Coexistence between Plasmids in Structured Biofilms: A Theoretical Model for Post-Segregational Killing Systems

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Abstract

COEXISTENCE BETWEEN PLASMIDS IN STRUCTURED BIOFILMS: A THEORETICAL MODEL FOR POST-SEGREGATIONAL KILLING SYSTEMS

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Currently there is a lack of understanding about how plasmids that contain Post-Segregational Killing (PSK+) systems and plasmids that do not contain such a system coexist in a population. In this thesis two spatially explicate models are presented to attempt to answer this gap in the literature. The first model is an extension on a previous single strain model, the second model has two different strains and allows for the plasmids to specialize to different bacterial strains. Weak coexistence was found in the single strain model. Coexistence was found in the second model but only across a small parameter space and only under conditions when a PSK+ plasmid is introduce by a process analogous to dispersal. The interesting implication of this result is that it suggests coexistence is possible with specialization, but that it requires dispersal from another locality for the coexistence process to be initiated.
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Contents

Abstract ii

Acknowledgments iii

Contents iv

List of Figures vii

List of Tables ix

I Introduction 1

1 Conjugation 2

2 Post Segregational Killing Systems and their Biology 3

3 Biofilms 10

4 Specialization 11

5 Theoretical Literature 12

II Models 17

6 Spatial Framework for the Biofilm 18
15.1 When would PSK- and PSK+ not coexist .............................. 73
15.2 Future Directions .............................................................. 74

16 Conclusion ...................................................................... 77

References ........................................................................ 78
List of Figures

1 Schematic of PSK+ System ................................................. 7
2 Schematic of Modeling Structure in Sato et al. ............................. 21
3 List of the Doublet Pairs in the Mochizuki Extension Model ............... 23
4 Transformation of States in the Mochizuki Extension Model .................. 25
5 Initialization Process of the Mochizuki Extension Model ....................... 31
6 Fitness of States in the Specialization Model ................................ 32
7 List of Doublet Pairs in the Specialization Model ........................... 33
8 Interactions of States in the Specialization Model ........................... 34
9 The Initialization Process of the Specialization Model ......................... 41
10 Scenarios for the Introduction of PSK+ in the Specialization Model ......... 42
11 Perturbations to a Point of Coexistence from the Mochizuki Extension Model: Example 1 .................................................. 50
12 Perturbations to a Point of Coexistence from the Mochizuki Extension Model: Example 2 .................................................. 51
13 Points of Coexistence for the Mochizuki Extension with Randomly Selected Parameters ................................................. 52
14 Perturbations to a Point of Coexistence from the Mochizuki Extension Model: Randomly Selected Parameters ............................... 53
15 Perturbations to a Point of Coexistence from the Specialization Model: Example 1 .................................................. 56
16 Perturbations to a Point of Coexistence from the Specialization Model: Example 2 .................................................. 57
17 Results of the Second Phase of Initialization of the Specialization Model ... 59
18 Results of the Mutation Scenarios of the Specialization Model ............ 61
List of Tables

1 Definitions Table of Variables. ................................................. 26
2 Values of the Parameters Used in this Thesis ............................ 45
3 The Parameter Values for the Points of Coexistence Found for the Specialization Model ................................................................. 57
4 Table of the Values used for the Mochizuki Extension Model ......... 119
5 Parameters Space of the Specialization Model ........................... 119
Part I

Introduction

Plasmids are extra chromosomal DNA that are found in bacteria. They range in size from some being only about 300 bp (Kado, 1998) in length to being about equal in size to the chromosome of a bacterium which is about 2,400 kb (Maclellan et al., 2004; Kado, 1998). Plasmids are transferred between cells in two ways, the first being vertical gene transfer and the other being horizontal gene transfer. Vertical gene transfer is when the plasmid is passed onto daughter cells. Horizontal transfer occurs in one of three different ways, conjugation, transformation, and transduction. Conjugation is when there is direct transfer of DNA between two bacterial cells. Transformation is when a bacterium picks up naked DNA from the environment and lastly transduction is when DNA is transferred via a bacteriophage. The typical mode of horizontal transfer of plasmids is conjugation through sex pili.

There are several mechanisms that help plasmids maintain themselves in bacteria. One of which is known as Post-Segregational Killing Systems (PSK) and is the focus of my research. This mechanism kills off or severely inhibits a daughter cell that does not inherit the plasmid. This is done by the plasmid encoding for toxins that can only be neutralized by less stable anti-toxins.

The goal of the research in this thesis is to examine whether plasmids with a PSK system (PSK+) and plasmids that do not have such a system (PSK-), can coexist with one another. In particular a previous paper (Mochizuki et al, 2007) studied whether a PSK- plasmid and a PSK+ plasmid could coexist in a population, but they did not consider the possibility that a bacterium may lack a plasmid. The first part of my thesis will examine a single strain of bacteria in which it
is possible that a bacterium does not harbour a plasmid, harbours a PSK- plasmid, or harbours a PSK+ plasmid. Thus it is possible in my model that a population may become fixed for bacteria that does not harbour a plasmid; this was not possible in the previous paper. The importance of adding a plasmidless bacteria to the model is that it could weaken the “fitness” of PSK- plasmids by introducing a mechanism such that PSK- plasmids are lost during segregation to the plasmidless state. The second part of my thesis will examine whether specialization facilitates the coexistence of PSK- and PSK+ plasmids. By specialization, it is meant that plasmids can have differing costs or benefits depending on which bacterial strain hosts them, such that a particular plasmid has a higher fitness in one strain versus the other and vice versa. The effects of specialization between different PSK systems and other plasmids has yet to be examined.

In the remainder of this introduction, I will first introduce and explain conjugation which is a major form of horizontal gene transfer. Secondly, I will provide background to PSK+ systems as well as alternative systems that support the persistence of plasmids. Thirdly, I will provide context and background to how bacterial populations are spatial structure. In particular, I will introduce the concept of a biofilm, which is a common form of spatial structure in bacteria. Fourth, I will provide background and motivation for specialization of plasmids to their host bacteria. Lastly, I will provide a brief summary of previous models of plasmid persistence in the absence and the presence of PSK+ systems.

1 Conjugation

Plasmids often transfer between cells via conjugation, which is the form of horizontal gene transfer modeled in my thesis. Conjugation requires the creation of a sex pili between the donor and the
recipient cell. Once the sex pili is connected to the recipient cell the two cells are brought closer to each other until the two cells make contact and their cell membranes fuse temporarily (Lawley et al., 2004). A copy of the plasmid is made by creating a nick in the plasmid and having a single strand of DNA from the plasmid sent to the donor cell. The single strand of DNA in both the donor and recipient cell becomes a double stranded DNA by the synthesis of a complementary strand. Once the plasmid is delivered to the recipient cell the sex pilus breaks off and conjugation is complete. If the recipient cell containing a plasmid prior to conjugation receives a plasmid during conjugation from the same incompatibility group, the two plasmids become unstable and one is eventually lost randomly via segregation due to the plasmids sharing similar proteins for replication (Lawley et al., 2004). Accordingly, a bacterium typically only contains a single plasmid from a particular incompatibility group.

2 Post Segregational Killing Systems and their Biology

It is in general difficult for plasmids to be maintained in a population for several reasons. For example, plasmids may not be beneficial to a bacterium and it will be lost via natural selection. Furthermore, when a plasmid is beneficial, it is often taken up by the chromosome as discussed below (Bergstrom et al., 2000). Additionally, plasmid size has an effect on maintenance (Smith and Bidochka, 1998). Small plasmids often do not encode the genes required for conjugation (Smith and Bidochka, 1998), therefore they require another mechanism for successful transfer. Small plasmids usually achieve this maintenance by increasing their copy number inside a bacterium, which increases the chance that both daughter cells inherit the plasmid vertically. Larger plasmids often encode genes that support conjugation, nevertheless large plasmids have lower copy number
and therefore can be lost by segregation with a relatively higher probability (Smith and Bidochka, 1998). Larger plasmids require other means besides conjugation since the level of conjugation is not enough to outdo the effects of the uptake of the bacteria into the chromosome or the loss of the plasmid through segregation (Bergstrom et al., 2000). In my thesis I explore cases when plasmids are both costly and beneficial, but I focus attention on plasmids that can conjugate and have lower copy number.

In order for plasmids to be maintained, plasmids employ several different stabilization systems such as PSK, partition systems (PAR), copy number control, and multimer resolution systems (MRS) (Dmowski and Jagura-Burdzy, 2013b). A PAR system is where the plasmid has a par site which allows the equal partitioning of plasmids into both daughter cells during cell division (Funnell and Slavcev, 2004). There are several different types of PAR systems, that are be generally known as “Pushing”, “Pulling”, “Tramming”, and “Diffusion-ratchet” (Schumacher, 2012; Baxter and Funnell, 2015). Copy number control is when plasmids have a regulatory system that controls the number of copies in the bacteria. Here, the plasmids will replicate until a certain number of plasmid copies are present in the bacterium (Krüger et al., 2004; del Solar and Espinosa, 2000). MRS is a more passive stabilization system (Dmowski and Jagura-Burdzy, 2013b). It works by breaking up multimer plasmids, which are plasmids that have undergone homologous recombination in a way that two or more copies of the same plasmid have become one plasmid (Hallet et al., 2004). Formation of multimer plasmids reduces the chance that both daughter cells inherit the plasmid following cell division (Summers et al., 1993; Summers and Sherrat, 1994). So breaking multimers up allows for the increase in copy number in the mother cell and increases the chance that a daughter cell will inherit the plasmids.

The last stabilization systems is Post-Segregational Killing Systems (PSK), which are some-
times known as Toxin-Antitoxin (TA) systems. This system is the focus of my thesis. Plasmids that contain such a system have genes that encode a toxin and an anti-toxin. The anti-toxin is unstable and degrades over time but the toxin is more stable and degrades at a slower rate. After cell division, toxins and anti-toxins from the mother cell are distributed to each daughter cell. Since the anti-toxin is unstable, it will degrade first and therefore the daughter cell may risk death from the action of the more stable toxin if the antitoxin is not continually expressed from the plasmid. In other words, the daughter cell will die from the effects of the toxin if the cell does not inherit the plasmid. PSK systems are quite diverse especially in terms of how the toxins interact, and new types are found nearly every year. Currently six different classes of PSK systems have been identified with four of them being discovered since 2013. The anti-toxins range from being a full fledged protein to an antisense RNA while the toxins range from CcdB which targets DNA gyrase which is important for cellular replication to YeeV which attacks MreB and FtsZ interfering with their polymerization and the bacteria cytoskeleton formation (Van Melderen and De Bast, 2009; Unterholzner et al., 2013). The parts of the cell that the toxins from a PSK system affect range from harming ATP synthesis, replication or most commonly translation (Unterholzner et al., 2013). The most common of these PSK systems is the type-II PSK system which is thought to constitute 30% of all PSK+ plasmids (Coray et al., 2017). Type II PSK involves an anti-toxin protein binding to the toxin protein which stops the toxin from attaching to its target (Figure 1). The toxins of type II often inhibit translation, some of which do so by cleaving the mRNA while others block DNA gyrase, and thus targeting more conserved parts of the cell (Mruk and Kobayashi, 2014; Coray et al., 2017). The other types of PSK systems work similarly with the antitoxin either prevent the toxin being made or preventing contact between the toxin and it’s target. This diversity of PSK systems suggest they are undergoing diversifying selection and there are processes supporting this
diversity.
Figure 1: The mechanism for a type II TA system. The blue circle is the plasmid while the black line is the chromosome of the bacteria. The green circular section is the anti-toxin and the red circular section is the toxin. In the bottom right the plasmid has been segregated out, which causes the antitoxins to decay and allow the toxin attacks its target. In this case, the target is the chromosome of bacteria and this kills the bacteria. On the left-hand side is the case where plasmid does not segregate out, therefore the bacteria persists.
PSK systems are commonly encoded on plasmids and are also often found in bacteria growing in stressful environments. These environments include but are not limited to environments that contain high concentrations of heavy metal or antibiotic drugs. In some environments, such as waste-treatment plant water, it has been found that nearly all plasmids in bacteria isolated from this environment have a stabilization system, this includes PSK, MRS, and PAR (Schlüter et al., 2008). While in other environments such as the human gut or ocean, stabilization systems are not found on all plasmids (Jones et al., 2010; Ma et al., 2012). TA systems seem to be fairly successful since they are present in nearly every species of bacteria (Hayes and van Melderen, 2011). TA systems are found in both gram-positive and gram-negative bacteria cells but less so in gram-positive cells (Dmowski and Jagura-Burdzy, 2013b). Of the TA types, type II is by far the most common, then type I, followed by type III. Very little is known about the other three due to their recent discovery and very little research done on them. Type II has at least twelve known sub-types (Hayes and van Melderen, 2011). Type I has at least sixteen known sub-types (Hayes and van Melderen, 2011). It has been proposed that the diversity and abundance of TA systems is associated with the targets of the systems (Hayes and van Melderen, 2011). There are only two known sub-types of type IV. As mentioned earlier there is only one known sub-type of type V TA system as well as one known sub-type of type VI system. It is possible that PSK+ systems are even more prevalent than current estimates due to how often new types of TA systems are found as well as new sub-types. Due to how common PSK systems are, there is an importance to understanding their population dynamics and also how they have not taken over completely in some environments but coexist with PSK- plasmids that may or may not contain other stability mechanisms.

PSK systems seem to be found on plasmids with low copy number. Copy number meaning the
number of plasmids in a single bacterial cell. Copy numbers can range from one or two plasmids to upwards of 15 copies of a plasmid in the bacterial cell. PSK systems are on plasmids that generally have less than 10 copies inside the bacteria (Wang 2017; Dmowski and Jagura-Burdzy, 2013a).

Despite PSK systems originally being found on plasmids it has been discovered that many PSK systems are located on chromosomes, which are referred to as chromosomals. A chromosomal is often used in the literature to describe a bacteria that has inserted a plasmid as part of its own DNA. This has often proven a problem for models studying plasmid maintenance (Bergstrom et al., 2000) due to the fact that if plasmids give too much of a fitness benefit they will be incorporated in bacteria chromosome. TA systems are often found on chromosomes and their purpose has been debated (Hayes and van Melderen, 2011). One purpose maybe that chromosomals could serve as an anti-addiction mechanism (Hayes and van Melderen, 2011). However, even relatively newly acquired chromosomals seem to not express the PSK phenotype (Coray et al., 2017). There are nine hypotheses postulated by Magnuson (2007) for what is the purpose of TA systems in chromosomes, including that they are junk DNA, they stabilize genomic “parasites” like transposons, that they are selfish alleles, used for gene regulation, as a form of growth control, programmed cell arrest which helps other cells, programmed cell death, and/or phage defense.

Thus far I have reviewed two modes of inheritance of plasmids: conjugation which is a form of horizontal gene transfer and vertical gene transfer via cell division. Conjugation requires spatial proximity between bacteria and in the next section, I focus on the spatial context of bacteria such that it allows for conjugation.
3 Biofilms

An important part of the biology of bacteria is that they often exist in biofilms instead of in well mixed environments. A biofilm is defined as a population of microorganisms, most often bacteria, which grows embedded in a matrix of extracellular parts (Stalder and Top, 2016). About 65 to 80% of human interactions with bacteria are with bacteria found in biofilms so it is important to understand what they are and how bacteria and plasmids act in a biofilm (Cook and Dunny, 2015). For example, 80% of the bacterial chronic inflammation and infectious disease involve biofilms (Barraud et al., 2009). Biofilms are created when planktonic, free floating bacteria attach themselves to a surface and begin to produce a matrix. The matrix produces a spatial structure that accompanies a loss of motility of the bacteria. The matrix is made up of, depending on the bacteria in the biofilm, DNA, proteins, polysaccharides as well as other cellular parts (Cook and Dunny, 2015).

Biofilms both support conjugation and are secondary products of conjugation supports biofilms. For example, conjugation create biomass, or structure, in the biofilm by the creation of sex pili (Cook and Dunny, 2015). These sex pili help build a biofilm by breaking off the cells and creating an extracellular matrix (Cook and Dunny, 2015). There are specific TA systems that are known to help with the production of biofilm since some of the proteins in a TA system also help make the external matrix of biofilms (Wang and Wood, 2011).

Plasmids have higher rates of conjugation within a biofilm than when outside of a biofilm (Cook and Dunny, 2015; Balcázar et al., 2015; Fux et al., 2005; Madsen et al., 2012); although there are some who disagree (Fox et al., 2008; Seoane et al., 2011). The reasoning for the higher rates of conjugation is due to several factors that play into the transfer rates. One is the age and condition of the biofilm; plasmids found in older and/or thicker biofilms were less likely to see an
increase in their conjugation rate. One possible reason for the increase in conjugation rate for some plasmid types is that the biofilm increases contact between cells and stabilize connections between cells (Stalder and Top, 2016). It should also be noted that biofilms can create regions within the biofilm where bacteria do not conjugate, which does not allow for a plasmid to sweep through a population (Stalder and Top, 2016). These pockets seem to be created through a combination of chemical and biological factors created from the heterogeneous mixture of bacteria and matrix in a biofilm (Stalder and Top, 2016). Interestingly, these regions may allow for specialization to evolve because they allow for isolated subpopulations.

4 Specialization

Plasmids can have differing cost to different bacteria species or even different strains of the same species of bacteria. This specialization can be facilitated by incompatibility groups, due to the restriction of a host range thus isolating one plasmid from another (Yano et al., 2013). This isolation allows the plasmids to evolve specialization such as by compensatory evolution (Loftie-Eaton et al., 2015) or encodes new functionality.

The concept of incompatibility groups is important in order to understand specialization. Incompatibility groups and specific plasmids have certain host range. Host range being the hosts that a plasmid may inhabit (De Gelder et al, 2007; Yano et al., 2013). Incompatibility groups determine in part the host range because bacteria that are hosts to certain plasmids will not be able to host plasmids from the same incompatibility group. Due to the shortness of time that the two plasmids from the same incompatibility group are in the same bacterium there is a lack of recombination between the two plasmids (Yano et al., 2013). This lack of recombination results in genetic
divergence which causes their host ranges to diverge from each other (Yano et al., 2013).

In terms of technical definitions for host range there are three: the range of hosts which the plasmid transfer to, the range of hosts which the plasmid can transfer to and replicate within, and the range of hosts which the plasmid can persist in the absence of selection (Yano et al., 2013; De Gelder et al., 2007). Here by selection, it is meant that a plasmids confers a beneficial effect on its host. Each of these definitions is a narrower definition than the previous in the list. The second of which is what will be used in my thesis.

To conclude, the opportunity for specialization occurs within biofilms due to spatial structure. Spatial structure allows for co-evolution between a plasmid and its host. This co-evolution can take several forms such as a plasmid confers a benefit to the host so that fitness is overall increased, or the plasmids takes over a chromosomal function such that fitness is maintained. In my thesis, the specialization model focuses on when the plasmid confers a benefit to its host but it also allows for instances where fitness is not increased or decreased. Furthermore, in the context of the mechanism that allows a plasmid to confer a benefit to one host can be absent when interacting with another host such that the plasmid is costly.

5 Theoretical Literature

Stewart and Levin (1977, 1979) produced a series of papers that is the basis of all theoretical work on plasmids. Their first paper focused on conjugative plasmids and what the required level of conjugation is needed to overcome segregational loss. They found that there is a broad range of parameters (conjugation rate, death, and cell division) where conjugative plasmids could be maintained. Nevertheless, there were concerns if the range of conditions that they studied were
biologically possible and therefore the maintenance of conjugative plasmids without selection remained uncertain. To help mitigate questions about the applicability of their 1977 model, Levin et al. (1979) conducted an empirical study of their model. They found that a plasmid could persist according to the predictions made by their previous models.

Van der Hoeven (1985) investigated the population dynamics of two or more competing plasmids from the same incompatibility group in a chemostat environment and a feast and famine environment based on Stewart and Levin (1977). Van der Hoeven (1985) found that two plasmids could be maintained but not three or more. If three or more plasmids were involved, the two plasmids with the highest and lowest conjugation rate respectively were maintained in the population as long as bacteria with the lower conjugation rate had a higher intrinsic growth rate. When plasmids were compatible two and three different plasmids could coexist; in fact one plasmid can facilitate the existence of another plasmid.

The first paper that modeled the effects of PSK systems was Mongold (1992). It was based off the model by Stewart and Levin (1977). Mongold (1992) found that PSK systems could only be maintained under unrealistically high conjugation rates. Furthermore, Mongold found no support that killing segregants is the selective pressure that allowed for the evolution of PSKs. Mongold (1992) often found no difference between with the work of Van der Hoeven (1985), in terms of equilibria which lead her concluded that PSKs are artifacts of gene transfer from chromosomes. Furthermore, Mongold stated there are two conditions for PSK to persist optimally. The first condition is when the competing plasmids are incompatible. Incompatibility increases the segregation rate thus allowing for PSK+ to have higher fitness than PSK- plasmids. The second condition is the presence of surface exclusion, such that conjugation is inhibited (Achtman et al., 1977). PSK+ plasmids that don’t conjugate offer no benefits. In general, Mongold was unable to find a clear
answer to the evolutionary benefits of PSK+ plasmids

Bergstrom et al. (2000) re-examined the Stewart and Levin (1977; 1979) models with the inclusion of chromosomals and showed that plasmids cannot coexist with plasmid free bacteria unless there was a hitchhiking effect. This was because if the cost of plasmids was high, plasmids would be segregated out of the population, and if a plasmid was beneficial it would become a chromosomal since it loses the cost of maintenance. The hitchhiking effect occurs when a plasmid conjugates with a passing bacterium which goes to a new location where the plasmid does not exist and this must repeatedly happen in order to maintain the plasmid. Bergstrom proposed a criterion that would allow for the maintenance of plasmids based on the study by Stewart and Levin (1977) which was the rate of conjugation was always less than the combined effect of selection against the plasmid and segregation.

Mochizuki et al. (2006) added the element of spatial structure to the model by Mongold (1992). They found that PSK systems can be maintained with spatial structure, but could not coexist with other plasmids, such that maintenance was bistable. The research found that in the absence of spatial structure PSK systems could coexist with other plasmids in a narrow range. This research did not include the concept of chromosomals, a cost on the growth of the bacteria, and surface exclusion. The most important element that the Mochizuki et al. (2006) found was that even at low population densities PSK+ plasmids can invade in the presence of a competitor which was not the case in Mongold (1992) and this was observed with biologically relevant transfer rates. The transfer rates or conjunction rates in a structured environment need only be higher than the cost of the PSK+ plasmid for PSK+ to establish themselves. This made Mochizuki et al. (2006) the first to showed PSK+ plasmids can be established in realistic conditions.

Bergstrom et al. (2000) was re-examined by Lili et al. (2007) which came to the opposite
conclusion. Lili et al. (2007) also argued against the criteria made by Bergstrom et al. (2000). This was due to Lili et al. (2007) assuming that certain variables such as cost and segregation were not constant as Bergstrom et al. (2000) assumed. But they also found even when cost and segregation remained constant the criteria from Bergstrom et al. (2000) did not hold true all of the time. They found that there is a possibility of plasmids being maintained outside of the hitchhiker effect that was proposed by Bergstrom et al. (2000).

Hill et al. (2016) examined the effect of source sink dynamics on plasmid persistence. They modeled two strains of bacteria, in which one strain acted as the source while the other acted as the sink. The strain of bacteria that acted as a sink would continuously lose the plasmid through segregation. The model did not include PSK systems but found that plasmids could be maintained in both strains of bacteria. The idea of a source and sink is analogous to the specialization of plasmids to a bacterial strain, in which the plasmid has specialized in one bacterial strain making it the source of the plasmid.

More recently, Lopatkin et al. (2017) examined how several plasmids interact with several different bacterial strains including the presence of antibiotic resistance. The study mainly focused on empirical measurements of plasmid persistence since this has been lacking in the literature. They found that the different plasmids were able to coexist in a well mixed environment. Coexistence also depended on the continued existence of the different species of bacteria.

Zwanzig et al. (2019) discussed the idea of compensatory evolution as a model of plasmid maintenance. Compensatory evolution is an idea that has gotten quite popular in the last several years with plasmid biologists which states that the chromosome of the bacteria or the plasmid itself undergoes mutation to reduce the cost that the plasmids confers on the bacteria. This paper examined which method of compensatory evolution is more likely to lead to the maintenance of the
plasmid especially when the plasmid is not beneficial to the bacteria at the beginning. Again, this research did not consider PSK effects on plasmid maintenance. They theorized that mutations to either the chromosome or the plasmid would have different end results. It turns out that mutations on the plasmid allowed for the plasmid to persist better in a population as the benefit against antibiotics is reduced. The mutations on the chromosome allowed for two different strains of bacteria, differentiated by the mutation, to persist. But no coexistence between different plasmids was found.

What ends up becoming apparent from this history of models is that each model covers one thing that another model lacked but in turn lacks something that previous models had. Many of the early models were dogged by the fact that conjugation was too low to sustain plasmids which led to the discussion about whether plasmids could possibly be maintained in bacteria at all (Bergstrom et al., 2000; Lili et al., 2007) this is because the rate of segregation inhibited the maintenance of the plasmids. Also, no model that investigates PSK systems has yet found an ability for PSK systems to coexist with other plasmids that do not contain a PSK system. The closest was Mochizuki et al. (2006) who found that PSK+ systems could coexist with PSK- with other plasmids in the absence of spatial structure in a small range. It should be reiterated that spatial structure is likely immensely important since most bacteria, especially those involved in antibiotic resistance, are found in biofilms which are spatially structured environments and antibiotic resistance is linked with PSK (Yang and Walsh, 2017). Biofilms have spatial structure unlike the well mixed medium that is assumed in many of the models. There also seems to be a lack of multi-plasmid models; of the ones that exist the Van der Hoeven (1985) model and the Hill et al. (2016) model do not include post-segregation killing systems. There are several more models but they are not as important because they focus on other aspects such as how TA systems evolved (Rankin et al., 2012), or are
only PSK- plasmids population persistence that are not critical for this research. There are several major gaps still left in the literature, one of which is how do PSK+ and PSK- plasmids coexist in biofilms and the other major question is how does specialization affect PSK+ plasmid dynamics.

**Part II**

**Models**

There are two models that are explored in this thesis. In both models there are two competing plasmids: PSK+ plasmid and PSK- plasmids. Furthermore, both models use the spatial modeling framework of Mochizuki et al. (2006). In the first model, I extend the Mochizuki et al. (2006) model by adding the possibility that there are plasmidless bacteria. The addition of plasmidless bacteria gives rise to the possibility that either a PSK- plasmid can be lost via segregate to the plasmidless state. The second model is a specialization model, such that there are two strains of bacteria and PSK- and PSK+ plasmids each specialize to one of the bacterial strains, respectively.

For both models there are several assumptions that remain constant. The first assumption is that the Post-Segregation Killing systems effects are 100% effective, such that if a daughter cell does not inherit PSK+ from the PSK+ parent, then the daughter cell dies. Furthermore, it is assumed there are no chromosomals. In both models it is assumed bacteria are living in a biofilm and therefore inhabiting a spatially structured environment. The spatial structure limits the interactions of a site to be between its neighboring bacteria. It is also assumed for both models that a PSK+ plasmid will overtake a PSK- plasmid bearing bacteria when the PSK+ plasmid conjugates into it due to properties of the PSK+ plasmid (Cooper and Heinenmann, 2000). By overtake, it is
meant that a bacteria that has both plasmids in it acts as if it only has a PSK+ plasmid under the assumption that PSK- is quickly lost from the bacteria via segregational loss. It is assumed that the number of plasmids in a bacterium is one immediately prior to DNA replication and cell division, which has been seen in nature (Volante et al., 2015). The plasmids are assumed to be from different incompatible groups.

6 Spatial Framework for the Biofilm

The spatial structure of a biofilm is captured using the doublet density pairs and conditional probability model of Sato et al. (1994) and Mochizuki et al. (2006). The combination of these two concepts approximate a grid that is placed on a torus with an infinity number of spaces.

Each space on the grid has four nearest neighbour spaces (n.n.). The number of n.n. is represented as $z$. These spaces each contain a site that can interact with an adjacent site (Fig. 2). These interactions can include birth, conjugation, as well as segregation. Birth increases the number of bacteria only when the mother cell is next to a vacant site. Conjugation only occurs between bacteria that are next to each other in the grid. Segregation is a constant rate but is associated with the birth of the cells.

To begin, the Sato et al. (1994) model will be presented to form a basis of understanding before presenting the extension of the Mochizuki et al. (2006) model and the specialization model. Sato et al. (1994) presents a spatial model of infectious disease in which there are three states for a cell on an infinite grid: vacant (0), occupied by a healthy host (+), and occupied by an infected host (-) which is represented by the set $S=\{0,+,\cdot\}$. Each of these states are called singlets ($\sigma$) such that $\sigma \in S$ and their density is $\rho_\sigma$. Each one of these singlets undergo a transition. Healthy and infected individuals undergo the transition,
A transition between singlets and doublet densities is given by

\[ +,- \rightarrow 0 \text{ at rate of } d, \]

where healthy and infected individuals turn into vacant sites at death rate at rate d. Vacant sites can become healthy individuals such that

\[ 0 \rightarrow + \text{ at rate of } m_{+} q_{+}/0, \]

where a vacant site turns into a healthy host at a rate of a healthy host’s birth rate of a healthy individual. For this to occur a vacant site needs to be next to a healthy host, which occurs with the probability \( q_{+}/0 \) and the healthy host then needs to give birth into the vacant site. Healthy individuals can become infected, such that

\[ + \rightarrow - \text{ at rate of } m_{-} q_{-}/+, \]

which corresponds to a healthy host turning into an infected host. For this to occur a healthy host needs to be next to an infected host with the probability \( q_{-}/+ \) and then be infected at rate \( m_{-} \).

Doublet density pairs are built off the combination of two singlets and the interactions of those singlets with each other and with their respective n.n. Building off the singlet densities, the doublet densities are \( \rho_{\sigma'\sigma} \) where \( \sigma' \) is the n.n. to \( \sigma \) and is also an element of \( S \), and it is assumed that \( \rho_{\sigma'\sigma} = \rho_{\sigma\sigma'} \). In Sato et al. the doublet pairs give rise to a system of six differential equations that govern the dynamics of doublet-pair densities.
As indicated by the \( q \) terms, conditional probability is a key aspect of the models. Conditional probability is used to determine the chance that \( \sigma' \) is interacting with another site that is not part of the focal doublet pair. In Sato et al. (1994) and Mochizuki et al. (2006) this is \( q_{\sigma''/\sigma'} \) which is read as the probability that a randomly chosen n.n. to \( \sigma' \) is \( \sigma'' \). \( q_{\sigma''/\sigma'} = \frac{\rho_{\sigma'' \sigma'}}{\rho_{\sigma'}} \) where the denominator is \( \rho_{\sigma'} = \sum_{\sigma'' \in S} \rho_{\sigma' \sigma''} \). Due to one of the neighbouring sites being occupied (doublet pairs) all \( q_{\sigma''/\sigma'} \) are scaled with \( \frac{z-1}{z} \) or \( \frac{3}{4} \) in these models since the number of neighbouring site is 4. Any interaction that \( \sigma' \) with it’s doublet paired site \( \sigma \) scaled with \( \frac{1}{z} \) or \( \frac{1}{4} \) due to it occupying 1 of the four neighbouring sites. There is also \( q_{\sigma''/\sigma' \sigma} \) where \( \sigma'' \) is another randomly chosen n.n. to \( \sigma' \). See figure 2 for an illustration of a section of the graph.

To aid further understanding, I will delve into an example from Sato et al. (1994), for the doublet density \( \rho_{+-} \). A doublet pair +- can change into a doublet pair \( \rho_{--} \). The probability individual is replaced by an offspring outside of the focal pair is 3/4 and the probability this interaction is with the type - given the +- focal pair is \( q_{-/+} \). Together with the rate of birth of - individual (\( m_- \)), the rate of change from the \( \rho_{+-} \) state to the \( \rho_{--} \) state is \( \frac{3}{4} q_{-/+} m_- \). It is also possible, in this setting for the - individual within the focal +- pair to give rise to an offspring that replaces the + individual. Following a similar logic as previously, this occurs at rate \( \frac{1}{4} m_- \). Either of the sites could die such that one changes to the state 0, and this occurs at the rate \( d \) per individual . Finally, the +- state is created when either a 0 in a -0 pair is adjacent to a +, which gives birth to an individual that replaces the zero, or a + in a ++ pair is adjacent to a - individual, which gives birth to - offspring that replaces the + individual. Together, these changes give rise to a differential equation for \( \rho_{+-} \), namely:

\[
\frac{d\rho_{+-}}{dt} = -[2d + m_- \{ \frac{1}{4} + \frac{3}{4} q_{-/-} \}] \rho_{+-} + \frac{3}{4} m_+ q_{+/-0} \rho_{-0} + \frac{3}{4} m_- q_{-/+0} \rho_{++}
\]

Please note that \( q_{\sigma''/\sigma' \sigma} \) where \( \sigma'' \) is another n.n. to \( \sigma' \) in the Sato paper can be simplified to
Figure 2: Each black cell is a space in the spatial structure that is filled with an element of S. The focal pair of spaces are $\sigma$ and $\sigma'$ which make up the density of $\sigma''$. Conditional probability only involves one of the focal pair where the identity of the spaces are unknown but each space interacts with the other.
$q_{\sigma''/\sigma'}$ (Mochizuki et al., 2006). So the above equation from Sato et al. (1994) can be simplified to:

$$\frac{d\rho_{++}}{dt} = -[2d + m_- \{ \frac{1}{z} + \frac{z-1}{z}q_{-/+} \}]\rho_{+-} + \frac{z-1}{z} m_+ q_{+/-0} \rho_{-0} + \frac{z-1}{z} m_- q_{-/+} \rho_{++}$$

7 Mochizuki Extension

There are four different states of a site in the Mochizuki extension: vacant (V), occupied by a plasmidless bacteria (0), a bacteria that contain a plasmid that is PSK- (1), and a bacteria that contains a plasmid that is PSK+ (2), such that $S=\{V,0, 1, 2\}$. These four states result into 10 different doublet pair densities (Fig.3). This differs from the Mochizuki model by allowing for the plasmidless state.
<table>
<thead>
<tr>
<th></th>
<th>Vacant (V)</th>
<th>Plasmidless (0)</th>
<th>PSK- (1)</th>
<th>PSK+ (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacant (V)</td>
<td>VV</td>
<td>V0</td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>Plasmidless (0)</td>
<td>00</td>
<td>01</td>
<td>02</td>
<td></td>
</tr>
<tr>
<td>PSK- (1)</td>
<td></td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>PSK+ (2)</td>
<td></td>
<td></td>
<td></td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 3: A list of the doublet pairs in the Mochizuki Extension Model and what each letter stands for. The doublet pairs are located in the upper triangle.
It is assumed that the conjugation rate, the cost, birth rate, death rate, and the segregation rate for PSK- and PSK+ plasmids are the same. PSK- and PSK+ plasmids differ in PSK+ plasmids can induce the death of a plasmidless daughter cell. In addition PSK+ and PSK- plasmids differ when PSK+ plasmid can conjugate into PSK- bacterium turning the bacterium into PSK+ bacterium but the reverse is not true. The states of this model can turn into other states as outlined below, which form the basis of a system of nonlinear differential equations that give the dynamics of doublet pair densities (Fig. 4). A list of variables presented in Fig. 4 and used below can be found in Table 1.
Figure 4: A diagram of how each state can transfer into an other state without the conditional probability. The types of sites are in the circles while the arrows indicate the direct interaction with the rate of change written along the arrow. The rate of change is a function of a combination of the parameters, death rate ($d$), cost ($c$), segregation rate ($\delta$), birth rate ($b$), and conjugation ($m$).
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho_i$</td>
<td>The density of $i$, which is an element of ${V,0,1,2}$ for the Mochizuki Extension model and element of ${V,A_0,A_1,A_2,B_0,B_1,B_2}$ for the Specialization model</td>
</tr>
<tr>
<td>V</td>
<td>A vacant site</td>
</tr>
<tr>
<td>0</td>
<td>A plasmidless bacteria</td>
</tr>
<tr>
<td>1</td>
<td>A bacteria that has a PSK- bacteria</td>
</tr>
<tr>
<td>2</td>
<td>A bacteria that has a PSK+ bacteria</td>
</tr>
<tr>
<td>$A_i$</td>
<td>A strain A bacteria in $i$ state</td>
</tr>
<tr>
<td>$B_i$</td>
<td>A strain B bacteria in $i$ state</td>
</tr>
<tr>
<td>$m$</td>
<td>The rate of conjugation (hour$^{-1}$)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>The rate of segregation loss (hour$^{-1}$)</td>
</tr>
<tr>
<td>$b$</td>
<td>The rate of birth (hour$^{-1}$)</td>
</tr>
<tr>
<td>$d$</td>
<td>The rate of death of a bacteria (hour$^{-1}$)</td>
</tr>
<tr>
<td>$c$</td>
<td>The cost of carrying a plasmid in the Mochizuki Extension Model</td>
</tr>
<tr>
<td>$c_i$</td>
<td>The cost of carrying a plasmid in the Specialization model where $i$ is an element of ${V,A_0,A_1,A_2,B_0,B_1,B_2}$</td>
</tr>
<tr>
<td>$z$</td>
<td>Is the number of neighbour sites next to an individual site</td>
</tr>
</tbody>
</table>

Table 1: Definitions Table of Variables.
Vacant sites can change into any other state by birth at rate \( b \) and adjusted by the conditional probability that the vacant site is next to one of the other states. So, for example, a vacant site can only become a plasmidless bacteria if that vacant site is next to a site that contains a plasmidless bacteria. It is assumed that birth rate (\( b \)) are equal across bacteria whether they have a plasmid or not. Overall, this gives rise to the following transitions,

\[ V \rightarrow 0, 1, 2, \text{ at rate } q_{j/0}b, \]

where \( j \) is an element of \( \{0, 1, 2\} \). The plasmidless bacteria can change into a vacant site by death at rate (\( d \)), such that,

\[ 0 \rightarrow V, \text{ at rate } d \]

Plasmidless bacteria change into either a bacteria with a PSK- plasmid or a PSK+ plasmid at a rate of conjugation known as \( m \) which is adjusted by the conditional probability that the plasmidless bacteria is next to one of those two states, such that

\[ 0 \rightarrow 1, 2 \text{ at rate } q_{i/0}m, \]

where \( i \) is an element of \( \{1, 2\} \). A bacteria that hosts PSK- plasmids can become a plasmidless bacteria at a segregation rate \( \delta \). It can turn into a vacant site at the rate of \( d+c \) where \( c \) is an additional cost of the plasmid that potentially increases its rate of mortality. A PSK- plasmid can become a bacteria that contains PSK+ by migration at a rate of \( m \), conditional on the probability a bacteria with a PSK- plasmid is next to a bacteria with a PSK+ plasmid. It is assumed that a PSK+ plasmid will out compete the PSK- plasmid following the assumption of Mochizuki et al. (2006). Overall, for state 1, the possible transitions and their rates are.
1→0, at rate $\delta$,

1→V at rate $d+c$,

1→2 at rate $q_{2/1}m$.

A bacteria with PSK+ plasmids can only become a vacant site at a combined rate of $d$, $c$, and $\delta$, such that

$$2\rightarrow V \text{ at rate } d+c+\delta.$$  

Using the above transition rates, a system of differential equations for the doublet pairs densities were derived. For example, the differential equation for the doublet-density of the pair $V1$ is

$$\frac{d\rho_{v1}[t]}{dt} = d\rho_{01}[t] + 2(c+d)\rho_{11}[t] + (c+d+\delta)\rho_{12}[t] + m\rho_{v0}[t] \left( \frac{3\rho_{01}[t]}{4(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{v0}[t])} \right) + 2b\rho_{vv}[t] \left( \frac{3\rho_{v1}[t]}{4(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vv}[t])} \right) - \rho_{v1}[t] \left( \frac{b}{4} + c + d + \delta \right)$$

$$- m\rho_{v1}[t] \left( \frac{3\rho_{12}[t]}{4(\rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{v1}[t])} \right) - b\rho_{v1}[t] \left( \frac{3\rho_{v0}[t]}{4(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vv}[t])} \right)$$

$$- b\rho_{v1}[t] \left( \frac{3\rho_{v1}[t]}{4(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vv}[t])} \right) - b\rho_{v1}[t] \left( \frac{3\rho_{v1}[t]}{4(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vv}[t])} \right)$$

Walking through this example, in the first line of this equation the death of a 0 state in a 01 pair will make the pair into a $v1$ pair. The death with addition of the cost of hosting a plasmid of either 1 state in a 11 pair, hence the 2 in front of the term, well create a $v1$ pair. While the segregation and death with the cost of maintenance of the plasmid of a 2 state in a 12 pair will also result in
a V1 pair due to segregation in a 2 state is the equivalent of a death. The next term is that the proportion of V1 state increases due to a PSK- plasmid conjugating into a 0 state in the V0 pair, this is conditioned with the probability that the 0 is next to a 1 that is the density o 01 state divided by all the possible combinations that the 0 state could be in and is multiplied by 3/4 due to that being the proportion of free available sites assuming that the simulation is occurring over a neat grid pattern across a torus.

The first term in the second line of the example equations is the rate of birth of a PSK+ into the vacant site of a VV pair which is conditioned by the probability of one of the two vacant sites, the reason for why it is doubled, to be next to a 1. The next term is the decline in the V1 pair through various means, either the birth of the 1 into the vacant site of the pair, the segregation of the 1 in the pair, or the death of the bacteria in the pair.

On the third line, it is the decrease of the V1 pair by the conjugation of a PSK+ plasmid into the bacteria that is hosting the PSK- plasmid based on the conditional probability of the PSK- bacteria being next to a PSK+ bacteria. As stated earlier, a bacteria that ends up with both types of plasmids should be treated effectively as a PSK+ bacteria due to the biological properties of a PSK+ plasmid. The second term on the third line is the birth of a plasmidless bacteria into the vacant site on the conditioned on whether the vacant site is next to a plasmidless bacteria which consequently decreases the proportion of V1 pairs in the whole population.

The last line is quite similar to the second term on the third line, first it is the birth of a PSK- plasmid carrying bacteria into the vacant site conditioned on whether the vacant site is next to a bacteria hosting a PSK- plasmid, and the last term is the birth of a PSK+ plasmid carrying bacteria into the vacant site of the pair conditioned on the probability of a PSK+ bacteria being next to the vacant site. Both of these terms in the last line decrease the density of the V1 pair.
Please see the Appendix A for the full list of equations for this model.

### 7.1 Simulation of the Mochizuki Extension

Simulation of the Mochizuki Extension was divided into three phases (Fig. 5). In the first phase, the population started with a completely vacant grid and a plasmidless bacteria was added to the grid as a V0 pair at a density of $10^{-7}$. The system of differential equations was run for $10^9$ time steps for a set of parameters $c$, $d$, $b$, $\delta$, and $m$. After this phase of initialization, which gives rise to an equilibrium density for the 0 state, the PSK- bacteria was added into the population as a 01 pair at a density of $10^{-7}$ and assuming the PSK- bacteria replaced plasmidless bacteria, such that the density for 00 pairs is reduced in density by a corresponding amount of $10^{-7}$. The system was again run for $10^9$ time steps to ensure a new equilibrium is reached. Next, PSK+ plasmids were added into the population as a 12 pair at a density $10^{-7}$ and with a corresponding reduction in the density of a 11 pair, which corresponds to the assumption that the PSK+ plasmid arise via a form of mutation from the PSK- state. The system of differential equations was run for another $10^9$ time steps to determine the equilibrium state of the system for a parameter set. The final step of this process was not run if PSK- plasmids were unable to establish themselves in the population in the second phase of the initialization.
8 Specialization

In the specialization model there are two strains of bacteria. One is the A genotype while the other is the B genotype. The PSK- plasmid being less costly in the A genotype than in the B genotype, while the PSK+ plasmid is less costly in the B genotype than in the A genotype (Fig. 6). The plasmids have different fitness costs in different host strains due to the plasmids being more specialized in one strain versus the other. A plasmid can conjugate into any of the bacterial strains. This creates nearly more than double the amount of equations than the Mochizuki Extension model with 28 equations. In this model, the genotype is annotated with the state the bacteria is in; with either 0, 1, or 2 (vacant sites are still just vacant) to read as the following: \{V, A_0, A_1, A_2, B_0, B_1, B_2\} with each being part of a doublet pair (Fig. 7). A graphical view of interactions between the states can be found in Figure 7. A chart of variables and their definitions is found in Table 1.
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$w_1$ =</td>
<td>$w_1$</td>
</tr>
<tr>
<td></td>
<td>$\land$</td>
<td>$\lor$ $\land$</td>
</tr>
<tr>
<td>1</td>
<td>$w_2$ &gt;</td>
<td>$w_3$</td>
</tr>
<tr>
<td></td>
<td>$\lor$ $\land$</td>
<td>$\land$</td>
</tr>
<tr>
<td>2</td>
<td>$w_4$ &lt;</td>
<td>$w_5$</td>
</tr>
</tbody>
</table>

Figure 6: The fitness that are required to find coexistence in the specialization model. Each $w$ is the realized fitness for the plasmid state in the bacterial strain.
<table>
<thead>
<tr>
<th></th>
<th>Vacant (V)</th>
<th>Strain A Plasmid-less (A0)</th>
<th>Strain A PSK- (A1)</th>
<th>Strain A PSK+ (A2)</th>
<th>Strain B Plasmid-less (B0)</th>
<th>Strain B PSK- (B1)</th>
<th>Strain B PSK+ (B2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacant (V)</td>
<td>VV</td>
<td>VA0</td>
<td>VA1</td>
<td>VA2</td>
<td>VB0</td>
<td>VB1</td>
<td>VB2</td>
</tr>
<tr>
<td>Strain A Plasmid-less (A0)</td>
<td>A0A0</td>
<td>A0A1</td>
<td>A0A2</td>
<td>A0B0</td>
<td>A0B1</td>
<td>A0B2</td>
<td></td>
</tr>
<tr>
<td>Strain A PSK- (A1)</td>
<td></td>
<td>A1A1</td>
<td>A1A2</td>
<td>A1B0</td>
<td>A1B1</td>
<td>A1B2</td>
<td></td>
</tr>
<tr>
<td>Strain A PSK+ (A2)</td>
<td></td>
<td>A2A2</td>
<td>A2B0</td>
<td>A2B1</td>
<td>A2B2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain B Plasmid-less (B0)</td>
<td></td>
<td>B0B0</td>
<td>B0B1</td>
<td>B0B2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain B PSK- (B1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B1B1</td>
<td>B1B2</td>
<td></td>
</tr>
<tr>
<td>Strain B PSK+ (B2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B2B2</td>
</tr>
</tbody>
</table>

Figure 7: A list of the doublet pairs in the Specialization Model and what each letter stands for. The top row and first column describe the state while the upper triangle is the doublet pairs.
Figure 8: The direct interactions between the seven different states in the Specialization Model. The states are inside the circles while lines connecting circles indicate a transition from one state to the other in the direction of the arrow at the rate indicated by either the conjugation rate \(m\), segregation \(\delta\), death rate \(d\), cost \(c_{A1}, c_{A2}, c_{B1}, c_{B2}\), birth rate \(b\) or some combination of them.
Vacant sites can become any other state by birth on the condition that it is next an occupied site. So for example a vacant site can only turn into a A genotype bacteria with a PSK- plasmid ($A_1$) if the vacant site is adjacent to an $A_1$. Therefore the transition of a vacant site to an occupied site is,

$$V \rightarrow A_0, A_1, A_2, B_0, B_1, B_2, \text{ at rate } q_j/v, b,$$

where $j$ is an element of \{A$_0$, A$_1$, A$_2$, B$_0$, B$_1$, B$_2$\}. A plasmidless A genotype can become an A genotype with a PSK- plasmid or a PSK+ plasmid at a conjugation rate $m$ on the condition that the $A_0$ state is next to a bacteria of any genotype with that plasmid. This also means a plasmidless A genotype bacteria can become a PSK+ plasmid carrying bacteria with the A genotype if the plasmidless A genotype bacteria is next to either a B genotype bacteria carrying the PSK+ plasmid or an A genotype bacteria carrying a PSK+ plasmid. Thus the rate of transition is

$$A_0 \rightarrow A_1, A_2, \text{ at rate } q_i/A_0 m,$$

where $i$ is an element of \{A$_1$, A$_2$, B$_1$, B$_2$\}. A plasmidless B genotype can become a B genotype with a PSK- plasmid or a PSK+ plasmid at a conjugation rate $m$ on the condition that the $B_0$ state is next to a bacteria of any genotype with that plasmid. This means that there is a chance for a plasmidless B genotype bacterium to become a B genotype bacterium hosting a PSK- plasmid if the plasmidless B genotype bacterium is next to either a B genotype bacterium that is carrying the PSK- plasmid or an A genotype bacterium that is carrying the PSK- plasmid in proportion to the conjugation rate. Thus a plasmidless B genotype acquires plasmids at the rate of
$B_0 \rightarrow B_1, B_2$, at rate $q_i/B_0 m$,

where $i$ is still an element of $\{A_1, A_2, B_1, B_2\}$. A plasmidless A genotype bacterium becomes a vacant site at the rate of death. A plasmidless B genotype bacterium becomes a vacant site at the rate of death. Thus the transition of plasmidless bacterium to a vacant site is

$$A_0, B_0 \rightarrow V, \text{ at rate } d.$$

An A genotype with a PSK- plasmid can become an A genotype with a PSK+ plasmid at the rate of conjugation ($m$) on the condition that it is found next to a bacteria of either genotype with the PSK+ plasmid. Thus the transition for an A genotype with a PSK- plasmid to one with a PSK+ plasmid is

$$A_1 \rightarrow A_2, \text{ at rate } q_k/A_1 m,$$

where $k$ is an element of $\{A_2, B_2\}$. An A genotype bacterium carrying a PSK- plasmid can become a plasmidless A genotype bacterium at a segregation rate of $\delta$. Thus the transition from a PSK- A genotype to plasmidless is

$$A_1 \rightarrow A_0, \text{ at rate } \delta.$$

An A genotype bacterium that contains a PSK- plasmid becomes a vacant site with a rate of $d$ plus the cost of a PSK- plasmid in an A genotype. Therefore the rate of transition is
$A_1 \rightarrow V$, at rate $d+cA_1$.

A B genotype bacteria hosting a PSK- plasmid takes on a PSK+ plasmid a conjugation rate ($m$) on the condition it is found next to a bacterium of either genotype with the PSK+ plasmid. The transition of $B_1$ state to $B_2$ is similar to $A_1$ to $A_2$,

$B_1 \rightarrow B_2$, at rate $q_{k/B1}m$,

where $k$ is an element of $\{A_2,B_2\}$. A PSK- plasmid that is contained within a B genotype bacterium becomes a plasmidless B genotype bacterium at a $\delta$. Thus like the segregation of PSK- plasmids from the B genotype is the similar to the segregation of PSK- plasmids from the A genotype,

$B_1 \rightarrow B_0$, at rate $\delta$.

A B genotype bacterium that hosts a PSK- plasmid can become a vacant site at a rate of death plus the cost of carrying a PSK- plasmid in a B genotype bacterium. The death of PSK- B genotype is similar to the PSK- A genotype,

$B_1 \rightarrow V$, at rate $d+cB_1$.

A site that contains an A genotype bacterium that hosts a PSK+ bacteria can become a vacant site at a rate of death plus $\delta$ plus the cost of carrying a PSK+ plasmid in a bacteria that has the A genotype. A B genotype bacterium that has the PSK+ plasmid can become a vacant site at a rate
of death plus $\delta$ plus the cost on a bacteria with the B genotype from carrying the PSK+ plasmid. Thus the rates of transition of PSK+ bacteria to vacant sites are

$$A_2 \rightarrow V, \text{at rate } d + c_{A2} + \delta,$$

$$B_2 \rightarrow V, \text{at rate } d + c_{B2} + \delta.$$}

One thing to note is that a genotype of a site in the grid can not directly change into the other genotype in this model unless it dies and becomes a vacant site first.

The model is then made up of 28 possible doublet pairs each with their own differential equation (Fig. 8). I will walk through an example equation of the model for the doublet density of $V_{B0}$ (For rest of the equations please see Appendix B).
\[
\frac{d}{dt} \rho_{vB0}[t] = 2d \rho_{BOB0}[t] + d \rho_{A0B0}[t] + (c_B + d) \rho_{BOB1}[t] + (c_A + d) \rho_{A1B0}[t] \\
+ (c_B + d + \delta) \rho_{BOB2}[t] + (c_A + d + \delta) \rho_{A2B0}[t] + \delta \rho_{vB1}[t] - d \rho_{vB0}[t] \\
+ 2b \rho_{VV}[t] \frac{3}{4} \left( \rho_{VV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) \\
\]

The first few of lines in the example equation deal with non-conditional process. The first terms are the death of either B0 in a B0B0 pair which will result in a VB0 pair, and the death of an A0 in a A0B0 pair. The cost and death rate of the B1 in the B0B1 pair also results in the VB0 pair. The cost and death rate of the A1 in the B0A1 results in the VB0 pair. The cost, death rate and the segregation rate of the B2 in the B0B2 pair and the A2 in the B0A2 pair results in the VB0 pair. Segregation of the plasmid in the B1 in the vVB1 pair will result in the VB0 pair. The last
non-conditional interaction is the death of the B0 in the VB0 pair which reduces the density of the vB0 since the death of B0 result in a VV pair.

The rest of the equation which is all conditioned on what is around the vB0 pair. Each of the conditional probability terms are scaled by $\frac{3}{4}$ due to 3 of the four n.n to a site are unaccounted for. The first conditional term is the rate of birth of a B0 into a vacant spot in a vacant-vacant pair. This can occur in either V in the VV pair hence the scale of 2 in front of the term. This is also the only conditional probability interaction that increases the VB0 pair. The next four lines are the conditional probability that a plasmid enters the B0 in the vB0 pair. This plasmid can come from either an A strain bacteria or a B strain bacteria. The next six lines are bacteria of any state (plasmidless, PSK- plasmid bearing, PSK+ plasmid bearing) being born into the vacant space of the vB0 pair. The second last line has the interesting distinction of have $\frac{1}{4}$ in the line. This $\frac{1}{4}$ represent the chance that the B0 in the focal vB0 pair gives birth into the V site in the vB0.

8.1 Initialization of the Specialization Model

The simulation of the specialization model was initialized through three phases much like the Mochizuki Extension model (Fig. 9) except there are three different routes in phase three (Fig. 10). All simulations began with a completely vacant grid and then equal amount of plasmidless bacteria of the A and B genotypes were added. The density for $\rho_{vA0}$ and $\rho_{vB0}$ were both $10^{-7}$ while the rest of the sites were left vacant at the start of the first phase. The parameters were also set before the first stage with the costs of $c_{A1}$, $c_{A2}$, $c_{B1}$, and $c_{B2}$ in the form displayed in Table 2. The phase ran for $10^9$ time steps.
Figure 9: The initialization process for the Specialization Model. Phase one is the introduction of plasmidless bacteria from both strains into the system. Phase two is the introduction of PSK-plasmids into both strands. After the second phase the route splits into two different phase threes. The first is the invasion method where strain B bacteria carrying PSK+ plasmids are added to a system of strain A bacteria carrying PSK- plasmids. The second is mutation where Phase two is stopped at 17000 time steps and PSK+ plasmids are added into either Strain A bacteria or Strain B bacteria carrying PSK- plasmids akin to mutation.
Figure 10: The several different paths examined in the specialization model. The black chromosome is for strain A, the green chromosome is for strain B bacteria. The orange circle is a PSK- plasmid while the blue circle is for PSK+ plasmids. On the left hand side is how the invasion or free ideal distribution scenario was simulated. In the invasion scenario the strain B bacteria carrying the PSK+ plasmid invades the population. In the middle is the scenario where a mutation for PSK+ happens on the plasmid being carried by the strain A bacteria. On the right hand side is the scenario where a mutation for PSK+ happens on the PSK- plasmid being carried by the Strain B bacteria.
In the second phase the densities from the end of first phase of initialization were used with small modification to add PSK- plasmids to bacteria of both A and B genotypes. The starting densities of $\rho_{A0A1}$ and $\rho_{B0B1}$ were both reset to $10^{-7}$ from 0, and $10^{-7}$ was subtracted from the densities of $\rho_{A0A0}$ and $\rho_{B0B0}$ respectively to keep the total density at 1. The second phase of the simulation was allowed to run for another $10^9$ time steps except in the mutation third stage. If the PSK- plasmids in either strain of bacteria went extinct before the stage was completed, the densities were not passed onto the next stage and the simulation was moved onto the next parameter set. It should be noted that all of the runs no matter the parameters in the second phase of the initialization ended up with only the Strain A bacteria and the PSK- plasmid. This was because PSK- plasmids were less costly in the Strain A bacteria causing the Strain B bacteria to go extinct due to competition.

In third phase of the initialization, there were several different paths that were simulated (Fig. 10). One of the three paths is the invasion scenario which simulated an invasion of strain B bacteria with PSK+ plasmid moving into a population which only has PSK- plasmids and the A bacterial strain. The densities from the previous stage were taken and then adjusted by letting the PSK+ hosting B strain bacteria enter into the population by taking $10^{-7}$ of the total density from $\rho_{A1A1}$ to $\rho_{A1B2}$. Once the densities were set, the phase was run for $10^9$ time steps. The densities after the third phase are the results.

The other two paths for the third phase of initialization both involve mutation from the PSK- state to the PSK+ state. Here the second phase of the initialization was stopped when the density of B1 was at its maximum. Once stopped, one of the PSK- plasmids was “mutated” in either the A or the B strain bacteria to become a PSK+ plasmid. By mutation I mean that the plasmid has acquired the PSK system. This could occur through recombination, such that it is fairly instantaneous.
Therefore the density of either $\rho_{A1A2}$ or $\rho_{B1B2}$ depending on which strain was being mutated is $10^{-7}$. This density is taken from either $\rho_{A1A1}$ or $\rho_{B1B1}$ depending on what strain is hosting the mutated plasmids the rest of the densities were unchanged from the truncated second phase. Once the mutation occurred the simulation was allowed to run for $10^9$ time steps. The mutation scenario should be seen as a theoretical experiment allowing for the best chance of the establishment of PSK+ plasmids.

9 Parameters Values for the Models

To ensure the robustness of the results parameters used in this thesis are based off biologically motivated and relevant values (Table 2). The high birth rate (0.8 hour$^{-1}$) is based on Harrison et al. (2015) for planktonic bacteria. At the low end (0.11 hour$^{-1}$) is based on Evan et al. (1997), but still approximately 20 times larger than their lowest value, such that the birth rates that were studied are near the intermediate to high range for bacteria.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Range of Values</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$</td>
<td>Cost to the Bacteria due to Carrying a Plasmid</td>
<td>-0.006-0.06</td>
<td>Loftie-Eaton et al., 2015; Porse et al., 2016</td>
</tr>
<tr>
<td>$d$</td>
<td>Rate of bacterial death</td>
<td>0.009-0.031</td>
<td>Hall et al., 2016; Pace 1988; Servais et al., 1985</td>
</tr>
<tr>
<td>$b$</td>
<td>Rate of bacterial birth</td>
<td>0.11-0.8</td>
<td>Harrison et al., 2015; Evans et al., 1991; Kovárová-Kovar and Egli, 1998; Fan et al., 2017; Brown et al., 1990</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Rate of Segregational loss of the plasmid</td>
<td>$10^{-4}$-$10^{-2}$</td>
<td>Hall et al., 2016; Loftie-Eaton et al., 2015; Porse et al., 2016 Harrison et al., 2015</td>
</tr>
<tr>
<td>$m$</td>
<td>Conjugation Rate of Plasmid between bacteria</td>
<td>$10^{-14}$-$10^{-1}$</td>
<td>Hall et al., 2016; Porse et al., 2016; Zhong et al., 2012</td>
</tr>
</tbody>
</table>

Table 2: A table of parameter value ranges that were used in the simulations in this thesis as well as the sources for those parameter values.
Death rates range from 0.009 day\(^{-1}\) (Pace, 1988) to 0.031 hour\(^{-1}\) (Servais et al. 1985). I chose to keep everything in hour\(^{-1}\) with 0.009 hour\(^{-1}\) (Hall et al., 2016) being the low end so that the simulation would be completed by 10\(^9\) time steps.

The cost of carrying a plasmid on a bacteria is often reported in percentages relative to a wild type (Hall et al., 2016; Porse et al., 2016; Louftie-Eaton et al., 2015). The range of values is large, but a plasmid is more often detrimental to its host and when it is beneficial it is marginally so. The highest benefit was about -0.03\% of the wild type (Louftie-Eaton et al., 2015) while the largest cost by a value 0.132 (Porse et al., 2016) or 14\% relative to a standard (Louftie-Eaton et al., 2015) while in Hall et al., (2016) it is up to 0.27 reduction in growth rate. Most costs reduce growth rate by a value around 10\(^{-2}\) (Porse et al., 2016). I came to decide that the costs plus the death rate should not be larger than the lowest birth rate, which resulted at the maximum cost of 0.06. In principle, plasmids can be beneficial to their host and thus incur a negative cost. I choose a cost of -0.006 since it is an order of magnitude smaller than the largest cost, which is consistent with plasmids tending to be on average deleterious compared to beneficial in nature.

The segregation rate range is straightforward at about 10\(^{-4}\) - 10\(^{-2}\) hour\(^{-1}\). Since almost all segregation rates fall under that range I used them without any changes.

The conjugation rate ranged from 10\(^{-14}\) hour\(^{-1}\) from Hall et al. (2016) to 10\(^{-9}\) hour\(^{-1}\) from Zhong et al. (2012). The value from Hall was in the units of cells/hour. In order to use this value in the model it has to be converted to hour\(^{-1}\). To convert the units, requires a simple multiplication by the maximum number of cells in the system (Zwanzig et al., 2019) so I decided this should allow the highest conjugation rates to be around 10\(^{-1}\). Combine this with the fact that biofilms are known to increase the conjugation rate I feel confident with this range (Molin and Tolker-Nielsen, 2003, Hausner and Wuertz, 1999, Sørensen et al., 2005, Savage et al., 2013). This means high
conjugation rates could be interpreted as biofilms that have more cells and lower conjugation rates are biofilms with less cells.

10 Numerical Analysis

The numerical analysis for this thesis was conducted in Mathematica version 11.1.1.0. I used the NDSolve function. Both the Mochizuki Extension and the Specialization models involve conditional probabilities, consisting of a ratio of the probability of a doublet pair combination over the probability of a certain cell state. The probability of a certain cell state can go to zero leading to a singular point if numerical inaccuracy in the evaluation of the numerical system retains a non-zero numerator.

This issue is known as stiffness. In principle, the numerator of a conditional probability is always less than or equal to the denominator because the denominator is a sum that includes the numerator. Accordingly, in the limit, the conditional probability should go to a value of zero or one. In reality, numerical inaccuracies can lead to slightly negative doublet densities, which can result in a singular point when summed together.

To accommodate stiffness I used the Methods option of “StiffnessSwitching”, a numerical method specifically designed by Mathematica to detect and accommodate stiffness during numerical integration. Furthermore, I used a working precision of 30, as well as representing each doublet pair density as an infinite precision number, which converts decimal numbers to their nearest rational equivalent, and helps preserve numerical accuracy. Rationalizing numbers reduces stiffness in the system by reducing rounding errors in the denominator of the conditional probabilities as
the denominator closes in on zero. The combined options ensure the highest amount of numerical accuracy possible to ensure the robustness of the results.

Part III

Results

11 Mochizuki Extension Model

I performed a comprehensive analysis of the biologically relevant parameters outlined in Table 2 along a multi-dimensional grid. For example, I simulated all combinations of the value $m \times 10^{-14}$ to $10^{-1}$ at regular and informative intervals across that range. Similarly, I simulated $\delta$ from $10^{-4}$ to $10^{-2}$ at regular and informative intervals (See Appendix C for all values used).

Given the non-linear nature and stiffness of the system I added values at points that I saw most critical. The most important result in the Mochizuki Extension Model is that across the range of parameters tested, PSK+ plasmids were the only type of plasmid that persisted in the population, with two exceptions. The two exceptions were when coexistence occurred between PSK- and PSK+ at parameter combinations {$b=0.4$, $c=0.06$, $d=0.009$, $m=0.1$, $\delta=0.05$} and {$b=0.11$, $c=0.06$, $d=0.011$, $m=0.096$, $\delta=0.05$}. For both cases coexistence was very weak and conjugation rates were very high with the densities of the two points of coexistence being Vacant=11.93%, Plasmidless=85.18%, PSK-=2.22%, and PSK+=0.66% in the first case and Vacant=10.13%, Plasmidless=87.05%, PSK-=2.13%, and PSK+=0.69% in the second cases.

These points of coexistence are restricted (Fig. 11 & Fig. 12). Figure 11 relates to point ($b=0.4$, $c=0.06$, $d=0.009$, $m=0.1$, $\delta=0.05$). Figure 11 shows how changes in either the positive or negative
direction of a parameter effects the equilibrium. For example, it shows that a small decrease in \( m \) results in a temporary increase of PSK- plasmids. Perturbations in the other parameters are shown where N/A means that the parameter is outside the parameter range as described in Table 2 or the system encounters problems with stiffness. Figure 12 relates to point \((b=0.11, c=0.06, d=0.011, m=0.096, \delta = 0.05)\). Figure 12 is similar to Figure 11 in how it is constructed. Again when \( m \) is decreased there is a temporary increase in PSK- plasmids. Both points of coexistence are lost nearly immediately with any small change to the parameters.

After finding coexistence using the grid method, I then used a different approach to assess the prevalence of coexistence. In particular, I randomly choose a value for each parameter uniformly across the intervals in Table 2, and generated \( 10^5 \) replicate parameter sets. Out of \( 10^5 \) parameter sets, 9 points of coexistence were found in which coexistence was established in \( 10^7 \) time steps (Fig. 13). The points of coexistence were found with high conjugation and segregation rates as listed in Table 2 (Fig. 13A). The death and birth rates of these points of coexistence tended to be towards the lower end of the range described in Table 2 (Fig. 13B). The densities of PSK- and PSK+ plasmids at coexistence were found to be small, typically less than 4% (Fig. 13C). To assess the extent of coexistence, I choose one of these nine points of coexistence with the following set of parameter values: \( b=0.112446, c=0.079886, d=0.0192604, m=0.0987338, \delta = 0.0859077 \). At this point the densities for the different states were Vacant=18.26%, Plasmidless=78.81%, PSK-=2.06%, PSK+=1.54%. I then perturbed the parameters at 0.5% increments until coexistence was lost (Fig. 14). Coexistence at this point was quickly lost for most parameters except for the cost of carrying a plasmid.
Figure 11: A diagram depicting the outcomes of small perturbations to the coexistence found at point with the following parameters: $b = 0.4$, $c = 0.06$, $d = 0.009$, $m = 0.1$, $\delta = 0.05$. The left hand side depicts perturbations that lower the parameters. The right hand side depicts the outcomes of a perturbations that increase the parameters. On the far sides of the diagram is an indication of whether PSK+ or PSK- plasmids increased in their density. N/A means that any perturbation was either outside the studied parameters or the system encounter issues with stiffness.
Figure 12: A diagram depicting the outcomes of small perturbations to the coexistence found at point with the following parameters: $b = 0.11, c = 0.06, d = 0.011, m = 0.096, \delta = 0.05$. The left hand side depicts perturbations that lower the parameters. The right hand side depicts the outcomes of a perturbations that increase the parameters. On the far sides of the diagram is an indication of whether PSK+ or PSK- plasmids increased in their density. N/A means that any perturbation was either outside the studied parameters or the system encounter issues with stiffness.
Figure 13: Points of Coexistence between plasmids through the random choosing of parameters for the Mochizuki Extension model. a) The birth (horizontal axis) and death (vertical axis) rates of the points of coexistence that were found. b) The conjugation (horizontal axis) and the segregation (vertical axis) rates of the points of coexistence. c) The density of the PSK+ (horizontal axis) and PSK- plasmids (vertical axis) at the points of coexistence.
Figure 14: A diagram depicting the outcomes of perturbations to the coexistence found at point
with the following parameters: $b = 0.112446, c = 0.079886, d = 0.0192604, m = 0.0987338, \delta = 0.0859077$. The left hand side depicts the size of perturbation needed to lose coexistence between
the plasmids when the parameter is lowered. The right hand side depicts the size of a perturbations
needed to lose coexistence between the plasmids when the parameter is increased. On the far sides
of the diagram is an indication of whether PSK+ or PSK- plasmids increased in their density.
12 Specialization Model

12.1 Invasion of the PSK+ Plasmid into a Population

Within the central core of the parameter space described in Table 2 a region of strong coexistence was found: \( b=0.4 \), \( c_{A1}=-0.002 \), \( c_{B1}=0.0001 \), \( c_{A2}=0.0001 \), \( c_{B2}=-0.0018575 \), \( d=0.0090585 \), \( m=0.00001 \), \( \delta =0.0005005 \) (Table 3, parameter set 1) such that the frequency of the Vacant Sites was 0.16, \( A_0=0.182 \), \( A_1=0 \), \( A_2=0.393 \), \( B_0=0 \), \( B_1=0.001 \), and \( B_2=0.404 \). Here I report the equilibrium point when where the frequencies of PSK- and PSK+ were nearly even. In a similar manner as the previous section, I perturbed the parameters to assess the breadth of parameter space where coexistence between PSK- and PSK+ plasmids persisted (Fig. 15). Whereas in the Mochizuki Extension, coexistence was nearly immediately lost, with specialization coexistence persisted across a wider breadth of parameter space, such that in Figure 15 I depict the endpoints where coexistence was lost and whether PSK- or PSK+ becomes the only plasmid within the population. Figure 15 illustrates that the system retain coexistence for relatively large changes in \( m \), smaller but large changes in \( b \) and \( d \), and narrow changes in \( \delta \). Furthermore, although not depicted in Figure 15, relatively small changes in the magnitude in the costs resulted in the lost of coexistence such that an increase of 2\% or decrease of .3\% resulted in the lost of coexistence. A decrease in cost resulted in only PSK+ plasmids in the population and an increase in cost resulted in only PSK- plasmids in the population.

Furthermore, similar to the Mochizuki Extension, I generated \( 10^5 \) random parameter sets by sampling parameter values uniformly across the intervals defined in appendix D. These intervals were based on the initial instance of coexistence found for the specialization model discussed above such that the range sampled for each parameter was approximately an order of magnitude above and below the point of coexistence. Out of \( 10^5 \) parameter sets 2 points were found where
coexistence occurred in $10^7$ time steps (parameter sets 2 & 3, Table 3). Examining Table 3, each parameter, except for birth, can vary by an order of magnitude and coexistence can still be present. Moreover, similar to the first parameter set coexistence was found to be strong for parameter sets 2 and 3. For parameter set 2, the frequency of PSK- was 0.43, and for PSK+ was 0.41, and for parameter set 3, PSK- was 0.39 and for PSK+ was 0.36 at equilibrium.

Similar to figure 15, I perturbed parameters from parameter set 3 from Table 3 to assess the relative breath of the parameter space in which coexistence persists (Fig. 16). The perturbations to the parameters were done in increments of 0.5%. Figure 16 illustrates that the system retains coexistence for relatively large changes in the birth rate and the conjugation rate, and narrow changes for segregation rate and death rate.
Figure 15: A diagram of the relative breadth of parameter space for coexistence between PSK+ and PSK-. The intersection of the lines (with end bars) depicts the equilibrium in which PSK+ and PSK- attain high frequencies of $\rho_{PSK^+}=0.39$ and $\rho_{PSK^-}=0.40$ ($b=0.4, d=0.0090585, c_{A1}=-0.002, c_{A2} = c_{B1}=0.0001, c_{B2}=-0.0018575, m=0.00001, \delta= 0.0005005$). In the upper panel, the length of the lines indicate the relative increase or decrease in parameter $m$ (horizontal axis) and parameter $\delta$ (vertical axis) which then results in the loss of coexistence. An indication of whether PSK+ or PSK- becomes the only type of plasmid that is present is then indicated. For example, when $m$ increases, PSK+ is lost, with only PSK- remaining. The bottom panel explores the effects of parameters $b$ and $d$ on coexistence. To help scale all panels in the figure, coexistence is lost when $m$ is increase by a factor of 80.
### Table 3: The Parameter Values for the Points of Coexistence Found for the Specialization Model

<table>
<thead>
<tr>
<th>Parameter Set</th>
<th>m</th>
<th>$\delta$</th>
<th>b</th>
<th>d</th>
<th>$c_A$</th>
<th>$c_B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00001</td>
<td>0.0005005</td>
<td>0.4</td>
<td>0.0090585</td>
<td>-0.002</td>
<td>0.0001</td>
</tr>
<tr>
<td>2</td>
<td>0.0000013</td>
<td>0.003228</td>
<td>0.26</td>
<td>0.0249</td>
<td>-0.0176</td>
<td>0.0056</td>
</tr>
<tr>
<td>3</td>
<td>0.00001</td>
<td>0.00423</td>
<td>0.74</td>
<td>0.0155</td>
<td>-0.014</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Figure 16: A diagram of the relative breadth of parameter space for coexistence between PSK+ and PSK-. The intersection of the lines (with end bars) depicts the equilibrium in which PSK+ and PSK- attain frequencies of $\rho_{PSK^+} = 0.36$ and $\rho_{PSK^-} = 0.39$ ($b = 0.74$, $d = 0.0155$, $c_A = -0.01$, $c_B = 0.003$, $c_{A2} = c_{B1} = 0.0053$, $c_{B2} = -0.0131$, $m = 0.00001$, $\delta = 0.00423$). In the upper panel, the length of the lines indicate the relative increase or decrease in parameter $m$ (horizontal axis) and parameter $\delta$ (vertical axis) which then results in the loss of coexistence. An indication of whether PSK+ or PSK- becomes the only type of plasmid that is present is then indicated. For example, when $m$ increases, PSK- is lost, with only PSK+ remaining. The bottom panel explores the effects of parameters $b$ and $d$ on coexistence. To help scale all panels in the figure, coexistence is lost when $b$ is increased by a factor of 221.
12.2 Mutation Scenario

In the second phase of initialization in the mutation scenario the B-strain bacteria goes extinct in the absence of a mutation from PSK- to PSK+ within the B strain (Fig. 17). This occurs because the PSK- plasmid is the only plasmid in the population at initialization of the second phase and that it has a deleterious effect in the B strain. Nevertheless, the PSK- plasmid has a positive effect in the A strain such that the A strain out competes the B strain resulting in the B strain going extinct.

I performed a theoretical experiment to assess the possibility of invasion of PSK+ through either the A strain or B strain via mutation. To potentially overcome the extinction of the B strain, a PSK- plasmid was mutated to the PSK+ state at time step 17 000, which is when the B-strain bacteria bearing the PSK- plasmid is at their peak density (Fig. 17). As noted earlier, a PSK- can transform into a PSK+ plasmid by mutation in either the background of an A-strain or B-strain bacteria. The mutation of a PSK- plasmid to a PSK+ plasmid was induced at time step 17,000 regardless of which strain of bacteria the mutation occurred. In the case when the mutation from the PSK- to PSK+ state occurred in the A strain, the B strain could receive PSK+ plasmids via conjugation and introducing the mutation when the B strain was at highest density allowed for the best chance of conjugation of PSK+ plasmids in the B strain.
Figure 17: On the left is the results of the specialized model after 45000 time steps in the second phase of initialization of the simulation. This trends hold consistent till the end of the run. On the right hand side is an zoom in view on the change in density of the PSK- plasmids in the B genotype before the introduction of PSK+ plasmids. This was seen in both the Sister Pair Mutation as well as the Invasion method for the Specialization Model.
12.2.1 Mutation in the A Strain Scenario

When the PSK- plasmid mutates to a PSK+ plasmid in the A strain, coexistence between PSK- and PSK+ was not found (results not shown). Correspondingly, the B strain went extinct. Generally this results indicate that conjugation of a PSK- plasmid that has been mutated to a PSK+ plasmid is not an effective pathway for the coexistence between PSK- and PSK+ plasmids. PSK- plasmids persisted because they are specialized to the A strain. This system like the invasion scenario had similar issues with stiffness so the starting parameters of the manual investigation were the same as the parameters where coexistence was first found in the invasion scenario. These parameters were then changed from the stating parameter \(b=0.4, d=0.009, c_{A1}=-0.002, c_{B1}=0.0001, c_{A2}=0.0001, c_{B2}=-0.0018575, \delta=0.005, m=0.00001\) to find coexistence but no point of coexistence was found.

12.2.2 Mutation in the B Strain Scenario

In the scenario where the mutation from a PSK- plasmid to a PSK+ plasmid occurred in the B strain, coexistence between PSK- and PSK+ plasmids was not found. Like all of the other Specialization model scenarios, the parameters were manually investigated. The starting parameters were the same parameters in the Mutation in the A Strain scenario.

In the present scenario either PSK- or PSK+ plasmids persisted without the other plasmid depending on the cost difference between \(c_{A1}\) and \(c_{B2}\), keeping in mind \(c_{A1}\) is always greater in magnitude than \(c_{B2}\). If the difference between \(c_{A1}\) and \(c_{B2}\) is small then PSK+ plasmids persisted, and if the difference is large PSK- plasmids persisted. However, it should be noted that there is transient coexistence between the plasmids for about 20,000,000 time steps, such that PSK+ plasmids are at very low densities (<1%) (Fig. 18B). PSK- plasmids in the B strain quickly go extinct (Fig. 18A), and after \(2 \times 10^7\) time steps the population is exclusively consists of the B-strain bacteria with PSK+ with some vacant sites (Fig. 18C).
Figure 18: Results of the PSK- to PSK+ mutation occurring in the B strain bacteria Scenario. The parameters are \( b = 0.4, \ d = 0.009, \ c_{A1} = -0.002, \ c_{B1} = 0.0001, \ c_{A2} = 0.0001, \ c_{B2} = -0.0018575, \delta = 0.005, \) and \( m = 0.00001 \) A) The collapse of PSK- strain B bacteria after 20,000 time steps. B) The change in density of the PSK+ after \( 1.5 \times 10^7 \) time steps, and C) The change in all densities over all of the time steps.
Part IV

Discussion

13 Mochizuki Extension

In the Mochizuki Extension model the most important result is that coexistence for the most part does not occur over the parameter space examined. In the majority of cases PSK+ plasmids take over the population. This result differs from Mochizuki et al. (2007) which found across nearly 50% of their parameter space PSK- plasmids persisted while in the other 50% resulted in PSK+ persisting at the exclusion of the other plasmid. The difference between my result and Mochizuki et al. (2007) is due to the introduction of plasmidless bacteria and a more natural initialization process than Mochizuki et al. (2007). Unlike Mochizuki et al. (2007), the Mochizuki Extension did not randomize the starting frequencies, in contrast plasmids in a process analogous to dispersal or mutation.

The persistence of PSK+ plasmids across all but a few regions of parameter space indicates that simple populations (populations with one bacterial strain) are unlikely to host PSK- plasmids. Simple populations are known to reduce the ability of two plasmids to coexist with each other especially if they are competing with one another (Kerr et al., 2002). The reason for the different results in the Mochizuki and in the Mochizuki Extension model is most likely the differences in how PSK- and PSK+ plasmid carrying cells transition to other states.

In the Mochizuki Model, a PSK- cell either becomes a PSK+ bacteria via conjugation, a vacant site through death, or another PSK- cell through birth (Fig. 19). This means that PSK- plasmids
are being maintained at a rate of \( q_{v/1}b - (q_{1/2}m + d) \) in the Mochizuki model. In contrast the Mochizuki Extension, a PSK- bacterium can become a plasmidless bacteria via segregation, can produce a PSK- bacterium if next to a vacant spot, or a vacant spot through death (Fig. 20).

In the Extension, PSK- are maintained at approximately a rate of \( q_{0/1}m + q_{v/1}b - (d + c + \delta + q_{1/2}m) \). The PSK+ bacteria in the Mochizuki Model can become either a vacant spot through death (compounded the carrying cost of the PSK system) or another PSK+ cell if adjacent to a vacant site. PSK+ plasmids are being maintained at a rate of \( b_{v/2}q - (d + c) \) in the Mochizuki model (after simplification). In the Mochizuki Extension model, a PSK+ bacteria can become either a vacant spot through death (again compounded by cost), segregation, or remain as a PSK+ bacteria, which can be made through either conjugation of the PSK+ plasmid into the plasmidless bacteria or birthed into a vacant spot (Fig. 20). This means PSK+ bacteria are maintained at a rate of \( q_{1/2}m + q_{0/2}m + q_{v/2}b - (d + c + \delta) \) in the Mochizuki Extension Model.
Figure 19: This depicts the outcomes of bacteria in the Mochizuki model ignoring conjugation. On the left hand side are the outcomes for the PSK- plasmid with the PSK- plasmid represented with the orange circle. The PSK- plasmid bearing bacteria only can become a vacant spot or a copy of itself outside of conjugation. On the right hand side is the outcomes for the PSK+ plasmid. The PSK+ plasmid is the blue circle in the bacteria cell. The PSK+ plasmid carrying bacteria can either become a vacant site or continue being a PSK+ plasmid carrying bacteria.

Together, this means that the Mochizuki model heavily depends on the balance of the conjugation rate and the cost of host killing, while the Extension relies on the conjugation and segregational loss of plasmids mediated through a plasmidless state which results in PSK- plasmids losing out to PSK+ plasmids. Empirical studies have demonstrated that PSK+ plasmids exclude other plasmids that do not contain PSK systems in a single bacterial strain population but with no spatial structure (Cooper and Heinenmann, 2000). So this would give the Mochizuki Extension model more support than the original Mochizuki model due to how much the PSK+ plasmids dominate in the Mochizuki Extension Model.

There were only a few cases where the PSK- plasmids were able to sustain against the invasion
Figure 20: The outcomes for PSK- (orange circular DNA) and PSK+ (blue circular DNA) cells in the Mochizuki Extension model. The PSK- cell can either remain itself or the plasmid can segregate out and become a PSK- plasmid after a cell division. New PSK- cells are created when they are birthed into a vacant spot. Vacant sites can become either plasmidless, PSK- containing, and PSK+ containing through conditional birth into the vacant site.
of PSK+. These results are outliers and caused by a high segregation rate and high conjugation rate. The high segregation rate decreases the fitness of the PSK+ plasmids by increasing the amount of vacant sites next to PSK+ plasmids. These vacant sites are created because the increase in the segregation rate results in more plasmidless daughter cells from PSK+ parent cells, which end up being killed by the TA system. In contrast with this, when a parent cell is PSK- a high segregation does not result in the death of a plasmidless daughter cell. Furthermore, this daughter cell is likely adjacent to a PSK- bacteria such that it can take up a PSK- plasmid via conjugation. The increase in plasmidless bacteria combined with a high conjugation rate in this structured environment allows PSK- plasmids to conjugate into the plasmidless bacteria, whereas the PSK+ plasmids have to give birth into the vacant spot. The high conjugation and segregation rates allows for quicker spread of the PSK- plasmids than the PSK+ plasmid.

In the outlier case where PSK- plasmids persist and PSK+ plasmids do not persist \((m=0.1, \delta = 0.05, c=0.06, d=0.011, b=0.11)\), the “fitness” of the PSK+ plasmids is less than the “fitness” of the PSK- plasmids due to the PSK system. The fitness of the PSK+ plasmid next to a vacant site is proportional to \(q_v/2b - (d + c + \delta)\), such that if \(q_v/2\) is equal to 1, fitness is -0.011 and if \(q_v/2\) is less than one the “fitness” drops even more. The fitness of the PSK- plasmid next to a plasmidless site is \(q_0/1m + q_v/1b - (d + c + \delta)\) and has a maximum of 0.089 depending on the conditional probabilities. If the \(q_v/1\) goes to 0 than the fitness of PSK- plasmids next to a plasmidless site would be worse than the PSK+ fitness with assumption that \(q_0/1\) is also 1 (since \(q_v/1\) would be 0). But it is unlikely that any conditional probability is 0 or 1 unless there has been an extinction of a plasmid.

Other results from the Mochizuki Extension model range from supporting previous research to clashing with previous research. PSK+ plasmids were able to sustain themselves in a single-strain
population even when costly to the bacteria host which is similar to the results from Mochizuki et al. (2007), but where the results of the Mochizuki Extension model differ from the original Mochizuki model is that there is a lack of instances were found where PSK- plasmids sustain themselves after the introduction of PSK+ plasmids. This difference could be due to numerous reasons. The first reason for the difference being that the Mochizuki Extension allows PSK- bacteria to become plasmidless bacteria due to segregational loss of the plasmid. This difference allows for PSK+ plasmids to conjugate into the plasmidless bacteria and increase the density of PSK+ plasmids (Mochizuki et al., 2007). This is in contrast with the Mochizuki model where in order for PSK+ bacteria to grow, they had to wait for a vacant spot to become available to give birth a new bacteria into that site with a PSK+ plasmid (Mochizuki et al., 2007). Another possibility is that Mochizuki et al. (2007) analysis only involved for 1,000,000 time steps which may be too short for PSK+ plasmid to take over the PSK- plasmid. Mochizuki et al. (2007) also did not test a specific mutation or invasion method of introducing PSK+ plasmids, but instead started with an already randomly mixed population of PSK- and PSK+ plasmids. In the Mochizuki Extension model, a more biological realistic approach to the building of the simulation was used.

14 Specialization Model

In the Specialization model the most important result was that coexistence was found. Furthermore, this coexistence was quite strong such that both PSK- and PSK+ plasmids were found at upwards of 40% of the population. An additional important consideration is that this coexistence only occurred under the invasion scenario.

Importantly, coexistence occurred with the central core of the parameter space as defined in
Table 2. While this suggests that specialization maybe a broadly applicable explanation for coexistence within this core region the breath of parameter space where coexistence persisted was limited, such that parameters could not be changed by more than 80% before coexistence was lost (Fig. 15). Furthermore, I only found 2 parameter sets out of $10^5$ random parameter sets near this core region where coexistence also occurs. Together, this suggests that although coexistence can occur, it is likely spotty such that there are narrow regions where coexistence occurs.

Although on the most important results of my thesis is establishing the potential strong coexistence between PSK- and PSK+ plasmids, another important result arises through the observation that this coexistence only occurs in the invasion scenario and not the mutation scenario. A prediction that arises from this observation is that if a PSK- plasmid coexists with a PSK+ plasmid and the PSK+ plasmid arose by common descent from the PSK- plasmid, it is likely that coexistence is transient. When the PSK+ plasmid is introduced by invasion via a process such as dispersal then PSK+ plasmids are not closely related in a genealogical sense to the PSK- plasmid. Together, these results from the basis of an empirical line of research. Namely, to assess whether coexisting PSK- and PSK+ systems are closely or distantly related genealogically. If they are closely related, then our results suggest coexistence is likely to be transient, whereas if they are not related, such that PSK+ plasmids were introduced by dispersal, then coexistence could persist in the long term. There is an argument that most coexistence is transient or that transient coexistence should not be discounted (Hastings, 2004). This leaves the mutation scenario as an interesting scenario to test empirically.

There are a few biological factors that were unaccounted for in my thesis. One is that plasmids have different partitioning systems. Another factor are chromosomals, which would allow bacteria to form a resistance to PSK+ plasmids (Bergstrom et al., 2000). There is also the possibility that
increasing n.n. sites will change dynamics by making interactions between states more relent on the conditional probability.

The results from the Specialization Model supports previous literature where more complex populations (more bacterial strains) supports coexistence between competitors (Kerr et al., 2002; Christensen et al., 1998). In this model the two plasmids are directly competing with each other. Complex populations in biofilms are known to increase the chance of coexistence between competitors compared to those complex populations that do not exist in a biofilm (Kerr et al., 2002; Christensen et al., 1998). This gives more support to the reason for coexistence existing in the Specialization model and not in the Mochizuki Extension Model.

An issue with the results is that coexistence between PSK- and PSK+ plasmids only occurred when the plasmids were beneficial to their host bacteria. This is in contrast with Lopatkin et al. (2017), which is the only other paper at the moment that has examined several different strains of bacteria with several different plasmids. They found coexistence when plasmids were costly to their host bacteria. In my thesis, the only plasmid that was costly and maintained for some of the parameter values was the PSK+ plasmids in A-strain bacteria. The PSK+ plasmids were marginally costly to the A-strain bacteria and the plasmid was maintained at low densities. The difference between the Loptikin et al. (2017), and the Specialization Model is most likely caused by the explicate use of spatial structure and the examination of PSK system. Neither spatial structure used nor were PSK systems investigated in Loptikin et al. (2017). However, Loptikin et al. (2017) also did empirical replications of their models where the results mirrored their model’s prediction. Another difference between Loptikin et al. (2017) and the Specialization Model is that Loptikin et al. (2017) started their simulation and empirical replications with equal amount of each bacterial strain and plasmids. All of these differences between my research and the research from Loptikin
et al. (2017) makes it difficult to compare the results.

Some of the other results of the invasion scenario are interesting. The first is that the results give some more support for the Hall et al.’s (2016) Source-Sink model. The source-sink model is where a plasmid can be maintained in a bacterial strain where the plasmid is not beneficial (the sink) due to conjugation of the plasmid from a bacterial strain where the plasmid is beneficial (Hall et al., 2016). This is demonstrated in the Invasion scenario where PSK+ plasmids were maintained in the B strain where the PSK+ plasmid was beneficial, a source, and the A strain where the PSK+ plasmid was costly, a sink (albeit at low densities of >1%).

The fact that the Invasion scenario leads to coexistence seems similar to the Bergstrom et al. (2000) results. However, Bergstrom et al. (2000) argued that the only way that plasmids can be maintained in a population is through a hitchhiking process in which that plasmids are maintained in a population by a process of repeated invasions of the plasmid. All models in this thesis differentiate from Bergstrom et al. (2000) with the fact the models presented here don’t have chromosomals which may explain the difference of that coexistence can happen after a one time invasion while the Bergstrom et al. (2000) suggest that repetitive invasions are need just to maintain plasmids in a population. This is due to the fact that chromosomals make the plasmids redundant and therefore extremely costly to maintain (Harrison and Brockhurst, 2012; Bergstrom et al., 2000). Bergstrom et al., (2000) also did not specifically investigate PSK but did suggest a PSK system could help in maintain plasmids due to reduction in plasmidless segregates.

Coexistence between PSK- and PSK+ plasmids was not found in the Mutation scenario where the mutation occurred in the A-strain bacteria. The most likely reason for the lack of coexistence is that the strain of bacteria that PSK+ plasmids are beneficial to, the B strain, goes extinct. The reason for the B strain going extinct is due to several factors. The first factor is that plasmidless
and PSK- carrying B-strain bacteria are out competed by the PSK- A-strain bacteria causing the B strain as a whole to be on decline. Secondly, once the PSK+ plasmids enter the population they enter through the A-strain bacteria. In the A strain, the PSK+ plasmids are out competed by A-strain bacteria with PSK- plasmids due to PSK- being more beneficial to the A strain than PSK+ plasmids. Thirdly, the conjugation rate is too low to bring PSK+ plasmids into the B strain from the A strain to prevent the extinction of the B strain bacteria where PSK+ plasmids are beneficial. These conditions make it impossible for the PSK+ plasmid to persist in this population unless it is more beneficial that the PSK- plasmids which would not lead to coexistence.

The extinction of the B strain bacteria creates competition between the two plasmids in a single strain of bacteria which makes this similar to the Mochizuki Extension model. The only difference between the Mochizuki Extension model and the current situation in the Specialization model is that the two plasmids have different costs in the host bacteria. Due to the PSK- plasmid being more beneficial to the A strain bacteria, PSK- plasmids are more likely to persist in the bacteria. The only cases where PSK+ plasmids persisted was when the benefit of the PSK- plasmid to the A strain bacteria was marginally better than the benefit of the PSK+ plasmid to the strain A bacteria. When the difference between the benefit is so small the stabilizing process of the PSK system was able to start benefiting the PSK+ plasmids, which allows the PSK+ plasmid to persist over the PSK- plasmid.

In the other scenario where a PSK- plasmid mutates into a PSK+ plasmid in the B strain, either the PSK+ plasmid or the PSK- plasmid end up becoming fixed in the population. The amount of the B-strain bacteria that the PSK+ plasmid can spread to when the PSK+ plasmid is introduced is much larger than the density at the beginning of the invasion scenario. This allows the PSK+ plasmids to spread quickly through the population of B-strain bacteria and create a critically large
foothold early on. This increases the density of B-strain bacteria due to the benefit the PSK+ plasmid gives to the B-strain bacteria. The PSK- plasmid and the A strain go extinct due to the marginal difference between the benefits of the PSK- plasmid to the A strain and the PSK+ plasmid to the B strain bacteria. This means that if the difference of benefits of the plasmids is small PSK+ plasmids exclusively persist in the population. PSK- plasmids were found to persist exclusively if the difference of benefits was large.

One issue with the mutation scenario is that the transformation from a PSK- plasmid to become a PSK+ plasmids is neither simple or well understood. In order to transition to a PSK+ plasmid, the PSK- must acquire both a Toxin gene and an anti-toxin gene (Rankin et al., 2017). The plasmid is more likely to be successful if it acquires an anti-toxin first since acquiring the toxin first would kill the plasmid’s host (Rankin et al., 2017). The mutation is a bit simpler if it is assumed that the PSK system is a type II, IV, V PSK systems due to the Anti-toxin system being a protein which theoretically can be used for other process in the bacterial host outside of blocking the toxins (Rankin et al., 2017). The issue is that most Anti-Toxins proteins are not known to do something outside of preventing the toxins from acting. There are some exceptions such as the type IV anti-toxin YeeU which is known to help increase global stabilization of internal homeostasis and the type V anti-toxin GohS which can be used as a sequence-specific endoribonuclease due to the unique way they work and the type II MqsR/MqsA (Masuda et al., 2012; Wang et al., 2012; Wang and Wood, 2011). This means that a more effective model for sister PSK- to PSK+ plasmid mutation would need several different types of plasmids that represent the stages between a pure PSK- plasmid and a PSK+ plasmid to be a more accurate representation of this evolution path. The issue would be the difficulty of modeling all of these stages together, which would require more differential equations and therefore cause more stiffness in the model due to the amount
of conditional probabilities. The other mechanism for the PSK- plasmid to mutate into a PSK+ plasmid is by the plasmids acquiring the required genes through transduction or transformation.

In the mutation scenario where the mutation occurs in the B strains, transient coexistence was found between the two plasmids that can last duration of $\text{>10}^7$ time steps. The PSK+ plasmids being at low densities of $<$1% of the population before a sudden and quick transition to PSK+ plasmids dominating the population. This leads to questions if there is actual coexistence of the plasmids in the population (Hastings, 2004). Since the length of the simulation, $\text{10}^7$ hours, is 114 years and the environment would more than likely change drastically which will result in changing costs of carrying the plasmids. Therefore the two plasmids could be considered functionally coexistent even though numerically they aren’t coexistent.

15 Things to Consider

15.1 When would PSK- and PSK+ not coexist

I mainly focused in my thesis on the coexistence of PSK- and PSK+ plasmids in the same environment. However, as indicated by the results, coexistence doesn’t always occur between these plasmids. So what are the reasons for the lack of coexistence between PSK- and PSK+ plasmids.

In the Mochizuki Extension model the lack of coexistence is due to a balance of segregational loss of the plasmids as well as the conjugation rate. This is because the cost of the two plasmids are the same and the two plasmids are competing for a place in the single bacterial strain. So in order for plasmids to coexist a fine balance has to be achieved between the segregation rate and the conjugation rate. Increasing the segregational loss of the plasmid would make the PSK system more “fit”.
In the Specialization model, the balance of the cost of carrying a plasmid with conjugation and segregation rates is the main factor determining the coexistence of plasmids. So, if one of the plasmids becomes consistently more fit on average across the set of frequencies of bacterial strains within a population as a function of its costs and a set of conjugation and segregation rates, it will be able to out compete the other plasmid.

15.2 Future Directions

There are a few things that my thesis does not take into consideration that has been considered in other models before. However, these aspects have not been investigated in context of a PSK system before either and spatial structure.

This research does not consider chromosomals. Chromosomals are bacteria that integrates DNA from a plasmid into their chromosome. The uptake of plasmids into the chromosome most likely leads the bacteria to become resistant to the toxins from the PSK+ system. Therefore, it would be expected that PSK systems would not be able to persist in chromosomal bacteria but PSK-plasmids may be able to persist depending on their cost to the bacteria (Bergstrom et al., 2000). However, some research has found that plasmid that encode for PSK+ systems can out compete chromosomals in an immunity arms race between the plasmid and the chromosome (Cooper et al., 2010; De Bast et al., 2008). This immunity arms race is characterized by the plasmid gaining more copies of different PSK systems at a faster rate compared to the chromosomals (Cooper et al., 2010; De bast et al., 2008). It is believed that plasmids gain PSK systems faster than a chromosome since they are actively competing against other plasmids to be maintained in the bacterium (Cooper et al., 2010).

Another concept that could affect coexistence is in the case where both plasmids have a stability
The stability system could be a different PSK system or an active partitioning system (such as Type I or Type II not to be confused with type I and type II PSK systems). A PSK-plasmid with a partitioning system would have a decreased rate of segregational loss. Depending on the reduction of segregation rate it could lead to coexistence or an increase chance of PSK-plasmid persistence. In the case where both plasmids have a PSK system, the likely outcome would be coexistence between the plasmids depending on the benefit/cost of the plasmids. The coexistence would also depended if both plasmids have an unique PSK system or if an arms race is occurring between the plasmids. Many large plasmids contain both partitioning and PSK systems so it would be important to research into it (Volante et al., 2015).

Another aspect that was not consider is that plasmids often have variable copy numbers in a bacteria cell. The copy number of a plasmid in a single cell they can range from 1 to even up to 200 or more copies (Vieira and Messing, 1982; Nordstrom, 2006). Larger plasmids, which is the case for most PSK system plasmids, usually have around 1-10 copies in a bacteria (Wang, 2017; Dmowski and Jagura-Burdzy, 2013a; Summers, 1996). This could lead to more chance of coexistence if the number of copies of PSK-plasmids in a single bacterial strain was larger than considered in this model since it will allow for a bacteria to having mixtures of the different plasmids. This then allows for the bacteria to conjugate both plasmids to neighboring bacteria. Coexistence could be possible between a costly and a beneficial plasmids where the costly plasmid is maintained in bacteria which has the beneficial plasmid as long as the costly plasmid isn’t so unfit to counteract the benefit of the beneficial plasmid. From empirical research (San Millan et al., 2014) different costly plasmids can have a epistatic effect on each other allowing for coexistence between the two plasmids.

One thing to note is that PSK systems in my thesis are assumed to be 100% effective in killing
daughter cells that do not inherit the plasmid (Brendler et al., 2004; Sayeed et al., 2000). PSK system are not always 100% effective (Wang et al., 2012; Guillet et al., 2019; Jensen and Gerdes, 1995; Lioy et al., 2012). Allowing for the PSK system to be less than 100% effective should allow for more plasmidless bacteria. This should allow for more PSK- plasmids to persist in the population as there is more plasmidless bacteria for PSK- plasmids to conjugate into. Furthermore, conjugation of a PSK+ plasmid into the PSK- bacterium will no longer change the PSK- bacterium to act like a PSK+ plasmid bacterium. Instead this bacterium that now contains both a PSK- and PSK+ plasmid will act differently since the daughter cells can either be PSK-, PSK+, plasmidless, vacant/dead or a bacterium that contains both plasmids. This new state will also allow for conjugation of either plasmid to neighboring bacteria. In my model it is also assumed that the daughter cells die instantly once the daughter cell is plasmidless when the parent cell is PSK+. This does not allow the chance for the plasmidless daughter cells to divide or to receive either PSK- or PSK+ plasmids through conjugation. If this plasmidless daughter cell receives a PSK+ plasmid it could be seen as cured and will no longer be killed by the toxins. The reason these phenomena were not explored in this thesis was due to the face that creating an additional bacterial strain would create more equations, and therefore would make the system potentially more stiff and harder to work with.

A major disagreement in the plasmid field is whether conjugation or compensatory evolution allows for persistence of plasmids (Hall et al., 2017). There is also a lack of research on plasmid coexistence especially in terms of different life strategies since most models assume competing plasmids are the same except in terms of costs or transfer rates (Hall et al., 2016; Mochizuki et al., 2007; Bergstrom et al., 2000). In this thesis I demonstrated that coexistence between plasmids is possible without compensatory evolution however both plasmids have to beneficial to a bacterial
strain. In the Mochizuki Extension, PSK+ plasmids can persist when the plasmids are costly to
the host as long as the competitor plasmid has the same carrying cost. Again this occurred without
the need for compensatory evolution. Future models should consider compensatory evolution and
directly compare it to a model without compensatory evolution.

16 Conclusion

PSK+ plasmids and PSK- plasmids most likely coexist in a population if two of the following are
achieved: a) a specialized bacteria that has a PSK+ plasmid is invading a single strain population
with the PSK- plasmid, and b) PSK- is more beneficial to the strain it is found in than PSK+ is in
that strain. If a PSK- plasmid mutates into PSK+ plasmid in a multi-strain population, depending
on which bacterial strain the mutation happens, either PSK- plasmids or PSK+ plasmids persist but
there was no coexistence. In a single bacterial strain system PSK+ plasmids were found to persist
over almost all of the parameter space that was examined in this thesis.
References


Appendices

Appendix A - Mochizuki Extension

\[
\frac{d\rho_{v0}[t]}{dt} = 2d\rho_{00}[t] + (c+d)\rho_{01}[t] + (c+d)\rho_{02}[t] + \delta\rho_{02}[t] + \delta\rho_{v1}[t] \\
+ 2b\rho_{VV}[t](\frac{3}{4}(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{VV}[t])) - d\rho_{v0}[t] \\
- m\rho_{v0}[t](\frac{3}{4}(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{v0}[t])) \\
- m\rho_{v0}[t](\frac{3}{4}(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{v0}[t])) \\
- b\rho_{v0}[t](\frac{3}{4}(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])) \\
- b\rho_{v0}[t](\frac{3}{4}(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])) \\
- b\rho_{v0}[t](\frac{3}{4}(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])) \\
- b\rho_{v0}[t](\frac{3}{4}(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t]))
\]
\[
\frac{dp_{v1}[t]}{dt} = d\rho_{01}[t] + 2(c+d)\rho_{11}[t] + (c+d)\rho_{12}[t] + \delta\rho_{12}[t]
\]
\[
+ m\rho_{v0}[t]\left(\frac{3}{4} \rho_0[t] \rho_{01}[t]\right)
\]
\[
+ 2b\rho_{VV}[t] \left(\frac{3}{4} \rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{VV}[t]\right)
\]
\[
- \rho_{v1}[t](c+d+\delta)
\]
\[
- m\rho_{v1}[t]\left(\frac{3}{4} \rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{v1}[t]\right)
\]
\[
- b\rho_{v1}[t]\left(\frac{3}{4} \rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{VV}[t]\right)
\]
\[
- b\rho_{v1}[t]\left(\frac{1}{4} + \frac{3}{4} \rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{VV}[t]\right)
\]
\[
- b\rho_{v1}[t]\left(\frac{3}{4} \rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{VV}[t]\right)
\]
\[
\frac{d\rho_{02}[t]}{dt} = d\rho_{02}[t] + (c + d)\rho_{12}[t] + 2(c + d)\rho_{22}[t] + 2\delta\rho_{22}[t] - \rho_{v2}[t](c + d + \delta) \\
+ m\rho_{0t}[t]\left(\frac{3}{4}\frac{\rho_{02}[t]}{(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{03}[t])}\right) \\
+ m\rho_{11}[t]\left(\frac{3}{4}\frac{\rho_{12}[t]}{(\rho_{00}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{11}[t])}\right) \\
+ 2b\rho_{VV}[t]\left(\frac{3}{4}\frac{\rho_{v2}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])}\right) \\
- b\rho_{02}[t]\left(\frac{3}{4}\frac{\rho_{v0}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])}\right) \\
- b\rho_{12}[t]\left(\frac{3}{4}\frac{\rho_{v1}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])}\right) \\
- b\rho_{22}[t]\left(\frac{3}{4}\frac{\rho_{v2}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])}\right)
\]

\[
\frac{d\rho_{01}[t]}{dt} = 2\delta\rho_{11}[t] - \rho_{01}[t](c + 2d + \delta) \\
+ 2m\rho_{00}[t]\left(\frac{3}{4}\frac{\rho_{01}[t]}{(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{03}[t])}\right) \\
- m\rho_{01}[t]\left(\frac{3}{4}\frac{\rho_{02}[t]}{(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{03}[t])}\right) \\
- m\rho_{01}[t]\left(\frac{1}{4}\frac{\rho_{01}[t]}{(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{03}[t])}\right) \\
- m\rho_{01}[t]\left(\frac{3}{4}\frac{\rho_{12}[t]}{(\rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{11}[t])}\right) \\
+ b\rho_{0t}[t]\left(\frac{3}{4}\frac{\rho_{v0}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])}\right) \\
+ b\rho_{v1}[t]\left(\frac{3}{4}\frac{\rho_{v0}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{vV}[t])}\right)
\]
\[
\frac{d\rho_{00}[t]}{dt} = \delta \rho_{01}[t] - 2d\rho_{00}[t]
\]

\[
- 2m\rho_{00}[t]\left(\frac{3}{4} \frac{\rho_{01}[t]}{(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{v0}[t])}\right)
\]

\[
- 2m\rho_{00}[t]\left(\frac{3}{4} \frac{\rho_{02}[t]}{(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{v0}[t])}\right)
\]

\[
+ b\rho_{v0}[t]\left(\frac{1}{4} + \frac{3}{4} \frac{\rho_{v0}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{VV}[t])}\right)
\]

\[
\frac{d\rho_{11}[t]}{dt} = m\rho_{01}[t]\left(\frac{1}{4} + \frac{3}{4} \frac{\rho_{01}[t]}{(\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{v0}[t])}\right)
\]

\[
- \rho_{11}[t](2(c + d) + 2\delta)
\]

\[
- 2m\rho_{11}[t]\left(\frac{3}{4} \frac{\rho_{12}[t]}{(\rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{v1}[t])}\right)
\]

\[
+ b\rho_{v1}[t]\left(\frac{1}{4} + \frac{3}{4} \frac{\rho_{v1}[t]}{(\rho_{v0}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{VV}[t])}\right)
\]
\[
\frac{d\rho_{02}[t]}{dt} = \delta \rho_{12}[t] - \rho_{02}[t](c + 2d + \delta) \\
+ 2m\rho_{00}[t]\left(\frac{3}{4} \frac{\rho_{02}[t]}{\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{00}[t]}\right) \\
- m\rho_{02}[t]\left(\frac{3}{4} \frac{\rho_{01}[t]}{\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{00}[t]}\right) \\
- m\rho_{02}[t]\left(\frac{1}{4} + \frac{3}{4} \frac{\rho_{02}[t]}{\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{00}[t]}\right) \\
+ m\rho_{01}[t]\left(\frac{3}{4} \frac{\rho_{12}[t]}{\rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{10}[t]}\right) \\
+ b\rho_{v0}[t]\left(\frac{3}{4} \frac{\rho_{v2}[t]}{\rho_{00}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{v0}[t]}\right) \\
+ b\rho_{v2}[t]\left(\frac{3}{4} \frac{\rho_{v0}[t]}{\rho_{00}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{v0}[t]}\right)
\]

\[
\frac{d\rho_{12}[t]}{dt} = m\rho_{01}[t]\left(\frac{3}{4} \frac{\rho_{02}[t]}{\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{00}[t]}\right) \\
+ m\rho_{02}[t]\left(\frac{3}{4} \frac{\rho_{12}[t]}{\rho_{00}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{00}[t]}\right) \\
+ 2m\rho_{11}[t]\left(\frac{3}{4} \frac{\rho_{12}[t]}{\rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{10}[t]}\right) \\
- \rho_{12}[t](2c + 2d + 2\delta) \\
- m\rho_{12}[t]\left(\frac{1}{4} + \frac{3}{4} \frac{\rho_{12}[t]}{\rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{10}[t]}\right) \\
+ b\rho_{v1}[t]\left(\frac{3}{4} \frac{\rho_{v2}[t]}{\rho_{00}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{v0}[t]}\right) \\
+ b\rho_{v2}[t]\left(\frac{3}{4} \frac{\rho_{v0}[t]}{\rho_{00}[t] + \rho_{v1}[t] + \rho_{v2}[t] + \rho_{v0}[t]}\right)
\]
\[
\frac{d\rho_{22}[t]}{dt} = -2(c + d)\rho_{22}[t] - 2\delta\rho_{22}[t] \\
+ m\rho_{02}[t] \left( \frac{1}{4} + \frac{3}{4} \right) \frac{\rho_{02}[t]}{\rho_{02}[t] + \rho_{01}[t] + \rho_{02}[t] + \rho_{10}[t]} \\
+ m\rho_{12}[t] \left( \frac{1}{4} + \frac{3}{4} \right) \frac{\rho_{12}[t]}{\rho_{01}[t] + \rho_{11}[t] + \rho_{12}[t] + \rho_{11}[t]} \\
+ b\rho_{22}[t] \left( \frac{1}{4} + \frac{3}{4} \right) \frac{\rho_{22}[t]}{\rho_{22}[t] + \rho_{12}[t] + \rho_{22}[t] + \rho_{12}[t]}
\]

\textbf{Appendix B - Specialization Equations}

\[
\frac{d\rho_{VV}[t]}{dt} = d\rho_{VA0}[t] + d\rho_{VB0}[t] + (c_{A1} + d)\rho_{VA1}[t] + (c_{B1} + d)\rho_{VB1}[t] \\
+ (c_{A2} + d)\rho_{VA2}[t] + (c_{B2} + d)\rho_{VB2}[t] + \delta\rho_{VA2}[t] + \delta\rho_{VB2}[t] \\
- 2bp_{VV}[t] \left( \frac{3}{4} \right) \frac{\rho_{VA0}[t]}{\rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t]} \\
- 2bp_{VV}[t] \left( \frac{3}{4} \right) \frac{\rho_{VB0}[t]}{\rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t]} \\
- 2bp_{VV}[t] \left( \frac{3}{4} \right) \frac{\rho_{VA1}[t]}{\rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB1}[t] + \rho_{VB1}[t] + \rho_{VB2}[t]} \\
- 2bp_{VV}[t] \left( \frac{3}{4} \right) \frac{\rho_{VB1}[t]}{\rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB1}[t] + \rho_{VB1}[t] + \rho_{VB2}[t]} \\
- 2bp_{VV}[t] \left( \frac{3}{4} \right) \frac{\rho_{VA2}[t]}{\rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t]} \\
- 2bp_{VV}[t] \left( \frac{3}{4} \right) \frac{\rho_{VB2}[t]}{\rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t]}
\]
\[
\frac{d \rho_{vB0}[t]}{dt} = 2 d \rho_{B0B0}[t] + d \rho_{A0B0}[t] + (c_{B1} + d) \rho_{B0B1}[t] + (c_{A1} + d) \rho_{A1B0}[t] + (c_{B2} + d) \rho_{B0B2}[t] \\
+ (c_{A2} + d) \rho_{A2B0}[t] + \delta \rho_{B0B2}[t] + \delta \rho_{A2B0}[t] + \delta \rho_{vB1}[t] - d \rho_{vB0}[t] \\
+ 2 b \rho_{vV}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \\
- m \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \\
- b \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \\
- b \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \\
- b \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \\
- b \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \\
- b \rho_{vB0}[t] \frac{1}{4} \left( \rho_{vB0}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \\
- b \rho_{vB0}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right)
\]
\[
\frac{d \rho_{VA0}[t]}{dt} = 2d \rho_{A0A0}[t] + d \rho_{A0B0}[t] + (c_{A1} + d) \rho_{A0A1}[t] - d \rho_{VA0}[t] \\
+ (c_{B1} + d) \rho_{A0B1}[t] + (c_{A2} + d) \rho_{A0A2}[t] + (c_{B2} + d) \rho_{A0B2}[t] + \delta \rho_{A0A2}[t] + \delta \rho_{A0B2}[t] + \delta \rho_{VA1}[t] \\
+ 2b \rho_{VV}[t] \frac{3}{4} \left( \rho_{VV}[t] + \rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t] \right) \\
- m \rho_{VA0}[t] \frac{3}{4} \left( \rho_{VA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \\
- m \rho_{VA0}[t] \frac{3}{4} \left( \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \\
- m \rho_{VA0}[t] \frac{3}{4} \left( \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \\
- m \rho_{VA0}[t] \frac{3}{4} \left( \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \\
- b \rho_{VA0}[t] \frac{4}{4} \left( \rho_{VV}[t] + \rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t] \right) \\
- b \rho_{VA0}[t] \frac{4}{4} \left( \rho_{VV}[t] + \rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t] \right) \\
- b \rho_{VA0}[t] \frac{4}{4} \left( \rho_{VV}[t] + \rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t] \right) \\
- b \rho_{VA0}[t] \frac{4}{4} \left( \rho_{VV}[t] + \rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t] \right) \\
- b \rho_{VA0}[t] \frac{1}{4} + \frac{3}{4} \left( \rho_{VV}[t] + \rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t] \right) \\
- b \rho_{VA0}[t] \frac{3}{4} \left( \rho_{VV}[t] + \rho_{VA0}[t] + \rho_{VA1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t] \right)
\]
\[
\frac{d\rho_{B1}[t]}{dt} = d\rho_{B0B1}[t] + d\rho_{A0B1}[t] + 2(c_B + d)\rho_{B1B1}[t] + (c_A + d)\rho_{A1B1}[t] \\
+ (c_B + d)\rho_{B1B2}[t] + (c_A + d)\rho_{A2B1}[t] + \delta\rho_{B1B2}[t] + \delta\rho_{A2B1}[t] \\
+ m\rho_{B1B0}[t]^3 \frac{\rho_{B0B1}[t]}{\rho_{A1B0}[t]} \\
+ m\rho_{B1B0}[t]^3 \frac{\rho_{A1B0}[t]}{\rho_{B0B0}[t]} \\
+ 2b\rho_{VV}[t]^3 \frac{\rho_{B1B2}[t]}{\rho_{A1B2}[t]} \\
- \rho_{B1}[t] \left( \frac{b}{4} + c_B + d + \delta \right) \\
- m\rho_{B1B1}[t]^3 \frac{\rho_{A0B1}[t]}{\rho_{A1B1}[t]} \\
- m\rho_{B1B1}[t]^3 \frac{\rho_{A1B1}[t]}{\rho_{B0B1}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A0B0}[t]}{\rho_{A1B1}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A1B0}[t]}{\rho_{B0B1}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A1B1}[t]}{\rho_{B0B0}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A0B1}[t]}{\rho_{A1B0}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A1B1}[t]}{\rho_{B1B1}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A1B2}[t]}{\rho_{B1B2}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A2B1}[t]}{\rho_{B2B1}[t]} \\
- b\rho_{B1B1}[t]^3 \frac{\rho_{A2B2}[t]}{\rho_{B2B2}[t]} \\
\]

97
\[
\frac{d\rho_{vA1}[t]}{dt} = d\rho_{A0A1}[t] + d\rho_{A1B0}[t] + 2(c_{A1} + d)\rho_{vA1}[t] + (c_{B1} + d)\rho_{A1B1}[t] \\
+ (c_{A2} + d)\rho_{A1A2}[t] + (c_{B2} + d)\rho_{A1B2}[t] + \delta\rho_{A1A2}[t] + \delta\rho_{A1B2}[t] \\
+ m\rho_{vA0}[t] \left( \frac{\rho_{A0A1}[t]}{4} \right) + m\rho_{vA0}[t] \left( \frac{\rho_{A0B1}[t]}{4} \right) + 2b\rho_{vV}[t] \left( \frac{b}{4} + c_{A1} + d + \delta \right) \\
- m\rho_{vA1}[t] \left( \frac{\rho_{A1A2}[t]}{4} \right) - m\rho_{vA1}[t] \left( \frac{\rho_{A1B2}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vA0}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) \\
- b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) \\
- b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right) - b\rho_{vA1}[t] \left( \frac{\rho_{vV}[t]}{4} \right)
\]
\[
\frac{d\rho_{vB2}[t]}{dt} = \frac{d\rho_{BOB2}[t]}{dt} + d\rho_{AOB2}[t] + c_{B1}\rho_{B1B2}[t] + d\rho_{B1B2}[t] + c_{A1}\rho_{A1B2}[t] + d\rho_{A1B2}[t] + 2(c_{B2} + d)\rho_{B2B2}[t] + 2\delta\rho_{B2B2}[t] + (c_{A2} + d)\rho_{A2B2}[t] + \delta\rho_{A2B2}[t]
\]

\[
+ m\rho_{vB0}[t] \left( \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \right) - \rho_{vB2}[t]c_{B2} - \rho_{vB2}[t]d - \rho_{vB2}[t]\delta
\]

\[
- b\rho_{vB2}[t] \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right)
\]

\[
- b\rho_{vB2}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right)
\]

\[
- b\rho_{vB2}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right)
\]

\[
- b\rho_{vB2}[t] \frac{1}{4} \left( 1 + \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \right)
\]

\[
- b\rho_{vB2}[t] \frac{3}{4} \left( \rho_{vA2}[t] \right)
\]

\[
- b\rho_{vB2}[t] \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right)
\]

99
\[
\frac{d\rho_{va2}[t]}{dt} = d\rho_{a0a2}[t] + d\rho_{a2b0}[t] + c_{a1}\rho_{a1a2}[t] + d\rho_{a1a2}[t] + c_{b1}\rho_{a2b1}[t] + d\rho_{a2b1}[t]
\]
\[
+ 2(c_{a2} + d)\rho_{a2a2}[t] + 2\delta\rho_{a2a2}[t] + (c_{b2} + d)\rho_{a2b2}[t] + \delta\rho_{a2b2}[t]
\]
\[
+ m\rho_{va0}[t] \frac{3}{4} \left( \rho_{va0}[t] + \rho_{a0a0}[t] + \rho_{a0a1}[t] + \rho_{a0a2}[t] + \rho_{a0b0}[t] + \rho_{a0b1}[t] + \rho_{a0b2}[t] \right)
\]
\[
+ m\rho_{va0}[t] \frac{3}{4} \left( \rho_{va0}[t] + \rho_{a0a0}[t] + \rho_{a0a1}[t] + \rho_{a0a2}[t] + \rho_{a0b0}[t] + \rho_{a0b1}[t] + \rho_{a0b2}[t] \right)
\]
\[
+ m\rho_{va1}[t] \frac{3}{4} \left( \rho_{va1}[t] + \rho_{a0a1}[t] + \rho_{a1a1}[t] + \rho_{a1a2}[t] + \rho_{a1b0}[t] + \rho_{a1b1}[t] + \rho_{a1b2}[t] \right)
\]
\[
+ m\rho_{va1}[t] \frac{3}{4} \left( \rho_{va1}[t] + \rho_{a0a1}[t] + \rho_{a1a1}[t] + \rho_{a1a2}[t] + \rho_{a1b0}[t] + \rho_{a1b1}[t] + \rho_{a1b2}[t] \right)
\]
\[
+ \left. \rho_{v2a2} \left( c_{a2} - \rho_{va2}[t]d - \rho_{v2a2}[t]\delta \right) \right. \]
\[
- b_{pva2}[t] \frac{3}{4} \left( \rho_{pv}[t] + \rho_{va0}[t] + \rho_{va1}[t] + \rho_{va2}[t] + \rho_{vb0}[t] + \rho_{vb1}[t] + \rho_{vb2}[t] \right)
\]
\[
- b_{pva2}[t] \frac{3}{4} \left( \rho_{pv}[t] + \rho_{va0}[t] + \rho_{va1}[t] + \rho_{va2}[t] + \rho_{vb0}[t] + \rho_{vb1}[t] + \rho_{vb2}[t] \right)
\]
\[
- b_{pva2}[t] \frac{3}{4} \left( \rho_{pv}[t] + \rho_{va0}[t] + \rho_{va1}[t] + \rho_{va2}[t] + \rho_{vb0}[t] + \rho_{vb1}[t] + \rho_{vb2}[t] \right)
\]
\[
- b_{pva2}[t] \frac{3}{4} \left( \rho_{pv}[t] + \rho_{va0}[t] + \rho_{va1}[t] + \rho_{va2}[t] + \rho_{vb0}[t] + \rho_{vb1}[t] + \rho_{vb2}[t] \right)
\]
\[
- b_{pva2}[t] \frac{1}{4} \left( \rho_{pv2}[t] + \rho_{va0}[t] + \rho_{va1}[t] + \rho_{va2}[t] + \rho_{vb0}[t] + \rho_{vb1}[t] + \rho_{vb2}[t] \right)
\]
\[
- b_{pva2}[t] \frac{3}{4} \left( \rho_{pv}[t] + \rho_{va0}[t] + \rho_{va1}[t] + \rho_{va2}[t] + \rho_{vb0}[t] + \rho_{vb1}[t] + \rho_{vb2}[t] \right)
\]

100
\[
\begin{align*}
&\frac{d\rho_{AIB1}[t]}{dt} = m\rho_{AIB0}[t]\left(\frac{3}{4}\left(\rho_{vA1}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]\right) - \rho_{AIB0}[t]c_{A1} - \rho_{AIB0}[t]2d - \rho_{AIB0}[t]\delta + \delta\rho_{AIB1}[t]\right) \\
&\quad+ m\rho_{AIB0}[t]\left(\frac{3}{4}\left(\rho_{vA1}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]\right) - \rho_{AIB0}[t]\frac{\rho_{BOB2}[t]}{\rho_{AIB0}[t]}\right) \\
&\quad- m\rho_{AIB0}[t]\left(\frac{3}{4}\left(\rho_{vA1}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]\right) - \rho_{AIB0}[t]\frac{\rho_{A1A2}[t]}{\rho_{AIB0}[t]}\right) \\
&\quad- m\rho_{AIB0}[t]\left(\frac{3}{4}\left(\rho_{vA1}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]\right) - \rho_{AIB0}[t]\frac{\rho_{A1A2}[t]}{\rho_{A1B2}[t]}\right) \\
&\quad- m\rho_{AIB0}[t]\left(\frac{3}{4}\left(\rho_{vA1}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]\right) - \rho_{AIB0}[t]\frac{\rho_{A1A2}[t]}{\rho_{A1B2}[t]}\right) \\
&\quad+ b\rho_{A1}[t]\left(\frac{3}{4}\left(\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t]\right) - \rho_{A1}[t]\frac{\rho_{vA1}[t]}{\rho_{vB0}[t]}\right) \\
&\quad+ b\rho_{vB}[t]\left(\frac{3}{4}\left(\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t]\right) - \rho_{vA1}[t]\frac{\rho_{vB1}[t]}{\rho_{vB2}[t]}\right)
\end{align*}
\]
\[
\frac{d\rho_{A0B1}[t]}{dt} = m\rho_{A0B0}[t] \left( \frac{3}{4} \left( \rho_{V\rho 0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) - \rho_{A0B1}[t] c_B^1 - \rho_{A0B1}[t] 2d - \rho_{A0B1}[t] \delta + \delta \rho_{A1B1}[t] \right)
\]

\[
- m\rho_{A0B1}[t] \left( \frac{3}{4} \rho_{A0A2}[t] \right)
\]

\[
- m\rho_{A0B1}[t] \left( \frac{3}{4} \rho_{A0A1}[t] \right)
\]

\[
- m\rho_{A0B1}[t] \left( \frac{3}{4} \rho_{A0A1}[t] \right)
\]

\[
- m\rho_{A0B1}[t] \left( \frac{3}{4} \rho_{A0B2}[t] \right)
\]

\[
+ b\rho_{V\rho_A}[t] \left( \frac{3}{4} \rho_{V\rho V}[t] + \rho_{V\rho 0}[t] + \rho_{V\rho 0}[t] + \rho_{V\rho A2}[t] + \rho_{V\rho B0}[t] + \rho_{V\rho B1}[t] + \rho_{V\rho B2}[t] \right)
\]

\[
+ b\rho_{V\rho B1}[t] \left( \frac{3}{4} \rho_{V\rho V}[t] + \rho_{V\rho 0}[t] + \rho_{V\rho 1}[t] + \rho_{V\rho A2}[t] + \rho_{V\rho B0}[t] + \rho_{V\rho B1}[t] + \rho_{V\rho B2}[t] \right)
\]
\[
\frac{d\rho_{BOB1}[t]}{dt} = 2m\rho_{BOB0}[t] \left( \frac{3}{4} \rho_{BOB1}[t] + \frac{\rho_{VOB0}[t]}{\rho_{AVB0}[t] + \rho_{AVB1}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]} \right)
\]
\[
+ 2m\rho_{BOB0}[t] \left( \frac{3}{4} \rho_{BOB1}[t] + \frac{\rho_{VOB0}[t]}{\rho_{AVB0}[t] + \rho_{AVB1}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]} \right)
\]
\[
- \rho_{BOB1}[t]c_{B1} - \rho_{BOB1}[t]2d - \rho_{BOB1}[t]\delta + 2\delta \rho_{B1B1}[t]
\]
\[
- m\rho_{BOB1}[t] \left( \frac{3}{4} \rho_{VOB0}[t] + \rho_{VOB0}[t] + \rho_{AVB0}[t] + \rho_{AVB1}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B1B2}[t] \right)
\]
\[
- m\rho_{BOB1}[t] \left( \frac{3}{4} \rho_{VOB0}[t] + \rho_{VOB0}[t] + \rho_{AVB0}[t] + \rho_{AVB1}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B1B2}[t] \right)
\]
\[
- m\rho_{BOB1}[t] \left( \frac{3}{4} \rho_{VOB0}[t] + \rho_{AVB0}[t] + \rho_{AVB1}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B1B2}[t] \right)
\]
\[
+ b\rho_{VB0}[t] \left( \frac{3}{4} \rho_{VOB0}[t] + \rho_{AVB0}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t] \right)
\]
\[
+ b\rho_{VB1}[t] \left( \frac{3}{4} \rho_{VOB0}[t] + \rho_{AVB0}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t] \right)
\]
\[
\frac{d\rho_{0A1}[t]}{dt} = 2m\rho_{0AO0}[t] \frac{\rho_{0A1}[t]}{4 (\rho_{0A0}[t] + \rho_{0AO0}[t] + \rho_{0A1}[t] + \rho_{0AO2}[t] + \rho_{0A0B0}[t] + \rho_{0A0B1}[t] + \rho_{0A0B2}[t])}
+ 2m\rho_{0AO0}[t] \frac{\rho_{0A2}[t]}{4 (\rho_{0A0}[t] + \rho_{0AO0}[t] + \rho_{0A1}[t] + \rho_{0AO2}[t] + \rho_{0A0B0}[t] + \rho_{0A0B1}[t] + \rho_{0A0B2}[t])}
- \rho_{0A1}[t] c_{A1} - \rho_{0A1}[t] 2d - \rho_{0A1}[t] \delta + 2\delta \rho_{1A1}[t]
- mp_{0A01}[t] \frac{\rho_{0A2}[t]}{4 (\rho_{0A0}[t] + \rho_{0AO0}[t] + \rho_{0A1}[t] + \rho_{0AO2}[t] + \rho_{0A0B0}[t] + \rho_{0A0B1}[t] + \rho_{0A0B2}[t])}
- mp_{0A01}[t] \frac{\rho_{0A1}[t]}{4 (\rho_{0A0}[t] + \rho_{0AO0}[t] + \rho_{0A1}[t] + \rho_{0AO2}[t] + \rho_{0A0B0}[t] + \rho_{0A0B1}[t] + \rho_{0A0B2}[t])}
- mp_{0A01}[t] \frac{1/4 + 3/4 (\rho_{0A0}[t] + \rho_{0AO0}[t] + \rho_{0A1}[t] + \rho_{0AO2}[t] + \rho_{0A0B0}[t] + \rho_{0A0B1}[t] + \rho_{0A0B2}[t])}{\rho_{0A1}[t]}
- mp_{0A01}[t] \frac{\rho_{0A1}[t]}{4 (\rho_{0A0}[t] + \rho_{0AO0}[t] + \rho_{0A1}[t] + \rho_{0AO2}[t] + \rho_{0A0B0}[t] + \rho_{0A0B1}[t] + \rho_{0A0B2}[t])}
- mp_{0A01}[t] \frac{\rho_{0A1}[t]}{4 (\rho_{0A0}[t] + \rho_{0AO0}[t] + \rho_{0A1}[t] + \rho_{0AO2}[t] + \rho_{0A0B0}[t] + \rho_{0A0B1}[t] + \rho_{0A0B2}[t])}
+ bp_{0A0}[t] \frac{\rho_{0V}[t]}{4 (\rho_{0V}[t] + \rho_{0A0}[t] + \rho_{0A1}[t] + \rho_{0A2}[t] + \rho_{0B0}[t] + \rho_{0B1}[t] + \rho_{0B2}[t])}
+ bp_{0A1}[t] \frac{\rho_{0V}[t]}{4 (\rho_{0V}[t] + \rho_{0A0}[t] + \rho_{0A1}[t] + \rho_{0A2}[t] + \rho_{0B0}[t] + \rho_{0B1}[t] + \rho_{0B2}[t])}
\]
\[
\frac{d\rho_{AOB0}[t]}{dt} = \delta \rho_{AOB1}[t] + \delta \rho_{A1B0}[t] - 2d\rho_{AOB0}[t]
\]

\[
- m \rho_{AOB0}[t] \left[ \begin{array}{l}
\frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \\
\frac{3}{4} (\rho_{vA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t]) \\
\frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \\
\frac{3}{4} (\rho_{vA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t]) \\
\frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \\
\frac{3}{4} (\rho_{vA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t]) \\
\frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \\
\frac{3}{4} (\rho_{vA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t]) \\
\frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \\
\rho_{vA0}[t]
\end{array} \right]
\]

\[
+ b \rho_{vA0}[t] \left[ \begin{array}{l}
\frac{3}{4} (\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t]) \\
\rho_{vA0}[t]
\end{array} \right]
\]

\[
+ b \rho_{vB0}[t] \left[ \begin{array}{l}
\frac{3}{4} (\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t]) \\
\rho_{vA0}[t]
\end{array} \right]
\]
\[
\frac{d\rho_{BOB0}[t]}{dt} = \delta \rho_{BOB1}[t] - 2d\rho_{BOB0}[t]
\]
\[
-2m\rho_{BOB0}[t]\left(\frac{3}{4}\rho_{BOB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]\right)
\]
\[
-2m\rho_{BOB0}[t]\left(\frac{3}{4}\rho_{BOB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]\right)
\]
\[
-2m\rho_{BOB0}[t]\left(\frac{3}{4}\rho_{BOB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]\right)
\]
\[
-2m\rho_{BOB0}[t]\left(\frac{3}{4}\rho_{BOB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]\right)
\]
\[
+ b\rho_{BOB0}[t]\left(\frac{1}{4} + \frac{3}{4}\rho_{Vv}[t] + \rho_{VvA0}[t] + \rho_{VvA1}[t] + \rho_{VvA2}[t] + \rho_{VvB0}[t] + \rho_{VvB1}[t] + \rho_{VvB2}[t]\right)
\]

\[
\frac{d\rho_{AOA0}[t]}{dt} = \delta \rho_{AOA1}[t] - 2d\rho_{AOA0}[t]
\]
\[
-2m\rho_{AOA0}[t]\left(\frac{3}{4}\rho_{AOA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t]\right)
\]
\[
-2m\rho_{AOA0}[t]\left(\frac{3}{4}\rho_{AOA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t]\right)
\]
\[
-2m\rho_{AOA0}[t]\left(\frac{3}{4}\rho_{AOA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t]\right)
\]
\[
-2m\rho_{AOA0}[t]\left(\frac{3}{4}\rho_{AOA0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t]\right)
\]
\[
+ b\rho_{AOA0}[t]\left(\frac{1}{4} + \frac{3}{4}\rho_{Vv}[t] + \rho_{VvA0}[t] + \rho_{VvA1}[t] + \rho_{VvA2}[t] + \rho_{VvB0}[t] + \rho_{VvB1}[t] + \rho_{VvB2}[t]\right)
\]
\[
\frac{d \rho_{A1B1}[t]}{dt} = m \rho_{A0B1}[t] \left( \frac{3}{4} \left( \rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \right) \\
+ m \rho_{A1B0}[t] \left( \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \right) \\
+ m \rho_{A1B0}[t] \left( \frac{1}{4} + \frac{3}{4} \left( \rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \right) \\
+ m \rho_{A0B1}[t] \left( \frac{1}{4} + \frac{3}{4} \left( \rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \right) \\
- c_{A1} \rho_{A1B1}[t] - c_{B1} \rho_{A1B1}[t] - 2d \rho_{A1B1}[t] - 2\delta \rho_{A1B1}[t] \\
- m \rho_{A1B1}[t] \left( \frac{3}{4} \left( \rho_{vA1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t] \right) \right) \\
- m \rho_{A1B1}[t] \left( \frac{3}{4} \left( \rho_{vB1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t] \right) \right) \\
- m \rho_{A1B1}[t] \left( \frac{3}{4} \left( \rho_{vB1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t] \right) \right) \\
- m \rho_{A1B1}[t] \left( \frac{3}{4} \left( \rho_{vB1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t] \right) \right) \\
+ b \rho_{vB1}[t] \left( \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \right) \\
+ b \rho_{vA1}[t] \left( \frac{3}{4} \left( \rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t] \right) \right)
\]
\[
\frac{d\rho_{B1B1}[t]}{dt} = m\rho_{B0B1}[t]\left(\frac{1}{4} + \frac{3}{4} \left(\rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t]\right)\right) \\
+ m\rho_{B0B1}[t]\left(\frac{1}{4} \left(\rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t]\right)\right) \\
- 2c_{B1}\rho_{B1B1}[t] - 2d\rho_{B1B1}[t] - 2\delta\rho_{B1B1}[t] \\
- 2m\rho_{B1B1}[t]\left(\frac{1}{4} \left(\rho_{vB1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]\right)\right) \\
- 2m\rho_{B1B1}[t]\left(\frac{1}{4} \left(\rho_{vB1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]\right)\right) \\
+ b\rho_{vB1}[t]\left(\frac{1}{4} + \frac{1}{4} \left(\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t]\right)\right)
\]

\[
\frac{d\rho_{A1A1}[t]}{dt} = m\rho_{A0A1}[t]\left(\frac{1}{4} + \frac{3}{4} \left(\rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]\right)\right) \\
+ m\rho_{A0A1}[t]\left(\frac{1}{4} \left(\rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]\right)\right) \\
- 2c_{A1}\rho_{A1A1}[t] - 2d\rho_{A1A1}[t] - 2\delta\rho_{A1A1}[t] \\
- 2m\rho_{A1A1}[t]\left(\frac{1}{4} \left(\rho_{vA1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t]\right)\right) \\
- 2m\rho_{A1A1}[t]\left(\frac{1}{4} \left(\rho_{vA1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t]\right)\right) \\
+ b\rho_{vA1}[t]\left(\frac{1}{4} + \frac{1}{4} \left(\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t]\right)\right)
\]
\[
\frac{d \rho_{A2B0}[t]}{dt} = m \rho_{A0B0}[t] \left( \frac{3}{4} \left( \rho_{V A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \rho_{A0A2}[t] \right)
\]

\[
+ \frac{3}{4} m \rho_{A0B0}[t] \left( \rho_{V A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t] \right) \rho_{A0B2}[t] \rho_{A0B1}[t]
\]

\[
- \rho_{A2B0}[t] c_{A2} - 2 d \rho_{A2B0}[t] - \delta \rho_{A2B0}[t] + \delta \rho_{A2B1}[t]
\]

\[
- m \rho_{A2B0}[t] \left( \frac{3}{4} \left( \rho_{V B0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \right)
\]

\[
- m \rho_{A2B0}[t] \left( \frac{3}{4} \left( \rho_{V B0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \right)
\]

\[
- m \rho_{A2B0}[t] \left( \frac{3}{4} \left( \rho_{V B0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \right)
\]

\[
- m \rho_{A2B0}[t] \left( \frac{3}{4} \left( \rho_{V B0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t] \right) \right)
\]

\[
+ m \rho_{A1B0}[t] \left( \frac{3}{4} \left( \rho_{V A1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t] \right) \right)
\]

\[
+ m \rho_{A1B0}[t] \left( \frac{3}{4} \left( \rho_{V A1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t] \right) \right)
\]

\[
+ b \rho_{V A2}[t] \left( \frac{3}{4} \left( \rho_{V V}[t] + \rho_{V A0}[t] + \rho_{V A1}[t] + \rho_{V A2}[t] + \rho_{V B0}[t] + \rho_{V B1}[t] + \rho_{V B2}[t] \right) \right)
\]

\[
+ b \rho_{V B0}[t] \left( \frac{3}{4} \left( \rho_{V V}[t] + \rho_{V A0}[t] + \rho_{V A1}[t] + \rho_{V A2}[t] + \rho_{V B0}[t] + \rho_{V B1}[t] + \rho_{V B2}[t] \right) \right)
\]
\[
\frac{d\rho_{AOB2}[t]}{dt} = m\rho_{AOB0}[t] \left( \frac{3}{4} \frac{\rho_{BOB2}[t]}{\rho_{AB0}[t]} + \frac{3}{4} \frac{\rho_{A0B0}[t]}{\rho_{AB20}[t]} \right) + 3 \rho_{AOB0}[t] \left( \frac{\rho_{BOB2}[t]}{\rho_{BOB0}[t]} + \frac{\rho_{A1B0}[t]}{\rho_{BOB0}[t]} \right) - \rho_{AOB2}[t] \rho_{B2} - 2d\rho_{AOB2}[t] - \delta\rho_{AOB2}[t] + \delta\rho_{A1B2}[t] \\
- m\rho_{AOB2}[t] \left( \frac{3}{4} \frac{\rho_{A0A0}[t] + \rho_{A0B1}[t]}{\rho_{AOB1}[t]} \rho_{AOA1}[t] \right) - m\rho_{AOB2}[t] \left( \frac{3}{4} \frac{\rho_{A0A0}[t] + \rho_{A0B1}[t]}{\rho_{AOB1}[t]} \rho_{A0A2}[t] \right) - m\rho_{AOB2}[t] \left( \frac{3}{4} \frac{\rho_{A0A0}[t] + \rho_{A0B1}[t]}{\rho_{AOB1}[t]} \rho_{A0A2}[t] \right) - m\rho_{AOB2}[t] \left( \frac{1}{4} + \frac{3}{4} \frac{\rho_{A0A0}[t] + \rho_{A0B1}[t]}{\rho_{AOB1}[t]} \rho_{AOB2}[t] \right) \\
+ m\rho_{AOB1}[t] \left( \frac{3}{4} \frac{\rho_{A0B1}[t] + \rho_{A1B1}[t]}{\rho_{A2B1}[t]} \rho_{B1B2}[t] \right) + m\rho_{AOB1}[t] \left( \frac{3}{4} \frac{\rho_{A0B1}[t] + \rho_{A1B1}[t]}{\rho_{A2B1}[t]} \rho_{A2B2}[t] \right) + m\rho_{AOB1}[t] \left( \frac{3}{4} \frac{\rho_{A0B1}[t] + \rho_{A1B1}[t]}{\rho_{A2B1}[t]} \rho_{B2B2}[t] \right) + m\rho_{AOB1}[t] \left( \frac{3}{4} \frac{\rho_{V}[t] + \rho_{A0B1}[t]}{\rho_{A0B1}[t]} \rho_{A0B2}[t] \right) + m\rho_{AOB1}[t] \left( \frac{3}{4} \frac{\rho_{V}[t] + \rho_{A0B1}[t]}{\rho_{A0B1}[t]} \rho_{A2B1}[t] \right) + m\rho_{AOB1}[t] \left( \frac{3}{4} \frac{\rho_{V}[t] + \rho_{A0B1}[t]}{\rho_{A0B1}[t]} \rho_{B1B2}[t] \right)
\]
\[
\frac{d\rho_{BOB2}[t]}{dt} = 2m\rho_{BOB0}[t] \left( \frac{3}{4} \rho_{B0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t] \right) + \frac{\rho_{BOB2}[t]}{\rho_{A2B0}[t]}
\]
\[
+ 2m\rho_{BOBO}[t] \left( \frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \right)
\]
\[
- \rho_{BOB2}[t] e_{B2} - 2d\rho_{BOB2}[t] - \delta \rho_{BOB2}[t] + \delta \rho_{B1B2}[t]
\]
\[
- m\rho_{BOB2}[t] \left( \frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \right)
\]
\[
- m\rho_{BOB2}[t] \left( \frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \right)
\]
\[
- m\rho_{BOB2}[t] \left( \frac{3}{4} (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t]) \right)
\]
\[
+ m\rho_{BOB1}[t] \left( \frac{3}{4} (\rho_{vB1}[t] + \rho_{AOB1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]) \right)
\]
\[
+ m\rho_{BOB1}[t] \left( \frac{3}{4} (\rho_{vB1}[t] + \rho_{AOB1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]) \right)
\]
\[
+ b\rho_{vB0}[t] \left( \frac{3}{4} (\rho_{vB1}[t] + \rho_{AOB1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]) \right)
\]
\[
+ b\rho_{vB2}[t] \left( \frac{3}{4} (\rho_{vB1}[t] + \rho_{AOB1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]) \right)
\]
\[
\frac{d\rho_{A0A2}[t]}{dt} = 2m\rho_{A0A0}[t] \frac{3}{4} (\rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t])
\]
\[
+ 2m\rho_{A0A0}[t] \frac{3}{4} (\rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t])
\]
\[
- \rho_{A0A2}[t]\rho_{A0A2}[t] - 2d\rho_{A0A2}[t] - \delta\rho_{A0A2}[t] + \delta\rho_{A1A2}[t]
\]
\[
- m\rho_{A0A2}[t] \frac{3}{4} (\rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t])
\]
\[
- m\rho_{A0A2}[t] \left( \frac{1}{4} + \frac{3}{4} (\rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]) \right)
\]
\[
- m\rho_{A0A2}[t] \frac{3}{4} (\rho_{vA0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t])
\]
\[
+ m\rho_{A0A1}[t] \frac{3}{4} (\rho_{vA1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t])
\]
\[
+ m\rho_{A0A1}[t] \frac{3}{4} (\rho_{vA1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t])
\]
\[
+ b\rho_{vA0}[t] \frac{3}{4} (\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t])
\]
\[
+ b\rho_{vA2}[t] \frac{3}{4} (\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t])
\]
\[
\frac{d\rho_{A2B1}[t]}{dt} = m\rho_{A0B1}[t]\left(\frac{3}{4} (\rho_{A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t])
+ m\rho_{A0B1}[t]\left(\frac{3}{4} (\rho_{A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t])
+ m\rho_{A2B0}[t]\left(\frac{3}{4} (\rho_{A0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t])
+ m\rho_{A2B0}[t]\left(\frac{3}{4} (\rho_{A0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t])
+ m\rho_{A1B1}[t]\left(\frac{3}{4} (\rho_{A1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t])
+ m\rho_{A1B1}[t]\left(\frac{3}{4} (\rho_{A1}[t] + \rho_{A0A1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t])
- c_A \rho_{A2B1}[t] - c_B \rho_{A2B1}[t] - 2d \rho_{A2B1}[t] - 2\rho \rho_{A2B1}[t]
- m\rho_{A2B1}[t]\left(\frac{1}{4} + \frac{3}{4} (\rho_{V1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t])
- m\rho_{A2B1}[t]\left(\frac{3}{4} (\rho_{V1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t])
+ b\rho_{V2}[t]\left(\frac{3}{4} (\rho_{VV}[t] + \rho_{V0}[t] + \rho_{V1}[t] + \rho_{V2}[t] + \rho_{V0B1}[t] + \rho_{V1B1}[t] + \rho_{V2B1}[t])
+ b\rho_{V1}[t]\left(\frac{3}{4} (\rho_{VV}[t] + \rho_{V0}[t] + \rho_{V1}[t] + \rho_{V2}[t] + \rho_{V0B1}[t] + \rho_{V1B1}[t] + \rho_{V2B1}[t])
\right)\right)\right)\right)
\]
\[
\frac{d \rho_{A1B2}[t]}{dt} = m \rho_{A1B0}[t] \frac{\rho_{B0B2}[t]}{4 (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t])} \\
+ m \rho_{A1B0}[t] \frac{\rho_{pA2B0}[t]}{4 (\rho_{vB0}[t] + \rho_{AOB0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t])} \\
+ m \rho_{AOB2}[t] \frac{\rho_{AOA1}[t]}{4 (\rho_{vA0}[t] + \rho_{AOA0}[t] + \rho_{A0A1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t])} \\
+ m \rho_{AOB2}[t] \frac{\rho_{AOB1}[t]}{4 (\rho_{vA0}[t] + \rho_{AOA0}[t] + \rho_{A0A1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t])} \\
+ m \rho_{A1B1}[t] \frac{\rho_{BOB2}[t]}{4 (\rho_{vB1}[t] + \rho_{AOB1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t])} \\
+ m \rho_{A1B1}[t] \frac{\rho_{B1B2}[t]}{4 (\rho_{vB1}[t] + \rho_{AOB1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{BOB1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t])} \\
- c_{A1} \rho_{A1B2}[t] - c_{B2} \rho_{A1B2}[t] - 2d \rho_{A1B2}[t] - 2 \delta \rho_{A1B2}[t] \\
- m \rho_{A1B2}[t] \left( \frac{1}{4} + \frac{3}{4} (\rho_{VA1}[t] + \rho_{AOA1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t]) \right) \\
- m \rho_{A1B2}[t] \frac{\rho_{A1A2}[t]}{4 (\rho_{vA1}[t] + \rho_{AOA1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t])} \\
+ b \rho_{VA1}[t] \frac{\rho_{vB2}[t]}{4 (\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t])} \\
+ b \rho_{VA1}[t] \frac{\rho_{vA1}[t]}{4 (\rho_{vV}[t] + \rho_{vA0}[t] + \rho_{vA1}[t] + \rho_{vA2}[t] + \rho_{vB0}[t] + \rho_{vB1}[t] + \rho_{vB2}[t])}
\]
\[
\frac{d\rho_{B1B2}[t]}{dt} = m\rho_{B0B1}[t]\left(\frac{3}{4} \left(\rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t]\right)
\right)
\]
\[
+ \rho_{B0B2}[t] \left(\frac{3}{4} \left(\rho_{vB0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t]\right)
\right)
\]
\[
\frac{d\rho_{A1A2}[t]}{dt} = m\rho_{A0A1}[t] \frac{\rho_{A0A2}[t]}{4} + m\rho_{A0A1}[t] \frac{\rho_{A0A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{4} + m\rho_{A0A2}[t] \frac{\rho_{A0A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{4} + m\rho_{A0A2}[t] \frac{\rho_{A0A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{4} + 2m\rho_{A1A1}[t] \frac{\rho_{A1A2}[t]}{4} + 2m\rho_{A1A1}[t] \frac{\rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t]}{4} + 2m\rho_{A1A1}[t] \frac{\rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t]}{4} + \frac{d\rho_{A1A2}[t]}{dt} \left( \frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{A1A2}[t]}{\rho_{A0A2}[t]} \right) \right) + \frac{d\rho_{A1A2}[t]}{dt} \left( \frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t]}{\rho_{A1B2}[t]} \right) \right) + b\rho_{A1}[t] \frac{3}{4} \left( \frac{\rho_{B1}[t]}{\rho_{A0}[t] + \rho_{A1}[t] + \rho_{A2}[t] + \rho_{B0}[t] + \rho_{B1}[t] + \rho_{B2}[t]} \right) + b\rho_{A2}[t] \frac{3}{4} \left( \frac{\rho_{B1}[t]}{\rho_{A0}[t] + \rho_{A1}[t] + \rho_{A2}[t] + \rho_{B0}[t] + \rho_{B1}[t] + \rho_{B2}[t]} \right)
\]
\[
\frac{d\rho_{A2B2}[t]}{dt} = -\rho_{A2B2}[t]\rho_{A2} - \rho_{A2B2}[t]\rho_{B2} - 2\rho_{A2B2}[t]d - 2\delta\rho_{A2B2}[t]
+ m\rho_{A0B2}[t]\left(\frac{3}{4} (\rho_{Va0}[t] + \rho_{AoA0}[t] + \rho_{AoA1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t])\right)
+ m\rho_{A0B2}[t]\left(\frac{1}{4} + \frac{3}{4} (\rho_{va0}[t] + \rho_{AOA0}[t] + \rho_{AOA1}[t] + \rho_{AOA2}[t] + \rho_{AOB0}[t] + \rho_{AOB1}[t] + \rho_{AOB2}[t])\right)
+ m\rho_{A2B0}[t]\left(\frac{1}{4} + \frac{3}{4} (\rho_{vb0}[t] + \rho_{AOB0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t])\right)
+ m\rho_{A2B0}[t]\left(\frac{3}{4} (\rho_{AOBO}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{BOB0}[t] + \rho_{BOB1}[t] + \rho_{BOB2}[t])\right)
+ m\rho_{A1B2}[t]\left(\frac{3}{4} (\rho_{VA1}[t] + \rho_{AOA1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t])\right)
+ m\rho_{A1B2}[t]\left(\frac{1}{4} + \frac{3}{4} (\rho_{VA1}[t] + \rho_{AOA1}[t] + \rho_{A1A1}[t] + \rho_{A1A2}[t] + \rho_{A1B0}[t] + \rho_{A1B1}[t] + \rho_{A1B2}[t])\right)
+ m\rho_{A2B1}[t]\left(\frac{1}{4} + \frac{3}{4} (\rho_{VB1}[t] + \rho_{AOB1}[t] + \rho_{A2B1}[t] + \rho_{BOB1}[t] + \rho_{BOB1}[t] + \rho_{B1B2}[t])\right)
+ m\rho_{A2B1}[t]\left(\frac{3}{4} (\rho_{AOB1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{BOB1}[t] + \rho_{BOB1}[t] + \rho_{B1B2}[t])\right)
+ b\rho_{VB2}[t]\left(\frac{3}{4} (\rho_{VV}[t] + \rho_{Va0}[t] + \rho_{V1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t])\right)
+ b\rho_{VA2}[t]\left(\frac{3}{4} (\rho_{VV}[t] + \rho_{Va0}[t] + \rho_{V1}[t] + \rho_{VA2}[t] + \rho_{VB0}[t] + \rho_{VB1}[t] + \rho_{VB2}[t])\right)
\]
\[
\frac{d\rho_{B2B2}[t]}{dt} = -2\rho_{B2B2}[t]c_{B2} - 2\rho_{B2B2}[t]d - 2\delta\rho_{B2B2}[t] \\
+ m\rho_{B0B2}[t]\left(\frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{B0}[t] + \rho_{A0B0}[t] + \rho_{A1B0}[t] + \rho_{A2B0}[t] + \rho_{B0B0}[t] + \rho_{B0B1}[t] + \rho_{B0B2}[t]}{\rho_{A2B0}[t]} \right) \right) \\
+ m\rho_{B0B2}[t]\left(\frac{3}{4} \left( \frac{\rho_{B1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]}{\rho_{B1B2}[t]} \right) \right) \\
+ m\rho_{B1B2}[t]\left(\frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{B1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]}{\rho_{B1B2}[t]} \right) \right) \\
+ m\rho_{B1B2}[t]\left(\frac{3}{4} \left( \frac{\rho_{B1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B1B2}[t]}{\rho_{B2B2}[t]} \right) \right) \\
+ b\rho_{B2}[t]\left(\frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{B1}[t] + \rho_{A0B1}[t] + \rho_{A1B1}[t] + \rho_{A2B1}[t] + \rho_{B0B1}[t] + \rho_{B1B1}[t] + \rho_{B2B2}[t]}{\rho_{B2B2}[t]} \right) \right)
\]

\[
\frac{d\rho_{A2A2}[t]}{dt} = -2\rho_{A2A2}[t]c_{A2} - 2\rho_{A2A2}[t]d - 2\delta\rho_{A2A2}[t] \\
+ m\rho_{A0A2}[t]\left(\frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{A0}[t] + \rho_{A0A0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{\rho_{A0B2}[t]} \right) \right) \\
+ m\rho_{A0A2}[t]\left(\frac{3}{4} \left( \frac{\rho_{B0}[t] + \rho_{A0B0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{\rho_{A0B2}[t]} \right) \right) \\
+ m\rho_{A1A2}[t]\left(\frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{B0}[t] + \rho_{A0B0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{\rho_{A0B2}[t]} \right) \right) \\
+ m\rho_{A1A2}[t]\left(\frac{3}{4} \left( \frac{\rho_{B0}[t] + \rho_{A0B0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{\rho_{A0B2}[t]} \right) \right) \\
+ b\rho_{A2}[t]\left(\frac{1}{4} + \frac{3}{4} \left( \frac{\rho_{B0}[t] + \rho_{A0B0}[t] + \rho_{A0A1}[t] + \rho_{A0A2}[t] + \rho_{A0B0}[t] + \rho_{A0B1}[t] + \rho_{A0B2}[t]}{\rho_{A0B2}[t]} \right) \right)
\]
Table 5: The table of parameter intervals used in the Specialization Model when randomly selecting the parameters. Parameters $y$ and $w$ were only used in this instance and were used to determine $c_{A2}$, $c_{B1}$, and $c_{B2}$ in relation to $c_{A1}$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low End of the Interval</th>
<th>High End of the Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m$</td>
<td>$10^{-6}$</td>
<td>$10^{-4}$</td>
</tr>
<tr>
<td>$\delta$</td>
<td>$5 \times 10^{-4}$</td>
<td>$5 \times 10^{-3}$</td>
</tr>
<tr>
<td>$b$</td>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>$d$</td>
<td>0.009</td>
<td>0.3</td>
</tr>
<tr>
<td>$c_{A1}$</td>
<td>-0.0002</td>
<td>-0.02</td>
</tr>
<tr>
<td>$y$</td>
<td>0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>$w$</td>
<td>0.9</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Appendix C- Values tested for the Mochizuki Extension in the Grid Method

Table 4: Table of the Values used for the Mochizuki Extension Model
Appendix D- Parameter Intervals for Specialization Model

The value of the other costs were calculated as $c_{B1} = c_{A2} = -c_{A1} \times y$ and $c_{B2} = c_{A1} \times w$ in relation to $c_{A1}$