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Population dynamics of viruses and defective interfering particles

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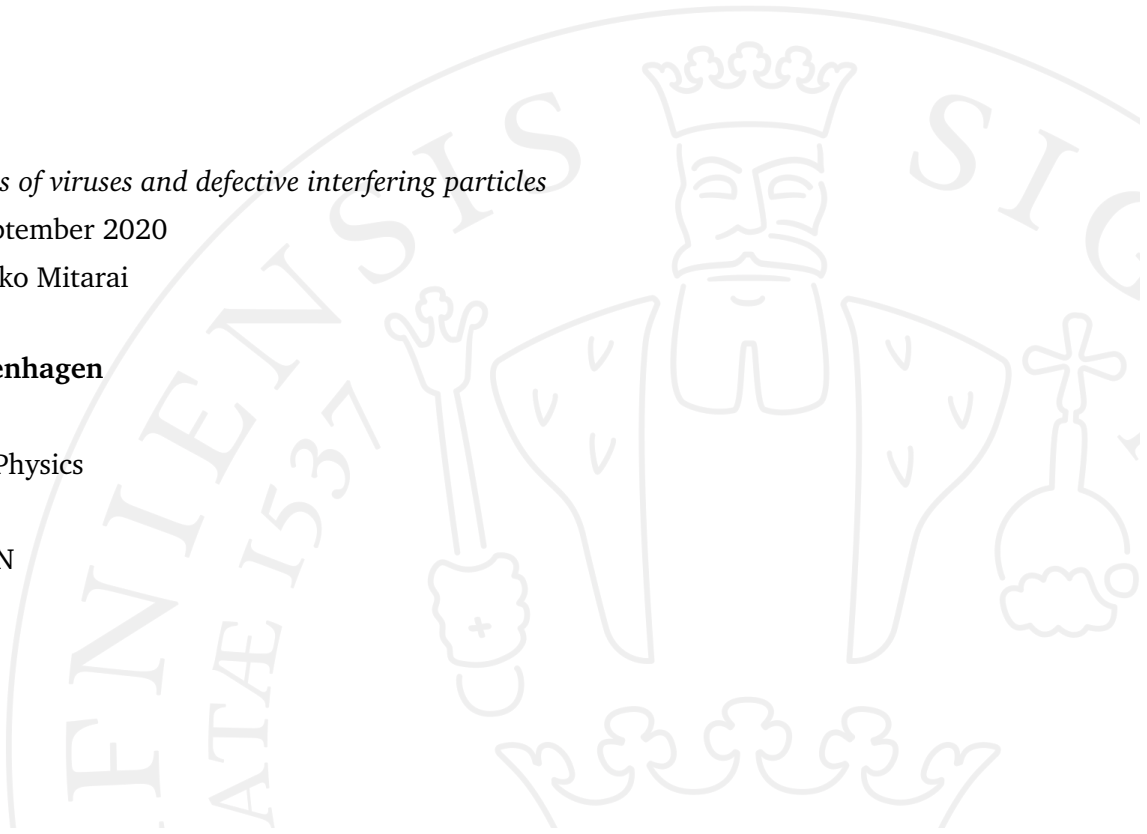

Abstract

Defective interfering particles or DIPs are viral mutants which interfere with standard viruses in order to make new copies: a DIP, in fact, requires the coinfection of a host cell by another virus, called helper virus, which provides viral nucleic acid that the DIP lacks. This doubly-infected host cell will produce a proportion of DIP copies and a proportion of helper-virus copies. We have investigated whether it is best for a DIP species if the doubly-infected cells produce mostly DIPs, mostly helper viruses or similar proportions of the two: in fact, since the replication of the DIP species requires both DIPs and helper viruses, it is not trivial what the best strategy is. We found that: when there is no flux, the cells which produce DIPs increase the fastest whether they produce DIPs and helpers in similar proportions or whether they produce mostly DIPs; under slow dilution, the DIP-genome level in the system is low only if the proportion of DIPs produced is very low or very high; under fast dilution, the proportion of DIPs produced has to surpass a threshold for the DIP species to survive. With a stochastic model, we verified that under slow dilution the DIP species is more at risk when the DIPs are produced in either low or high proportions: in this case, demographic stochasticity led the DIP species to extinction more rapidly, compared to when helpers and DIPs are produced in similar proportions.

A system of cells affected by two DIP species and two helper-virus species sees two competitions: one between the two DIP species and one between the two helper species. We studied how these competitions are affected by the proportions of DIPs produced by the cells infected by a DIP and a helper: we found that specific proportions determine a winning DIP species and a winning helper species. However, if no winner is determined, the levels of free DIPs, free-helper viruses and infected cells oscillate. The probability of extinction of a DIP species driven by demographic stochasticity is affected by this oscillating dynamics: this probability oscillates with the same frequency.

The simulation of a lattice of cells affected by a DIP species and a helper-virus species, where the doubly-infected cells produce only DIPs and no helper,

showed the propagation of a leading front of helper infection followed by a front of DIP infection. The DIP-infection front could not stop the helper-infection front. Almost all cells ended up infected with both species; a small minority ended up infected only by a DIP; an even smaller minority remained uninfected. Whether or not some regions of the lattice were without cells, the infection dynamics appeared almost identical.

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1 Introduction

1.1 What is a virus?

Viruses are obligate intracellular parasites [1], i.e. microparasites that can only grow and reproduce inside host cells. After they infect a cell, they can steer the cellular machinery to produce copies of the same virus.
A virus is made up of a DNA or RNA genome inside a protein shell called a capsid. Some viruses also have an external membrane envelope [2].

Viruses are very diverse, and different viruses can infect different types of hosts; there exist viruses that infect [2]:

- animal cells
- plant cells
- bacteria
- archaea

The viruses that infect bacteria or archaea are typically called bacteriophages or simply phages.
In figure 1.1 are illustrations and micrographs of various types of virus.

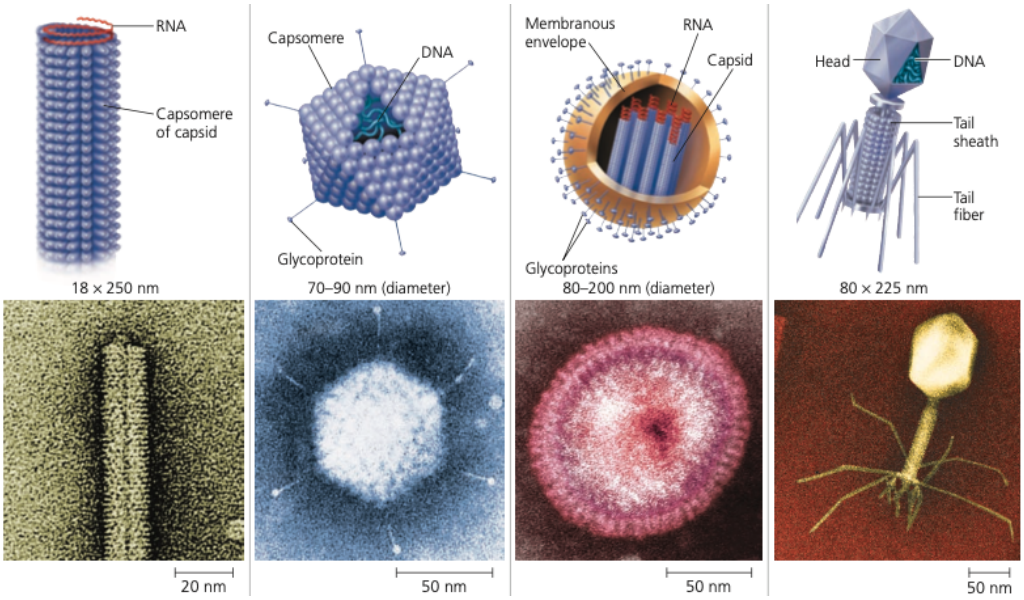


Figure 1.1.: Examples of viruses infecting different types of hosts and their structures. (a) Tobacco mosaic virus (plant virus). (b) Adenovirus, (c) Influenza virus (animal viruses). (d) Bacteriophage T4. Credit to [2] for the illustration.

1.2 How do viruses operate?

Although viruses can be very diverse, they replicate following a general pattern consisting of 5 steps [3]:

1. **Attachment**: The virus recognises and binds to a host cell via a receptor molecule on the cell surface.

2. **Entry**: The virus or just the viral nucleic acid enters the cell.

3. **Synthesis**: The host cell copies the viral genome, expresses the viral genes and makes new viral proteins.

4. **Assembly**: The host cell assembles new viral particles from the viral genome copies and the viral proteins.

5. **Release**: The cell releases the newly formed viruses.

Figure 1.2 shows a visual representation of these 5 steps.

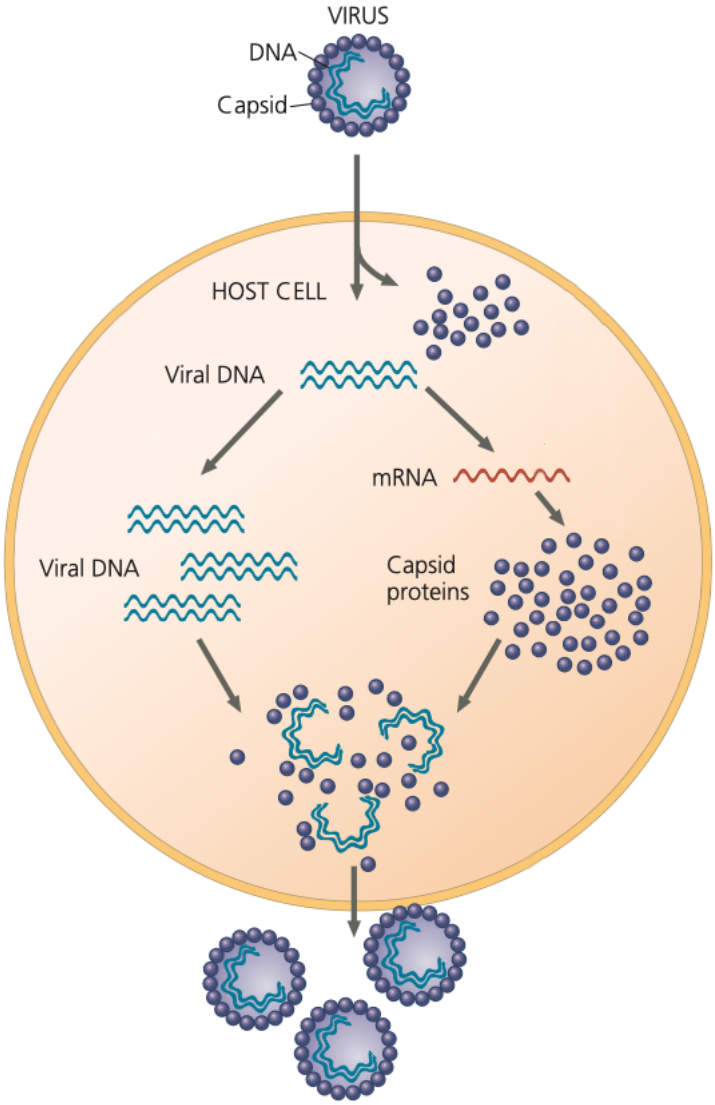


Figure 1.2.: Simplified replicative cycle of a virus. The virus attaches to a cell, enters it and releases its genome (**attachment** and **entry**). The host replicates the viral genome and also makes new capsid proteins, which are encoded in the viral genome (**synthesis**). Viral genomes and capsid proteins self-assemble into new virus particles, which exit the cell (**assembly** and **release**). Credit to [2] for the illustration.

1.3 Different host types and their viruses

As we mentioned above, a virus may infect animal cells, plant cells, bacteria or archaea. The main difference between these cells is [2]:

- animal and plant cells are *eukaryotic*, i.e. they contain membrane-bound organelles, including a nucleus which contains the cell genome;

- bacteria and archaea are *prokaryotic* cells, i.e. they do not contain any membrane-bound organelle, so their genome is not surrounded by a nuclear membrane.

Another difference is that prokaryotic cells typically have a diameter of $0.5 - 5\mu m$, much smaller than the $10 - 100\mu m$ diameter of many eukaryotic cells [2]. (As a comparison, viruses are even smaller, where their sizes range between $20 - 200nm$ [2].)

For the scope of this thesis, we illustrate the replication strategy of one type of animal virus, the retrovirus, and the three replication strategies of bacteriophages.

1.3.1 Retroviruses and their replication strategy

Among the animal viruses are many strategies of viral replication. We will focus on one type of virus: the **retrovirus**.

A retrovirus is an RNA virus which contains and encodes an enzyme called reverse transcriptase: inside the host cell, this enzyme converts the viral RNA into DNA, which will be incorporated into the cell DNA [4]. One example of a retrovirus is HIV (Human Immunodeficiency Virus), a virus that causes AIDS (Acquired Immunodeficiency Syndrome) [2].

The retroviral replicative cycle is the following. After a retrovirus enters a host cell, its reverse transcriptase molecules catalyse the synthesis of viral DNA. Next, the newly made viral DNA enters the cell's nucleus and integrates into the cellular DNA. The integrated viral DNA, called a provirus, never leaves the host's genome, remaining a permanent resident of the cell. Now that the viral genome is integrated into the host's genome, the host transcribes RNA molecules and synthesises capsid proteins and reverse transcriptases and will assemble them into new viruses. Finally, the cell will release the newly made viruses [2]. In figure 1.3 is a representation of this process.

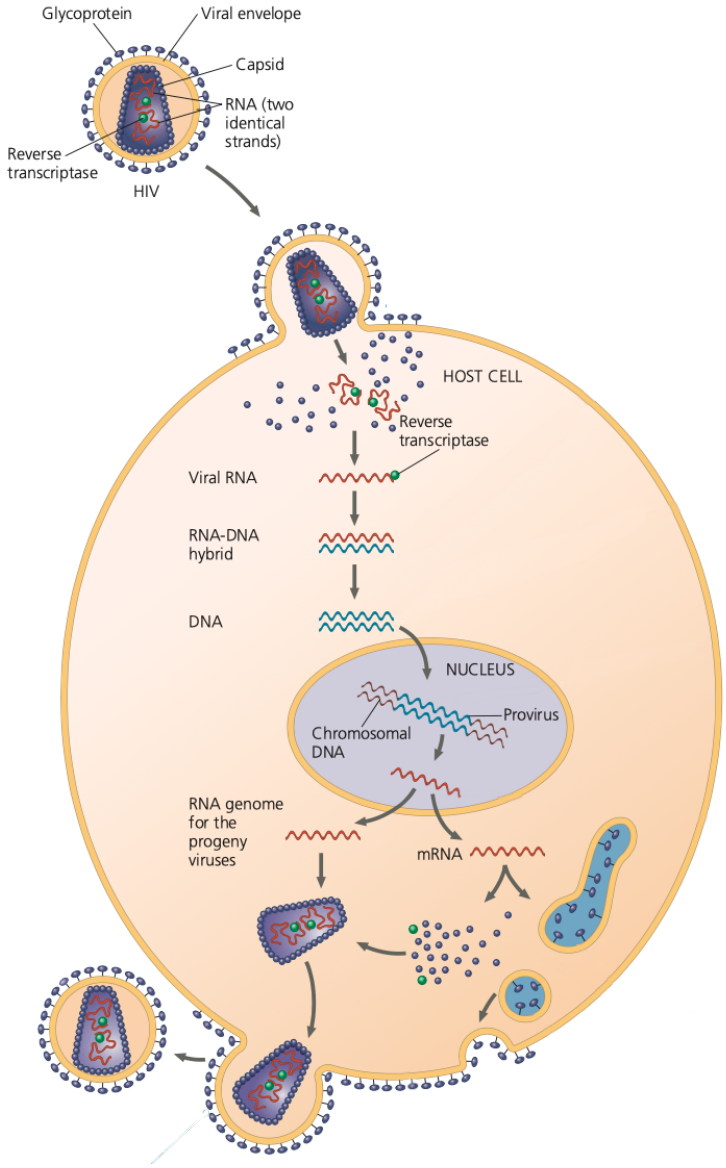


Figure 1.3.: Simplified replicative cycle of HIV. The envelope enables the virus to bind to receptors on the cell. The virus fuses with the cell's membrane. The capsid proteins are removed, releasing the RNA and reverse transcriptases. The reverse transcriptases catalyse the synthesis of viral DNA from the viral RNA. The viral DNA is incorporated as a provirus into the cell's DNA. Proviral genes are transcribed into RNA molecules, which serve as genomes for progeny viruses, and capsid proteins and reverse transcriptases are synthesised. New viruses are assembled and bud from the host cell. Credit to [2] for the illustration.

1.3.2 The replication strategies of bacteriophages

Bacteriophages are the most understood of all viruses. Phages can replicate with three different strategies:

- **Virulent phages** replicate only by a lytic cycle. During a lytic cycle, the viral genes immediately turn the host cell into a phage-producing factory, and the cell soon lyses (breaks open) and releases its virus progeny. An example of virulent phage is the phage $T4$ which infects the bacterium *Escherichia coli* [2].

- **Temperate phages** can use both a lytic cycle or a lysogenic cycle. The lysogenic cycle allows replication of the phage genome without tearing apart the host. Once the phage genome has entered the host, either the lytic cycle or lysogenic cycle can be induced. During a lysogenic cycle, the phage genome can be incorporated into the bacterial chromosome. Once integrated into the bacterial chromosome, the viral genome is called a prophage. (While a retroviral provirus never leaves the host's genome, a prophage instead leaves the host's genome at the start of a lytic cycle.) Every time the cell gets ready to divide, it replicates the phage genome along with its own chromosome so that each daughter cell inherits the phage genome. A single infected cell can give rise to a large population of bacteria carrying the phage genome. This mechanism allows temperate phages to propagate without killing the host cells on which they rely. The term lysogenic means that the phage genome is capable of generating active phages that lyse their host cells, i.e. that the phage genome can be induced to initiate a lytic cycle. One example of a temperate phage is the λ phage, which also infects *E. coli* [2].

- **Chronic phages** are non-lytic, i.e. they do not kill the infected host cell. Infected host cells continue to grow and to divide indefinitely while at the same time assembling and secreting viral particles. Chronic phages and retroviruses are comparable, in that the infected host cell produces and releases new copies of the virus without being destroyed, as instead occurs at the end of the lytic cycle of a virulent or temperate phage. An example of chronic phage is the phage $M13$, which also infects *E. coli* [5].

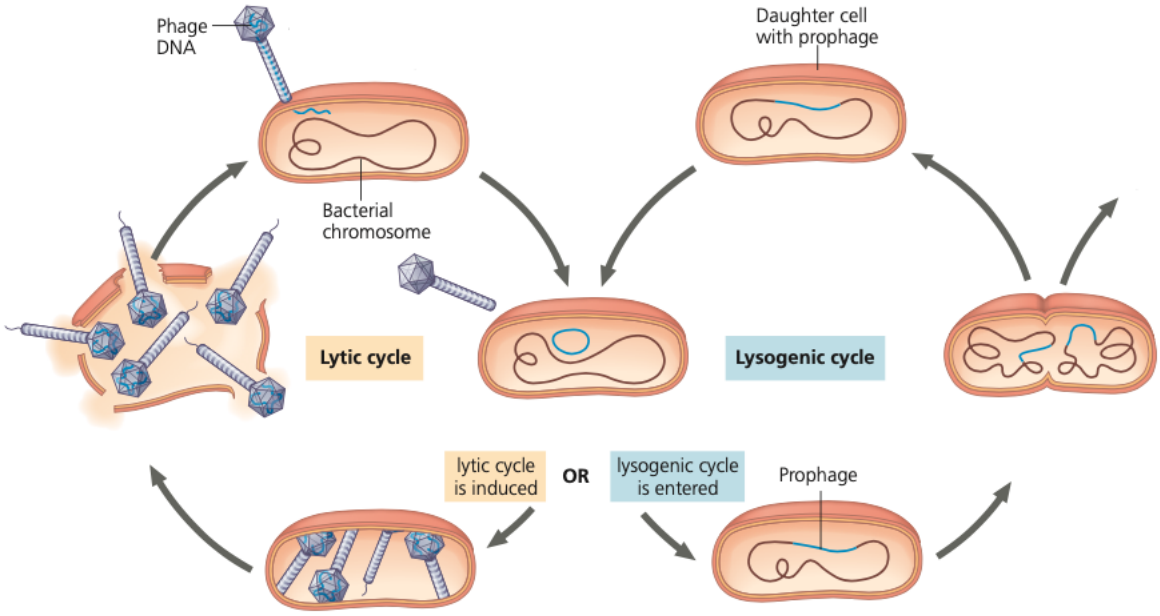


Figure 1.4.: The lytic and lysogenic cycles of the temperate phage λ. After penetrating the cell and circularizing (this circle of DNA is called *plasmid*), the λ DNA can immediately initiate the production of a large number of progeny phages (lytic cycle) or integrate into the bacterial chromosome (lysogenic cycle). Credit to [2] for the illustration.

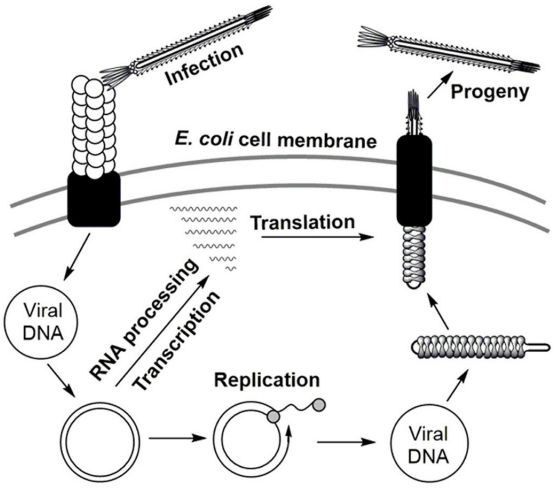


Figure 1.5.: Simplified replicative cycle of the chronic phage $M13$. After the viral genome has entered the cell and has formed a plasmid, new copies of the phage are created and released from the *E. coli* without lysis of the cell. Credit to [6] for the illustration.

1.4 Special types of viruses: defective interfering particles and pirate phages

Defective interfering particles, or DIPs, are animal or plant viral mutants that cannot hijack cellular machinery on their own. The defect of the DIPs consists in the lack of some of their nucleic acid. They must be complemented by a standard virus, which we call helper virus. DIPs replicate at the expense of this helper virus, thus interfering with it, and operate as parasites of viruses [7].

The interference by DIPs is observed as the hampering of the absolute number of standard virus copies created by hosts infected by both the DIP and the standard virus, compared to the number created by hosts infected only by the standard virus.
HIV and the influenza virus are examples of virus that can be a DIP [8, 9]. When it comes to influenza, for example, the majority of influenza viruses are non-infectious [10]. As Von Magnus demonstrated in 1954, the non-infective influenza viruses can hamper the replication of the infectious viruses [10]. Because of the significant lessening of infectious virus production caused by DIPs, they have been suggested as antiviral agents, to be an alternative to vaccination or treatment with other antivirals [10]. For example, DIPs were proposed as a way to control the HIV/AIDS pandemic [9]. These engineered DIPs are called therapeutic interfering particles or TIPs and have been predicted to reduce viral levels in patients and viral transmission events across populations [11].

Pirate phages, also known as satellite phages, are similar to DIPs but are instead bacteriophages. Pirate phages infect bacteria and hijack standard phages, which we call helper phages.
The term piracy refers to the fact that the so-called pirate phages use the structural proteins encoded by the helpers for the assembly of their own copies [12, 13]. When the pirate genome is alone in the host, the host does not produce new copies of the pirate phage. However, suppose the helper phage genome is also present in the host: in that case, the pirate can take advantage of the helper phage structural proteins to make new pirates, thus interfering with the production of copies of the helper.
There exist pirates of both temperate phages and chronic phages. If we con-

sider the pirate phage of a temperate phage, when the pirate genome is alone in the host, it behaves as a prophage.

The best-studied example of phage piracy is that of the pirate P4 and the temperate helper P2 [12]. The P2 phage encodes most of the proteins that make up the P4 capsid: the P4 genome lacks the information for these structural proteins, but also for the lysis of the host cell [14, 15].
If we instead talk about chronic phages, satellite phages can appear in stocks of phage M13 [16].

1.5 Motivations behind this thesis

DIPs are attractive because of their potential as alternative antiviral agents. However, what is the best strategy for a DIP species to disrupt the production of helper viruses? Let us focus on the following quantity: *the probability that a newly produced virus, made by a cell infected by both a DIP and its helper, is a new DIP*. If this probability is close to 0, then the production of DIPs will be low; at the same time, if this probability is close to 1, the production of helpers by these doubly-infected cells will be low, and the DIPs need helpers to produce new DIP copies. Thus, both low and high probability appear disadvantageous for the DIP species, for two different reasons. Then **is there an optimal range of values of this probability for the propagation of the DIP? If there is, what is it? And more in general, how do the DIP and helper population dynamics change with this parameter?**

Now, we can imagine a system of cells and viruses which is replenished with new, uninfected cells more or less rapidly and the cells and viruses are diluted more or less rapidly. **Does the optimal range of values for the probability vary under such varying flux conditions? And how do the population dynamics change?**
We can also imagine a system of cells and viruses with not only one DIP species and one helper species, but two closely-related DIP species and two closely-related helper species with superinfection exclusion: superinfection exclusion means that the first infection of a cell by a DIP prevents the infection by the other DIP (and analogously for the two helpers). **What are the population dynamics, in this different scenario?**

Finally, **how might the propagation of a DIP species and its helper species look like in space?**
We try to answer these questions throughout this thesis.

1.6 Outline of the thesis

We performed a qualitative study of the population dynamics of DIPs and helper viruses infecting the same animal cells. Our models do not apply precisely to the case of pirate phages and helper phages infecting the same bacteria, because cell growth is not contemplated. However, the models can still be informative for the context of the interference between pirate and helper phages.
The thesis is divided into 4 parts. Each part studies different types of models:

1. *well-mixed* models with 1 DIP species and 1 helper-virus species;

2. a *well-mixed* model with 2 superinfection-exclusive DIP species and 1 helper-virus species;

3. *well-mixed* models with 2 superinfection-exclusive DIP species and 2 superinfection-exclusive helper virus species;

4. a *spatial* model with 1 DIP species and 1 helper-virus species.

Parts 1 and 3, where we study well-mixed models, are themselves divided into three sections.

a) First, we use a *deterministic* model where we do not consider influx or outflow of the elements in the system.

b) Next, we use another *deterministic* model with an inflow of new, uninfected cells and the dilution of all elements of the system.

c) Finally we have a *stochastic* model with an inflow of uninfected cells and the dilution of the elements.

In part 2, we use only a *deterministic* model with an inflow of new, uninfected cells and the dilution of all elements of the system.

The *well-mixed deterministic* models (models 1a, 1b, 2, 3a, 3b) are used to investigate the dynamics between cells, DIPs and helper viruses. The models describe systems of infinite volume with ordinary differential equations.

The *well-mixed stochastic* models (models 1c, 3c) are used to investigate the extinction of viruses. The models describe systems of finite volume that advance according to the Gillespie algorithm.

The *spatial* model (model 4) is used to investigate the spreading of a DIP species and a helper-virus species. This is an agent-based model of a 2D lattice of cells.

2 The interference of one DIP species with one helper-virus species

2.1 For a rapid I_{HP} increase, high DIP production is not necessary

The first model we consider is deterministic, and it is described by ordinary differential equations. The model is of a system of host cells that can be infected by a DIP and by a helper virus. This system has no flux: no element enters the system, and no element is removed from the system.

2.1.1 Description of the elements in the model and their interactions

Here we describe the cells and viruses in the model and their interactions. The elements of the system are described as concentrations. The free viruses are described by

- P, the concentration of free DIPs
- H, the concentration of free helper viruses
- $V = H + P$, the concentration of all free viruses

The cells are described by

- B, the concentration of uninfected cells

- I_P, the concentration of cells infected only by the DIP
- I_H, the concentration of cells infected only by the helper
- I_{HP}, the concentration of cells infected by both the DIP and the helper
- $T = B + I_H + I_P + I_{HP}$, the concentration of all cells

The cells and viruses interact in various ways. One or more parameters accompany each type of interaction.

- **Absorption of a virus by a cell**: each cell can absorb any virus. If a cell is not already infected by the type of virus it absorbed, then it becomes infected by it. The rate of absorption is $\eta \left[\frac{1}{concentration \cdot time}\right]$.
- **Production of a virus by a cell**: the cells I_H can produce helper viruses; the cells I_{HP} can produce both DIPs and helper viruses. When an I_{HP} produces a new virus, it is a DIP with probability f; it follows that the probability for this newly made virus to be a helper is $1 - f$. The rate of virus production is $\beta \left[\frac{1}{time}\right]$.

2.1.2 The ODEs of the model

We start with an initial concentration of uninfected cells, free DIPs and free helper viruses. We then let the system evolve.

The equations describing the evolution of the system are

$$\frac{dB}{dt} = -\eta V B \tag{2.1}$$

$$\frac{dI_P}{dt} = \eta P B - \eta H I_P \tag{2.2}$$

$$\frac{dI_H}{dt} = \eta HB - \eta PI_H \tag{2.3}$$

$$\frac{dI_{HP}}{dt} = \eta PI_H + \eta HI_P \tag{2.4}$$

$$\frac{dP}{dt} = \beta f I_{HP} - \eta PT \tag{2.5}$$

$$\frac{dH}{dt} = \beta \left[I_H + (1-f) I_{HP} \right] - \eta HT \tag{2.6}$$

We can simplify the model by rescaling it. We can rescale time and concentration by using the parameters η and β.
We define

$$t' \equiv \frac{t}{1/\beta} \tag{2.7}$$

$$B' \equiv \frac{B}{\beta/\eta} \tag{2.8}$$

and so on for all other concentrations.

The unit of t' is

$$\frac{1}{\beta} \tag{2.9}$$

while the unit of the concentrations primed is

$$\frac{\beta}{\eta} \tag{2.10}$$

The parameters η and β have to be rescaled too, and they are obviously substituted by

$$\eta' \equiv \frac{\eta}{\eta} = 1 \tag{2.11}$$

$$\beta' \equiv \frac{\beta}{\beta} = 1 \tag{2.12}$$

(The reader can find in the appendix A examples of values of the parameters η and β found in the literature for various viruses.)

Now we drop the index $'$ for simplicity. The equations therefore become

$$\frac{dB}{dt} = -VB \tag{2.13}$$

$$\frac{dI_P}{dt} = PB - HI_P \tag{2.14}$$

$$\frac{dI_H}{dt} = HB - PI_H \tag{2.15}$$

$$\frac{dI_{HP}}{dt} = PI_H + HI_P \tag{2.16}$$

$$\frac{dP}{dt} = fI_{HP} - PT \tag{2.17}$$

$$\frac{dH}{dt} = [I_H + (1-f)I_{HP}] - HT \tag{2.18}$$

Thus now f is the only parameter.

As we said earlier, we start with only uninfected cells and free DIPs and helper viruses. As we can see from the equations, all cells are doomed to become infected by both a DIP and a helper.

2.1.3 Results

Let us focus on the DIP and the parameter f. We wanted to investigate which values of f are better for the DIP: is it better for the DIP if f is close to 0; close to 1; or maybe about 0.5? I.e., is it better for the DIP if the I_{HP} cells produce more DIPs, more helper viruses or balanced amounts of the two? It is not trivial to identify which strategy is better:

- if f is close to 0, the I_{HP} produce mainly helper viruses, and it is good for the DIPs if there are plenty of helpers, but this comes at the expense of the production of DIPs themselves;

- if f is close to 0, the I_{HP} produce mainly DIPs, and it is good if many DIPs are produced, but this comes at the expense of the production of helper viruses.

So what is better for the DIPs? If the I_{HP} cells produce mostly DIPs; if they produce mostly helpers; or if they produce DIPs and helpers in similar proportions?

(Obviously we did not consider $f = 0$, as in that case the I_{HP} cells produce no DIP.)
The way we measure the quality of the parameter f from the perspective of the DIP is by measuring how fast 90% of the cells become I_{HP}: as we said, all cells are going to become I_{HP}, and the I_{HP} cells are the "mothers" of the DIPs, so the sooner the cells become I_{HP}, the sooner all cells produce DIPs.

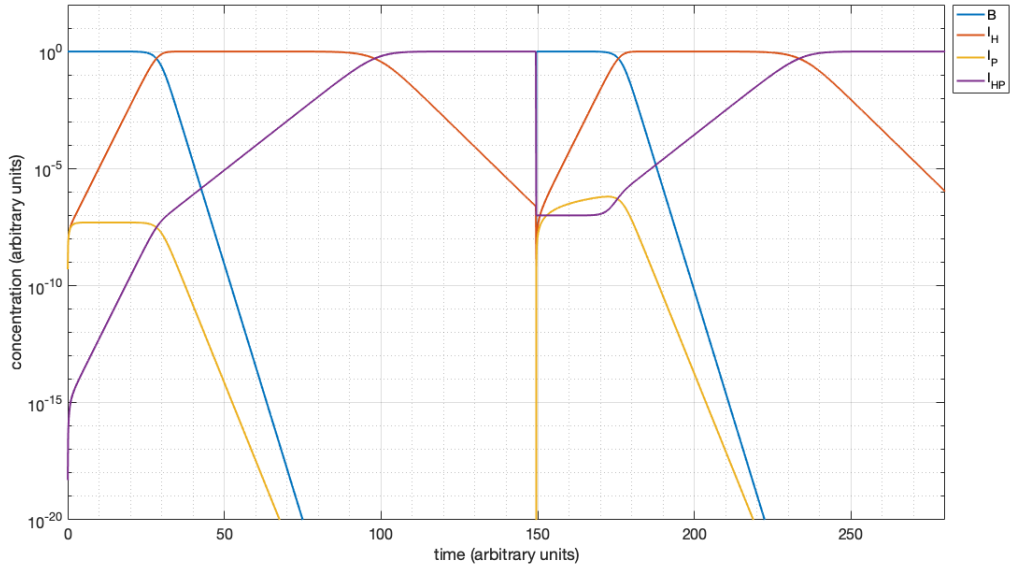
The initial concentrations of the cells are

- $B(t=0)=1$
- $H(t=0)=0.5\times10^{-7}$
- $P(t=0)=0.5\times10^{-7}$
- all other concentrations are zero at $t=0$

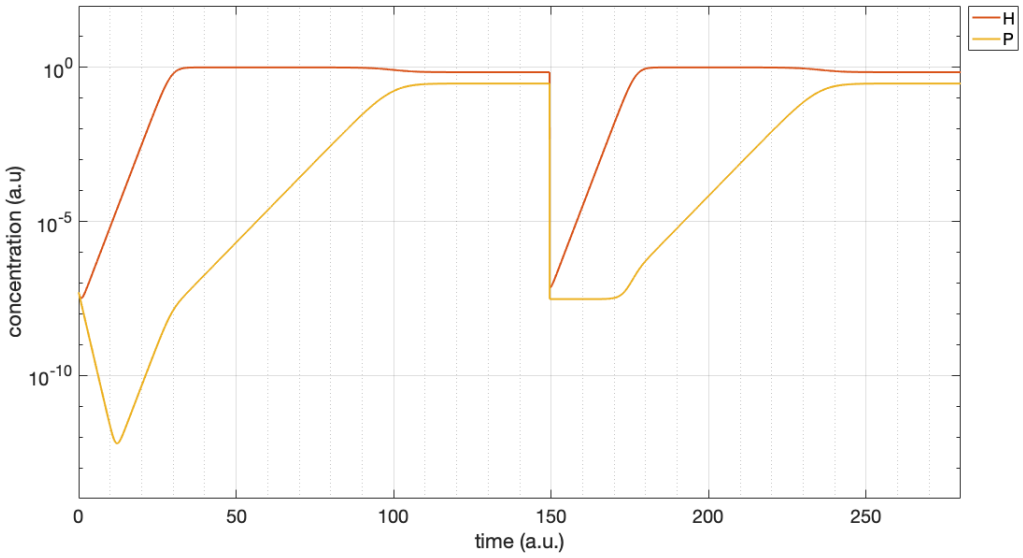
We let the system evolve until it reached saturation. Once saturation was reached, we imagined taking a small sample of the saturated system, precisely 1 part in 10^7, and injecting it onto another system made of only uninfected cells (i.e. type-B cells). Then the second system would evolve and reach saturation itself. We can see an example of the time evolution of the concentrations in figure .

The time when we start measuring how long it takes for 90% of the cells to become I_{HP} is **the moment of injection**. We did this because we wanted the I_{HP} cells to drive the dynamics. In fact, the droplet we inject onto the second system is made of I_{HP} with P and H at equilibrium between absorption and production by the I_{HP} cells.

We call the length of time we measure $T_{90\%}$.



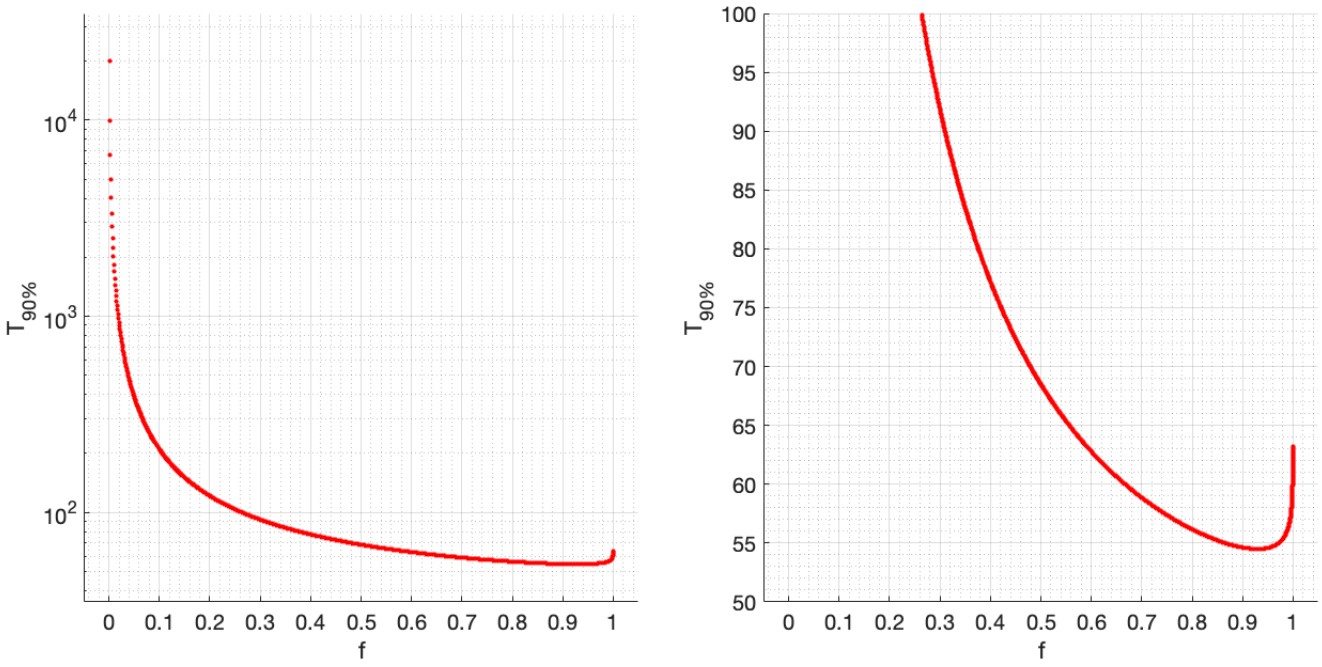
(a)



(b)

Figure 2.1.: Time evolution of the concentrations of cells and viruses. The parameter f here is $f = 0.3$. The plots show the concentrations in the first system up until $t = 150$; then they show the concentrations in the second system. At $t = 150$ the first system is saturated, and at that moment the injection onto the second system is done. $t = 150$ is an arbitrary time that we chose once the first system was saturated. (a) Cell concentrations vs time. (b) Virus concentrations vs time.

$T_{90\%}$ has been studied for $f \in]0, 1]$. We see the result of this study in figure 2.2.



(a) (b)

Figure 2.2.: $T_{90\%}$ vs. f. (a) All the values of $T_{90\%}$ we obtained. (b) A closer look at the minimum.

There are two distinct behaviours for $T_{90\%}$, as we can see in figure 2.2a. In the first $\sim 20\%$ of the domain of f, $T_{90\%}$ varies rapidly and spans two orders of magnitude; in comparison, in the rest of the domain $T_{90\%}$ is almost constant. One thing worth noting is that $T_{90\%}$ increases again very close to $f = 1$, but not significantly. A conclusion we can make is that low production of DIPs (low f) is worse than low production of helpers (high f) when thinking about the growth of I_{HP}. Another conclusion regards the fastest growth of these cells: the minimum of $T_{90\%}$ is for $f \simeq 0.93$, i.e. very close to 1 (figure 2.2b); therefore, the I_{HP} prefer a low production of helpers (high f), instead of a similar production between helpers and pirates ($f \simeq 0.5$). This may be because the I_H already provide helpers.

2.2 Cell-inflow and dilution interfere with the DIP-helper interaction

Next, we look at another deterministic model. This model is an expansion of the previous model: now there is also an influx of uninfected cells and the dilution of the viruses and cells. We studied this model because the influx of cells and the outflow of the components must enforce a dynamic balance between the components of the system. The components of the system, i.e. the cells and the viruses, are the same as in the previous model. Once again, among the processes, we have the absorption of a free virus by a cell and the release of a virus by a cell. The two processes are described by the rates η and β once again. In order to describe the addition of cells of type B, we introduce the rate of influx of B-type cells $\mathcal{C}$ $\left[\frac{cells}{time}\right]$. To describe the outflow of the components of the system, we introduce the rate of dilution γ $\left[\frac{1}{time}\right]$.

2.2.1 The ODEs of the model

The ODEs which describe the time evolution of the system are

$$\frac{dB}{dt} = \mathcal{C} - \eta V B - \gamma B \tag{2.19}$$

$$\frac{dI_H}{dt} = \eta H B - \eta P I_H - \gamma I_H \tag{2.20}$$

$$\frac{dI_P}{dt} = \eta P B - \eta H I_P - \gamma I_P \tag{2.21}$$

$$\frac{dI_{HP}}{dt} = \eta P I_H + \eta H I_P - \gamma I_{HP} \tag{2.22}$$

$$\frac{dH}{dt} = \beta\left[I_H + (1-f)I_{HP}\right] - \eta HT - \gamma H \tag{2.23}$$

$$\frac{dP}{dt} = \beta f I_{HP} - \eta PT - \gamma P \tag{2.24}$$

Once again we can rescale the model just like before by introducing t' (2.7) and B' (2.8) and so on for the other concentrations. This means that the parameters η and β are substituted again by $\eta' = 1$ (2.11) and $\beta' = 1$ (2.12), while $\mathcal{C}$ and γ are substituted by

$$\mathcal{C}' \equiv \frac{\mathcal{C}\cdot\eta}{\beta^2} \tag{2.25}$$

$$\gamma' \equiv \frac{\gamma}{\beta} \tag{2.26}$$

Once again, we remove the index $'$ for simplicity. The rescaled equations are

$$\frac{dB}{dt} = \mathcal{C} - VB - \gamma B \tag{2.27}$$

$$\frac{dI_H}{dt} = HB - PI_H - \gamma I_H \tag{2.28}$$

$$\frac{dI_P}{dt} = PB - HI_P - \gamma I_P \tag{2.29}$$

$$\frac{dI_{HP}}{dt} = PI_H + HI_P - \gamma I_{HP} \tag{2.30}$$

$$\frac{dH}{dt} = \left[I_H + (1-f)I_{HP}\right] - HT - \gamma H \tag{2.31}$$

$$\frac{dP}{dt} = fI_{HP} - PT - \gamma P \tag{2.32}$$

Thus now we have 3 parameters: $\mathcal{C}$, γ and f.

Our question is: under different flux conditions, does the same value of f affect the system differently?
Before we look at the results of the study, we need to define two quantities.

- We define the ***DIP genome*** or ***P genome*** as $g_P \equiv P + I_P + I_{HP}$. It is the total concentration of cells and viruses containing the genome of the DIP.

- Analogously, we define the ***helper genome*** or ***H genome*** as $g_H \equiv H + I_H + I_{HP}$. This is the total concentration of cells and viruses containing the genome of the helper virus.

g_P and g_H are useful quantities to study the disappearance of the DIP or the helper from the system. In this model, if one of the two genomes reaches the value 0, then the corresponding virus cannot come back and it is extinct, because the only thing that enters the system is uninfected cells, not viruses.
We used initial conditions just like in the previous model, i.e. only uninfected cells, DIPs and helper viruses are present at the start. Given a combination of the parameters $\mathcal{C}$, γ and f, we let the system evolve.
The initial conditions are

- $B(t=0) = 1$

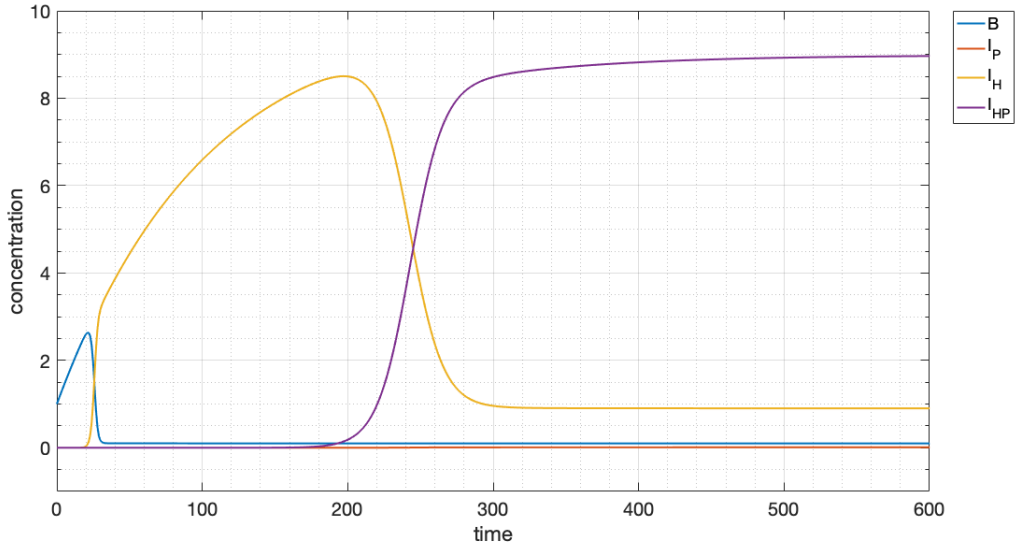
- $H(t=0) = 0.5 \times 10^{-7}$

- $P(t=0) = 0.5 \times 10^{-7}$

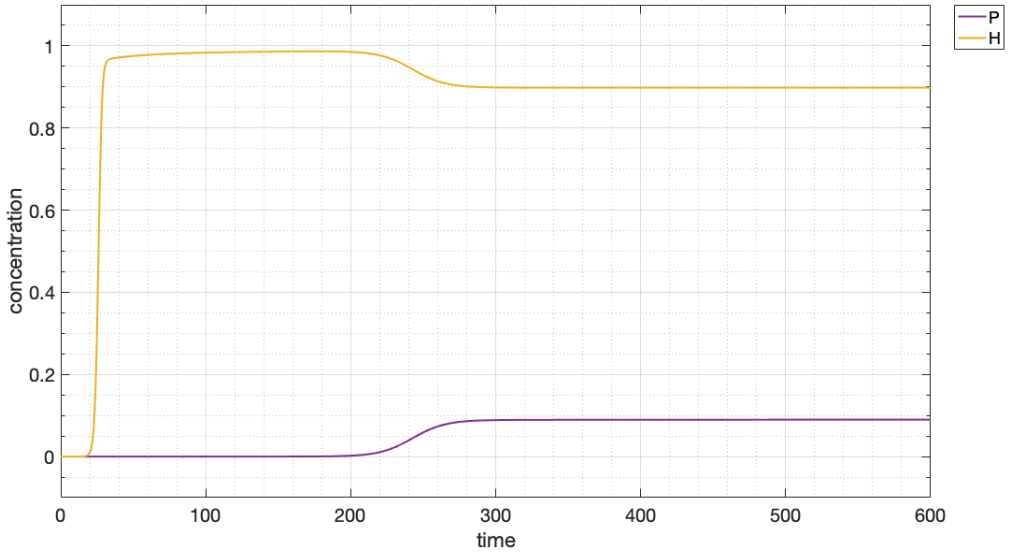
- all other concentrations are zero at $t = 0$

2.2.2 Results

We can see an example of the time evolution of the system in figure 2.3. As it turns out, the components reach a steady-state (except for the case $f \simeq 1$, which we will look at later).



(a)



(b)

Figure 2.3.: Example of evolution of the system. Here the parameters are $f = 0.1$, $\mathcal{C} = 0.1$, $\gamma = 0.01$ (a) Time evolution of the cell levels. (b) Time evolution of the virus levels.

What we did was studying the steady-state concentrations of the genomes g_P and g_H, and also of the viruses and of the cell types, whilst scanning the parameters $\mathcal{C}$, γ and f. We scanned

- f in the interval $]0, 1[$
- $\mathcal{C}$ and γ in the interval $[0.01, 1]$

First, we look in figure 2.4 at the steady-state g_P and g_H levels for three values of f: 0.1, 0.5, 0.9.

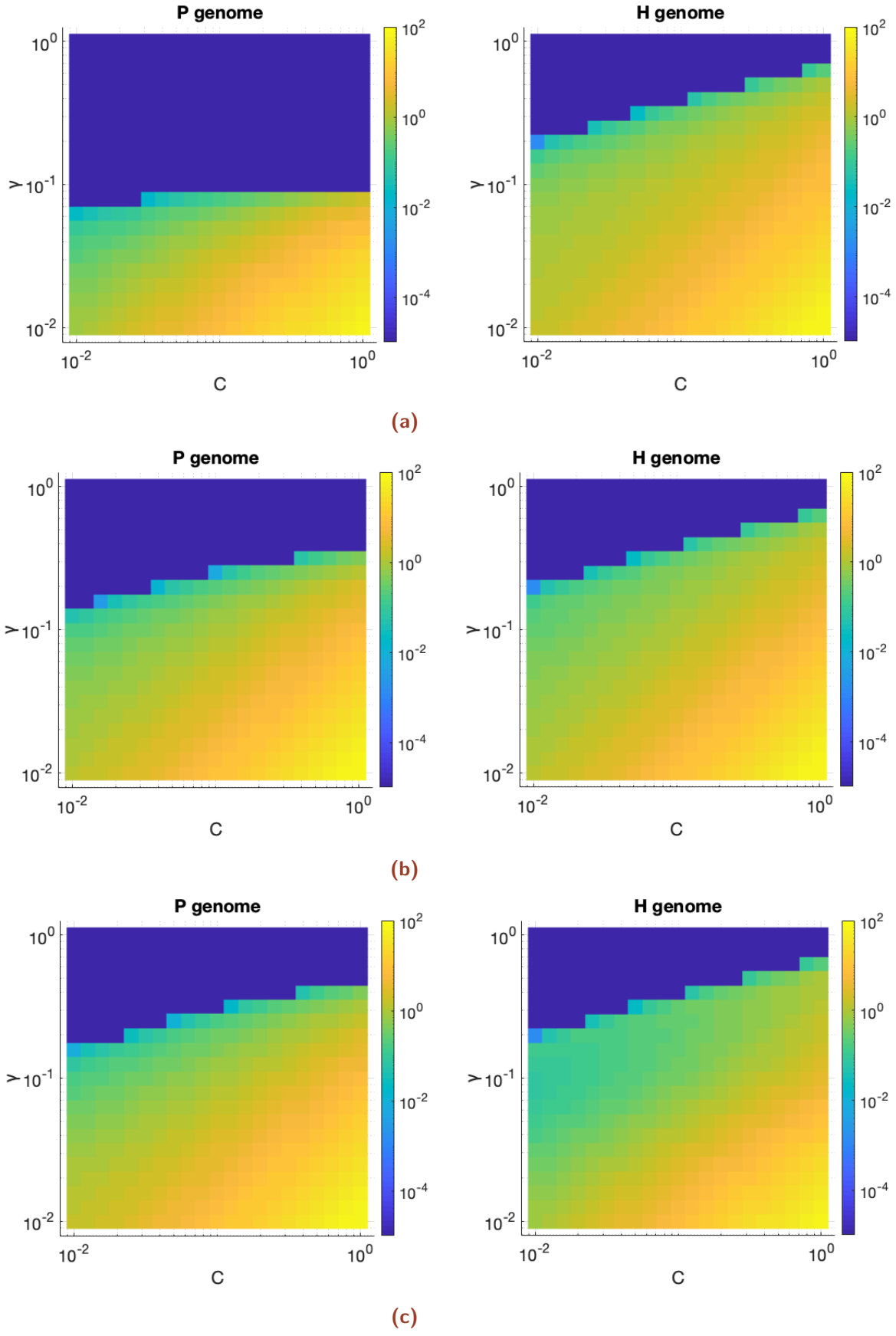


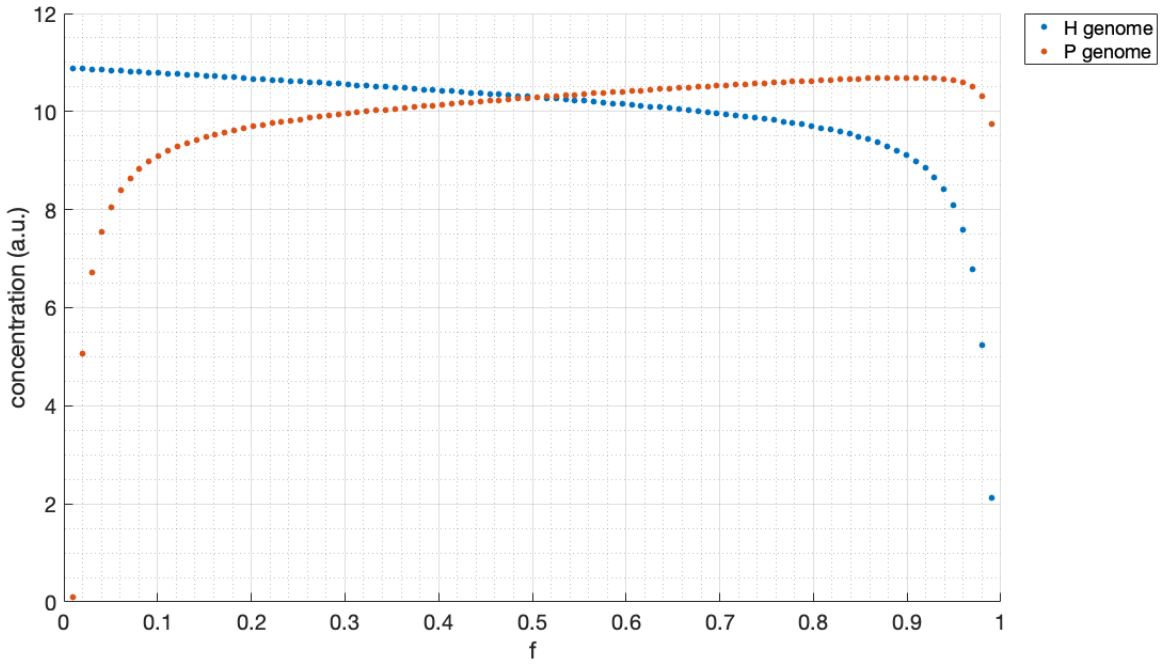
Figure 2.4.: Steady-state levels of g_P and g_H for three values of the parameter f: (a) $f = 0.1$; (b) $f = 0.5$; (c) $f = 0.9$.

We can observe that with intense dilution, i.e. high γ, the two genomes generally disappear.
With low f, $f = 0.1$ in figure 2.4a, part of the $(\mathcal{C}, \gamma)$ parameter space sees the extinction of the DIP and the survival of the helper. Let us focus our attention on this region of the $(\mathcal{C}, \gamma)$ parameter space, but let us use $f = 0.9$ (figure 2.4c). The same region does not give the extinction of the DIP. And if we compare figure 2.4c with figure 2.4b, we can see that the region of survival of the DIP (and of the helper) is pretty much the same. Thus, it would appear that, for the DIP, $f \geq 0.5$ is better than $f < 0.5$.
Let us now consider two specific points of the $(\mathcal{C}, \gamma)$ parameter space:

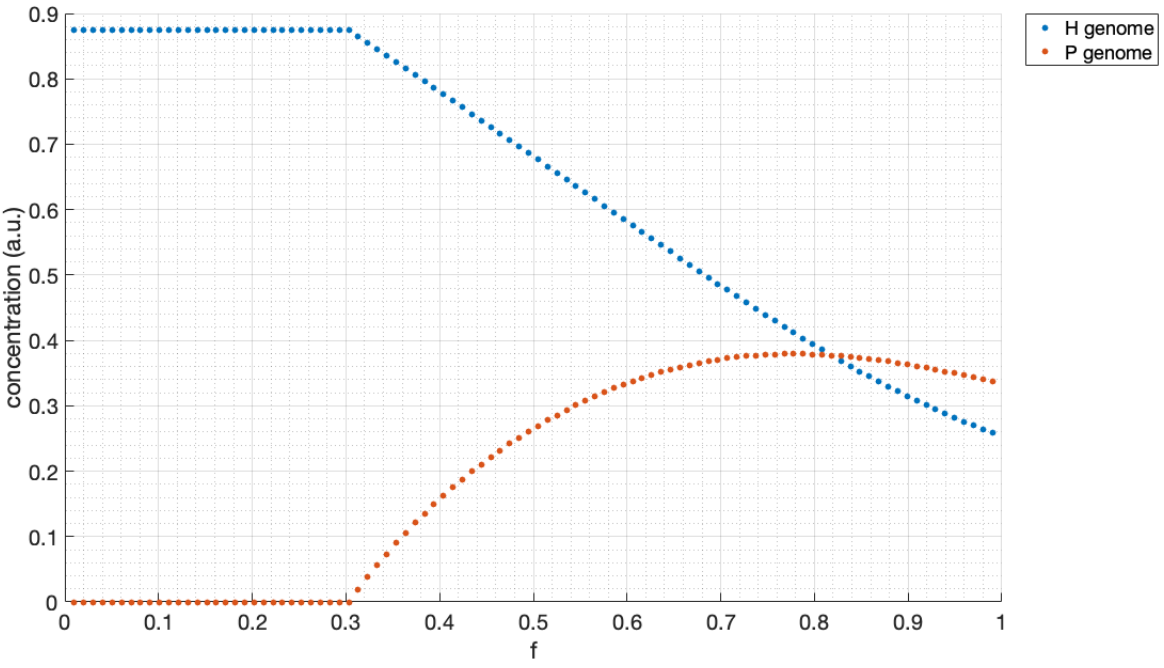
- $(\mathcal{C}, \gamma) = (0.1, 0.01)$ - Here the DIP does not go extinct for $f = 0.1$ or $f = 0.9$.

- $(\mathcal{C}, \gamma) = (0.1, 0.2)$ - Here $\mathcal{C}$ is the same, but the dilution rate γ is higher, and the DIP goes extinct for $f = 0.1$ but not for $f = 0.9$.

We want to look at how the steady-state levels of the two genomes change with the parameter f in these two points. We see the results in figure 2.5.



(a)



(b)

Figure 2.5.: The steady-state levels of the two genomes vs f, in both cases with $\mathcal{C} = 0.1$, but with two different dilution rates γ. (In figure B.2 in the appendices we show the steady-state levels of only the infected cells vs f for the two dilution rates.) (a) $\gamma = 0.01$ (slower dilution) (b) $\gamma = 0.2$ (faster dilution).

- With slower dilution (figure 2.5a) $f \simeq 0$ and $f \simeq 1$ both lead to low levels of g_P, while only $f \simeq 1$ leads to a low level of g_H. In the rest of the domain of f, the two genome levels are consistently $\simeq 10$, and they have the same value for $f \simeq 0.5$.

- With faster dilution (figure 2.5b), for f up to ~ 0.3, the DIP goes extinct; instead, $f \simeq 1$ is not a problem either for the DIP genome or the helper genome. Also, the two genome levels do not cross at $f \simeq 0.5$, but at $f \simeq 0.8$.

Now we will try to explain why $f \simeq 1$ does not lead to a drop of the two genome levels when the dilution is faster.

- The I_H cells produce only helpers.

- The I_{HP} cells mostly produce DIPs and very few helpers, because $f \simeq 1$.

- To obtain I_H, only one "step" is necessary: $B + H \to I_H$.

- Instead, to obtain I_{HP}, two "steps" are necessary: $B + H \to I_H$ and then $I_H + P \to I_{HP}$,
  or alternatively $B + P \to I_P$ and then $I_P + H \to I_{HP}$

The faster dilution hinders the processes of the system because it removes cells and viruses which could interact with one another. Because the I_{HP} formation requires two steps, instead of the only step necessary to form I_H, the I_{HP} formation is disadvantaged compared to the I_H formation. This disadvantage indirectly hinders the creation of DIPs. Thus, a faster dilution balances a high value of f: in fact, with faster dilution (figure 2.5b) not all cells become I_{HP} before they are diluted; instead, with slower dilution (figure 2.5a), the cells have time to become I_{HP} before they are diluted, so the I_{HP} cells become the dominant type of cells. This implies that with slower dilution, the I_{HP} cells are the predominant makers of helpers, whilst the I_H do not have much influence in the helper production. There is symmetry in the productions of DIPs and helpers because it is essentially only the I_{HP} cells to produce both of them; so the two steady-state genome levels cross at $f \simeq 0.5$. Instead, with faster dilution, the I_H cells have a bigger role in the helper production, so the symmetry in the productions of DIPs and helpers disappears: the two

steady-state genome levels cross at a higher f, $f \simeq 0.8$. The I_{HP} cells need to make more DIPs than helpers in order to balance the helper production by the I_H cells and for the two genome levels to be equal.

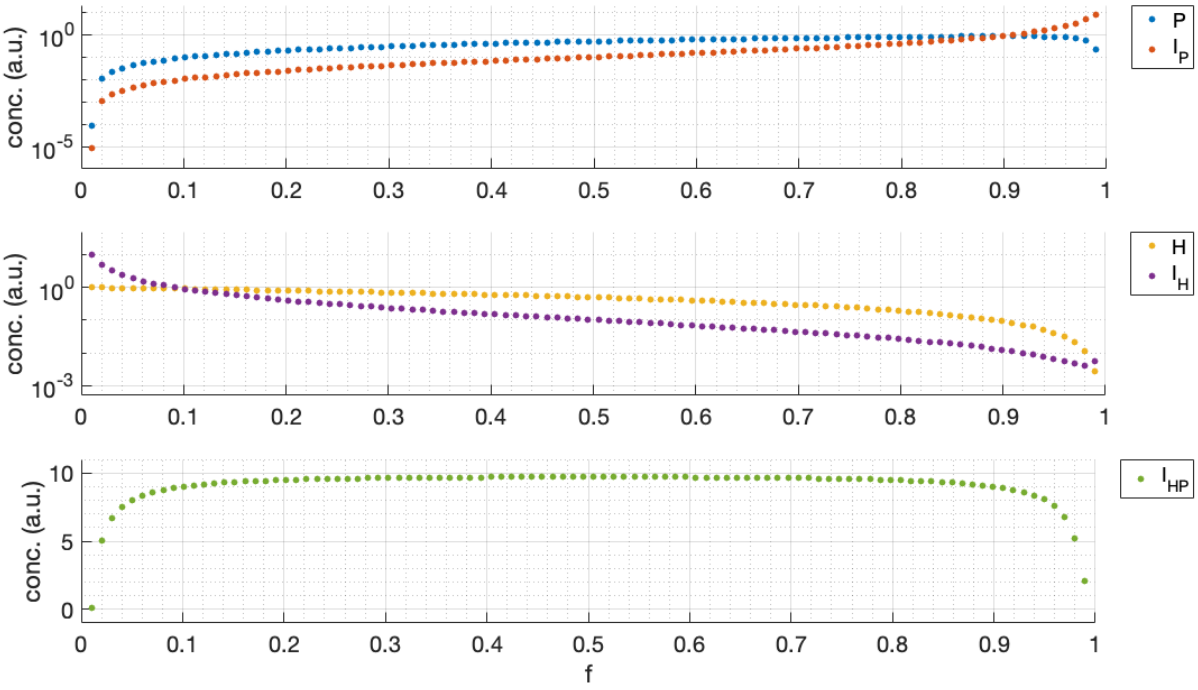
If we break down the steady-state genome levels seen in figure 2.4 into the distinct levels of viruses and infected cells, we find (see figure B.1 in appendices) that the steady-state genome is mostly made of

- I_{HP} and I_H for $f = 0.1$
- I_{HP} for $f = 0.5$
- I_{HP} and I_P for $f = 0.9$.

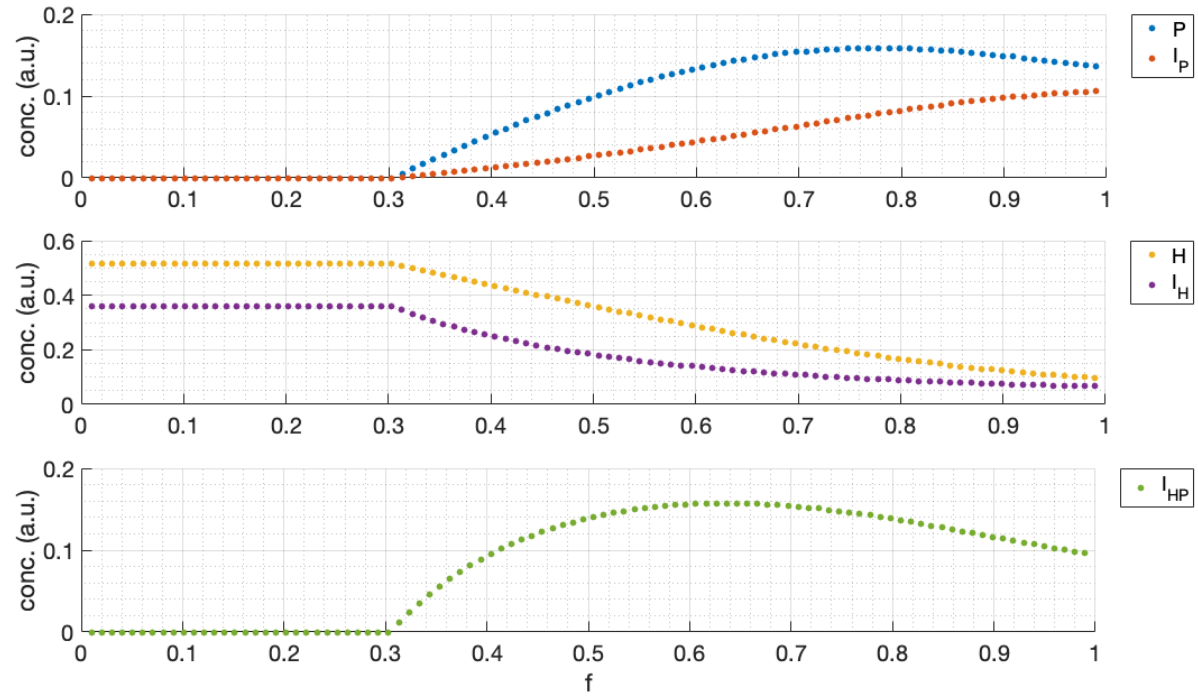
Now let us instead break down the steady-state genome levels shown in figure 2.5 into individual types of virus or cell. Once again, we are looking at a case of slower dilution and one of faster dilution. This breakdown is in figure 2.6.

- In the case of slower dilution (figure 2.6a), I_{HP} is the dominant type of cell if $f \neq 0$ and $f \neq 1$. If $f \simeq 0$ and $f \simeq 1$, I_H and I_P become the dominant cells respectively. We can see this even better in figure B.2a.

- In the case of faster dilution (figure 2.6b), where the DIPs survive ($f \gtrsim 0.3$), I_H and I_P maintain levels similar to that of I_{HP} for the reason described before, i.e. the faster dilution hinders the formation of the more complex species I_{HP}. We can see even better the similar levels of these species in figure B.2b.



(a)



(b)

Figure 2.6.: Steady-state levels of the viruses and infected cells vs f, with two different rates of dilution γ. $\mathcal{C}$ is the same in the two cases. (a) $(\mathcal{C}, \gamma) = (0.1, 0.01)$ (slower dilution). (b) $(\mathcal{C}, \gamma) = (0.1, 0.2)$ (faster dilution). In figure B.2 in the appendices is a closer look at the levels of the infected cells.

2.2.3 What if the I_{HP} cells produce only DIPs?

Let us now consider the special case $f = 1$. If $f = 1$, new helper viruses are produced only by the I_H cells, and the I_{HP} cells only make DIPs. As we just saw, $f \simeq 1$ allows for the survival of the DIPs if the dilution is fast enough. One more thing we found was that in a region of the $(\mathcal{C}, \gamma)$ parameter space, the levels of viruses and cells oscillate. Let us take a look at this.
While studying this model, we saw that the levels in the system usually reach a steady-state. However, the levels may oscillate instead. We noticed that this occurred for $f \simeq 1$ when there is a slow influx of cells and slow dilution of cells and viruses. More precisely, when $f = 1$, we saw oscillations for

- $\mathcal{C} \lesssim 3 \times 10^{-2}$
- $\gamma \lesssim 3 \times 10^{-2}$

In figure 2.7 we see, for $\mathcal{C} \in [0.01, 1]$ and $\gamma \in [0.01, 1]$, the region of the $(\mathcal{C}, \gamma)$ parameter space that gives oscillations when $f = 1$.

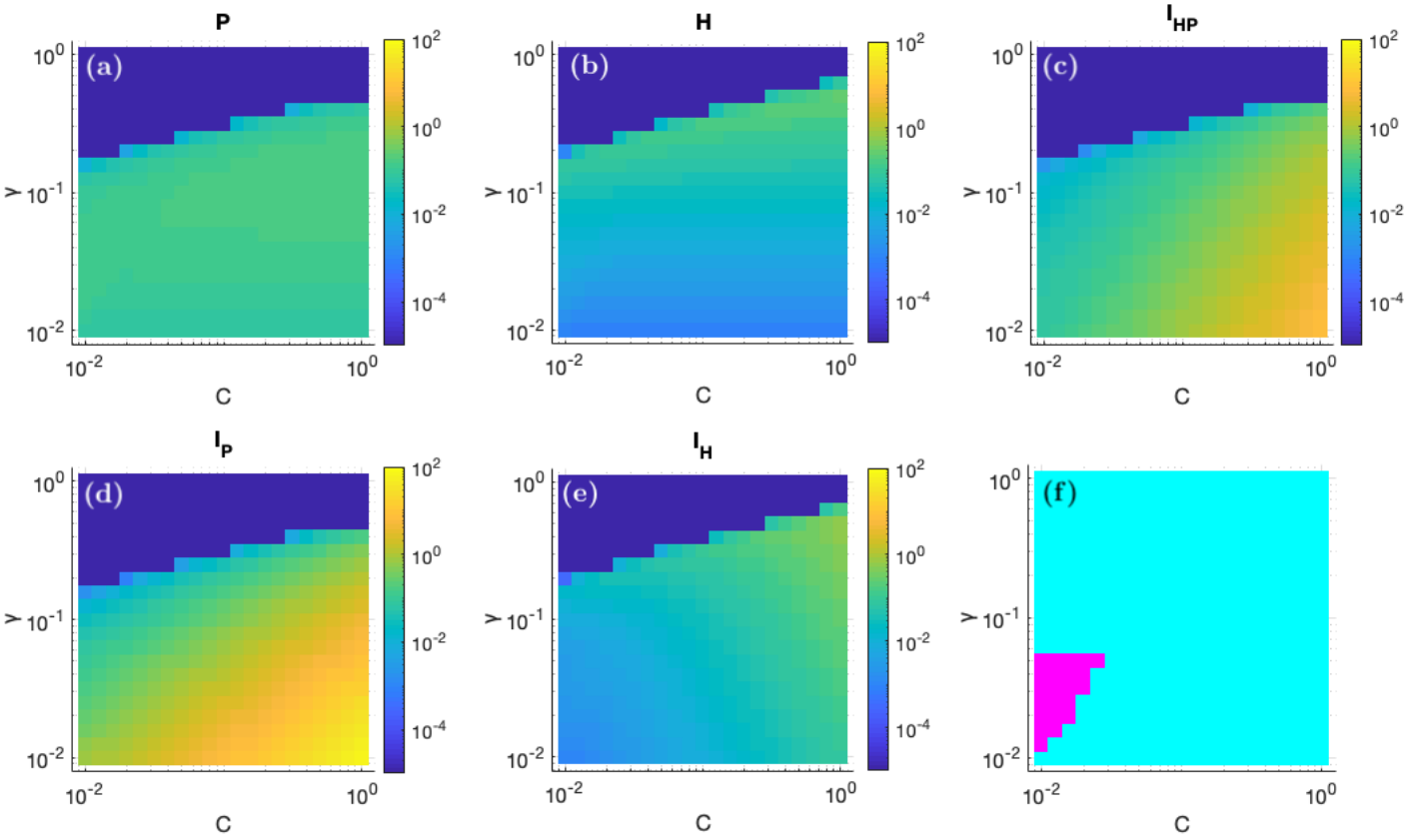
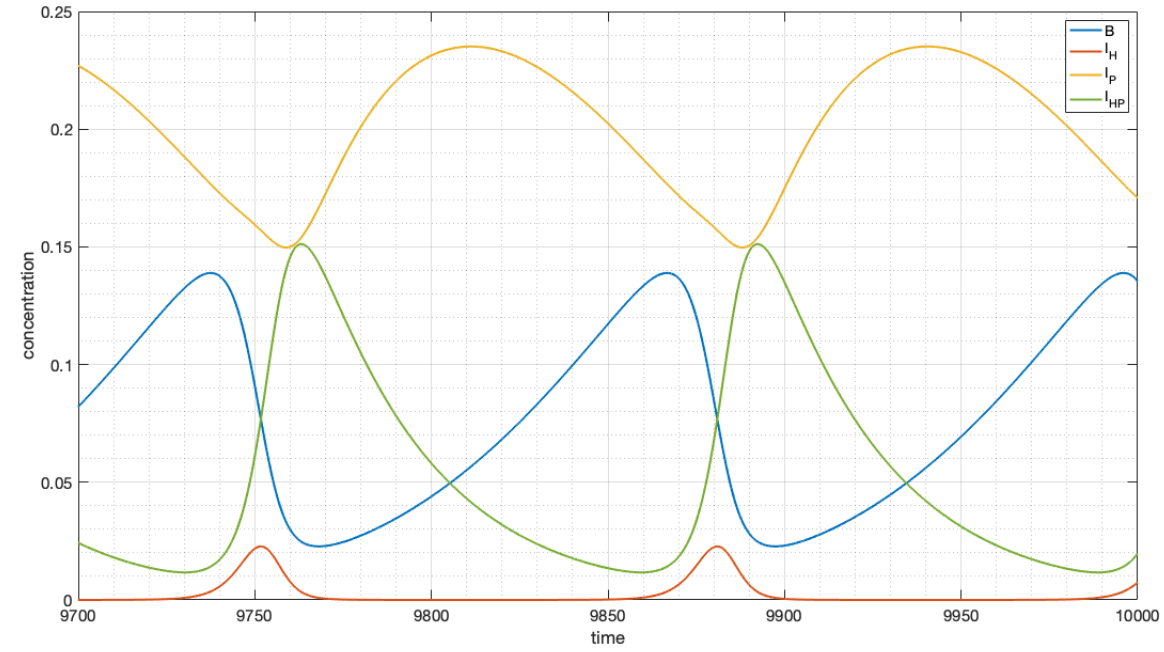
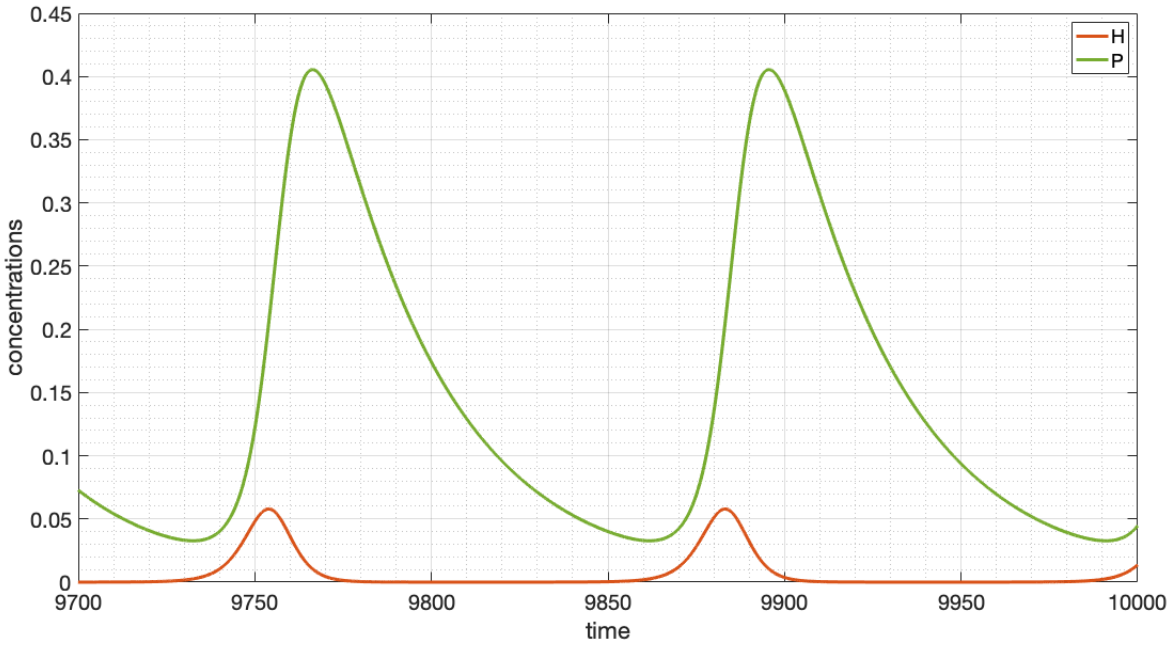


Figure 2.7.: In figure 2.7f, in magenta is the $(\mathcal{C}, \gamma)$ region that gives oscillation of the virus and cell levels, and in cyan is the region which gives a steady-state. In figures 2.7a-e are the steady-state levels when there is a steady-state, and the average levels when the levels oscillate. There is no discontinuity between the steady-state levels and the average levels. Also, the region with oscillations is a region of low average I_{HP} and I_H, i.e. the makers of DIPs and helpers respectively.

In figure 2.8 is an example of oscillating levels (the parameters are $(\mathcal{C}, \gamma) = (0.01, 0.03)$).



(a)



(b)

Figure 2.8.: Oscillating levels of cells and viruses. $(\mathcal{C}, \gamma) = (0.01, 0.03)$. (a) Cell concentrations. (b) Virus concentrations.

Let us try to explain why the levels oscillate. We will start from a trough of I_{HP}.

- Because I_{HP} is low, few P are produced.

- I_H is not much lower than I_{HP}. These cells produce H viruses, which in turn transform B cells into more I_H cells.

- At the same time, some H viruses produced by I_H turn some I_P into I_{HP}; this reaction $H + I_P \rightarrow I_{HP}$ occurs more frequently than the reaction $H + B \rightarrow I_H$ because there are more I_P cells than B cells.

- The I_{HP} surpass the B: now the absorption of H by I_{HP} becomes more frequent than the absorption of H by B. Thus the reaction $H + B \rightarrow I_H$ is neutralised, and I_H decreases, also because the I_{HP} are producing P, which turn I_H into I_{HP}.

- I_{HP} increases due to the reaction $H + I_P \rightarrow I_{HP}$ until the I_H become very low.

- Once I_H has become very low, the formation of I_{HP} becomes rare because the few I_H produce few H, which could transform I_P into I_{HP}.

- So, the dilution of I_{HP} becomes more frequent than its formation and I_{HP} starts to decrease.

- With lower I_{HP} and I_H, the B cells are infected less, so B manages to grow.

- Eventually, B becomes high enough compared to I_P and I_{HP}: the B cells become easier targets for the H produced by the I_H, and more and more B are transformed into additional I_H. So now we are back at the beginning.

A scheme of these upregulations and downregulations is in figure 2.9.

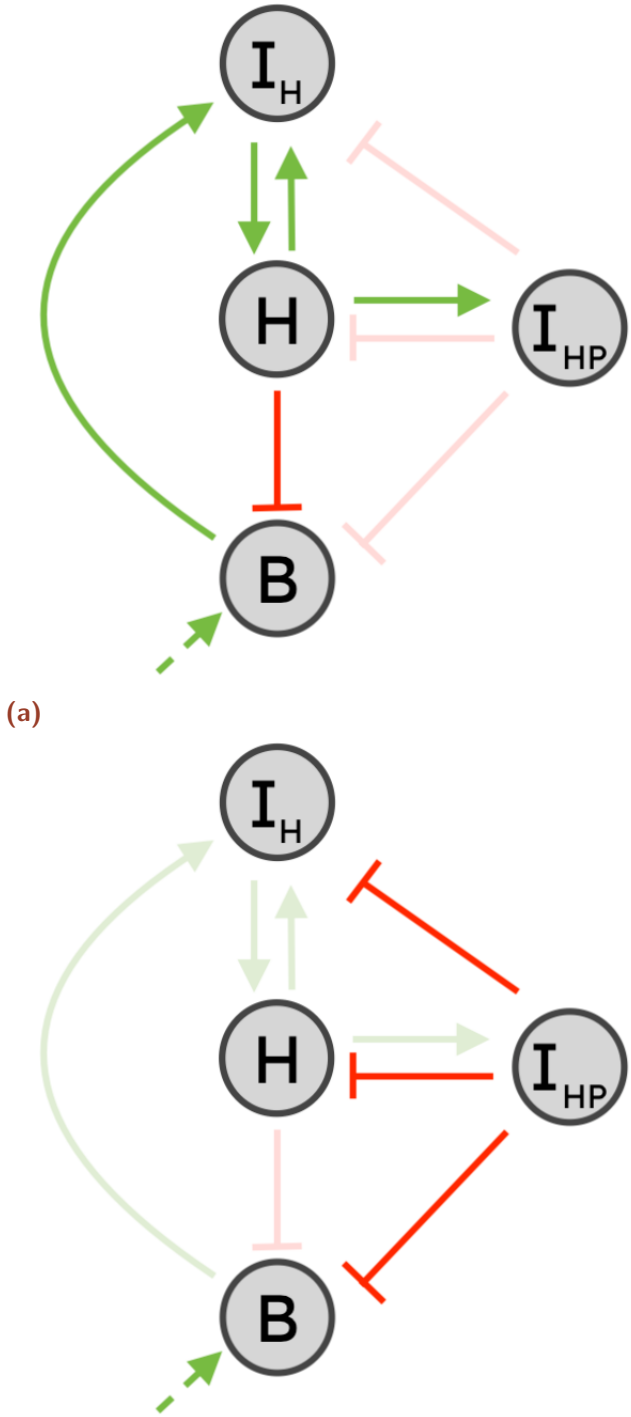


Figure 2.9.: Upregulations and downregulations between some of the components of the system (a) When the levels of I_H and I_{HP} are comparable, I_H manages to increase thanks to the positive feedback loop between I_H and H. At the same time, I_{HP} increases. (b) Once the level of I_{HP} is high enough, it downregulates the level of H, I_H and B, which later leads to a decrease of I_{HP} itself due to negative feedback loops. Eventually, I_{HP} has decreased so much that I_H can increase again.

2.3 Under low dilution, the DIP and helper species are safest when produced in similar proportions

The models we have considered so far assumed that the volume of the system was infinite: this way, the cells and the viruses, which are discrete quantities, can be treated as continuous quantities through their concentrations.
However, if we choose to study a system of finite volume, the model has to change: in fact, now the concentrations are not continuous quantities any longer. If the volume of the system is finite, we should study the absolute amounts of the cells and viruses, and these are discrete quantities.
Now that we want to study a system of finite volume, we have to take one more thing into account: now we cannot ignore the effects of noise, more precisely *demographic stochasticity*. Demographic stochasticity describes the random fluctuations in a population's size occurring because each person's birth and death is a discrete and probabilistic event. Demographic stochasticity tends to average out in large populations and has a greater impact on small populations [17–19]. Now, we did not study people, but cells and viruses: thus

- the births correspond to the introduction of new uninfected cells and release of new viruses by cells, while
- the deaths correspond to the dilution of cells and viruses and absorption of viruses by cells.

2.3.1 The new stochastic model

Let us now look at the details of the model. As we said, we do not use concentrations any longer, but absolute amounts; therefore we define the quantities below.

- $\widetilde{P}$: number of DIPs
- $\widetilde{H}$: number of helper viruses

- $\widetilde{B}$: number of uninfected cells
- $\widetilde{I}_P$: number of cells infected by the DIP only
- $\widetilde{I}_H$: number of cells infected by the helper virus only
- $\widetilde{I}_{HP}$: number of cells infected by both types of virus.

Now that we have introduced the components of the system let us focus on the processes that can happen. Previously, ODEs described the time evolution of the system; we cannot do that any longer, because now the system is finite and the components are in finite amounts. The Gillespie algorithm [20] now handles the time evolution of the system. The rates for the Gillespie algorithm derive from the addends of the ordinary differential equations 2.27-2.32. Below we list these rates and their processes, which we indicate as chemical reactions; we wrote the rates on top of the reaction arrows. Note that the rates are affected by the volume v of the system, because the variables are not concentrations like before, but absolute amounts.

- *Introduction of one uninfected cell*

$$\varnothing \xrightarrow{c \cdot v} \widetilde{B} \tag{2.33}$$

- *Absorption of a virus by a cell*

$$\widetilde{P} + \widetilde{B} \xrightarrow{\eta/v \cdot \widetilde{P} \cdot \widetilde{B}} \widetilde{I}_P \tag{2.34}$$

$$\widetilde{H} + \widetilde{B} \xrightarrow{\eta/v \cdot \widetilde{H} \cdot \widetilde{B}} \widetilde{I}_H \tag{2.35}$$

$$\widetilde{P} + \widetilde{I}_P \xrightarrow{\eta/v \cdot \widetilde{P} \cdot \widetilde{I}_P} \widetilde{I}_P \tag{2.36}$$

$$\widetilde{H} + \widetilde{I}_P \xrightarrow{\eta/v \cdot \widetilde{H} \cdot \widetilde{I}_P} \widetilde{I}_{HP} \tag{2.37}$$

$$\widetilde{P} + \widetilde{I}_H \xrightarrow{\eta/v \cdot \widetilde{P} \cdot \widetilde{I}_H} \widetilde{I}_{HP} \tag{2.38}$$

$$\widetilde{H} + \widetilde{I}_H \xrightarrow{\eta/v \cdot \widetilde{H} \cdot \widetilde{I}_H} \widetilde{I}_H \tag{2.39}$$

$$\widetilde{H} + \widetilde{I}_{HP} \xrightarrow{\eta/v \cdot \widetilde{H} \cdot \widetilde{I}_{HP}} \widetilde{I}_{HP} \tag{2.40}$$

$$\widetilde{P} + \widetilde{I}_{HP} \xrightarrow{\eta/v \cdot \widetilde{P} \cdot \widetilde{I}_{HP}} \widetilde{I}_{HP} \tag{2.41}$$

- *Production of a virus*

$$\widetilde{I}_{HP} \xrightarrow{f \cdot \beta \cdot \widetilde{I}_{HP}} \widetilde{I}_{HP} + \widetilde{P} \tag{2.42}$$

$$\widetilde{I}_{HP} \xrightarrow{\beta \cdot \widetilde{I}_H + (1-f)\beta \cdot \widetilde{I}_{HP}} \widetilde{I}_{HP} + \widetilde{P} \tag{2.43}$$

- *Dilution of a virus or cell*

$$\widetilde{P} \xrightarrow{\gamma \cdot \widetilde{P}} \varnothing \tag{2.44}$$

$$\widetilde{H} \xrightarrow{\gamma \cdot \widetilde{H}} \varnothing \tag{2.45}$$

$$\widetilde{B} \xrightarrow{\gamma \cdot \widetilde{B}} \varnothing \tag{2.46}$$

$$\widetilde{I}_P \xrightarrow{\gamma \cdot \widetilde{I}_P} \varnothing \tag{2.47}$$

$$\widetilde{I}_H \xrightarrow{\gamma \cdot \widetilde{I}_H} \varnothing \tag{2.48}$$

$$\widetilde{I}_{HP} \xrightarrow{\gamma \cdot \widetilde{I}_{HP}} \varnothing \tag{2.49}$$

Once again, we rescale the system. We redefine t' (2.7) and define v'

$$v' = \frac{v}{\eta/\beta} \tag{2.50}$$

The parameters are rescaled according to (2.11) ($\eta' = 1$), (2.12) ($\beta' = 1$), (2.25) ($\mathcal{C}'$), (2.26) (γ'). We drop the index $'$ for simplicity.

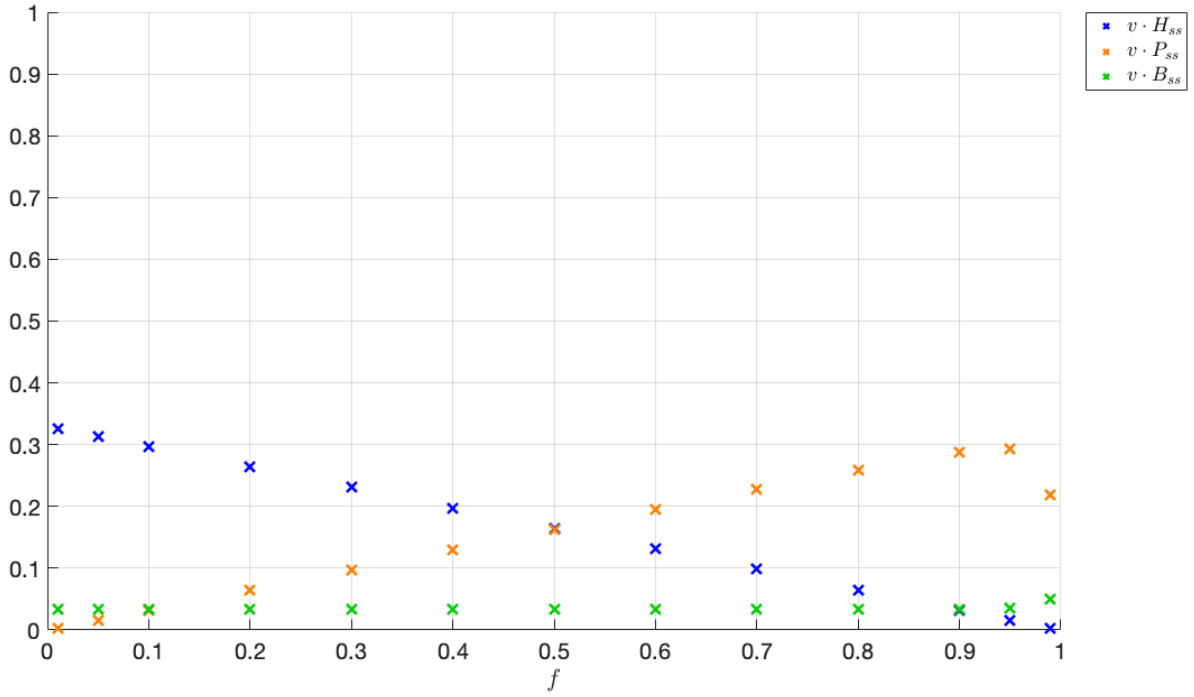
Thus now we have 4 parameters: $\mathcal{C}$, γ, v and f. What we wanted to do was investigate which values of f are best for the DIPs in this type of system, where demographic stochasticity plays an important role. We thus fixed the three remaining parameters. $\mathcal{C}$ and γ are $\mathcal{C} = 0.1$ and $\gamma = \frac{1}{300}$ (we used a dilution rate lower than those scanned in section 2.2 so that the dilution has little interference with the dynamics between cells and viruses). We will look at the chosen value of v in a moment: first, we will discuss the initial conditions for this model.

We wanted to focus on the effects of stochasticity on the system: therefore we used initial conditions that reflect dynamic-equilibrium levels in a system of infinite volume. So the initial amounts are the product of the steady-state concentrations of the infinite-volume system times the volume of this system, rounded to the nearest integer. The steady-state concentration derive from the model in section 2.2. We chose our volume v so that the initial amounts would be low, and the effect of the noise significant. Our choice was

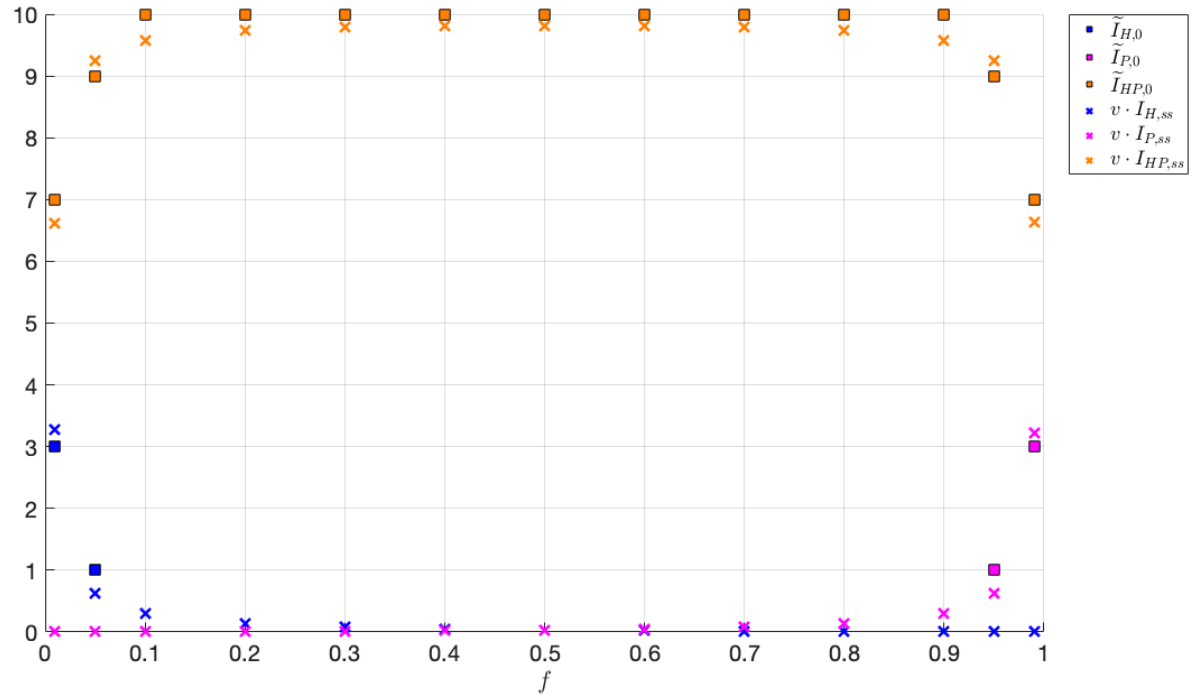
$$v = 0.33. \tag{2.51}$$

In figure 2.10 are the product of v times the infinite-volume steady-state concentrations, and their roundings, for a series of values of f:

$$f \in \{0.01,\ 0.05,\ 0.1,\ 0.2,\ 0.3,\ 0.4,\ 0.5,\ 0.6,\ 0.7,\ 0.8,\ 0.9,\ 0.95,\ 0.99\} \tag{2.52}$$



(a)



(b)

Figure 2.10.: v times the infinite-volume steady-state concentrations, and their roundings to the nearest integer, i.e. the initial amounts for this stochastic model. (a) Initial amounts of DIPs and helper viruses. v times the infinite-volume steady-state virus concentrations are < 0.5 every time. Therefore the initial amounts of viruses will be 0. (b) Initial amounts of cells. The initial amounts of cells infected by only one virus are 0 for some values of f.

The list of initial amounts of cells and viruses is in table B.1 in the appendices. The initial state includes

- only $\widetilde{I}_{HP}$ for $0.1 \leq f \leq 0.9$
- mostly $\widetilde{I}_{HP}$ with some $\widetilde{I}_H$ if $f < 0.1$
- mostly $\widetilde{I}_{HP}$ with some $\widetilde{I}_P$ if $f > 0.9$.

An example of time evolution of the system is in figure 2.11 ($f = 0.1$).

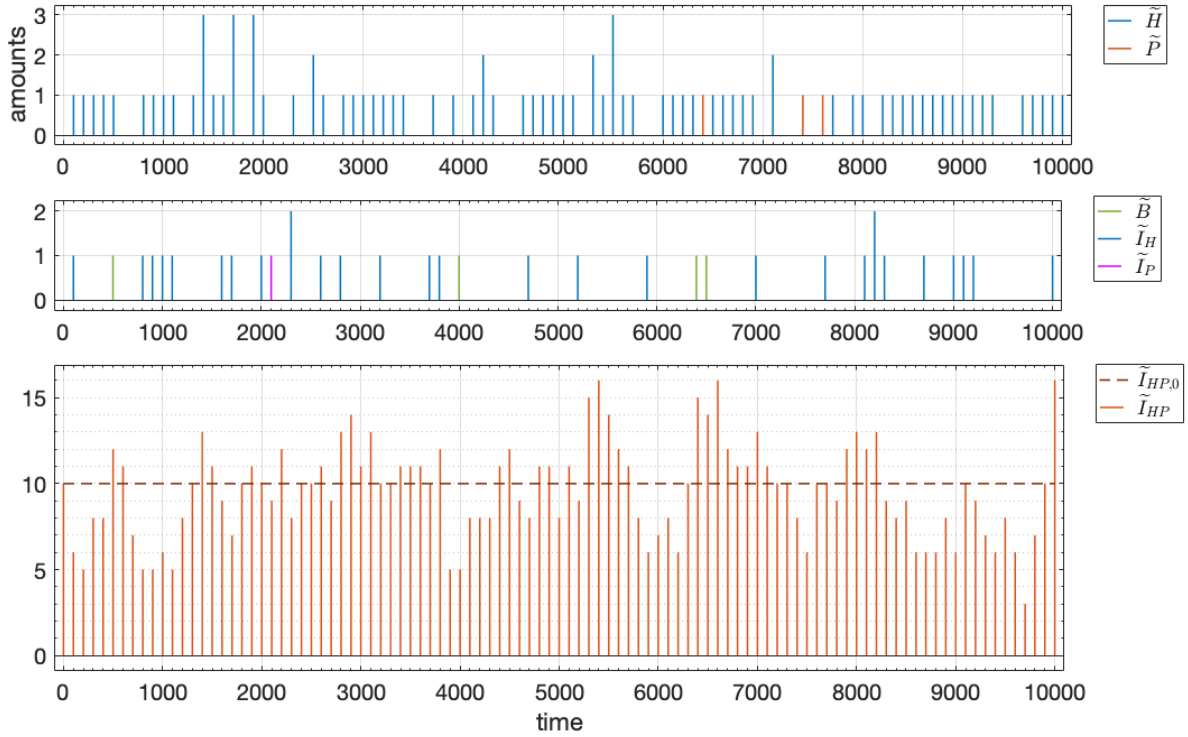


Figure 2.11.: An example of time-evolution of the numbers of viruses and cells. Here $f = 0.1$. The amounts are plotted every 100 seconds.
The oscillation due to stochasticity of $\widetilde{I}_{HP}$ are about the initial amount $\widetilde{I}_{HP,0}$, amount which reflects the dynamic equilibrium of the infinite-volume system. The other initial amounts were 0 (see table B.1).

As we said, we want to study what values are better for the DIPs in this kind of system. However, how do we measure how good the value of f is for the DIP? By studying the extinction of the DIP itself. This system has low amounts, and the demographic stochasticity could lead the DIP to extinction.

We wanted to see how differently the stochasticity would lead the DIP to extinction depending on the value of f, so this is what we studied.

2.3.2 Results

In figure 2.12 is an example of time evolution of the system with extinction of the DIP species. (In the appendices, with figure B.3 the reader can see an example of time evolution where the DIP and the helper species go extinct simultaneously).

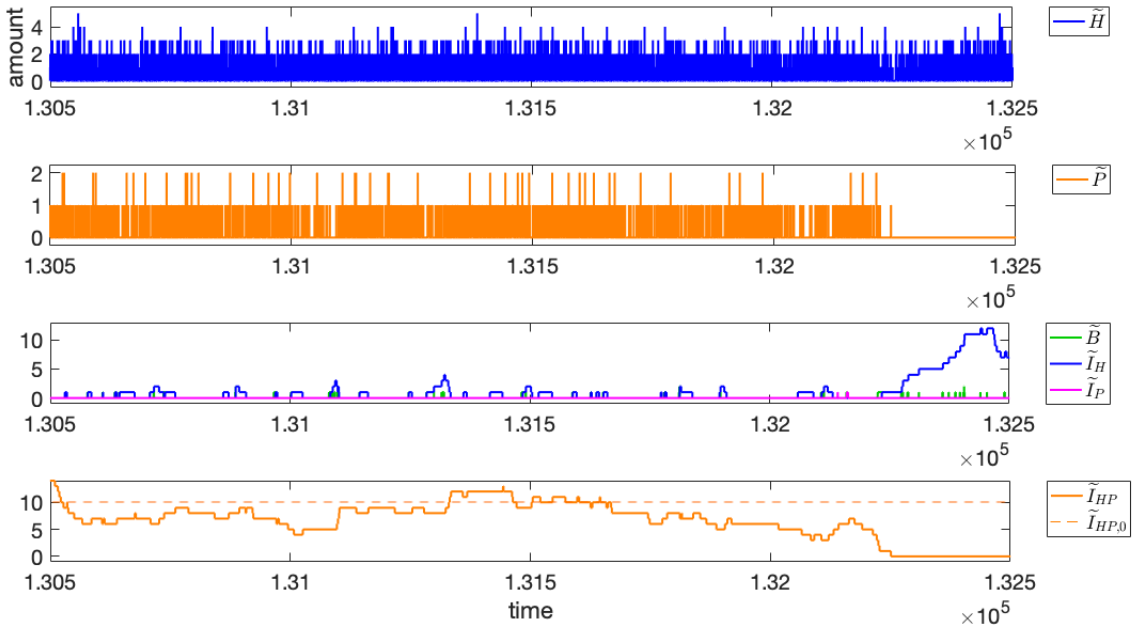


Figure 2.12.: Example of time evolution of the system with extinction of the DIP. In this example $f = 0.1$.

We let the system evolve according to the model until the discrete DIP genome $\widetilde{g}_P$

$$\widetilde{g}_P \equiv \widetilde{P} + \widetilde{I}_P + \widetilde{I}_{HP} \tag{2.53}$$

reached the value 0.
We define T_P as the time of extinction of the DIP. We measured it with 40000 runs of the model and studied its statistics. This was done for each value of f listed in (2.52).

Let us look at an example of statistics of T_P: in figure 2.13 are the statistics of T_P for $f = 0.5$.

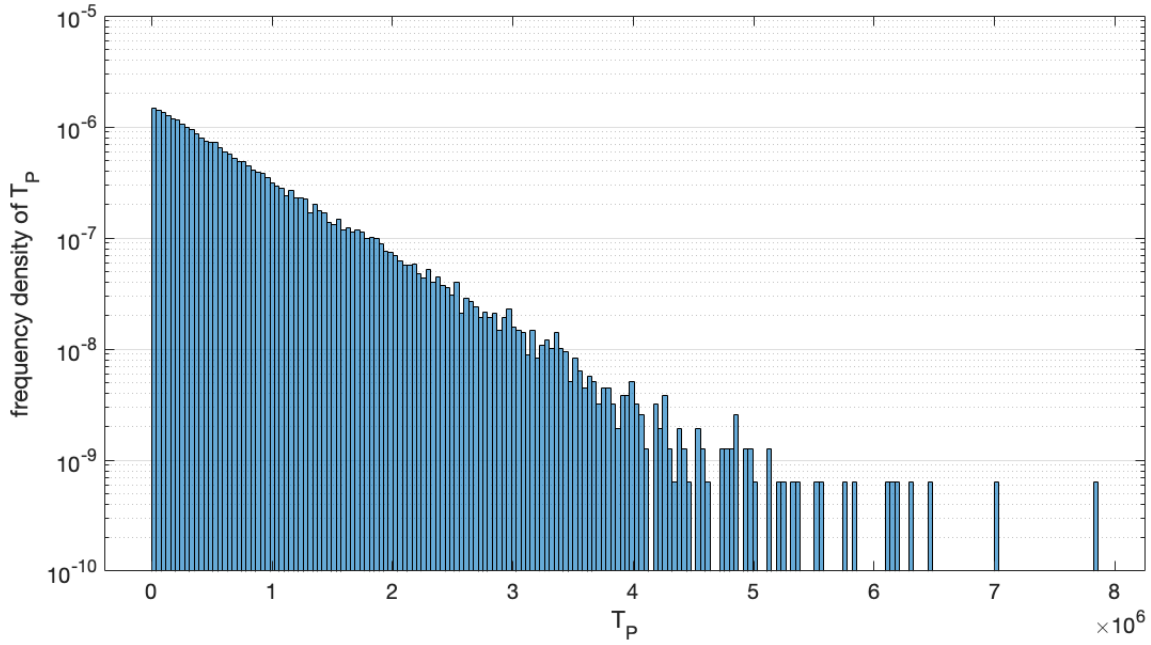


Figure 2.13.: Statistics of T_P with 40000 iterations of the model. Here $f = 0.5$. The vertical scale is logarithmic. The columns represent the frequency density of the times in the bins, i.e. the frequency normalised by (*bin width*)$\times$(*total number of events*).

For each value of f we considered, T_P is Poisson-distributed, i.e. T_P follows the decaying-exponential distribution

$$h(T_P) = r_P \cdot \exp(-r_P T_P) \tag{2.54}$$

We can find the rate r_P of the distribution with a linear fit of the logarithms of the frequency densities. The fitting function is

$$l(T_P) \equiv \log(h(T_P)) = \log(r_P) - r_P T_P \tag{2.55}$$

So we searched r_P, i.e. the rate of disappearance of the DIP for the already mentioned values of f (list (2.52)). The results are in figure 2.14.

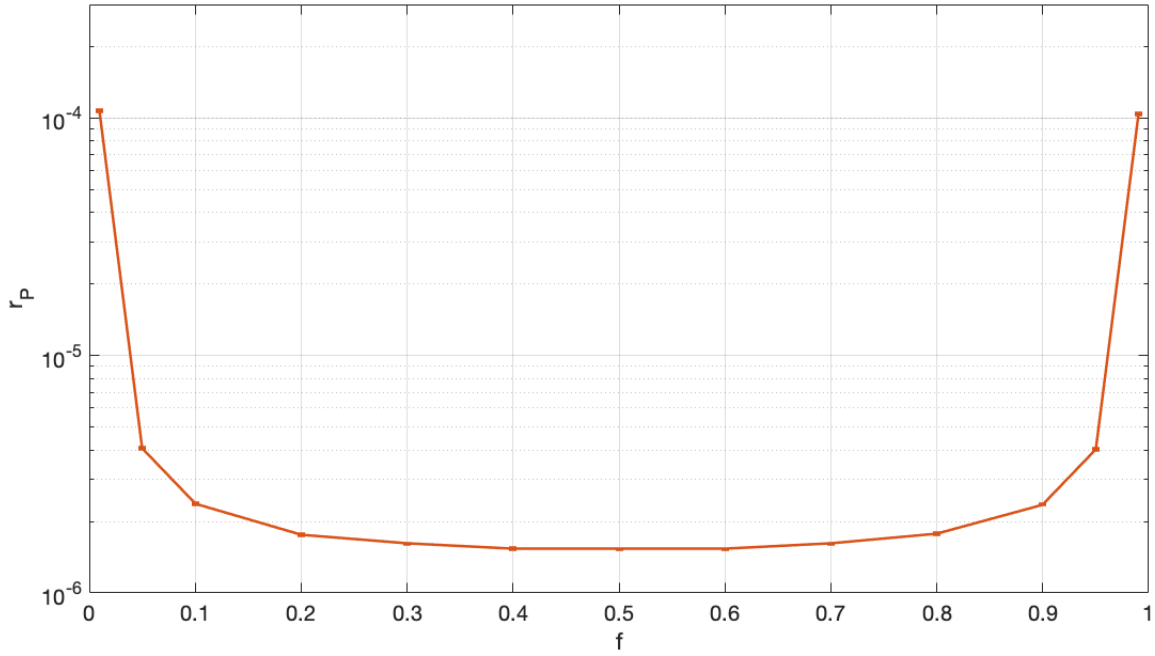


Figure 2.14.: r_P vs. f

r_P is consistently $\simeq 2 \times 10^{-6}$ in the range $0.1 \leq f \leq 0.9$, while it increases rapidly and significantly as f approaches 0 and 1.
r_P shows symmetry around $f = 0.5$. Thus the rate of extinction of the DIP is the same whether the probability f that the I_{HP} just produced a DIP has a certain value or the probability $1 - f$ that the I_{HP} just produced a helper virus has the *same* value.

It is possible that the helper virus goes extinct before the DIP. In the appendices (figure B.4), we show the example of a system where this occurs.

Thus, during the 40000 system simulations for each value of f, we recorded how frequently the helper went extinct before the DIP, and also the time when the helper went extinct, which we call T_H.
Does the value of f affect how frequently the helper goes extinct before the DIP does? What is the distribution of T_H? Moreover, how does it depend on f?
In figure 2.15 is the relative frequency that the helper goes extinct before or simultaneously as the DIP, for each value of f. We call this relative frequency $\mathcal{R}$.

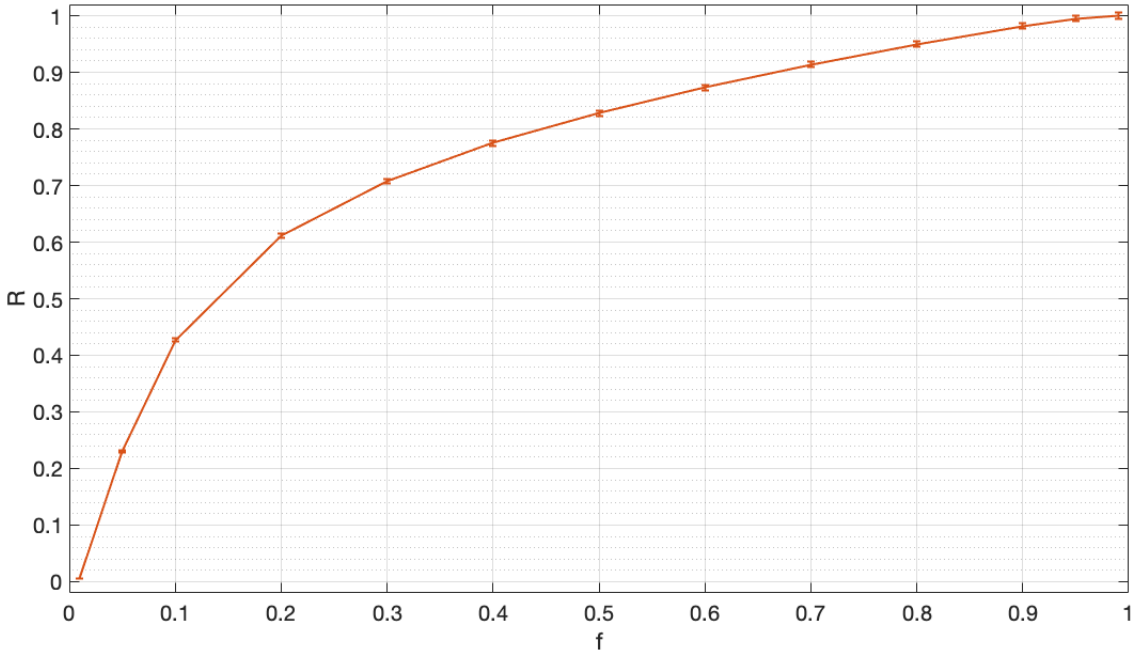


Figure 2.15.: The relative frequency $\mathcal{R}$ vs. f. $\mathcal{R}$ is the fraction of times when the helper virus goes extinct before or simultaneously as the DIP in the 40000 simulations.

The relative frequency $\mathcal{R}$ monotonically increases with f, and while it is $\simeq 0$ for $f = 0.01$, it is already more than 60% for $f = 0.2$.

Next we show how $\mathcal{R}$ breaks down into the relative frequency of helper extinction *before* DIP extinction and the relative frequency of helper-DIP simultaneous extinction. We call these $\mathcal{R}_{helper\ before\ DIP}$ and $\mathcal{R}_{simultaneous}$. $\mathcal{R}_{h.\ before\ DIP} + \mathcal{R}_{sim.} = \mathcal{R}$. We also plot $(1 - \mathcal{R})$, i.e. the relative frequency of events when the DIP goes extinct while the helper is still present in the system. The three quantities are in figure 2.16.

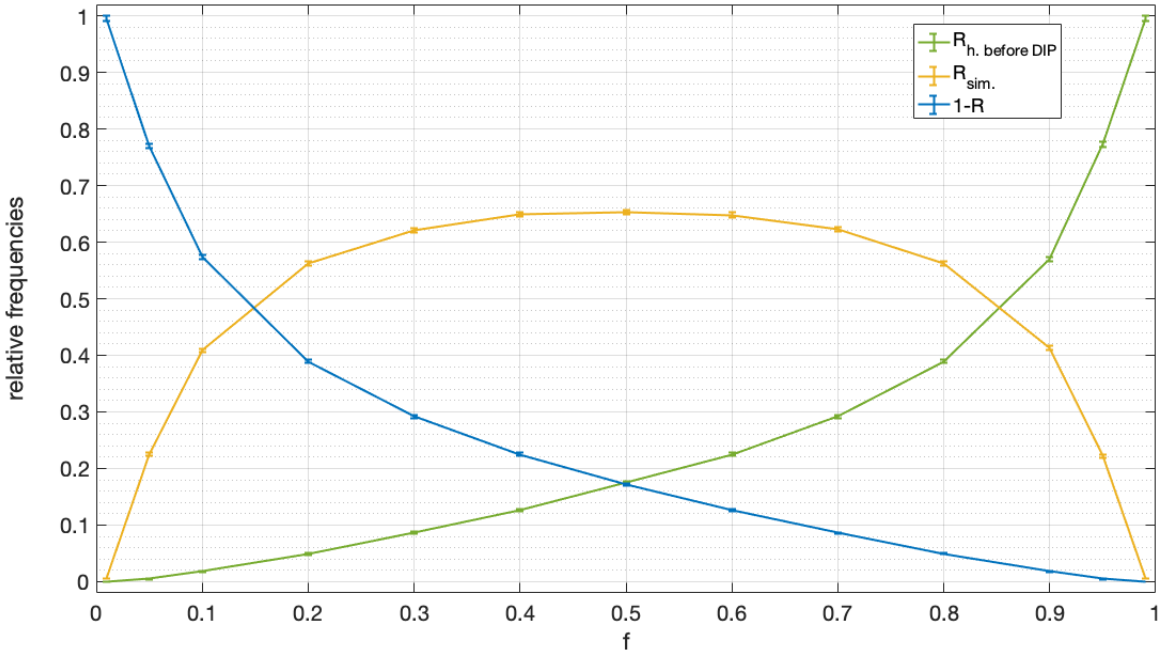


Figure 2.16.: The relative frequencies $\mathcal{R}_{h.\ before\ DIP}$, $\mathcal{R}_{sim.}$ and $(1-\mathcal{R})$ vs. f. $\mathcal{R}_{h.\ before\ DIP}$ is the relative frequency of events when the helper goes extinct before the DIP does. $\mathcal{R}_{sim.}$ is the relative frequency of events when the helper and the DIP go extinct simultaneously. $(1-\mathcal{R})$ is the relative frequency of events when the DIP goes extinct while the helper is still present in the system.

We can see that for $f \leq 0.8$ the helper goes extinct before the DIP ($\mathcal{R}_{h.\ before\ DIP}$) more often than simultaneously ($\mathcal{R}_{sim.}$).
We can also see that $\mathcal{R}_{h.\ before\ DIP}$ and $(1-\mathcal{R})$ are specular to each other. (We remind the reader that $(1-\mathcal{R})$ is the relative frequency that the DIP goes extinct while the helper is still in the system.) This symmetry is due to the low levels of I_P and I_H: in fact, the DIP and the helper are asymmetrical in that I_P and I_H function differently (I_P is sterile, I_H produces helpers); since the levels of I_P and I_H are scarce, the asymmetry is weakened.
Next, we look at the distribution of T_H, i.e. the time when the helper goes extinct if it goes extinct before the DIP. It turns out that T_H follows a Poissonian distribution just like T_P does (we show an example of a distribution of T_H in the appendices in figure B.5). From

$$h(T_H) = r_H \cdot \exp(-r_H T_H) \tag{2.56}$$

we obtain

$$l(T_H) = \log(h(T_H)) = \log(r_H) - r_H T_H \tag{2.57}$$

and we use it to fit the frequency densities to obtain the extinction rate of the helper, which we call r_H. We show r_H plotted against f in figure .

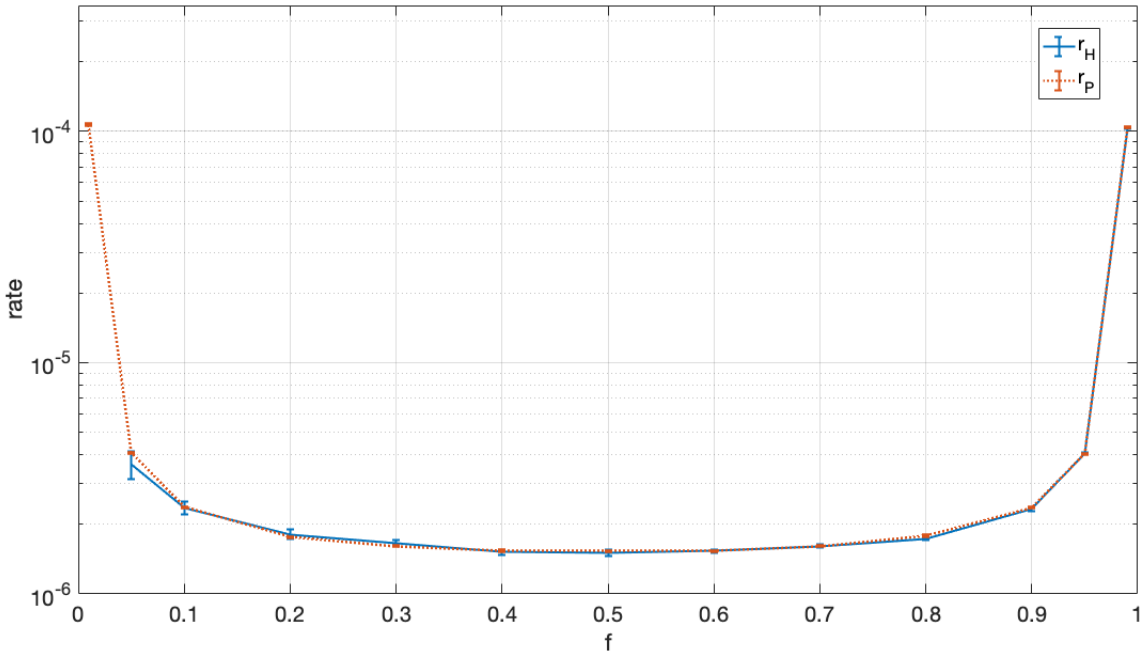


Figure 2.17.: r_H vs f. We plot also r_P.

r_P is specular to r_H because the amounts of I_P and I_H are scarce. Therefore the asymmetry between DIP and helper is weakened.

The last thing we investigated with this model was for how long the DIP survives in the system after the extinction of the helper virus. For the cases where the helper goes extinct before the DIP, we define $\Delta T \equiv T_P - T_H$. An example of statistics of ΔT (for $f = 0.5$) is shown in the appendices in figure B.6.

Also this quantity follows a Poisson distribution. Thus after introducing

$$h(\Delta T) = r_* \cdot \exp(-r_* \Delta T) \tag{2.58}$$

we fitted the frequency densities with

$$l(\Delta T) = \log(h(\Delta T)) = \log(r_*) - r_* \Delta T \tag{2.59}$$

The resulting rates r_* are in figure 2.18.

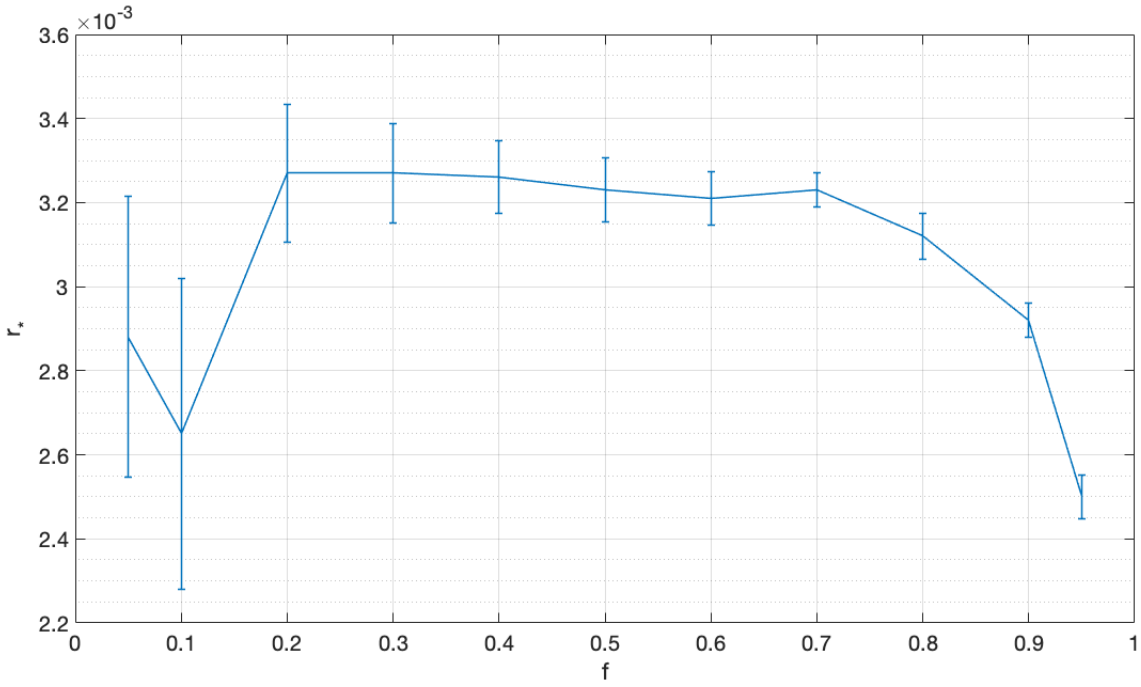


Figure 2.18.: r_* vs. f. r_* is the rate of extinction of the DIP *since the extinction of the helper*.

r_* is consistently about 3.25×10^{-3} for $0.2 \leq f \leq 0.7$. Closer to 0 and 1 r_* drops a little, but the change is not by orders of magnitude like for the rates r_P and r_H (see figure 2.17).
The fact that the rate r_* decreases for f closer to 0 and 1 means that the moment the helper goes extinct more DIPs are left in the system, compared to the case $0.2 \lesssim f \lesssim 0.7$.

3 The interference of one DIP species with two helper-virus species

Let us consider a system of cells with two standard virus species; let us assume there is also a DIP species that can be helped by both. What are the dynamics going to be?

3.1 When two helper species compete for the same DIP species, one wins

We use a deterministic model of well-mixed cells and viruses, with an influx of uninfected cells and the dilution of all cells and viruses, similar to the model in section 2.2. In that model, there was only one helper species; now we have two, and we will distinguish them with the indices *1* and *2*. Thus now we have the species P, B, I_P but also

- H_1 and H_2, the free helper viruses
- I_{H_1} and I_{H_2}, the cells infected by a helper virus
- I_{H_1P} and I_{H_2P}, the cells infected by both a helper and a DIP

We assume that there is superinfection exclusion. *Superinfection exclusion* or *homologous interference* is defined as the ability of an established virus infection to interfere with a secondary infection by the same virus or by a closely related virus [**Tscherne2007SuperinfectionFrom**, 21]. Superinfection exclusion has been described for several virus-host systems, including viruses that cause serious diseases in humans, animals, and plants [21]. Because of

this assumption, in our model, once a cell has absorbed its first helper virus, any subsequent absorption of a helper will not have any effect. Therefore we cannot have a cell $I_{H_1H_2}$ or $I_{H_1H_2P}$.

3.1.1 The ODEs of the model

The equations of the model are

$$\frac{dB}{dt} = \mathcal{C} - \eta V B - \gamma B \tag{3.1}$$

$$\frac{dI_{H_1}}{dt} = \eta H_1 B - \eta P I_{H_1} - \gamma I_{H_1} \tag{3.2}$$

$$\frac{dI_{H_2}}{dt} = \eta H_2 B - \eta P I_{H_2} - \gamma I_{H_2} \tag{3.3}$$

$$\frac{dI_P}{dt} = \eta P B - \eta \left(H_1 + H_2\right) I_P - \gamma I_P \tag{3.4}$$

$$\frac{dI_{H_1P}}{dt} = \eta P I_{H_1} + \eta H_1 I_P - \gamma I_{H_1P} \tag{3.5}$$

$$\frac{dI_{H_2P}}{dt} = \eta P I_{H_2} + \eta H_2 I_P - \gamma I_{H_2P} \tag{3.6}$$

$$\frac{dH_1}{dt} = \beta \left[I_{H_1} + (1 - f_1) I_{H_1P}\right] - \eta H_1 T - \gamma H_1 \tag{3.7}$$

$$\frac{dH_2}{dt} = \beta \left[I_{H_2} + (1 - f_2) I_{H_2P}\right] - \eta H_2 T - \gamma H_2 \tag{3.8}$$

$$\frac{dP}{dt} = \beta \left(f_1 I_{H_1 P} + f_2 I_{H_2 P} \right) - \eta P T - \gamma P \tag{3.9}$$

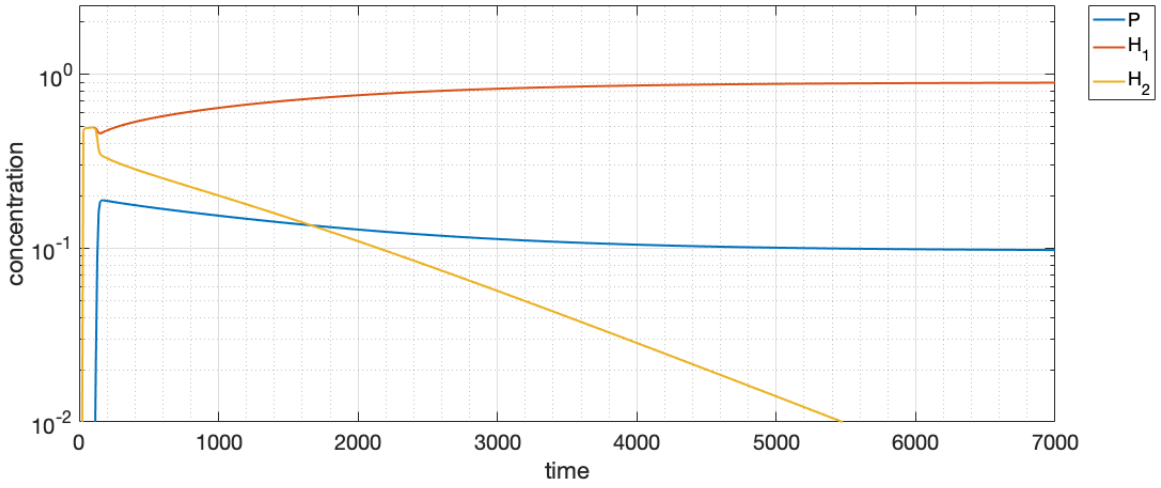
where $V \equiv P + H_1 + H_2$, f_1 is the probability of a virus just made by a $I_{H_1 P}$ cell to be a DIP and analogously for f_2 and the $I_{H_2 P}$ cells.
We rescale time and the concentrations like in section 2.1: we use t' (see eq. (2.7)) and B' (2.8) and so on for the other concentrations. So once again the parameters η, β, $\mathcal{C}$ and γ are substituted by η' (eq. (2.11)), β' (2.12), $\mathcal{C}'$ (2.25), γ' (2.26). Now we remove the $'$ to simplify,

We simulated a system that in the beginning is only made of uninfected cells and free viruses: the initial conditions are

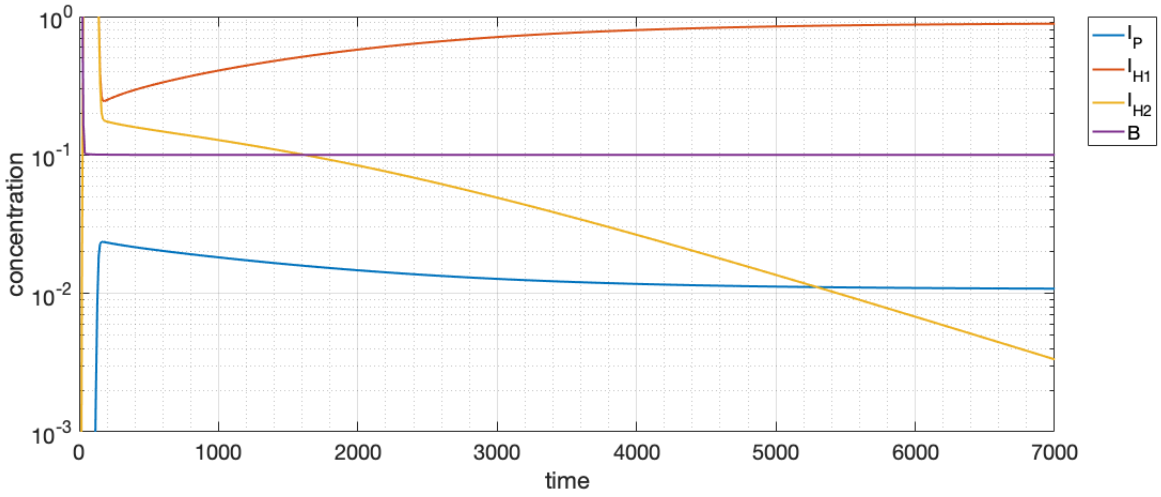
- $B(t = 0) = 1$
- $H_1(t = 0) = 0.25 \times 10^{-7}$
- $H_2(t = 0) = 0.25 \times 10^{-7}$
- $P(t = 0) = 0.25 \times 10^{-7}$
- all other concentrations are zero at $t = 0$

3.1.2 Results

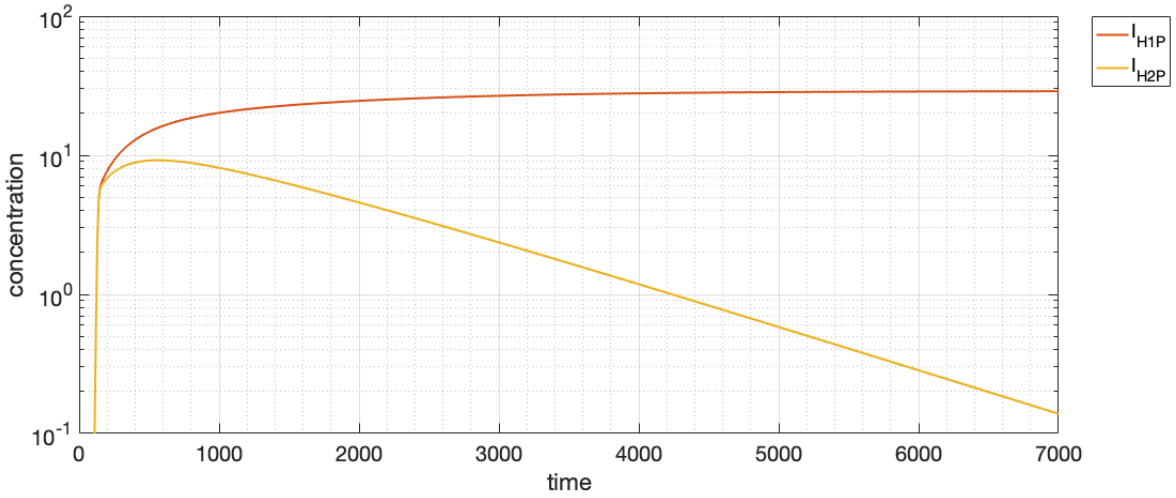
This system evolves in the following way: the system reaches a steady state where the helper virus with the higher parameter f has gone extinct. We can see an example in figure 3.1: in that case, $f_1 = 0.1$ and $f_2 = 0.3$, $f_2 > f_1$ and the helper *2* goes extinct.



(a)



(b)



(c)

Figure 3.1.: Time evolution of the viruses and cells of a system with one DIP species and two helper species. $f_1 = 0.1$, $f_2 = 0.3$. (a) The free viruses. Helper virus *2* goes extinct. (b) The cells that are uninfected or infected by only one type of virus. (c) The cells infected by both the DIP and one type of helper.

So the rule for the extinction of one of the two species of helper is:

- if $f_1 > f_2$, helper virus 1 goes extinct
- if $f_2 > f_1$, helper virus 2 goes extinct

In figure 3.2 is evidence of this.

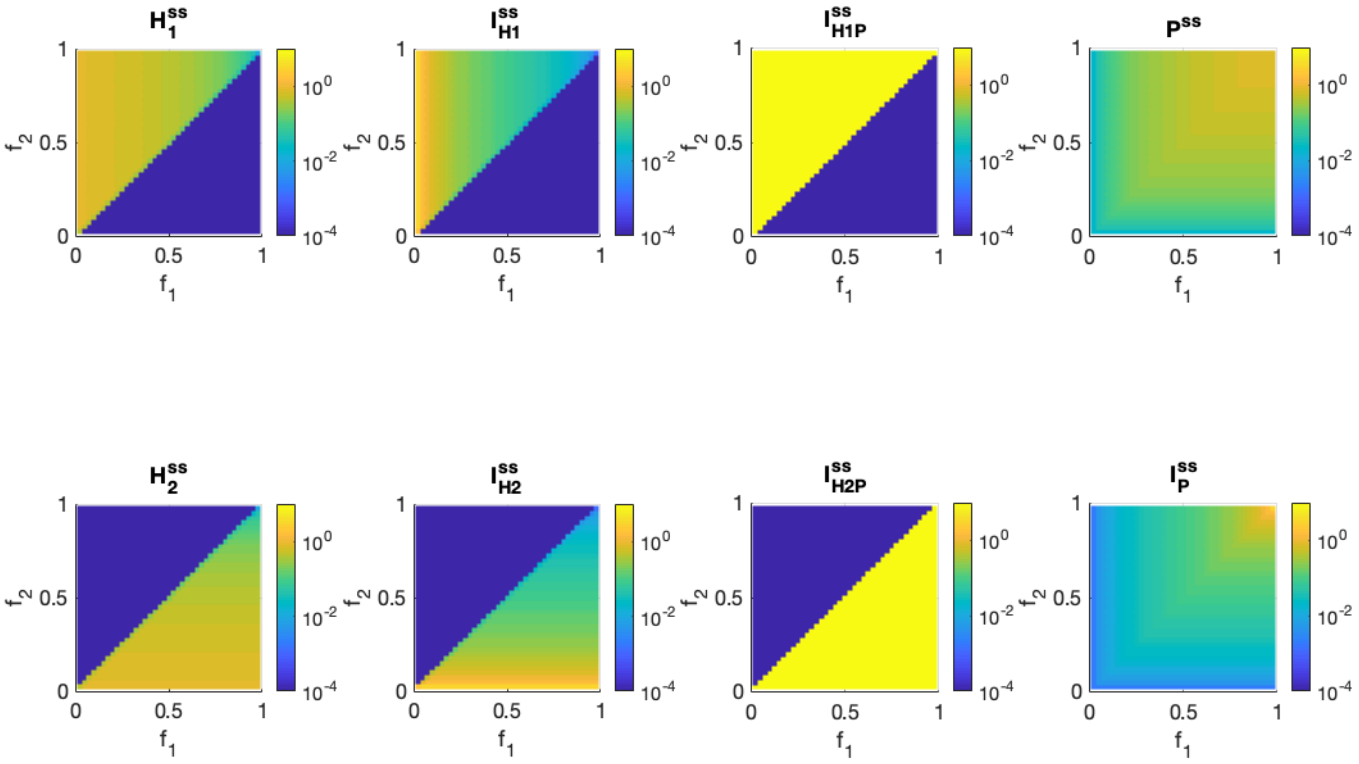


Figure 3.2.: The steady-state levels of the viruses and infected cells vs. (f_1, f_2). Beneath the parameter-space diagonal, i.e. the region $f_1 > f_2$, helper virus *1* goes extinct (and vice versa in the region $f_2 > f_1$, where helper virus *2* goes extinct).

We do not consider the case $f_1 = f_2$, because in that case, the two types of helper virus would effectively be the same: in fact in our model what distinguishes the two species is the parameter f, i.e. how the species of helper virus interacts with the DIP.

4 The interference of two DIP species with two helper-virus species

We just saw that the destiny of a system with two helper species and one DIP species is determined. However, what happens if there is not only one DIP species, but there are two?
Once again we have the species H_1, H_2, B, I_{H_1} and I_{H_2}. But as we have two types of DIP, which we label *a* and *b*, now we have also the following species:

- P_a and P_b, the free DIPs
- I_{P_a} and I_{P_b}, the cells infected by one type of DIP
- $I_{H_1P_a}$, $I_{H_1P_b}$, $I_{H_2P_a}$ and $I_{H_2P_b}$, the cells infected by one type of DIP and one type of helper virus.

Because in our model we assume superinfection exclusion, once a cell has absorbed a DIP, the subsequent absorption of another DIP has no effect. Therefore a cell cannot be $I_{P_aP_b}$. In general, a cell cannot have more than two simultaneous infections, and if a cell has two infections, they must be a DIP infection and a helper infection.
In this model we have four species of cells infected by a helper virus and a DIP, thus now we have four parameters f, one for each of these doubly-infected species. We will write these four parameters as a 2×2 matrix, which we call $\bar{f}$:

$$\bar{f} = \begin{pmatrix} f_a^1 & f_b^1 \\ f_a^2 & f_b^2 \end{pmatrix} \tag{4.1}$$

The questions are: now that the DIP species are two, is there once again a clear loser between the two helper species, like in section 3?
And what about the DIP species? Will one of them go extinct? Or will they coexist?
Finally, do the answers to these questions depend on the matrix $\bar{f}$?

4.1 Won competitions and ongoing competitions between DIP species and between helper species

We start with a deterministic model, with well-mixed viruses and cells, and no flux. This model is similar to that of section 2.1.

4.1.1 The ODEs of the model

The equations of the model are

$$\frac{dB}{dt} = \eta V B \tag{4.2}$$

$$\frac{dI_{H_1}}{dt} = \eta H_1 B - \eta \left(\sum_i P_i \right) I_{H_1} \tag{4.3}$$

$$\frac{dI_{H_2}}{dt} = \eta H_2 B - \eta \left(\sum_i P_i \right) I_{H_2} \tag{4.4}$$

$$\frac{dI_{P_a}}{dt} = \eta P_a B - \eta \left(\sum_i H_i \right) I_{P_a} \tag{4.5}$$

$$\frac{dI_{P_b}}{dt} = \eta P_b B - \eta \left(\sum_i H_i \right) I_{P_b} \tag{4.6}$$

$$\frac{dI_{H_1P_a}}{dt} = \eta P_a I_{H_1} + \eta H_1 I_{P_a} \tag{4.7}$$

$$\frac{dI_{H_1P_b}}{dt} = \eta P_b I_{H_1} + \eta H_1 I_{P_b} \tag{4.8}$$

$$\frac{dI_{H_2P_a}}{dt} = \eta P_a I_{H_2} + \eta H_2 I_{P_a} \tag{4.9}$$

$$\frac{dI_{H_2P_b}}{dt} = \eta P_b I_{H_2} + \eta H_2 I_{P_b} \tag{4.10}$$

$$\frac{dH_1}{dt} = \beta \left[I_{H_1} + \sum_i (1 - f_i^1) I_{H_1P_i} \right] - \eta H_1 T \tag{4.11}$$

$$\frac{dH_2}{dt} = \beta \left[I_{H_2} + \sum_i (1 - f_i^2) I_{H_2P_i} \right] - \eta H_2 T \tag{4.12}$$

$$\frac{dP_a}{dt} = \beta \sum_i f_a^i I_{H_iP_a} - \eta P_a T \tag{4.13}$$

$$\frac{dP_b}{dt} = \beta \sum_i f_b^i I_{H_iP_b} - \eta P_b T \tag{4.14}$$

where

$$V \equiv H_1 + H_2 + P_a + P_b \tag{4.15}$$

and

$$T \equiv B + I_{H_1} + I_{H_2} + I_{P_a} + I_{P_b} + I_{H_1P_a} + I_{H_1P_b} + I_{H_2P_a} + I_{H_2P_b}. \qquad (4.16)$$

We do the rescaling of time and concentrations according to eq. (2.7) and eq. (2.8). The parameters η and β are substituted by η' (eq. (2.11)) and β' (2.12). We drop the $'$ for simplicity.
We start with a system of uninfected cells, free helper viruses of both types and free DIPs of both types: the initial conditions are

- $B = 1$
- $H_1 = H_2 = P_a = P_b = 0.25 \times 10^{-7}$
- all other quantities equal to 0

After choosing the values for the parameter matrix $\bar{f}$, we let the system advance.

4.1.2 Results

This type of system reaches saturation, i.e. all cells are eventually infected by a helper and a DIP. An example of time evolution up until saturation is shown in figure 4.1.

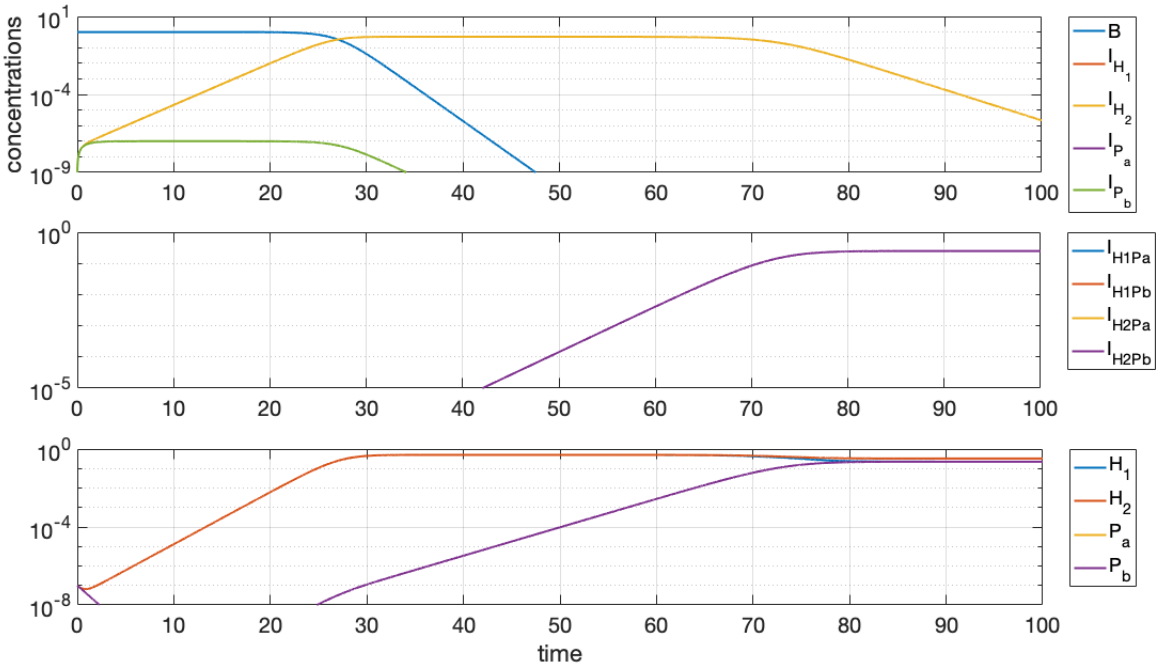


Figure 4.1.: Time evolution of the virus and cell levels up until saturation. The parameters of the matrix $\bar{f}$ are: $f_a^1 = 0.6$, $f_b^1 = 0.5$, $f_a^2 = 0.3$, $f_b^2 = 0.4$.

Once saturation was reached, we took a small sample of the saturated system (1 part in 10^7) and injected it onto a new system made of only uninfected cells, with concentration $B = 1$. We then let this new system advance, until it too reached saturation (figure 4.2).

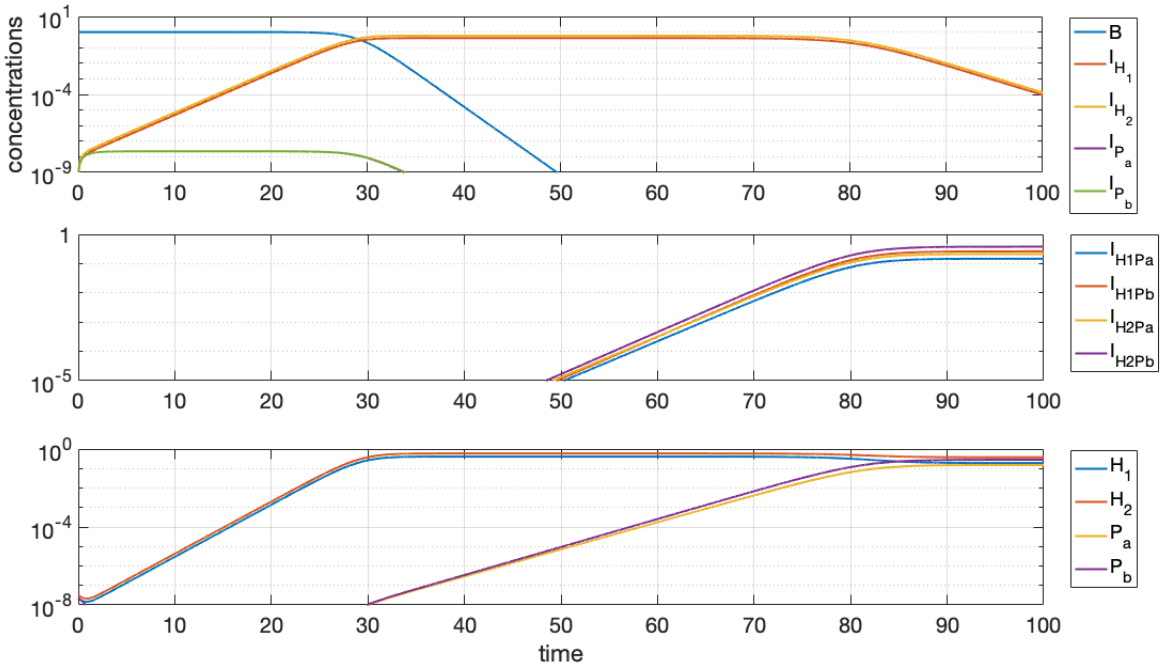


Figure 4.2.: Time evolution of the virus and cell levels in the second system, i.e. the system that received the injection from the original system. Time starts at the moment of injection. This figure is a follow-up to figure 4.1, i.e. a part of the saturated system in figure 4.1 was injected to form the second system. The matrix $\bar{f}$ is the same as in figure 4.1: $f_a^1 = 0.6$, $f_b^1 = 0.5$, $f_a^2 = 0.3$, $f_b^2 = 0.4$.

Next, we took a small sample of the second system and injected it onto a third system, once again made of only uninfected cells with concentration $B = 1$. And so on.

What we noticed was that, for certain choices of $\bar{f}$, the saturation levels of $I_{H_1P_a}$, $I_{H_2P_a}$, $I_{H_1P_b}$ and $I_{H_2P_b}$ would approach specific values more and more with each new injection, and eventually the saturation levels would converge to these values (figure 4.3). However, for other choices of $\bar{f}$, the saturation levels of those four species would not converge but oscillate (figure 4.4).

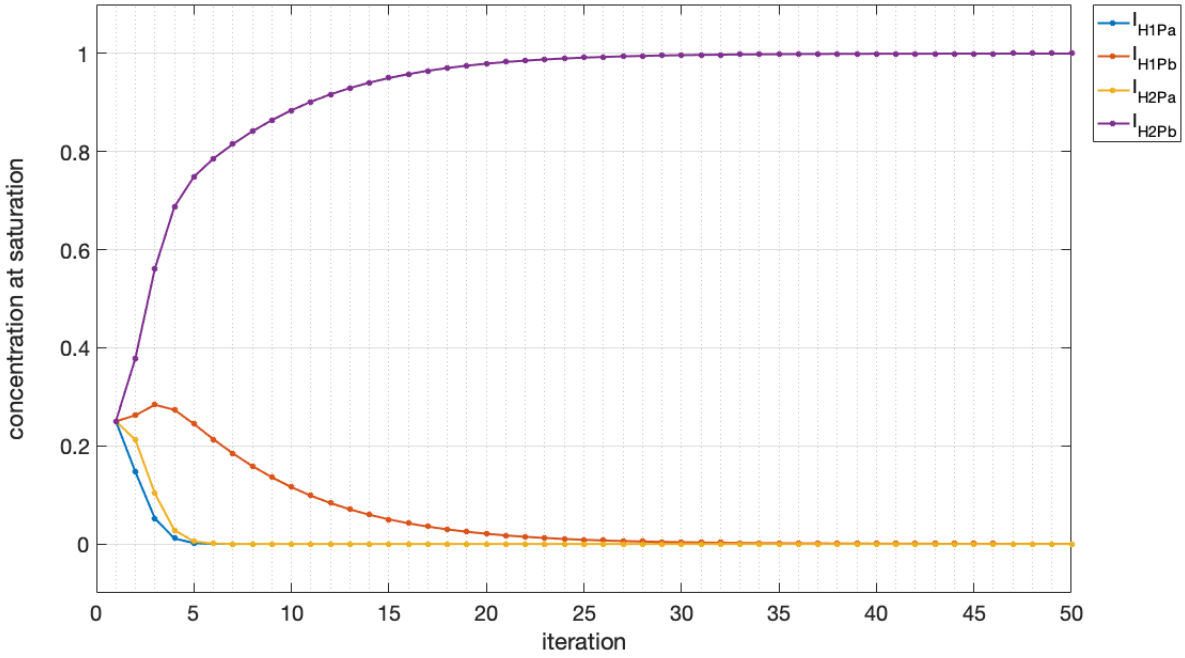


Figure 4.3.: Example of converging saturation levels. The matrix $\bar{f}$ here is: $f_a^1 = 0.6$, $f_b^1 = 0.5$, $f_a^2 = 0.3$, $f_b^2 = 0.4$. On the x-axis is the order of the systems: at $x = 1$ are the saturation levels of the original system; at $x = 2$ are the saturation levels of the second system, i.e. the one that received the first injection; and so on.

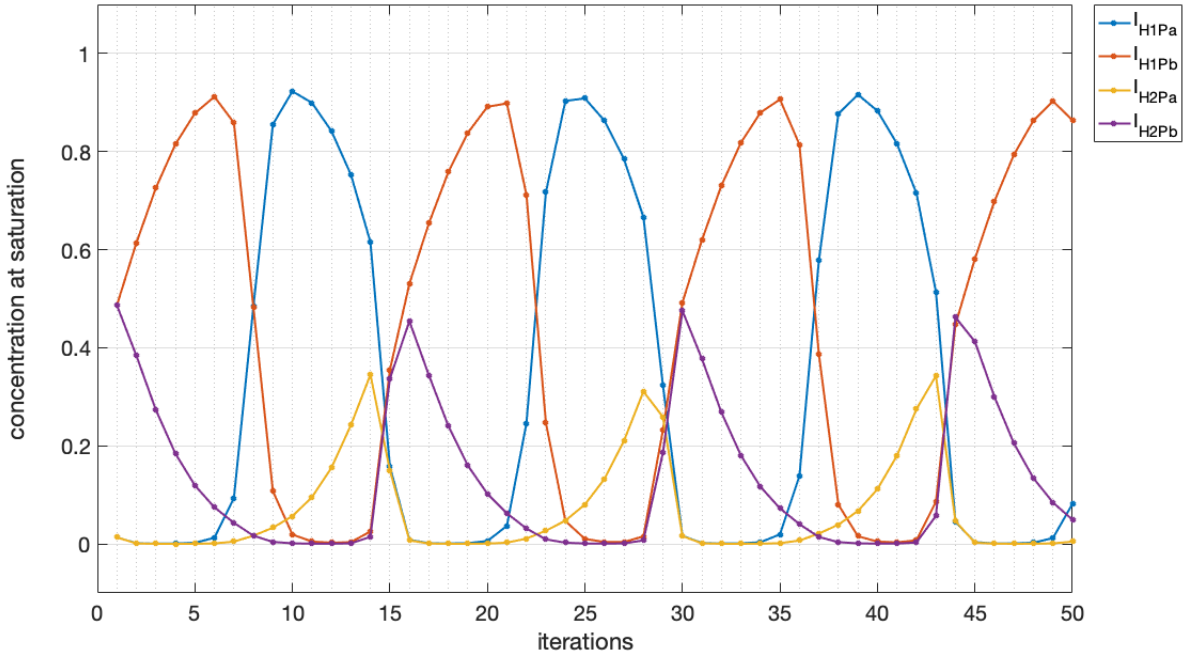


Figure 4.4.: Example of oscillating saturation levels. The matrix $\bar{f}$ here is $f_a^1 = 0.6$, $f_b^1 = 0.5$, $f_a^2 = 0.3$, $f_b^2 = 0.7$. On the x-axis is the order of the systems: at $x = 1$ are the saturation levels of the original system; at $x = 2$ are the saturation levels of the second system, i.e. the one that received the first injection; and so on.

We found that the convergence and oscillation follow this rule:
if the condition

$$\begin{cases} f_i^1 > f_j^1 \\ f_i^1 > f_i^2 \\ \\ f_j^2 > f_j^1 \\ f_j^2 > f_i^2 \quad with \;\; i,j \in \{a,b\},\; i \neq j \end{cases} \tag{4.17}$$

is satisfied, then the saturation levels oscillate; otherwise, they converge. The parameters $\bar{f}$ that give oscillation do so because they cannot determine a winning helper or a winning DIP.

Let us focus on the doubly-infected cells, the I_{HP}-like cells.

- If we compare two such species that make the same helper virus, the species making more DIPs wins.

- If we compare two I_{HP}-like species that make the same DIP, the one making more helpers wins.

We can imagine that there are 4 duels:

1. two duels between I_{HP}-like species making the same helper

   a) $I_{H_1P_a}$ vs. $I_{H_1P_b}$

   b) $I_{H_2P_a}$ vs. $I_{H_2P_b}$

2. and two duels between I_{HP}-like species making the same DIP

   a) $I_{H_1P_a}$ vs. $I_{H_2P_a}$

   b) $I_{H_1P_b}$ vs. $I_{H_2P_b}$

Duels (1a) and (1b) are won by the species with the higher f. Duels (2a) and (2b) are won by the species with the lower f. If in a duel the two species have the same f, then there is a tie.

If two species making the same DIP (e.g. $I_{H_1P_a}$ and $I_{H_2P_a}$) win the duels (1a) and (1b), then we know that the winning DIP is the DIP made by both (P_a). If one of the duels (1a) and (1b) is a tie, the winner of the other duel determines the winning DIP, i.e. the DIP it produces. If both duels (1a) and (1b) are ties, we cannot determine a winning DIP by comparing the I_{HP}-like species that produce the same helpers. However, if we find a winning DIP, we are in a situation like that in section 3: we have two helper species and one DIP species. We compare the two I_{HP}-like species left: the species with the lower f wins, and it determines the winning helper, i.e. the helper it produces. So we have found a winning DIP and a winning helper.

Alternatively, if two species making the same helper virus (e.g. $I_{H_1P_a}$ and $I_{H_1P_b}$) win the duels (2a) and (2b), then the winning helper is the one both species produce (H_1). If one of these two duels is a tie, the winner of the other

duel determines the winning helper, i.e. the helper it produces. If both duels (2a) and (2b) are ties, we cannot determine a winning helper by comparing the I_{HP}-like species that produce the same DIP. However, if we find a winning helper, we are in a situation similar to that in section 3: we have *one helper species* and *two DIP species*. In this case, we compare the two I_{HP}-like species left, and the one with the higher f wins, and it determines the winning helper, i.e. the one it produces. So we have found a winning helper and a winning DIP.

Let us look at figure 4.3. In this case, $f_a^1 > f_a^2$, $f_b^1 > f_b^2$. The winners in the duels (2a) and (2b) are $I_{H_2P_a}$ and $I_{H_2P_b}$, thus the winning helper virus is H_2. Now we just have to compare f_a^2 and f_b^2. The parameter that gives more DIPs determines the winner: $f_b^2 > f_a^2$, therefore P_b is the winner.

Oscillations occur when a different DIP species wins each of the duels (1a) and (1b), and at the same time, a different helper species wins each of the duels (2a) and (2b) (condition (4.17)).

The rules to find a winning DIP species and a winning helper species are in figure 4.5 (in the appendices, in figure C.1, is the complete set of rules, which contemplates the case of a tie between I_{HP}-like species).

METHOD A

• Compare the I_{HP}-like species making the same helper:

I_{H1Pa} vs I_{H1Pb}
I_{H2Pa} vs I_{H2Pb}

I.e., look at the rows of the matrix $\bar{f}$.

$$\bar{f} = \begin{pmatrix} f_a^1 & f_b^1 \\ f_a^2 & f_b^2 \end{pmatrix}$$

In a row, the species with higher f wins.

Example:

$$\bar{f} = \begin{pmatrix} 0.6 & 0.7 \\ 0.5 & 0.2 \end{pmatrix}$$

First row: I_{H1Pb} wins.
Second row: I_{H2Pa} wins.

If the two species that win make the same DIP (same column), this DIP is the winning DIP.
Otherwise, this method does not determine a winning DIP; use method B.

Example:
In the previous example, the winning I_{HP}-like species do not make the same DIP ⇒ this method does not determine a winning DIP.

• Now compare the two winning I_{HP}-like species:
the one with lower f wins;
the helper it makes is the winning helper.

Example:
Imagine that in the previous example I_{H1Pb} and I_{H2Pb} had won.
$f_b^1 > f_b^2$ ⇒ I_{H2Pb} would win ⇒ the winning helper would be H_2.

(a)

METHOD B

• Compare the I_{HP}-like species making the same DIP:

I_{H1Pa} vs I_{H2Pa}	I_{H1Pb} vs I_{H2Pb}

I.e., look at the columns of the matrix $\bar{f}$.

$$\bar{f} = \begin{pmatrix} f_a^1 & f_b^1 \\ f_a^2 & f_b^2 \end{pmatrix}$$

In a column, the species with lower f wins.

Example:

$$\bar{f} = \begin{pmatrix} 0.6 & 0.7 \\ 0.5 & 0.2 \end{pmatrix}$$

First column: I_{H2Pa} wins.
Second column: I_{H2Pb} wins.

If the two species that win make the same helper (same row), this helper is the winning helper.
Otherwise, this method does not determine a winning helper; use method A.

Example:
In the previous example, the winning I_{HP}-like species make the same helper (H_2) ⇒ H_2 is the winning helper.

• Now compare the two winning I_{HP}-like species:
the one with higher f wins;
the DIP it makes is the winning DIP.

Example:
In the previous example, I_{H2Pa} and I_{H2Pb} won.
$f_a^2 > f_b^2$ ⇒ I_{H2Pa} wins ⇒ the winning DIP is P_a.

(b)

Figure 4.5.: Set of rules to find the winning DIP and the winning helper virus. There are two alternative methods. (a) According to method A, one starts by looking for the winning DIP. (b) According to method B, one starts by looking for the winning helper virus.

4.2 Cell-influx and dilution can lead to an endless viral competition

Suppose we change the previous model by preventing saturation with an influx of infected cells and the dilution of cells and viruses. Will there be oscillatory behaviour?

4.2.1 The ODEs of the model

The equations of the model are

$$\frac{dB}{dt} = C - \eta V B - \gamma B \tag{4.18}$$

$$\frac{dI_{H_1}}{dt} = \eta H_1 B - \eta \left(\sum_i P_i \right) I_{H_1} - \gamma I_{H_1} \tag{4.19}$$

$$\frac{dI_{H_2}}{dt} = \eta H_2 B - \eta \left(\sum_i P_i \right) I_{H_2} - \gamma I_{H_2} \tag{4.20}$$

$$\frac{dI_{P_a}}{dt} = \eta P_a B - \eta \left(\sum_i H_i \right) I_{P_a} - \gamma I_{P_a} \tag{4.21}$$

$$\frac{dI_{P_b}}{dt} = \eta P_b B - \eta \left(\sum_i H_i \right) I_{P_b} - \gamma I_{P_b} \tag{4.22}$$

$$\frac{dI_{H_1P_a}}{dt} = \eta P_a I_{H_1} + \eta H_1 I_{P_a} - \gamma I_{H_1P_a} \tag{4.23}$$

$$\frac{dI_{H_1P_b}}{dt} = \eta P_b I_{H_1} + \eta H_1 I_{P_b} - \gamma I_{H_1P_b} \tag{4.24}$$

$$\frac{dI_{H_2P_a}}{dt} = \eta P_a I_{H_2} + \eta H_2 I_{P_a} - \gamma I_{H_2P_a} \tag{4.25}$$

$$\frac{dI_{H_2P_b}}{dt} = \eta P_b I_{H_2} + \eta H_2 I_{P_b} - \gamma I_{H_2P_b} \tag{4.26}$$

$$\frac{dH_1}{dt} = \beta \left[I_{H_1} + \sum_i (1 - f_i^1) I_{H_1P_i} \right] - \eta H_1 T - \gamma I_{H_1} \tag{4.27}$$

$$\frac{dH_2}{dt} = \beta \left[I_{H_2} + \sum_i (1 - f_i^2) I_{H_2P_i} \right] - \eta H_2 T - \gamma I_{H_2} \tag{4.28}$$

$$\frac{dP_a}{dt} = \beta \sum_i f_a^i I_{H_iP_a} - \eta P_a T - \gamma I_{P_a} \tag{4.29}$$

$$\frac{dP_b}{dt} = \beta \sum_i f_b^i I_{H_iP_b} - \eta P_b T - \gamma I_{P_b} \tag{4.30}$$

This is an extension of the model in section 4.1. Here we reintroduce the parameters $\mathcal{C}$ and γ. Once again, we rescale the model according to 2.7 and 2.8. The parameters η, β, $\mathcal{C}$ and γ are substituted by η' (eq. (2.11)), β' (2.12), $\mathcal{C}'$ (2.25), γ' (2.26). We drop the $'$ for simplicity.
Once again the system is initially made of only uninfected cells and free DIPs and helper viruses.

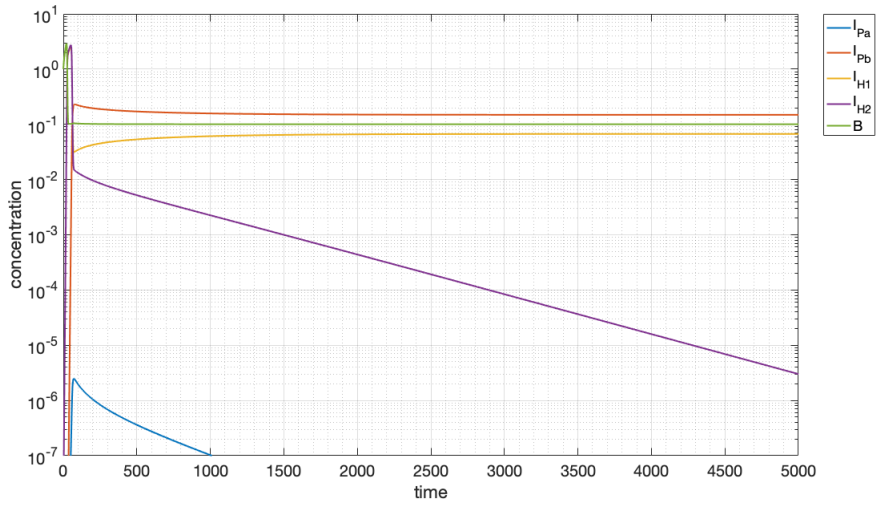
The initial conditions are

- $B = 1$
- $H_1 = 0.229 \times 10^{-7}$
- $H_2 = 0.242 \times 10^{-7}$
- $P_a = 0.247 \times 10^{-7}$
- $P_b = 0.264 \times 10^{-7}$
- all other quantities equal to 0 at $t = 0$

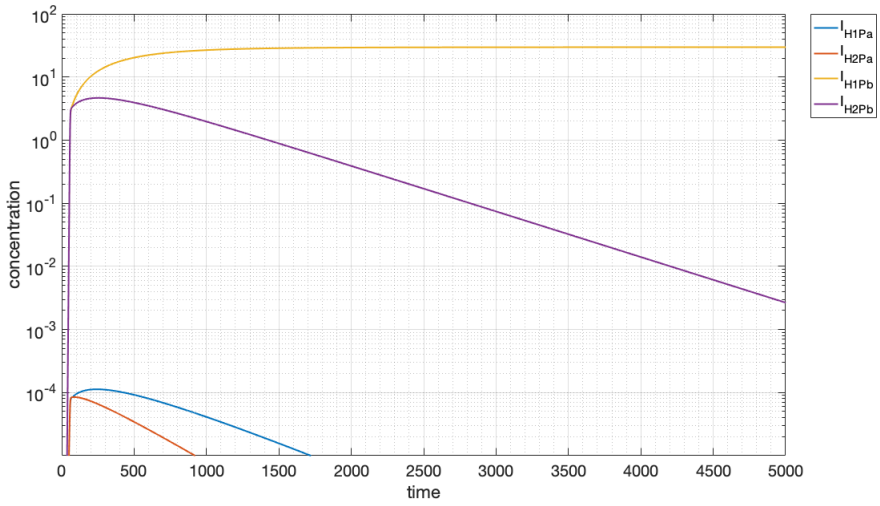
We will explain in a moment why the initial concentrations of free DIPs and helper viruses are all slightly off 0.25×10^{-7} and not equal to each other.

4.2.2 Results

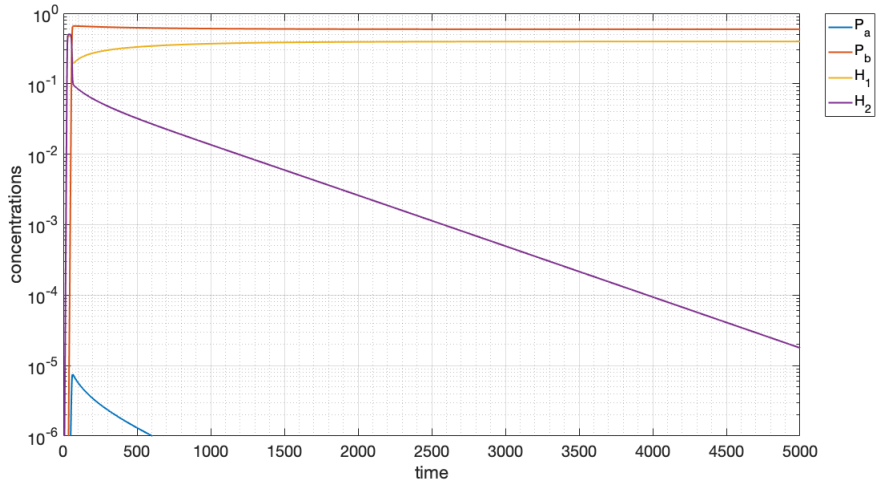
It turns out that the concentrations of cells and viruses in the systems described by this model can oscillate; the levels either oscillate or reach a steady state. In figure 4.7 is an example of oscillating levels and in figure 4.6 is an example of steady-state levels.



(a)

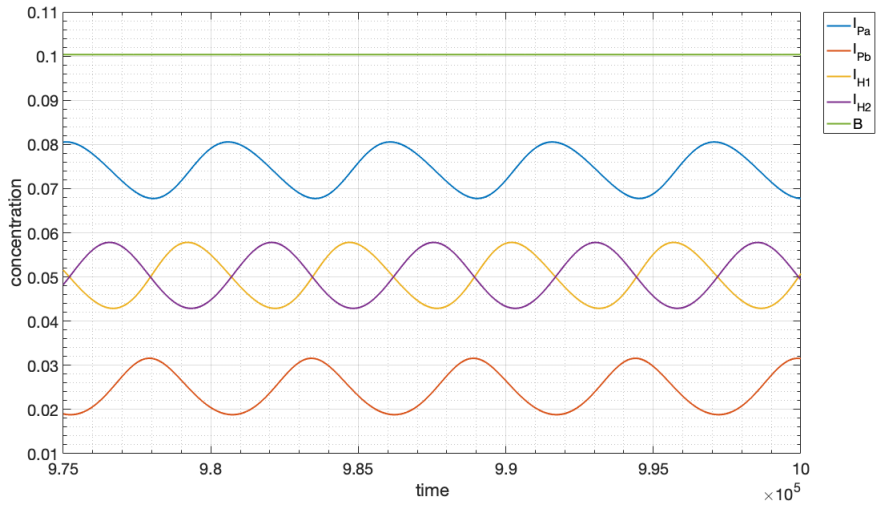


(b)

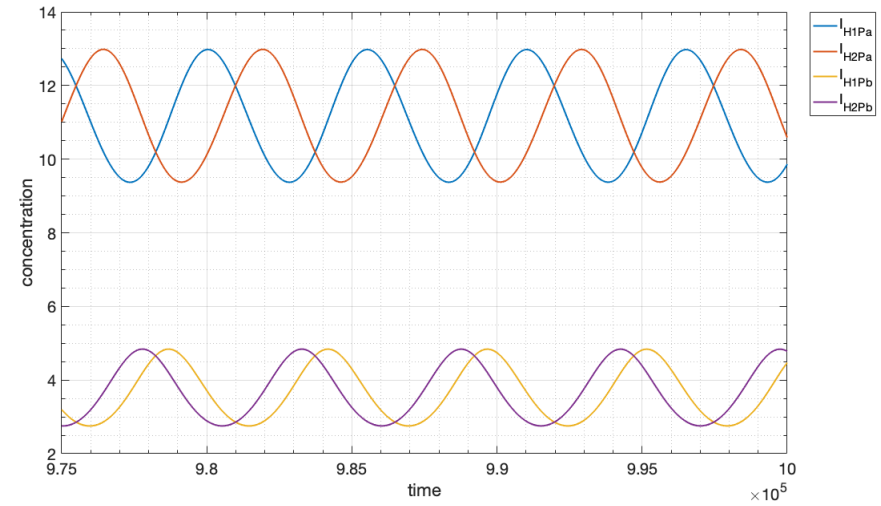


(c)

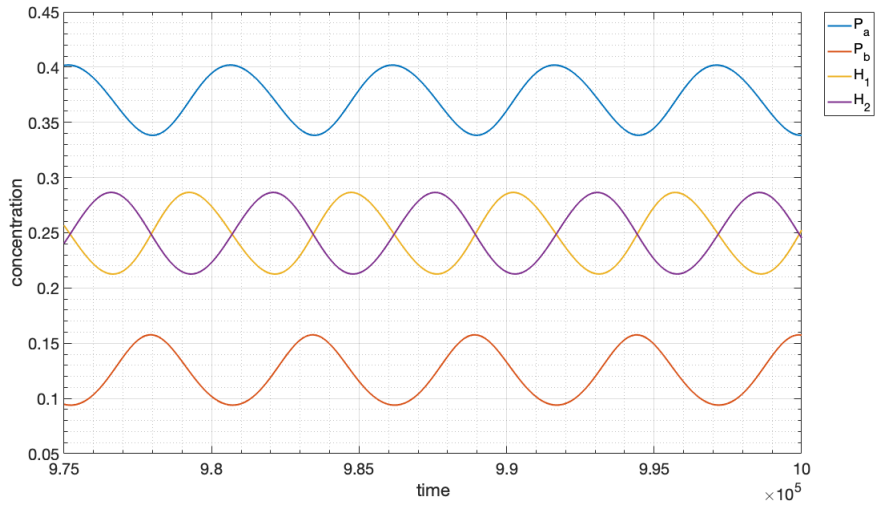
Figure 4.6.: The values of the matrix $\bar{f}$ are: $f_a^1 = 0.6$, $f_b^1 = 0.2$, $f_a^2 = 0.4$, $f_b^2 = 0.8$. The system reaches a steady state. (a) Levels of the uninfected and singly-infected cells. (b) Levels of the doubly-infected cells. (c) Levels of the free helper viruses and DIPs.



(a)



(b)



(c)

Figure 4.7.: The values of the matrix $\bar{f}$ are: $f_a^1 = 0.6$, $f_b^1 = 0.2$, $f_a^2 = 0.4$, $f_b^2 = 0.8$. The levels of cells and viruses oscillate. (a) Levels of the uninfected and singly-infected cells. (b) Levels of the doubly-infected cells. (c) Levels of the free helper viruses and DIPs.

We found that the levels of cells and viruses oscillate if the condition (4.17) is met, i.e. the condition found for the model in section 4.1 that allows for the saturation levels to oscillate. If the condition is not met, the system goes to a steady state.

Now we will explain why we used initial conditions with levels of helper viruses and DIPs similar to each other but not equal. We found that if

$$\begin{cases} f_a^1 = f_b^2 \\ f_a^2 = f_b^1 \\ P_a(t=0) = P_b(t=0) \\ H_1(t=0) = H_2(t=0) \end{cases} \tag{4.31}$$

the levels always reach a steady state; so, even if condition (4.17) is met, the levels do not oscillate but reach a steady state. In the appendices, in figures C.2 and C.3, are the time-evolutions of the levels for a matrix $\bar{f}$ with values $f_a^1 = f_b^2$, $f_a^2 = f_b^1$; however, in the two figures we have the two different types of initial conditions:

- in figure C.2, the initial conditions satisfy $H_1(t=0) \neq H_2(t=0)$, $P_a(t=0) \neq P_b(t=0)$ and the levels oscillate;
- in figure C.3, the initial conditions satisfy $H_1(t=0) = H_2(t=0)$, $P_a(t=0) = P_b(t=0)$ and the levels reach a steady state.

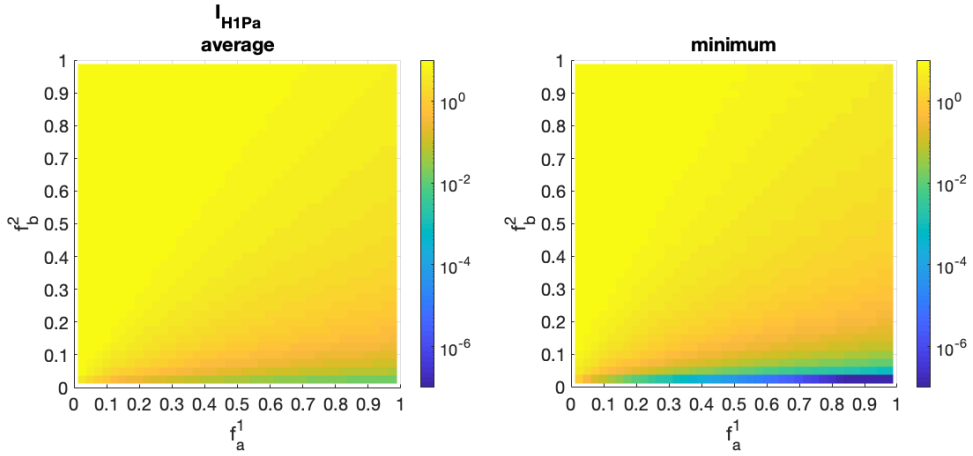
4.2.3 Analysis of the oscillating levels

Now, the system described by this model is a system of infinite volume. However, if the volume is finite, and the DIPs and helpers are few, the extinction of a DIP or helper species is possible. If the levels in the system oscillate, does the oscillation affect the extinction? We will study viral extinction in a system with two DIP species and two helper species in section 4.3. Now we continue to use the current model to study something that may be useful for the extinction study, i.e. the minima and averages of the oscillating levels.

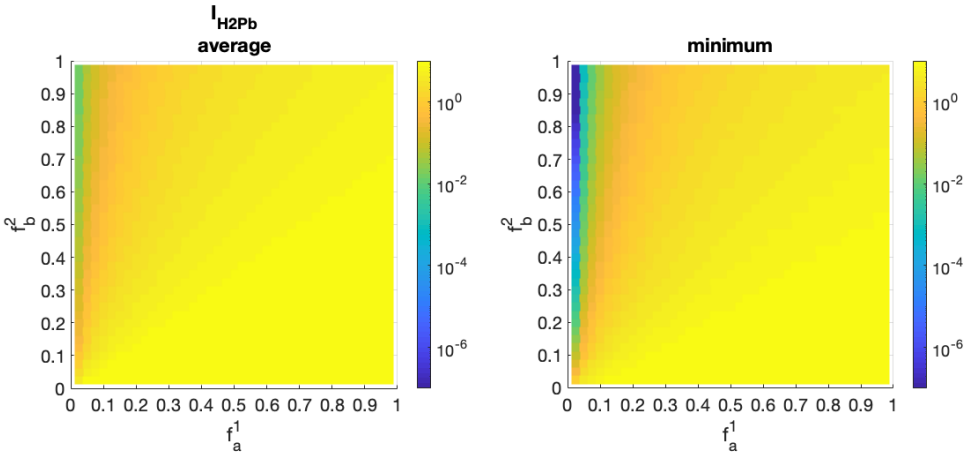
To simplify the system, we set f_b^1 and f_a^2 in the matrix $\bar{f}$ to zero:

$$\bar{f}_* = \begin{pmatrix} f_a^1 & 0 \\ 0 & f_b^2 \end{pmatrix} \tag{4.32}$$

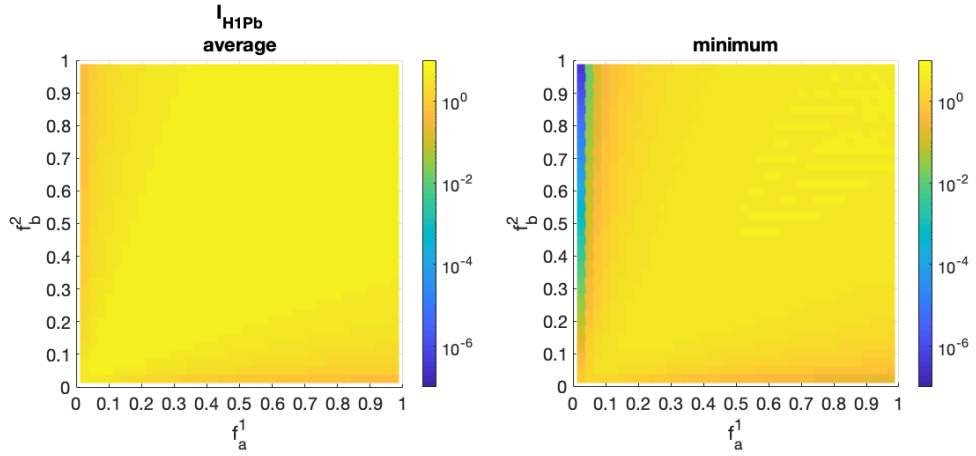
This means that the species $I_{H_1P_b}$ and $I_{H_2P_a}$ will produce only helper viruses and no DIP. We also fixed $f_a^1 \neq 0$ and $f_b^2 \neq 0$. Because the matrix $\bar{f}_*$ satisfies condition (4.17), the levels of the system will oscillate.
We focus on the levels of the doubly-infected cells because they are the dominant species in the system. We searched how the minima and averages of the four doubly-infected cell species depend on the parameters f_a^1 and f_b^2 (figure 4.8).



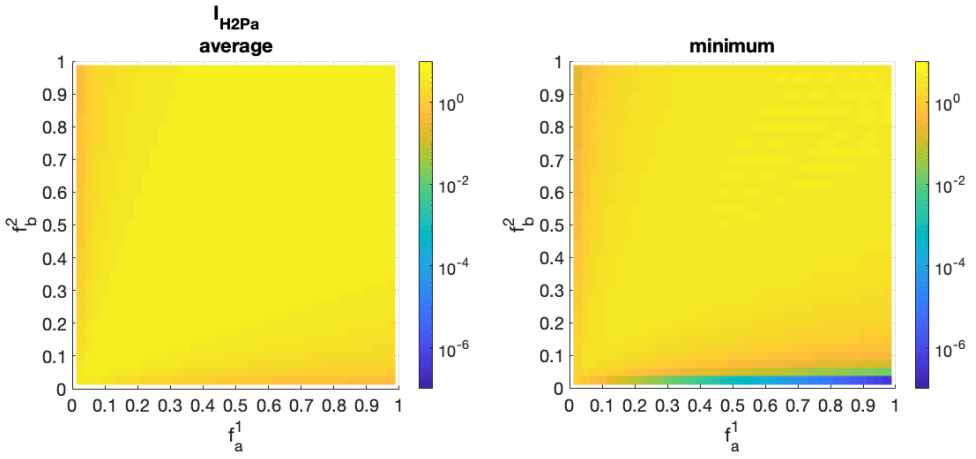
(a)



(b)



(c)



(d)

Figure 4.8.: The averages and minima of the doubly-infected cells vs. (f_a^1, f_b^2): (a) $I_{H_1P_a}$; (b) $I_{H_2P_b}$; (c) $I_{H_1P_b}$; (d) $I_{H_2P_a}$. Notice the symmetry between (a) and (b) and the symmetry between (c) and (d).

The system is symmetrical to the double switch of the indices $1 \longleftrightarrow 2$ and $a \longleftrightarrow b$, so clearly the plots for $I_{H_1P_a}$ are symmetrical to those for $I_{H_2P_b}$, and clearly the plots for $I_{H_1P_b}$ are symmetrical to those for $I_{H_2P_a}$.
It turns out that $f_a^1 \simeq 0$ and $f_b^2 \simeq 0$ give the lowest averages and minima: more precisely

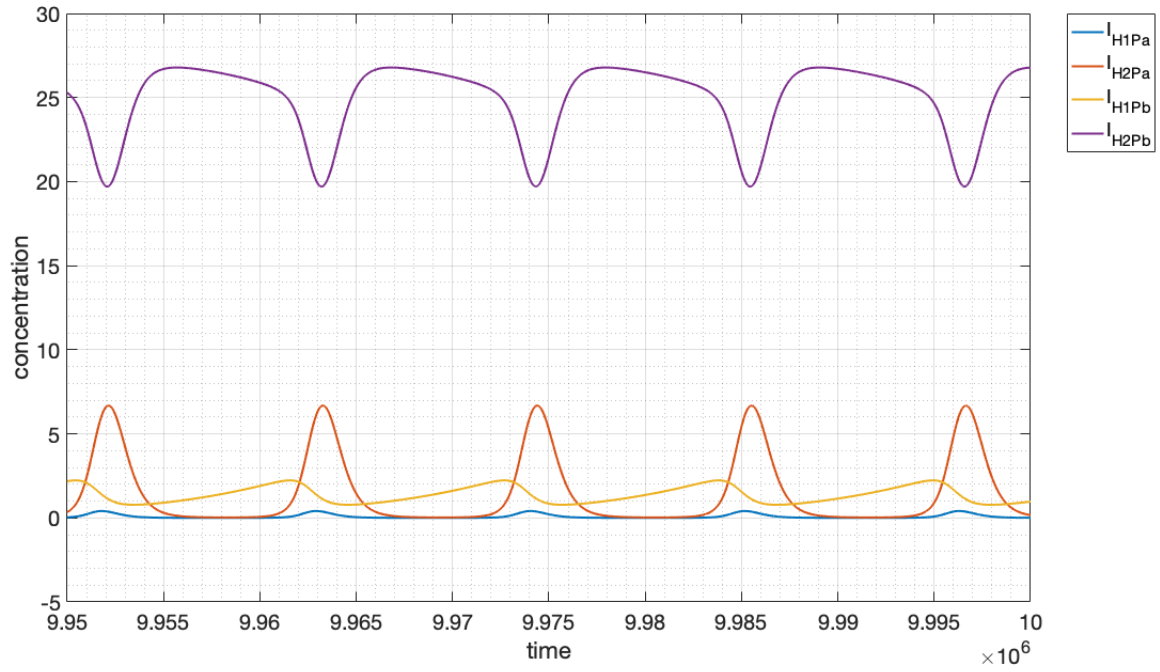
- if $f_a^1 \simeq 0$, the average of $I_{H_2P_b}$ and the minima of $I_{H_1P_b}$ and $I_{H_2P_b}$ are lower than on the rest of the (f_a^1, f_b^2) parameter space;

- if $f_b^2 \simeq 0$, the average of $I_{H_1P_a}$ and the minima of $I_{H_1P_a}$ and $I_{H_2P_a}$ are lower than on the rest of the (f_a^1, f_b^2) parameter space.

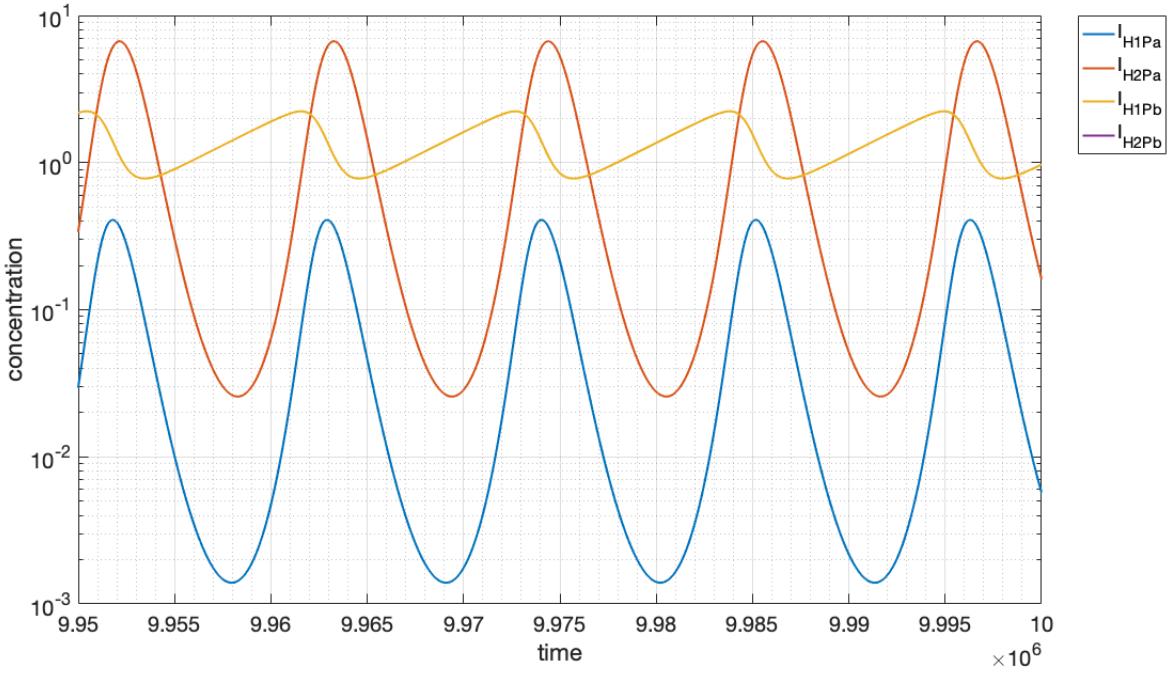
From these results we suspect that in a finite-volume system with few viruses and cells, the extinction of a DIP or helper species would be more affected by the oscillations if $f_a^1 \simeq 0$ or $f_b^2 \simeq 0$. In fact, under these conditions, the dynamics of the system would lead certain doubly-infected cell species to reach lower minima while oscillating than in the rest of the (f_a^1, f_b^2) parameter space; and the lower the numbers of a species, the more the species is going to be affected by demographic noise. Demographic noise could then push certain doubly-infected cell species to 0, and the doubly-infected cells are the only makers of DIPs and the primary makers of helpers (since they are the dominant cell species).
The fact that, if $f_a^1 \simeq 0$ or $f_b^2 \simeq 0$, $I_{H_2P_b}$ and $I_{H_1P_a}$ respectively have low averages, possibly makes these two cell species more likely to be pushed to 0 by demographic noise.

Notice also that $f_a^1 \simeq 0$ would appear to put at risk the DIP species *b*, or at least more at risk than the DIP species *a*: in fact, we saw that if $f_a^1 \simeq 0$, the average and minimum of $I_{H_2P_b}$ are significantly low. Somebody might find this unintuitive: maybe one would think that this condition would be dangerous or more dangerous to DIP *a* since it means that the species $I_{H_1P_a}$ makes mostly helpers of species *1* and scarcely DIPs *a*. (We can make the analogous argument for $f_b^2 \simeq 0$: this condition would appear to put at risk DIP *a*, or at least more at risk than DIP *b*.)

We show an example of oscillations with $f_b^2 \simeq 0$ in figure 4.9, where the average of $I_{H_1P_a}$ and the minima of $I_{H_1P_a}$ and $I_{H_2P_a}$ are particularly low.



(a)



(b)

Figure 4.9.: Oscillating levels of the doubly-infected cells, with the following values for the matrix $\bar{f}_*$: $f_a^1 = 0.9$, $f_b^2 = 0.05$. With $f_b^2 \simeq 0$, the average of $I_{H_1P_a}$ and the minima of $I_{H_1P_a}$ and $I_{H_2P_a}$ are significantly lower than the levels of $I_{H_1P_b}$ and $I_{H_2P_b}$. (a) Linear scale. (b) Logarithmic scale.

4.3 Oscillating dynamics affect the probability of viral extinction

We now want to consider a finite-volume system with low numbers of cells, DIPs and helper viruses, where the DIP species are two, and the helper species are two. We keep in mind the deterministic model in section 4.2 and derive a new, stochastic model similar to that in section 2.3.

4.3.1 The new stochastic model

Our system will not evolve according to some ODEs, but according to the Gillespie algorithm. We now define the variables in the system, i.e. the finite amounts of cells and viruses.

- $\widetilde{P_a}$, $\widetilde{P_b}$: amounts of free DIPs;
- $\widetilde{H_1}$, $\widetilde{H_2}$: amounts of free helper viruses;
- $\widetilde{B}$: amount of uninfected cells;
- $\widetilde{I}_{P_a}$, $\widetilde{I}_{P_b}$: amounts of cells infected only by a DIP;
- $\widetilde{I}_{H_1}$, $\widetilde{I}_{H_2}$: amounts of cells infected only by a helper virus;
- $\widetilde{I}_{H_1P_a}$, $\widetilde{I}_{H_2P_a}$, $\widetilde{I}_{H_1P_b}$, $\widetilde{I}_{H_2P_b}$: amounts of cells infected by both a DIP and a helper virus.

Next, we show the possible events and their Gillespie rates. The rates derive from the terms of the model equations in section 4.2. Note that v is the volume of the system.

- *Introduction of an uninfected cell*

$$\varnothing \xrightarrow{c \cdot v} \widetilde{B} \tag{4.33}$$

- *Absorption of a virus by a cell*

$$\widetilde{P}_{a,b} + \widetilde{B} \xrightarrow{\eta/v \cdot \widetilde{P}_{a,b} \cdot \widetilde{B}} \widetilde{I}_{P_{a,b}} \tag{4.34}$$

$$\widetilde{H}_{1,2} + \widetilde{B} \xrightarrow{\eta/v \cdot \widetilde{H}_{1,2} \cdot \widetilde{B}} \widetilde{I}_{H_{1,2}} \tag{4.35}$$

$$\widetilde{P}_{a,b} + \widetilde{I}_{P_{a,b}} \xrightarrow{\eta/v \cdot \widetilde{P}_{a,b} \cdot \widetilde{I}_{P_{a,b}}} \widetilde{I}_{P_{a,b}} \tag{4.36}$$

$$\widetilde{H}_{1,2} + \widetilde{I}_{P_{a,b}} \xrightarrow{\eta/v \cdot \widetilde{H}_{1,2} \cdot \widetilde{I}_{P_{a,b}}} \widetilde{I}_{H_{1,2}P_{a,b}} \tag{4.37}$$

$$\widetilde{P}_{a,b} + \widetilde{I}_{H_{1,2}} \xrightarrow{\eta/v \cdot \widetilde{P}_{a,b} \cdot \widetilde{I}_{H_{1,2}}} \widetilde{I}_{H_{1,2}P_{a,b}} \tag{4.38}$$

$$\widetilde{H}_{1,2} + \widetilde{I}_{H_{1,2}} \xrightarrow{\eta/v \cdot \widetilde{H}_{1,2} \cdot \widetilde{I}_{H_{1,2}}} \widetilde{I}_{H_{1,2}} \tag{4.39}$$

$$\widetilde{H}_{1,2} + \widetilde{I}_{H_{1,2}P_{a,b}} \xrightarrow{\eta/v \cdot \widetilde{H}_{1,2} \cdot \widetilde{I}_{H_{1,2}P_{a,b}}} \widetilde{I}_{H_{1,2}P_{a,b}} \tag{4.40}$$

$$\widetilde{P}_{a,b} + \widetilde{I}_{H_{1,2}P_{a,b}} \xrightarrow{\eta/v \cdot \widetilde{P}_{a,b} \cdot \widetilde{I}_{H_{1,2}P_{a,b}}} \widetilde{I}_{H_{1,2}P_{a,b}} \tag{4.41}$$

- *Production of a virus*

$$\widetilde{I}_{H_{1,2}P_{a,b}} \xrightarrow{f \cdot \beta \cdot \widetilde{I}_{H_{1,2}P_{a,b}}} \widetilde{I}_{H_{1,2}P_{a,b}} + \widetilde{P}_{a,b} \tag{4.42}$$

$$\widetilde{I}_{H_{1,2}P_{a,b}} \xrightarrow{\beta \cdot \widetilde{I}_{H_{1,2}} + (1-f)\beta \cdot \widetilde{I}_{H_{1,2}P_{a,b}}} \widetilde{I}_{H_{1,2}P_{a,b}} + \widetilde{P}_{a,b} \tag{4.43}$$

- *Dilution*

$$\widetilde{P}_{a,b} \xrightarrow{\gamma \cdot \widetilde{P}_{a,b}} \varnothing \tag{4.44}$$

$$\widetilde{H}_{1,2} \xrightarrow{\gamma \cdot \widetilde{H}_{1,2}} \varnothing \tag{4.45}$$

$$\widetilde{B} \xrightarrow{\gamma \cdot \widetilde{B}} \varnothing \tag{4.46}$$

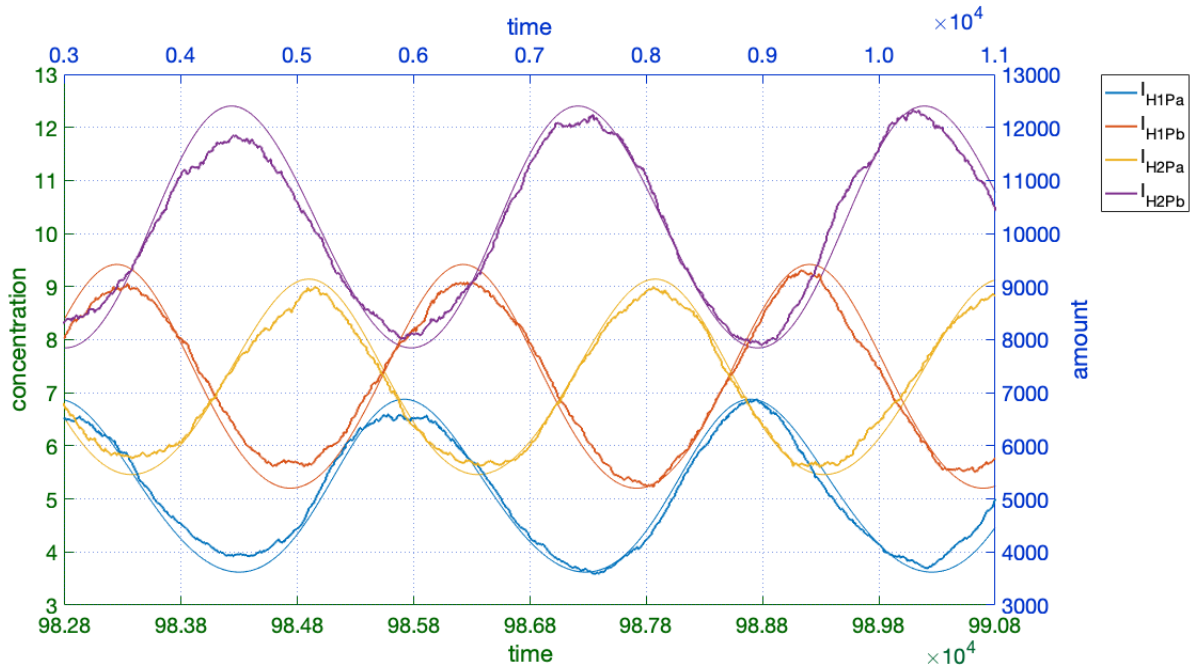
$$\widetilde{I}_{P_{a,b}} \xrightarrow{\gamma \cdot \widetilde{I}_{P_{a,b}}} \varnothing \tag{4.47}$$

$$\widetilde{I}_{H_{1,2}} \xrightarrow{\gamma \cdot \widetilde{I}_{H_{1,2}}} \varnothing \tag{4.48}$$

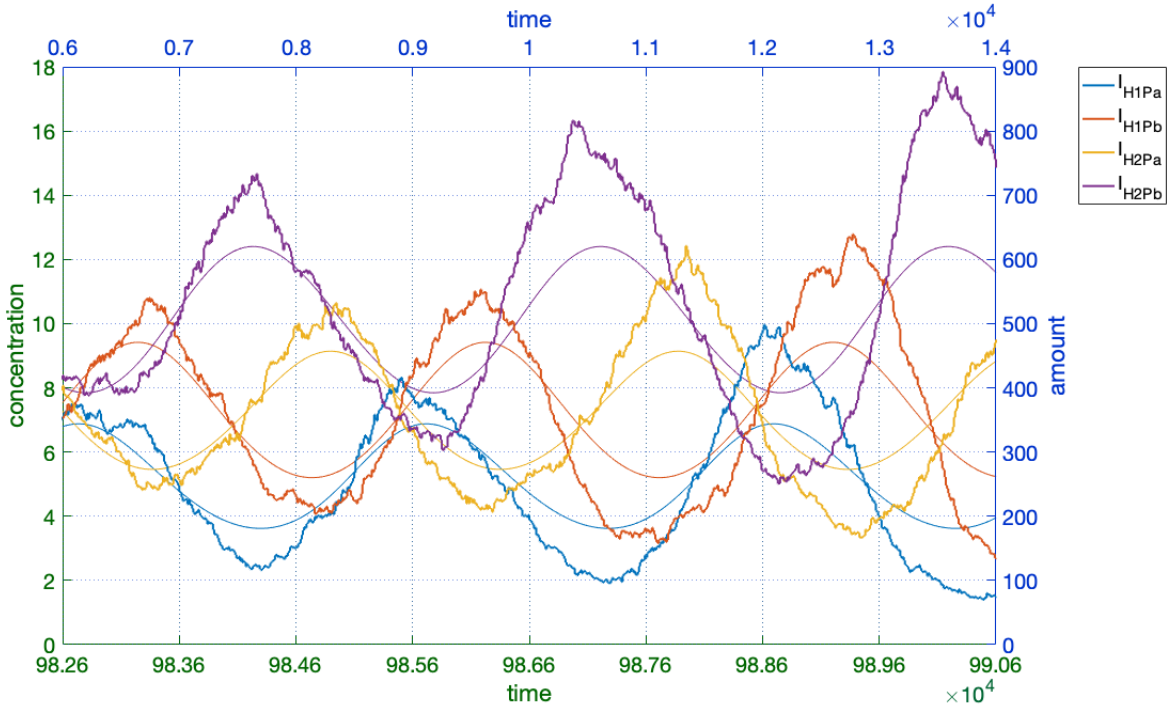
$$\widetilde{I}_{H_{1,2}P_{a,b}} \xrightarrow{\gamma \cdot \widetilde{I}_{H_{1,2}P_{a,b}}} \varnothing \tag{4.49}$$

We will use the matrix $\bar{f}_*$ (eq. 4.32), i.e. we fix $f_b^1 = f_a^2 = 0$.

Now let us discuss the initial conditions. We made this model to study how the extinction of DIP species or helper species is affected by the oscillations seen in section 4.2. So we want the current, stochastic model to reproduce the oscillations found in section 4.2. Thus, for each choice of $\bar{f}_*$, the initial conditions will be the product of v times the concentrations from the deterministic model at a chosen time.
However, this model has a big difference compared to the model in section 4.2: because this is a finite-volume system, there will be demographic noise. Moreover, because demographic noise disturbs small systems more significantly, the smaller the volume v, the more influential is the demographic noise, as we can see in the example of figure 4.10.



(a)



(b)

Figure 4.10.: Comparison between the levels of doubly-infected cells in two systems with different volumes. We chose the doubly-infected cells as they are the dominant species. In both systems, the values of the matrix $\bar{f}_*$ are: $f_a^1 = 0.7$, $f_b^2 = 0.5$. The time-evolution of the amounts in the finite-volume system overlays the time-evolution of the concentrations in the infinite-volume system, i.e. the system described by the model in section 4.2. (a) The volume of this system is $v = 1000$. The time evolution is similar to that of the infinite-volume system. (b) The volume of this system is $v = 50$. In this smaller system, the demographic stochasticity is more influential, and the finite-volume levels deviate more from the infinite-volume levels.

4.3.2 Study of the first extinction

The first thing we measure is which DIP/helper virus goes extinct first. We measure this for certain values of the matrix $\bar{f}_*$: we fix $f_b^2 = 0.5$, and f_a^1 will be scanned according to

$$f_a^1 \in \{0.1,\ 0.2,\ 0.3,\ 0.4,\ 0.5,\ 0.6,\ 0.7,\ 0.8,\ 0.9\} \tag{4.50}$$

Now we want to be more detailed about the initial conditions. We kept in mind that the doubly-infected cells (the I_{HP}-like cells) are the dominant species. We wanted to use initial conditions that would not put the I_{HP}-like species with the lowest minimum at a disadvantage. Thus we chose a time when this I_{HP} species has a maximum, and we used the concentrations (from the model in section 4.2) of virus and cells at this time.
The species with the lowest minimum is

- $I_{H_1P_b}$ for $f_a^1 < 0.5$;
- $I_{H_1P_a}$ for $f_a^1 > 0.5$;
- for $f_a^1 = 0.5$, the four I_{HP}-like species have the same minimum.

Because we want to see extinction, we chose to use the volume $v = 10$. This volume is even smaller than that in figure 4.10b, thus:

1. the levels will be even lower;
2. the demographic stochasticity will be even more influential.

These two aspects will lead to more likely extinctions.

In table C.1 in the appendices we show the resulting initial conditions for each value of f_a^1. We remind the reader that they correspond to the product of v times the concentrations just mentioned, i.e. the concentrations (from the model in section 4.2) when the I_{HP} species with the lowest minimum has a peak. The product is rounded to the closest integer.

For each value of f_a^1 we ran 2000 simulations according to the model until either a DIP species or helper species went extinct. In figure 4.11 we show how frequently each of these species went extinct first for each f_a^1.

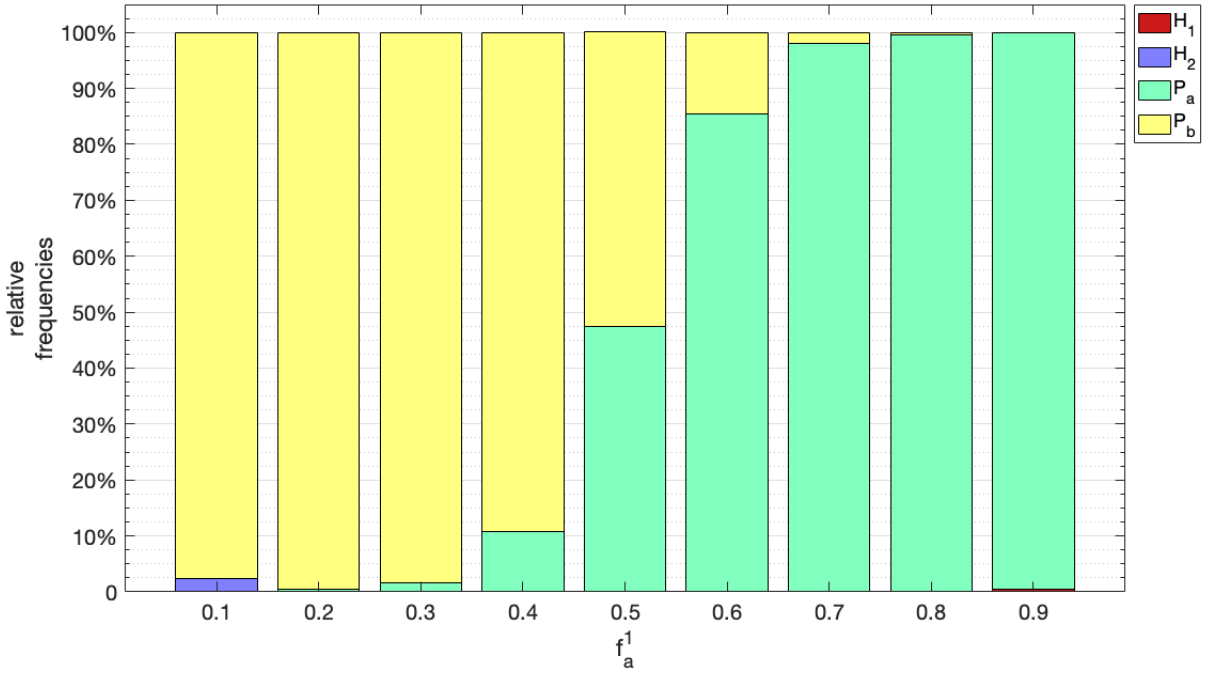


Figure 4.11.: Relative frequencies of the first viral species to disappear from the system. For each f_a^1, 2000 simulations were run.

These simulations confirmed the suspicion mentioned at the end of section 4.2, i.e. $f_a^1 \simeq 0$ puts the DIP species *b* at a risk, not the DIP species *a*. For $f_a^1 < 0.5$, DIP *b* was the first to go extinct, and for $f_a^1 > 0.5$ DIP *a* was the first to go extinct; instead, for $f_a^1 = 0.5$ the two frequencies are $\sim 50\%$. Also notice that the extinction frequencies of the two DIP species are symmetrical.
Another thing to notice is that the helper species went extinct first only with the extremal values of f_a^1, and even then they went extinct much less frequently than the DIP species.

4.3.3 Results

Now we want to see the effect of the oscillatory dynamics seen in section 4.2 on the time of extinction of a DIP or helper species. It is reasonable to think that whenever the amount of one of the four I_{HP}-like species is at a trough of its oscillation, it has a higher chance to reach zero due to demographic stochasticity than at any other moment of its oscillation. The I_{HP}-like cells are the dominant components in the system, and if one of them reaches zero, there may be a disruption of the system. This disruption may lead to the extinction of a DIP or helper species.
We made a precise choice of the matrix $\bar{f}_*$: we chose $f_a^1 = 0.1$ and $f_b^2 = 0.5$. With this choice of parameters, it is the DIP species *b* to go extinct most frequently. We will focus on the time of extinction of this species.
We chose to consider three different volumes: $v = 10$, $v = 50$, $v = 100$. The value of the volume may affect the extinction time of DIP species *b*.
As initial conditions, once again, we used the product of v times the concentrations when the I_{HP}-like species with the lowest minimum is at its maximum. This product is rounded to the nearest integer. (With our choice of $\bar{f}_*$, the I_{HP}-like species with the lowest minimum is $I_{H_1P_b}$.) In table C.2 in the appendices are the initial conditions for the three different values of v.

For each value of v, we simulated the system 40000 times up until a DIP or helper species went extinct.

DIP species *b* was indeed the first viral species to go extinct most of the times for all three values of v (figure 4.12). The helper species *2* went extinct first sometimes only with the smallest volume, $v = 10$. For the larger volumes, every single time it was DIP species *b* to go extinct first.

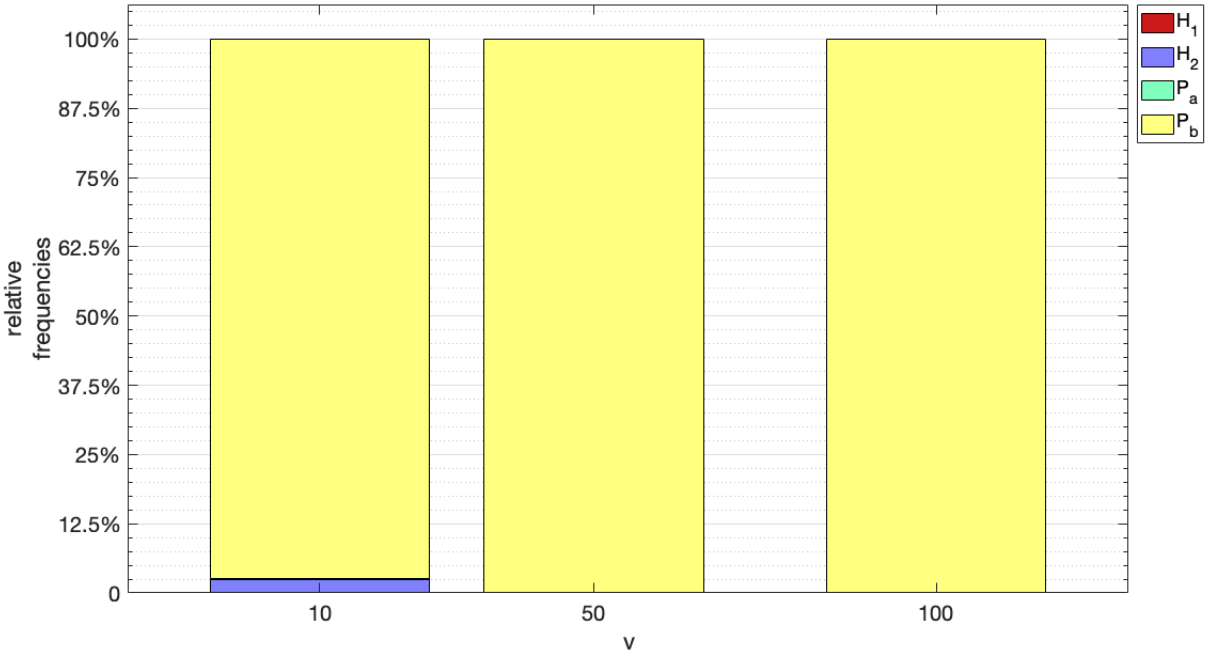


Figure 4.12.: Relative frequencies of the first viral species to go extinct for different values of v. The number of simulations for each v is 40000.

Now we will look at the distribution of the extinction time of DIP species *b* for the three different v. We call this time T_{ext}. In figure 4.13 are the distributions of T_{ext} for the three values of v.

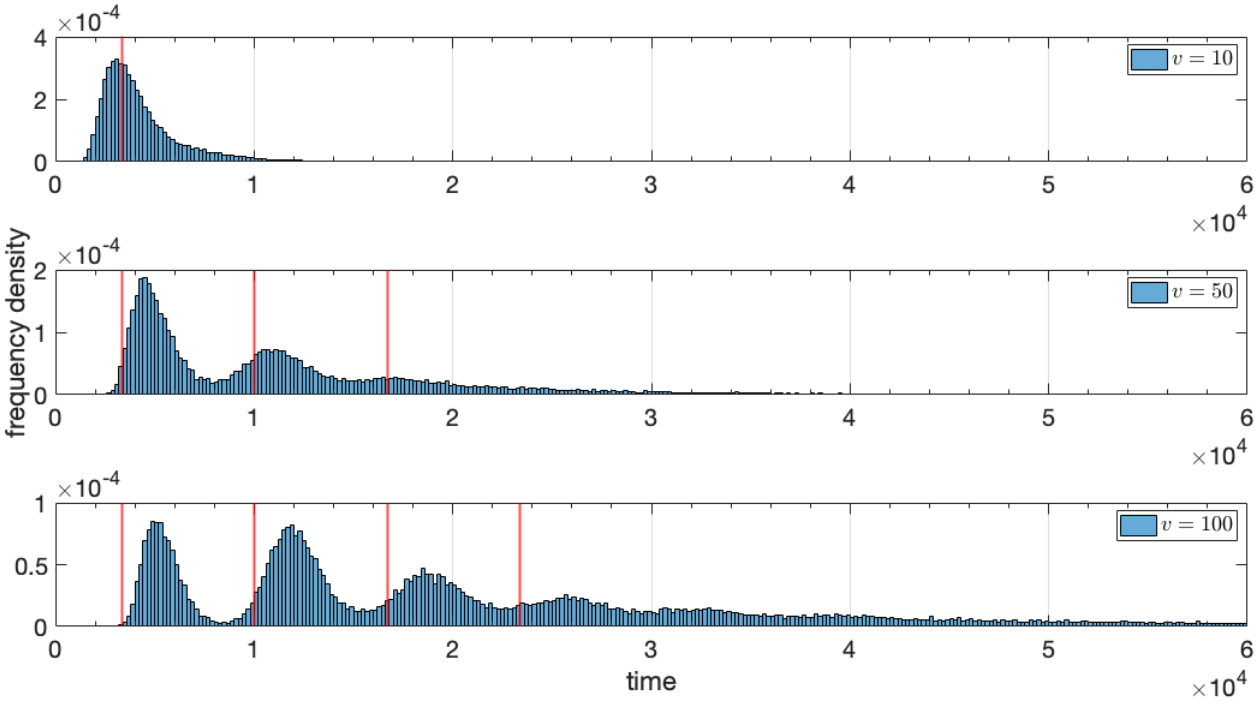


Figure 4.13.: The distribution of T_{ext}, i.e. the time of extinction of the DIP species b, for the three values of v.

For the lowest volume, $v = 10$, the distribution of T_{ext} has only one peak. The larger the volume, the more peaks there are.

We can see one more thing: the peak for $v = 10$ is not at a random time, but instead it coincides with half a period of the oscillations in the infinite system: the period of that system for $f_a^1 = 0.1$ and $f_b^2 = 0.5$, which we call ΔT_{osc}, was $T_{osc} \simeq 6.68 \times 10^3$ (found by using the model in section 4.2). We said that the current simulations started at a peak of $I_{H_1P_b}$. Thus the extinction of DIP b tended to happen at the trough of $I_{H_1P_b}$.

For the two larger volumes, it appears the first peak of the T_{ext} distribution is delayed after the trough of $I_{H_1P_b}$. Additionally, the delay for $v = 100$ appears longer than the delay for $v = 50$. However, it looks like the peaks appear with a specific frequency. As we can see in figures 4.14 and , the peaks appear at regular intervals which are equal to ΔT_{osc}.

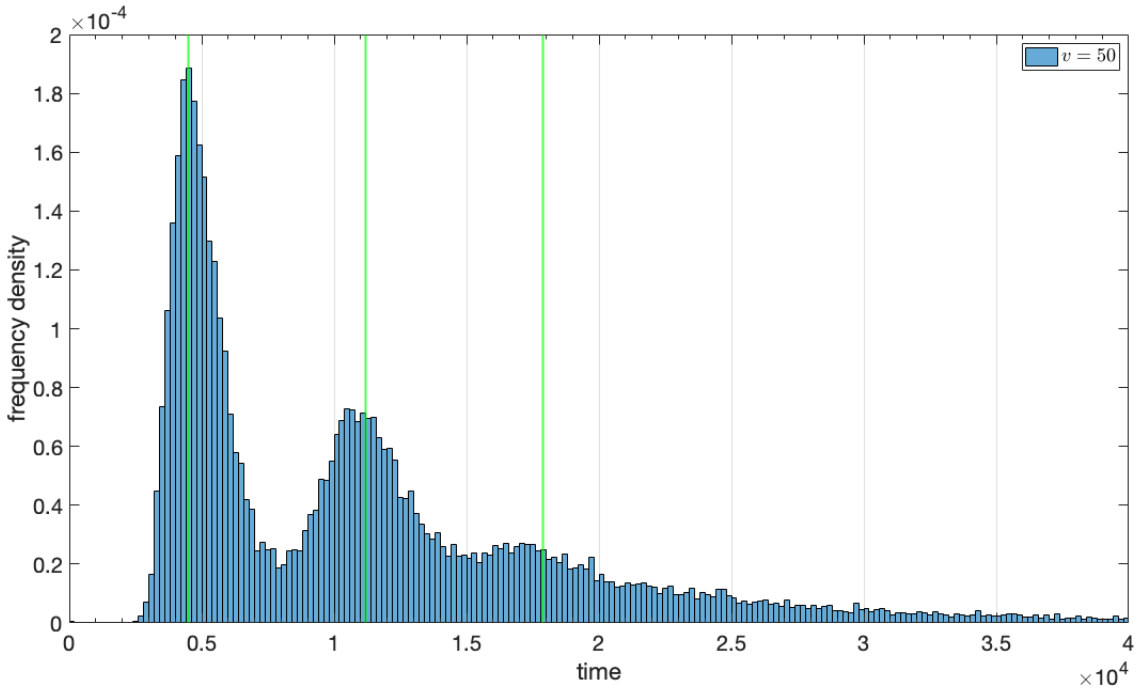


Figure 4.14.: Distribution of T_{ext} for $v = 50$. If we define T_f as the centre of the modal bin in the first extinction peak, the green vertical lines are placed in $T_f + k \cdot \Delta T_{osc}$, with $k \in \{0, 1, 2\}$. The following peaks approximately match the green lines. Thus the peaks of extinction have approximately the same frequency as the oscillations in the deterministic system (section 4.2).

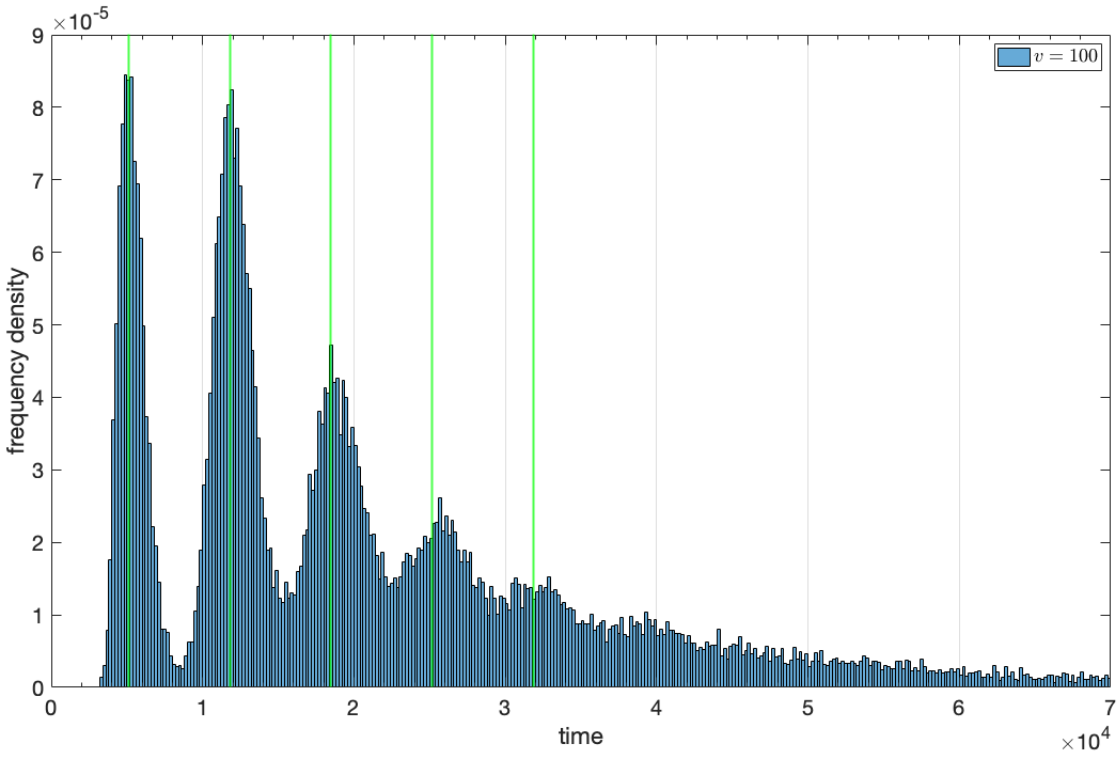


Figure 4.15.: Distribution of T_{ext} for $v = 100$. If we define T_f as the centre of the modal bin in the first extinction peak, the green vertical lines are placed in $T_f + k \cdot \Delta T_{osc}$, with $k \in \{0, 1, 2, 3, 4\}$. The following peaks approximately match the green lines. Thus the peaks of extinction have approximately the same frequency as the oscillations in the deterministic system (section 4.2).

Thus, the larger the volume v, the longer the delay of the extinction events of DIP species b after each trough of $I_{H_1P_b}$. However, the peaks of the extinction events occur with a specific frequency (and thus with the same delay after each trough of $I_{H_1P_b}$).

5 One DIP species interfering with one helper-virus species in space

5.1 The DIP propagation does not halt the helper-virus propagation

So far, we have used models of well-mixed systems to study the interplay of DIPs and helpers and the extinction of viruses. Now we want to move on and study something different: the spreading of a DIP species and a helper species in space.

5.1.1 The lattice model

We now introduce a 2D square lattice where each site is a cell, and each cell can be infected by a DIP and a helper virus. We will not treat the viruses as particles like in the previous models; instead, the viruses will only be apparent in the infected states of the cells.
Each cell can be in one of four different states.

- state B: the cell is not infected
- state I_P: the cell is infected by the DIP only
- state I_H: the cell is infected by the helper only
- state I_{HP}: the cell is infected by both the DIP and the helper

This is an agent-based model, where the agents are the cells. If a cell is I_H or I_{HP}, it can infect adjacent cells. Due to time constraints, we assume that the I_{HP} cells only release DIP progeny, i.e. that their parameter f is $f = 1$. In this model, this is the worst possible value of f for the spreading of the helper species: once a cell becomes I_{HP}, this state is final, and now this cell only produces DIPs.
The cells can infect other cells in the following ways.

- an I_H cell can turn a B neighbour into I_H
- an I_H cell can turn an I_P neighbour into I_{HP}
- an I_{HP} cell can turn a B neighbour into I_P
- an I_{HP} cell can turn a I_H neighbour into I_{HP}

The algorithm that regulates the progression of the system is the following:

1. We start at time $t = 0$.
2. We randomly pick a cell (which we call cell *1*). We randomly select one neighbour (cell *2*). If cell *1* can infect cell *2*, the infection occurs. Then we pick another random cell and another random neighbour; we check if the new cell *1* can infect the new cell *2*, and so on until we have picked N pairs of cells, where N is the number of sites in the lattice. Then, we increase the time t by 1 unit.
3. Repeat the previous step.

We used a large square lattice with side $L = 800$ and thus $N = L \times L = 640000$ sites. The lattice has open boundary conditions.

The initial conditions we used are meant to represent a glass completely covered in cells; we imagine that we deposit a drop containing DIPs and helper viruses onto the cells, and rapidly tilt the glass so that the viruses can infect a straight line of cells from side to side of the glass, covering 800 sites (cells). The 800 cells that become covered by the drop become either I_P or I_H and essentially divide the glass into two halves.

We imagine that the viruses in the drop are $\sim 1.5\%$ helpers and the rest DIPs. We made this choice to show the ease of DIPs to spread even with few helpers.

5.1.2 Results

Now the system is ready to advance. The advancement of such system is in figure 5.1.
Two fronts develop: a leading front of I_H state followed by a front of I_{HP} state. In other words, there is a leading front of helper infection followed by a front of DIP infection. We remind the reader that $f = 1$: because there is a leading front of helper infection with $f = 1$, we expect that there would be this leading front of helper infection also with $f < 1$.
The DIP infection does not manage to overtake and stop the helper infection. Occasionally, the DIP-infection front manages to catch up and wrap some uninfected B cells that become I_P surrounded by I_{HP} cells (figure 5.4a). Sometimes, the DIP-infection front manages to wrap enough B cells, that inside the wrapped I_P cells, there may also be some uninfected B cells.

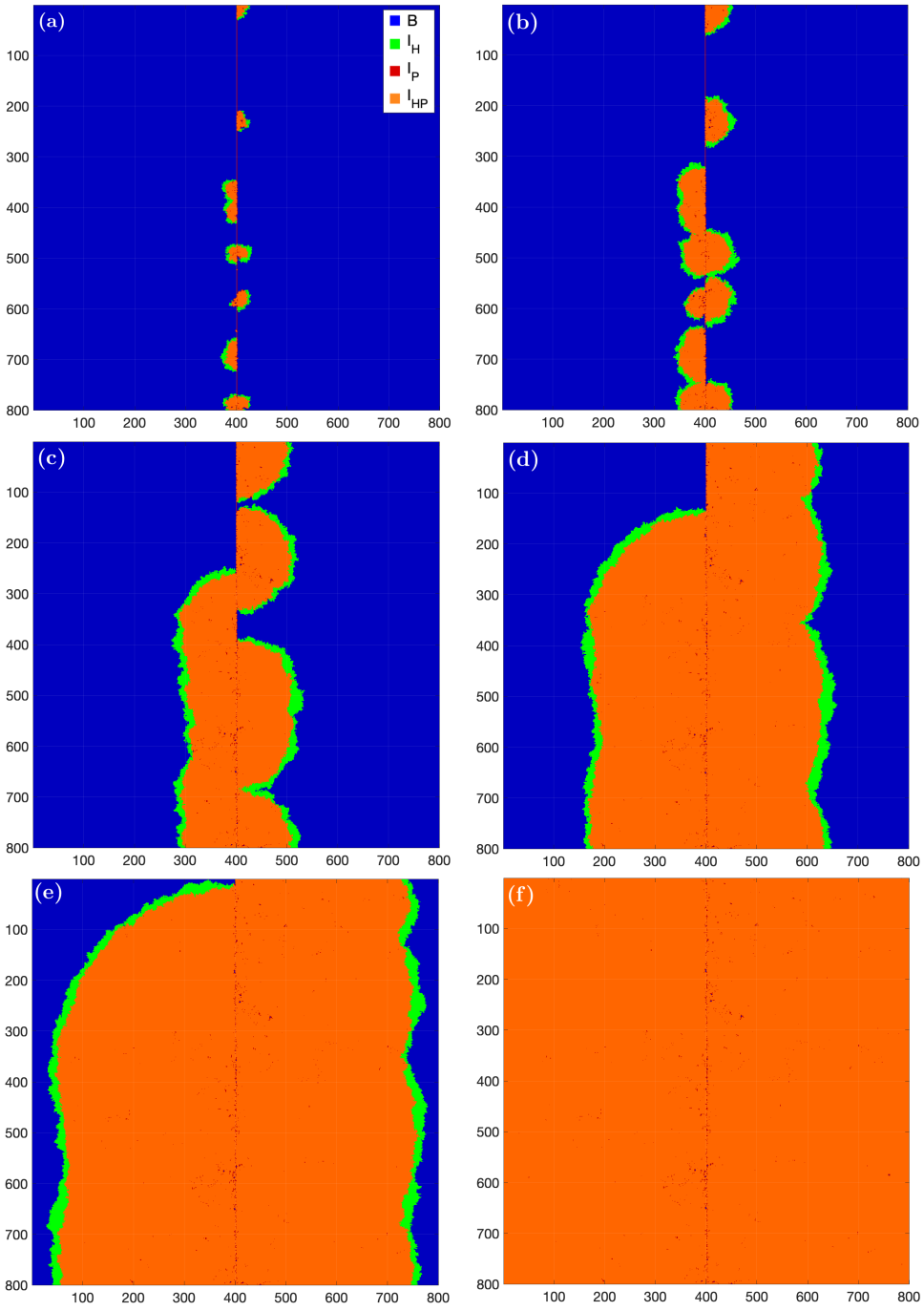


Figure 5.1.: Snapshots of the time-evolution of the system. (a) $t = 50$. (b) $t = 100$. (c) $t = 200$. (d) $t = 400$. (e) $t = 600$. (f) $t = 950$. Notice the front of I_H state followed by the front of I_{HP} state. The cells are not moving; the infections are propagating.

5.2 A partially-empty lattice does not change the infection dynamics

We investigated whether a lattice with empty regions (i.e. regions without cells) could allow the DIP-infection front to stop the helper-infection front.

5.2.1 Description of the new lattices

We made two new lattices: one with circular empty regions, and one with square empty regions. We made this choice to see whether different curvatures of the empty regions could affect the system differently.
For these two lattices, we made the following choices:

1. if the first lattice has a circular empty region centered in site (i, j), the second lattice has a square empty region centered in site (i, j);

2. if the circular empty region with centre (i, j) (in the first lattice) has a diameter equal to n sites, the square empty region with centre (i, j) (in the second lattice) has a side equal to n sites;

3. in both cases we used the same initial conditions used for the lattice in section 5.1: i.e., the initial I_P and I_H cells are in the same positions;

4. in both cases we used the same seed used for the lattice in section 5.1 for the random number generator.

We made these four choices to see the differences in time-evolution due to the different lattices.

5.2.2 Results

The time-evolutions of the systems with circular and square empty regions are in figures 5.2 and D.1 (in the appendices) respectively. Also with empty regions, a first front of I_H develops, and is followed by a front of I_{HP}. Moreover, also

with empty regions, the DIP infection does not manage to stop the helper infection.

Just like without empty regions, the DIP-infection front can catch up to the helper-infection front and wrap some uninfected B cells, turn them into I_P cells and these I_P cells end up surrounded by I_{HP} cells. Moreover, just like without empty regions, sometimes the DIP-infection front can wrap so many uninfected B cells that inside the trapped I_P cells are also some trapped B cells.

It appears that, if the DIP-infection front catches up to the helper-infection front when the helper-infection front has hit a flat edge of an empty region, the DIP-infection front may wrap B cells more easily; thus the structure of trapped I_P cells surrounding trapped B cells may appear more easily. This phenomenon is shown in figure 5.4.

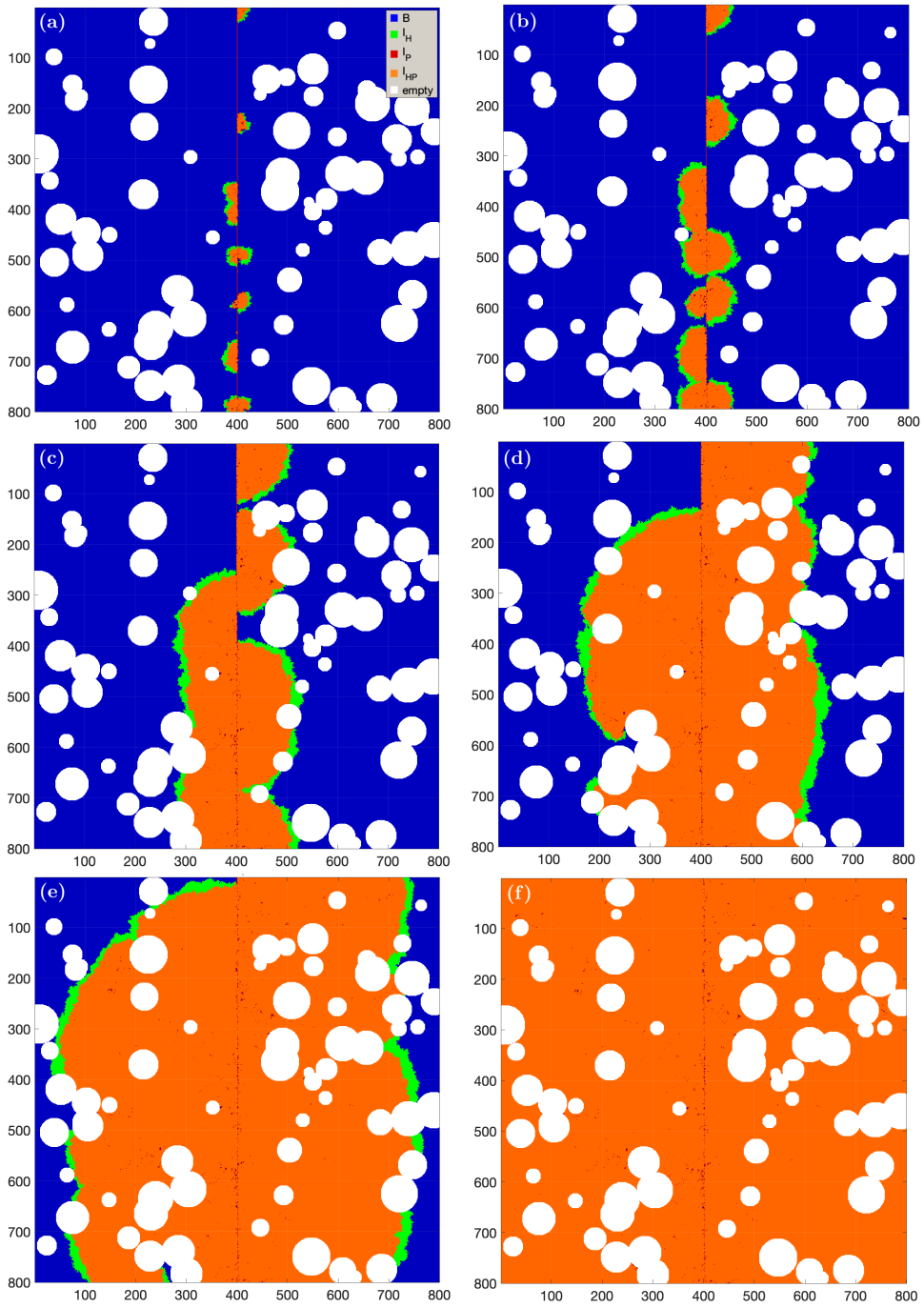


Figure 5.2.: Snapshots of the time-evolution of the system with circular empty-regions. (a) $t = 50$. (b) $t = 100$. (c) $t = 200$. (d) $t = 400$. (e) $t = 600$. (f) $t = 950$. Once again a front of the I_H state develops and is followed by a front of the I_{HP} state.

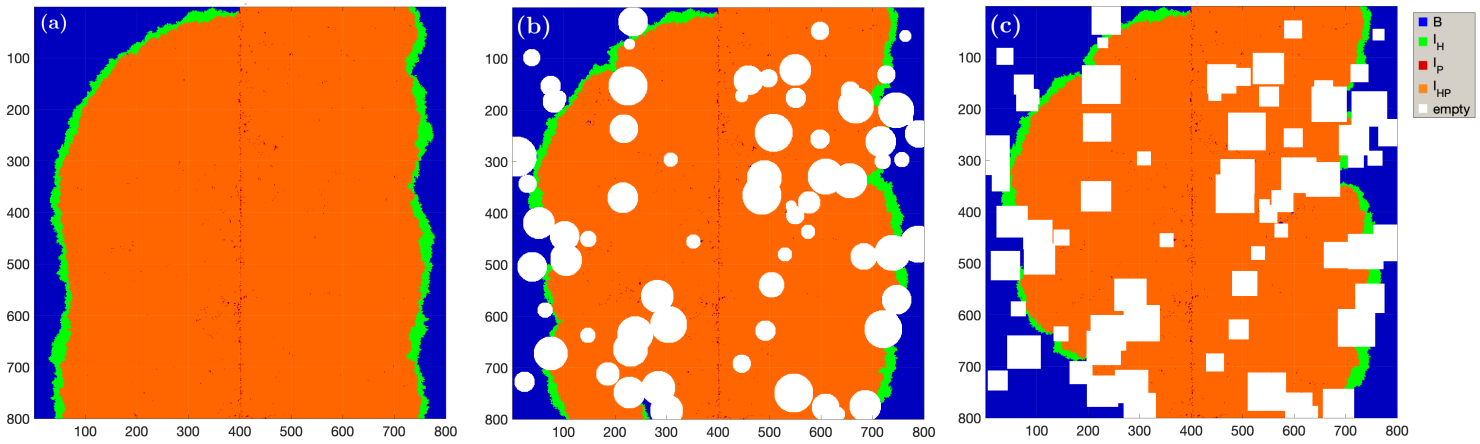


Figure 5.3.: Snapshots of the three systems at $t = 600$. (a) Lattice with no empty regions. (b) Lattice with circular empty regions. (c) Lattice with square empty regions. With and without empty regions, the development of the three systems is very similar. Therefore, neither type of obstacles interfered majorly with the spread of the infections. (There is a difference in figure (c), in the bottom-left corner, but that is because there is a "peninsula" of cells touched by a "sea" of empty sites that is not present in the other two lattices.)

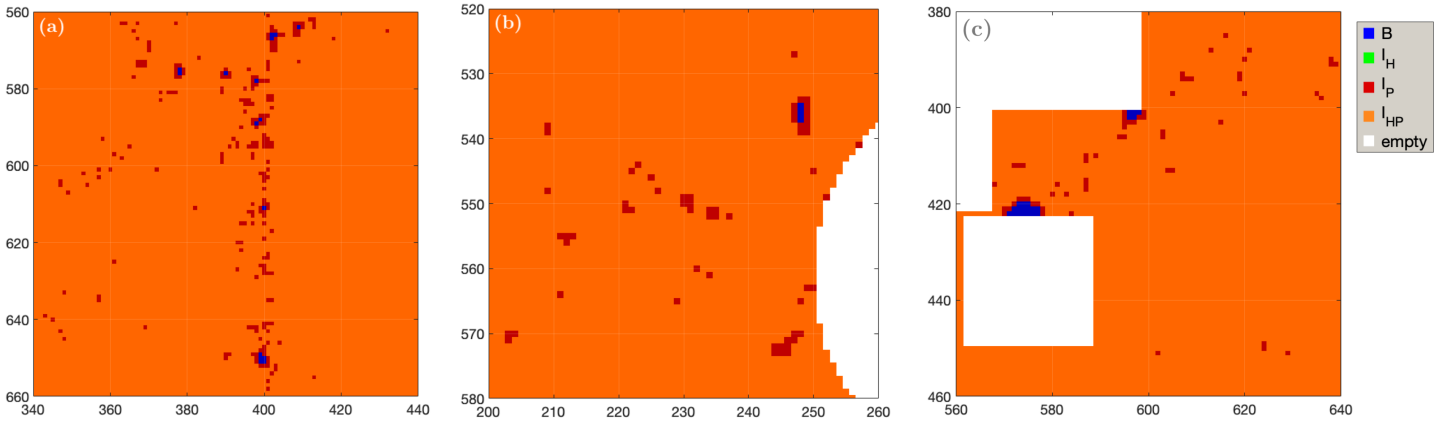


Figure 5.4.: Examples of I_P cells surrounded by I_{HP} cells, and of B cells surrounded by I_P cells. These snapshots are at $t = 950$, i.e. once the lattice is no longer developing. (a) Close-up of the lattice with no empty regions. (b) Close-up of the lattice with circular empty regions. (c) Close-up of the lattice with square empty regions. Against two flat edges of empty regions, I_{HP}-cells are surrounding I_P-cells which themselves are surrounding B-cells.

6 Conclusions

We have performed a qualitative study of the population dynamics of DIPs and helper viruses which infect the same cells.

We began with only one DIP species and one helper species. We saw that, without flux, the transformation of the cells into I_{HP} cells takes a similar amount of time whether the I_{HP} cells make DIPs and helpers in similar proportions of whether they produce mostly DIPs: this is because the I_H cells too produce new helper viruses. Instead, the transformation of the cells takes longer if the I_{HP} cells produce mostly helpers.

We saw that if we introduce an influx of uninfected cells and the dilution of the cells, DIPs and helpers, then a dynamic balance develops. This dynamic balance is different depending on how fast the dilution is. If the dilution is relatively slow, then the steady-state level of the I_{HP} cells is unchanging for different proportions of DIPs produced by the I_{HP} cells, unless these cells produce a great majority of either DIPs or helpers. Instead, if the dilution is relatively fast, the formation of I_{HP} cells is hampered by dilution itself. This causes the DIP species not to be able to survive unless the proportion of DIPs made by the I_{HP} cells is higher than a threshold. Another consequence is that even if the I_{HP} cells produce only DIPs, with fast dilution the DIP and helper species manage to survive, because not all I_H cells become I_{HP} cells before being diluted. This causes the I_H cells and I_{HP} cells to take the respective roles of helper-virus producers and DIP producers.

We then saw that the DIP species goes extinct much more rapidly if the I_{HP} cells produce either mostly DIPs or mostly helpers. The helper species can go extinct before the extinction of the DIP species or at the same time: there is a direct proportionality between the frequency of this type of event and the proportion of DIPs produced by the I_{HP} cells. When the helper species goes extinct before the DIP species, this extinction happens much more rapidly when the I_{HP} cells produce mostly DIPs. Once the helper species has gone extinct before the DIP species, the DIP species manages to survive in the system for a longer time if the I_{HP} were not producing mostly DIPs or helpers: in fact, if the I_{HP} cells were producing similar proportions of DIPs and helpers, at the

time of the helper extinction there are more DIPs left in the system.
We saw that in a system with two helper species and one DIP species which can use both helper species, there is a clear winner between the helper species, i.e. the one which is produced in a greater proportion by the cells infected by both this helper and the DIP. However, if the DIP species are two, things become more interesting. In fact, depending on the proportions of DIPs and helper viruses produced by the doubly-infected cells, not always there is a winner between the helper species or a winner between the DIP species; and if there are no clear winners, the DIP and helper levels and the levels of infected cells oscillate (if there is an influx of uninfected cells and the dilution of DIPs, helpers and cells). If each DIP species can take advantage of only one helper species and not the same helper species, a DIP species has the lowest levels when the other DIP species is produced in low proportions. This DIP species with the lowest level becomes the most likely to go extinct first among the DIP and helper species. Additionally, if there are oscillating dynamics, the probability of extinction of this DIP species oscillates with the same frequency as the oscillating levels.
We finally considered a spatial model with one DIP species and one helper species, where the I_{HP} cells produce only DIPs. Two infection fronts developed, a leading helper-infection front followed by a DIP-infection front. Most cells ended up infected by both the DIP and the helper, but a few ended up infected only by the DIP, and even fewer ended up not infected at all. These uninfected cells were protected by a layer of DIP-infected cells, which cannot infect. As the very last thing, we found that a cell-lattice with empty regions did not hamper the infection propagation. However, it appeared that the flat surface of an empty region might lead to a more likely formation of only-DIP infected cells or uninfected cells.

6.1 Perspectives

There are features which we did not investigate due to time constraints; however, it would be interesting to include these in a future study.

One could create a lattice model with one DIP species and one helper species where the I_{HP} cells produce DIPs with varying proportions. It would be interesting to study how fast the DIP infection propagates depending on f.

Another lattice model could include two superinfection-exclusive DIP species and two superinfection-exclusive helper species. We studied the competitions between DIP species and between helper species in a well-mixed model, but we do not know how space would affect these two competitions. In this spatial model, could the virus and cells levels oscillate?

One could create models where the cells divide: such models would better depict systems of bacteria with pirate and helper phages. The growth rate of a cell would depend on its infection state: the infection of a host can slow down its growth [22]. Pirate and helper phages would spread not only through infection but also through growth of their hosts. However, cell growth could become a disadvantage for the pirate: pirate replication requires two infections, helper replication only one. If a doubly-infected cell, which makes pirates, grows more slowly than a cell infected only by a helper, the pirate would be at a disadvantage. This disadvantage would affect the population dynamics.

In a model, whether it is well-mixed or spatial, one could also model the cell receptors. Let us consider a system with multiple DIP species and multiple helper species. Each helper species can only use one type of receptor [23], but a DIP species could possibly take advantage of more than one helper species. This would make this DIP species able to use more than one receptor, as opposed to the helper viruses which can only use one receptor. This advantage of the DIPs would counterweigh their fundamental disadvantage, i.e. the fact that they need the coinfection of a helper virus in a cell to create new copies of themselves. Thus this would be an additional ingredient for the dynamics between DIPs and helper viruses.

Appendices

Values of η and β

A

Here we include the values found in the literature of the rates η (rate of virus absorption by a cell) and β (rate of virus release by a cell) for three viral species.

Virus	η	β
HIV-1 (retrovirus)	$0.65 \times 10^{-3}\ \mu l \cdot day^{-1}$ [24]	$850\ day^{-1}$ [24]
SHIV-KS661 (retrovirus)	$8.61 \times 10^{-11}\ ml \cdot day^{-1}$ [25]	$3.24 \times 10^{4}\ day^{-1}$ [25]
M13 (chronic phage)	$1.6 \times 10^{5}\ \mu m^{3} \cdot h^{-1}$ [26]	$1.66 \times 10^{3}\ h^{-1}$ [27]

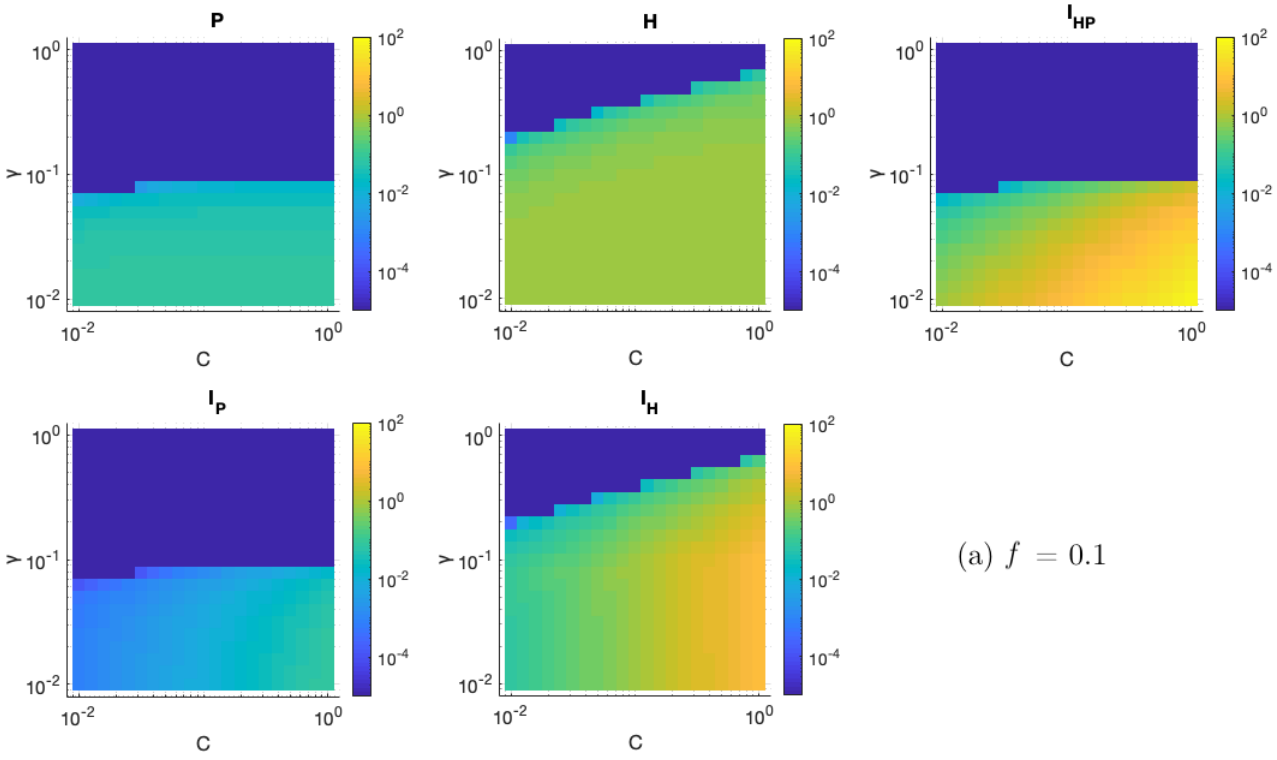
Table A.1.: Values of η and β for a few example viruses. SHIV-KS661 is a simian-human immunodeficiency virus [25]. M13 is a bacteriophage with a satellite/pirate variant [16].

B

Additional figures and tables - Chapter 2

B.1 Steady-state levels of viruses and infected cells (follow-up to figure 2.4)

Here we break down figure 2.4 into distinct steady-state levels of viruses and infected cells.



(a) $f = 0.1$

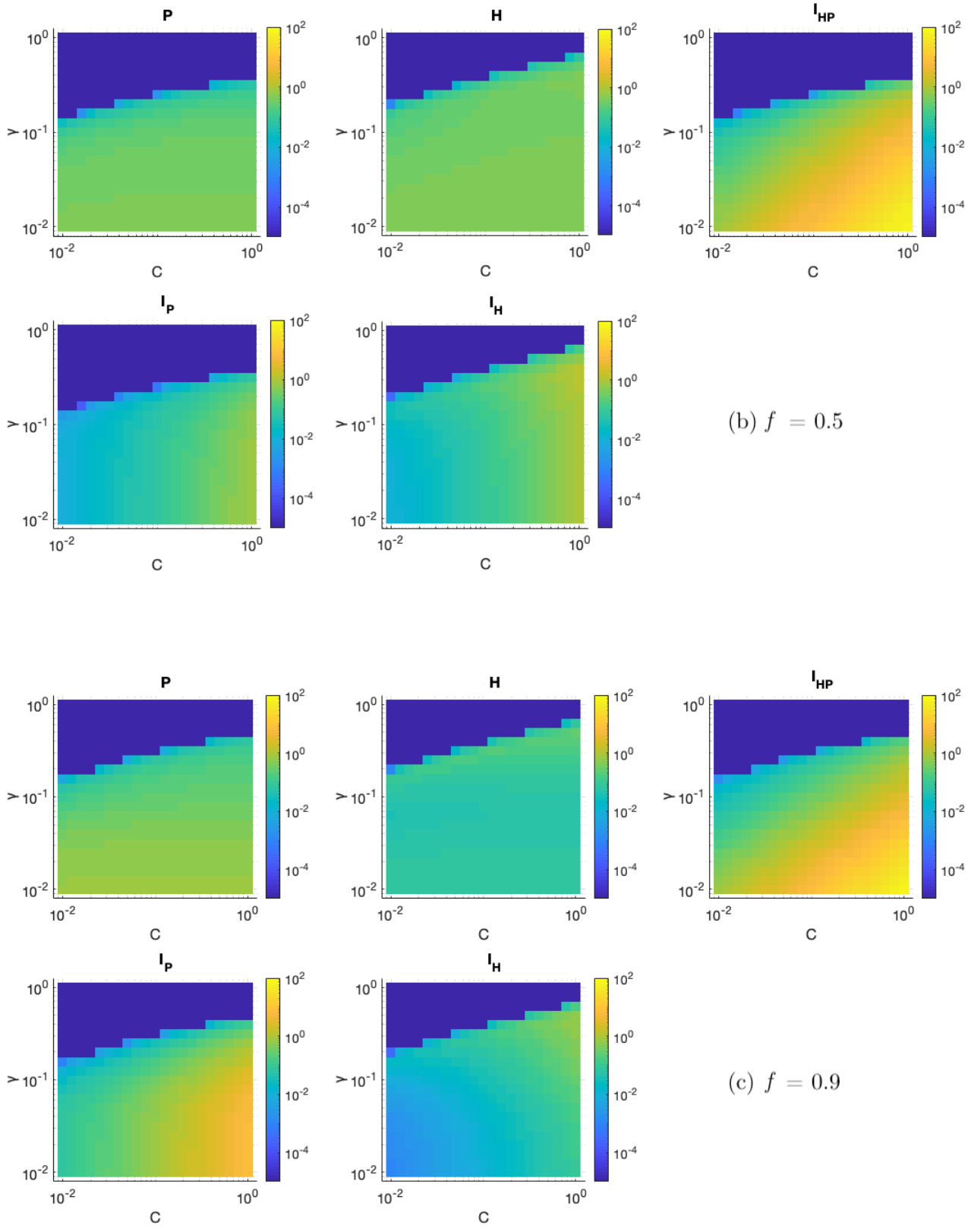
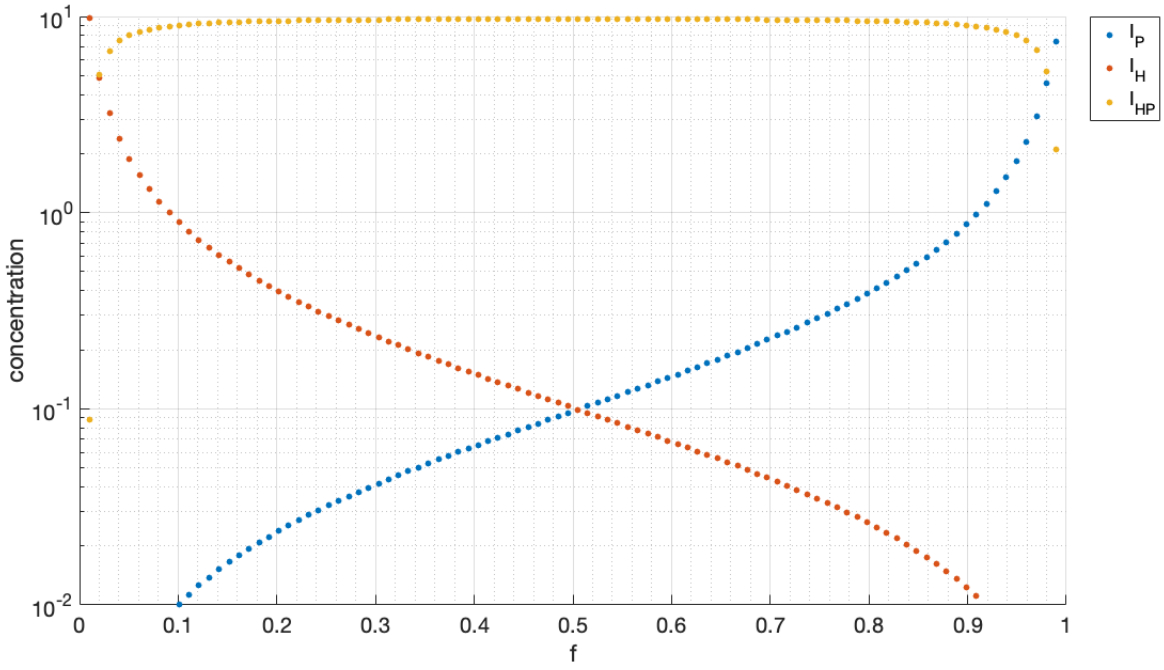
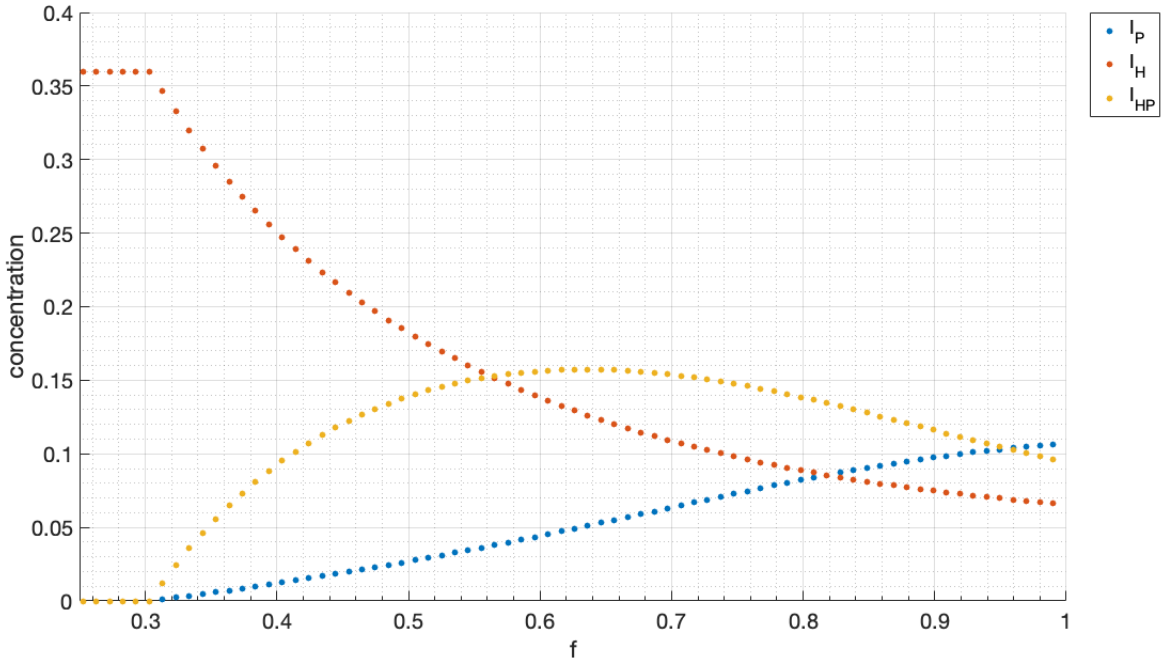


Figure B.1.: Steady-state concentrations of the viruses and infected cells for different values of f. Compare with figure 2.4. (a) $f = 0.1$. (b) $f = 0.5$. (c) $f = 0.9$.

B.2 Steady-state levels of the infected cells for two different dilution rates (follow-up to figure 2.5)



(a)



(b)

Figure B.2.: Steady-state levels of *only* the infected cells vs f, with two different rates of dilution γ. $\mathcal{C}$ is the same in the two cases. See figure 2.5 for the genome levels. (a) $(\mathcal{C}, \gamma) = (0.1, 0.01)$ (slower dilution) (b) $(\mathcal{C}, \gamma) = (0.1, 0.2)$ (faster dilution)

B.3 Initial conditions for the stochastic model in section 2.3.1

Here we show the initial conditions used for the model described in section 2.3.1.

f	0.01	0.05	0.1, ... , 0.9	0.95	0.99
$\widetilde{P}_0$	0	0	0	0	0
$\widetilde{H}_0$	0	0	0	0	0
$\widetilde{B}_0$	0	0	0	0	0
$\widetilde{I}_{P,0}$	0	0	0	1	3
$\widetilde{I}_{H,0}$	3	1	0	0	0
$\widetilde{I}_{HP,0}$	7	9	10	9	7

Table B.1.: The initial amounts of viruses and cells for each value of f.

B.4 Example of time evolution of a system with 1 DIP species and 1 helper species where the two species go extinct simultaneously (compare with figure 2.12)

Here we show an example of time evolution of a system that advances according to the model in section 2.3.1: in this example the DIP and helper species go extinct simultaneously.

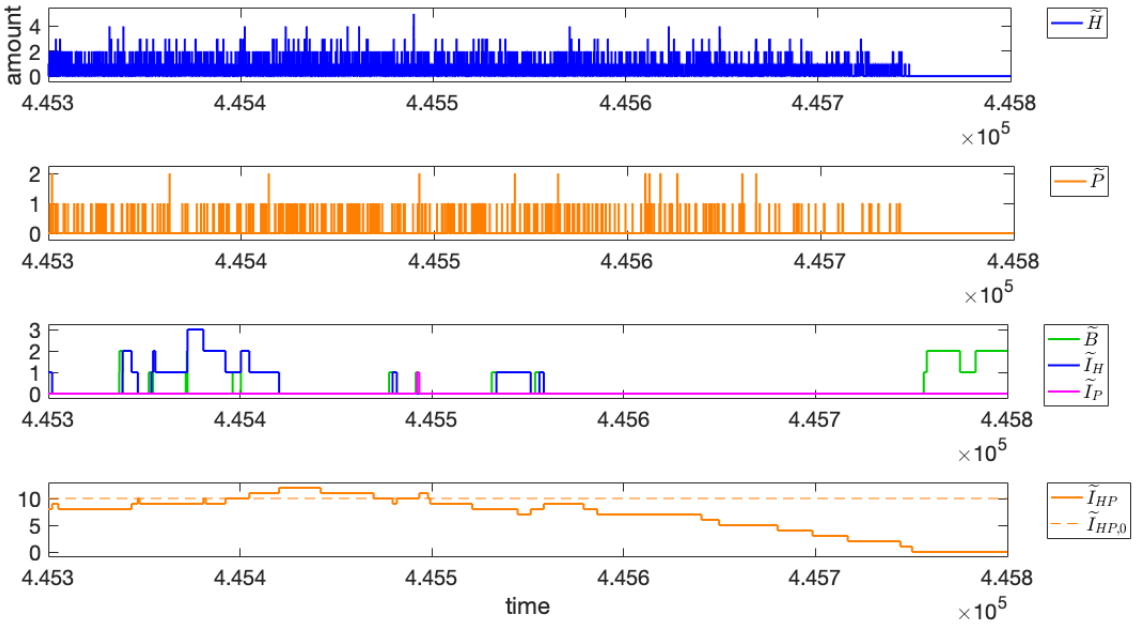


Figure B.3.: Another example of time evolution of the system with extinction of the DIP. In this example $f = 0.1$, just like for figure 2.12. Here the DIP and the helper virus go extinct at the same time: in fact, $\widetilde{I}_{HP}$ reaches 0 when $\widetilde{I}_P$, $\widetilde{I}_H$, $\widetilde{P}$, $\widetilde{H}$ are already all 0.

B.5 Example of time evolution of a system with one DIP species and one helper species where the helper species goes extinct before the DIP species (compare with figures 2.12 and B.3)

Here we show the time evolution of a system that advances according to the model in section 2.3.1: in this example, the helper goes extinct before the DIP does.

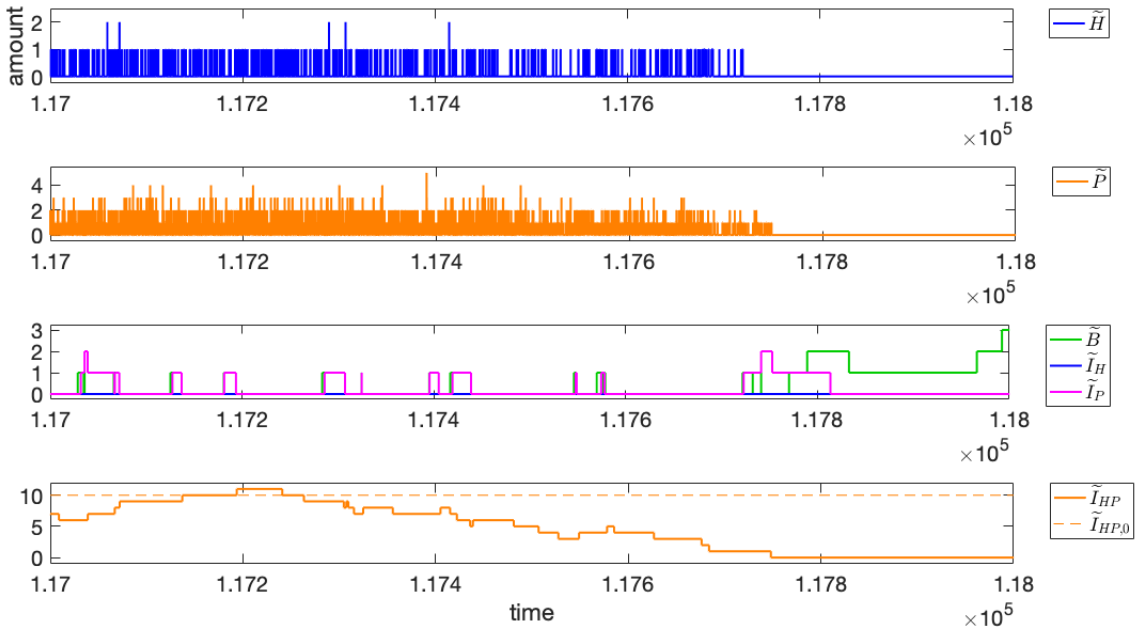


Figure B.4.: In this example the helper virus goes extinct before the DIP does. Here $f = 0.9$.

B.6 Example of distribution of T_H (section 2.3.2)

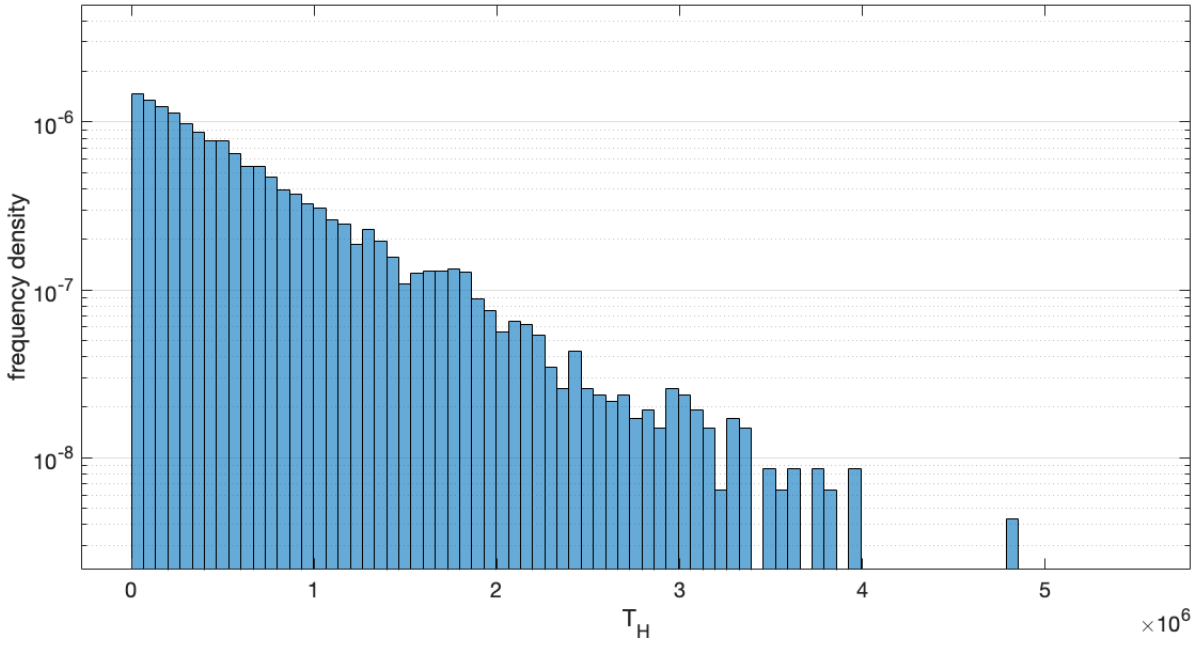


Figure B.5.: The statistics of T_H for $f = 0.5$. The vertical scale is logarithmic. (The total frequency is less than 40000, in fact $\mathcal{R} < 1$ for $f = 0.5$. For $f = 0.5$ the frequency was 33118, corresponding to $\mathcal{R} \simeq 0.83$.)

B.7 Example of distribution of ΔT (section 2.3.2)

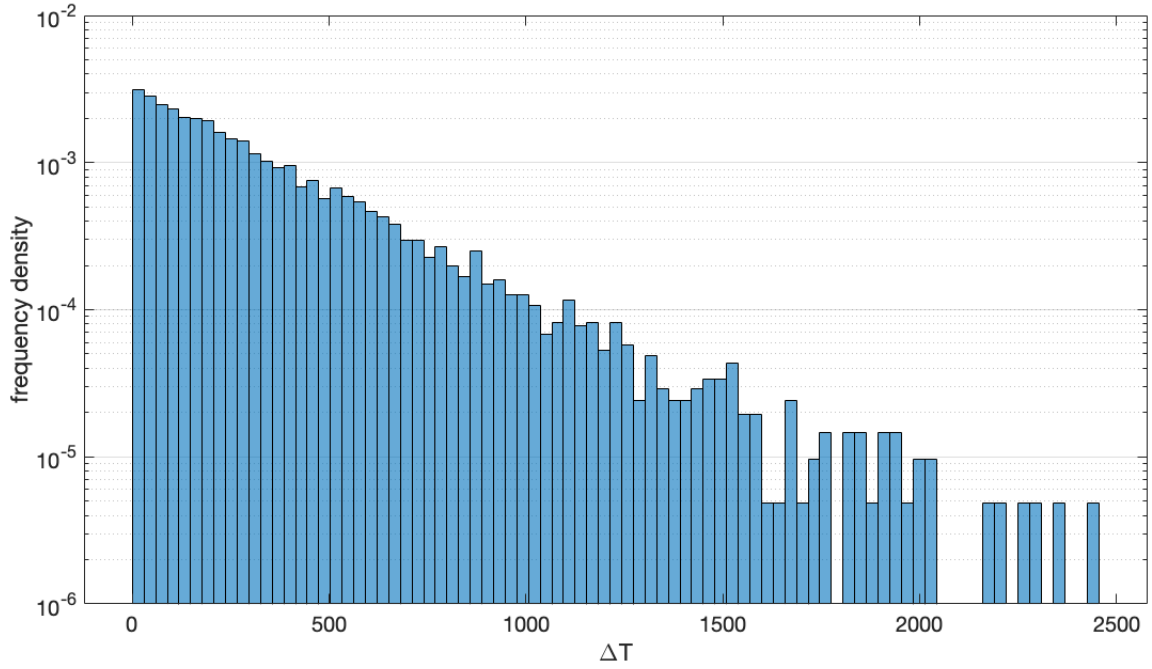


Figure B.6.: The statistics of ΔT for $f = 0.5$. The vertical scale is logarithmic. (The total frequency is less than 40000, in fact $\mathcal{R} < 1$ for $f = 0.5$. For $f = 0.5$ the frequency was 33118, corresponding to $\mathcal{R} \simeq 0.83$.)

C

Additional figures and tables - Chapter 4

C.1 Complete set of rules to find a winning DIP species and a winning helper species (expansion of figure 4.5)

METHOD A

• Compare the I_{HP}-like species making the same helper:

I_{H1Pa} vs I_{H1Pb}
I_{H2Pa} vs I_{H2Pb}

You do this by looking at the rows of the matrix $\bar{f}$.

$$\bar{f} = \begin{pmatrix} f_a^1 & f_b^1 \\ f_a^2 & f_b^2 \end{pmatrix}$$

In a row, the species with higher f wins. If f is the same, it's a tie.

i. If the two duels are won by two species making the same DIP (same column), this DIP is the winning DIP.
ii. If one duel is a tie, the DIP made by the winner of the other duel is the winning DIP.
iii. If both duels are ties, *use method B.*
iv. If the two duels are won by two species making different DIPs (different columns), *use method B.*

• Now you can find the winning helper by comparing the two I_{HP}-like species left: the one with lower f wins and the helper virus it produces is the winning helper.
If the two I_{HP}-like species left have a tie, compare the two losing species from the first part of this method: the species with lower f wins and the helper it produces is the winning helper.

(a)

METHOD B

• Compare the I_{HP}-like species making the same DIP:

I_{H1Pa}	I_{H1Pb}
VS	VS
I_{H2Pa}	I_{H2Pb}

You do this by looking at the columns of the matrix $\bar{f}$.

$$\bar{f} = \begin{pmatrix} f_a^1 & f_b^1 \\ f_a^2 & f_b^2 \end{pmatrix}$$

In a column, the species with lower f wins. If f is the same, it's a tie.

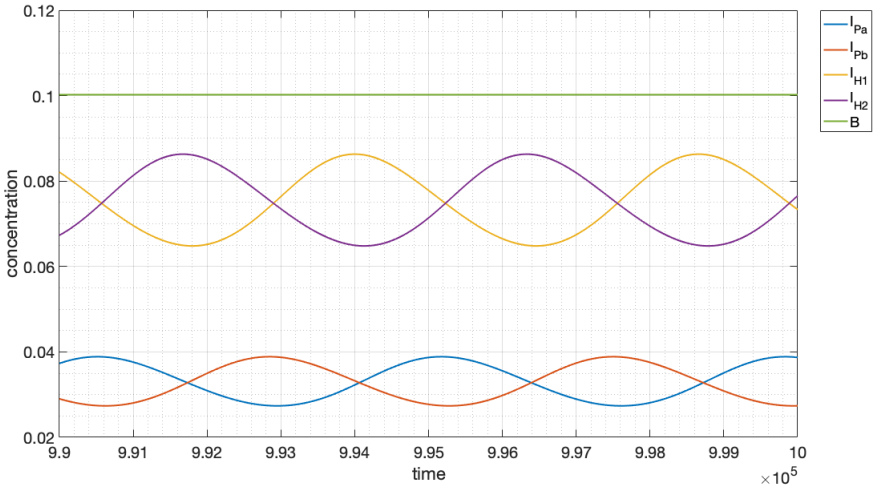
i. If the two duels are won by two species making the same helper, this helper is the winning helper.
ii. If one duel is a tie, the helper made by the winner of the other duel is the winning helper.
iii. If both duels are ties, *use method A.*
iv. If the two duels are won by two species making different helpers (different rows), *use method A.*

• Now you can find the winning DIP by comparing the two I_{HP}-like species left: the one with higher f wins and the DIP it produces is the winning DIP.
If the two I_{HP}-like species left have a tie, compare the two losing species from the first part of this method: the species with higher f wins and the DIP it produces is the winning DIP.

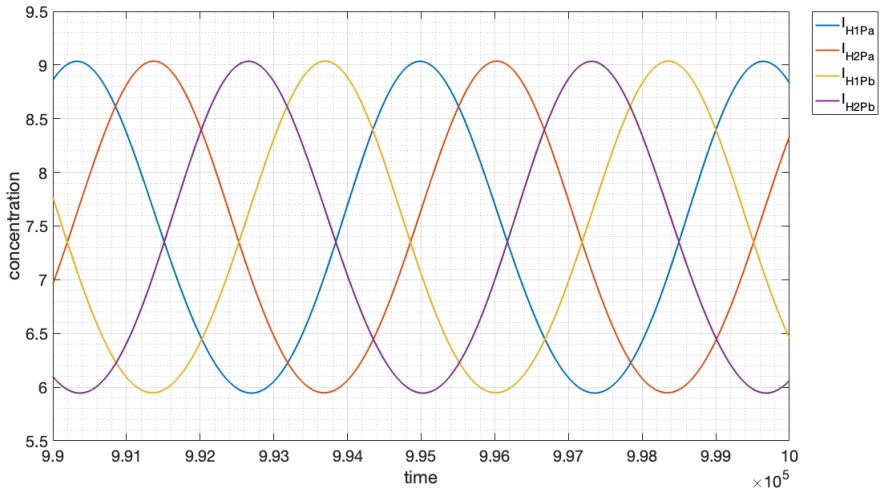
(b)

Figure C.1.: Complete set of rules to find the winning DIP and the winning helper virus: here we contemplate the case of a tie between I_{HP}-like species. There are two alternative methods. (a) According to method A, one starts by looking for the winning DIP. (b) According to method B, one starts by looking for the winning helper virus.

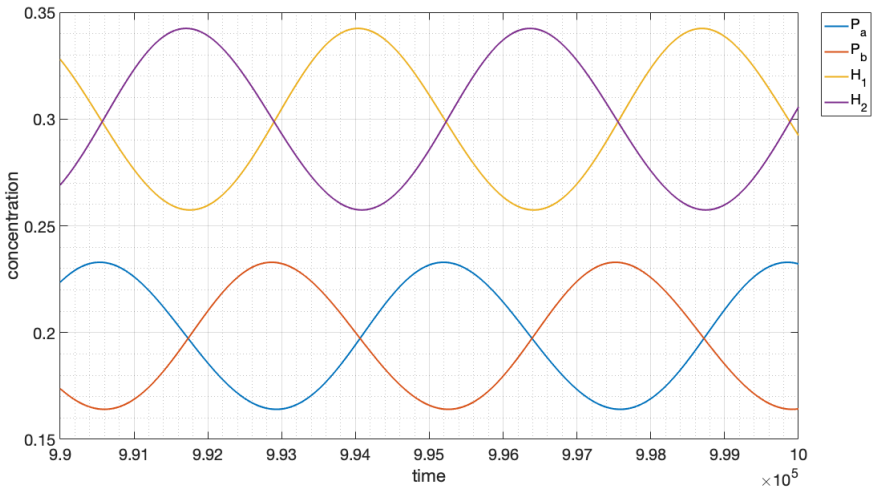
C.2 Examples of oscillating levels and steady-state levels based on the initial conditions when $f_a^1 = f_b^2$ and $f_b^1 = f_a^2$ (section 4.2.2)



(a)

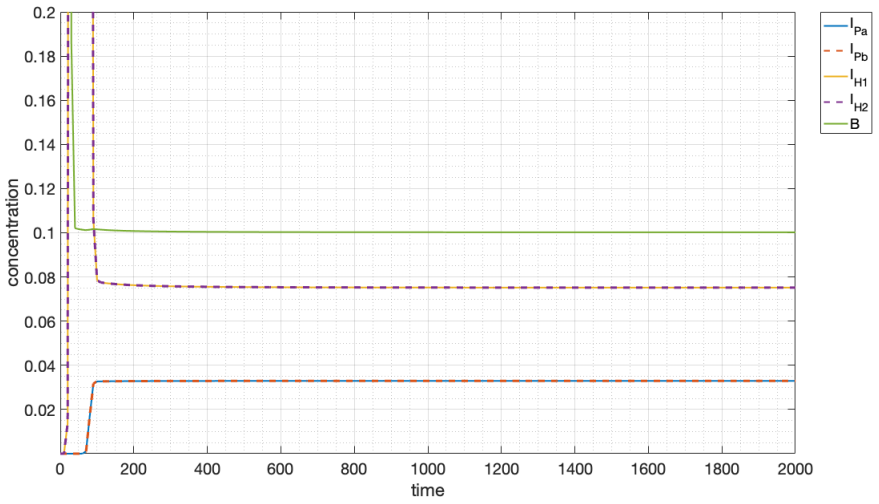


(b)

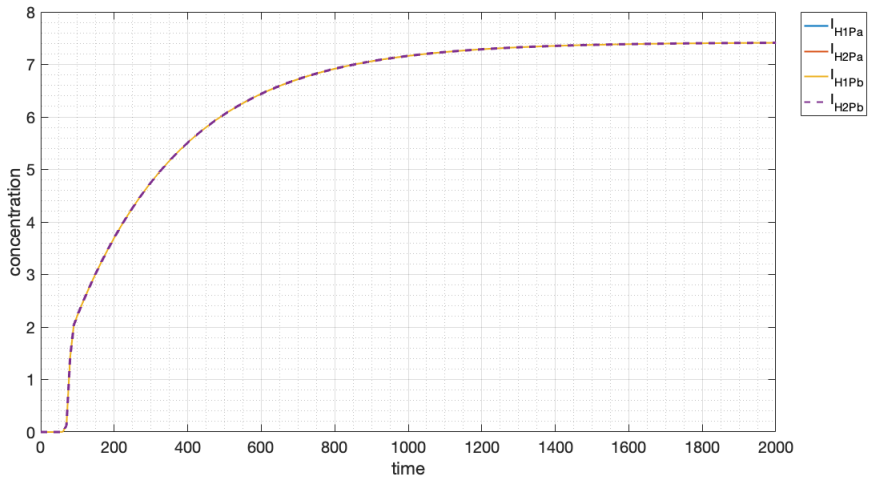


(c)

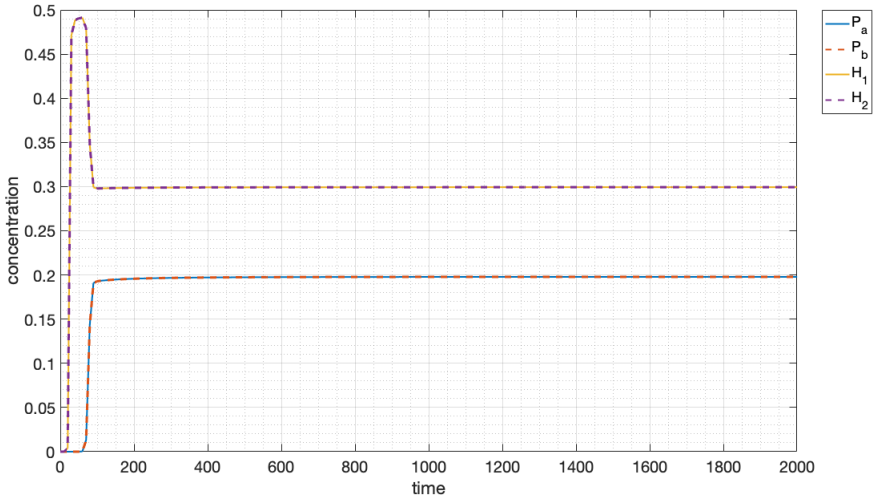
Figure C.2.: The levels of cells and viruses oscillate. The matrix $\bar{f}$ has values $f_a^1 = f_b^2 = 0.6$, $f_b^1 = f_a^2 = 0.2$. The initial conditions are $H_1(t=0) = 0.229 \times 10^{-7}$, $H_2(t=0) = 0.242 \times 10^{-7}$, $P_a(t=0) = 0.247 \times 10^{-7}$, $P_b(t=0) = 0.264 \times 10^{-7}$. Thus $H_1(t=0) \neq H_2(t=0)$, $P_a(t=0) \neq P_b(t=0)$. (a) The levels of the uninfected and singly-infected cells. (b) The levels of the doubly-infected cells. (c) The levels of the free helper viruses and DIPs.



(a)



(b)



(c)

Figure C.3.: The levels of cells and viruses reach a steady state, even though the condition (4.17) is met: in fact, the values of the matrix f are $f_a^1 = f_b^2 = 0.6$, $f_b^1 = f_a^2 = 0.2$. The initial conditions are $P_a(t=0) = P_b(t=0) = 0.3 \times 10^{-7}$ and $H_1(t=0) = H_2(t=0) = 0.2 \times 10^{-7}$.

C.3 Initial conditions for the study of the first viral extinction (section 4.3.2)

f_a^1	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
$\widetilde{P}_{a,0}$	1	1	1	1	1	2	2	2	2
$\widetilde{P}_{b,0}$	0	1	1	1	2	1	1	1	2
$\widetilde{H}_{1,0}$	8	6	5	4	4	3	3	3	2
$\widetilde{H}_{2,0}$	2	2	3	3	4	4	4	4	4
$\widetilde{B}_0$	1	1	1	1	1	1	1	1	1
$\widetilde{I}_{P_a,0}$	0	0	0	0	0	0	0	0	0
$\widetilde{I}_{P_b,0}$	0	0	0	0	0	0	0	0	0
$\widetilde{I}_{H_1,0}$	9	4	3	2	1	1	1	1	1
$\widetilde{I}_{H_2,0}$	2	2	2	2	1	1	1	1	1
$\widetilde{I}_{H_1P_a,0}$	157	117	88	68	54	80	69	60	52
$\widetilde{I}_{H_2P_a,0}$	36	53	61	64	64	82	81	80	78
$\widetilde{I}_{H_1P_b,0}$	78	85	86	85	82	66	67	68	67
$\widetilde{I}_{H_2P_b,0}$	17	38	59	78	95	67	79	89	99

Table C.1.: The initial amounts of DIPs, helper viruses and cells, for each value of f_a^1. Notice that the I_{HP}-type cells are dominating.

C.4 Initial conditions for the study of the viral extinction-time distribution (section 4.3.3)

v	10	50	100
$\widetilde{P}_{a,0}$	1	3	5
$\widetilde{P}_{b,0}$	0	1	3
$\widetilde{H}_{1,0}$	8	38	76
$\widetilde{H}_{2,0}$	2	8	15
$\widetilde{B}_0$	1	5	10
$\widetilde{I}_{P_a,0}$	0	0	1
$\widetilde{I}_{P_b,0}$	0	0	0
$\widetilde{I}_{H_1,0}$	9	45	89
$\widetilde{I}_{H_2,0}$	2	9	18
$\widetilde{I}_{H_1P_a,0}$	157	786	1572
$\widetilde{I}_{H_2P_a,0}$	36	180	359
$\widetilde{I}_{H_1P_b,0}$	78	388	775
$\widetilde{I}_{H_2P_b,0}$	17	87	175

Table C.2.: The initial amounts of DIPs, helper viruses and cells for each value of v.

D Additional figures - Chapter 5

D.1 Time-evolution of the system with square empty regions (section 5.2.2)

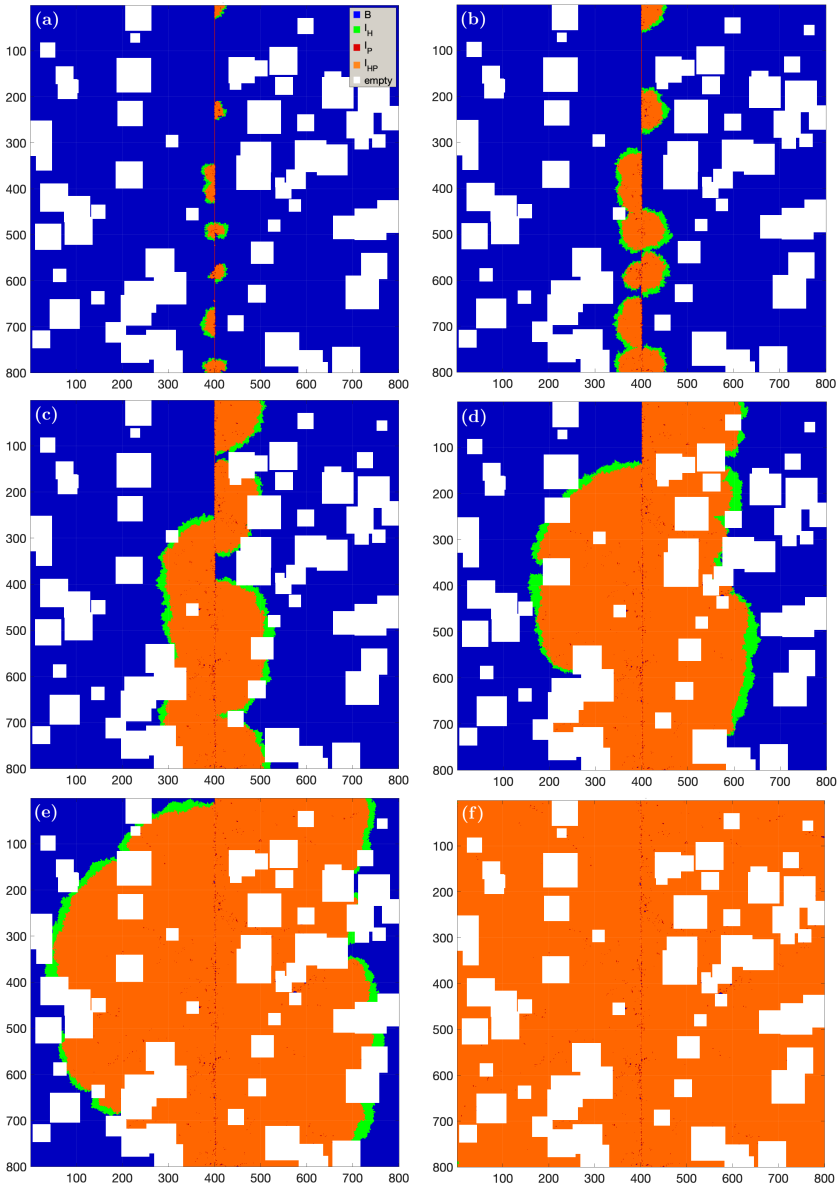


Figure D.1.: Snapshots of the time-evolution of the system with square empty-regions. (a) $t = 50$. (b) $t = 100$. (c) $t = 200$. (d) $t = 400$. (e) $t = 600$. (f) $t = 950$. Once again a front of the I_H state develops and is followed by a front of the I_{HP} state.

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