done outside of the initial spot radius, in order to focus on the effect of the spreading.

Somewhat counterintuitively, for the helper phage spreading, we observe that the SaPI-helper strategy is always as good as or even better than the P4-P2 strategy (Fig. 3a left and b left). Because the pirate phage cannot spread by themselves, the system self-organize so that the helper take over the spreading front. Once the helper take over the front, it is better for helper prophage that pirate phage cannot lyse the helper lysogen. The spreading is inhibited when $API_P \sim 100$ and $API_H \sim 0.01$, where the helper phage fails to take over the front. However this seems to happen for similar range of API's for both of the strategies; simply the initial helper phage number is too low to grow and spread significantly outside of the initial spot before nutrient runs out. For the pirate phage spreading, Fig. 3 shows that the P4-P2 strategy is better when the initial pirate phage input is API_P small and the helper phage input API_H is large. In this case the abundant helper phages convert bacteria into helper lysogens first, therefore for the pirate phage production it is necessary that the pirate phage can lyse the helper phage to produce pirate phage ($\alpha_P < 1$, $f_P > 0$). When API_P is moderately high, the SaPI-helper strategy do as well or better than the P4-P2 strategy in the pirate phage spreading, as we saw in Fig. 2.

Phase diagram of the phage gene spreading in strategy space

So far, we observed there are pros and cons in the SaPI-helper strategy and the P4-P2 strategy in the phage gene spreading, depending on the initial condition. Next, we analyzed the dependence on the parameters that govern the pirate-helper interaction, α_H, $f_P^{(H)}$, α_P

and f_P. We did this with two different initial conditions based on Fig. 3: $API_H = API_P = 1$ where the SaPI-strategy is better for the pirate phage gene spreading (Fig. 4ab), and $API_H = 50$ and $API_P = 0.05$ where the P4-P2 strategy is better (Fig. 4cd). Fig. 4ac are "SaPI-helper" phase diagram, where the parameters that are not varied in the plot are fixed to the default value for the SaPI-helper system ($\alpha_P = 1$ for top panels, and $\alpha_H = 0.15$ and $f_P^{(H)} = 0.9$ for bottom pannels). The stars in these figures indicate the default parameter for the SaPI-helper system. Similary, the "P4-P2" phase diagram (Fig. 4bd) uses $\alpha_P = 0.4$ and $f_P = 1$ for top panels, while $\alpha_H = 0.15$ and $f_P^{(H)} = 0.001$ are used for bottom panels, and the stars indicate the default parameter for the P4-P2 system.

For all the cases, α_H primarily determines the helper phage spreading, which is natural because helper phages are at the front of the spreading for both of the initial condition, hence the interaction with the pirate phage matters less. Naturally intermediate level of α_H is the best for having high level of helper prophages, since it allows both production of helper phages to spread and the lysogenization of the helper phages. α_P, that determines the frequency for pirate phage to lyse helper lysogen, has weak effect on the helper phage spreading, and as expected low α_P and high f_P reduces the helper phage spreading.

Interesting patterns can be seen in the pirate phage gene spreading. For $API_P = API_H = 1$ case with $\alpha_P = 1$ (right top panel of Fig. 4a), the pirate phage production relies on the high $f_P^{(H)}$, and it is more effective for low α_H. However, if $\alpha_H = 0$ there will not be any double lysogen left. Hence there is a peak at low but finite α_H and at high $f_P^{(H)}$. Since there are enough pirate phages from the start, strong interference with $f_P^H = 0.9$ is effective enough for production of the new pirate phages, hence $\alpha_P = 1$ maximizes the pirate prophages at the end (right bottom panel of Fig. 4a). The location of the stars in the diagram Fig. 4a shows that the SaPI strategy is close to the global optimum for the pirate phage spreading for this initial condition.

The P4-P2 statategy is less optimal for pirate phage gene spreading with $API_P = API_H = 1$, as we already have seen in Fig. 2. Fig. 4b top panel shows that it is better to have higher $f_P^{(H)}$, though the ability of the pirate phage to lyse the helper phage and take over ($\alpha_P = 0.4$, $f_P = 1$) makes wider range of $f_P^{(H)}$ values acceptable to for the spreading. Clearly, $f_P^{(H)} = 0.001$ (location of the star) is too low. However, given that $\alpha_H = 0.15$ and $f_P^{(H)} = 0.001$, the pirate prophage spreading works the best when $f_P = 1$ and α_P intermediate, allowing large production of pirate phages by infecting helper lysogen and some formation of double lysogens. Interestingly, the P4-P2 strategy (the star in Fig. 4b right

bottom) is close to this local optimum.

In the $API_P = 0.05$ and $API_H = 50$ case, most of the uninfected bacteria under the initial spot are converted to the helper lysogen first. Therefore, it becomes better to be able to lyse the helper lysogen and produce pirate phage, which gives maximum at finite α_P and $f_P = 1$ for pirate prophage spreading in Fig. 4cd right bottom panels. Still, being able to interfere with the lysis caused by helper phage (high $f_P^{(H)}$) helps as shown Fig. 4cd right top panels. Also, we see that the SaPI-helper strategy is close to the local optimum given $\alpha_P = 1$ (the star in Fig. 4c right top). Given $f_P^{(H)}$ is high as in SaPI-helper case, α_P does not need to be that low to be optimal (Fig. 4c right bottom). Overall, the SaPI-helper strategy (stars in Fig. 4c) is not the best but not too far from the optimum performance. Fig. 4d shows that the P4-P2 strategy could do better by having somewhat higher $f_P^{(H)}$, but again the value of α_P and f_P is close to the optimum for the given value of α_H and $f_P^{(H)}$.

DISCUSSION

The pirate-helper phage system demonstrates the fascinating richness of the interaction between phages in nature. At the same time, given the increased number of possible infection scenarios, it becomes significantly complex to analyze the population dynamics. Still, the SaPI-helper system and the P4-P2 system pose interesting examples, in that their spreading among the lysogen populations are completely opposite (Fig. 1). The present analysis of spreading among the uninfected lawn showed that the both strategies appear to be somewhat optimized. The SaPI-helper strategy was found to be the global optimum for $API_P = API_H = 1$ case, while the P4-P2 strategy was a local optimum, i.e., the values of α_P and f_P was the best for the pirate phage gene spreading given that P4 cannot take over much upon helper lysis. When $API_P = 0.05$ and $API_H = 50$, it helps significantly for the pirate phage to be able to lyse helper lysogen and produce pirate phages, making the P4-P2 strategy better than the SaPI-helper strategy. Still, having high value of $f_P^{(H)}$ can help, therefore the SaPI-helper strategy is a local optimum for the given $\alpha_P = 1$, and also the P4-P2 strategy could be optimized better if it had somewhat higher $f_P^{(H)}$. It is interesting to note that $f_P^{(H)}$ of P4-P2 system changes close to 1 upon mutation of P2 gene A or *rep* gene of the host bacterium [15]; co-evolution of the whole system is important when considering how the pirate phage strategies can evolve.

Another point that the present analysis clarified is the weak trade-off between the helper

phage spreading and the pirate phage spreading in the spatially structured lawn of uninfected cells. Even if the interference by pirate phage upon helper burst is strong as $f_P^{(H)} \sim 1$, as long as the helper phages come to the front of spreading this does not inhibit the helper phage spreading, and pirate phage can spread as well by following the front. Because the pirate phage cannot spread by themselves, it is relatively easy for the helper phage to take over the front. The pirate-behind-helper configuration makes the most of the pirate phage lysogens be double lysogens with the helper. However, this contradicts somewhat to the observation that very few SaPIs are found as a double lysogen with its own helper phage [2]. The scarcity of SaPI-helper double lysogen could be due to strong selection pressure against being a helper phage because SaPI is inhibiting the helper phage so much upon induction of the double lysogen [2]. This possibility can be analyzed by extending the present population dynamics model to include the mutations and simulate longer time scale.

In this paper, we focused on the spreading of the pirate prophage among the uninfected population, but it should be noted that this is not the only factor that select the parameters in the pirate-helper system. For example the frequency of lysogenization for helper phage α_H may be selected mainly by the frequency of the host population collapse [33] or by competition with other temperate phages [34], independent of the interaction with the pirate phage, even though the value of α_H do affect the outcome of the pirate-helper system strongly. In addition, there are many other factors that are not considered here that should contribute to the selection of the pirate phage strategies. One of them is the fact that many pirate phages have multiple helper phages. For example, SaPIvol1 can use both phage $\phi 11$ and phage 80α as helper phage [11], while P4 can use not only P2 but also the phage 186 [35]. Naturally, the pirate-helper interaction parameters vary depending on the combination. The frequency of lysogenization α_H for phage 80α was found to be quite low ($10^{-5} \sim 10^{-3}$) [36], which will change the outcome even if other parameters were identical. More interestingly, the P4 phage cannot lyse the phage 186 lysogen, i.e., $\alpha_P = 1$, while interference upon the phage 186 bursting P4 lysogen is moderately strong ($f_P^{(H)} \sim 0.6$ to 0.9)[35], which appear to be closer to the SaPI-heler strategy. It is difficult to tell how often a given pirate phage uses the found helper phage in natural environments, even though that could affect how much the system had evolved for better spreading. It should also be noted that using multiple helper phages is beneficial by itself, since it greatly increases the host range of the pirate phages. However, at the same time, the selection for the ability for a pirate phage to interact with multiple helper phages may put some limit in optimizing the interaction with a specific

helper phage due to the molecular differences of helper phages. In addition, the pirate phage may deliver the gene beneficial to the host bacteria as SaPIs carry pathogenic genes, and this can significantly contribute to spreading of the pirate phage gene, too.

Overall, this paper sheds light on one of the many facets of the art of war among bacteria and phages. Considering the ubiquity of the pirate phage, their impact on the ecology and evolution of the microbial system should be significant. Clearly, further study is required to understand the rich population dynamics of pirate-helper systems.

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FIGURE LEGENDS

FIG. 1. **Strategies of SaPI-helper system and P4-P2 system.** (a) Pirate phage can only become lysogen when infecting a host alone, hence it cannot propagate on a lawn of uninfected host. (b) Helper phage is temperate, with frequency of lysogenization α_H. Typically helper phage forms a turbid plaque on a lawn of uninfected host. (c) In the SaPI-helper system (e.g. SaPIbov1-ϕ11), infection of pirate phage SaPI particle to a helper lysogen results in formation of double lysogen (frequency of lysogenization of pirate phage upon infection of helper lysogen α_P is one). Hence SaPI cannot propagate on a lawn of helper lysogen. When a pirate phage infects a SaPI lysogen and choose the lytic pathway with probability $1 - \alpha_H$, the majority of the produced particles are pirate phaes (fracion of pirate phage upon helper lysis decision $f_P^{(H)} \geq 0.9$), which inhibits the helper phage propergation on a pirate lysogen lawn. (d) In the P4-P2 system, infection of P4 phage to a P2 lysogen can results in the lysis with probability $1 - \alpha_P \sim 0.6$, and upon burst almost all of the phages produced are P4 (fraction of pirate phage upon pirate lysis decision $f_P \sim 1$), allowing pirate phage propagation on a helper lysogen lawn. On the other hand, P4 cannot take over much of P2 when P2 infection lyses a P4 lysgen ($f_P^{(H)} \sim 0.001$), which allows helper phage propagation on a pirate lysogen lawn.

FIG. 2. **Simulated plaque formation by (a-g) SaPI-helper and (h-n) P4-P2 system.** (a) and (h) shows the final configuration, where miclocolonies are darker for the higher fraction of the lysogens with pirate prophage $L_P + L_{PH}$. The outer edge of the plaques consists of helper-only lysogens L_H, which can be distinguished from the uninfected lawn due to smaller sizes of microcolonies. (b-g) and (i-n) represents the time evolution of the populations. The unit is the number of the cells / phage particles per $18\mu m^2$, corresponding to the number per microcolony. The profiles at 1h, 3h, 5h, and 7h are shown by solid line, coarse dashed line, fine dashed line, and dash-dotted line, respectively.

FIG. 3. **The initial condition dependence of the helper and pirate phage gene spreading.** The average phage input in the initial spot, API_H and API_P is varied, and the plaque formation is simulated by using (a) the SaPI-helper system's parameter set ($\alpha_H = 0.15$, $f_P^{(h)} = 0.9$, $\alpha_P = 1$) or (b) the P4-P2 system's parameter set ($\alpha_H = 0.15$, $f_P^{(h)} = 0.001$, $\alpha_P = 0.4$, $f_P = 1$). The number of lysogen cells with helper phage prophage $L_H + L_{PH}$ (pirate phage prophage $L_P + L_{PH}$) integrated over the space out side of the intial spot ($r > 1$mm) and normalized to the maximum observed value 7.1×10^7 (6.3×10^7) are visualized by color code in figure left panels [right panels], respectively, where the higher value is shown with lighter color.

FIG. 4. **The parameter dependence of the helper and pirate phage gene spreading.** The number of lysogen cells with helper phage gene $L_H + L_{PH}$ (pirate phage gene $L_P + L_{PH}$) integrated over the space out side of the intial spot ($r > 1$mm) and normalized to the maximum observed value [5.7×10^7 for $API_H = API_P = 1$ case (a,b) , 8.7×10^7 for $API_H = 50, API_P = 0.05$ case (c,d) (3.1×10^7 for $API_H = API_P = 1$ case (a,b) , 3.8×10^7 for $API_H = 50, API_P = 0.05$ case (c,d))] are visualized by color code, respectively, where the higher value is shown with lighter color. To compare with SaPI-helper system, $\alpha_P = 1, \alpha_H = 0.15$ and $f_P^{(h)} = 0.9$ are used in (a,c) unless the parameter is varied in the plot, and the green stars indicate the default parameter set used for SaPI-helper system (Note that the result with $\alpha_P = 1$ is independent of the value of f_P). Similarly, to compare with P4-P2 system, $\alpha_P = 0.4, f_P = 1, \alpha_H = 0.15$ and $f_P^{(H)} = 0.001$ are used in (b,d) unless the parameter is varied in the plot, and the red stars indicate the default parameter set used for P4-P2 system. The $L_P + L_{PH}$ value in the right bottom panel in (b) is relatively low, so the color is rescaled so that 0.2 appears the lightest.

TABLES

TABLE I. **Default parameters used**

	Meaning	Default value	References, comments
η_H	Helper phage adsorption rate	$3.3 \times 10^3 \mu\mathrm{m}^3/\mathrm{h}$	P2 [32]
η_P	Pirate phage adsorption rate	$13 \times 10^3 \mu\mathrm{m}^3/\mathrm{h}$	P4 [32]
τ	latency time at fast growth	0.75 h	P2 latency 30min[14]$\sim$ 48min [32] P4 latency on P2 lysogen 60 min [14, 32]
β	Total burst size	100	P2 on uninfected host: 60[37]$\sim$ 160[32] P4 on P2 lysogen: 100[15]$\sim$300[32] ϕ11 on uninfected host: $\sim$100 [38]
α_H	Lysogenization frequency for a helper phage	0.15 for both systems	P2: $0.15 \sim 0.2$ [37] ϕ11: ~ 0.14 [39]
α_P	Lysogenization frequency when a pirate phage infects a helper lysogen	0.4 for P4-P2 1 for SaPI-helper	$0.3 \sim 0.5$ [13] 1 [5]
$f_P^{(H)}$	fraction of pirate phage from a helper phage lysing a pirate lysogen	0.001 for P4-P2 0.9 for SaPI-helper	Yield is less than one per cell [15] 0.9-0.99 for SaPI-helper systems [12]
f_P	fraction of pirate phage from a pirate phage lysing a helper lysogen	1 (n/a for SaPI)	$10^{-5} \sim 10^{-3}$ P2 and 100 P4 [14] P2 level possibly due to the induction
D_H	Helper phage diffusion constant	$10^4 \mu\mathrm{m}^2/\mathrm{h}$	4-fold less than the value used for λ [24]. λ and P2 silmiar size [32] but λ was selected for bigger plaque [40].
D_P	Pirate phage diffusion constant	$D_H \times \eta_P/\eta_H$	The tail of P2 and P4 are identical. The difference in the adsorption likely due to the difference in diffusion.
D_n	Nutrient diffusion constant	$4 \times 10^5 \mu m^2/h$	[24]
g_{max}	Fastest growth rate	2/h	Typical *E. coli* growth in rich medium
δ	Phage decay rate	0.001/h	Small in the time scale of interest.
N_0	Initial nutrient level	$30/\mu\mathrm{m}^2$	[24]
K_s	Constant for Monod growth	$N_0/5$	Depends on the nutrient.
M	number of intermediate steps	10	30% variation in latancy time [24].

FIGURES

Figure 1

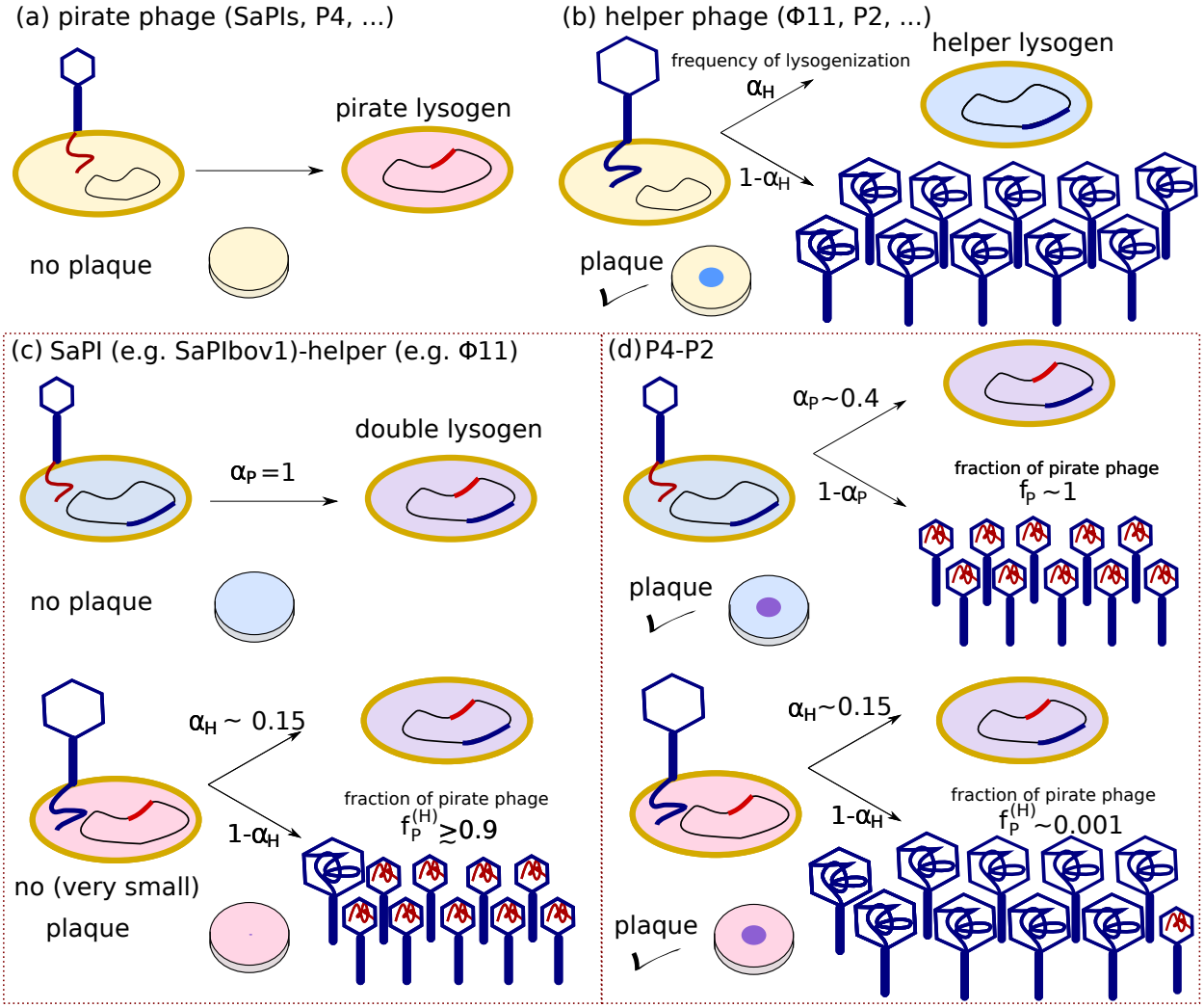


Figure 2

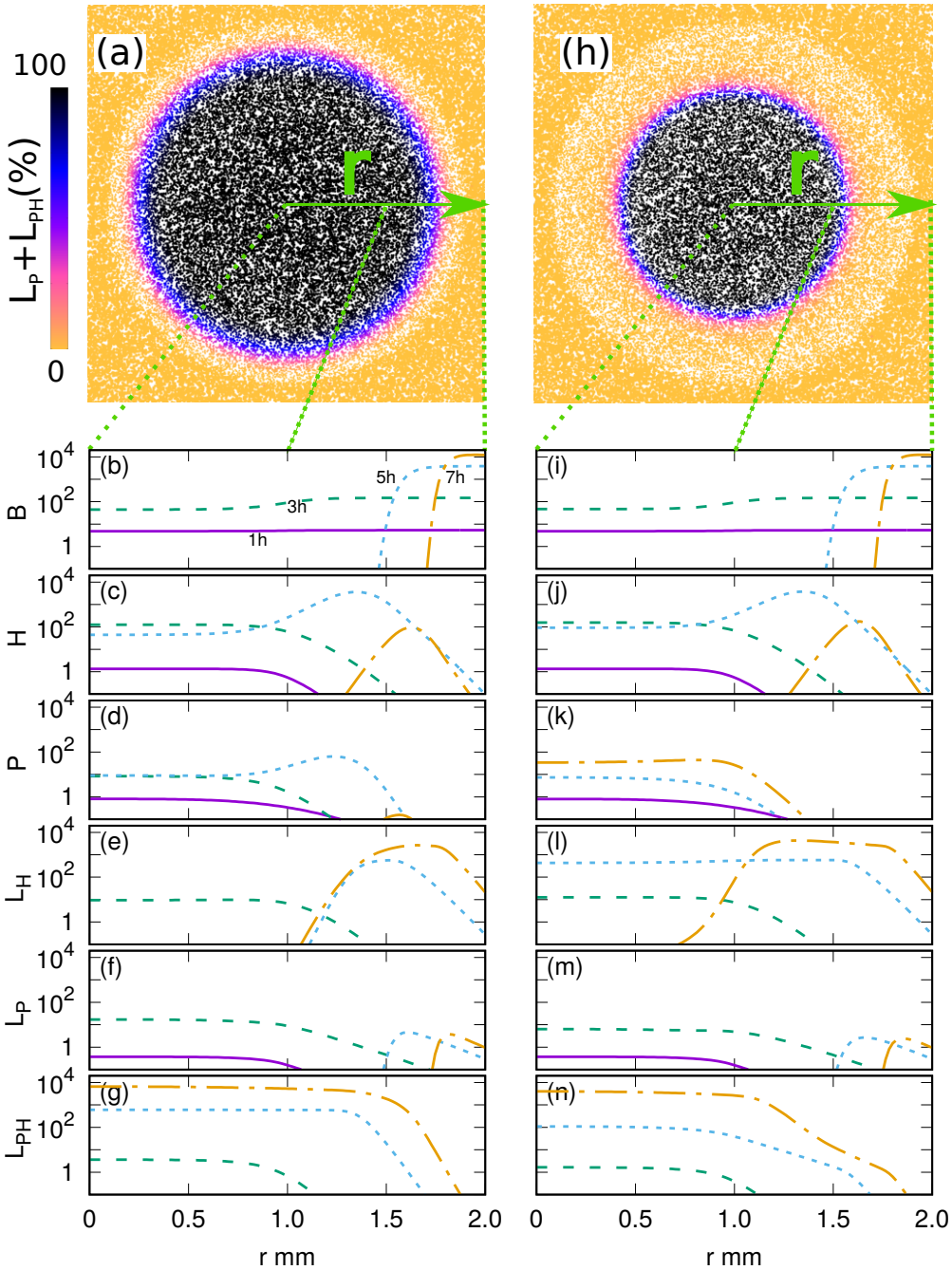

(a)
(h)
100
0
L_P+L_{PH}(%)
r
(b)
(c)
(d)
(e)
(f)
(g)
(i)
(j)
(k)
(l)
(m)
(n)
B
H
P
L_H
L_P
L_{PH}
10^4
10^2
1
1h
3h
5h
7h
0
0.5
1.0
1.5
2.0
r mm

Figure 3

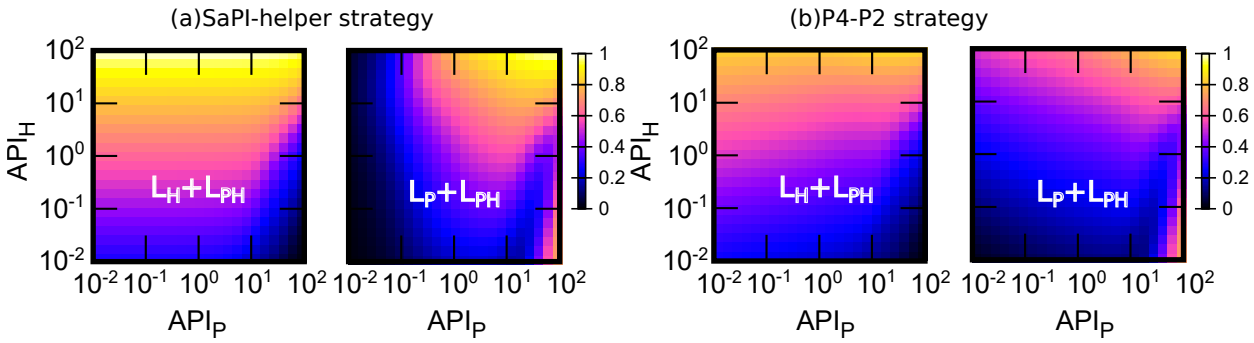
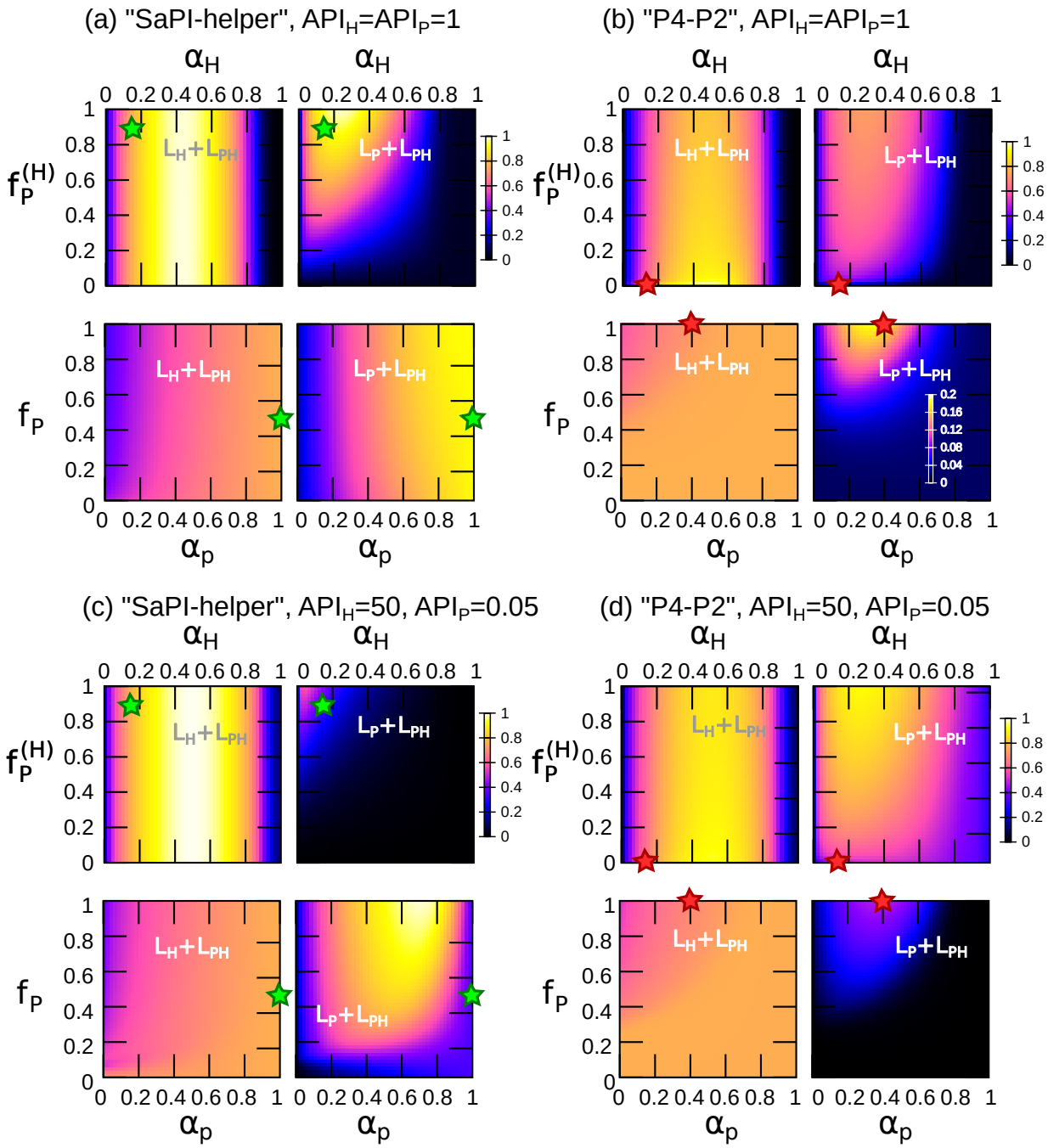

(a)SaPI-helper strategy
(b)P4-P2 strategy
API_H
API_P
L_H+L_{PH}
L_P+L_{PH}
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
1
0.8
0.6
0.4
0.2
0

Figure 4


(a) "SaPI-helper", API_H=API_P=1
(b) "P4-P2", API_H=API_P=1
(c) "SaPI-helper", API_H=50, API_P=0.05
(d) "P4-P2", API_H=50, API_P=0.05
αH
αp
f_P^(H)
f_P
L_H+L_PH
L_P+L_PH