BACTERIAL EVOLUTION AND ANTIBIOTIC PRODUCTION

THE EVOLUTION OF ANTIBIOTIC PRODUCTION RATE IN A SPATIAL MODEL OF BACTERIAL COMPETITION

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Lay Abstract

We looked at computational models of bacterial interaction involving producers, non-producers, and susceptible cell types that interacted in a manner similar to the game “rock-paper-scissors”. We determined that the system is stable for the long term for a given set of parameters, otherwise susceptible cells win as not enough antibiotic is being produced, or too much is being produced, significantly inhibiting the growth of producers. Moreover, we found that these systems can evolve, tending towards one production rate, in order to better allow the system to survive. Non-producers also evolve, tending to low production rates instead. These results have implications in understanding bacteria that cannot be cultured and perhaps aiding in the discovery of novel antibiotics.

Abstract

Bacteria occupy a wide range of niches with many different types coexisting. They compete directly, with some capable of producing antibiotics that kill other members of the niche. Despite this, long term survival of these ecosystems is possible. Here, we consider a lattice-based three-component system with antibiotic producers, non-producers (or cheaters), and susceptible cells competing. In our system, there is a metabolic cost tied to production rate, resulting in a decrease in growth rate for the producers. Non-producers behave as cheaters that gain the benefit of an antibiotic without the cost of producing it themselves. The susceptible cells are a faster growing different species. The model behaves in a fashion similar to the game “rock-paper-scissors", because producers beat susceptible cells, non-producers beat producers, and susceptible cells beat non-producers. We consider two spatial lattice models, one in which there is a nearest neighbour interaction between cells, and one in which the long-range diffusion of the antibiotic is explicitly included. We consider the parameter space in which the three cell types can coexist (taking into account cost and production rate), and determine the regions in which production rate is too high or too low to allow coexistence. We determine that antibiotic producers will evolve to an optimal production rate and that low-rate producers can outcompete complete cheaters (non-producers). We finally illustrate that the introduction of a fourth “resistant” cell type allows the system to survive with four members for some parameters. In other cases, addition of the resistant cells causes the extinction of the producers, which eventually favours the susceptible cells.

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Chapter 1

The Ecology and Importance of Antibiotic Production and Bacterial Cooperation

1.1 Motivation: the Importance of Antibiotics Resistance

Antibiotics are undoubtedly one of the most important medical discoveries of the 20th century. Since the discovery of penicillin in 1928 (Fleming 1929), countless lives have been saved through the use of this class of drugs. They exist in many forms, with many categories but the general idea is simple – prevention and treatment of bacterial infection. In general, antibiotics have two modes of action. They could be bactericidal, resulting in death of the target cell. These function through methods such as destroying the cell wall or damaging integral enzymes. On the other hand, antibiotics can be bacteriostatic, simply arresting cell growth and division.

However, despite the importance of these drugs, new discoveries are not being made very quickly. Regulations and lack of economic incentives mean that development by pharmaceutical companies is quite slow. However, antibiotic resistance is on the rise. Overuse, inappropriate prescribing, and agricultural use lead to the emergence of novel strains that cannot be targeted by our present repertoire of antibiotics. Therefore, we are seeing more and more species that are much harder to deal with today than in the past. Of especial clinical relevance are Methicillin-Resistant *Staphylococcus aureus* and Vancomycin-Resistant Enterococci, which are found largely in hospitals, where immunocompromised patients may be affected (Ventola 2015).

Most of the antibiotics currently in use are derived from bacteria living in complex environments in the soil in which many bacterial species are interacting with one another, as will

be described in more detail below. This raises many interesting problems in ecology and evolution. How does antibiotic production affect the conditions for survival and coexistence of diverse communities of bacteria? When is it evolutionary favourable for an organism to produce an antibiotic? When would we expect resistance mechanisms to be selected in competing species? In this thesis, we turn to computational methods and models to give insights into how these bacteria coexist and interact so that we may better understand them. Through this understanding, we may be able to culture them or use them to our benefit in the fight against antibiotic resistance.

1.2 Antibiotic Producers and Sources

The discovery of penicillin in 1928 from a *Penicillium* fungus demonstrated that useful antibiotics can be found in nature. Since then many classes and types of these drugs have been found in plants, animals, and bacteria. In fact, microbes constitute one of the largest sources of antibiotics in our medical repertoire. Thus, the study of the ecology and lives of these organisms can provide incredible utility in combatting antibiotic resistance, as well as finding novel drugs capable of curing as-of-yet incurable illnesses.

The *Streptomyces* is a gram positive genus of bacteria and one of the most notable producers of antibiotics. Since the discovery of streptothricin and streptomycin in the 1940s, many groups have increased efforts to find antibiotics from the genus. Consequently, between 1955 and 1962 80% of antibiotics were obtained from actinomycetes, with the plurality coming from *Streptomyces.* Moreover, modelling based on the *Streptomyces* antibiotics discovered between 1941 and 1997 places conservative estimates at 150,000 unique compounds possibly being produced by the genus, with the vast majority as of yet undiscovered (Watve et al. 2001).

Thus, many important antibiotics have been contributed from *Streptomyces*, with a plethora still in use today. Streptomycin, actinomycin, erythromycin, and vancomycin are widely popular examples that come from the actinomycete group in general (Clardy 2005). Streptomycin is quite cost-effective and appears on the WHO Model List of Essential Medicines, which describes the minimum medicine needs for a basic health-care system (WHO 2015). However, with the growing dangers of antibiotic resistance, its efficacy and use is at a decline. Vancomycin is also one of the most important antibacterial drugs. It is used as a last-line treatment for many more serious infections (Boneca and Chiosis 2003). However, with the appearance of many vancomycin resistant strains as well, it too is in danger of losing its utility (Di Pentima and Chan 2010).

These examples illustrate the need for novel antibiotics sources. Although actinomycetes represent a massive source of our antimicrobial compounds, their efficacy has become overall diminished due to the presence of resistant bacterial strains. Therefore, it is important to consider other bacterial sources that may not be as widely studied. An example of this are the microcystins, which are hepatotoxins produced by cyanobacteria. These have been demonstrated effective against a variety of bacterial (Zurawell et al. 2005; Singh et al. 2011). However, due to the complexity of culturing cyanobacteria, these products are difficult to work with and result in very expensive compounds (Welker et al. 2012).

Additionally, myxobacteria are a group of soil bacteria that present a potential as a source of antibiotics. Epothilone is presently the only clinically used compound derived from these bacteria, although a plethora of secondary metabolites of varying antibacterial efficacy have been isolated from the group (Wenzel and Müller 2009). Bacillus have also been proven to produce

antibiotics (Chen et al. 2008; Atallah 2012), with surfactin being one of the most widely used (Baruzzi et al. 2011).

Many of the bacteria described live in niches that are very complex and occupied by many members. Work from the 1990s demonstrated that 1g of deciduous forest soil contains ~1.5 x 10^{10} bacteria spread across ~4,000 different species (Torsvik et al. 1990). Biogeographical analysis indicates further biodiversity: communities vary widely based on geographical location and factors such as pH (Fierer and Jackson 2006). Consequently, it is difficult to culture many of these species by themselves, let alone recreating the complex multispecies environments found in nature. While it has been demonstrated that some species can be cultured using older techniques, such as solid media and petri dishes (Joseph et al. 2003), other methods must be used to further study these systems. In addition to being sources of antibiotics, it is becoming more evident that soil bacteria may be sources of resistance (D'Costa et al. 2007), further necessitating the study of these environments.

Therefore, it is worth reducing these systems to simpler models, allowing analysis of interactions both *in vivo* (with fewer members, giving a gist of large scale dynamics), and *in silico*, allowing for consideration of niches that may be completely impossible to grow in culture. Consequently, this thesis utilizes these computational methods to study the environments occupied by antibiotic producers and their competitors.

1.3 The Role of Secreted Molecules in Bacterial Interactions

Ultimately, it is evident that the study of microbial ecology is of great importance. Understanding the niches that these cells live in can help with culturing them, or purifying their secretions, possibly leading to the discovery of novel drugs. Indeed, while antibiotics are largely

sourced from bacteria, other drugs such as antifungals and anticancer agents have been discovered in microbes as well. Thus, it is important to consider the dynamics of the secondary metabolites and enzymes secreted when bacteria interact. Concentration and efficacy of these chemicals are partly governed by diffusion, which has been studied in the context of bacterial interactions fairly extensively.

A major study by Vetsigian et al (2011) looked to characterize pairwise interactions between *Streptomyces* strains. They grew a strain on a medium, and then removed the strain, being left with conditioned medium. They then grew another strain on the conditioned medium to determine how the secretions from the first strain affected the second. They found that 45% of interactions were inhibitory with 19% inducing growth. It appears that in the matrix they generated the inhibitory interactions were quite broad, with isolates affecting almost everyone or almost no one. It has further been shown that Streptomycetes react to antibiotics produced by others (Vetsigian et al. 2011).

When encountering secretions of other bacteria, antibiotic production is ramped up in some cases as a defense mechanism, and some cells begin producing substances that inhibit production of antibiotics in other cells (Abrudan et al. 2015). Tyc et al. (2014) also looked at production of metabolites using *S. aureus* and *Escherichia coli* as indicator organisms. They found that antimicrobial activity can occur in both monoculture and mixed cultures of different species but sometimes the interactions can cause a loss of antimicrobial activity against the indicator organism. This may be due to the suppressive interactions within the culture. The opposite was also possible, indicating that signaling to produce antibiotics can also occur.

As antibiotics can diffuse into the surrounding area, resistant species that are close to antibiotic producers can benefit from the presence of the producers. In this sense, the antibiotics

are public goods which can be defined as those "which all enjoy in common in the sense that each individual's consumption of such a good leads to no subtractions from any other individual's consumption of that good" (Samualson 1954). As such, it is difficult to see when it is beneficial to cooperate with the colony and when it is better to become a cheater and gain the benefit by not producing any public good (Levin 2014). In communal environments where a mutually beneficial public good is involved, cheaters can arise that gain the group benefit without paying a cost of contributing. In this thesis, the most important kind of cheaters are non-producers - *i.e.* individuals of an antibiotic producing species that stop producing the antibiotic but retain their intrinsic resistance to the antibiotic produced by their own species. Non-producers of all types can arise via several means, including deleterious mutations in the genes responsible for production and secretion, loss of a plasmid containing the relevant genes, or quantitative changes in the production mechanism that lead cells to produce less of a substance (Allison et al. 2014; Chao and Levin 1981). In all these cases, there is expected to be a reduced metabolic cost in the non-producers that will give them an advantage relative to the producers.

This difference in metabolic cost between non-producers and producers has been demonstrated well by Drescher et al. (2014). They looked at producers and non-producers of chitinase, a secreted enzyme that breaks down chitin, allowing uptake of its monomers for use within the cell. These authors found that cells deficient in production of this public good enzyme had a higher basal growth rate (~0.5% higher) than the producers. When grown in chitin with knockout mutants, it was found that producers decreased in frequency if they were seeded at high frequencies to begin with. Increasing biofilm production by introducing a gene, thereby limiting public good access only to producers, allowed the producers to outcompete the cheaters as the

diffusion of the liberated chitin monomers was limited and more accessible to the producers than non-producers.

Allison et al. (2014) demonstrated the specific advantage that non-producers have over producers when a public good is involved. They looked at a protease producing strain of *Psuedomonas fluorescens* under well-mixed and spatially structured conditions. In well-mixed experiments when starting with both producing and non-producing strains in equal concentrations, cheaters eventually took over the system. However, when starting in spatial conditions with equal distributions of both, it was found that both cell types were maintained, although producers at lower concentrations. They also found that cheaters emerged spontaneously and through different pathways. These results indicate that lower diffusion benefits producers more than cheaters, as the resource is more available to the organism incurring the cost.

Dobay et al. (2014) considered cheaters and cooperators benefitting from some generic "public good." This simulation utilized cells that physically grow and divide wherein the molecule taken up generates a benefit to any cells that utilizes it. The production of each molecule has a cost. Cells were allowed to move, as well as grow and die. The model also took into account durability and diffusion of the beneficial molecule. They found that lower diffusion resulted in less cooperation; *i.e.* cheaters have less access to the benefit and grow slower. However, at higher diffusion rates cooperation was favoured only with cell diffusion occurring as well. High public goods durability also negatively affected the cheaters.

Borenstein et al. (2013) looked at diffusion of a molecule produced by cooperators with cheaters in the system. They used a computer model with all sites occupied, with cells replacing other cells. It was found that these two species systems could not exist indefinitely, as there was

no selection bias when a species became rarer. Instead, there was a critical value of diffusion and cost at which the chance of each species reaching fixation was equal. Moreover, they found that similar to other studies, long diffusion favoured the cheater, while limiting diffusion to short distances favoured the producer.

One thing that distinguishes antibiotic production from some other kinds of public good scenarios is that the antibiotic is only of benefit when there are sensitive cells present. Thus the study of the evolutionary interaction between producers and non-producers only makes sense in the presence of a third type of cell that is sensitive. Other kinds of molecules secreted by bacteria do have a direct benefit, such as proteases (Allison et al. 2014) and chitinase (Drescher et al. 2014) (described in the models above) and these cases do not depend on the existence of a third species. Most of the above models considered this sort of public good, and while different from the producer-susceptible-non-producer case, they provided excellent insights into cooperation and diffusion of molecules. In this thesis we are specifically interested in the interaction of antibiotic producers and non-producers in the presence of sensitive cells. This problem involves (at least) three species, as described above. Therefore, we will now review studies and models of three species interactions.

1.4 Three-Species Interactions

Many types of three species interactions can be described by a rock-paper-scissors model that mimics the children's game in which rock beats scissors, scissors beat paper, and paper beats rock, creating a cyclic hierarchy. It has long been established that such systems can be used to model ecological interactions, including lizards (Sinervo and Lively 1996), salmon (Guill et al. 2011), and bacteria, such as *E. coli* bacteria (Durrett and Levin 1997; Kerr, M. a. Riley, et al.

2002). Over the years a variety of these models have been established, with differing degrees of complexity and biological relevance.

The simplest possible rock-paper-scissors model involves cyclic interaction between three cell types. In such a case, Species A beats Species B; Species B beats Species C; and Species C beats Species A. Often times interaction occurs in a nearest-neighbour fashion, where cells kill each other on a grid with a given probability depending on the defined cycle. Such a model was described by Reichenbach (2007). This model opts for the simplest case of these dynamics, with each cell having identical growth and death rate. A key observation in this model is that, in the well-mixed case, a mixture with equal concentrations of the three species is unstable. Large oscillations in the three concentrations build up, until one of the three becomes extinct by chance. After this, one of the remaining two species outcompetes the other. In a spatial model, however, where interactions only occur between close neighbours, it is possible for all three species to coexist due to the presence of spiral wave patterns. The spiral waves become less stable when the diffusion rate of cells is increased. Thus, the conclusion was that maintaining spatial structure is important to allow these sorts of systems to thrive.

Systems of these types generally behave with what is often described as “cyclic dominance”. These interactions result in periods where one species dominates the system for short cycles before being brought down by another, as each of the three has a weakness and advantage. One group demonstrated that this is possible with a simple quorum sensing model, wherein bacteria secrete a repressive molecule that beats a different bacterial type. They considered three species with three suppressors, creating a rock-paper-scissors game. In this case, each molecule occupied one site and a certain number needed to surround a cell to affect it (Gómez Esteban and Rodríguez-Patón 2011). This model was in the same vein as the

Reichenbach model as cells impacted each other directly and were identical aside from the target of their effect.

Another model considered an approach with a rock paper scissors effect in a radius that also included secretion of a breakdown enzyme – also in a radius. They found that intrinsic resistance with cell diffusion (i.e. well mixed) resulted in collapse of the system. However, in a breakdown model, the system was maintained even with diffusion of cells. Introduction of cheaters also resulted in a stable system. When a cheater of their fastest growing strain was introduced (with small fitness advantage), it replaced the parent stably. The slower growing cheaters could not invade except with high growth advantage, resulting in dominating the community or generating a four species community. They were also able to stabilize their system with more species producing more antibiotics (Kelsic et al. 2015).

Other groups looked at variants of these symmetrical rock-paper-scissors models. Kerr et al. (2002) considered a model with actual toxins, rather than just cyclic killing (**Figure 1.1a**). They created a nearest neighbour model to study interactions in colicinogenic bacteria, which have been widely used as a model system for studying bacterial interactions. Their model was composed of three cell types: colicin sensitive, colicin resistant, and colicin producing. Thus, sensitive displaced resistant due to difference in growth rate; resistant displaced colicinogenic due to difference in growth rate; and coliciongenic displaced sensitive via killing. Consequently the system functioned due to differences in growth rather than just nearest-neighbour killing, creating a model distinct from the simple case described by Reichenbach.

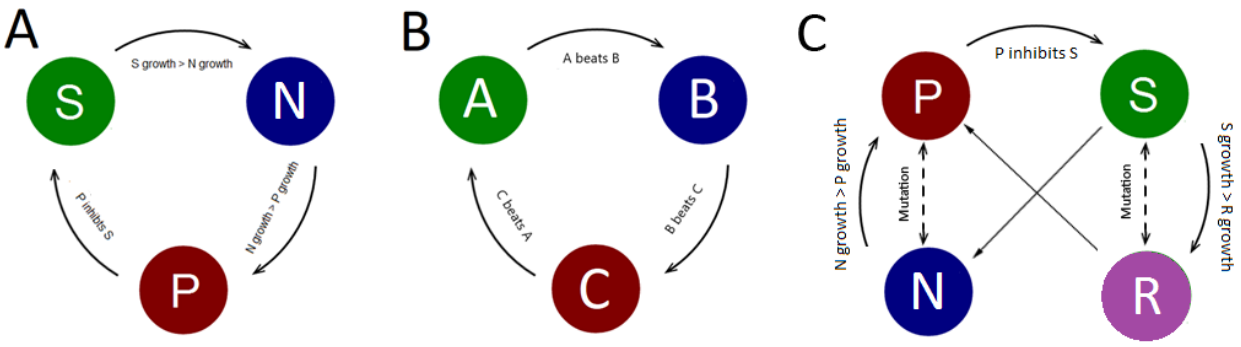

Figure 1.1: Three models of three-species bacterial competition. A) Our model and the colicin model, where the advantage between P and S is the production of antibiotics, causing detriment to S. B) The symmetric model of competition, where species beat each other based on inherent advantage. Every cell type has the same level of advantage as every other, but affecting a different species. C) The introduction of the resistant cell type as a mutant of the susceptible type.

They found that diversity is maintained for a large amount of parameter space in non-mixed systems, and that the system is not stable in well mixed conditions. Of note was the fact that the producer species was not maintained at lower concentrations. They also grew bacterial colonies of the same types as in the simulations on a single plate and found that diversity was maintained under non-mixed conditions. If the growth conditions were perturbed through mixing, one species always won out, as in their computational results (Kerr, M.A. Riley, et al. 2002).

More complicated models were also often considered. Czárán et al. looked at many different sensitive, resistant, and producer colonies, with different interactions possible. At each time point a cell could mutate to any of the three states, or combine with a neighbouring cell to obtain all of its resistance and toxin genes, or simply kill the neighbouring cell (based on its own state rather than probability; the model allows for no empty sites). This model resulted in eventual equilibrium (Czárán et al. 2002). A complex model proposed by Allison (Allison 2005) took into account the cost of extracellular enzyme production via uptake of carbon and nitrogen

(for amino acids), diffusion of broken down resource, and abundance of products in the environment (in which case resources were spent on growth instead of foraging). The model accounted for diffusion of enzymes and diffusion of resources (enzyme products). It was found once again that large diffusion rates favoured cheaters and also that higher production rate (in this model higher production had a higher cost) favoured cheaters as well. Stability was possible over a wide range of parameters. In most cases cheaters were maintained at higher levels than producers.

Interestingly, very few groups considered models with diffusion of molecules when considering rock-paper-scissors systems and antibiotics. One group considered a model of colicin-producing bacteria, similar to that of Kerr (2002), but allowing the colicin molecule to diffuse. They found that stability was maintained for larger lattice sizes but not for smaller ones, in which case the resistant strain dominated the system. They argued that long-range diffusion causes an effect similar to mixing and that in larger lattices the sensitive strains had more room to escape from the diffusing molecules (MacMartin and Rychtár 2007). On the other hand, introduction of diffusion to a colicin model by another group did not allow for long-term stability whereas a nearest-neighbour model did (Aristotelous and Durrett 2014).

Similarly, there appears to be a dearth of literature on mutations in rock-paper-scissors models. A more recent paper by Nahum et al. (2011) looked at the evolution of resistance in a well-based model that was similar to nearest neighbour interactions. They allowed for a certain volume of bacteria to be transferred to a well of the nearest neighbour and allowed resistant cells to mutate in growth rate. They found that the resistant cells tended towards the lowest possible growth rate, as this allowed producers to thrive and protect them. Moreover, stability was maintained in the long term.

1.5 Aims

Ultimately, three component systems have been studied in considerable depth using exact rock-paper-scissors models, and variations thereof. As such, a variety of things are clear with regards to these systems that seem to be true in almost all cases. The first is the necessity of spatial structure. Indeed, almost universally it seems that without structure and in cases where mixing is allowed it is almost impossible to maintain diversity. Further, it appears that these systems are generally stable, with cyclic dominance occurring in local patches, but all three species being present in the long term when averaged over large areas.

However, we do not know everything there is to know, especially in certain biologically relevant cases. While we do know that diffusion of a common good generally favours cheaters in common goods simulations, only a few cases has been found wherein a model implements diffusion in a rock-paper-scissors model of bacterial competition. Also, evolution has been sorely neglected in this field, with a limited number of studies considering the evolution of resistance, but as far as we can tell no one has studied the evolution of production and its correlation to cost.

As such, the aims of this thesis are as follows:

1. Construction of a rock-paper-scissors model of bacterial interaction with diffusion of antibiotics, with members being producers, non-producers, and susceptible cells. This will allow the study of these systems under more physically real conditions, allowing us to gain insight into the biological workings of these niches.
2. Consider the correlation between cost and production rate and determine the range of production rates under which the system can survive. When producing antibiotics there is an inherent metabolic cost associated with the actual construction of the molecules. This

will provide understanding of parameters and circumstances under which these environments might thrive, die, or hang on by a hair. It will provide in-depth understanding about what might happen to particular strains.

3. Study the evolution of production rates through mutation. This will provide understanding of the evolution of these systems. Where mutations are allowed the producer cell types should move towards an optimal production rate. This would give insight into the sorts of environments possibly required to culture these cells or why certain functions disappear in cultured producers.
4. Implement a fourth, resistant cell type in these systems. The resistant type functions similarly to a cheating non-producer but is biologically distinct. This cell type is a susceptible cell that gains resistant, rather than an inherently resistant cell that loses antibiotic production ability. This will demonstrate whether four-member systems are viable and what the effect of an emerging resistant bacteria might be.

Thus, the ultimate point of this thesis is to create a model that is closer to biological reality than previously studied. This will help with understanding these bacterial niches, including physical dynamics and effects of change and perturbation. While the model consists of only three or four cell types compared to the thousands found in these niches in the environment, the hope is that valuable insight can be gleaned in this field. In this way perhaps we can better understand microbial ecology, necessities for successful culture, and use this knowledge in the discovery of novel drugs.

Chapter 2

Mathematical Models of Interacting Bacterial Species

2.1 Mathematical Modelling of Interacting Species

Previously, I described many of the spatial models that have been used to study bacterial interactions *in silico.* However, they draw much of their basis from the work of earlier mathematicians that used ordinary differential equations (ODEs) to describe well-mixed systems. In this section I describe two historical models. The first is a symmetrical model, wherein each species affects another in a simple rock-paper-scissors cycle. The second describes a system closer to what is found in nature, as described by authors studying colicin. Finally, I describe our model that draws inspiration from the colicin model (**Figure 1.1**).

One of the earliest papers describing interactions between three competing species comes from May and Leonard (1975). The most general equation covering *n* species can be written:

$$\frac{dN_i(t)}{dt} = r_i N_i(t)\left[1 - \sum_{j=1}^{n} \alpha_{ij} N_j(t)\right] \quad (1)$$

Here, $N_i(t)$ is the density (number of individuals per unit area) of species *i* at time *t*, r_i is the growth rate of species i, and α_{ij} is the coefficient describing the detriment species j has on the growth rate of species i. For three equivalent species in a rock-paper-scissors arrangement, the equation set is as follows:

$$\frac{dN_1(t)}{dt} = N_1[1 - N_1 - \alpha N_2 - \beta N_3] \quad (2)$$

$$\frac{dN_2(t)}{dt} = N_2[1 - \beta N_1 - N_2 - \alpha N_3] \quad (3)$$

$$\frac{dN_3(t)}{dt} = N_3[1 - \alpha N_1 - \beta N_2 - N_3] \quad (4)$$

The following assumptions have been made:

- all three have equal growth rates which can therefore be reduced to 1 through scaling by t;
- all species affect themselves via a carrying capacity $\alpha_{1\,1} = \alpha_{2\,2} = \alpha_{3\,3} = 1$;
- all three species affect one another in a cyclic fashion: $\alpha_{1\,2} = \alpha_{2\,3} = \alpha_{3\,1} = \alpha$; $\alpha = \alpha_{3\,2} = \alpha_{1\,3} = \beta$).

Three different outcomes are possible for this system at equilibrium, according to the values of α and β:

1. if $\alpha + \beta < 2$, there is a stable equilibrium solution with all three species coexisting;
2. if $\alpha + \beta > 2$, but either $\alpha < 1$ or $\beta < 1$, there is a stable limit cycle with all species present;
3. if $\alpha > 1$ and $\beta > 1$, there are non-periodic cycles with ever increasing cycle time (May and Leonard 1975).

To understand these three cases, it should be remembered that the strength of inhibition of each species on itself is $\alpha_{ii} = 1$. This represents a carrying capacity, or limitation of space/resources. If there were three independent species that did not interact in any way other than through the carrying capacity, we would have $\alpha = \beta = 1$. The first two outcomes require at least one of α and β to be less than 1, *i.e.* the effect of one species on another is less than the effect on itself. In all the systems that we will study in this thesis, there is a space limitation that gives a carrying capacity for the total population, for example in lattice models, there can be at most one individual per site, so the total density of all species cannot be greater than 1. Given this

constraint, the only way to have α or β less than 1 is to have some beneficial interactions between species in addition to the unavoidable inhibitory interaction that simply comes from competition for space. In this thesis, we will be considering cases where there are additional *inhibitory* interactions between some of the species on top of the competition for space, and we will not consider cases with beneficial interactions. Hence it is the third outcome of the May-Leonard model that is most relevant here.

The third outcome involves a heteroclinic cycle that moves around the boundaries of the phase space (further described in Zeeman (1993) and Wolkowicz (2006)). The system spends most of its time very close to one or other of single-species equilibrium points, and very occasionally switches from one equilibrium to the next. This is only possible because the model is deterministic and allows arbitrarily small densities of each species to recover and become large again. In finite size populations, this cannot occur because there can never be fewer than one individual in a population, and once a species is extinct it cannot reappear. In finite populations, the heteroclinic orbit cannot occur, and the system is bound to go to one or other of the three single-species equilibrium states.

We thus conclude that deterministic, well-mixed models will be insufficient to describe the phenomena that we are interested in this thesis and that it will be necessary to study models with stochastic fluctuations due to finite population sizes and spatial effects. One well-studied model that does this is that of Reichenbach *et al.* (2007, 2008) The ODEs for this model may be written as below (changing some symbols to facilitate comparisons with other models in this chapter):

$$\frac{dN_1}{dt} = rN_1(1-T) - \sigma N_1 N_3 \qquad (5)$$

$$\frac{dN_2}{dt} = rN_2(1-T) - \sigma N_2 N_1 \quad (6)$$

$$\frac{dN_3}{dt} = rN_3(1-T) - \sigma N_3 N_2 \quad (7)$$

where $T = N_1 + N_2 + N_3$ is the total population density. This model is very similar to the case of the May-Leonard model with the heteroclinic orbit. By separating out the effect of carrying capacity in the 1-T term, it can be seen that the terms involving σ are further inhibitory interactions in addition to the effects of carrying capacity. The inhibitions occur in a cyclic fashion: 1 inhibits 2, 2 inhibits 3, and 3 inhibits 1, as in **Figure 1.1**. The outcome is that the deterministic infinite system will follow a heteroclinic orbit around the boundaries of the phase space, and that that a finite size population would tend to one or other of the equilibria with only a single species present. The important contribution of Reichenbach *et al.* (2007) is to develop a lattice model whose well-mixed limit is equivalent to the ODEs of equations 5-7, but which shows interesting spiral wave solutions in the spatial case. Spiral wave solutions are stable for long times with finite mean frequencies of each of the three species. This model clearly demonstrates that in stochastic spatial models, there are stable long term solutions where three species in a rock-paper-scissors arrangement can coexist.

Another case of three species interactions is the production of colicin, which has been studied by Kerr et al. (2002) as described in the previous chapter. That study draws inspiration from the work of Durrett and Levin (1997). In this case the system is composed of three E. coli types: a full producer (population P; lowest birth rate r_P), a non-producing cheater (population N; intermediate birth rate r_N), and a susceptible type (population S; highest birth rate r_S). P and N

are both resistant to the colicin. The symbols have again been changed to facilitate comparison within this chapter. The well-mixed system can be described by a set of ODEs:

$$\frac{dP}{dt} = r_P P(1-T) - P \quad (8)$$

$$\frac{dN}{dt} = r_N N(1-T) - N \quad (9)$$

$$\frac{dS}{dt} = r_S S(1-T) - S - \sigma SP \quad (10)$$

$T = P + N + S$ is the total population density, as with equations 5-7. In 8-10 it is important that $r_P < r_N < r_S$, and that only the S species is inhibited by the colicin production of P, whereas all the growth rates are the same in 5-7, and each species is inhibited by the next species in the cycle. Additionally, there is a linear death rate for each species in 8-10 (*i.e.* the $-P$ term in 8, *etc.*). This is essential in this model because otherwise T tends to 1 (or the lattice becomes completely full when we do the spatial simulation) and no further growth is possible. The equivalent term was not essential in 5-7 because there were inhibitory terms causing death in all three species. In 8-10, the death rates have been set to 1, and the time is scaled relative to the death rates, as we do in all the models in this thesis. The version of the model discussed by Durrett and Levin (1997) also allows for different death rates in each species, but this is not essential if the three growth rates are different.

The essential property of 8-10 is that if we begin from any state with non-zero densities of all three species, the system converges to a steady state with only S present. The presence of N initially is important for the above conclusion, because P can sometimes beat S if N is not present and if there is sufficient P initially to have a significant detrimental effect on S. If we begin with

a mixture of *P* and *S* only, the system tends either to a state with only *P* or a state with only *S*, depending on how much *P* is initially present. However, if there is even a small amount of *N* present initially, then *N* always outgrows *P* when *P* reaches a high density, and the system moves towards a state with high *N*. However, the state with a high *N* density is itself not stable, because it is outgrown by *S*. Hence, the only stable state, if we begin with all three present, is the state with only *S*. A specific numerical example of this will be given in the following section for the model used in this thesis, which behaves similarly to the Durrett and Levin (1997) model in equations 8-10 in this respect. The important conclusion is that antibiotic producers will always be destroyed by the evolution of non-producing cheaters according to well mixed models.

Durrett and Levin (1997) have shown that in a spatial version of the colicin model, it is possible for all three species to coexist for certain ranges of the rate parameters. In the spatial model, cyclic dominance occurs locally, with *P* tending to beat *S*, which tends to beat *N*, which tends to beat *P*... etc. Patches of the three species move across the lattice and replace one another, but all three remain present in the long term. This result is similar to the Reichenbach (2007) model discussed above, in that it is the spatial pattern formation that allows the three species to coexist, and only one species would survive in the well-mixed case. However, the patterns of spiral waves that arise with 5-7 are qualitatively different from the patterns of random patches that arise with 8-10. Many examples of spatial patterns of the random-patch type will be given in the results of this thesis.

2.2 Three-Species Models Studied in this thesis: ODEs

The three species models we study in this thesis are similar to the model of Durrett and Levin described in the previous section. The ODEs for the well mixed model in our case can be written:

$$\frac{dP}{dt} = (r_1 - ac)P(1-T) - P \quad (11)$$

$$\frac{dN}{dt} = r_1 N(1-T) - N \quad (12)$$

$$\frac{dS}{dt} = (r_2 - A)S(1-T) - S \quad (13)$$

$$\frac{dA}{dt} = aP - bA \quad (14)$$

Here we are explicitly assuming that *P* and *N* belong to the same species, which has a growth rate r_1. The growth rate of *P* relative to *N* is reduced by the cost of antibiotic production *ac*, where *a* is the rate of antibiotic production by *P* cells, and *c* is the metabolic cost per unit of production. We count these two factors as separate parameters because we are interested in studying the evolution of the rate of antibiotic production. *P* cells are able to adjust the rate of production, *a*, in order to balance the metabolic cost to themselves with the strength of the effect on sensitive species. It is assumed that *c* is a fixed property of the metabolic pathway for the antibiotic that cannot be adjusted by *P* (unless it evolves a completely different antibiotic molecule).

The parameter r_2 is the growth rate of sensitive cells (species 2) in absence of the antibiotic. The effect of the antibiotic is to reduce the growth rate of *S* in proportion to the antibiotic concentration *A*. We thus model a bacteriostatic case (where antibiotic reduces growth rate), rather than a bacteriocidal case (where antibiotic increases death rate). The antibiotic is produced by *P* at rate *a*, and breaks down chemically at rate *b*, as described by equation 14. In the spatial version of the models, described below, we will model the diffusion of the antibiotic from the *P* cells into the surroundings. Therefore it will be important that each individual *S* cell is

affected by the antibiotic concentration at its own spatial position. Initially, however, we can consider the well-mixed case in which the antibiotic concentration is equal everywhere. We will further assume that production and breakdown of antibiotic is rapid in comparison to cell growth and death, so that equation 14 comes to equilibrium: $A = aP/b$. If this is the case, 13 and 14 can be replaced by a single equation

$$\frac{dS}{dt} = \left(r_2 - \frac{aP}{b} \right) S(1-T) - S \qquad (15)$$

The three equations 11,12 and 15 describe a three species model that is rather similar to the Durrett and Levin (1997) model, but which includes parameters *a*, *b* and *c*, which describe the properties of the antibiotic more explicitly.

We will now consider the dynamical behaviour of equations 11,12 and 15 in the well-mixed case in order to show that there is no stable state where all three species are present, and that the system converges to a state with only *S* present, if we begin from any initial state with all three present.

Firstly, it is clear from equations 11,12 and 15 that there are stable equilibrium solutions for each of the three species when there is only one species present. For example, if only *P* is present, then *T* = *P*, and the equilibrium density of *P* (from 11) is:

$$P = 1 - \frac{1}{r_1 - ac} \qquad (16)$$

Similarly, the one species equilibria for *N* and *S* are

$$N = 1 - \frac{1}{r_1} \qquad (17)$$

$$S = 1 - \frac{1}{r_2} \tag{18}$$

Secondly, we consider what happens when only two species present. If only *P* and *N* are present, the two growth rates are constant, and *N* grows faster than *P*. Therefore the system converges to the equilibrium with only *N* (equation 17). If only *N* and *S* are present, there is no antibiotic, so the growth rate of *S* is just r_2, which is faster than r_1. Therefore, the system converges to the state with only *S* (equation 18). If *P* and *S* are present, there is a particular density of $P = P_0$ where the growth rates of *P* and *S* are equal:

$$r_1 - ac = r_2 - aP_0 / b\,, \tag{19}$$

from which

$$P_0 = b\left(\frac{r_2 - r_1}{a} + c\right). \tag{20}$$

The corresponding density $S = S_0$ at this point can be found from equation 11:

$$T = P_0 + S_0 = 1 - \frac{1}{r_1 - ac}, \tag{21}$$

$$S_0 = 1 - \frac{1}{r_1 - ac} - b\left(\frac{r_2 - r_1}{a} + c\right). \tag{22}$$

The point P_0, S_0 is an unstable fixed point. Trajectories in the *P*,*S* plane (with $N = 0$) are shown in **Figure 2.1**. The two stable single-species fixed points (equations 16 and 18) are shown as black circles, and the unstable fixed point is shown as a star. All trajectories lead to one of the two stable points. A very similar result is seen in the model of Durrett and Levin (1997).

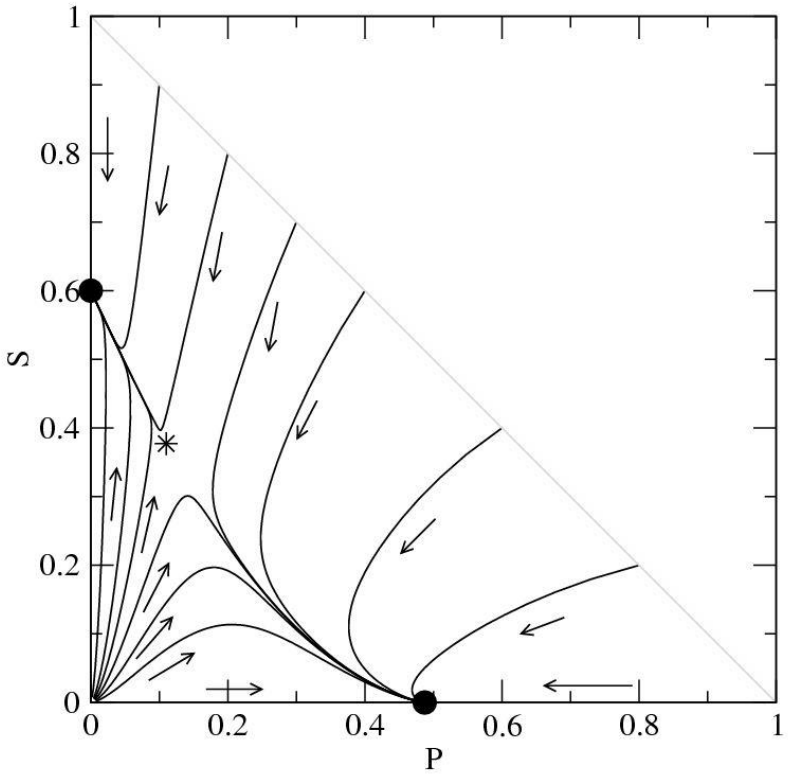

Figure 2.1: Trajectories of the well-mixed model in P,S space with N = 0. Stable single-species fixed points shown as black circles, unstable fixed point shown as a star. Parameters: r_1 = 2.0, r_2 = 2.5, a = 50, b = 10, c = 0.001.

The unstable fixed point only exists if $S_0 > 0$ (in equation 22). This condition is true for values of a between a_{min} and a_{max}, where a_{min} and a_{max} are the two roots of equation 22. When a approaches either of these two limits, the position of the unstable point approaches the stable P fixed point, and the fixed point for P then becomes unstable. The behaviour as a function of a is summarized in **Figure 2.2**. The fixed point for S exists for all a and is independent of a (equation 18). The fixed point for P exists for $a_{min} < a < a_{max}$, and is a decreasing function of a in this range (equation 16). The reason that P can only outcompete S in the intermediate range is that if a is too low, the there is too little antibiotic to inhibit the growth of S and if a is too high, the metabolic cost to P outweighs the benefit of inhibiting S. Note that in the intermediate range,

both solutions are stable and the *P* solution will only arise if there is sufficient *P* present initially. A small amount of *P* cannot invade a population of *S* in the well-mixed case.

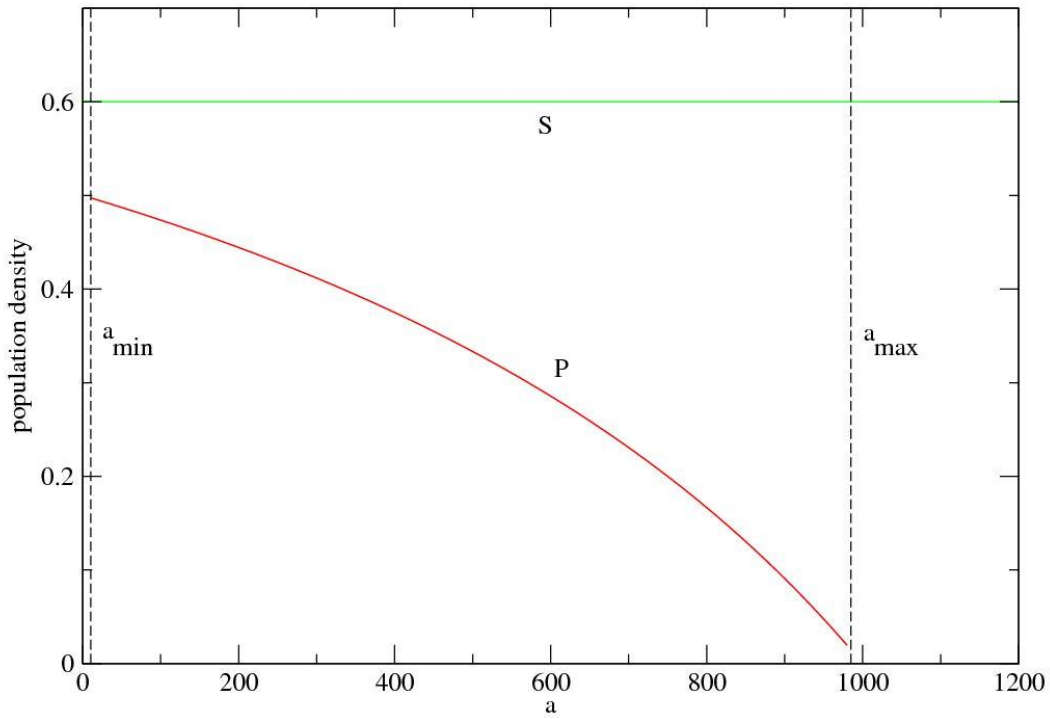

Figure 2.2: Stable fixed points for *S* and *P* in the well-mixed model with $N = 0$ Paramaters: $r_1 = 2.0$, $r_2 = 2.5$, $b = 10$, $c = 0.001$. For these parameters $a_{min} = 10.26$ and $a_{max} = 984.69$.

The dynamics of the well mixed model when all three populations are present initially is substantially different from the case when only *P* and *S* are present. It is convenient to consider scaled variables $P' = P/(P+N+S)$ and $N' = N(P+N+S)$. This reduces the three dimensional phase space to a two-dimensional diagram, with the three single-species fixed points at the corners of a triangle (**Figure 2.3**). The fixed point for only-*S* (equation 18) is at the origin in this diagram. The unstable fixed point is on the boundary between *S* and *P* (shown by a star). The only-*S* point is the only stable fixed point when *N* is present. The only-*P* point is stable in

absence of *N* but is unstable to invasion by *N*. The only-*N* point is unstable to invasion by *S*. Trajectories starting close to the unstable P_0,S_0 fixed point are shown in **Figure 2.3**. These either approach the only-*S* point directly, or they follow a circuit that passes close to the only-*P* and only-*N* fixed points but eventually ends up at the only-*S* point.

The conclusion is that antibiotic production should not be evolutionarily stable according to this well-mixed model because the producers are always invaded by non-producing cheats, and these are themselves then outcompeted by the susceptible species. Understanding the evolution of antibiotic production therefore requires us to account for spatial interactions. This is the main focus of this thesis. The spatial version of the *P, N, S* model will be described in the following section.

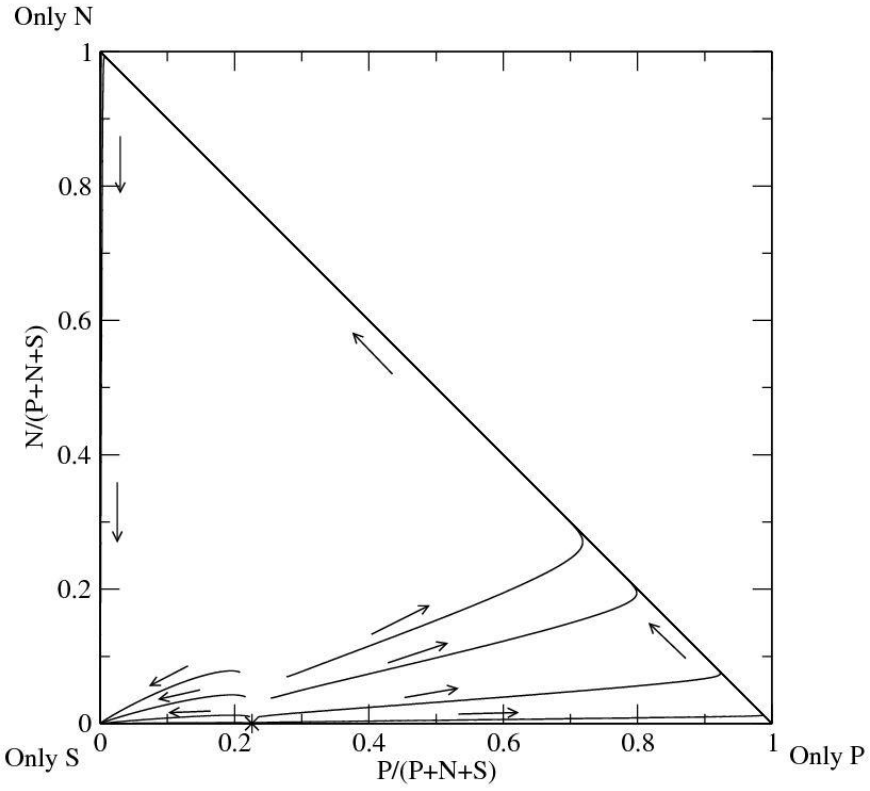

Figure 2.3: Trajectories of the well-mixed model with all three species present. Parameters: r_1 = 2.0, r_2 = 2.5, a = 50, b = 10, c = 0.001.

2.3 Lattice Models Studied in this Thesis

For our diffusion model we consider a square lattice in which each site can either be occupied by a cell or vacant. Cells may be either of two possible species, and each species has two possible cell types, making four types in all (**Figure 1.1c**).

Species 1 is either a producer (P) or a non-producer (N). The growth rate of a non-producer is

$$r_N = r_1$$

whereas the growth rate of a producer is reduced due to the cost of antibiotic production:

$$r_P = r_1 - ac.$$

The non-producer is a cheater that gains the benefit of the antibiotic but pays no cost of production. The non-producer is intrinsically resistant to the antibiotic, as it belongs to Species 1.

Species 2 is a competitor species which is susceptible (S) to antibiotics. The growth rate of the susceptible in absence of the antibiotic is r_2, where $r_2 > r_1$. The growth rate of the susceptible is reduced in proportion to the local concentration, A, of antibiotic

$$r_S = r_2 - A.$$

The resistant cell type will be considered later on in the thesis. It is a mutant of species 2 and has a lower growth rate due to the cost of resistance. Its growth rate is

$$r_R = r_2 - C_R,$$

where C_R is the cost of resistance. The death rate, v, is the same for of all cell types, and we may take $v = 1$, as a definition of the time scale of the model. Therefore, 1 time unit is the mean

lifetime of a cell. Throughout this thesis $\delta t = 0.01$; as such, 1 time unit is equal to 100 increments of δt.

The parameter *d* controls the diffusion rate of the antibiotic and the parameter *b* controls antibiotic breakdown. The model proceeds in time steps, δt. Birth and death of cells are dealt with as stochastic events. Diffusion of antibiotic is treated deterministically.

The simulation occurs algorithmically as follows:

Initially, the lattice is populated randomly with each of the three cell types, until it is ~15% full.

1. Each site on the lattice is visited in a pseudorandom sequence, making sure each site is visited once. If it is not empty, cells reproduce with probability $r_i \delta t$, where *i* is the type of cell being considered.
    a. A site is chosen at random from the eight surrounding neighbours
    b. If the site is empty and the reproduction check is passed, the selected site is filled with the type of the parent. If the site is not empty, cell division does not occur.
2. Each site is visited in a sequential manner. If the cell type is a producer, the amount of antibiotic at that site, $A(x,y)$, is increased by $a\delta t$.
3. Each site is visited in a sequential manner. If it contains antibiotic, the amount that will diffuse is calculated and stored. This is equivalent to $A(x,y)d\delta t$. This amount is subtracted from the site.
4. Each site is visited in a sequential manner and 1/8 of the previously calculated amount is then added to each of the sites in the Moore neighbourhood. This allows for diffusion to occur all at once throughout the lattice.

5. Each site is visited in a sequential manner. The amount of antibiotic at each site is reduced by an amount $A(x,y)b\delta t$ due to chemical breakdown.
6. Each site is visited in a pseudorandom sequence, making sure each site is visited once. Each cell has a probability $v\delta t$ to die, and thus become an empty site.

We also looked at a nearest neighbour model as computational limitations caused the diffusion model to take too long to run. The nearest neighbour model behaves similarly to the diffusion model. However, one main difference is a change in how susceptible cell growth is treated. Instead of being impacted by antibiotics at a susceptible cell's own site, it is instead affected by the Moore neighbourhood, which are the eight surrounding cells:

$$r_s = r_2 - \frac{an_P}{9b}$$

In this case, a is the production rate, n_P is the number of producers in the Moore neighbourhood, b is the breakdown rate. Thus, a susceptible cell has a decreased growth rate for each producer in its vicinity. In this way the model behaves as the diffusion model did with the antibiotics limited to only the sites nearest to the producers. This allows for two fewer iterations over the lattice, resulting in a much faster run time for the simulations.

The models were designed in this way to correspond to the well-mixed case described above. If mixing were introduced into either model, the fully well-mixed case would behave exactly as described by the ODEs. In that case every cell is presumed to be interacting with every other cell and antibiotics homogenously occupy the system.

Chapter 3

Results for Two and Three-Member Interactions

3.1 Minimum Growth Rate for a Single Cell Type

We began analysis of the spatial diffusion system using just the producers, in order to determine the minimum possible growth rate of the producer, and thus the maximum production rate. We ran simulations for production rates starting at 0, increasing in increments of 10 until the system could no longer survive. Each simulation was initialized on a 252x252 lattice, with cost penalty $c = 0.001$ and allowed to run for 100 time units. At a production rate of approximately 650, the system could no longer survive (**Figure 3, dashed red line**). Continuation past this point may have allowed survival in the short term, but the stochastic nature of the simulation meant that for extended runs the system may have died by chance. This placed the minimum growth rate required for a cell type to survive alone at $r \approx 1.35$. Intuitively one might assume that any number above 1 would allow the cells to thrive as the death rate is overcome by the birth rate. However, the spatial nature of the simulation prevents this. Instead, there is a competition for space between members of the same species. Therefore, at low growth rates being next to an occupied site increased the chances that a cell might be dead by the time the next cycle of reproduction occurred. In the well-mixed case, any $r_P > 1.0$ would be viable for producers alone to survive.

In addition to this, equilibrium concentration of producers decreased with antibiotic production rate. Intuitively, if growth rate is lower compared to death rate, relatively fewer cells are born than die, thus leaving more empty space within the lattice. At 0 antibiotic production the lattice was the most full but only at a concentration of approximately 0.43.

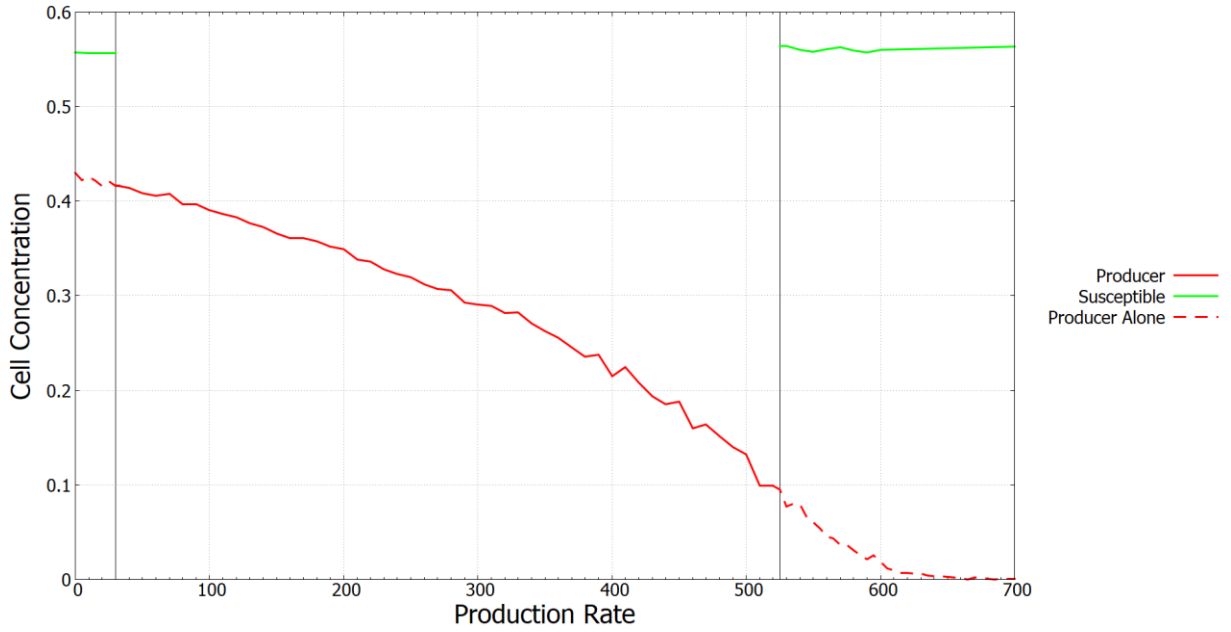

Figure 3.1: Outcomes of competition between susceptible cells and producers of a given rate. Simulation began with random configuration of equal concentration of both types and ran until equilibrium was reached, with surviving cells depicted at each production rate. Parameters: $b = 10$, $c = 0.001$, $d = 50$, $\Delta t = 0.01$, lattice 256x256.

3.2 Interactions with Two Cell Types

Next, we considered interactions of two cell types in our spatial diffusion model. The outcomes of most of the combinations could be deduced intuitively:

- Producers competing with cheaters would result in the cheaters winning, as long as the producers are producing a nonzero amount of antibiotics and the production penalty is above 0
- Susceptible cells competing with non-producers would result in susceptibles winning due to the inherent higher growth rate

Consequently, the only interaction of note was the competition of the producers and the susceptible cell type. The outcome of this interaction is not immediately obvious as the

production of antibiotics results in an unknown that could have different outcomes. As such, we ran simulations on a 256x256 size lattice for 5000 time units each, increasing the production rate from 0 to 700 by increments of 10 and keeping $c = 0.001$ throughout the simulation. We found that coexistence was not possible for any production rate (**Figure 3, solid red and green lines**). Instead, the graph was split into three distinct regions corresponding to low, intermediate, and high production rates.

In the low production rate range, for a between 0 and 30, only the susceptible cells survived. The existence of this region can be attributed simply to insufficient production of antibiotics. While the detriment due to production was fairly low, the actual amount of molecules was unable to overcome the difference in growth between the producer and susceptible cell type. As such, the producer was unable to compete and ended up going extinct.

Following this, there existed a region between production rates of 30 and 510 where the producers were capable of overcoming the susceptible cells. In this region the production rate was high enough to decrease the susceptible growth rate to a level that allowed the producers to compete. As such, sites were cleared out to allow the producers to move into areas previously occupied by the susceptible cells (**Figure 3.2**). While cells began in a random configuration, clustering quickly occurred, with the clusters growing and destroying patches of susceptible cells. However, increasing production rate of antibiotics resulted in decreasing equilibrium concentration of producers. At the lowest survivable production rate the producers were already winning. As such, any increase in production had no benefit aside from perhaps speed of reaching equilibrium. Consequently, increases in production rate resulted in a detriment to growth exclusively – whether the production rate was 30 or 500, the susceptible cells were equally dead. On the other hand, producer growth rate was decreased, preventing the lattice from

filling up (**Figure 3.1**).

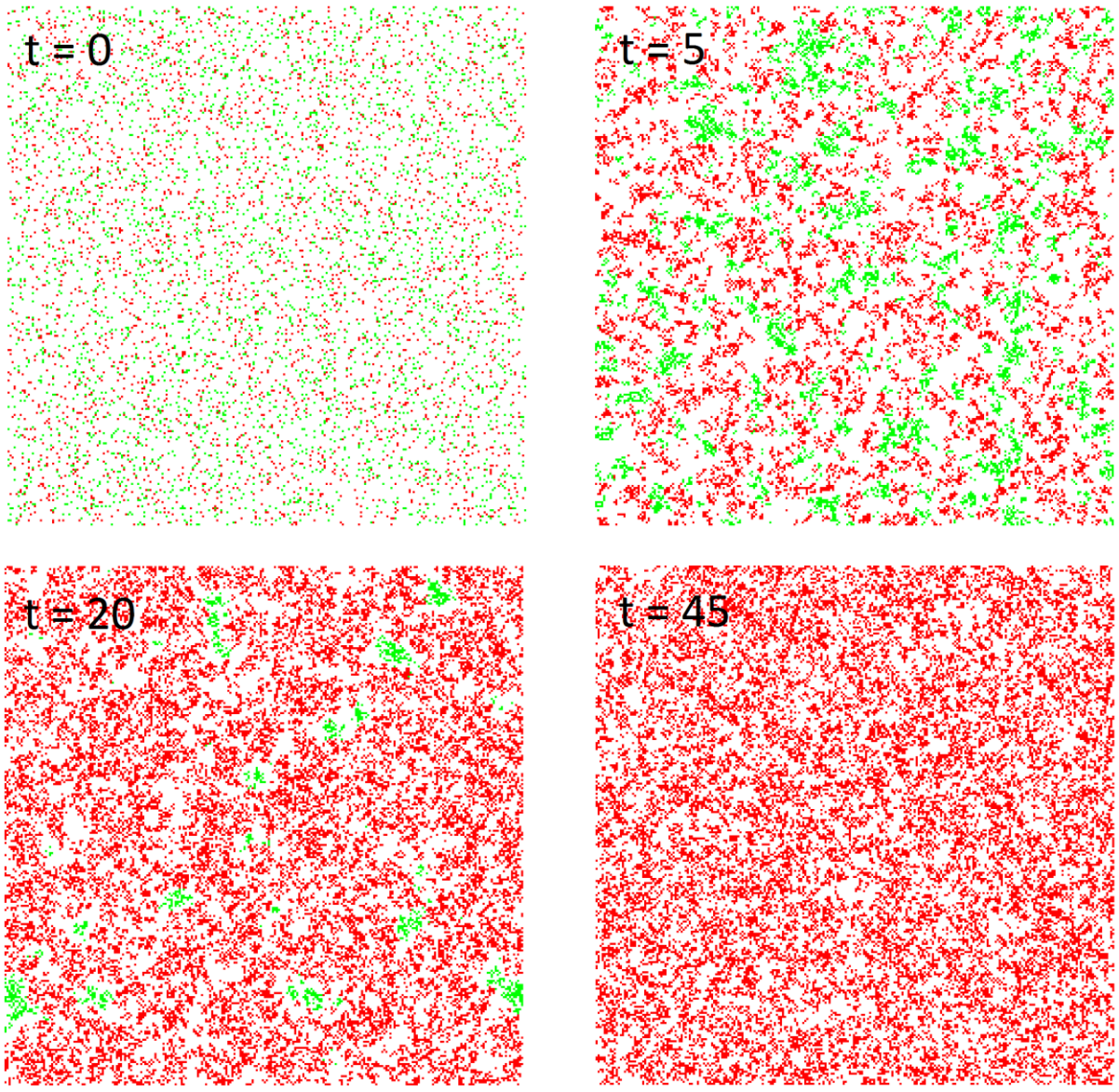

Figure 3.2: Progression of susceptible-producer competition over time. Clustering of like cells initially occurred, resulting in producers growing out and destroying patches of susceptible cells. This simulation occurred in the intermediate region where producers could actually win. Parameters: a = 200, b =10, c = 0.001, d = 50.

As the starting production rate in the simulations was increased past a rate of 510, the susceptible cells once again took over the system. Here, the production rate was so high that the

penalty for the producers brought the growth rate below what was required to survive – the penalty *ac* was too high for the producers to benefit from the effect of the antibiotic. Thus, despite incredibly high production rates that significantly decreased the susceptible cells' growth rates to a manageable level, the difference in growth once again became too high as the producers' growth rate was negatively affected as well. Interestingly, this third region occurred much earlier than when the producers were allowed to exist alone. This is attributable to the existence of the susceptible cell, which occupied space as well. Consequently, while production rates of up to 700 allowed for the producers to survive alone, when susceptible cells were present, the decrease in growth rate brought on by production rates above 510 prevented the producers from competing for space with the faster-growing susceptible cells.

The range in which the producer wins is narrower than in the well-mixed case as well. However, in the well-mixed case either the producer or the susceptible cell type can win in the region depending on the initial concentration of each cell type. In the lattice model the only stable outcome for this region is the producer winning, even when starting with very small starting concentrations. In this case the colonies start small and spread outwards as they increase, eventually taking over the system (**Figure 3.2**).

3.3 Interactions with Three Cell Types

After understanding the dynamics in interactions between two cell types, we expanded our diffusion model to consider the full three-type system. To do this we introduced a non-producer to the previously mentioned two-species system, as described above. We began in the same way as in the two-cell type simulations, measuring equilibrium concentrations of all three cell types at increasing production rates (**Figure 3.3**). Simulations were run for 5,000 time units

on a 1024x1024 lattice, which was larger than the two-cell type system; production rate was incremented by 10 as before.

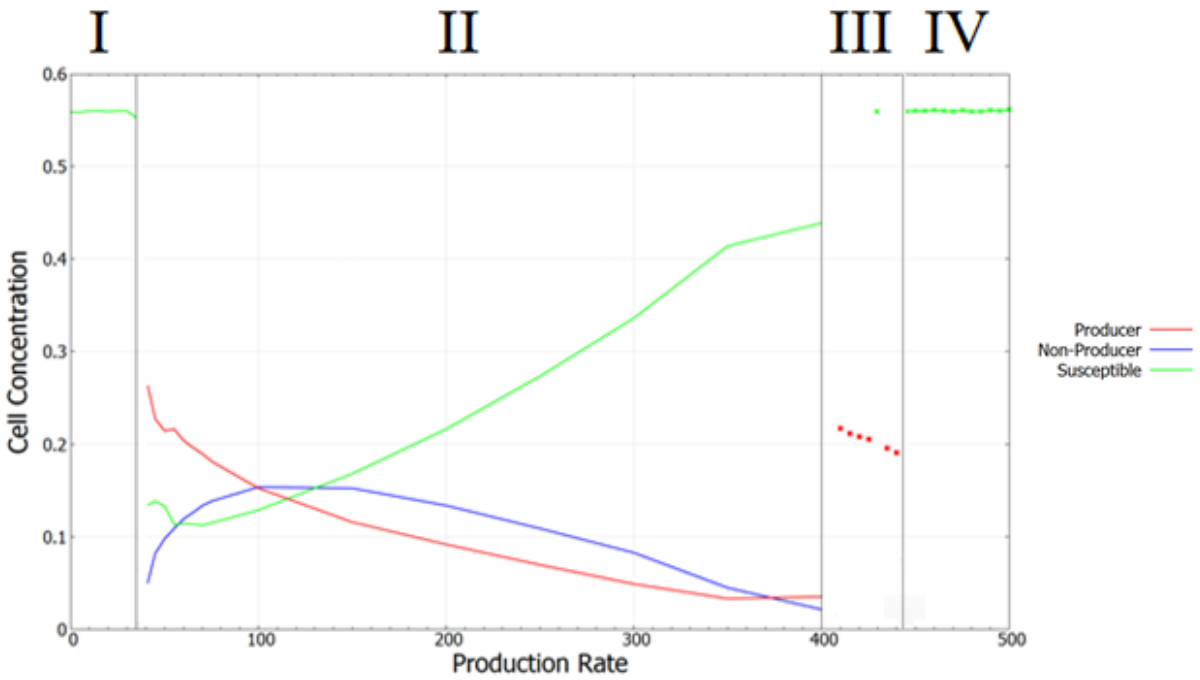

Figure 3.3: Outcomes of competition between susceptible cells, producers of a given rate, and non-producers. Simulation began with random configuration of equal concentration of both types and ran for 5000 time units or until equilibrium was reached with one cell type left. Depicted are equilibrium concentrations for single type or average over time for cases where all three survived. Three distinct regions emerge I) Susceptibles win due to low production rates II) All three types coexist III) Region of stochastic outcomes in which the producer usually wins IV) Susceptibles win due to high penalty for high production rate. Parameters: $b = 10$, $c = 0.001$, $d = 50$, $\Delta t = 0.01$, lattice 1024x1024.

In this case we see the formation of the same three distinct regions as before, with the addition of a fourth region (Region III) that is unstable and did not previously exist (**Figure 3.3**). Region I corresponded to the first region in the two-cell type simulation. Here, production rate was too low to overcome the difference in growth rate between the producer and susceptible cell type. As such, the producers were unable to survive, despite the fact that there was only a low decrease in growth rate. Region IV was also the same as in the two-cell simulation, with the susceptible cell types winning. Indeed, production was very high but despite this, the decrease in

producer growth rate was too much for the producers to win. Interestingly, the addition of the non-producer increased the size of the regions where the susceptible cells were able to take over the system. This was due to increased competition for the producers. The addition of the non-producers added a competitor for space, limiting the total number or producers in the system. This decrease in producers resulted in a lower antibiotic concentration throughout the lattice. As a result, the susceptible cells were able to better compete, making the range in which producers survived with non-producers (**Figure 3.3**) narrower than when non-producers were not present (**Figure 2.1, solid red and green lines**).

The other two regions were different between the two types of simulations. Region II corresponded approximately to the region where the producers had won (although due to the presence of non-producers was narrower). In this case the non-producers created a system that allowed all three cell types to coexist. Region III occurred towards the end of this region of coexistence. Here, producers had a chance to win, due to stochasticity of the system. As such, in this region either producers or susceptible cells had the chance to win.

Equilibrium concentrations of the three cell types also varied extensively with the production rate. In a similar fashion to the two-cell system, the producer decreased in concentration with increasing production rate. The reason for this was similar to before – the lowest production rate was already low enough to win over the susceptible cells. As a result, any increase in production rate simply served to penalize the producers. However, non-producers saw an initial increase in equilibrium concentration until production rate 110, before beginning to drop. At this point the non-producer equilibrium concentration also started being higher than the producer equilibrium concentration and remained as such almost until the end of Region II. This follows logically as non-producers cheat – they benefit from high production rates that detriment

the producers. The initially increasing concentration of the non-producers corresponds to a low benefit to these cheaters, allowing the producers to remain relatively competitive in comparison. As production rate increased, more antibiotics were allowed to diffuse, creating a larger buffer zone for the non-producers to exist in. Thus, they only had to compete with the producers in these areas and higher production rates served the dual purpose of decreasing producer growth (thereby allowing easier competition) and increased inhibition zone area (allowing for more real estate free of susceptible cells).

The susceptible cells benefited even more than the non-producers. While the other two cell types decreased in concentration with increasing production rate, susceptible cells showed a constant increase. This was again due to the detriment of high production rates to producers. The decrease in producer growth caused a decrease in their concentration. This caused fewer producers to exist in the system, thus decreasing the amount of antibiotic produced on a whole, despite increased production rates.

We further analyzed the dynamics within the three regions by looking at time series of individual runs (**Figure 3.4**). For the initial region, it is apparent that the barrier to survivability was too low of a production rate (**Figure 3.4a**). In this case the susceptible cells simply grew without hindrance, as if the producers were not there. The other two cell types had a slight spike in growth due to the empty space at the start of the simulation before being outcompeted very quickly by the faster growing susceptible cell type.

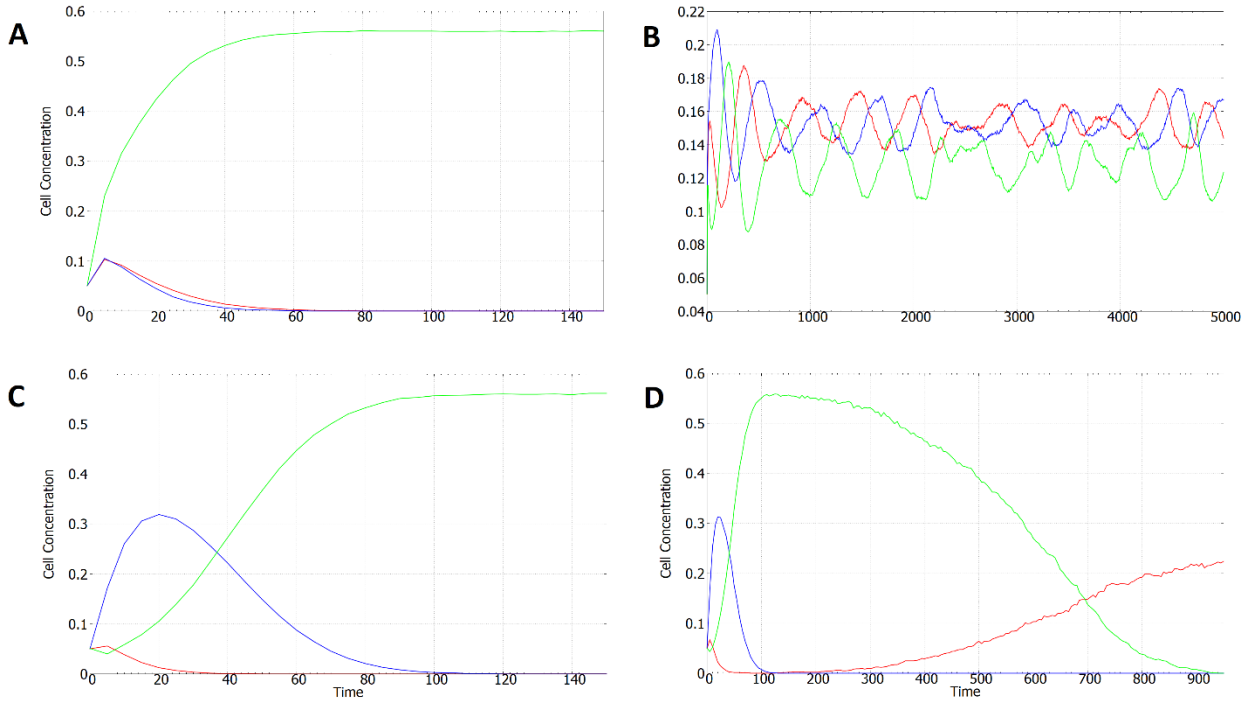

Figure 3.4: Single outcomes for different production rates in a three cell type system. Simulation began with random configuration of equal concentration of both types and ran for 5000 time units or until equilibrium was reached with one cell type left. Depicted are time courses of the different runs. A) Outcome in region A with low production rates, resulting in producer victory (a = 20) B) Long term stability with three cell type coexistence in region B (a = 100) C) Outcome in region C with high production rate and penalty resulting in susceptible victory (a = 500) D) Outcome in region C with producer victory due to stochastic and lattice. Parameters: b = 10, c = 0.001, d = 50, Δt = 0.01, lattice 1024x1024.

The dynamics of the intermediate region were the most interesting (**Figure 3.4b**). Here, we see the aforementioned cyclic dominance and interaction in a rock-paper-scissors fashion. Each of the three cell types were seen dominating the system as time progressed, in a cyclic manner. However, the increase in those resulted in non-producers to begin acting almost uncontested. As those increased in concentration, producers decreased, allowing susceptible cells to begin taking over as there was a lower concentration of antibiotics in the system. This cycling occurred indefinitely and is shown on the lattice model in **Figure 3.5**.

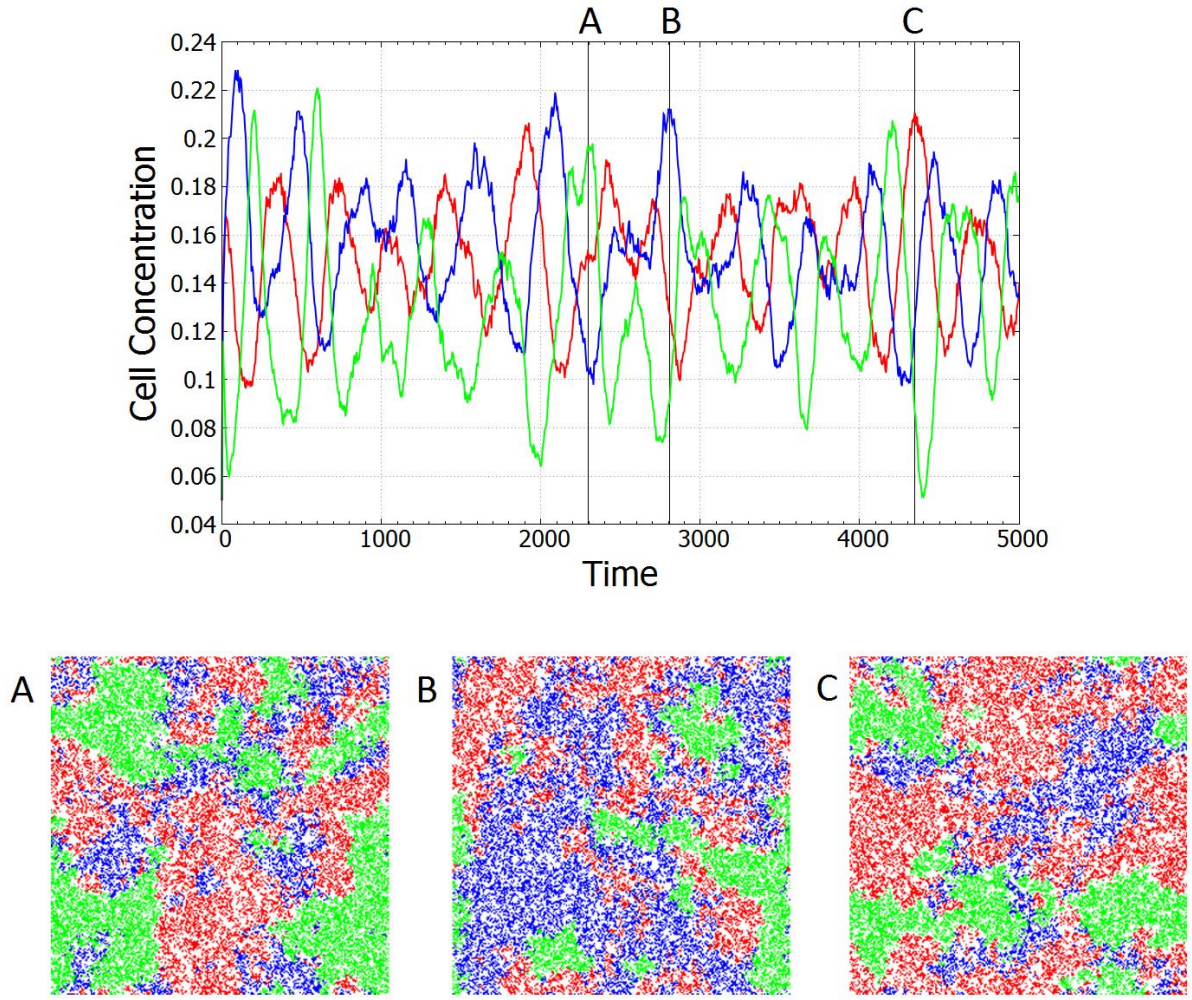

Figure 3.5: Demonstration of cyclic dominance in space and time. The graph shows a time course for the stable region. Lower panels correspond to regions where the different cell types are the most numerous. A) Susceptible cells dominating (t = 2295) B) Non-producers dominating (t = 2810) C) Producers dominating (t = 4345). Simulations were started with random starting conditions with each cell occupying ~5% of the lattice. Parameters: a = 100, b = 10, d = 50, lattice: 256x256.

Region IV had susceptible cells fixing within the system. Once again the production rate decreased the producer growth by too much to overcome the susceptible growth, despite high antibiotic concentration (**Figure 3.4c**). In this case the producers initially inhibited the susceptible cell growth with high production, allowing the non-producers to spike in

concentration. However, this allowed the susceptible type to compete, causing it to ultimately win out as the producers hit zero concentration.

Region III occurred immediately following the region of stability. This area was a sort of unstable region wherein either the producers or susceptible cells could win (**Figure 3.4d**). This occurred because of stochastic effects due to the finite size of the lattice. In this case producers were capable of winning if the non-producers were the first cell type to go extinct. Being left with no competition, they were capable of rebounding and taking over the system. On the other hand, there was a chance for the producers to go extinct first. In this case, the outcome was entirely identical to Region IV.

3.4 Analysis of Parameter Space: Cost vs. Production Rate

Eventually our diffusion model became limited by our computing power. The actual simulation of diffusion required additional iterations over the lattice, causing a very large amount of time to be required for larger lattices. Consequently, we switched to a nearest neighbour model. This prevented the extra computation required for the diffusion of molecules. Instead, susceptible cells were affected by their Moore neighbourhood. Production rate determined the effect that each producer in the neighbourhood had on a central susceptible cell. This greatly sped up the simulations, allowing many runs to be conducted in a shorter period of time.

The increase in computational power allowed us to consider many runs for a given set of parameters. Whereas in the diffusion model 24 hours would allow us one run on a 256x256 lattice for 2000 time units, the nearest neighbour model allowed approximately 100 runs on a 1024x1024 lattice in the same span of time. We used this massive speed up to consider a wide range of parameter space and create a phase diagram illustrating the outcomes at various regions,

comparing production cost penalty and production rate. Each coordinate in **Figure 3.6** represents the most likely outcome after 100 runs.

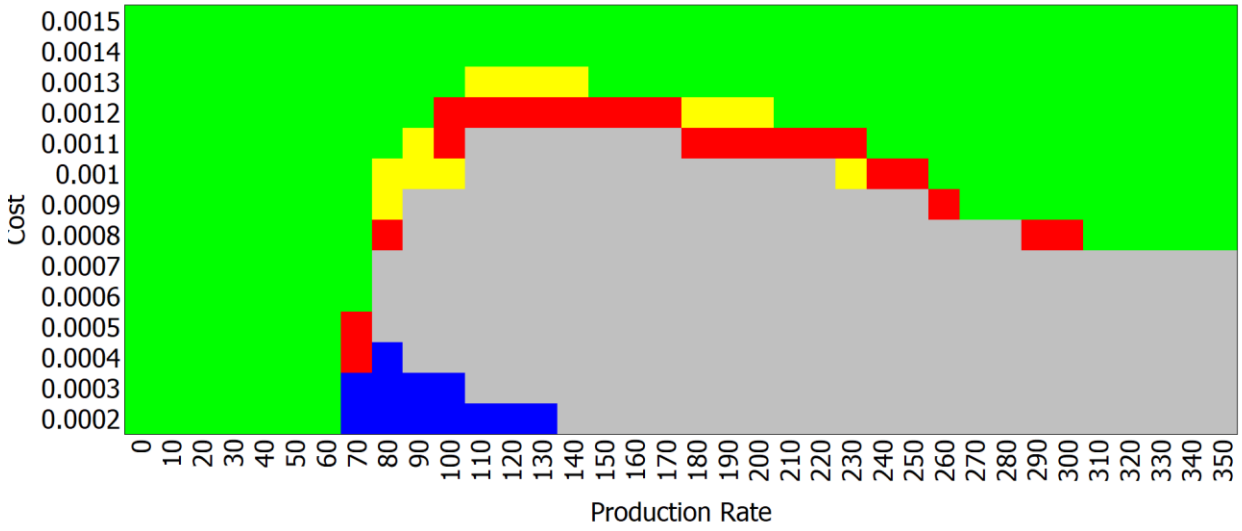

Figure 3.6: Phase diagram illustrating the outcomes of 10,000 iterations for a given cost and production rate. Simulations were started with random configurations consisting of 15% of each cell type. Equilibrium outcomes key: Green is susceptible, red producer, blue non-producer, grey stable region, yellow producer plus susceptible. Parameters: b = 10, lattice size 1024x1024, Δt = 0.01.

The results here were largely as expected. Low production rates created regions where susceptible cells dominated the system, as the amount of antibiotics was not able to overcome the difference in growth rate. At higher costs, these regions widened. Moreover, past a cost of 0.0013, only one outcome was possible: susceptible cells winning. This, again, followed logically, as here the inherent cost was simply too high, regardless of production rate to have any effect on the system. Consequently, the producers simply died allowing the susceptible cells to take over the lattice.

A wide range of parameter space also allowed for coexistence of all three cell types. This was generally flanked by regions of producers winning or ambiguous outcomes. On both the higher and lower production rate sides of the stable region, this was due to the same reason

(**Figure 3.7a and b**). The combination of production rate and cost allowed the producers to almost go extinct, causing a spike in non-producers. This allowed the susceptibles to compete easily, causing the non-producers to go extinct. Following this, the producers had free reign. The results were very similar to the stochastic Region III seen in the diffusion model (**Figure 3.4d**). This demonstrates that this region is not necessarily stochastic. It lends credence to the fact that it was not always observed due to the small number of runs in the diffusion model. The continuous persistence of this region across a wide range of parameter space and many runs indicates that this is in fact a true region. However, some stochasticity was observed even across 100 runs as the lattice is still finite in size. While producers winning was the most likely outcome in these regions by a large margin, there were some cases where susceptible cells were able to win. There were also many places where the outcomes were ambiguous at first glance, resulting in the persistence of both producers and susceptible cells over the course of the runs. Further consideration of these runs demonstrated a similar curve shape to that of producers winning. The trajectory of the producers was up, while susceptible cells down. This indicated that given more time these ambiguous regions would end up with producers winning.

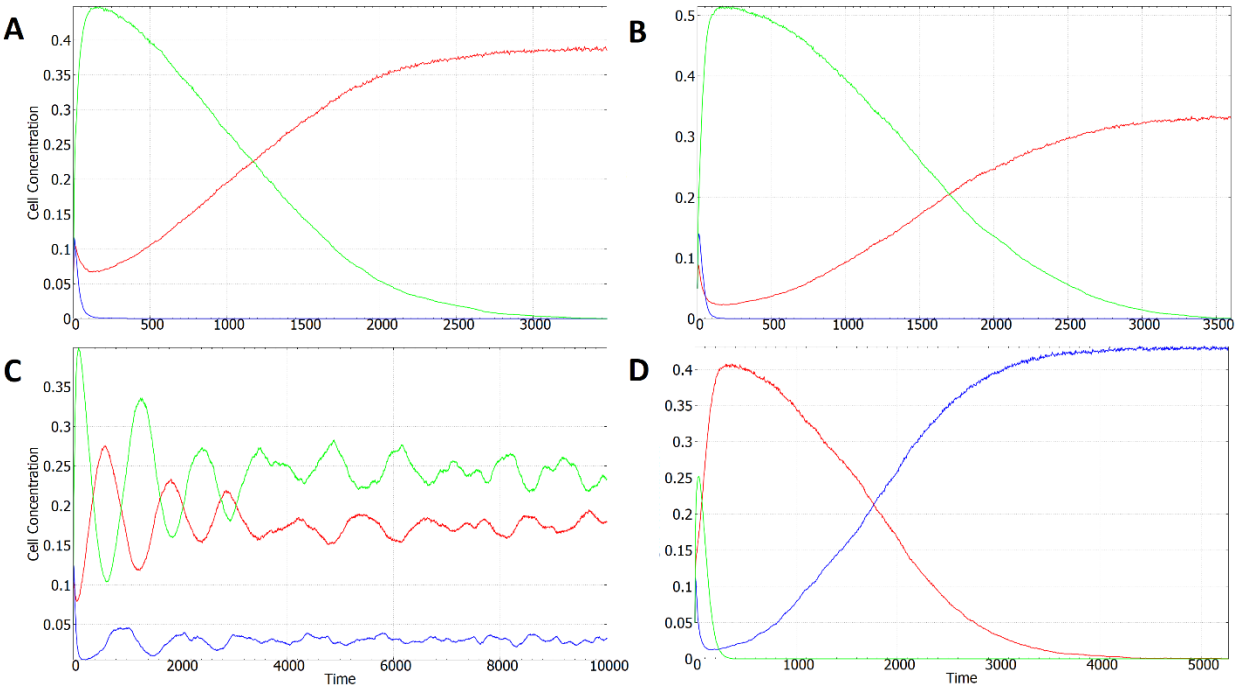

Figure 3.7: Unusual regions in phase space. A) Producers winning where c = 0.0011 and a = 100 B) Producers winning where c = 0.0011 and a = 210 C) Three cell stability where c = 0.001 and a = 150 D) Cheaters winning where c = 0.0002 and a = 90. Parameters: b = 10, lattice size 1024x1024.

The stable region behaved similarly to the stable region in the diffusion model. The system was stable in the long term, with all three cell types increasing and decreasing in concentration depending on the concentration of the opponent cell (**Figure 3.7c**). However, in this case the fluctuations were not as pronounced, owing largely to the lack of diffusion. This made all interactions local, preventing the long distance effects responsible for the large fluctuations in the previous model. This caused there to be little change in the most dominant cell at any given time. Instead, all three were maintained at relatively stable concentrations throughout, with susceptibles having the highest concentration, followed by producers, then non-producers. This was a reasonable outcome as non-producers did not benefit from long range diffusion protecting them from susceptible cells. Consequently they were kept down throughout the run of the simulation.

The most unexpected outcome was of non-producers being able to win, which was unobserved in the smaller parameter space of the previous model (**Figure 3.7d**). Here, the time course showed a similar curve to the previous ones, although with the opposite outcome. The production cost here was so low that a fairly low production rate allowed for the producers to compete very well with the susceptible cells. Consequently, the producers spiked while the susceptible cells went extinct. The non-producers meanwhile approached zero. When the susceptible cells disappeared, the cheaters rebounded as they still had a slight growth advantage.

3.5 Analysis of Equilibrium Concentrations for a Given Cost

We next conducted an experiment identical to the one for the diffusion model, considering production rates across a given cost. We looked at a cost of 0.001 and again incremented production rates by 10, measuring the equilibrium concentrations of each cell type (**Figure 3.8**). The main difference was the highly decreased concentration in non-producers. For all parameters non-producers existed at a much lower concentration than the other cell types. This followed logically for this model as the lack of diffusion decreased the benefit to the non-producers; when diffusion is limited the benefit is concentrated locally and as such, the producers gain more benefit than the non-producers. Aside from that, the trajectory of the cell types was similar as well. Producers and non-producers decrease steadily, while susceptible cell types increase in equilibrium concentration. Other regions are similar as well: low and high production regions have susceptible cells coming out on top. Region III also appears present, but

as previously discussed appears to be a real region rather than stochastic.

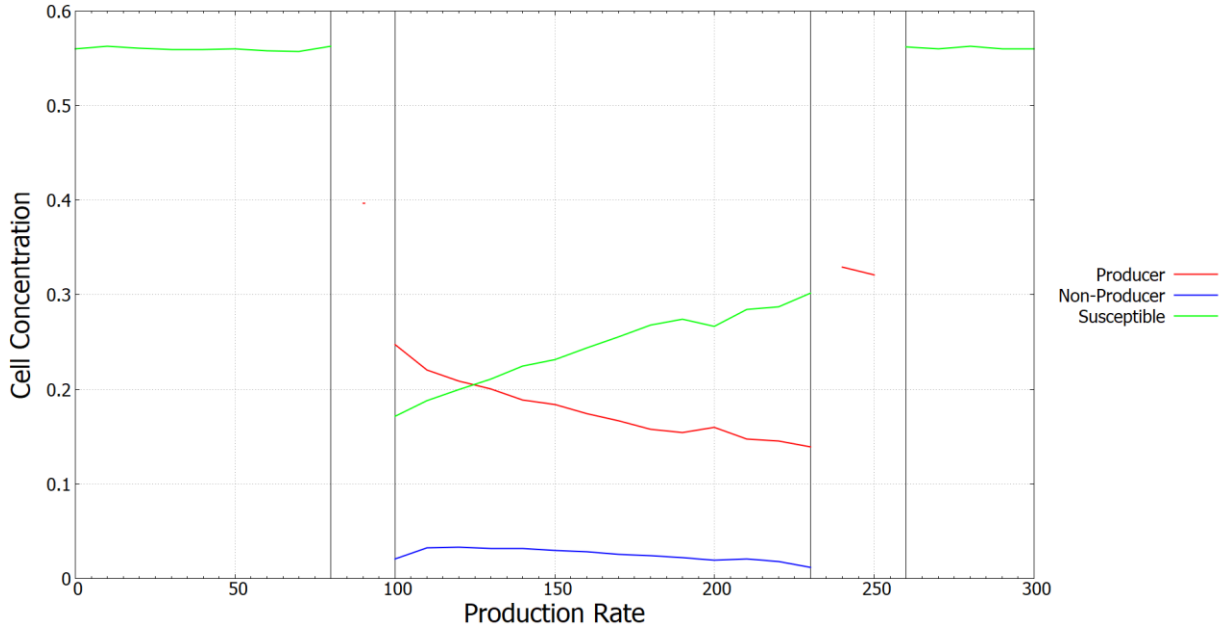

Figure 3.8: Outcomes of competition between susceptible cells, producers of a given rate, and non-producers in the nearest neighbour model. Simulation began with random configuration of equal concentration of both types and ran for 10,000 time units or until equilibrium was reached with one cell type left. Depicted are equilibrium concentrations for single type or average over time for cases where all three survived. Four distinct regions emerge, similarly to the diffusion model. Parameters: b = 10, c = 0.001, Δt = 0.01, lattice 1024x1024.

Generating the phase diagram gave a considerable amount of data that further allowed us to look into probabilities of given outcomes. We looked at specific outcomes across a cost of 0.001 and determined each of the probabilities across 100 runs (**Figure 3.9**). The results demonstrate the probabilities of given outcomes, corresponding to the four regions. Production rates initially have 100% susceptible outcomes. Following this, there is a transition region allowing producers to succeed, followed by a region where all three exist 100% of the time. This was followed by another transition region and a region wherein the susceptible cells win 100% of the time. Evidentially, some stochasticity still exists as the lattice is still limited in size.

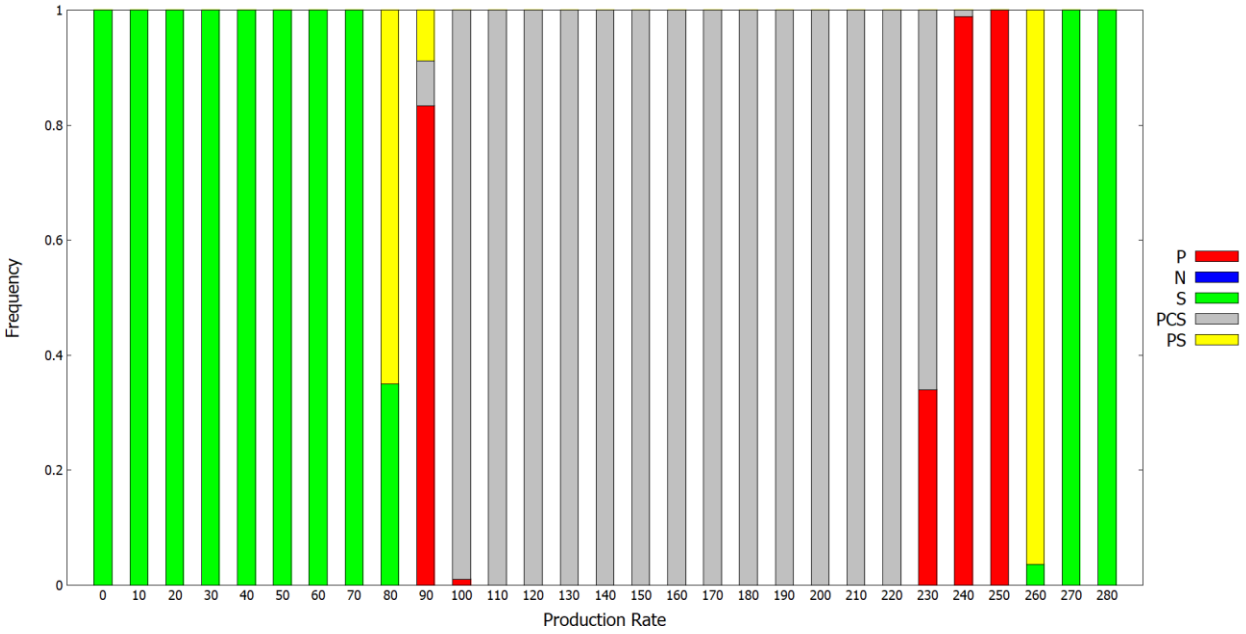

Figure 3.9: Probabilities of given outcomes after 10,000 time units in a nearest neighbour model. Simulation began with random configuration of equal concentration of producers, non-producers, and susceptible cells. Each simulation was repeated 100 times. Parameters: $b = 10$, $c = 0.001$, $\Delta t = 0.01$. Producers in red, cheaters in blue, susceptibles in green, triple coexistence in grey, producer-susceptible coexistence in yellow.

In the unstable region it appeared as if the producers could become most populous if the non-producers went extinct before them. As such, we wanted to determine what would happen if a constant supply of non-producers was provided to the system. We ran simulations allowing producers and non-producers to mutate between one another. This allowed for a small supply of non-producers should only producers and susceptible cells remain. The result was a disappearance of the transition zones where producers are capable of winning (**Figure 3.10 and 3.11**). These are instead replaced by susceptible cells winning or slightly increased regions of three cell stability. This demonstrated the important effects of the non-producers on this system. Maintenance of non-producers allowed the system to proceed to its natural conclusion, with the influx of non-producers limiting the viable range of the producers.

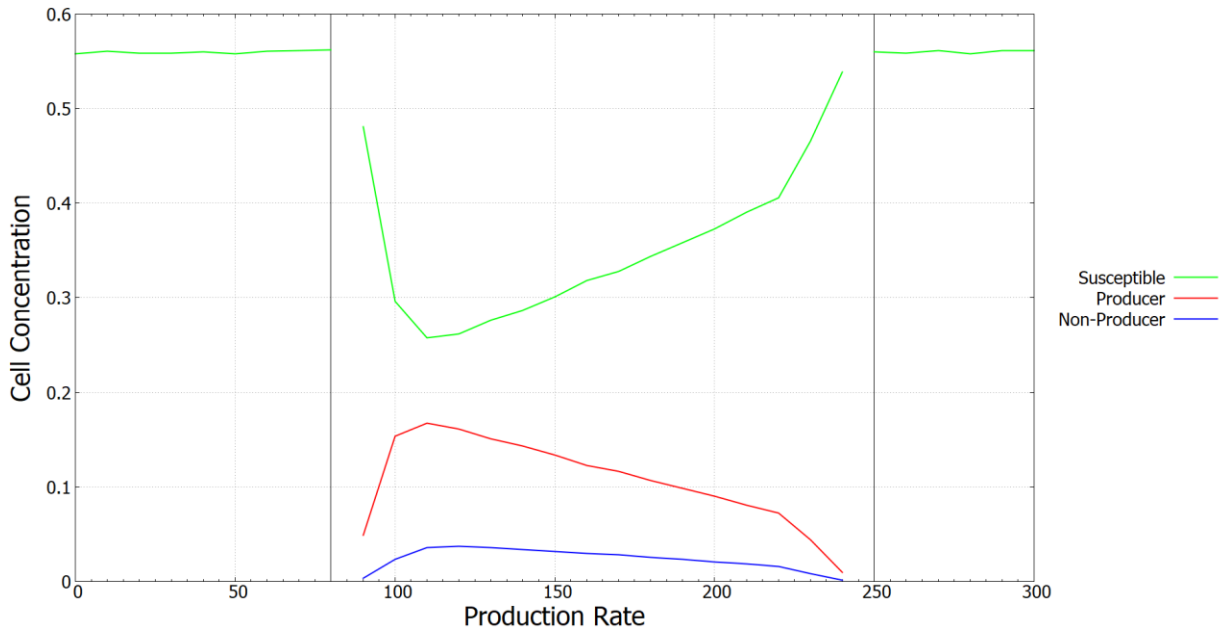

Figure 3.10: Outcomes of competition between susceptible cells, producers of a given rate, and non-producers in the nearest neighbour model. However, in this case, producers and non-producers were allowed to mutate back and forth. Simulation began with random configuration of equal concentration of both types and ran for 10,000 time units or until equilibrium was reached with one cell type left. Depicted are equilibrium concentrations for single type or average over time for cases where all three survived. Three distinct regions emerge, similarly to the diffusion model. Parameters: b = 10, c = 0.001, Δt = 0.01, mutation rate = 0.0001, lattice 1024x1024

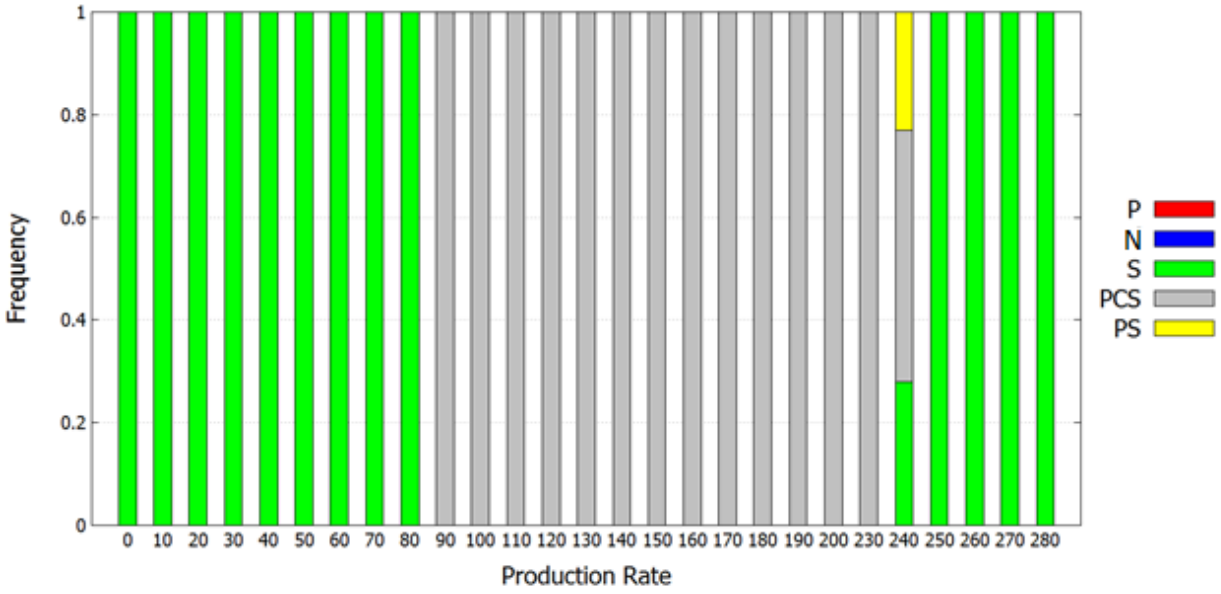

Figure 3.11: probabilities of given outcomes after 10,000 time units in a nearest neighbour model with producer-non-producer mutations. Simulation began with random configuration of equal concentration of producers, non-producers, and susceptible cells. However, in this case, producers and non-producers were allowed to mutate back and forth. Each simulation was repeated 100 times. Parameters: $b = 10$, $c = 0.001$, $\Delta t = 0.01$, mutation rate = 0.0001, lattice 1024x1024. Producers in red, cheaters in blue, susceptibles in green, triple coexistence in grey, producer-susceptible coexistence in yellow

Chapter 4

Introduction of Mutations that Affect Production Rate

4.1 Mutations in the Diffusion Model

After understanding the dynamics of three interacting cell types, we sought to expand upon the model by introducing mutations. This seemed like the logical step as in living organisms functions can be lost, gained or modified. This can occur in a number of ways in bacteria, for example mutation and horizontal gene transfer can result in a gain of function. On the other hand deletion or mutation can cause a loss of function (**Figure 1.1c**). Consequently, our model takes into account mutation in order to determine whether evolution of production rate could occur. As previously mentioned, the producer and non-producer are the same species in our model – the non-producer is simply a 0 production rate producer. Therefore, we allow producers and non-producers to mutate in the same way. Cells have birth occurring the same as before. If the birth check is passed, there is a probability, *m*, that a cell will mutate upon reproduction. This means that, the offspring becomes a producer of any production rate between 0 and 200, binned into groups of production rate 10 (i.e. 0, 10, 20, 30… 200). Consequently, the new cell is a mutant that is different than its parent. In this way, non-producers and producers can change between one another. When considering resistant and susceptible cells, those can change between one another as well. We first considered the diffusion model.

For these experiments we began with a 256x256 lattice, mutation rate of 0.0001. Susceptible and 0-producers were initialized to fill 15% of the lattice. Producers of rates 10-200 filled the lattice equally, also summing to 15%. The time course demonstrates the evolution of

the system (**Figure 4.1**). In general, most of the producer categories fluctuated at very low concentrations. However, intermediate production rates between 30 and 50 were maintained at much higher concentrations.

The susceptible concentrations were maintained at fairly high concentrations, although fluctuations did occur more erratically and with much greater amplitudes than the non-mutating systems described above. Non-producers occurred in a similar fashion. Ultimately, producers of rate 50 continued on an upwards trajectory from the start, with fluctuations getting larger as time progressed. They were maintained at a concentration higher than other producer types. Eventually, the non-producers hit a concentration of zero, allowing the producers of rate 50 to spike immensely, resulting in the extinction of the susceptible cells. As soon as the susceptible cells went extinct, the 50 producers began a rapid decline. The lack of susceptible cells meant no competition for low producers and we saw a spike in producers of rate 10 and 20. However, this gave the opportunity for cheaters to re-emerge as there was no point in maintaining any production rate while with no susceptible cells present. Indeed, having a non-zero production rate was only detrimental at this point. Every producer went extinct, allowing the 0 producers to be only cell type left in the system.

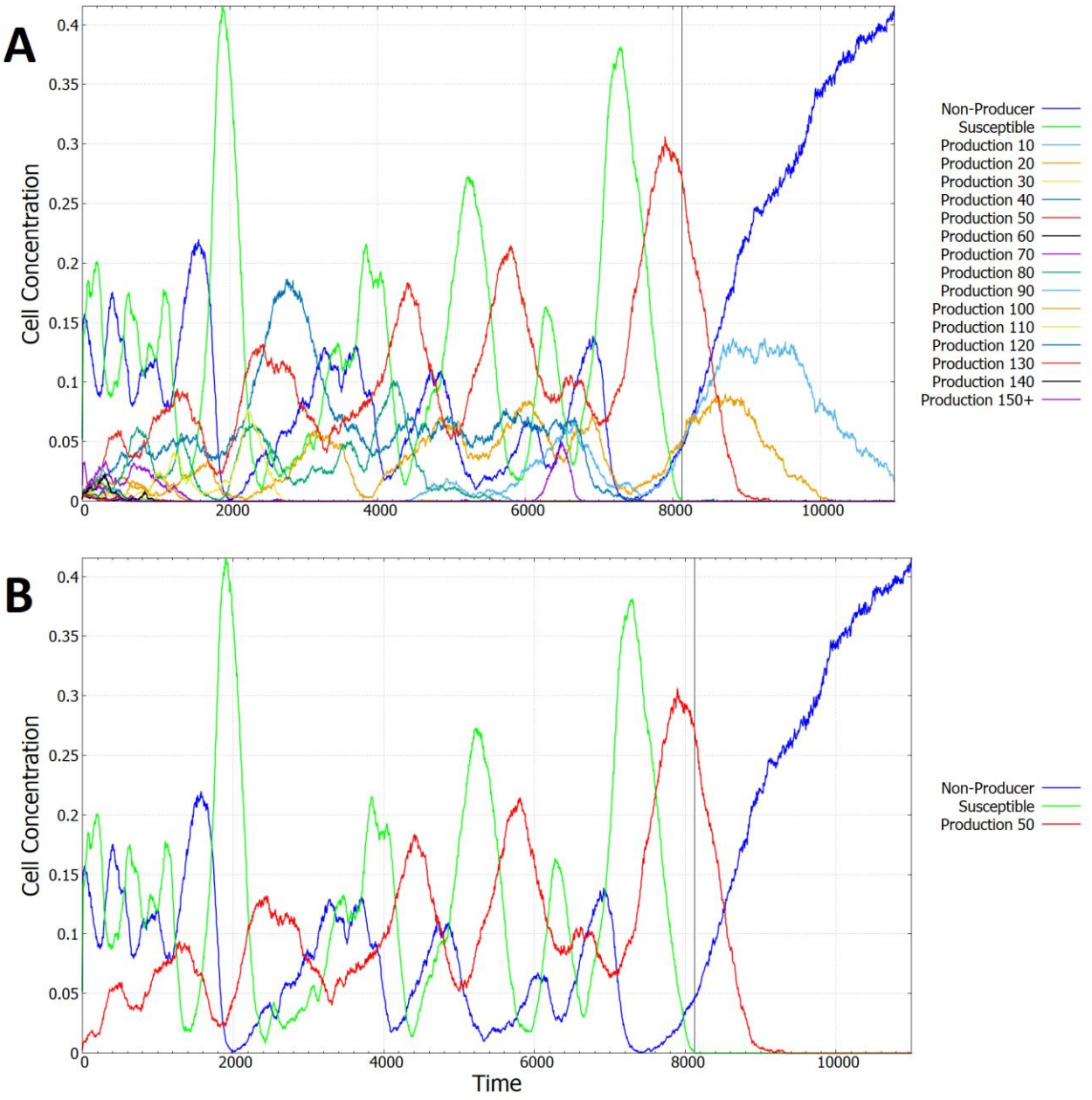

Figure 4.1: Time course for three cell type simulations with mutations. Simulation began with random configuration of equal concentration of three cell types (with the producer category composed of rates between 0 and 200 in equal proportion) and ran until equilibrium was reached or one cell type was left. Depicted are time courses of the different runs with mutations turned on. A) Shows all producer categories B) Removes low concentration producers for visibility. Parameters: b = 10, c = 0.001, d = 50, Δt = 0.01, lattice 256x256, m = 0.0001. Vertical line indicates point where susceptible cell concentration = 0.

This system illustrated evolution of production rate. Over time, the producers of an optimal production rate (in this case 50) were evolved towards. This production rate proved perhaps too optimal – they were capable of causing their opponent to go fully extinct. After this, we saw a second evolution of production rate – with no susceptible cell types left, the system tried low production rates such as 10 and 20 before simply switching production rate off entirely as it served no purpose any longer (**Figure 4.2**).

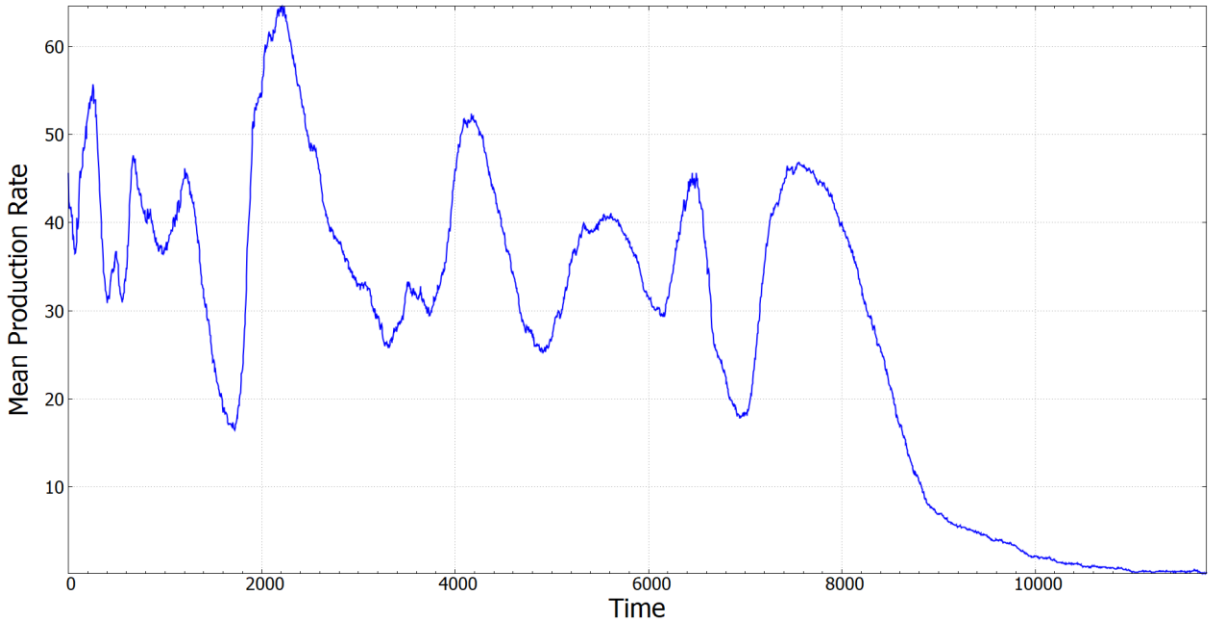

Figure 4.2: Time course showing mean production rate for three cell type simulations with mutations. Simulation began with random configuration of equal concentration of three cell types (with the producer category composed of rates between 0 and 200 in equal proportion) and ran until equilibrium was reached or one cell type was left. Parameters: b = 10, c = 0.001, d = 50, Δt = 0.01, lattice 256x256, m = 0.0001

However, we considered that the erratic fluctuations may have been the result of size limitations in our lattice. Therefore, we tried running the simulation again on a grid of size 1024x1024, for an extended period of time. The results were inconclusive, although they did show signs of evolution (**Figure 4.3**). Here, we see evidence of evolution of production rate once again. However, the system appears to persist in the long term. Instead of non-producers, the role

appears to be taken by producers of rate 20, which are balanced by producers of rate 70. The susceptible cells are maintained at a concentration intermediate to the two. The fluctuations in general appear smaller. It is not clear whether or not the system has stabilized and whether or not the state would have persisted indefinitely. This simulation pushed us close to the limit of our computational power and caused us to return to the faster nearest-neighbour model instead.

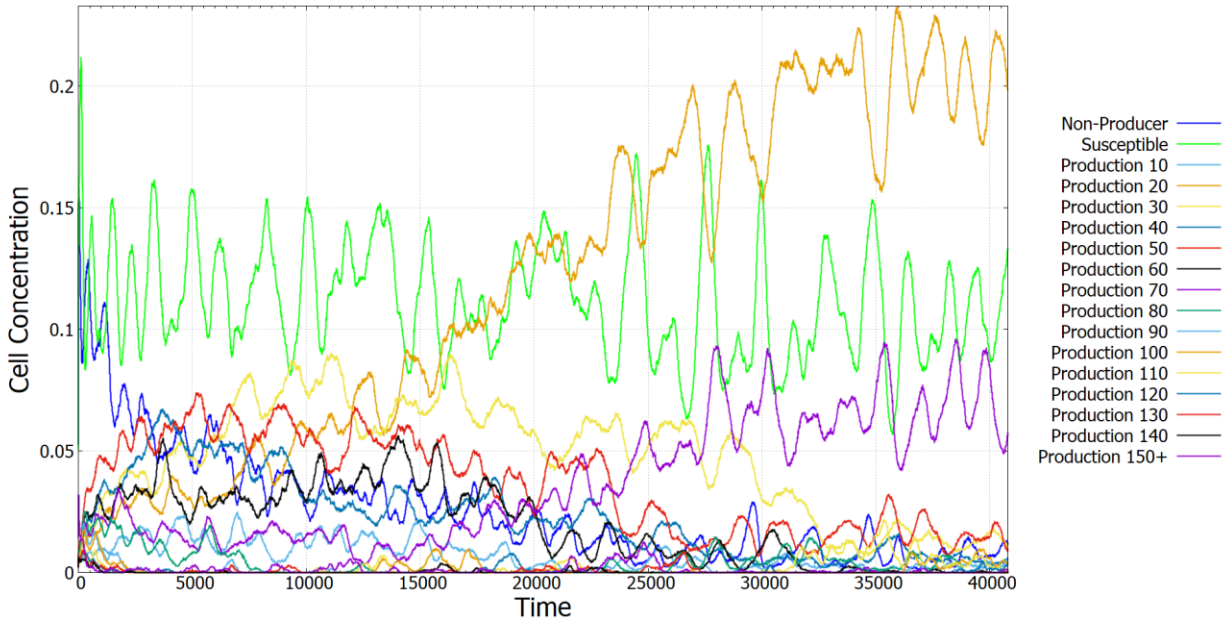

Figure 4.3: Time course for three cell type simulations with mutations. Simulation began with random configuration of equal concentration of three cell types (with the producer category composed of rates between 0 and 200 in equal proportion) and ran until equilibrium for 40,000 time units. Depicted are time courses of the different runs. Parameters: b = 10, c = 0.001, d = 50, Δt = 0.01, lattice 1024x1024, m = 0.0001.

4.2 Mutations in the Nearest Neighbour Model

We next wanted to determine the truth behind the evolution we observed in the diffusion model. The nearest neighbour model allowed us to look at mutations in a larger lattice (1024x1024) for a longer time to limit stochastic effects. We began by looking at whether or not a different cell type can take the niche of the non-producer. Thus, we began a simulation starting with producers of rate 110, in a three-cell type nearest neighbour system. After 5,000 time units we allowed producers to mutate between their initial production rate and a production rate of 50. After a short time, the non-producers went fully extinct. Instead, the production rate 50 cells were maintained at a concentration similar to the production rate 110 cells (**Figure 4.4**). This demonstrated the ability of members of these systems to change in order to fill specific roles. In this case the non-producers were weaker than the low production rate cells. The lack of production cost was not able to overcome the benefit obtained by producing a low amount of antibiotics. Instead, the cells of production rate 50 took the role of non-producers – they produced a small amount of antibiotics to protect themselves, while still gaining the benefit of the higher production rate cells. This system was capable of surviving in the long term as well.

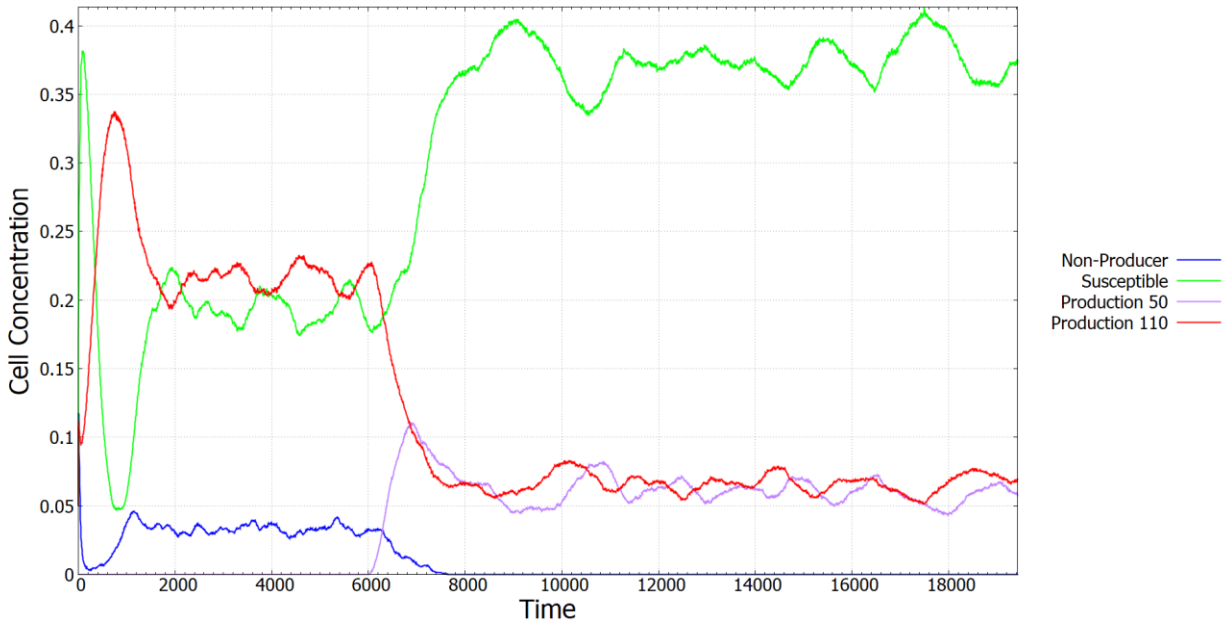

Figure 4.4: time series of cell concentrations with mutations between producers of rate 110 and 50. Simulation began with random configuration of equal concentration of three cell types and ran for 19,000 time units, with mutations turned on at 5,000 time units. Parameters: b = 10, c = 0.001, Δt = 0.01, mutation rate = 0.0001, lattice size 1024x1024.

After discovering that the non-producer niche can adapt and be replaced, we wanted to determine if production rate can evolve when given more freedom. Thus, we ran simulations where producers and non-producers were allowed to mutate to production rates of multiples of 10, with minimum and maximum production rate being 0 and 200 respectively. For the initial simulation, we looked at evolution starting from a low production rate. We initialized a lattice with producers of rate 0 to act as non-producers as well as susceptible cells and producers of rate 150. After 1000 time units, mutations were turned on. The result was a complete extinction of the 0 production rate cell type. These were replaced by cells within many production categories between 10 and 90. The highest frequency producer was rate of 110, which was higher than the next highest category by a large factor. Starting at a production rate of 200 also caused similar results. In this case, rate 110 producers were maintained as the highest too (**Figure 4.5**).

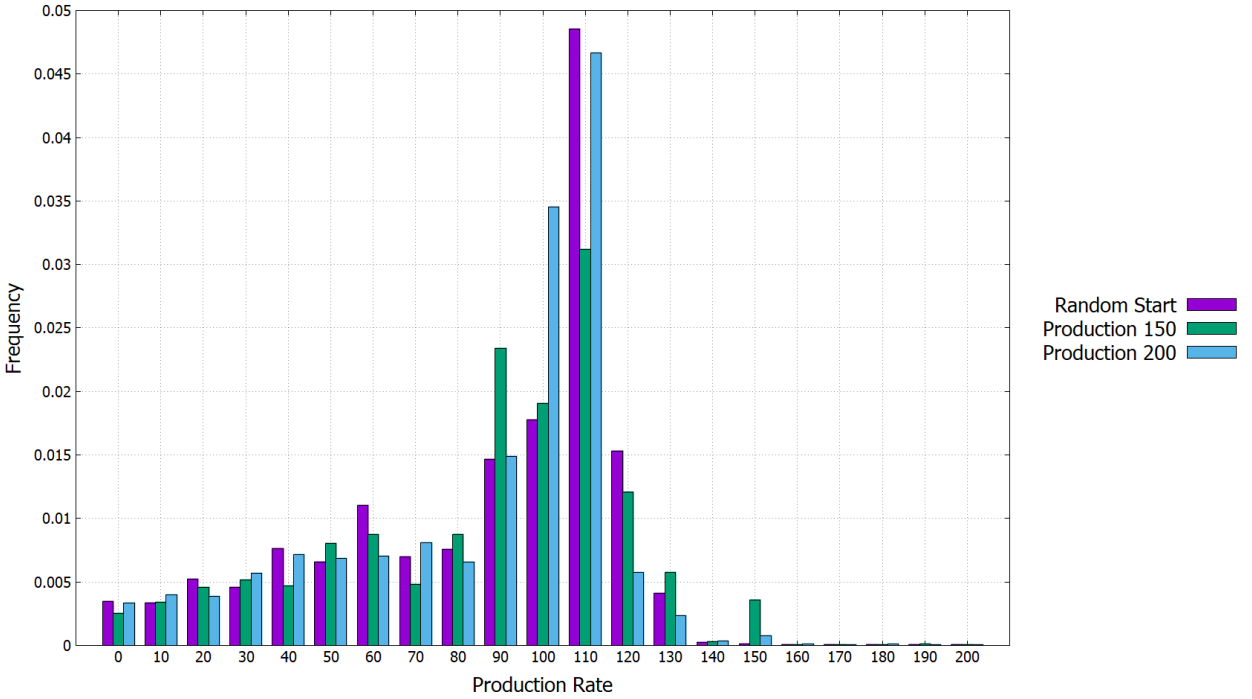

Figure 4.5: Mean frequencies in mutation simulations. For production rates 150 and 200, simulations were started with non-producers, susceptibles, and producers of the given rate and allowed to mutate after 1000 time units. In the random start, the simulation started with non-producers, susceptibles, and producers randomly distributed between rates of 0 and 200. Simulations were run for 100,000 time units and an average was take over the second half of the simulation. Parameters: b = 10, c = 0.001, Δt = 0.01, mutation rate = 0.0001, lattice 1024x1024.

We also wanted to determine if random production rates would converge on a single category if allowed to evolve. Thus, we initialized a lattice with producers of rate 0, susceptible cells, and producers of random rate categories, in equal proportion. The system was allowed to mutate immediately. The results were similar to the previous starts. Non-producers went extinct and were replaced by an aggregate of low producing cells (**Figure 4.5**). Most interestingly, the highest frequency cells were once again of production rate 110. The time course is shown in **Figure 4.6**. As such, it appears that when allowed to evolve, both the producer and non-producer niches become occupied by more optimal cell types. Indeed, it appears that producing even a small amount of antibiotics is more beneficial than being pure non-producers. It also appears that the producers have an optimal rate that allows them to survive better than others. This rate is

likely high enough to keep the susceptible cells at bay, while not incurring the too much of a penalty or benefit to non-producers. In this set of experiments, it appears that this rate is somewhere around 110. This further lends credence to the lattice effects on our diffusion model. When that model was expanded, although the simulation had not fully taken off, it appeared that the producers evolved towards and optimal production rate. On the other hand, the cheaters were also replaced by low-producers as well.

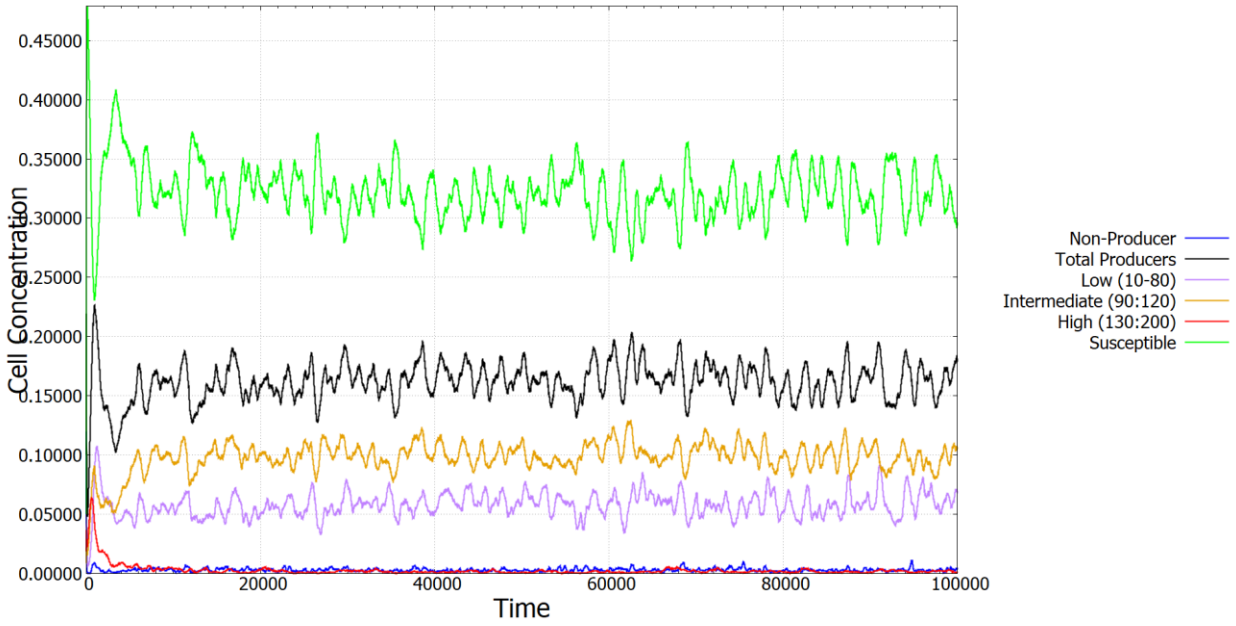

Figure 4.6: time series of cell concentrations with mutations between producers all rate categories. Simulation began with random configuration of equal concentration of three cell types and ran for 100,000 time units, with mutations turned on from the start. Parameters: b = 10, c = 0.001, Δt = 0.01, mutation rate = 0.0001, lattice 1024x1024.

4.3 Introduction of a Resistant Cell Type

Since we characterized the three-species system and evolution thereof, we began expanding our model to include a fourth, resistant cell type. This variant is a mutant of the susceptible cell type. It is not susceptible to antibiotics but incurs a penalty, which is the cost of resistance. Therefore, it functions similarly to the non-producer. Due to the increased speed of the nearest neighbour model, we continued using it for the remainder of our experiments.

We began by testing the survivability of a four-cell type system. Can such a system even exist? Intuitively, with no mutations, two cells behaving identically cannot coexist, as the faster growing one will simply take up the niche. Therefore, we began with a three component system with mutations. We allowed producers and non-producers to mutate between each other, and resistant and susceptible cells to mutate between each other. We began with only the three original types and turned mutations on after 5000 time units, with mutation rate 0.0001. We considered the equilibrium concentrations at various growth rates of the resistant cell types, beginning with producer and non-producer growth rate of 2.0 and susceptible growth rate of 2.5 (**Figure 4.7**). The remaining parameters were selected based on stability in the three cell type case.

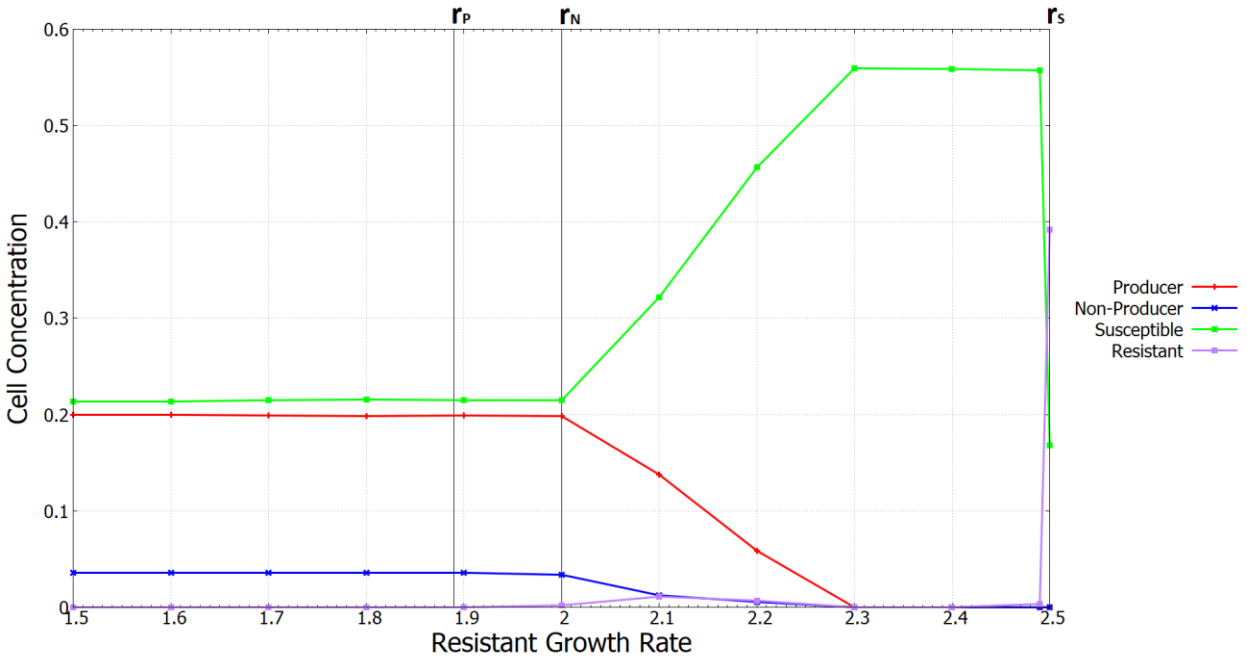

Figure 4.7: Equilibrium concentrations of four cell types. Mutations between producer/non-producer and susceptible/resistant were turned on after 5000 time units. Producer and non-producer growth rate = 2.0, susceptible growth rate = 2.5, a = 110 b = 10, c = 0.001, Δt = 0.01, m = 0.0001, lattice 1024x1024

Growth rates below 2.0 caused a near-zero equilibrium concentration of the resistant cell type, as expected. At this growth rate the new cell type was unable to compete with any of the existing cell types

and was therefore functionally useless. At growth rate of 2.0 (equal to the cheater), the results were similar to lower growth rates. This follows logically as the non-producer was well established at this point. With an identical growth rate, the resistant cell type did not have any advantage to overcome it. In addition to this, the resistant cells were naturally born into neighbourhoods close to the susceptible cells. This meant they had to compete with high-growth cells, whereas non-producers were born next to producers, which had a lower growth rate than them (**Figure 4.8**).

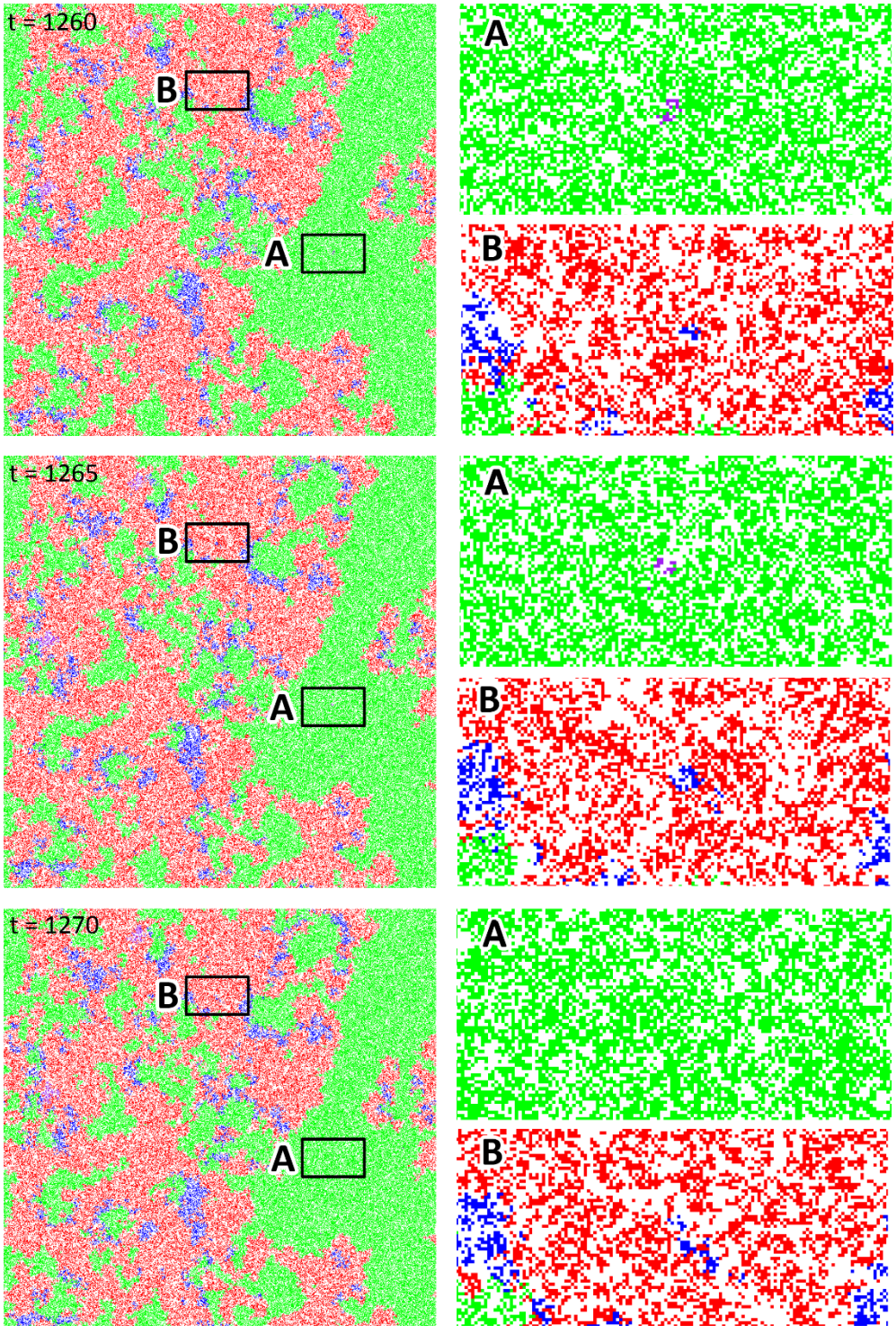

Figure 4.8: A comparison of non-producer and resistant cell growth over time where growth of $r_R = r_N = 2.0$. Left panels show overall progress of the simulation. Right panels show a zoomed in section where A) Resistant types are born in an environment of susceptible cells and unable to survive B) Non-producers are born in an environment of producers and are capable of surviving in the same time frame. Nearest-neighbour model Parameters: $a = 110$ $b = 10$, $c = 0.001$, $\Delta t = 0.01$, $m = 0.0001$, lattice 1024x1024

Changes began occurring between production rates 2.1 and 2.3. Steadily, the producer ends up decreasing in equilibrium concentration with increasing resistant growth rate, while the susceptible cell type ends up increasing. Interestingly, the non-producer and resistant types are maintained at similar concentrations to each other. The cause of this is the decrease in producers in the lattice. The higher growth rate of the resistant cells causes a decrease in producer concentration. This allows the susceptible cells to be the most dominant by far. This in turn keeps both cheater and non-producer concentrations low. At a growth rate above 2.3, the system cannot survive. The susceptible cells simply dominate. The high-growth resistant cells kill the non-producers and producers, resulting in the susceptible cells competing only with the resistant. Due to difference in growth, the susceptible cells simply win out. At a growth rate of 2.5, where resistant and susceptible cells are equal, both are capable of surviving, with resistant at a higher concentration. The resistant cells are capable of competing with the producers, hence are capable of occupying space faster. Therefore by the end of the simulation they overtake the susceptible cells despite equal growth.

Chapter 5
Discussion and Future Work

In this thesis we have constructed two models of bacterial interaction. Drawing on previous models of colicin and rock-paper-scissors types systems, we have created both a nearest-neighbour and diffusion model that allows for the interaction of producers, susceptible cells, and non-producers. Therefore, we were able to study *in silico* systems that are very complex in real life and possibly those that cannot be cultured. While we have reduced these environments into just three cell types, we believe that the model can provide insight into the nature of these systems. Moreover, we believe we are the first to study the evolution of production rate under conditions where the cost to the producer is proportional to the production rate.

Our initial results, looking at three cell types interacting, demonstrate the capabilities of these biological systems to thrive. While it may seem intuitive that the producers should simply win, rather than have environments with many interacting species, we have shown that this is not necessarily the case. In simulations with only producers and susceptible cells, as well as in three-cell simulations, we have shown that production can be too high or too low. In these cases, only susceptible cells survive, as the producers cannot compete. The lower-production regions illustrate the necessity to produce sufficient amounts of antibiotics – low production rates do not allow the producers to overcome the susceptible cells' higher growth rate. On the other hand, producing too much antibiotic causes a massive penalty in growth brought on by higher metabolic cost or more resources required. This causes the producers' growth rate to decrease below what is viable. Hence, while susceptible cells may be negatively affected by high concentrations of antibiotics, the producers are not growing fast enough to take advantage,

resulting in low absolute numbers of producers, and ultimately too little of the substance in the system to affect the susceptible cells to any significant degree. While *in vivo* experiments need to be done to verify these results, it seems logical that real bacteria would behave in this way as well. We know that bacterial toxins require a minimum inhibitory concentration to have an effect, and it is likely that high-production low-growth cells would likely go extinct due to inability to compete.

The system with only producer and susceptible cells had no conditions under which it was stable. Instead, the most biologically relevant situation occurs with the addition of the non-producer, allowing for all three cell types to coexist for long runs of the simulation. This case is a demonstration of how these niches can survive in real environments, and suggests two conditions for these systems to thrive. The first is an intermediate production rate of antibiotics – too high or low a production rate results in susceptible cells winning, as discussed previously. The wide range of possible production rates demonstrates that this does not need to be exceedingly specific, allowing some variation between and within systems. The second prerequisite is the existence of a third cell type that modulates the system. This allows cyclic dominance to occur, preventing one cell type from ever taking over. Biologically, it is easy to imagine such a case as well. We know cheaters arise due to mutation, horizontal gene transfer, or some other loss of function and such a non-producing cell can easily take up this niche. Moreover, we know that many cell types coexist in nature and thus a cell of an intermediate growth rate can also take up this role, even if not from the same species. The nearest neighbour model also allowed us many runs in order to demonstrate the most probably outcomes over a large sample size. The occurrence of regions where producers won close to 100% of the time indicates that it is possible to produce antibiotics in a sort of "sweet spot" to allow one species to win out, although in

reality this would eventually lead to non-producers becoming the dominant strain due to mutation as they would have no competition.

The decreasing equilibrium concentration of producers when running simulations at increasing production rates foreshadowed that an optimal production rate might exist. While the diffusion model was limited in demonstrating this due to computational constraints, introducing mutations into the nearest neighbour model demonstrated the way that these systems can evolve. Pure non-producers became replaced by a different kind of cheaters, instead becoming producers of low production rate. In biological systems mutations can cause gain of function or a decrease in function. As such, it is possible that a lower production rate creates the benefit of combating susceptible types to an extent, while still gaining the benefit of increased growth and synergizing with the higher production rate of non-cheaters.

In addition to this, it seems that producers in general will evolve to an optimal production rate. It was seen that high production was almost entirely eliminated – producing too much is too costly to be maintained even in a mixed pool of producers. On the other hand, those producing too little took on the role of cheaters, replacing complete non-producers. Instead, the system tended to a production rate towards the low end of what is viable for survivability. In all starting cases the system evolved towards the same production rate that allowed the system to survive and those producers became the most numerous. It can be expected that in real environments something similar may occur, with evolution driving producers to be the most competitive and allow the niches to survive with multiple cell types.

The introduction of the resistant cell type demonstrated that four cell types can survive, but increasing the growth rate of the resistant type decreases equilibrium concentrations of the producer. Moreover, for high growth rates it appears as if the susceptible cell type is favoured. In

a biological system this follows logically as well. If a resistant mutant were to evolve, it benefits the original phenotype in that it is capable of competing with the producers. However, the work on resistant cell types is very preliminary and these four-member systems must be considered more in depth. Many of the experiments done for the three-member systems should be repeated to better understand the dynamics.

Moreover, there is consideration to study other types of resistant cell types. Here, we considered only an intrinsically resistant variant that has a decreased growth rate, and a full resistance. Other considerations could be made, such as linking resistance to a cost and allowing it to operate on a gradient, similar to what we did in this thesis for production rate. Additionally, in biological systems there are other methods of resistance. It is worth studying this cell type also as a producer that secretes a substance that attenuates the effect of the antibiotics, allowing the diffusion model to be reused with two diffusible substances. There is also the case for intrinsic degraders, as some cells can take up antibiotics and break them down, such as in the case of β-lactamase producers. In this way the resistant types act as sinks, removing some of the antibiotics in the system and likely greatly benefiting the susceptible cells.

It also remains to be seen whether or not these models can be generalized to many species. Arguably, as long as rock-paper-scissors interactions are happening the system should thrive but actually demonstrating this would be useful in expanding the model. Indeed, we know that millions of cells across thousands of species can occupy the same niches so expanding the model would add biological validity.

Ultimately, we have demonstrated that bacterial niches composed of bacteria that should ostensibly kill each other are capable of surviving. It seems as if these systems need to occupy a specific range of parameter space but that when this sweet spot is reached, the niche can live

indefinitely. Moreover, it appears that these systems can evolve to an optimal production and cheater category, attenuating some of the effects of unnecessarily high production cost. Interestingly, cheaters are heavily punished for being complete non-producers. Instead, the cheaters produce a small amount of antibiotics to better compete with the susceptible cells. Hopefully the results contained in this work aid with the understanding of real bacterial systems, providing insight into the environments in which these organisms live. This can provide utility in better culturing techniques and can maybe lead to the discovery of novel antibiotics for human use.

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